Hilary Marusak *Editor*

Cannabis and the Developing Brain

Springer

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About the Editor

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Contributors

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Marc D. Ferger is a physician-scientist at the Departments of Child and Adolescent Psychiatry at the Universities of Ulm and Cologne, Germany, and a lecturer at Ulm University's Institute of Clinical Pharmacology. His research focuses on the endocannabinoid system in stress-related disorders, particularly nonsuicidal self-injury and PTSD in youth. He aims to advance pharmacological treatments through translational research, bridging preclinical studies and clinical applications. Marc has led early career initiatives in Germany's Society of Child and Adolescent Psychiatry and serves as an ambassador for the Association of Child and Adolescent Mental Health.

Jacqueline-Marie N. Ferland is an Assistant Professor in the Department of Psychiatry at the Icahn School of Medicine in New York. Dr. Ferland's research leverages translational preclinical models to determine mechanisms that contribute to addiction vulnerability with a focus on cannabis use disorder. Using multidisciplinary techniques ranging from robust behavioral profiling to in-depth molecular assessments, her team seeks to understand cell-specific mechanisms that drive maladaptive behaviors and the impact developmental cannabis exposure has on these outcomes. Dr. Ferland has 20 peer-reviewed publications in nationally recognized scientific journals including *Molecular Psychiatr*y and *JAMA Psychiatry* and has received funding from the National Institutes of Health to investigate the impact of adolescent THC exposure on cell-specific mechanisms underlying cannabis-induced cognitive deficits.

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Jodi M. Gilman, Ph.D., is an Associate Professor in Psychiatry at Harvard Medical School and the Director of Neuroscience at the Massachusetts General Hospital Center for Addiction Medicine, and the Director of the Postdoctoral Division at Massachusetts General Hospital. She received her Ph.D. in Neuroscience from Brown University. Her research uses multimodal imaging, behavioral and cognitive testing to understand the biological, psychological, and clinical aspects of addiction. Specifically, she studies the effects of cannabis use on addiction and other health outcomes. Her goal is to conduct research that can inform decision-making among the public, patients, and clinicians regarding evidence-based decisions to use or not use cannabis for recreational or medical purposes.

Connor J. Haggarty, Ph.D., is a postdoctoral scholar at Wayne State University. He began his research career completing a Ph.D. in cognitive neuroscience at Liverpool John Moores University, UK. Using the research skills acquired in his training, Dr. Haggarty then relocated to Sweden where he began working on a clinical trial into the efficacy of FAAH inhibition (to increase the levels of the endocannabinoid, anandamide) and prolonged exposure therapy for the treatment of PTSD. He then moved to Chicago to work with Harriet de Wit looking at the role of various drugs in social motivation and behavior, here running projects with methamphetamine, MDMA, and LSD. More recently, Dr. Haggarty began a postdoctoral position with Dr. Rabinak at Wayne State University. Here, we run several studies looking at the role of cannabinoids and prolonged exposure therapy in the treatment of PTSD.

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Iveta Kopil, M.S., serves as the Director of Operations for the Office of Women's Health at Wayne State University. She has over 12 years of clinical research experience and is very passionate in helping women, especially during the time of pregnancy and the perinatal period. Through her leadership position as a director, she has been serving the Detroit community by promoting access to social and health support services and resources.

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Joseph Lanigan holds a B.A. in Psychology and Medicine, Health, and Society from Vanderbilt University. As a research assistant in the Schaefer Lab, he studied the link between cannabis use and mental health outcomes, focusing on twin studies. He also worked at Vanderbilt's Social Emotional NeuroScience Endocrinology Lab, assisting with data collection on social and emotional functioning in children with autism and facilitating intervention programs to improve their social interaction. Joseph is currently pursuing higher education with the goal of becoming a licensed mental healthcare provider.

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Krista M. Lisdahl, a Professor of Psychology and Neuroscience, is the Director of the University of Wisconsin-Milwaukee's Brain Imaging and Neuropsychology (BraIN) Laboratory. Her primary research focus has been on examining risk and resilience factors that predict the onset of adolescent substance use, as well as characterizing the effects of chronic cannabis and poly-substance use on neurocognition during adolescence. Her research also attempts to explain individual differences by examining whether genetics, sex-at-birth, or lifestyle factors moderate these effects. Dr. Lisdahl's work has been nationally recognized and utilized by public health prevention and policy efforts.

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Georgia Watt is a postdoctoral researcher in the field of behavioural neuroscience. Her research focuses on evaluating the therapeutic potential of medicinal marijuana for neuropsychiatric and neurodegenerative conditions.

Halle V. Weimar is a postdoctoral fellow at the University of Idaho. She earned her Ph.D. in Neuroscience from Washington State University. Her doctoral work examined the impacts of in utero cannabis vapor exposure on emotional behavior, drug-seeking behavior, and neurobiology in rat offspring. Her research on the impacts of prenatal cannabis exposure has resulted in two peer-reviewed publications to date, one using a passive vapor model and another establishing a volitional, response-contingent vapor self-administration model.

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propose enhancements, including motion correction and subject education. Stefania is dedicated to integrating environmental and neurobiological research to inform mental health care.

Clara G. Zundel, Ph.D., is a behavioral neuroscientist whose research focuses on uncovering the neurobehavioral mechanisms linking environmental toxicants (e.g., air pollution) to increased risk for psychiatric disease, across the lifespan. Dr. Zundel is currently a postdoctoral research fellow at Wayne State University School of Medicine in the Department of Psychiatry and Behavioral Neurosciences.

Part I
Foundations of Cannabis Science and the Developing Brain

Chapter 1
Introduction to Cannabis and the Developing Brain



Hilary Marusak

1 Introduction to the Topic

Cannabis is one of the most widely used psychoactive substances worldwide, with its use accelerating amidst shifting landscapes of legalization and evolving public perceptions. While cannabis and its components are increasingly studied for potential medical applications, particularly in treating conditions like epilepsy and chronic pain, its effects on developing brains and bodies remain less understood.

Unique challenges arise when cannabis is used during critical windows of neurodevelopment, such as the perinatal period and adolescence. These stages represent heightened susceptibility to external influences, making it crucial to understand the potential risks and implications of cannabis exposure.

This book provides a comprehensive exploration of cannabis as a complex plant, examining its historical context, key chemical components, and basic neurobiology. It delves into the endocannabinoid system's role in brain development and psychiatric vulnerability, the consequences of prenatal cannabis exposure, and the effects of adolescent use. In addition, topics such as cannabis use disorder (CUD), secondhand cannabis exposure, impacts on seizure disorders and neonatal pain

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management, psychotic disorders, and insights from animal models and twin studies are discussed.

Finally, the book offers practical insights for clinicians on how to navigate conversations about cannabis use and provides a framework for understanding its implications for public policy, education, and future research.

2 Overview of the Book

Cannabis and the Developing Brain offers a comprehensive exploration of the intricate relationship between cannabis use and neurodevelopment, combining foundational science with practical insights to guide education, policy, and clinical practice. The book is organized into six interconnected parts, each examining a distinct dimension of cannabis use and its impact on the developing brain.

Chapters are authored by leading or emerging experts in pharmacology, psychiatry, neuroscience, and cannabis science, featuring studies in both human populations and animal models. Contributions also include insights from clinicians specializing in substance use and psychiatric outcomes during adolescence and pregnancy, as well as experts in cannabis education and policy. Together, the chapters present the latest scientific findings, contextualized within historical, social, and clinical frameworks, providing a multidimensional perspective on this rapidly evolving field.

2.1 Part 1: Foundations of Cannabis Science and the Developing Brain

The book begins by establishing a foundation for understanding cannabis and its complex relationship with brain development. It begins with an introduction to cannabis and its history (Chap. 2), followed by an overview of the endogenous cannabinoid signaling system (Chap. 3), a vital network for neurodevelopment. The subsequent chapters explore how the endocannabinoid system influences early brain development (Chap. 4) and continues to shape neurodevelopment during postnatal periods (Chap. 5). This section provides readers with the necessary scientific background to appreciate the complexities of cannabis' effects on the brain.

2.2 Part 2: Prenatal and Early Life Effects

This section examines the consequences of prenatal and early life exposure to cannabinoids. Chapters investigate the behavioral and neurodevelopmental effects of prenatal cannabis exposure (Chaps. 6, 7 and 8), including insights from animal

models and the implications of routes of administration. Further, it explores the therapeutic applications of cannabinoids in neonatal pain management (Chap. 9) and pediatric epilepsy treatment (Chap. 10), offering a balanced view of both risks and potential benefits.

2.3 Part 3: Adolescence as a Sensitive Period

Adolescence is a critical window of vulnerability to cannabis exposure. This section highlights the effects of cannabis on cognitive development (Chap. 12) and the heightened risks of cannabis use disorder during this developmental stage (Chap. 13). Insights from animal studies (Chap. 11) shed light on the mechanisms underlying these vulnerabilities. Additional focus is given to the interplay between early cannabis use and developmental brain disorders like schizophrenia and autism spectrum disorder (Chap. 14).

2.4 Part 4: Psychiatric Risks and Vulnerabilities

This section investigates the links between cannabis use and psychiatric risks, emphasizing the role of the endocannabinoid system in contributing to mental health vulnerabilities (Chap. 15). Subsequent chapters explore cannabis use and its associations with psychiatric symptoms in adolescence (Chap. 16), suicidal thoughts and behaviors (Chap. 17), and the risk of psychotic disorders (Chap. 18). Challenges in disentangling causality from confounding factors are discussed, with particular attention to the utility of twin studies (Chap. 19).

2.5 Part 5: Social and Environmental Contexts

Cannabis use does not occur in isolation but is deeply embedded within social and environmental contexts. This section examines how factors like childhood maltreatment (Chap. 20), secondhand cannabis smoke exposure (Chap. 21), and minority stress (Chap. 22) interact with cannabis use to shape physical, neurodevelopmental, and mental health outcomes.

2.6 Part 6: Education, Policy, and Practice

The final section provides a forward-looking perspective, connecting science to education, clinical practice, and public policy. It includes strategies for engaging teens, parents, and healthcare providers in discussions about cannabis (Chap. 24), as

well as a review of cannabis education and reproductive health (Chaps. 23 and 25, respectively). The book concludes with an author's review of cannabis policy in the age of legalization (Chap. 26) and an analysis of how scientific findings summarized in this book can inform public policy (Chap. 27). This includes a reflection of broader implications of this work and future research directions.

Chapter 2
Cannabis: A History



Connor J. Haggarty and Christine Rabinak

Abbreviations

CB1/CB2	Cannabinoid receptors 1 and 2
CBD	Cannabidiol
CBG	Cannabigerol
CBGA	Cannabigerolic acid
CBN	Cannabinol
D9-THC	D9-Tetrahydrocannabinol
ECS	Endocannabinoid System
THC	Tetrahydrocannabinol
THCA	Tetrahydrocannabinolic acid

1 Components of Cannabis

Cannabis sativa L., commonly known as marijuana (also colloquially known as pot, weed, grass, dope, reefer, Mary Jane, Devil's lettuce, ganja, skunk), comprises a myriad of components that contribute to its complex nature. This versatile plant contains various cannabinoids, terpenes, and other organic compounds, each playing a distinct role in its physiological effects and therapeutic potential. In fact, because of these various properties, the plant has been used recreationally and medicinally for millennia prior to any empirical studies into its various components and effects being ever discovered. While cannabinoids like tetrahydrocannabinol (THC) and cannabidiol (CBD) are often the focus of attention, the plant's diverse array of terpenes and flavonoids also contribute significantly to its aroma, flavor, and overall pharmacological profile. Together, these components interact synergistically in what is commonly referred to as the "entourage effect," influencing the

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plant's diverse range of effects on the body and mind. Below we discuss some of the most used compounds and their behavioral profiles (Fig. 2.1).

1.1 Cannabis Sativa, Indica, and Ruderalis

Not only does the cannabis plant contain around 200 different compounds and terpenes each with unique properties, but there are also different strains of the plant itself. Despite much discussion on how related these plant strains are, there are several differences in the way they grow and the psychoactive properties of these different strains which highlight the unique taxonomy of each plant. *Cannabis Sativa*, for example, grows significantly larger (up to 12′) than the other strains and contains fewer branches and flowers for longer periods than indica varieties, making it easier to harvest. *Cannabis Sativa* is associated with uplifting, energizing effects and is typically used during the day to promote wakefulness, creativity, and enhance mood (National Academies of Sciences 2017). *Cannabis Indica* on the other hand is characterized as shorter, bushier plants with significantly less flowering time. Morphologically, the buds on these strains are much denser and often contain higher levels of CBD relative to THC, whereas the opposite is true for *Cannabis Sativa* strains, which contain higher levels of THC relative to CBD. *Cannabis Indica* is often referred to as "couchlock" as it is known for its highly relaxing properties,

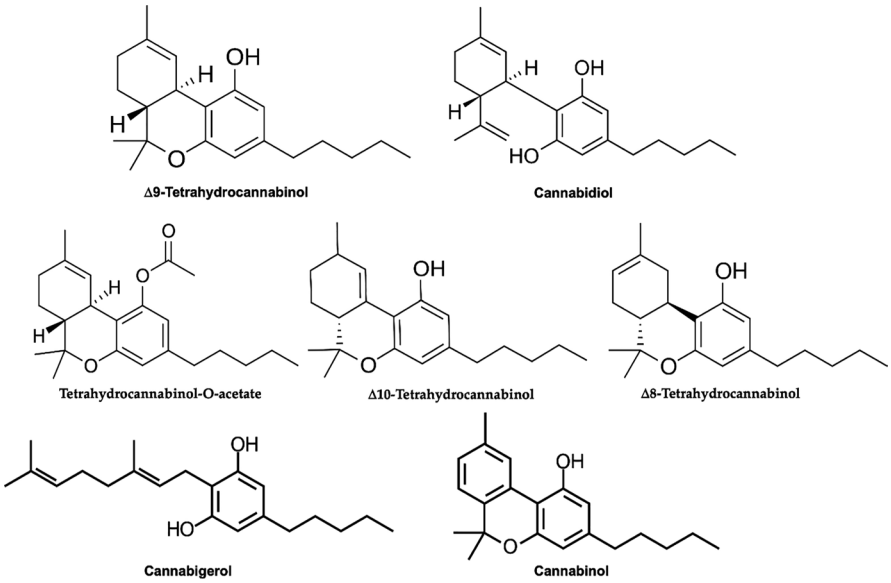


Fig. 2.1 Chemical structure of D9-THC, CBD, THC-O, D10-THC, D8-THC, CBG, and CBN Structural differences and binding potential to CB1/CB2 receptors in the body result in varied effects of these compounds

causing individuals to become sleepy and relaxed, this strain is often used at night to promote relaxation and sleep. Some recorded uses for *Cannabis Indica* include the alleviation of insomnia, anxiety, muscle spasms and chronic pain, potential increases in appetite, and relief of nausea (National Academies of Sciences 2017). This strain is often used by patients with a chronic/terminal illness such as HIV/AIDS and cancer to reduce pain and promote appetite (Mack and Joy 2000). *Cannabis Ruderalis* is a lesser known and used strain. It is significantly smaller than both Sativa and Indica varieties and contains far less THC, which may be why it is less commonly used in recreation or medical use. *Cannabis Ruderalis* is reported to contain high concentrations of CBD and is self-flowering, which, unlike other strains, means that it is ready to harvest once it is matured and not through more complex growing mechanisms. *Cannabis Ruderalis* is often used in hybridization and breeding between strains to promote faster flowering and researchers are beginning to explore the therapeutic potential of this strain, however research in this area is scarce (Small 2015).

1.2 Tetrahydrocannabinol

THC is the primary intoxicating constituent of cannabis. While the regulated isomer D9-THC is the most discussed, this is just one psychoactive isomer, and each isomer has unique properties that affect individuals differently. Despite being widely available recreationally and medicinally in the United States, only one cannabis-derived product, Epidiolex® (CBD), and three synthetic cannabis-related drug products—Marinol (dronabinol), Syndros (dronabinol), and Cesamet (nabilone)—are currently FDA approved and are used specifically for the reduction of nausea and vomiting (associated with chemotherapy treatment), for appetite loss (for individuals with HIV/AIDS) and as an anticonvulsant (associated with Lennox-Gastaut syndrome, a rare treatment resistant childhood epilepsy). Anecdotally, D9-THC has been used recreationally for millennia with records of its use dating back to ancient China, around 2000 B.C (Crocq 2020). Often the cannabis plant was used for its strength and durability as fibers for clothing, but the medicinal properties are further reaching and versatile.

THC itself is subcategorized with D9-THC being the federally regulated psychoactive component due to its high concentration in the cannabis plant. However, there are several federally unregulated varieties, including D8-THC, D10-THC, D11-THC, D6a-THC, and THC-O-acetate. Most of these varieties are less potent than D9-THC and are found in smaller quantities but have unique characteristics. For example, D8-THC, which is sold over the counter, is suggested to provide a clearer-headed high with less anxiety or paranoia that some cannabis users' experience (Kruger and Kruger 2023). Little is known about D10-THC except that its chemical structure closely resembles that of D9-THC but with a double bond on the 10th (not the 9th) position isomer. It is commonly considered an impurity from the synthesis of D8-THC produced from CBD (Haghdoost et al. 2023) however, recent patents

suggest it can also be synthesized directly from D9-THC by migration of the double bond isomer (Adair et al. 2020). On the other hand, THC-O-acetate is a semisynthetic compound, meaning that something has been added to the natural compound to make this one. Specifically, THC-O-acetate is produced through the acetylation of D9-THC and D8-THC and is considered incredibly potent but with a delayed onset. Research is still ongoing for this constituent, so it is not possible to ascertain the relative benefits or risks of such a product. However, considering the anecdotal evidence of its heightened potency, caution is recommended when using such a product. More research is required to understand how THC affects people differently and the potential risk factors for developing adverse side effects.

1.3 Cannabidiol

Cannabidiol (CBD) is touted as a panacea curing aches, pains, anxiety, and all manner of other ailments. In recent years, CBD products have been sold online and in stores around the world with all manner of medicinal properties listed. CBD is a victim of the digital era, influencers across the world push products with CBD in them with varied claims of their success in curing all sorts of problems. However, science does not strictly support any of these claims. We know very little about CBD and indeed not enough to support how it is sold. What we do know is that CBD does not appear to bind to cannabinoid receptors in the brain as THC does, making it less intoxicating, but also meaning its direct effects are unknown (Thomas et al. 2007). In fact, it is important to note that CBD (Epidiolex® is only FDA-approved for Lennox-Gaustaut syndrome, a treatment-resistant pediatric epilepsy (Patra et al. 2019; Rosenberg et al. 2023). It works to treat seizures by reducing hippocampal hyperactivity via modulation of the inhibitory-excitatory ratio in these neurons. A recent review of (Paton 1975) available products containing CBD reported a major concern in the labeling of these products with 18% over-labeled CBD concentration, 58% under-labeled CBD concentration and 35% of products also containing THC (Spindle et al. 2022). Legally, CBD-containing products can have up to 0.3% THC to qualify as an unregulated hemp product, and while most of these products do contain some level of THC, it is often not reported. With little regulation, it is difficult to "police" the increasing interest and availability of CBD products.

Despite the unproven claims that CBD can cure all manner of conditions on its own, it is important to consider the role that it is having as many individuals report positive effects and most importantly what its role is in cannabis consumption and the subsequent effects. While D9-THC is the primary intoxicating component in cannabis, this makes the study of CBD more complicated. After all, researchers will often use questionnaires such as the drug effects questionnaire (Morean et al. 2013) to gauge the subjective experiences participants are having on a drug and use this information to inform behavioral changes. In fact, CBD may specifically be an important entourage molecule (Ferber et al. 2008) adding to or modulating some of the effects of D9-THC dominant strains. Research into CBD using Epidiolex® is

ongoing to determine the potency of effects and the expected benefits in adults as well as infants. The effects of CBD are discussed in Sect. 2.1.

1.4 Cannabigerol/Cannabinol

Cannabigerol (CBG) and Cannabinol (CBN) are lesser-known compounds but have been shown to have unique properties that affect individuals differently. Both cannabinoids are precursors, meaning that they are intermediate compounds in the pathway leading to active cannabinoids in the cannabis plant. Subsequently, this makes them important in the potency and relative concentrations of compounds in the plant. CBG is often referred to as the "mother cannabinoid" because it is the precursor to other cannabinoids such as THC and CBD. CBG is found in smaller quantities than THC and CBD, but it has been gaining significant attention given its potential therapeutic properties. To date, CBG has been linked to anti-inflammation (Lowin et al. 2023), neuroprotective properties (Echeverry et al. 2021), antibacterial properties (Nachnani et al. 2021) and appetite stimulation (Brierley et al. 2016). CBN is derived from cannabigerolic acid (CBGA), a precursor cannabinoid, and it is often formed through the degradation of THC and CBD when exposed to oxygen and heat, which results in CBN (García-Valverde et al. 2022). This process of extraction explains why poorly stored cannabis tends to have higher levels of CBN. Unlike other cannabinoids (THC excluded), CBN has mild psychoactive properties, is well known for its sedative effects, (Lavender et al. 2023) and is often consumed as a sleep aid. Other potential applications for CBN include pain relief (Ghovanloo et al. 2024), appetite stimulation (Farrimond et al. 2012), and antibacterial effects (Appendino et al. 2008).

1.5 Terpenes

Terpenes are a diverse class of organic compounds produced by a wide variety of plants including cannabis. They are responsible for the characteristics of different strains of a plant and subsequently affect the flavor, smell, and even the therapeutic potential of cannabinoids. It is believed these terpenes may not directly impact the therapeutic potential of cannabis but rather provide an entourage effect enhancing the properties of cannabinoids (Sommano et al. 2020; Hanuš and Hod 2020; LaVigne et al. 2021). The terpenes are the reason there are so many "flavors" and types of cannabis, as often these are naturally occurring but can be manipulated through hybridization and genetic modifications (Parsons et al. 2019; Booth et al. 2020; Ingvardsen and Brinch-Pedersen 2023). Myrcene is one of the most abundant terpenes in cannabis. It has an earthy, musky aroma with hints of cloves. It is believed to have relaxing and sedative effects and may contribute to "couchlock" (a state of idleness, laziness or inactivity) often associated with *Cannabis indica*

strains (Surendran et al. 2021). Limonene gives off a citrusy aroma, reminiscent of lemons and is commonly found in strains that produce uplifting and energizing effects. Limonene is also believed to have mood-enhancing properties and may help reduce stress and anxiety (Lorigooini et al. 2021). Pinene has a pine-like aroma. It comes in two varieties: alpha-pinene and beta-pinene. Alpha-pinene is also found in pine trees, while beta-pinene has a spicier scent. Pinene is associated with increased alertness and may help counteract some of the short-term memory impairment associated with THC consumption (Salehi et al. 2019). Linalool has a floral aroma with hints of lavender. It's known for its calming and relaxing effects and may help alleviate anxiety and stress. Linalool is also found in lavender and is commonly used in aromatherapy (dos Santos et al. 2022). Caryophyllene has a spicy, peppery aroma with hints of wood and cloves. It's unique among terpenes because it can also bind to cannabinoid receptors, particularly the CB2 receptor, which is why it's sometimes referred to as a "cannabinoid." Caryophyllene is believed to have anti-inflammatory and analgesic properties (Fidyt et al. 2016). Given that this is just a small fraction of the ~140 terpenes reported in cannabis, it is apparent that the concentration and combination of these can result in a wide variety of effects not just related to scent and flavor.

2 Mechanisms of Cannabis

The endocannabinoid system (ECS) is a fascinating biological system found in all vertebrate animals, including humans. It plays a crucial role in regulating various physiological processes such as mood, memory, appetite, pain sensation, and even fertility. The ECS consists of three main components: endocannabinoids (ECBs), receptors, and enzymes. The two primary ECBs circulating in the body are anandamide (AEA) and 2-arachidonoylglycerol (2-AG). These molecules are essential for the normal functioning of the ECS, even in the absence of cannabis exposure. Recent evidence suggests that the circulating levels of ECBs vary between individuals, which influence stress resilience and pain perception (Hill et al. 2023; Spagnolo et al. 2016; Zabik et al. 2022; Mayo et al. 2020). Genetic polymorphisms such as C385A, are known to affect the degradation of AEA by reducing the activity of fatty acid amide hydrolase (FAAH), an enzyme responsible for breaking down AEA (Zabik et al. 2022; Conzelmann et al. 2012; Mayo et al. 2022).

2.1 Cannabis in the Brain

Understanding the effects of cannabis on the brain is crucial due to its widespread use and increasing legalization for both recreational and medicinal purposes. Exogenous cannabinoids in cannabis, which are introduced to the body via consumption (through smoking, vaping, ingesting), modulate the ECS and the

circulating ECBs within this system. Upon ingestion, Δ9-THC binds to receptors in the ECS, influencing neuronal signaling and subsequent neurotransmitter release. Understanding the mechanisms by which cannabis compounds affect brain function is essential for assessing both their therapeutic potential and the risks associated with their use.

The ECS consists of cannabinoid type 1 (CB1) and type 2 (CB2) receptors (Fig. 2.2). CB1 receptors are primarily found in brain regions associated with cognition, such as attention, perception, memory, language, and thinking (Freund et al. 2003; Haspula and Clark 2020; Howlett et al. 2004; Tsou et al. 1998; Zou and Kumar 2018). CB2 receptors are mainly located in peripheral tissues, such as the gastrointestinal tract, liver, and adipose tissue, where they regulate functions like appetite, metabolism, and energy balance (Galiègue et al. 1995; Munro et al. 1993; Spigelman 2010). More recently, CB2 receptors have also been identified in the central nervous system, where they are thought to modulate pain management and immune responses without producing psychoactive effects (Pandey et al. 2009; Ashton and Glass 2007; Atwood and Mackie 2010).

Several clinical trials have shown the benefits of pharmacologically manipulating the ECS without the use of cannabis. For instance, studies have demonstrated reduced stress, anxiety, and emotional reactivity when manipulating ECBs like AEA and 2-AG (Lutz 2009). Unlike other neurotransmitter systems, ECBs are not stored in presynaptic vesicles but are synthesized on demand when the system is activated. This unique "made-to-order" feature ensures that the ECS produces and utilizes ECBs only as needed, preventing overstimulation and waste. However, genetic polymorphisms can influence the quantity, availability, and activity of ECBs, leading to individual differences in ECS function (Conzelmann et al. 2012; Carey et al. 2015).

Now, let's consider how exogenously derived cannabinoids, such as those in cannabis, interact with the ECS. Cannabis floods this system in a nonspecific manner. Δ9-THC partially binds to CB1 receptors in the brain and affects many of the cognitive processes mentioned above in a dose-dependent manner. While the ECS is beneficial in regulating stress and emotional responses, cannabis does not discriminate in its activation of these roles. Consequently, users may experience euphoria, reduced stress, and relaxation regardless of the context. However, this indiscriminate activation can lead to side-effects such as increased anxiety, impaired short-term memory, difficulty concentrating, and altered time perception (Hall and Degenhardt 2009; Volkow et al. 2014). Long-term effects of cannabis use include increased risk of anxiety, depression, cognitive impairments, dependence, and respiratory conditions (Crean et al. 2011; Karila et al. 2014; Kroon et al. 2021). Although research on the risk factors for these long-term effects is still ongoing, scientists are investigating how ECS function and ECB availability may influence sensitivity to cannabis and its short- and long-term effects. This raises the question of whether cannabis is a suitable alternative to current medications. While there are many potential benefits, side effects often pose challenges to its approval and use. Alternative solutions could involve targeted activation of CB1 or CB2 receptors in

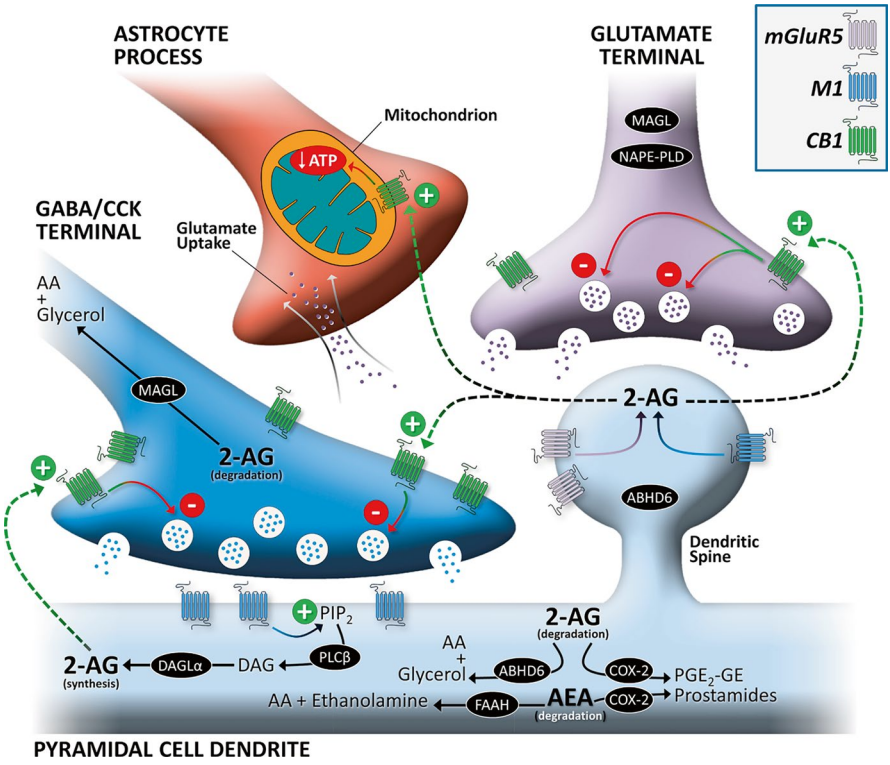


Fig. 2.2 Schematic depicting the positioning of components of the endocannabinoid system at the synapse. Diagram displaying both inhibitory and excitatory terminals synapsing onto the dendritic shaft of a typical cortical pyramidal neuron. The augmented quantity of CB1 receptors on the CCK/GABA terminal signifies the heightened density of CB1 receptors observed on these axon terminals. Various molecular components are labeled, including AA (arachidonic acid), ABHD6 (alpha/beta domain-containing hydrolase 6), 2-AG (2-arachidonoyl glycerol), ATP (adenosine triphosphate), CB1 (CB1 cannabinoid receptor), CCK (cholecystokinin), COX-2 (cyclooxygenase-2), DAG (diacylglycerol), DAGLα (diacylglycerol lipase α), GABA (γ-aminobutyric acid), M1 (M1 muscarinic receptor), MAGL (monoacylglycerol lipase), mGluR5 (metabotropic glutamate receptor 5), FAAH (fatty acid amidohydrolase), NAPE-PLD (N-arachidonoyl phosphatidyl ethanolamine-preferring phospholipase D), PGE2-GE (prostaglandin E2 glycerol ester), PIP2 (phosphatidyl inositol bis-phosphate), PLCβ (phospholipase C β) (Lu and Mackie 2016). *Reproduced with permission from Elsevier*

the brain to minimize psychoactive side effects (Zou and Kumar 2018). AEA, for example, has been proposed as a promising target for stress- and trauma-related conditions. This is particularly exciting because AEA has no known subjective effects, meaning that it doesn't interfere with clinical trials, so it does not hinder clinical trials, nor is it likely result to in unwanted side effects (Mayo et al. 2022).

Cannabis has the potential for dependence, but its addictive properties differ from those of substances like opioids or nicotine. In addition, the euphoric feelings associated with consumption themselves become addictive. Anecdotally, for

example, consider individuals with heightened anxiety, such as those with social anxiety or generalized anxiety disorder; these conditions produce high levels of anxious arousal during many ordinary situations, but if cannabis were able to provide some anxiolytic effects, then one might use it as a crutch to minimize negative effects. The act of taking cannabis then becomes habit through regular use, subsequently resulting in a motivation to use it regularly to counteract the anxiety. Also, the more a psychoactive substance is used, the more likely side effects are to occur through withdrawal (Zehra et al. 2018). It is possible to argue that drugs with no known physiological addiction mechanism are more difficult to treat. Therefore, many researchers over the world are working to determine how best to combat cannabis use disorder. Current advances suggest blocking of CB1 receptors in the brain, or indeed manipulation of ECBs (Haney et al. 2023).

2.2 Cannabis in the Body

As mentioned earlier, ECS receptors are not exclusive to the CNS. Many areas of the peripheral nervous system (PNS), including peripheral nerves, the gastrointestinal tract, and adipose tissue, are rich in CB2 receptors. These receptors are likely involved in immune responses and have effects that are distinct from the psychoactive effects of CB1 receptors found in the brain (Bie et al. 2018). So, what does cannabis do to the PNS? Below, we explore some of these potential effects.

2.2.1 Pain

Pain is a complex physiological mechanism beginning with activation of peripheral nerve fibers. The Aδ and C-nociceptor fibers do not directly encode pain; they merely send signals that the brain interprets as pain (Garland 2012). The subjective experience of pain is only formed when these nociceptive signals reach the brain. While this opens multiple possibilities for pain treatment, it also presents challenges. For instance, traditional pain medications block these signals at the level of the spinal cord, reducing the frequency and intensity of the signals reaching the brain. However, this approach merely masks the pain rather than addressing its root cause. There are also conditions, such as fibromyalgia, where individuals experience pain without peripheral nerve stimulation. Fibromyalgia, often triggered by catastrophic injury or prolonged intense pain, is believed to involve chemical changes in the brain that result in the persistent activation of pain regions, even after the initial physical damage has healed (Staud 2002). Cannabis has been suggested as an alternative to opioids for pain relief, though this remains speculative (Bains and Mukhdomi 2024). While THC binds to CB1 receptors in the PNS and can trigger the release of inflammatory factors (Miller et al. 2020), evidence linking cannabis to consistent pain relief is still inconclusive. For example, a large-scale study examining chronic pain and drug use found that approximately 60% of participants

used cannabis to manage their pain, yet there was no significant reduction in pain levels reported (Campbell et al. 2018). Interestingly, the cannabis users in this study were those experiencing the highest levels of pain. A meta-analysis of 79 clinical trials, involving 6462 patients, did not show a reduction in acute pain (Whiting et al. 2015). These findings suggest that cannabis may be more effective in treating specific types of pain, such as inflammatory pain, though its impact on neuropathic pain has also shown promise in some studies (McQuay 2010). It is important to note that many individuals using cannabis for chronic pain may benefit more from its effects on mood and sleep rather than direct pain relief (Ware et al. 2010).

2.2.2 Appetite

Certain conditions and medications can suppress appetite. Cannabis is well known for inducing the "munchies," a side effect that can be beneficial for individuals with treatment-resistant nausea or appetite loss. For instance, the synthetic THC drug, Dronabinol (sold as Marinol® and Syndros®) is FDA-approved for appetite stimulation in cancer patients and those with HIV-/AIDS-related anorexia. This suggests that the ECS system plays a significant role in regulating appetite. Research shows that manipulating ECB levels can increase appetite (Järbe and DiPatrizio 2005; Kirkham 2005; Kirkham 2009; Lambert and Muccioli 2007). In the CNS, the hypothalamus and the release of dopamine are responsible for cannabis-induced appetite increases (Di Marzo et al. 2001; Kirkham and Williams 2001). In the PNS, CB1 receptors in the gastrointestinal tract influence gut motility and the secretion of appetite-stimulating hormones like ghrelin (Pagotto et al. 2006). CB1 receptors in adipose tissue also affect lipid metabolism and energy storage, which indirectly promote appetite by signaling the brain to increase food intake when energy reserves are low (Bellocchio et al. 2008; DiPatrizio and Piomelli 2012).

2.2.3 Muscle Relaxation

Several conditions, such as Parkinson's disease, Huntington's disease, and multiple sclerosis, are characterized by involuntary tremors due to the degeneration of dopamine-producing neurons in the substantia nigra, which affects the motor cortex and basal ganglia (Helmich et al. 2012). Anecdotal reports suggest that cannabis can help control these tremors (Arjmand et al. 2015). In the PNS, CB1 receptors at neuromuscular junctions modulate the release of acetylcholine, a neurotransmitter responsible for muscle contractions (Ashton and Milligan 2008; Nielsen et al. 2019). Although Δ9-THC drugs have not been formally approved for these conditions, cannabis is often included under medical marijuana regulations for the treatment of motor-related issues (What medical conditions are eligible? [Internet] 2024). Stress- and trauma-related conditions can also lead to restless leg syndrome (RLS), characterized by tingling, pain, and involuntary leg movements, especially

at rest. Clinical trials have shown that cannabis can reduce the symptoms and duration of RLS while promoting sleep (Koppel et al. 2014; Samaha et al. 2020; Ghorayeb 2021).

3 A History of Cannabis Use

3.1 Recreational Cannabis Use

Cannabis has been used recreationally for thousands of years. Its origins are believed to be in Central Asia, with evidence of use dating back to 2700 BC, where it was valued both for its medicinal and psychoactive properties (Charitos et al. 2021). The earliest known classification of cannabis appears in the *Shennong Bencaojing, an ancient Chinese pharmacopeia with over 365 entries* on *plants and their uses. The text* describes *cannabis (referred to as Má,* 麻) as a treatment for ailments like constipation, rheumatic pain, and disorders related to the female reproductive system. It also mentions its psychoactive effects, describing a "lightness" and euphoria, suggesting it was used both for medicinal and ritualistic purposes. Around that same time, cannabis is mentioned in ancient Hindu texts known as the Vedas (c. 2000–1000 BC) (Touw 1981). Specifically, the *Atharva Veda,* a text on health, healing and rituals, references cannabis as a component of "Soma," a ritual drink and as "Bhanga," one of five sacred plants. *Bhanga* is revered for its medicinal properties and was considered a source of happiness and liberation. According to Hindu mythology, these sacred plants were brought down from the Himalayas by the god Shiva, and cannabis became a part of religious ceremonies like Diwali and Kumbha Mela. Similarly, in other ancient Asian civilizations, cannabis was used for various purposes, including in clothing, medicine, and rituals (Brand and Zhao 2017). Recent archeological discoveries in eastern Asia unearthed traces of cannabis in pots and braziers estimated to be 3500 years old, suggesting its use in burial rituals (Ren et al. 2019). In addition, clay artifacts from this period show evidence of hemp fibers being worked into the pottery to increase strength. In ancient Greece, the historian Herodotus (c. fifth century BC), described ritualistic cannabis use among the Scythians, an ancient civilization in modern-day Iran. They reportedly threw cannabis seeds onto hot stones to produce a "hallucinogenic vapor" (Magazine and Solly 2024).

Throughout history, various cultures have documented the use of cannabis. By the tenth century, it was mentioned frequently in Islamic texts from the Middle East. The *Avicenna*, a cornerstone of Islamic medicine (c. 980–1037 AD), noted the use of cannabis to treat headaches, joint pain, infections, gout, and uterine pain (Mahdizadeh et al. 2015). From the Middle East, explorers introduced cannabis to Africa, beginning with the Islamic nations such as Egypt, which had extensive trade routes. Centuries later, the Dutch East India Company brought cannabis seeds to southern Africa when establishing a colony there (Duvall 2019). Today, South

Africa is one of the few nations in continental Africa where cannabis is legalized for both recreational and medicinal use.

In modern times, cannabis is widely used across the globe. Estimates suggest that around 209 million people use cannabis recreationally, making it one of the most popular non-prescription drugs (World Drug Report 2022 2024). In a 2022 survey by the Substance Abuse and Mental Health Services Administration, 128.96 million Americans reported using cannabis at some point in their lives, with 53.18 million using it in the past year (National Survey on Drug Use and Health 2024). Cannabis remains the most commonly used illicit substance, with opioid misuse (excluding illegally produced fentanyl) affecting 9.42 million people. This number of cannabis users has increased significantly, rising from 37.57 million 5 years earlier, as cannabis legalization expands (National Survey on Drug Use and Health 2024). Alongside this rise in use, rare but persistent side effects such as psychosis (Forti et al. 2019) and anxiety (Myran et al. 2024) have become more common. However, it's important to note that the individuals most at risk for these side effects are not fully understood, with evidence suggesting that adolescent use or prenatal exposure may be significant predictors of future mental health issues (Hengartner et al. 2020; Evanski et al. 2024; Jacobus and Tapert 2014). Legalization may impact usage rate. In Europe, where few countries have legalized recreational cannabis, usage remains lower. In 2022, less than 12% of individuals in each country reported cannabis (e.g., Czech Republic: 11.1%; Italy: 10.8%; France: 10.6%; Netherlands: 10.2%; Germany: 8.8%) (Statista 2024a).

3.2 Cannabis Use in Research

Over the past 80 years, cannabis research has made significant strides. Interest in studying cannabis was sparked by the discovery and synthesis of CBD by Robert Cahn (Hengartner et al. 2020), followed by the identification of THC by Raphael Mechoulam, often called the "grandfather" of cannabis research (Mechoulam and Shvo 1963; Mechoulam et al. 1970). Since then, over 35,000 scientific papers on cannabis have been published (as estimated from PubMed). These early studies laid the foundation for our understanding of cannabis, the ECS, and their interactions (Pertwee 1988; Paton 1975; Maickel 1973). While cannabis research has grown exponentially, progress in uncovering its pharmacotherapeutic benefits has been slow. Below is a timeline of key developments in cannabis research over the last 80 years. In the nineteenth century, W.B. O'Shaughnessy, during his time in India, documented the effects of cannabis on the digestive and cerebral systems (O'Shaughnessy 1843). His experiments included administering cannabis to dogs and children to observe its effects. While he noted intoxication, he also reported that symptoms subsided without pain or convulsions during the peak effects. O'Shaughnessy's observations, which covered conditions like tetanus, rheumatological issues, cholera, and infantile convulsions, suggest that cannabis was being considered as a therapeutic agent long before modern research. In the late

nineteenth century, a team at Cambridge University in the United Kingdom applied fractional distillation to the cannabis plant, isolating a viscous red liquid called the "red oil." This substance, found to be highly active even in small doses, induced intoxication and sleep (Wood et al. 1896). It is believed to be the first instance of THC and other cannabinoids being isolated, although the researchers did not fully understand the composition of this "red oil" at the time. They named one of the crystalline components "cannabinol" (CBN), which was initially classified as a narcotic. Research into cannabis at Cambridge University ceased after the death of one of these seminal researchers and the emigration of another.

In the 1930s, British chemist Robert Sidney Cahn successfully extracted CBN from *Cannabis indica,* marking the first time a single cannabinoid had been isolated, and its chemical structure identified (Cahn 1933). During the 1940s, Roger Adams in the United States and Alexander Todd in the United Kingdom independently studied the active components of CBN (Adams et al. 1940; Todd 1946). Both research groups discovered CBD, although its structure remained unclear at the time. The breakthrough came in 1964, when Raphael Mechoulam identified and synthesized D9-THC, the main intoxicating component of cannabis (Mechoulam and Shvo 1963). This discovery opened new avenues for researchers to understand how cannabis affects the brain and body. Advances in nuclear magnetic resonance (NMR) spectroscopy allowed Mechoulam to determine the chemical structure of both THC and CBD. Initial studies on D9-THC were conducted using rhesus macaques (Mechoulam et al. 1970; Grunfeld and Edery 1969; Scheckel et al. 1968; Thompson 1994).

In the following decade, researchers discovered several naturally occurring cannabinoids in the plant. These compounds, known as acid-cannabinoids occur naturally and are converted into the cannabinoids used recreationally and for research through a process called decarboxylation This process, typically achieved through heating (e.g., smoking, vaporizing, or cooking), transforms tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) into THC and CBD, respectively (Turner et al. 1980). At the time, it was believed that cannabis's psychoactive effects of cannabis were due to its high lipophilicity, meaning its ability to dissolve in fats. This led to skepticism about the existence of specific cannabinoid receptors. However, research in the early 1980s confirmed that cannabinoids act in a highly stereospecific manner (Mechoulam 1989). In 1988, a study identified a cannabinoid-specific receptor in the rodent brain, now known as the CB1 receptor, paper (Devane et al. 1988). This finding led researchers to hypothesize that the brain produces its own cannabinoids, paving the way for further discoveries about the ECS. In 1992, Mechoulam's lab used mass spectrometry to identify the first ECB, AEA (Devane et al. 1992). The CB2 receptor was discovered later, in the spleen of a rat. CB2 receptors were found to be primarily involved in the immune system, explaining some of cannabis's anti-inflammatory, analgesic, and immunosuppressive effects (Munro et al. 1993). In 1995, Mechoulam's group identified another ECB, 2-AG, which binds to both CB1 and CB2 receptors. An independent research group later confirmed the presence of 2-AG in the brain (Mechoulam et al. 1995; Sugiura et al.

1995). Ongoing research aims to better understand how AEA and 2-AG interact with THC and their roles in maintaining brain and body functions.

With these discoveries, cannabis research has become more active than ever. Since 2010, over 70% of all published papers on cannabis and cannabinoids have been released, and this number continues to grow annually (Fig. 2.3). Looking ahead, cannabis research will likely focus on several key areas: (1) Understanding the long-term effects of cannabis use, including its safety and efficacy; (2) Investigating the pharmacotherapeutic benefits of cannabis, with ongoing trials for conditions like PTSD, anxiety, depression, pain, autism spectrum disorders; (3) Examining the side effects of early cannabis exposure, whether in-utero or in childhood and adolescence; and (4) Exploring the role of the ECS in healthy development and how THC and ECBs interact in this system.

Cannabis research, particularly in the United States, faces a unique challenge. The cannabis used recreationally today is vastly different from that used in clinical research. Cannabis originally sourced for the National Institute on Drug Addiction (NIDA) contains about 5% THC, a concentration typical of the 1970s when the supply chain was established. However, modern cannabis, available over the counter in dispensaries, often contains more than 15% THC, with some strains reaching up to 30% THC, and pure concentrates as high as 95% THC (NIDA 2016; Cash et al. 2020). This difference, due to decades of selective breeding and advanced cultivation techniques, creates challenges when comparing research findings to the experiences of modern users (Ingvardsen and Brinch-Pedersen 2023). It's worth noting that most beneficial findings from past research were based on lower THC

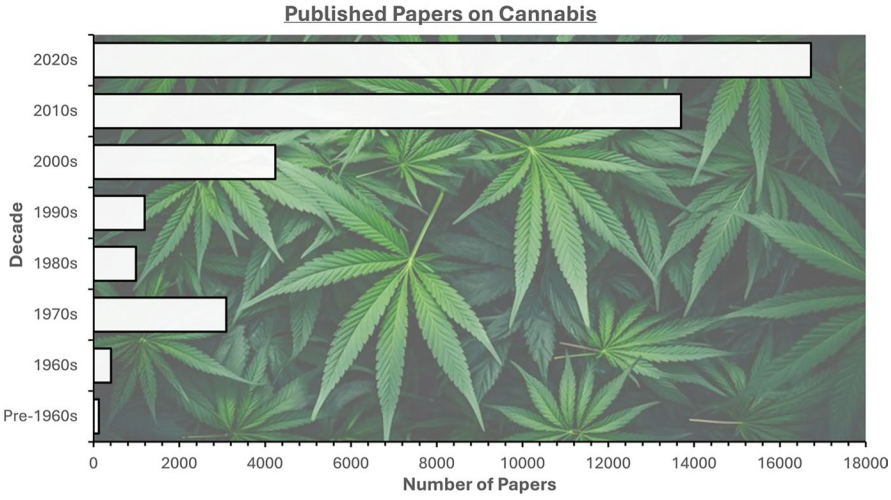


Fig. 2.3 Bar chart showing the number of published papers on *Cannabis Sativa/Indica* since the twentieth century. While research has increased, over 70% of papers on the topic have been published since 2010 showing a greater interest in studying cannabis compared to in the beginning. A dip in publications in the 1980s and 1990s is likely due to worldwide restrictions and legal status that came about in the 1970s for many countries (*data sourced from PubMed*)

concentrations (around 5%), as required in United States clinical studies. As recreational use continues to rise, researchers are working to conduct appropriate clinical trials to provide evidence-based guidance on the use or risks of cannabis.

4 Legalization and Criminalization of Cannabis

Cannabis is one of the most widely used psychoactive substances globally, with recent estimates suggesting 209 million users worldwide, despite varying legalization statuses (World Drug Report 2022 2024). Legalization has certainly contributed to this number, but it's important to recognize that cannabis has been widely used even before legalization, meaning the actual number of users may be even larger.

4.1 In the Americas

The United States has been a global leader in the movement toward cannabis legalization (State Medical Cannabis Laws 2024). In 2024, it was announced that cannabis may be rescheduled to a Schedule III drug, indicating that it is no longer considered a highly addictive substance with no medical benefits (Office of Public Affairs 2024). This rescheduling marks a monumental shift, as it will pave the way for more research into cannabis treatments and make it easier to compare modern recreational cannabis with its medical counterparts. Historically, however, the United States took a strict stance on cannabis. The 1937 Marijuana Tax Act effectively criminalized possession and imposed heavy taxes on its sale, production, and transfer. Later, the "War on Drugs" led to the Controlled Substances Act of 1970, which classified cannabis as a Schedule I drug, alongside lysergic acid diethylamide (LSD) and 3,4-methylenedioxymethamphetamine (MDMA), substances that are also now being studied in clinical trials. This classification severely hindered research into the long-term effects and potential medical benefits of cannabis. Oregon became the first US state to decriminalize cannabis possession in 1973, reducing penalties for small amounts intended for personal use. California and Alaska followed suit in the late 1970s. In 1996, California made another significant leap by introducing Proposition 215, legalizing medical cannabis. Other states like Oregon, Alaska, Washington, Maine, Colorado, and Nevada soon passed similar medical cannabis laws. In 2009, the Department of Justice announced it would not be prosecuting individuals for complying with state medical cannabis laws.

The "new era" of cannabis began in 2012 with the legalization of recreational use in Colorado and Washington. This movement quickly spread, and by 2024, 24 US states had legalized both recreational and medical cannabis, while 17 states had legalized cannabis solely for medical use. US territories, including Guam and the Northern Mariana Islands, have also legalized cannabis for both medical and recreational use, with Puerto Rico and the US Virgin Islands permitting medical use only.

With the federal rescheduling of cannabis, more states are expected to legalize recreational use over time.

Criminalization of cannabis has had a disproportionate impact on minority communities. Black Americans and Latinos have been incarcerated at higher rates than their white counterparts, despite similar usage levels (American Civil Liberties Union 2024). Nonviolent drug offenses have led to millions of arrests, costing vast sums of money to enforce prohibition and manage incarceration. Recent cannabis law reforms have resulted in expungements of nonviolent cannabis-related offenses. States like California have even written provisions for expungements into their legalization laws, reflecting a shift in public and governmental attitudes. In Canada, medical cannabis has been legal since 2001, and recreational cannabis was legalized nationwide in 2018 with the passage of the Cannabis Act (Cannabis legalization in CanadaIAlberta.ca 2024). While legalization is national, individual provinces regulate production, sales, and distribution. The cannabis industry has become a major economic force in Canada with an estimated value of $10.8 billion CAD in 2023 (Government of Canada SC 2023).

In Latin America, approaches to cannabis legalization vary. Uruguay was the first country to fully legalize recreational use in 2013 (Queirolo et al. 2019). Cannabis can be grown at home or supplied through cannabis social clubs, although restrictions apply to the number or plants and members Mexico decriminalized small quantities of cannabis in 2009, and in 2021, the Supreme Court ruled the prohibition of cannabis unconstitutional, opening the door for legalization (Cannabis law and legislation in Mexico 2024). Columbia legalized medical cannabis in 2015 and has established a robust framework for cultivation, production and export, given its favorable climate (Global Initiative 2024). Argentina (MOfMC et al. 2023) and Peru (Cannabis law and legislation in Peru 2024) have legalized medical cannabis, while Brazil permits the importation of cannabis-based products for medical use, typically for terminally ill patients (Cannabis law and legislation in Brazil 2024). Chile allows individuals to grow small amounts of cannabis for personal use, although sale and distribution remain illegal (Cannabis law and legislation in Chile 2024).

4.2 In Europe

Cannabis laws in Europe vary widely by country. Germany, the largest medical cannabis market in the world, legalized medical use in 2017, and most cases are covered by medical insurance—something rare in countries with private healthcare systems (POLITICO 2024). As of April 2024, Germany has also legalized cannabis for personal cultivation and consumption through nonprofit "cannabis clubs." Other European countries, including Italy (Cannabis law and legislation in Italy 2024), the Netherlands (Knottnerus et al. 2023), Greece (Stevens 2024), Poland (Cannabis law and legislation in Poland 2024), and the United Kingdom (Cannabis law and legislation in the UK 2024), have legalized medical cannabis, though prescribing it can be restricted by stringent regulations. For example, in the United Kingdom, only

specialist consultants can prescribe cannabis, not general practitioners. In the European Union (EU), cannabis policies are influence by cross-country regulations, particularly for agriculture and trade. Some EU countries, such as Spain (Cannabis law and legislation in Spain 2024) and Portugal (Cannabis law and legislation in Portugal 2024), have decriminalized recreational use, although it remains illegal. Luxembourg, in 2023, became the first EU member to legalize recreational cannabis, but with strict guidelines on personal use and cultivation (Cannabis Science Tech 2023). Malta and the Netherlands also have legalized recreational use, though regulations in the Netherlands limit the sale of cannabis to coffee shops where it must be consumed on-site (Marijuana Moment 2024).

4.3 In the Rest of the World

Cannabis legalization outside of the Americas and Europe is a mixed picture. Israel has long been a pioneer in medical cannabis research, with legalization for medical use dating back to the early 1990s (NIDA 2024). While recreational cannabis remains illegal, enforcement is lax, and it is estimated that up to 27% of adults in Israel have used cannabis (Statista 2024b). Thailand became the first Southeast Asian country to legalize medical cannabis in 2019, followed by recreational use in 2022, however, it was recently reported that this may be repealed (Thepgumpanat and Wongcha-um 2024). Conversely, many Asian countries impose severe penalties for drug offenses, including capital punishment in Singapore for possession of 500 g (about 1.1 lb) or more of cannabis (Central Narcotics Bureau of Singapore 2024). China imposes strict prison sentences for cannabis possession (Leafwell 2024). In India, despite its traditional use in religious and cultural rituals, cannabis remains illegal under the Narcotic Drugs and Psychotropic Substances Act of 1985, which prohibits production, possession, and sale (Chithra et al. 2023).

In Africa, Lesotho became the first country to legalize cannabis cultivation in 2017, primarily for export (Sensi Seeds 2023). Zimbabwe followed suit in 2018, legalizing cannabis for medical and scientific purposes (Rusenga et al. 2024). South Africa decriminalized private cannabis use, possession, and cultivating, though sale and distribution remain illegal. Medical cannabis is also legal in South Africa, but access is highly limited (Cannabis law and legislation in South Africa 2024).

In Oceana, Australia legalized medical cannabis in 2016, with regulation delegated to individual territories. In July 2024, the Australian Capital Territory (ACT) became the first region to legalize recreational cannabis, though the sale of cannabis remains illegal (Control (ODC) O of D 2021). New Zealand legalized medical cannabis in 2020, and while a national referendum on recreational use was narrowly defeated, debates continue in parliament (New Zealand Police 2024). While cannabis legalization is expanding worldwide, it presents many challenges, including addressing the potential harms and side effects of long-term use. Research into cannabis remains heavily regulated, even as recreational use grows. Individuals should exercise caution and consult a medical professional if concerned about adverse effects.

5 Conclusions

In this overview, we have traced the history of cannabis, highlighting its use in medicine, rituals, and recreation for thousands of years. It wasn't until the twentieth century that researchers began to uncover the science behind cannabis, understanding how it affects the brain and body, along with its potential benefits and risks. While this discussion explores many of the potential medical applications, it's important to acknowledge the significant gaps in our understanding. There is still much to learn about the long-term effects of cannabis, individual differences in how people respond to it, and the susceptibility of certain individuals to develop adverse effects, including cannabis use disorder. More research is needed to fully assess the safety of cannabis and its viability as a replacement for some medications that carry harmful side effects. As the global conversation around cannabis continues, countries regularly reassess its legal status and explore the role it may plan in the future of medicine and society.

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Dedication We dedicate this chapter to Raphael Mechoulam (1930–2023) whose discoveries paved the way for researchers everywhere.

References

National Academies of Sciences E, Division H and M, Practice B on PH and PH, Agenda C on the HE of MAER and R. Therapeutic Effects of Cannabis and Cannabinoids. In: The Health Effects of Cannabis and Cannabinoids: The Current State of Evidence and Recommendations for Research [Internet]. National Academies Press (US); 2017 [cited 2024 Jul 5]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK425767/

Mack A, Joy J. Marijuana and AIDS. In: Marijuana as Medicine? The Science Beyond the Controversy [Internet]. National Academies Press (US); 2000 [cited 2024 Jul 5]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK224400/

Small E. Evolution and classification of Cannabis sativa (Marijuana, Hemp) in relation to human utilization. Bot Rev. 2015;81(3):189–294.

Crocq MA. History of cannabis and the endocannabinoid system. Dialogues Clin Neurosci. 2020 Sep;22(3):223–8.

Kruger DJ, Kruger JS. Consumer experiences with Delta-8-THC: medical use, pharmaceutical substitution, and comparisons with Delta-9-THC. Cannabis Cannabinoid Res. 2023 Feb;8(1):166–73.

Haghdoost M, Brumar D, Geiling B, Brunstetter M, Bonn-Miller MO. Chemistry, crystal structure, and in vitro receptor binding of Δ10-THC isomers. Cannabis Cannabinoid Res. 2023;8(S1):S1–10.

Adair C, Geiling B, Haghdoost MM. Methods for preparing cannabinoids by base-promoted double-bond migration. WO2020248059A1. 2020;

Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG. Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. Br J Pharmacol. 2007 Mar;150(5):613–23.

Patra PH, Barker-Haliski M, White HS, Whalley BJ, Glyn S, Sandhu H, et al. Cannabidiol reduces seizures and associated behavioral comorbidities in a range of animal seizure and epilepsy models. Epilepsia. 2019;60(2):303–14.

Rosenberg EC, Chamberland S, Bazelot M, Nebet ER, Wang X, McKenzie S, et al. Cannabidiol modulates excitatory-inhibitory ratio to counter hippocampal hyperactivity. Neuron. 2023;111(8):1282–1300.e8.

Spindle TR, Sholler DJ, Cone EJ, Murphy TP, ElSohly M, Winecker RE, et al. Cannabinoid content and label accuracy of hemp-derived topical products available online and at National Retail Stores. JAMA Netw Open. 2022;5(7):e2223019.

Morean ME, de Wit H, King AC, Sofuoglu M, Rueger SY, O'Malley SS. The drug effects questionnaire: psychometric support across three drug types. Psychopharmacol Berl. 20121228th ed. 2013;227(1):177–92.

Ferber SG, Feldman R, Makhoul IR. The development of maternal touch across the first year of life. Early Hum Dev. 2008;84(6):363–70.

Lowin T, Tigges-Perez MS, Constant E, Pongratz G. Anti-inflammatory effects of Cannabigerol in rheumatoid arthritis synovial fibroblasts and peripheral blood mononuclear cell cultures are partly mediated by TRPA1. Int J Mol Sci. 2023;24(1):855.

Echeverry C, Prunell G, Narbondo C, de Medina VS, Nadal X, Reyes-Parada M, et al. A comparative in vitro study of the neuroprotective effect induced by Cannabidiol, Cannabigerol, and their respective acid forms: relevance of the 5-HT1A receptors. Neurotox Res. 2021;39(2):335–48.

Nachnani R, Raup-Konsavage WM, Vrana KE. The pharmacological case for Cannabigerol. J Pharmacol Exp Ther. 2021 Feb;376(2):204–12.

Brierley DI, Samuels J, Duncan M, Whalley BJ, Williams CM. Cannabigerol is a novel, well-tolerated appetite stimulant in pre-satiated rats. Psychopharmacology. 2016;233(19):3603–13.

García-Valverde MT, Sánchez-Carnerero Callado C, Díaz-Liñán MC, Sánchez de Medina V, Hidalgo-García J, Nadal X, et al. Effect of temperature in the degradation of cannabinoids: from a brief residence in the gas chromatography inlet port to a longer period in thermal treatments. Front Chem. 2022;10:1038729.

Lavender I, McCartney D, Marshall N, Suraev A, Irwin C, D'Rozario AL, et al. Cannabinol (CBN; 30 and 300 mg) effects on sleep and next-day function in insomnia disorder ("CUPID" study): protocol for a randomised, double-blind, placebo-controlled, cross-over, three-arm, proof-of-concept trial. BMJ Open. 2023;13(8):e071148.

Ghovanloo MR, Effraim PR, Tyagi S, Zhao P, Dib-Hajj SD, Waxman SG. Functionally-selective inhibition of threshold sodium currents and excitability in dorsal root ganglion neurons by cannabinol. Commun Biol. 2024;7:120.

Farrimond JA, Whalley BJ, Williams CM. Cannabinol and cannabidiol exert opposing effects on rat feeding patterns. Psychopharmacology. 2012;223(1):117–29.

Appendino G, Gibbons S, Giana A, Pagani A, Grassi G, Stavri M, et al. Antibacterial cannabinoids from Cannabis sativa: a structure−activity study. J Nat Prod. 2008;71(8):1427–30.

Sommano SR, Chittasupho C, Ruksiriwanich W, Jantrawut P. Cannabis Terpenes Mol Basel Switz. 2020;25:24.

Hanuš LO, Hod Y. Terpenes/Terpenoids in Cannabis: are they important? Med Cannabis Cannabinoids. 2020;3(1):25–60.

LaVigne JE, Hecksel R, Keresztes A, Streicher JM. Cannabis sativa terpenes are cannabimimetic and selectively enhance cannabinoid activity. Sci Rep. 2021;11(1):8232.

Parsons JL, Martin SL, James T, Golenia G, Boudko EA, Hepworth SR. Polyploidization for the genetic improvement of Cannabis sativa. Front Plant Sci [Internet]. 2019 [cited 2024 Jul 23];10. Available from: https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2019.00476/full

Booth JK, Yuen MMS, Jancsik S, Madilao LL, Page JE, Bohlmann J. Terpene synthases and terpene variation in Cannabis sativa1[OPEN]. Plant Physiol. 2020;184(1):130–47.

Ingvardsen CR, Brinch-Pedersen H. Challenges and potentials of new breeding techniques in Cannabis sativa. Front Plant Sci [Internet]. 2023 [cited 2024 Jul 23];14. Available from: https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2023.1154332/full

Surendran S, Qassadi F, Surendran G, Lilley D, Heinrich M. Myrcene—what are the potential health benefits of this flavouring and aroma agent? Front Nutr. 2021;(19):699666.

Lorigooini Z, Boroujeni SN, Sayyadi-Shahraki M, Rahimi-Madiseh M, Bijad E, Amini-khoei H. Limonene through attenuation of neuroinflammation and nitrite level exerts antidepressant-like effect on mouse model of maternal separation stress. Behav Neurol. 2021;2021:8817309.

Salehi B, Upadhyay S, Erdogan Orhan I, Kumar Jugran A, Jayaweera SLD, Dias DA, et al. Therapeutic potential of α- and β-Pinene: a miracle gift of nature. Biomol Ther. 2019;9(11):738.

dos Santos ÉRQ, Maia JGS, Fontes-Júnior EA, Maia C, do SF. Linalool as a therapeutic and medicinal tool in depression treatment: a review. Curr Neuropharmacol. 2022;20(6):1073–92.

Fidyt K, Fiedorowicz A, Strządała L, Szumny A. β-caryophyllene and β-caryophyllene oxide—natural compounds of anticancer and analgesic properties. Cancer Med. 2016;5(10):3007–17.

Hill MN, Haney M, Hillard CJ, Karhson DS, Vecchiarelli HA. The endocannabinoid system as a putative target for the development of novel drugs for the treatment of psychiatric illnesses. Psychol Med. 2023;53(15):7006–24.

Spagnolo PA, Ramchandani VA, Schwandt ML, Kwako LE, George DT, Mayo LM, et al. FAAH gene variation moderates stress response and symptom severity in patients with posttraumatic stress disorder and comorbid alcohol dependence. Alcohol Clin Exp Res. 2016;40(11):2426–34.

Zabik NL, Iadipaolo AS, Marusak HA, Peters C, Burghardt K, Rabinak CA. A common genetic variant in fatty acid amide hydrolase is linked to alterations in fear extinction neural circuitry in a racially diverse, nonclinical sample of adults. J Neurosci Res. 2022;100(3):744–61.

Mayo LM, Asratian A, Lindé J, Holm L, Nätt D, Augier G, et al. Protective effects of elevated anandamide on stress and fear-related behaviors: translational evidence from humans and mice. Mol Psychiatry. 2020;25(5):993–1005.

Conzelmann A, Reif A, Jacob C, Weyers P, Lesch KP, Lutz B, et al. A polymorphism in the gene of the endocannabinoid-degrading enzyme FAAH (FAAH C385A) is associated with emotional-motivational reactivity. Psychopharmacology. 2012;224(4):573–9.

Mayo LM, Rabinak CA, Hill MN, Heilig M. Targeting the endocannabinoid system in the treatment of posttraumatic stress disorder: a promising case of preclinical-clinical translation? Biol Psychiatry. 2022;91(3):262–72.

Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev. 2003;83(3):1017–66.

Haspula D, Clark MA. Cannabinoid receptors: an update on cell signaling, pathophysiological roles and therapeutic opportunities in neurological, cardiovascular, and inflammatory diseases. Int J Mol Sci. 2020;21(20):7693.

Howlett AC, Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Porrino LJ. Cannabinoid physiology and pharmacology: 30 years of progress. Neuropharmacology. 2004;47(Suppl 1):345–58.

Tsou K, Brown S, Sañudo-Peña MC, Mackie K, Walker JM. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience. 1998;83(2):393–411.

Zou S, Kumar U. Cannabinoid receptors and the endocannabinoid system: signaling and function in the central nervous system. Int J Mol Sci. 2018;19(3):833.

Galiègue S, Mary S, Marchand J, Dussossoy D, Carrière D, Carayon P, et al. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem. 1995;232(1):54–61.

Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature. 1993;365(6441):61–5.

Spigelman I. Therapeutic targeting of peripheral cannabinoid receptors in inflammatory and neuropathic pain states. In: Kruger L, Light AR, editors. Translational pain research: from mouse to man [Internet]. Boca Raton: CRC Press/Taylor & Francis; 2010. [cited 2024 Jul 2] (Frontiers in Neuroscience). Available from: http://www.ncbi.nlm.nih.gov/books/NBK57261/.

Pandey R, Mousawy K, Nagarkatti M, Nagarkatti P. Endocannabinoids and immune regulation. Pharmacol Res Off J Ital Pharmacol Soc. 2009;60(2):85–92.

Ashton JC, Glass M. The cannabinoid CB2 receptor as a target for inflammation-dependent neurodegeneration. Curr Neuropharmacol. 2007;5(2):73–80.

Atwood BK, Mackie K. CB2: a cannabinoid receptor with an identity crisis. Br J Pharmacol. 2010;160(3):467–79.

Lutz B. Endocannabinoid signals in the control of emotion. Curr Opin Pharmacol. 2009;9(1):46–52.

Carey CE, Agrawal A, Zhang B, Conley ED, Degenhardt L, Heath AC, et al. Monoacylglycerol lipase (MGLL) polymorphism rs604300 interacts with childhood adversity to predict cannabis dependence symptoms and amygdala habituation: evidence from an endocannabinoid system-level analysis. J Abnorm Psychol. 2015;124(4):860–77.

Hall W, Degenhardt L. Adverse health effects of non-medical cannabis use. Lancet. 2009;374(9698):1383–91.

Volkow ND, Baler RD, Compton WM, Weiss SRB. Adverse health effects of marijuana use. N Engl J Med. 2014;370(23):2219–27.

Crean RD, Crane NA, Mason BJ. An evidence-based review of acute and long-term effects of Cannabis use on executive cognitive functions. J Addict Med. 2011;5(1):1.

Karila L, Roux P, Rolland B, Benyamina A, Reynaud M, Aubin HJ, et al. Acute and long-term effects of Cannabis use: a review. Curr Pharm Des. 2014;20(25):4112–8.

Kroon E, Kuhns L, Cousijn J. The short-term and long-term effects of cannabis on cognition: recent advances in the field. Curr Opin Psychol. 2021;38:49–55.

Zehra A, Burns J, Liu CK, Manza P, Wiers CE, Volkow ND, et al. Cannabis addiction and the brain: a review. J Neuroimmune Pharmacol. 2018;13(4):438–52.

Haney M, Vallée M, Fabre S, Collins Reed S, Zanese M, Campistron G, et al. Signaling-specific inhibition of the CB1 receptor for cannabis use disorder: phase 1 and phase 2a randomized trials. Nat Med. 2023;29(6):1487–99.

Lu HC, Mackie K. An introduction to the endogenous cannabinoid system. Biol Psychiatry. 2016;79(7):516–25.

Bie B, Wu J, Foss JF, Naguib M. An overview of the cannabinoid type 2 (CB2) receptor system and its therapeutic potential. Curr Opin Anaesthesiol. 2018;31(4):407–14.

Garland EL. Pain processing in the human nervous system: a selective review of nociceptive and biobehavioral pathways. Prim Care. 2012;39(3):561–71.

Staud R. Evidence of involvement of central neural mechanisms in generating fibromyalgia pain. Curr Rheumatol Rep. 2002;4(4):299–305.

Bains S, Mukhdomi T. Medicinal cannabis for treatment of chronic pain. In: StatPearls [Internet]. Treasure Island: StatPearls Publishing; 2024. [cited 2024 Jul 23]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK574562/.

Miller HP, Bonawitz SC, Ostrovsky O. The effects of delta-9-tetrahydrocannabinol (THC) on inflammation: a review. Cell Immunol. 2020;352:104111.

Campbell G, Hall WD, Peacock A, Lintzeris N, Bruno R, Larance B, et al. Effect of cannabis use in people with chronic non-cancer pain prescribed opioids: findings from a 4-year prospective cohort study. Lancet Public Health. 2018;3(7):e341–50.

Whiting PF, Wolff RF, Deshpande S, Di Nisio M, Duffy S, Hernandez AV, et al. Cannabinoids for medical use: a systematic review and meta-analysis. JAMA. 2015;313(24):2456–73.

McQuay HJ. More evidence cannabis can help in neuropathic pain. CMAJ Can Med Assoc J. 2010;182(14):1494–5.

Ware MA, Wang T, Shapiro S, Robinson A, Ducruet T, Huynh T, et al. Smoked cannabis for chronic neuropathic pain: a randomized controlled trial. CMAJ Can Med Assoc J. 2010;182(14):E694–701.

Järbe TUC, DiPatrizio NV. Delta9-THC induced hyperphagia and tolerance assessment: interactions between the CB1 receptor agonist delta9-THC and the CB1 receptor antagonist SR-141716 (rimonabant) in rats. Behav Pharmacol. 2005;16(5–6):373–80.
Kirkham TC. Endocannabinoids in the regulation of appetite and body weight. Behav Pharmacol. 2005;16(5–6):297–313.
Kirkham TC. Cannabinoids and appetite: food craving and food pleasure. Int Rev Psychiatry Abingdon Engl. 2009;21(2):163–71.
Lambert DM, Muccioli GG. Endocannabinoids and related N-acylethanolamines in the control of appetite and energy metabolism: emergence of new molecular players. Curr Opin Clin Nutr Metab Care. 2007;10(6):735–44.
Di Marzo V, Goparaju SK, Wang L, Liu J, Bátkai S, Járai Z, et al. Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature. 2001;410(6830):822–5.
Kirkham TC, Williams CM. Endogenous cannabinoids and appetite. Nutr Res Rev. 2001;14(1):65–86.
Pagotto U, Marsicano G, Cota D, Lutz B, Pasquali R. The emerging role of the endocannabinoid system in endocrine regulation and energy balance. Endocr Rev. 2006;27(1):73–100.
Bellocchio L, Cervino C, Pasquali R, Pagotto U. The endocannabinoid system and energy metabolism. J Neuroendocrinol. 2008;20(6):850–7.
DiPatrizio NV, Piomelli D. The thrifty lipids: endocannabinoids and the neural control of energy conservation. Trends Neurosci. 2012;35(7):403–11.
Helmich RC, Hallett M, Deuschl G, Toni I, Bloem BR. Cerebral causes and consequences of parkinsonian resting tremor: a tale of two circuits? Brain. 2012;135(11):3206–26.
Arjmand S, Vaziri Z, Behzadi M, Abbassian H, Stephens GJ, Shabani M. Cannabinoids and tremor induced by motor-related disorders: friend or foe? Neurotherapeutics. 2015;12(4):778–87.
Ashton JC, Milligan ED. Cannabinoids for the treatment of neuropathic pain: clinical evidence. Curr Opin Investig Drugs Lond Engl 2000. 2008;9(1):65–75.
Nielsen S, Murnion B, Campbell G, Young H, Hall W. Cannabinoids for the treatment of spasticity. Dev Med Child Neurol. 2019;61(6):631–8.
What medical conditions are eligible? [Internet]. [cited 2024 Jul 23]. Available from: https://www.michigan.gov/cra/faq/program-list/applicant-questions/what-medical-conditions-are-eligible
Koppel BS, Brust JCM, Fife T, Bronstein J, Youssof S, Gronseth G, et al. Systematic review: efficacy and safety of medical marijuana in selected neurologic disorders. Neurology. 2014;82(17):1556–63.
Samaha D, Kandiah T, Zimmerman D. Cannabis use for restless legs syndrome and uremic pruritus in in patients treated with maintenance dialysis: a survey. Can J Kidney Health Dis. 2020;7:2054358120954944.
Ghorayeb I. Cannabis for restless legs syndrome. Adv Exp Med Biol. 2021;1297:173–81.
Charitos IA, Gagliano-Candela R, Santacroce L, Bottalico L. The Cannabis spread throughout the continents and its therapeutic use in history. Endocr Metab Immune Disord Drug Targets. 2021;21(3):407–17.
Touw M. The religious and medicinal uses of Cannabis in China, India and Tibet. J Psychoact Drug. 1981;13(1):23–34.
Brand EJ, Zhao Z. Cannabis in Chinese medicine: are some traditional indications referenced in ancient literature related to cannabinoids? Front Pharmacol. 2017;8:108.
Ren M, Tang Z, Wu X, Spengler R, Jiang H, Yang Y, et al. The origins of cannabis smoking: chemical residue evidence from the first millennium BCE in the Pamirs. Sci Adv. 2019;5(6):eaaw1391.
Magazine S, Solly M. Smithsonian Magazine. [cited 2024 Jul 8]. The First Evidence of Smoking Pot Was Found in a 2,500-Year-Old Pot. Available from: https://www.smithsonianmag.com/smart-news/2500-year-old-chinese-cemetery-offers-earliest-physical-evidence-cannabis-smoking-180972410/
Mahdizadeh S, Khaleghi Ghadiri M, Gorji A. Avicenna's canon of medicine: a review of analgesics and anti-inflammatory substances. Avicenna J Phytomed. 2015;5(3):182–202.

Duvall CS. A brief agricultural history of cannabis in Africa, from prehistory to canna-colony. EchoGéo [Internet]. 2019; [cited 2024 Jul 9];(48). Available from: https://journals.openedition.org/echogeo/17599

World Drug Report 2022 [Internet]. [cited 2024 Jul 3]. Available from: https://www.unodc.org/unodc/en/data-and-analysis/world-drug-report-2022.html

National Survey on Drug Use and Health [Internet]. [cited 2024 Jul 9]. Available from: https://www.samhsa.gov/data/data-we-collect/nsduh-national-survey-drug-use-and-health

Forti MD, Quattrone D, Freeman TP, Tripoli G, Gayer-Anderson C, Quigley H, et al. The contribution of cannabis use to variation in the incidence of psychotic disorder across Europe (EU-GEI): a multicentre case-control study. Lancet Psychiatry. 2019;6(5):427–36.

Myran DT, Harrison LD, Pugliese M, Tanuseputro P, Gaudreault A, Fiedorowicz JG, et al. Development of an anxiety disorder following an emergency department visit due to cannabis use: a population-based cohort study. eClinicalMedicine [Internet]. 2024. [cited 2024 Jul 9];69. Available from: https://www.thelancet.com/journals/eclinm/article/PIIS2589-5370(24)00034-8/fulltext

Hengartner MP, Angst J, Ajdacic-Gross V, Rössler W. Cannabis use during adolescence and the occurrence of depression, suicidality and anxiety disorder across adulthood: findings from a longitudinal cohort study over 30 years. J Affect Disord. 2020;272:98–103.

Evanski JM, Zundel CG, Baglot SL, Desai S, Gowatch LC, Ely SL, et al. The first "hit" to the endocannabinoid system? Associations between prenatal Cannabis exposure and frontolimbic white matter pathways in children. Biol Psychiatry Glob Open Sci. 2024;4(1):11–8.

Jacobus J, Tapert SF. Effects of Cannabis on the adolescent brain. Curr Pharm Des. 2014;20(13):2186–93.

Statista [Internet]. [cited 2024a Jul 9]. Cannabis use frequency in Europe 2022. Available from: https://www.statista.com/statistics/473371/frequency-cannabis-use-european-union-eu/

Mechoulam R, Shvo Y. Hashish. I The structure of cannabidiol. Tetrahedron. 1963;19(12):2073–8.

Mechoulam R, Shani A, Edery H, Grunfeld Y. Chemical basis of hashish activity. Science. 1970;169(3945):611–2.

Pertwee RG. The central neuropharmacology of psychotropic cannabinoids. Pharmacol Ther. 1988;36(2–3):189–261.

Paton WD. Pharmacology of marijuana. Annu Rev Pharmacol. 1975;15:191–220.

Maickel RP. Pharmacology of Marihuana (Cannabis sativa). Am Biol Teach. 1973;35(7):398–404.

O'Shaughnessy WB. On the preparations of the Indian Hemp, or Gunjah. Prov Med J Retrosp Med Sci. 1843;5(123):363–9.

Wood TB, Spivey WTN, Easterfield TH. XL.—Charas. The resin of Indian hemp. J Chem Soc Trans. 1896;69(0):539–46.

Cahn RS. 326. Cannabis indica resin. Part IV. The synthesis of some 2: 2-dimethyldibenzopyrans, and confirmation of the structure of cannabinol. J Chem Soc Resumed. 1933;1(0):1400–5.

Adams R, Pease DC, Cain CK, Baker BR, Clark JH, Wolff H, et al. Conversion of Cannabidiol to a product with Marihuana activity. A type reaction for synthesis of analogous substances. Conversion of Cannabidiol to Cannabinol. J Am Chem Soc. 1940;62(8):2245–6.

Todd AR. Hashish Experientia. 1946;2(2):55–60.

Grunfeld Y, Edery H. Psychopharmacological activity of the active constituents of hashish and some related cannabinoids. Psychopharmacologia. 1969;14(3):200–10.

Scheckel CL, Boff E, Dahlen P, Smart T. Behavioral effects in monkeys of racemates of two biologically active marijuana constituents. Science. 1968;160(3835):1467–9.

Thompson RA. Emotion regulation: a theme in search of definition. Monogr Soc Res Child Dev. 1994;59(2–3):25–52.

Turner CE, Elsohly MA, Boeren EG. Constituents of Cannabis sativa L. XVII. A review of the natural constituents. J Nat Prod. 1980;43(2):169–234.

Mechoulam R. Chapter 2 alkaloids in *Cannabis sativa* L. In: Brossi A, editor. The alkaloids: chemistry and pharmacology [Internet]. Academic Press; 1989. p. 77–93. [cited 2024 Jul 19]. Available from: https://www.sciencedirect.com/science/article/pii/S0099959808602278.

Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol. 1988;34(5):605–13.
Devane WA, Hanuš L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science. 1992;258(5090):1946–9.
Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol. 1995;50(1):83–90.
Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, et al. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun. 1995;215(1):89–97.
NIDA. Abuse NI on D. Marijuana Plant Material Available from the NIDA Drug Supply Program|National Institute on Drug Abuse (NIDA) [Internet]. 2016 [cited 2024 Jul 17]. Available from: https://nida.nih.gov/research/research-data-measures-resources/nida-drug-supply-program-dsp/nida-drug-supply-program-dsp-ordering-guidelines/marijuana-plant-material-available
Cash MC, Cunnane K, Fan C, Romero-Sandoval EA. Mapping cannabis potency in medical and recreational programs in the United States. PLoS One. 2020;15(3):e0230167.
State Medical Cannabis Laws [Internet]. [cited 2024 Sep 13]. Available from: https://www.ncsl.org/health/state-medical-cannabis-laws#undefined
Office of Public Affairs|Justice Department Submits Proposed Regulation to Reschedule Marijuana|United States Department of Justice [Internet]. 2024 [cited 2024 Jul 23]. Available from: https://www.justice.gov/opa/pr/justice-department-submits-proposed-regulation-reschedule-marijuana
American Civil Liberties Union. Report: The War on Marijuana in Black and White [Internet]. [cited 2024 Jul 23]. Available from: https://www.aclu.org/publications/report-war-marijuana-black-and-white
Cannabis legalization in Canada|Alberta.ca [Internet]. 2024 [cited 2024 Sep 13]. Available from: https://www.alberta.ca/cannabis-legalization-in-canada
Government of Canada SC. The daily—five years since legalization, what have we learned about cannabis in Canada? [Internet] 2023 [cited 2024 Jul 23]. Available from: https://www150.statcan.gc.ca/n1/daily-quotidien/231016/dq231016c-eng.htm
Queirolo R, Rossel C, Álvarez E, Repetto L. Why Uruguay legalized marijuana? The open window of public insecurity. Addict Abingdon Engl. 2019;114(7):1313–21.
Cannabis law and legislation in Mexico [Internet]. [cited 2024 Sep 13]. Available from: https://cms.law/en/int/expert-guides/cms-expert-guide-to-a-legal-roadmap-to-cannabis/mexico
Global Initiative. Cannabis legalization in Colombia: Exploring the potential impacts on organized crime [Internet]. [cited 2024 Sep 13]. Available from: https://globalinitiative.net/analysis/cannabis-legalization-in-colombia-exploring-the-potential-impacts-on-organized-crime/
Leone MOfMC, Ramirez CA, Mosteirin MJ. Lexology. 2023 [cited 2024 Sep 13]. A general introduction to cannabis law in Argentina. Available from: https://www.lexology.com/library/detail.aspx?g=e2b0152f-3de0-446f-bfdc-305bd08da082
Cannabis law and legislation in Peru [Internet]. [cited 2024 Sep 13]. Available from: https://cms.law/en/int/expert-guides/cms-expert-guide-to-a-legal-roadmap-to-cannabis/peru
Cannabis law and legislation in Brazil [Internet]. [cited 2024 Sep 13]. Available from: https://cms.law/en/int/expert-guides/cms-expert-guide-to-a-legal-roadmap-to-cannabis/brazil
Cannabis law and legislation in Chile [Internet]. [cited 2024 Sep 13]. Available from: https://cms.law/en/int/expert-guides/cms-expert-guide-to-a-legal-roadmap-to-cannabis/chile
POLITICO [Internet]. 2024 [cited 2024 Sep 13]. Germany's dodged weed legalization. Available from: https://www.politico.eu/article/germany-pot-legalization-cannabis-black-market-marijuana-dealers-bureaucracy/
Cannabis law and legislation in Italy [Internet]. [cited 2024 Sep 13]. Available from: https://cms.law/en/int/expert-guides/cms-expert-guide-to-a-legal-roadmap-to-cannabis/italy

Knottnerus JA, Blom T, van Eerden S, Mans JHH, van de Mheen D, de Neeling JND, et al. Cannabis policy in The Netherlands: rationale and design of an experiment with a controlled legal ('closed') cannabis supply chain. Health Policy. 2023;129:104699.
Stevens B. "New era in Greek healthcare" as first medical Cannabis prescriptions issued [Internet]. Business of Cannabis 2024 [cited 2024 Sep 13]. Available from: https://businessofcannabis.com/new-era-in-greek-healthcare-as-first-medical-cannabis-prescriptions-issued/
Cannabis law and legislation in Poland [Internet]. [cited 2024 Sep 13]. Available from: https://cms.law/en/int/expert-guides/cms-expert-guide-to-a-legal-roadmap-to-cannabis/poland
Cannabis law and legislation in the UK [Internet]. [cited 2024 Sep 13]. Available from: https://cms.law/en/int/expert-guides/cms-expert-guide-to-a-legal-roadmap-to-cannabis/united-kingdom
Cannabis law and legislation in Spain [Internet]. [cited 2024 Sep 13]. Available from: https://cms.law/en/int/expert-guides/cms-expert-guide-to-a-legal-roadmap-to-cannabis/spain
Cannabis law and legislation in Portugal [Internet]. [cited 2024 Sep 13]. Available from: https://cms.law/en/int/expert-guides/cms-expert-guide-to-a-legal-roadmap-to-cannabis/portugal
Cannabis Science Tech [Internet]. 2023 [cited 2024 Sep 13]. Luxembourg Legalizes Cannabis. Available from: https://www.cannabissciencetech.com/view/luxembourg-legalizes-cannabis
Marijuana Moment. Malta Issues EU's First Licenses Allowing Nonprofit "Cannabis Associations" To Begin Growing Marijuana - Marijuana Moment [Internet]. [cited 2024 Sep 13]. Available from: https://www.marijuanamoment.net/malta-issues-eus-first-licenses-allowing-nonprofit-cannabis-associations-to-begin-growing-marijuana/
NIDA. Abuse NI on D. Israel National Cannabis Policy|National Institute on Drug Abuse (NIDA) [Internet]. [cited 2024 Sep 13]. Available from: https://nida.nih.gov/international/abstracts/israel-national-cannabis-policy
Statista [Internet]. [cited 2024b Jul 23]. Countries with the highest cannabis use rates in 2020. Available from: https://www.statista.com/statistics/1015296/rate-of-cannabis-use-worldwide-country/
Thepgumpanat P, Wongcha-um P. Thailand to ban recreational cannabis use by year-end, health minister says. Reuters [Internet]. 2024. [cited 2024 Sep 13]; Available from: https://www.reuters.com/world/asia-pacific/thailand-ban-recreational-cannabis-use-by-year-end-says-health-minister-2024-02-29/
Central Narcotics Bureau of Singapore|Singapore's Anti-Drug Laws on Cannabis [Internet]. [cited 2024 Sep 13]. Available from: https://www.cnb.gov.sg/educational-resources/myths-and-facts-about-drugs/cannabis/singapore's-anti-drug-laws-on-cannabis
Leafwell [Internet]. [cited 2024 Sep 13]. Is Marijuana Legal in China? Available from: https://leafwell.com/blog/is-marijuana-legal-in-china
Chithra NK, Bojappen N, Vajawat B, Pai NM, Gowda GS, Moirangthem S, et al. Legalization of recreational Cannabis: is India ready for it? Indian J Soc Psychiatry. 2023;39(4):325.
Sensi Seeds [Internet]. 2023 [cited 2024 Sep 13]. Cannabis in Lesotho—Laws, Use, History, and More Info. Available from: https://sensiseeds.com/en/blog/countries/cannabis-in-lesotho-laws-use-history/
Rusenga C, Klantschnig G, Carrier N, Howell S. Business as usual? Cannabis legalisation and agrarian change in Zimbabwe. J Peasant Stud. 2024;51(4):982–1001.
Cannabis law and legislation in South Africa [Internet]. [cited 2024 Sep 13]. Available from: https://cms.law/en/int/expert-guides/cms-expert-guide-to-a-legal-roadmap-to-cannabis/south-africa
Control (ODC) O of D. Medicinal cannabis|Office of Drug Control (ODC) [Internet]. Office of Drug Control (ODC); 2021 [cited 2024 Sep 13]. Available from: https://www.odc.gov.au/medicinal-cannabis
New Zealand Police [Internet]. [cited 2024 Sep 13]. Cannabis and the law. Available from: https://www.police.govt.nz/advice-services/drugs-and-alcohol/cannabis-and-law

Chapter 3
Overview of the Endogenous Cannabinoid Signaling System



Todd M. Stollenwerk, César E. Martinez Ramirez, and Cecilia J. Hillard

1 Introduction

The endocannabinoid system is an endogenous signaling system composed classically of the CB1 and CB2 cannabinoid receptors, their endogenous ligands, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and the synthesizing and degrading enzymes of these ligands. Within the brain, the endocannabinoid system modulates synaptic transmission and corresponding neuronal activity. Due to the widespread expression of its components throughout the brain, the endocannabinoid signaling system plays an important role in maintaining homeostasis and modulating a broad range of behaviors.

2 The Cannabinoid Receptors

There are two major cannabinoid receptors: the CB1 receptor, which is found primarily in the central nervous system (CNS), and the CB2 receptor, which is most highly expressed on immune cells.

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Both receptors are G protein-coupled receptors (GPCRs) and are primarily coupled to G_i and G_o proteins. As such, both CB1 and CB2 receptors inhibit adenylyl cyclase to decrease intracellular cAMP. They also share additional signaling pathways, including activation of the MAPK/ERK signaling pathway (Kobayashi et al. 2001) and the phospholipase C (PLC)-inositol 1,4,5-triphosphate (IP_3) pathway to increase intracellular Ca^{2+} (Zoratti et al. 2003). They also have been shown to recruit β-arrestin signaling (Ibsen et al. 2019). While CB1 receptors are able to couple to potassium channels, this does not occur with CB2 receptors (Felder et al. 1995).

The structures of the CB1 and CB2 receptors are unusual compared to many GPCRs in that the N-terminal and the second extracellular loop form a lid over the orthosteric pocket (Shao et al. 2016), which isolates the ligand binding pocket from the extracellular space and suggests that ligands enter the binding pocket in an unconventional manner. In agreement with this notion, molecular dynamics simulations predict that ligands enter into the binding pocket through the membrane bilayer (Hurst et al. 2010). This interaction mechanism is consistent with the high degree of lipophilicity of cannabinoid receptor ligands.

2.1 Localization of the CB1 Receptor

The CB1 receptor is the most abundant GPCR in the brain. There is particularly dense expression in the cortex, basal ganglia, hippocampus, and cerebellum (Lu and Mackie 2016). CB1 receptors are abundantly expressed in cholecystokinin (CCK)-expressing GABAergic interneurons (Katona et al. 1999; Marsicano and Lutz 1999); however, they are largely absent from other interneuron subtypes, such as parvalbumin- and calretinin-positive interneurons. The CB1 receptor is also expressed to a lower degree in glutamatergic neurons in cortical regions and the hippocampus (Marsicano and Lutz 1999). Despite lower levels of expression, CB1 receptors in these glutamatergic neurons play important roles, including protection against excitotoxicity (Marsicano et al. 2003), feedback inhibition of glutamatergic synaptic activity (Marinelli et al. 2009), and the modulation of various behaviors that are described below. There is also evidence for the expression of CB1 receptors in cholinergic (Nyíri et al. 2005b), serotonergic (Häring et al. 2015; Häring et al. 2007), and noradrenergic (Carvalho et al. 2010) neurons. In addition to their expression on neurons, CB1 receptors are expressed to variable degrees on glial cells, which is addressed in more detail below.

The CB1 receptor is expressed at high density in axon terminals, consistent with their role in regulating presynaptic neurotransmitter release (Nyíri et al. 2005a). At axon terminals, the CB1 receptor is clustered at the membrane-associated periodic skeleton (MPS), a highly organized structure that organizes transmembrane proteins to facilitate signal transduction (Zhou et al. 2019). CB1 receptors are recruited to the MPS in a ligand-dependent manner.

In addition to their location at the axon terminal, CB1 receptors have also been found to be present on neuronal mitochondria, where they inhibit cellular

respiration and modify neurotransmitter release (Bénard et al. 2012). Studies have suggested that some of the functions that were initially attributed to CB1 receptors at the axon terminal may in fact be related to signaling through mitochondrial CB1 receptors; however, their role in modulating behavior remains incompletely understood.

2.2 CB2 Receptor Localization and Expression

The CB2 receptor is most abundantly present on cells of the immune system, with highest expression in B lymphocytes and natural killer cells, followed by monocytes, macrophages, neutrophils, and T cells (Galiègue et al. 1995). As such, the CB2 receptor is most highly expressed in immune tissues, such as the spleen, tonsils, and thymus. It is absent in most other tissues, but has been found in the pancreas, lung, and uterus. There is also evidence that the CB2 receptor plays a role in fertility (Battista et al. 2012) with expression in both male (Grimaldi et al. 2009) and female (El-Talatini et al. 2009) reproductive tissues.

The CB2 receptor is also expressed in the brain, albeit at extremely low levels in the healthy brain. Initial studies looked at the expression of CB2 receptors in microglia, given their role as the major immune cells within the brain. It was found that resting, isolated microglia express very low levels of CB2 receptor, but expression increases with microglial activation (Carlisle et al. 2002). Further studies have shown that CB2 receptor expression is increased in certain pathological states associated with increased neuroinflammation, including Alzheimer's disease (Lopez et al. 2018).

The expression of the CB2 receptor in neurons has been a controversial topic, plagued by conflicting results and complicated by poor-quality antibodies with inadequate specificity to make definitive conclusions (Atwood and Mackie 2010). However, growing high-quality evidence has suggested that the CB2 receptor is expressed at low levels in neurons. This is supported by experiments using in situ hybridization (Li and Kim 2015), particularly in dopaminergic neurons (Zhang et al. 2014, 2022), as well as by experiments showing that increasing signaling through the CB2 receptor affects firing of dopamine neurons in the ventral tegmental area (VTA) (Ma et al. 2015).

2.3 Allosteric Modulation

The CB1 and CB2 receptors have both orthosteric and allosteric binding sites, and there are endogenous compounds that bind to each of these sites. Binding of a ligand to an orthosteric binding site can engage signaling through the receptor (e.g., agonist or partial agonist), occupy the site without causing significant conformational changes (e.g., antagonist), or stabilize the receptor in an inactive conformation,

thereby preventing constitutive activity (e.g., inverse agonist). Examples of orthosteric ligands for the CB1 and CB2 receptors include the endocannabinoids 2-AG and AEA and the phytocannabinoid Δ^9-tetrahydrocannabinol (THC) that is primarily responsible for the psychogenic effects of cannabis plant preparations and extracts.

Allosteric binding sites are regions outside of the orthosteric binding site that, when occupied, can alter orthosteric ligand signaling. When ligands bind to these sites, they can enhance (positive allosteric modulator, PAM) or inhibit (negative allosteric modulator, NAM) signaling caused by binding of an orthosteric agonist through several mechanisms.

The phytocannabinoid cannabidiol (CBD) has been shown to act as an NAM, thereby reducing or inhibiting the pharmacological effects of THC at the CB1 receptor. CBD has a low-binding affinity for the orthosteric binding site of the CB1 receptor (Devane et al. 1988) and exerts its inhibitory effects by binding to an allosteric binding site (Jakowiecki et al. 2021), thereby functioning as an NAM of both β-arrestin recruitment and G protein-mediated signaling (Laprairie et al. 2015). Similar effects have been seen at the CB2 receptor (Martínez-Pinilla et al. 2017), indicating that CBD is a NAM at both the CB1 and CB2 receptors.

There are also endogenous allosteric modulators for the CB1 receptor, including cholesterol (Hua et al. 2020) and pregnenolone, which acts as an NAM that is biased towards inhibiting THC-mediated β-arrestin recruitment (Raux et al. 2022; Vallée et al. 2014).

3 AEA and 2-AG: Endogenous Agonists of the Cannabinoid Receptors

The best characterized endogenous agonists of the cannabinoid receptors, called "endocannabinoids," are AEA and 2-AG. A central feature of the endocannabinoids is that they are not stored in vesicles, as are other neurotransmitters, but rather are synthesized on demand through Ca^{2+}-dependent processes in response to stimuli.

The two endogenous agonists of the cannabinoid receptor have unique properties. 2-AG has lower affinity for the CB1 receptor than AEA, but it has higher efficacy (Hillard 2000). 2-AG binding to the CB1 receptor recruits greater β-arrestin signaling than AEA (Ibsen et al. 2019), which is consistent with findings that 2-AG is more likely to cause receptor desensitization and downregulation (Lichtman et al. 2010). There is considerable evidence that 2-AG production is stimulated by postsynaptic activity and thereby modulates synaptic activity in a phasic manner, described further below. AEA synthesis is largely constitutive, which underlies the notion that AEA is a tonic activator of the CB1 receptor in some brain regions (discussed further below). Importantly, 2-AG also serves as the major source of free arachidonic acid in the brain to support synthesis of prostaglandins and other arachidonates, such that inhibition of 2-AG metabolism to arachidonic acid

significantly reduces concentrations of proinflammatory arachidonates in the brain (Nomura et al. 2011).

3.1 AEA Synthesis and Degradation

Synthesis of AEA can occur by multiple enzymatic pathways in the brain, which each utilize N-arachidonoyl phosphatidyl ethanolamine (NAPE) as a substrate (Maccarrone 2017). These pathways include (1) NAPE-specific phospholipase D (NAPE-PLD), resulting in AEA and phosphatidic acid (Schmid et al. 1983); (2) NAPE-specific phospholipase C (NAPE-PLC), resulting in phosphorylated AEA, which is converted to AEA by a phosphatase (Liu et al. 2006); (3) dual hydrolysis of the acyl groups by alpha/beta domain-containing hydrolase 4 (ABHD4), resulting in glycerophospho-AEA, converted to AEA by glycerophosphodiesterase I (GDE-1) (Simon and Cravatt 2010); and (4) hydrolysis of one acyl group by phospholipase A1 (PLA1) or PLA2, followed by lyso-NAPE-PLD to release AEA (Nyilas et al. 2008). NAPE-PLD, the most well-studied synthetic enzyme for AEA, is localized on the endoplasmic reticulum of presynaptic terminals, in close association with axonal calcium stores (Nyilas et al. 2008).

AEA is primarily degraded by the enzyme fatty acid amine hydrolase (FAAH) (Cravatt et al. 1996), which degrades multiple fatty acid amides, including oleoylethanolamide (OEA) and palmitoylethanolamide (PEA). N-acylethanolamine-hydrolyzing acid amidase (NAAA) (Tsuboi et al. 2005) and cyclooxygenase-2 (COX-2) (Woodward et al. 2008) can also contribute to the degradation of AEA.

FAAH is primarily located in neurons that are postsynaptic to neurons expressing presynaptic CB1 receptors (Gulyas et al. 2004) and therefore may regulate the amount of AEA that is released from the postsynaptic neuron rather than participate in its degradation at the CB1 receptor. FAAH is an integral membrane protein that is localized primarily to intracellular membranes and not the plasma membrane (Hillard et al. 1995). There is a relatively prevalent single nucleotide polymorphism in the FAAH gene in humans that results in instability of the FAAH protein (Chiang et al. 2004), leading to increased circulating AEA concentrations (Spagnolo et al. 2016). This genetic variation has proven useful in understanding the contribution of AEA to behaviors and psychiatric symptoms, which is discussed further below.

3.2 2-AG Synthesis and Degradation

2-AG is synthesized by the sequential hydrolysis of an SN2-arachidonoyl-containing phosphatidyl inositol bis-phosphate (often 1-stearoyl-2-arachidonoyl-sn-glycerol) by a phospholipase Cβ (PLCβ), followed by hydrolysis of the resulting diacylglycerol by diacylglycerol lipase (DAGL) (Jung et al. 2007). There are two isoforms of

this enzyme, DAGLα and DAGLβ, of which DAGLα appears to contribute most to the 2-AG production that regulates synaptic plasticity in the CNS (Gao et al. 2010; Tanimura et al. 2010). DAGLβ is expressed in the developing CNS (Bisogno et al. 2003) as well as in microglia (Viader et al. 2016).

There is evidence that DAGLα gene expression can be modulated by environmental conditions (Marco et al. 2014). DAGLα activity can also be regulated by posttranslational modifications. Phosphorylation by calmodulin-dependent protein kinase II (CamKII) reduces enzymatic activity and decreases 2-AG synthesis (Murray et al. 2021; Shonesy et al. 2013). However, phosphorylation by cAMP-dependent protein kinase (PKA) enhances DAGLα activity and increases 2-AG synthesis (Shonesy et al. 2020).

A second pathway for 2-AG synthesis involves the cleavage of a phosphatidyl inositol precursor by a phospholipase A1, resulting in the formation of lysophosphatidyl inositol, followed by hydrolysis of the phosphate ester by a lyso-PLC (Higgs and Glomset 1994); however, the importance of this pathway in the brain is unclear.

2-AG inactivation involves hydrolysis to arachidonic acid and glycerol. This hydrolysis is catalyzed by three different serine hydrolase enzymes: monoacylglycerol lipase (MAGL), alpha/beta domain-containing hydrolase 6 (ABHD6), and alpha/beta domain-containing hydrolase 12 (ABHD12) (Blankman et al. 2007). 2-AG can also be oxidized by COX-2 and hydrolyzed by FAAH, although this is not likely to be an important mechanism in vivo. MAGL appears to account for the majority of 2-AG hydrolysis in the brain (Blankman et al. 2007), and is primarily localized to the synaptic terminals (Gulyas et al. 2004; Ludányi et al. 2011), suggesting a role of MAGL in limiting 2-AG signaling at CB1 receptors.

3.3 Binding and Transport of the Endocannabinoids

Due to the hydrophobic nature of AEA and 2-AG, it has been hypothesized that they require transporters to be able to diffuse in the cytosol and across the synapse (Kaczocha and Haj-Dahmane 2022). One class of proteins that has been implicated is the fatty acid binding proteins (FABPs) (Xu et al. 2019). AEA and 2-AG bind to several of the FABPs, including FABP5, which is present in the adult brain (Kaczocha et al. 2012). There is evidence that FABP5 may be functionally important in both the release and inactivation of endocannabinoids (Haj-Dahmane et al. 2018; Miller et al. 2020). Another protein that may be important for intracellular transport of endocannabinoids is the sterol carrier protein 2 (SCP-2), which binds to AEA and 2-AG with high affinity (Hillard et al. 2017). It is present in the brain and is enriched in synaptosomes (Myers-Payne et al. 1996), suggesting a role in regulating endocannabinoid signaling at the synapse.

4 The Endocannabinoid System in the Regulation of Synaptic Transmission

The fact that the CB1 receptor is located most prominently in the axon terminals suggested a role of the receptor in regulating synaptic signaling by modulating neurotransmitter release. In addition, the presence of 2-AG synthesizing enzymes, particularly DAGLα, in the postsynaptic terminal suggests a role for the 2-AG/CB1 receptor signaling pair in retrograde regulation of synaptic activity. Binding of agonists to the CB1 receptor modulates neurotransmitter release through inhibiting the opening of voltage-dependent calcium channels in the axon terminal and activation of potassium channels, resulting in hyperpolarization. The ultimate effect of these processes is a decrease in presynaptic vesicular release probability with resulting decreases in synaptic neurotransmission (Kano et al. 2009).

Three basic forms of endocannabinoid-mediated effects on synaptic transcription have been described: (1) depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE); (2) metabotropic receptor-induced suppression of inhibition (MSI) and metabotropic-induced suppression of excitation (MSE); and (3) endocannabinoid-mediated long-term depression (LTD). These forms of synaptic transmission have been reviewed extensively previously (Kano et al. 2009; Winters and Vaughan 2021) and are described briefly here.

4.1 DSI and DSE

Key evidence for a role of endocannabinoids in DSI was based on the finding that depolarization of postsynaptic neurons results in a transient suppression of presynaptic neurotransmitter release from GABAergic neurons (Pitler and Alger 1992), an effect which is dependent on GPCR signaling (Pitler and Alger 1994). It was later found that this effect, known as depolarization-induced suppression of inhibition (DSI), is dependent upon endocannabinoid binding to the CB1 receptor (Ohno-Shosaku et al. 2001; Wilson and Nicoll 2001). Similar postsynaptic depolarization-induced inhibition of presynaptic glutamate release through a CB1 receptor-mediated mechanism, known as depolarization-induced suppression of excitation (DSE), was also discovered (Kreitzer and Regehr 2001). In DSI and DSE, depolarization results in elevations of calcium in the postsynaptic neuron, which leads to 2-AG synthesis by DAGL. 2-AG release and transsynaptic trafficking is a current topic of investigation, but functional studies in brain slices indicated that 2-AG rapidly diffuses to the axon terminal, adsorbs into the plasma membrane, and binds and activates CB1 receptors. This subsequently results in impaired neurotransmitter release from the presynaptic axon terminal. More recently, DSI has been found to play an important role in modulating inhibitory synapses in the hippocampus during behavior (Dudok et al. 2024).

4.2 MSI and MSE

Metabotropic-induced suppression of inhibition or excitation occurs after binding of a neurotransmitter to a postsynaptic GPCR that couples to activation of G_q or another heterotrimeric G protein that activates PLCβ to produce diacylglycerol. The resulting diacylglycerol is then deacylated by DAGL to produce 2-AG, as described above. The subsequent diffusion of 2-AG, binding to CB1 receptors, and inhibition of neurotransmitter release is thought to occur through the same mechanisms as described above for DSI and DSE. A variety of GPCRs can induce this signaling, including mGluR1, mGluR5, M1, and M3 muscarinic receptors, orexinA, CCK_A, and α_1-adrenergic receptors.

4.3 LTD

LTD is a long-lasting form of synaptic plasticity that can occur through multiple processes, including through the action of endocannabinoid/CB1 receptor signaling. LTD can occur at the synapse being stimulated (homosynaptic LTD) or at an adjacent synapse (heterosynaptic LTD). Homosynaptic LTD is prominent at glutamatergic synapses in the striatum and is evoked by persistent low-frequency stimulation (Gerdeman et al. 2002; Robbe et al. 2002). Heterosynaptic LTD is most prominent at GABAergic synapses in the hippocampus, which results in removal of inhibition and an associated increase in dendritic excitability. This process is associated with the maturation of cortical circuits (Jiang et al. 2010).

5 The Endocannabinoid System in Glial Cells

5.1 Astroglial Endocannabinoid System

A common structural feature in the brain is a tripartite synapse, which consists of astroglial processes in close proximity to neuronal presynaptic and postsynaptic terminals. Astrocytes play a crucial role in modulating synaptic transmission and plasticity at the tripartite synapse (Martinez Ramirez et al. 2023). Astrocytes express CB1 receptor on their plasma membrane, which can respond to endocannabinoids produced from the postsynaptic terminal (Covelo et al. 2021). For example, at tripartite synapses involving hippocampal pyramidal neurons, activation of the astroglial CB1 receptor increases cytosolic calcium, triggering astrocytic glutamate release and activation of neuronal NMDA receptors (Navarrete and Araque 2008, 2010). Notably, astroglial CB1 receptors are important for long-term object recognition memory as they mediate basal levels of synaptic D-serine (Robin et al. 2018), which is a necessary co-agonist of the NMDA receptors at CA3-CA1 synapses important for induction of long-term potentiation (LTP) (Robin et al. 2018).

Furthermore, in mice with CB1 receptor deletion in astrocytes, the systemic injection of D-serine reversed memory deficits (Robin et al. 2018).

5.2 *Lateral Synaptic Regulation*

Since a single astrocyte can contact many synapses (Oberheim et al. 2009), they serve as a bridge to allow crosstalk between distant synapses in a process known as lateral synaptic regulation (Covelo and Araque 2016). This process was first shown in the CA1 region of the hippocampus, where astroglial CB1 receptor activation at one tripartite synapse induces gliotransmitter glutamate release at a distant tripartite synapse, which subsequently activates presynaptic type-1 metabotropic glutamate receptors (mGluR1) to produce lateral synaptic potentiation (LSP) (Covelo et al. 2021; Navarrete and Araque 2010). This process has been shown to play a role in amygdala-related behavioral outcomes. For example, activation of central amygdala (CeM) astrocytes by endocannabinoids differentially regulates excitatory and inhibitory synapses, decreases firing rate of CeM neurons, and reduces fear expression (Martin-Fernandez et al. 2017), demonstrating that the astroglial CB1 receptor mediates LSP with specific synapses that ultimately affect circuit-level behavioral output in the amygdala.

5.3 *Astroglial Mitochondrial CB1 Receptors*

In addition to expression at the plasma membrane, the CB1 receptor is expressed in the mitochondrial membrane of astrocytes (Gutiérrez-Rodríguez et al. 2018; Serrat et al. 2021). In fact, astrocytic mitochondrial CB1 (mtCB1) receptors play an important role in lateral synaptic regulation (Csordás et al. 2018; Gutiérrez-Rodríguez et al. 2018; Serrat et al. 2021). mtCB1 receptor activation was shown to be required for LSP between two distant synapses in the CA1 region of the hippocampus (Serrat et al. 2021). Specifically, mtCB1 receptor mediates uptake of calcium from the endoplasmic reticulum through the mitochondrial calcium uniporter, and this process is required for LSP (Serrat et al. 2021). In addition, mtCB1 receptor signaling regulates astroglial glucose metabolism, which mediates social behavior (Jimenez-Blasco et al. 2020). In this study, mtCB1 receptor activation with THC resulted in diminished release of lactate from astrocytes to neurons, which in turn increased neuronal oxidative stress, ultimately leading to impaired social behavior (Jimenez-Blasco et al. 2020). While there is substantial evidence of CB1 receptor expression in astrocytes, there is a lack of evidence supporting CB2 receptor expression in astrocytes under physiological conditions (Eraso-Pichot et al. 2023). On the other hand, CB2 receptor expression has been reported in human astrocytes derived from human fetal brain tissue (Sheng et al. 2005) and in the cerebellar granule layer in postmortem studies from patients with spinocerebellar ataxias (Rodríguez-Cueto et al. 2014).

5.4 Microglial Endocannabinoid System Involved in Immune Function

The endocannabinoid system, specifically the CB2 receptor, plays a key role in regulating microglial phenotypes. Microglia have highly ramified motile processes which surveil brain parenchyma for changes to homeostasis (Augusto-Oliveira et al. 2021). Once tissue damage or pathogens are detected, microglia retract their processes and migrate to the site of injury (Komorowska-Müller and Schmöle 2020). A pro-inflammatory microglial phenotype is induced to eliminate a pathogen or remove damaged tissue. In vitro studies with lipopolysaccharide (LPS) or interferon gamma stimulation induce this pro-inflammatory microglial state, which is characterized by secretion of pro-inflammatory cytokines, such as TNFα, IL-1β, and iNOS (Maresz et al. 2005). In the presence of inflammatory stimuli, in vitro studies show that CB2 receptor activation induces an anti-inflammatory microglial phenotype, characterized by decreased release of inflammatory mediators, such as TNFα and IL1β, and increased release of anti-inflammatory mediators, such as IL-4 and IL-10 (Tang and Le 2016). CB2 receptor activation can also reduce phagocytic activity through IL1RL1 suppression (Du et al. 2020; Nguyen et al. 2019). Thus, CB2 receptor activation mediates a shift from an inflammatory to an anti-inflammatory microglial phenotype.

5.5 Microglial Endocannabinoid System Involved in Synaptic Remodeling

Under physiological conditions, there is low basal expression of CB2 receptor in microglia as revealed by immunofluorescent staining (López et al. 2018) and electron microscopy (Ruiz de Martin Esteban et al. 2022) in transgenic mice that co-express GFP with the CB2 receptor. There is growing evidence supporting a role of microglial CB2 receptor in modulating synaptic strength and memory processes. For example, there is evidence that microglial CB2 receptor plays a role in contextual fear memory (Li and Kim 2017). CB2 receptor knock-out mice exhibit reduced brain-derived neurotrophic factor (BDNF) and glucocorticoid receptor (NR3C1) gene expression, as well as a reduced number of synapses in the dentate gyrus, which were all associated with impaired aversive memory consolidation (García-Gutiérrez et al. 2013). Moreover, CB2 receptor knockout mice exhibit reduced mushroom spine density in CA3 pyramidal neurons, reduced thin spine density in CA1 pyramidal neurons, and impaired LTP at CA1 Schaffer collateral synapses (Li and Kim 2016). These findings suggest that CB2 receptor signaling is involved in the formation and maturation of dendritic spines.

There is also evidence that microglial CB1 receptors have an important physiological role in social memory. In mice with a 16p11.2 duplication (16p11dup), a chromosomal change associated with increased risk for autism spectrum disorder,

and adolescent THC exposure, there was increased microglial apoptosis in the medial prefrontal cortex (mPFC), reduced excitability of pyramidal tract (PT) neuron in the mPFC, and social memory deficits at adulthood (Hasegawa et al. 2023). Genetic deletion of microglial CB1 receptor prior to adolescent THC exposure reduced apoptotic microglia in the mPFC and rescued social memory deficits in 16p11dup mice (Hasegawa et al. 2023). It is still unknown whether PT neurons are selectively affected in THC-exposed, 16p11dup mice through aberrant microglial synaptic pruning.

6 The Endocannabinoid System and Stress Responses

The endocannabinoid system plays a crucial role in modulating the effects of stress on the brain as well as modulating other stress-responsive systems, including the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis (deRoon-Cassini et al. 2020; Hill et al. 2018; Morena et al. 2016). Stress induces changes in endocannabinoid signaling generally through a two-phase response. First is a rapid phase in which increased corticotropin-releasing hormone (CRH) leads to a decrease in AEA, which initiates the stress response. This is followed by a delayed phase in which increased glucocorticoids mediate an increase in 2-AG to terminate the stress response.

6.1 Corticotropin-Releasing Hormone (CRH) and AEA

The effects of stress on AEA are rapid and precede activation of the HPA axis (Hill et al. 2009). Consistent with this, it has been found that CRH binding to its receptor, the CRH type 1 receptor (CRHR1), on glutamatergic neurons in the basolateral amygdala (BLA) results in increased FAAH activity, leading to increased AEA hydrolysis and decreased AEA content (Gray et al. 2015). The result of these biochemical events is hypothesized to be increased glutamatergic signaling onto efferent neurons in the amygdala projecting to the hypothalamus. These findings are in accord with data that inhibition of FAAH reduces stress responses (Patel and Hillard 2006) and that FAAH knock-out mice are protected from behavioral and morphological effects of chronic, severe stress (Hill et al. 2013).

6.2 Glucocorticoids and 2-AG

Glucocorticoids are released from the adrenal gland following activation of the HPA axis in stress. Glucocorticoids rapidly increase 2-AG through mechanisms that are independent of the genomic effects of the glucocorticoid receptor (GR). Evidence

from the hypothalamus demonstrates that binding of glucocorticoids to a membrane-bound GR leads to G-protein activation and the subsequent production of endocannabinoids (Di et al. 2003, 2005) through the recruitment of Gαs-cAMP-PKA signaling (Di et al. 2009; Malcher-Lopes et al. 2006). This leads to functional changes in neurotransmission, including decreased glutamate release onto hypothalamic magnocellular neurons (Di et al. 2003, 2005) and suppression of GABAergic currents in the BLA (Di et al. 2016), which are dependent upon the glucocorticoid-induced endocannabinoid signaling. This signaling is required for glucocorticoid-mediated feedback of the HPA axis in both the hypothalamus (Evanson et al. 2010) and prefrontal cortex (Hill et al. 2011), although the mechanism linking glucocorticoids to 2-AG mobilization is likely different.

6.3 *Endocannabinoid Signaling and Sympathetic Responses to Stress*

The endocannabinoid system has been shown to play an important role in the regulation of sympathetic nervous system responses to stress. CB1 receptors are expressed on the terminals of sympathetic neurons (Pfitzer et al. 2005) and on adrenal medullary cells (Niederhoffer et al. 2001), and CB1 receptor agonists inhibit the release of norepinephrine and epinephrine from these cells, respectively. The locus coeruleus is the site of noradrenergic neurons that innervate the forebrain to regulate arousal, attention, and vigilance. Input to this region is negatively regulated by endocannabinoid signaling (Wyrofsky et al. 2019), suggesting that the endocannabinoid system plays an important role in buffering against overactivation of noradrenergic signaling.

AEA has been shown to modulate vasoconstriction through binding to peripheral CB1 receptors (Wagner et al. 1997) as well as modulate the baroreflex through activation of CB1 receptors within the nucleus tractus solitarius (Seagard et al. 2004). In addition, there is evidence that CB1 receptor signaling is involved in controlling the cardiovascular responses to stress through modulating activity of the bed nucleus of the stria terminalis (Gomes-de-Souza et al. 2020), a brain area important for the integration of stress-related behaviors.

6.4 *Effects of Chronic Stress on Endocannabinoid Signaling*

Exposure to chronic stress alters endocannabinoid signaling in the brain.

When there is repeated exposure to the same stressor, it is beneficial for stress responses to habituate. Sensitization of endocannabinoid signaling appears to be important for this process. Repeated exposure to the same stressor results in increased 2-AG in the amygdala (Hill et al. 2010; Patel et al. 2005, 2009; Rademacher

et al. 2008), prefrontal cortex (Dubreucq et al. 2012; Patel et al. 2005), hippocampus (Dubreucq et al. 2012), and hypothalamus (Dubreucq et al. 2012; Patel et al. 2004). The elevation of 2-AG after repeated stress exposure is more robust than that seen in acute stress. This appears to be mediated by a reduction in MAGL-mediated hydrolysis (Sumislawski et al. 2011), and may account for attenuation of the glucocorticoid response following repeated exposure to the same stressor (Patel et al. 2004).

There is also a decrease in AEA content in the amygdala (Hill et al. 2010, 2013; Patel et al. 2005; Rademacher et al. 2008), hippocampus (Dubreucq et al. 2012; Hill et al. 2010), hypothalamus (Dubreucq et al. 2012; Hill et al. 2010), and prefrontal cortex (Hill et al. 2010; Rademacher et al. 2008), mediated by an increase in FAAH activity (Hill et al. 2013), as is seen following acute stress, but again is amplified. Interestingly, chronic, non-habituating stress results in less reliable changes in either 2-AG (Bortolato et al. 2007; Hill et al. 2005, 2008; Lomazzo et al. 2015; Wang et al. 2010) or AEA, although 2-AG has been found to decrease, rather than increase, in the hippocampus following chronic, unpredictable stress (Hill et al. 2005; Lomazzo et al. 2015; Zhong et al. 2014).

Chronic stress also results in downregulation of CB1 receptor signaling in a variety of brain regions including the hippocampus (Hill et al. 2005, 2008), amygdala (Hill et al. 2013), striatum (Hill et al. 2008; Rossi et al. 2008), nucleus accumbens (Wang et al. 2010), and hypothalamus (Wamsteeker et al. 2010), which may be mediated by chronic elevations in glucocorticoids. However, there is an increase in CB1 receptor expression in the prefrontal cortex following chronic stress (Bortolato et al. 2007; Hill et al. 2008; Lee and Hill 2013; McLaughlin et al. 2013; Zoppi et al. 2011) for reasons that remain unclear.

7 Regulation of Behavior by the Endocannabinoid System

As a major regulator of synaptic function, the endocannabinoid system is situated to regulate a wide variety of behaviors. While an extensive review of these behaviors is beyond the scope of this chapter, a summary of the role of the endocannabinoid system in modulating behaviors is described here.

7.1 Anxiety

Anxiety is a behavioral state associated with the anticipation of potential future threats, which allows the organism to act in a way that allows them to avoid potentially harmful or dangerous situations. The endocannabinoid system has been shown to play an important role in modulating these behaviors (Lutz et al. 2015).

Genetic deletion of FAAH, which results in impaired degradation of AEA, leads to increased AEA concentrations in the brain and corresponding decreases in

anxiety-like behavior in animals (Moreira et al. 2008). Similarly, pharmacological inhibition of FAAH reduces anxiety behaviors (Busquets-Garcia et al. 2011; Kathuria et al. 2003; Moreira et al. 2008; Patel and Hillard 2006). In humans, a polymorphism in the FAAH gene (rs324429, in which cysteine 385 is changed to alanine) was discovered, which results in destabilization of the FAAH enzyme and a corresponding increase in AEA signaling. Both humans and mice with this allele ($FAAH^{A/A}$) show a decrease in anxiety-like behavior (Dincheva et al. 2015).

Regarding 2-AG, studies have found that decreasing 2-AG through genetic deletion of DAGL, the synthetic enzyme for 2-AG, results in increased anxiety behavior (Jenniches et al. 2016; Shonesy et al. 2014). In addition, reducing 2-AG through overexpression of MAGL in hippocampal glutamatergic neurons results in increased anxiety behavior (Guggenhuber et al. 2015). On the other hand, increasing 2-AG through pharmacological inhibition of MAGL results in decreased anxiety behaviors (Busquets-Garcia et al. 2011; Kinsey et al. 2011).

In summary, acute increases in endocannabinoid signaling are associated with decreased anxiety, whereas blockade of these pathways results in increased anxiety.

7.2 Fear

Fear is a behavioral response to specific and actual threatening stimuli. Fear can be innate or can be acquired when a stimulus is learned to be associated with a specific threat. There is extensive evidence that the endocannabinoid system is involved in modulating fear behaviors (Lutz et al. 2015).

Cued fear conditioning is the most common model that is used to study fear circuits. In this protocol, a neutral stimulus is paired with a fear-inducing stimulus. The neutral stimulus is known as the conditioned stimulus (CS) and can be an acoustic, visual, tactile, gustatory, or olfactory cue. The fear-inducing stimulus, such as a foot shock, is known as the unconditioned stimulus (US). Over time, the subject learns to associate the US with the CS, and the CS alone can trigger a fear response.

There is strong evidence that endocannabinoid signaling is important in the extinction of a fear memory, which occurs with exposure to the conditioned stimulus in the absence of the unconditioned stimulus, resulting in a decrease in CS-stimulated fear expression. The first studies found that decreased signaling through the CB1 receptor, either through genetic deletion of the CB1 receptor or through pharmacologic antagonism, resulted in impaired extinction of fear memory in mice (Marsicano et al. 2002). Growing evidence has indicated that intact signaling of 2-AG at CB1 receptors is necessary for appropriate extinction of fear memories. For example, decreasing 2-AG through genetic deletion or pharmacologic inhibition of DAGL consistently impairs fear extinction (Cavener et al. 2018; Jenniches et al. 2016; Ramos-Medina et al. 2024). Interestingly, enhancement of 2-AG signaling through inhibition of MAGL also results in impaired fear extinction (Hartley et al. 2016; Llorente-Berzal et al. 2015; Mizuno et al. 2022). This suggests

that 2-AG is necessary for appropriate processing of fear memories, and 2-AG concentrations that are either too high or too low may impair fear extinction.

The effects of endocannabinoid signaling on fear extinction are dependent on brain region. For example, CB1 receptor antagonism in the central amygdala results in impaired short-term extinction, whereas antagonism in the basolateral amygdala results in impaired long-term extinction (Kamprath et al. 2011). Antagonism of CB1 receptors in the infralimbic region of the medial prefrontal cortex also impairs fear extinction (Lin et al. 2009). Enhancing AEA signaling in the basolateral amygdala through inhibition of FAAH facilitates fear extinction (Gunduz-Cinar et al. 2013). In humans and mice with the $FAAH^{A/A}$ allele, which results in increased AEA due to impaired FAAH-mediated degradation, there is enhanced fear-extinction learning (Dincheva et al. 2015), suggesting that AEA signaling, in particular, at CB1 receptors may enhance the extinction of fear memories.

7.3 Stress Responsive Behavior

There is evidence that the endocannabinoid system plays a significant role in modulating behavioral responses during times of stress. It is hypothesized that stress produces anxiety through a rapid reduction in AEA signaling, resulting in a transient decrease in CB1 receptor signaling that facilitates the emergence of an anxiety state (Morena et al. 2016). This is supported by findings that brain concentrations of AEA are negatively correlated with anxiety behaviors (Bluett et al. 2014), and elevating AEA through the inhibition of FAAH reduces anxiety following stress (Bluett et al. 2014; Gray et al. 2015; Haller et al. 2009). On the other hand, blockade of CB1 receptor signaling results in an increase in anxiety induced by stress (Gamble-George et al. 2013).

Evidence for the role of AEA signaling in modulating stress-induced anxiety in humans has come through studies in individuals with a common polymorphism in the FAAH gene, where the $FAAH^{A/A}$ allele is associated with decreased FAAH activity and elevated AEA. In these individuals, there is evidence of reduced anxiety responses to stressors (Spagnolo et al. 2016) and decreased negative affective responses to stress (Mayo et al. 2020). As such, the endocannabinoid system appears to play an important role in buffering against anxiety induced by stress.

Chronic unpredictable stress (CUS) can result in a wide variety of behavioral disturbances, including increased anxiety, depression, and chronic pain. Pharmacological inhibition of FAAH or MAGL, which increase concentrations of AEA and 2-AG, respectively, have been shown to improve anxiety and depressive behaviors in CUS models (Lomazzo et al. 2015; Zhang et al. 2015). Likewise, inhibition of FAAH has been shown to improve chronic nociceptive changes that occur following CUS (Lomazzo et al. 2015).

7.4 Learning and Memory

There is evidence that appropriate endocannabinoid signaling is important in the acquisition of new memories. For example, decreasing 2-AG signaling through inhibition of its biosynthetic enzyme DAGL results in impairments in integration of new information in a special memory task (Schurman et al. 2019). On the other hand, enhancing AEA signaling by genetic deletion (Varvel et al. 2006; Varvel et al. 2007) or pharmacologic inhibition (Mazzola et al. 2009) of FAAH has been shown to enhance memory acquisition. However, there is consistent evidence that activation of the endocannabinoid system with exogenous ligands impairs memory acquisition. For example, administration of CB1 receptor agonists before training impairs acquisition of memory in a number of behavioral tasks (Da and Takahashi 2002; Lichtman et al. 1995; Pamplona and Takahashi 2006).

The role of endocannabinoid signaling appears to be particularly important in facilitating memory consolidation in highly arousing situations. Glucocorticoids recruit endocannabinoid signaling within the basolateral amygdala to enhance memory consolidation (Campolongo et al. 2009). Additional studies have found a crucial role for AEA in potentiating memory consolidation through CB1 receptor activation in corticolimbic circuits (Morena et al. 2014).

8 Conclusion

There has been great progress in elucidating the components and regulation of the endocannabinoid system and its functions. The endocannabinoid system has widespread impact, including maintaining homeostasis, modulating neuronal signaling, and regulating a wide range of behaviors. As exogenous cannabinoids, such as the components of the cannabis plant, act through this system, it is unsurprising that they may have broad ranging effects. This is particularly salient given the growing understanding of the role of endocannabinoid system dysregulation in a variety of psychiatric disorders. Further advancements in our understanding of this system may lead to novel treatments for psychiatric disorders and better modulation of their risk factors.

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References

Atwood BK, Mackie K. CB2: a cannabinoid receptor with an identity crisis. Br J Pharmacol. 2010;160:467–79.

Augusto-Oliveira M, Arrifano GP, Delage CI, Tremblay ME, Crespo-Lopez ME, Verkhratsky A. Plasticity of microglia. Biol Rev Camb Philos Soc. 2021;34:217–50.

Battista N, Meccariello R, Cobellis G, Fasano S, Di Tommaso M, Pirazzi V, Konje JC, Pierantoni R, Maccarrone M. The role of endocannabinoids in gonadal function and fertility along the evolutionary axis. Mol Cell Endocrinol. 2012;355:1–14.

Bénard G, Massa F, Puente N, Lourenço J, Bellocchio L, Soria-Gómez E, Matias I, Delamarre A, Metna-Laurent M, Cannich A, et al. Mitochondrial CB_1 receptors regulate neuronal energy metabolism. Nat Neurosci. 2012;15:558–64.

Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, Matias I, Schiano-Moriello A, Paul P, Williams EJ, et al. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol. 2003;163:463–8.

Blankman JL, Simon GM, Cravatt BF. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol. 2007;14:1347–56.

Bluett RJ, Gamble-George JC, Hermanson DJ, Hartley ND, Marnett LJ, Patel S. Central anandamide deficiency predicts stress-induced anxiety: behavioral reversal through endocannabinoid augmentation. Transl Psychiatry. 2014;4:e408.

Bortolato M, Mangieri RA, Fu J, Kim JH, Arguello O, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D. Antidepressant-like activity of the fatty acid amide hydrolase inhibitor URB597 in a rat model of chronic mild stress. Biol Psychiatry. 2007;62:1103–10.

Busquets-Garcia A, Puighermanal E, Pastor A, de la Torre R, Maldonado R, Ozaita A. Differential role of anandamide and 2-arachidonoylglycerol in memory and anxiety-like responses. Biol Psychiatry. 2011;70:479–86.

Campolongo P, Roozendaal B, Trezza V, Hauer D, Schelling G, McGaugh JL, Cuomo V. Endocannabinoids in the rat basolateral amygdala enhance memory consolidation and enable glucocorticoid modulation of memory. Proc Natl Acad Sci USA. 2009;106:4888–93.

Carlisle SJ, Marciano-Cabral F, Staab A, Ludwick C, Cabral GA. Differential expression of the CB2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. Int Immunopharmacol. 2002;2:69–82.

Carvalho AF, Mackie K, Van Bockstaele EJ. Cannabinoid modulation of limbic forebrain noradrenergic circuitry. Eur J Neurosci. 2010;31:286–301.

Cavener VS, Gaulden A, Pennipede D, Jagasia P, Uddin J, Marnett LJ, Patel S. Inhibition of diacylglycerol lipase impairs fear extinction in mice. Front Neurosci. 2018;12:479.

Chiang KP, Gerber AL, Sipe JC, Cravatt BF. Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. Hum Mol Genet. 2004;13:2113–9.

Covelo A, Araque A. Lateral regulation of synaptic transmission by astrocytes. Neuroscience. 2016;323:62–6.

Covelo A, Eraso-Pichot A, Fernández-Moncada I, Serrat R, Marsicano G. CB1R-dependent regulation of astrocyte physiology and astrocyte-neuron interactions. Neuropharmacology. 2021;195:108678.

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature. 1996;384:83–7.

Csordás G, Weaver D, Hajnóczky G. Endoplasmic reticulum–mitochondrial contactology: structure and signaling functions. Trends Cell Biol. 2018;28:523–40.

Da S, Takahashi RN. SR 141716A prevents delta 9-tetrahydrocannabinol-induced spatial learning deficit in a Morris-type water maze in mice. Prog Neuro-Psychopharmacol Biol Psychiatry. 2002;26:321–5.

deRoon-Cassini TA, Stollenwerk TM, Beatka M, Hillard CJ. Meet your stress management professionals: the endocannabinoids. Trends Mol Med. 2020;26:953–68.

Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol. 1988;34:605–13.

Di S, Malcher-Lopes R, Halmos KC, Tasker JG. Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. J Neurosci Off J Soc Neurosci. 2003;23:4850–7.

Di S, Malcher-Lopes R, Marcheselli VL, Bazan NG, Tasker JG. Rapid glucocorticoid-mediated endocannabinoid release and opposing regulation of glutamate and γ-aminobutyric acid inputs to hypothalamic magnocellular neurons. Endocrinology. 2005;146:4292–301.

Di S, Maxson MM, Franco A, Tasker JG. Glucocorticoids regulate glutamate and GABA synapse-specific retrograde transmission via divergent nongenomic signaling pathways. J Neurosci Off J Soc Neurosci. 2009;29:393–401.

Di S, Itoga CA, Fisher MO, Solomonow J, Roltsch EA, Gilpin NW, Tasker JG. Acute stress suppresses synaptic inhibition and increases anxiety via endocannabinoid release in the basolateral amygdala. J Neurosci. 2016;36:8461–70.

Dincheva I, Drysdale AT, Hartley CA, Johnson DC, Jing D, King EC, Ra S, Gray JM, Yang R, DeGruccio AM, et al. FAAH genetic variation enhances fronto-amygdala function in mouse and human. Nat Commun. 2015;6:6395.

Du JJ, Liu ZQ, Yan Y, Xiong J, Jia XT, Di ZL, Ren JJ. The cannabinoid WIN 55,212-2 reduces delayed neurologic sequelae after carbon monoxide poisoning by promoting microglial M2 polarization through ST2 signaling. J Mol Neurosci. 2020;70:422–32.

Dubreucq S, Matias I, Cardinal P, Häring M, Lutz B, Marsicano G, Chaouloff F. Genetic dissection of the role of cannabinoid type-1 receptors in the emotional consequences of repeated social stress in mice. Neuropsychopharmacology. 2012;37:1885–900.

Dudok B, Fan LZ, Farrell JS, Malhotra S, Homidan J, Kim DK, Wenardy C, Ramakrishnan C, Li Y, Deisseroth K, et al. Retrograde endocannabinoid signaling at inhibitory synapses in vivo. Science. 2024;383:967–70.

El-Talatini MR, Taylor AH, Elson JC, Brown L, Davidson AC, Konje JC. Localisation and function of the endocannabinoid system in the human ovary. PLoS One. 2009;4:e4579.

Eraso-Pichot A, Pouvreau S, Olivera-Pinto A, Gomez-Sotres P, Skupio U, Marsicano G. Endocannabinoid signaling in astrocytes. Glia. 2023;71:44–59.

Evanson NK, Tasker JG, Hill MN, Hillard CJ, Herman JP. Fast feedback inhibition of the HPA axis by glucocorticoids is mediated by endocannabinoid signaling. Endocrinology. 2010;151:4811–9.

Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O, Lai Y, Ma AL, Mitchell RL. Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Mol Pharmacol. 1995;48:443–50.

Galiègue S, Mary S, Marchand J, Dussossoy D, Carrière D, Carayon P, Bouaboula M, Shire D, Le Fur G, Casellas P. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem. 1995;232:54–61.

Gamble-George JC, Conger JR, Hartley ND, Gupta P, Sumislawski JJ, Patel S. Dissociable effects of CB1 receptor blockade on anxiety-like and consummatory behaviors in the novelty-induced hypophagia test in mice. Psychopharmacology. 2013;228:401–9.

Gao Y, Vasilyev DV, Goncalves MB, Howell FV, Hobbs C, Reisenberg M, Shen R, Zhang MY, Strassle BW, Lu P, et al. Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. J Neurosci. 2010;30:2017–24.

García-Gutiérrez MS, Ortega-Álvaro A, Busquets-García A, Pérez-Ortiz JM, Caltana L, Ricatti MJ, Brusco A, Maldonado R, Manzanares J. Synaptic plasticity alterations associated with memory impairment induced by deletion of CB2 cannabinoid receptors. Neuropharmacology. 2013;73:388–96.

Gerdeman GL, Ronesi J, Lovinger DM. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. Nat Neurosci. 2002;5:446–51.

Gomes-de-Souza L, Costa-Ferreira W, Oliveira LA, Benini R, Crestani CC. Cannabinoid receptor type 1 in the bed nucleus of the stria terminalis modulates cardiovascular responses to stress via local N-methyl-D-aspartate receptor/neuronal nitric oxide synthase/soluble guanylate cyclase/protein kinase G signaling. J Psychopharmacol. 2020;34:429–40.

Gray JM, Vecchiarelli HA, Morena M, Lee TTY, Hermanson DJ, Kim AB, McLaughlin RJ, Hassan KI, Kuhne C, Wotjak CT, et al. Corticotropin-releasing hormone drives anandamide hydrolysis in the amygdala to promote anxiety. J Neurosci. 2015;35:3879–92.

Grimaldi P, Orlando P, Di Siena S, Lolicato F, Petrosino S, Bisogno T, Geremia R, De Petrocellis L, Di Marzo V. The endocannabinoid system and pivotal role of the CB2 receptor in mouse spermatogenesis. Proc Natl Acad Sci USA. 2009;106:11131–6.

Guggenhuber S, Romo-Parra H, Bindila L, Leschik J, Lomazzo E, Remmers F, Zimmermann T, Lerner R, Klugmann M, Pape HC, et al. Impaired 2-AG signaling in hippocampal glutamatergic neurons: aggravation of anxiety-like behavior and unaltered seizure susceptibility. Int J Neuropsychopharmacol. 2015;19

Gulyas AI, Cravatt BF, Bracey MH, Dinh TP, Piomelli D, Boscia F, Freund TF. Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. Eur J Neurosci. 2004;20:441–58.

Gunduz-Cinar O, MacPherson KP, Cinar R, Gamble-George J, Sugden K, Williams B, Godlewski G, Ramikie TS, Gorka AX, Alapafuja SO, et al. Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. Mol Psychiatry. 2013;18:813–23.

Gutiérrez-Rodríguez A, Bonilla-Del Río I, Puente N, Gómez-Urquijo SM, Fontaine CJ, Egaña-Huguet J, Elezgarai I, Ruehle S, Lutz B, Robin LM, et al. Localization of the cannabinoid type-1 receptor in subcellular astrocyte compartments of mutant mouse hippocampus. Glia. 2018;66:1417–31.

Haj-Dahmane S, Shen RY, Elmes MW, Studholme K, Kanjiya MP, Bogdan D, Thanos PK, Miyauchi JT, Tsirka SE, Deutsch DG, et al. Fatty-acid-binding protein 5 controls retrograde endocannabinoid signaling at central glutamate synapses. Proc Natl Acad Sci USA. 2018;115:3482–7.

Haller J, Barna I, Barsvari B, Gyimesi Pelczer K, Yasar S, Panlilio LV, Goldberg S. Interactions between environmental aversiveness and the anxiolytic effects of enhanced cannabinoid signaling by FAAH inhibition in rats. Psychopharmacology. 2009;204:607–16.

Häring M, Marsicano G, Lutz B, Monory K. Identification of the cannabinoid receptor type 1 in serotonergic cells of raphe nuclei in mice. Neuroscience. 2007;146:1212–9.

Häring M, Enk V, Aparisi Rey A, Loch S, Ruiz de Azua I, Weber T, Bartsch D, Monory K, Lutz B. Cannabinoid type-1 receptor signaling in central serotonergic neurons regulates anxiety-like behavior and sociability. Front Behav Neurosci. 2015;9:235.

Hartley ND, Gunduz-Cinar O, Halladay L, Bukalo O, Holmes A, Patel S. 2-arachidonoylglycerol signaling impairs short-term fear extinction. Transl Psychiatry. 2016;6:e749.

Hasegawa Y, Kim J, Ursini G, Jouroukhin Y, Zhu X, Miyahara Y, Xiong F, Madireddy S, Obayashi M, Lutz B, et al. Microglial cannabinoid receptor type 1 mediates social memory deficits in mice produced by adolescent THC exposure and 16p11.2 duplication. Nat Commun. 2023;14

Higgs HN, Glomset JA. Identification of a phosphatidic acid-preferring phospholipase A1 from bovine brain and testis. Proc Natl Acad Sci USA. 1994;91:9574–8.

Hill MN, Patel S, Carrier EJ, Rademacher DJ, Ormerod BK, Hillard CJ, Gorzalka BB. Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. Neuropsychopharmacology. 2005;30:508–15.

Hill MN, Carrier EJ, McLaughlin RJ, Morrish AC, Meier SE, Hillard CJ, Gorzalka BB. Regional alterations in the endocannabinoid system in an animal model of depression: effects of concurrent antidepressant treatment. J Neurochem. 2008;106:2322–36.

Hill MN, McLaughlin RJ, Morrish AC, Viau V, Floresco SB, Hillard CJ, Gorzalka BB. Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic-pituitary-adrenal axis. Neuropsychopharmacology. 2009;34:2733–45.

Hill MN, McLaughlin RJ, Bingham B, Shrestha L, Lee TT, Gray JM, Hillard CJ, Gorzalka BB, Viau V. Endogenous cannabinoid signaling is essential for stress adaptation. Proc Natl Acad Sci USA. 2010;107:9406–11.

Hill MN, McLaughlin RJ, Pan B, Fitzgerald ML, Roberts CJ, Lee TTY, Karatsoreos IN, Mackie K, Viau V, Pickel VM, et al. Recruitment of prefrontal cortical endocannabinoid signaling by glucocorticoids contributes to termination of the stress response. J Neurosci Off J Soc Neurosci. 2011;31:10506–15.

Hill MN, Kumar SA, Filipski SB, Iverson M, Stuhr KL, Keith JM, Cravatt BF, Hillard CJ, Chattarji S, McEwen BS. Disruption of fatty acid amide hydrolase activity prevents the effects of chronic stress on anxiety and amygdalar microstructure. Mol Psychiatry. 2013;18:1125–35.

Hill MN, Campolongo P, Yehuda R, Patel S. Integrating endocannabinoid signaling and cannabinoids into the biology and treatment of posttraumatic stress disorder. Neuropsychopharmacology. 2018;43:80–102.

Hillard CJ. Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. Prostaglandins Other Lipid Mediat. 2000;61:3–18.

Hillard CJ, Wilkison DM, Edgemond WS, Campbell WB. Characterization of the kinetics and distribution of N-arachidonylethanolamine (anandamide) hydrolysis by rat brain. Biochim Biophys Acta. 1995;1257:249–56.

Hillard CJ, Huang H, Vogt CD, Rodrigues BE, Neumann TS, Sem DS, Schroeder F, Cunningham CW. Endocannabinoid transport proteins: discovery of tools to study sterol carrier Protein-2. Methods Enzymol. 2017;593:99–121.

Hua T, Li X, Wu L, Iliopoulos-Tsoutsouvas C, Wang Y, Wu M, Shen L, Brust CA, Nikas SP, Song F, et al. Activation and signaling mechanism revealed by cannabinoid receptor-G(i) complex structures. Cell. 2020;180:655–665.e618.

Hurst DP, Grossfield A, Lynch DL, Feller S, Romo TD, Gawrisch K, Pitman MC, Reggio PH. A lipid pathway for ligand binding is necessary for a cannabinoid G protein-coupled receptor. J Biol Chem. 2010;285:17954–64.

Ibsen MS, Finlay DB, Patel M, Javitch JA, Glass M, Grimsey NL. Cannabinoid CB1 and CB2 receptor-mediated arrestin translocation: species, subtype, and agonist-dependence. Front Pharmacol. 2019;10:350.

Jakowiecki J, Abel R, Orzeł U, Pasznik P, Preissner R, Filipek S. Allosteric modulation of the CB1 cannabinoid receptor by Cannabidiol-A molecular modeling study of the N-terminal domain and the allosteric-orthosteric coupling. Molecules. 2021;26

Jenniches I, Ternes S, Albayram O, Otte DM, Bach K, Bindila L, Michel K, Lutz B, Bilkei-Gorzo A, Zimmer A. Anxiety, stress, and fear response in mice with reduced endocannabinoid levels. Biol Psychiatry. 2016;79:858–68.

Jiang B, Huang S, de Pasquale R, Millman D, Song L, Lee HK, Tsumoto T, Kirkwood A. The maturation of GABAergic transmission in visual cortex requires endocannabinoid-mediated LTD of inhibitory inputs during a critical period. Neuron. 2010;66:248–59.

Jimenez-Blasco D, Busquets-Garcia A, Hebert-Chatelain E, Serrat R, Vicente-Gutierrez C, Ioannidou C, Gomez-Sotres P, Lopez-Fabuel I, Resch-Beusher M, Resel E, et al. Glucose metabolism links astroglial mitochondria to cannabinoid effects. Nature. 2020;583:603–8.

Jung KM, Astarita G, Zhu C, Wallace M, Mackie K, Piomelli D. A key role for diacylglycerol lipase-alpha in metabotropic glutamate receptor-dependent endocannabinoid mobilization. Mol Pharmacol. 2007;72:612–21.

Kaczocha M, Haj-Dahmane S. Mechanisms of endocannabinoid transport in the brain. Br J Pharmacol. 2022;179:4300–10.

Kaczocha M, Vivieca S, Sun J, Glaser ST, Deutsch DG. Fatty acid-binding proteins transport N-acylethanolamines to nuclear receptors and are targets of endocannabinoid transport inhibitors. J Biol Chem. 2012;287:3415–24.

Kamprath K, Romo-Parra H, Häring M, Gaburro S, Doengi M, Lutz B, Pape HC. Short-term adaptation of conditioned fear responses through endocannabinoid signaling in the central amygdala. Neuropsychopharmacology. 2011;36:652–63.

Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. Physiol Rev. 2009;89:309–80.

Kathuria S, Gaetani S, Fegley D, Valiño F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A, et al. Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med. 2003;9:76–81.

Katona I, Sperlágh B, Sík A, Käfalvi A, Vizi ES, Mackie K, Freund TF. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci. 1999;19:4544–58.

Kinsey SG, O'Neal ST, Long JZ, Cravatt BF, Lichtman AH. Inhibition of endocannabinoid catabolic enzymes elicits anxiolytic-like effects in the marble burying assay. Pharmacol Biochem Behav. 2011;98:21–7.

Kobayashi Y, Arai S, Waku K, Sugiura T. Activation by 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand, of p42/44 mitogen-activated protein kinase in HL-60 cells. J Biochem. 2001;129:665–9.

Komorowska-Müller JA, Schmöle A-C. CB2 receptor in microglia: the Guardian of self-control. Int J Mol Sci. 2020;22:19.

Kreitzer AC, Regehr WG. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. Neuron. 2001;29:717–27.

Laprairie RB, Bagher AM, Kelly ME, Denovan-Wright EM. Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. Br J Pharmacol. 2015;172:4790–805.

Lee TT, Hill MN. Age of stress exposure modulates the immediate and sustained effects of repeated stress on corticolimbic cannabinoid CB_1 receptor binding in male rats. Neuroscience. 2013;249:106–14.

Li Y, Kim J. Neuronal expression of CB2 cannabinoid receptor mRNAs in the mouse hippocampus. Neuroscience. 2015;311:253–67.

Li Y, Kim J. Deletion of CB2 cannabinoid receptors reduces synaptic transmission and long-term potentiation in the mouse hippocampus. Hippocampus. 2016;26:275–81.

Li Y, Kim J. Distinct roles of neuronal and microglial CB2 cannabinoid receptors in the mouse hippocampus. Neuroscience. 2017;363:11–25.

Lichtman AH, Dimen KR, Martin BR. Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. Psychopharmacology. 1995;119:282–90.

Lichtman AH, Blankman JL, Cravatt BF. Endocannabinoid overload. Mol Pharmacol. 2010;78:993–5.

Lin HC, Mao SC, Su CL, Gean PW. The role of prefrontal cortex CB1 receptors in the modulation of fear memory. Cereb Cortex. 2009;19:165–75.

Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, Chan AC, Zhou Z, Huang BX, Kim HY, et al. A biosynthetic pathway for anandamide. Proc Natl Acad Sci USA. 2006;103:13345–50.

Llorente-Berzal A, Terzian AL, di Marzo V, Micale V, Viveros MP, Wotjak CT. 2-AG promotes the expression of conditioned fear via cannabinoid receptor type 1 on GABAergic neurons. Psychopharmacology. 2015;232:2811–25.

Lomazzo E, Bindila L, Remmers F, Lerner R, Schwitter C, Hoheisel U, Lutz B. Therapeutic potential of inhibitors of endocannabinoid degradation for the treatment of stress-related hyperalgesia in an animal model of chronic pain. Neuropsychopharmacology. 2015;40:488–501.

Lopez A, Aparicio N, Pazos MR, Grande MT, Barreda-Manso MA, Benito-Cuesta I, Vazquez C, Amores M, Ruiz-Perez G, Garcia-Garcia E, et al. Cannabinoid CB2 receptors in the mouse brain: relevance for Alzheimer's disease. J Neuroinflammation. 2018;15:158.

López A, Aparicio N, Pazos MR, Grande MT, Barreda-Manso MA, Benito-Cuesta I, Vázquez C, Amores M, Ruiz-Pérez G, García-García E, et al. Cannabinoid CB2 receptors in the mouse brain: relevance for Alzheimer's disease. J Neuroinflammation. 2018;15:*158*.

Lu HC, Mackie K. An introduction to the endogenous cannabinoid system. Biol Psychiatry. 2016;79:516–25.

Ludányi A, Hu SS, Yamazaki M, Tanimura A, Piomelli D, Watanabe M, Kano M, Sakimura K, Maglóczky Z, Mackie K, et al. Complementary synaptic distribution of enzymes responsible for synthesis and inactivation of the endocannabinoid 2-arachidonoylglycerol in the human hippocampus. Neuroscience. 2011;174:50–63.

Lutz B, Marsicano G, Maldonado R, Hillard CJ. The endocannabinoid system in guarding against fear, anxiety and stress. Nat Rev Neurosci. 2015;16:705–18.

Ma L, Jia J, Liu X, Bai F, Wang Q, Xiong L. Activation of murine microglial N9 cells is attenuated through cannabinoid receptor CB2 signaling. Biochem Biophys Res Commun. 2015;458:92–7.

Maccarrone M. Metabolism of the endocannabinoid anandamide: open questions after 25 years. Front Mol Neurosci. 2017;10:166.
Malcher-Lopes R, Di S, Marcheselli VS, Weng F-J, Stuart CT, Bazan NG, Tasker JG. Opposing crosstalk between leptin and glucocorticoids rapidly modulates synaptic excitation via endocannabinoid release. J Neurosci Off J Soc Neurosci. 2006;26:6643–50.
Marco EM, Echeverry-Alzate V, López-Moreno JA, Giné E, Peñasco S, Viveros MP. Consequences of early life stress on the expression of endocannabinoid-related genes in the rat brain. Behav Pharmacol. 2014;25:547–56.
Maresz K, Carrier EJ, Ponomarev ED, Hillard CJ, Dittel BN. Modulation of the cannabinoid CB2 receptor in microglial cells in response to inflammatory stimuli. J Neurochem. 2005;95:437–45.
Marinelli S, Pacioni S, Cannich A, Marsicano G, Bacci A. Self-modulation of neocortical pyramidal neurons by endocannabinoids. Nat Neurosci. 2009;12:1488–90.
Marsicano G, Lutz B. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. Eur J Neurosci. 1999;11:4213–25.
Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglgänsberger W, et al. The endogenous cannabinoid system controls extinction of aversive memories. Nature. 2002;418:530–4.
Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, Azad SC, Cascio MG, Gutiérrez SO, van der Stelt M, et al. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science. 2003;302:84–8.
Martinez Ramirez CE, Ruiz-Perez G, Stollenwerk TM, Behlke C, Doherty A, Hillard CJ. Endocannabinoid signaling in the central nervous system. Glia. 2023;71:5–35.
Martínez-Pinilla E, Varani K, Reyes-Resina I, Angelats E, Vincenzi F, Ferreiro-Vera C, Oyarzabal J, Canela EI, Lanciego JL, Nadal X, et al. Binding and signaling studies disclose a potential allosteric site for cannabidiol in cannabinoid CB(2) receptors. Front Pharmacol. 2017;8:744.
Martin-Fernandez M, Jamison S, Robin LM, Zhao Z, Martin ED, Aguilar J, Benneyworth MA, Marsicano G, Araque A. Synapse-specific astrocyte gating of amygdala-related behavior. Nat Neurosci. 2017;20:1540–8.
Mayo LM, Asratian A, Lindé J, Holm L, Nätt D, Augier G, Stensson N, Vecchiarelli HA, Balsevich G, Aukema RJ, et al. Protective effects of elevated anandamide on stress and fear-related behaviors: translational evidence from humans and mice. Mol Psychiatry. 2020;25:993–1005.
Mazzola C, Medalie J, Scherma M, Panlilio LV, Solinas M, Tanda G, Drago F, Cadet JL, Goldberg SR, Yasar S. Fatty acid amide hydrolase (FAAH) inhibition enhances memory acquisition through activation of PPAR-alpha nuclear receptors. Learn Mem. 2009;16:332–7.
McLaughlin RJ, Hill MN, Dang SS, Wainwright SR, Galea LAM, Hillard CJ, Gorzalka BB. Upregulation of CB1 receptor binding in the ventromedial prefrontal cortex promotes proactive stress-coping strategies following chronic stress exposure. Behav Brain Res. 2013;237:333–7.
Miller S, Daily L, Dharla V, Gertsch J, Malamas MS, Ojima I, Kaczocha M, Ogasawara D, Straiker A. Endocannabinoid metabolism and transport as targets to regulate intraocular pressure. Exp Eye Res. 2020;201:108266.
Mizuno I, Matsuda S, Tohyama S, Mizutani A. The role of the cannabinoid system in fear memory and extinction in male and female mice. Psychoneuroendocrinology. 2022;138:105688.
Moreira FA, Kaiser N, Monory K, Lutz B. Reduced anxiety-like behaviour induced by genetic and pharmacological inhibition of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) is mediated by CB1 receptors. Neuropharmacology. 2008;54:141–50.
Morena M, Roozendaal B, Trezza V, Ratano P, Peloso A, Hauer D, Atsak P, Trabace L, Cuomo V, McGaugh JL, et al. Endogenous cannabinoid release within prefrontal-limbic pathways affects memory consolidation of emotional training. Proc Natl Acad Sci USA. 2014;111:18333–8.
Morena M, Patel S, Bains JS, Hill MN. Neurobiological interactions between stress and the endocannabinoid system. Neuropsychopharmacology. 2016;41:80–102.

Murray CH, Gaulden AD, Kawa AB, Milovanovic M, Caccamise AJ, Funke JR, Patel S, Wolf ME. CaMKII modulates diacylglycerol lipase-α activity in the rat nucleus accumbens after incubation of cocaine craving. eNeuro. 2021;8:ENEURO.0220.

Myers-Payne SC, Fontaine RN, Loeffler A, Pu L, Rao AM, Kier AB, Wood WG, Schroeder F. Effects of chronic ethanol consumption on sterol transfer proteins in mouse brain. J Neurochem. 1996;66:313–20.

Navarrete M, Araque A. Endocannabinoids mediate neuron-astrocyte communication. Neuron. 2008;57:883–93.

Navarrete M, Araque A. Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. Neuron. 2010;68:113–26.

Nguyen T, Thomas BF, Zhang Y. Overcoming the psychiatric side effects of the cannabinoid CB1 receptor antagonists: current approaches for therapeutics development. Curr Top Med Chem. 2019;19:1418–35.

Niederhoffer N, Hansen HH, Fernandez-Ruiz JJ, Szabo B. Effects of cannabinoids on adrenaline release from adrenal medullary cells. Br J Pharmacol. 2001;134:1319–27.

Nomura DK, Morrison BE, Blankman JL, Long JZ, Kinsey SG, Marcondes MC, Ward AM, Hahn YK, Lichtman AH, Conti B, et al. Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. Science. 2011;334:809–13.

Nyilas R, Dudok B, Urbán GM, Mackie K, Watanabe M, Cravatt BF, Freund TF, Katona I. Enzymatic machinery for endocannabinoid biosynthesis associated with calcium stores in glutamatergic axon terminals. J Neurosci. 2008;28:1058–63.

Nyíri G, Cserép C, Szabadits E, Mackie K, Freund TF. CB1 cannabinoid receptors are enriched in the perisynaptic annulus and on preterminal segments of hippocampal GABAergic axons. Neuroscience. 2005a;136:811–22.

Nyíri G, Szabadits E, Cserép C, Mackie K, Shigemoto R, Freund TF. GABAB and CB1 cannabinoid receptor expression identifies two types of septal cholinergic neurons. Eur J Neurosci. 2005b;21:3034–42.

Oberheim NA, Takano T, Han X, He W, Lin JH, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, et al. Uniquely hominid features of adult human astrocytes. J Neurosci. 2009;29:3276–87.

Ohno-Shosaku T, Maejima T, Kano M. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. Neuron. 2001;29:729–38.

Pamplona FA, Takahashi RN. WIN 55212-2 impairs contextual fear conditioning through the activation of CB1 cannabinoid receptors. Neurosci Lett. 2006;397:88–92.

Patel S, Hillard CJ. Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. J Pharmacol Exp Ther. 2006;318:304–11.

Patel S, Roelke CT, Rademacher DJ, Cullinan WE, Hillard CJ. Endocannabinoid signaling negatively modulates stress-induced activation of the hypothalamic-pituitary-adrenal Axis. Endocrinology. 2004;145:5431–8.

Patel S, Roelke CT, Rademacher DJ, Hillard CJ. Inhibition of restraint stress-induced neural and behavioural activation by endogenous cannabinoid signalling. Eur J Neurosci. 2005;21:1057–69.

Patel S, Kingsley PJ, Mackie K, Marnett LJ, Winder DG. Repeated homotypic stress elevates 2-arachidonoylglycerol levels and enhances short-term endocannabinoid signaling at inhibitory synapses in basolateral amygdala. Neuropsychopharmacology. 2009;34:2699–709.

Pfitzer T, Niederhoffer N, Szabo B. Search for an endogenous cannabinoid-mediated effect in the sympathetic nervous system. Naunyn Schmiedeberg's Arch Pharmacol. 2005;371:9–17.

Pitler TA, Alger BE. Postsynaptic spike firing reduces synaptic GABAA responses in hippocampal pyramidal cells. J Neurosci. 1992;12:4122–32.

Pitler TA, Alger BE. Depolarization-induced suppression of GABAergic inhibition in rat hippocampal pyramidal cells: G protein involvement in a presynaptic mechanism. Neuron. 1994;13:1447–55.

Rademacher DJ, Meier SE, Shi L, Ho WS, Jarrahian A, Hillard CJ. Effects of acute and repeated restraint stress on endocannabinoid content in the amygdala, ventral striatum, and medial prefrontal cortex in mice. Neuropharmacology. 2008;54:108–16.

Ramos-Medina L, Rosas-Vidal LE, Patel S. Pharmacological diacylglycerol lipase inhibition impairs contextual fear extinction in mice. Psychopharmacology. 2024;241:569–84.

Raux PL, Drutel G, Revest JM, Vallée M. New perspectives on the role of the neurosteroid pregnenolone as an endogenous regulator of type-1 cannabinoid receptor (CB1R) activity and function. J Neuroendocrinol. 2022;34:e13034.

Robbe D, Kopf M, Remaury A, Bockaert J, Manzoni OJ. Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. Proc Natl Acad Sci USA. 2002;99:8384–8.

Robin LM, Oliveira da Cruz JF, Langlais VC, Martin-Fernandez M, Metna-Laurent M, Busquets-Garcia A, Bellocchio L, Soria-Gomez E, Papouin T, Varilh M, et al. Astroglial CB(1) receptors determine synaptic D-serine availability to enable recognition memory. Neuron. 2018;98(935–944):e935.

Rodríguez-Cueto C, Benito C, Fernández-Ruiz J, Romero J, Hernández-Gálvez M, Gómez-Ruiz M. Changes in CB_1 and CB_2 receptors in the post-mortem cerebellum of humans affected by spinocerebellar ataxias. Br J Pharmacol. 2014;171:1472–89.

Rossi S, De Chiara V, Musella A, Kusayanagi H, Mataluni G, Bernardi G, Usiello A, Centonze D. Chronic psychoemotional stress impairs cannabinoid-receptor-mediated control of GABA transmission in the striatum. J Neurosci. 2008;28:7284–92.

Ruiz de Martin Esteban S, Benito-Cuesta I, Terradillos I, Martinez-Relimpio AM, Arnanz MA, Ruiz-Perez G, Korn C, Raposo C, Sarott RC, Westphal MV, et al. Cannabinoid CB(2) receptors modulate microglia function and amyloid dynamics in a mouse model of Alzheimer's disease. Front Pharmacol. 2022;13:841766.

Schmid PC, Reddy PV, Natarajan V, Schmid HH. Metabolism of N-acylethanolamine phospholipids by a mammalian phosphodiesterase of the phospholipase D type. J Biol Chem. 1983;258:9302–6.

Schurman LD, Carper MC, Moncayo LV, Ogasawara D, Richardson K, Yu L, Liu X, Poklis JL, Liu QS, Cravatt BF, et al. Diacylglycerol lipase-alpha regulates hippocampal-dependent learning and memory processes in mice. J Neurosci. 2019;39:5949–65.

Seagard JL, Dean C, Patel S, Rademacher DJ, Hopp FA, Schmeling WT, Hillard CJ. Anandamide content and interaction of endocannabinoid/GABA modulatory effects in the NTS on baroreflex-evoked sympathoinhibition. Am J Phys Heart Circ Phys. 2004;286:H992–H1000.

Serrat R, Covelo A, Kouskoff V, Delcasso S, Ruiz-Calvo A, Chenouard N, Stella C, Blancard C, Salin B, Julio-Kalajzic F, et al. Astroglial ER-mitochondria calcium transfer mediates endocannabinoid-dependent synaptic integration. Cell Rep. 2021;37:110133.

Shao Z, Yin J, Chapman K, Grzemska M, Clark L, Wang J, Rosenbaum DM. High-resolution crystal structure of the human CB1 cannabinoid receptor. Nature. 2016;540:602–6.

Sheng WS, Hu S, Min X, Cabral GA, Lokensgard JR, Peterson PK. Synthetic cannabinoid WIN55,212-2 inhibits generation of inflammatory mediators by IL-1beta-stimulated human astrocytes. Glia. 2005;49:211–9.

Shonesy BC, Wang X, Rose KL, Ramikie TS, Cavener VS, Rentz T, Baucum AJ 2nd, Jalan-Sakrikar N, Mackie K, Winder DG, et al. CaMKII regulates diacylglycerol lipase-α and striatal endocannabinoid signaling. Nat Neurosci. 2013;16:456–63.

Shonesy BC, Bluett RJ, Ramikie TS, Báldi R, Hermanson DJ, Kingsley PJ, Marnett LJ, Winder DG, Colbran RJ, Patel S. Genetic disruption of 2-arachidonoylglycerol synthesis reveals a key role for endocannabinoid signaling in anxiety modulation. Cell Rep. 2014;9:1644–53.

Shonesy BC, Stephenson JR, Marks CR, Colbran RJ. Cyclic AMP-dependent protein kinase and D1 dopamine receptors regulate diacylglycerol lipase-α and synaptic 2-arachidonoyl glycerol signaling. J Neurochem. 2020;153:334–45.

Simon GM, Cravatt BF. Characterization of mice lacking candidate N-acyl ethanolamine biosynthetic enzymes provides evidence for multiple pathways that contribute to endocannabinoid production in vivo. Mol BioSyst. 2010;6:1411–8.

Spagnolo PA, Ramchandani VA, Schwandt ML, Kwako LE, George DT, Mayo LM, Hillard CJ, Heilig M. FAAH gene variation moderates stress response and symptom severity in patients with posttraumatic stress disorder and comorbid alcohol dependence. Alcohol Clin Exp Res. 2016;40:2426–34.

Sumislawski JJ, Ramikie TS, Patel S. Reversible gating of endocannabinoid plasticity in the amygdala by chronic stress: a potential role for monoacylglycerol lipase inhibition in the prevention of stress-induced behavioral adaptation. Neuropsychopharmacology. 2011;36:2750–61.

Tang Y, Le W. Differential roles of M1 and M2 microglia in neurodegenerative diseases. Mol Neurobiol. 2016;53:1181–94.

Tanimura A, Yamazaki M, Hashimotodani Y, Uchigashima M, Kawata S, Abe M, Kita Y, Hashimoto K, Shimizu T, Watanabe M, et al. The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase alpha mediates retrograde suppression of synaptic transmission. Neuron. 2010;65:320–7.

Tsuboi K, Sun YX, Okamoto Y, Araki N, Tonai T, Ueda N. Molecular characterization of N-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. J Biol Chem. 2005;280:11082–92.

Vallée M, Vitiello S, Bellocchio L, Hébert-Chatelain E, Monlezun S, Martin-Garcia E, Kasanetz F, Baillie GL, Panin F, Cathala A, et al. Pregnenolone can protect the brain from cannabis intoxication. Science. 2014;343:94–8.

Varvel SA, Cravatt BF, Engram AE, Lichtman AH. Fatty acid amide hydrolase (−/−) mice exhibit an increased sensitivity to the disruptive effects of anandamide or oleamide in a working memory water maze task. J Pharmacol Exp Ther. 2006;317:251–7.

Varvel SA, Wise LE, Niyuhire F, Cravatt BF, Lichtman AH. Inhibition of fatty-acid amide hydrolase accelerates acquisition and extinction rates in a spatial memory task. Neuropsychopharmacology. 2007;32:1032–41.

Viader A, Ogasawara D, Joslyn CM, Sanchez-Alavez M, Mori S, Nguyen W, Conti B, Cravatt BF. A chemical proteomic atlas of brain serine hydrolases identifies cell type-specific pathways regulating neuroinflammation. elife. 2016;5:e12345.

Wagner JA, Varga K, Ellis EF, Rzigalinski BA, Martin BR, Kunos G. Activation of peripheral CB1 cannabinoid receptors in haemorrhagic shock. Nature. 1997;390:518–21.

Wamsteeker JI, Kuzmiski JB, Bains JS. Repeated stress impairs endocannabinoid signaling in the paraventricular nucleus of the hypothalamus. J Neurosci Off J Soc Neurosci. 2010;30:11188–96.

Wang W, Sun D, Pan B, Roberts CJ, Sun X, Hillard CJ, Liu QS. Deficiency in endocannabinoid signaling in the nucleus accumbens induced by chronic unpredictable stress. Neuropsychopharmacology. 2010;35:2249–61.

Wilson RI, Nicoll RA. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature. 2001;410:588–92.

Winters BL, Vaughan CW. Mechanisms of endocannabinoid control of synaptic plasticity. Neuropharmacology. 2021;197:108736.

Woodward DF, Liang Y, Krauss AH. Prostamides (prostaglandin-ethanolamides) and their pharmacology. Br J Pharmacol. 2008;153:410–9.

Wyrofsky RR, Reyes BAS, Zhang XY, Bhatnagar S, Kirby LG, Van Bockstaele EJ. Endocannabinoids, stress signaling, and the locus coeruleus-norepinephrine system. Neurobiol Stress. 2019;11:100176.

Xu H, Diolintzi A, Storch J. Fatty acid-binding proteins: functional understanding and diagnostic implications. Curr Opin Clin Nutr Metab Care. 2019;22:407–12.

Zhang HY, Gao M, Liu QR, Bi GH, Li X, Yang HJ, Gardner EL, Wu J, Xi ZX. Cannabinoid CB2 receptors modulate midbrain dopamine neuronal activity and dopamine-related behavior in mice. Proc Natl Acad Sci USA. 2014;111:E5007–15.

Zhang Z, Wang W, Zhong P, Liu SJ, Long JZ, Zhao L, Gao HQ, Cravatt BF, Liu QS. Blockade of 2-arachidonoylglycerol hydrolysis produces antidepressant-like effects and enhances adult hippocampal neurogenesis and synaptic plasticity. Hippocampus. 2015;25:16–26.

Zhang HY, De Biase L, Chandra R, Shen H, Liu QR, Gardner E, Lobo MK, Xi ZX. Repeated cocaine administration upregulates CB(2) receptor expression in striatal medium-spiny neurons that express dopamine D(1) receptors in mice. Acta Pharmacol Sin. 2022;43:876–88.

Zhong P, Wang W, Pan B, Liu X, Zhang Z, Long JZ, Zhang HT, Cravatt BF, Liu QS. Monoacylglycerol lipase inhibition blocks chronic stress-induced depressive-like behaviors via activation of mTOR signaling. Neuropsychopharmacology. 2014;39:1763–76.

Zhou R, Han B, Xia C, Zhuang X. Membrane-associated periodic skeleton is a signaling platform for RTK transactivation in neurons. Science. 2019;365:929–34.

Zoppi S, Pérez Nievas BG, Madrigal JL, Manzanares J, Leza JC, García-Bueno B. Regulatory role of cannabinoid receptor 1 in stress-induced excitotoxicity and neuroinflammation. Neuropsychopharmacology. 2011;36:805–18.

Zoratti C, Kipmen-Korgun D, Osibow K, Malli R, Graier WF. Anandamide initiates Ca(2+) signaling via CB2 receptor linked to phospholipase C in calf pulmonary endothelial cells. Br J Pharmacol. 2003;140:1351–62.

Chapter 4
The Endocannabinoid System and the Early Development of the Brain



Shiwani Thapa and Anna Bukiya

1 Introduction

The *Cannabis sativa* plant has been used since ancient times for medicinal, agricultural, and recreational purposes (Gaoni and Mechoulam 1964; Mechoulam 1986; Booth 2003). Modern understanding of its physiological effects began to emerge in the mid-twentieth century with the identification of its primary psychoactive compound, Δ9-tetrahydrocannabinol (THC) (Mechoulam 1986). Subsequent discoveries included the identification of the first cannabinoid receptor (CB1), and then the endogenously produced cannabinoids such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG) (Devane et al. 1988; Matsuda et al. 1990; Herkenham et al. 1990; Devane et al. 1992; Sugiura et al. 1995; Mechoulam et al. 1995). These endocannabinoids are synthesized on demand from lipid precursors within cellular membranes (Correa et al. 2016). AEA, a member of the N-acylethanolamine (NAE) family, is primarily synthesized by N-acyl phosphatidylethanolamine specific phospholipase D (NAPE-PLD) (Liu et al. 2006), with additional pathways involving α, β hydrolase domain 4 (ABHD4) (Simon and Cravatt 2008), phosphatase PTPN22 (Liu et al. 2008), and glycerophosphodiesterase (GDE1) (Liu et al. 2008). The synthesis of 2-AG involves phospholipase C activation followed by diacylglycerol lipase (DAGLα/β)-mediated release (Bisogno et al. 2003; Liu et al. 2006). Following their production, endocannabinoids undergo rapid degradation by hydrolytic enzymes. AEA is primarily degraded by fatty acid amide hydrolase (FAAH), with additional involvement from enzymes such as cyclooxygenase-2 (COX 2) (Maccarrone et al. 1998; Burstein et al. 2000; Kozak et al. 2002; Ross et al. 2002).

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Conversely, 2-AG is predominantly degraded by monoacylglycerol lipase (MAGL) (Blankman et al. 2007; Muccioli et al. 2007).

The cannabinoid receptors (CBs) 1 and 2 are extensively studied Gi/o protein-coupled receptors (Matsuda et al. 1990; Slipetz et al. 1995; Shire et al. 1996; Mukhopadhyay et al. 2000; Psychoyos et al. 2012). CB1 receptors are widely distributed in various neuronal populations, and glial cells, as well as in peripheral organs (Fernández-Ruiz et al. 1999; Oliveira da Cruz et al. 2016; Galiègue et al. 1995; Barutta et al. 2018). In contrast, CB2 receptors are found in glial cells, specific brain regions, and primarily in peripheral tissues, playing a crucial role in immune function (Galiègue et al. 1995; Van Sickle et al. 2005; Onaivi et al. 2006; Cabral et al. 2008; Galve-Roperh et al. 2009; Rom and Persidsky 2013; Lee et al. 2016; Li and Kim 2017; Barutta et al. 2018). In addition to canonical cannabinoid receptors, G protein-coupled receptors like GPR55 and GPR119 are also recognized components of the endocannabinoid system (Godlewski et al. 2009). Stimulation of cannabinoid receptors triggers diverse signaling pathways, influencing processes such as adenylate cyclase inhibition, mitogen-activated protein kinase (MAPK) signaling activation, and modulation of ion channel activity (Matsuda et al. 1990; Slipetz et al. 1995; Felder et al. 1995; Bondarenko et al. 2018). Activation of CB1 receptors has been shown to induce MAPK signaling pathways, including extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), and p38, which regulate cell proliferation, cell cycle control, and cell death (Turu & Hunyady 2010; Howlett et al. 2010). The specific pathways activated by cannabinoid receptors can vary depending on the cell type and the type of stimulus (Bouaboula et al. 1995; Galve-Roperh et al. 2006; Flores-Otero et al. 2014).

The interactions between endocannabinoids, cannabinoid receptors, and their signaling pathways highlight their importance in maintaining health and addressing various disease states. The critical importance of the endocannabinoid system has been described throughout the lifespan, starting from fertilization, and embryo implantation to lasting into aging. During early development, the endocannabinoid system regulates gametogenesis, embryo implantation, nervous system development, and peripheral organogenesis (Correa et al. 2016; Bukiya 2019). Later, the endocannabinoid system governs neurotransmission, pain modulation, immune regulation, and metabolic processes (Katona et al. 1999; Katona & Freund 2008; Gerdeman & Lovinger 2001; Clapper et al. 2010; Montecucco et al. 2012). It also regulates cardiovascular function, reproductive processes, and even bone health, highlighting its broad impact throughout the body (Tam et al. 2008; Maccarrone et al. 2015; Kaur et al. 2016; Lu & Anderson 2017).

In the brain development, the endocannabinoid system guides critical processes across distinct stages, from the embryonic to the fetal phase, including the perinatal period. The embryonic stage is characterized by rapid cell division and neural structure formation. In humans, this period spans from conception to the end of the eighth week of gestation. The fetal stage follows, it extends from the ninth week of gestation until birth, focusing on cellular division, organ growth, and maturation (Elphick & Egertová 2001; Moore and Persaud 2003). To gain a comprehensive understanding of the role of the endocannabinoid system and its components in

brain development from embryonic to fetal and perinatal stages, numerous studies have been conducted across different species. Rodent species are predominantly used due to their size, ease of maintenance, cost-effectiveness, ability to produce larger litters, and availability for genetic manipulations. Importantly, the shorter gestation periods in rodents facilitate more research output.

The comparison of gestation periods between laboratory animals and humans is informed by the developmental milestones. For instance, the first trimester of human pregnancy, crucial for neural tube formation and early brain structure establishment, aligns approximately with gestational days 1–9 in rats and mice (Bayer et al. 1993; Pressler & Auvin 2013). Similarly, the second trimester in humans, marked by accelerated neuronal differentiation and synaptogenesis, corresponds to gestational days 10–19 in rats and mice (Bayer et al. 1993; Pressler & Auvin 2013). Finally, the third trimester in humans, focusing on extensive synaptogenesis, myelination, and the maturation of higher cognitive functions, parallels the late gestational period and perinatal phase in rodents, approximately from gestational day 20 to postnatal day 10 in rats and mice (Romijn et al. 1991; Pressler & Auvin 2013). These parallels underscore fundamental similarities in neurodevelopmental processes across species, aiding in interpreting experimental findings and their application to understanding the role of endocannabinoid signaling in brain development.

In this chapter, we will review available literature that describes the contribution of the endocannabinoid system to fetal brain development (Fig. 4.1). First, we will review the distribution and role of endocannabinoid system components and signaling in neurogenesis. Next, we will explore its role in gliogenesis following a similar order of presentation. Finally, we will delve into its involvement in cerebrovascular

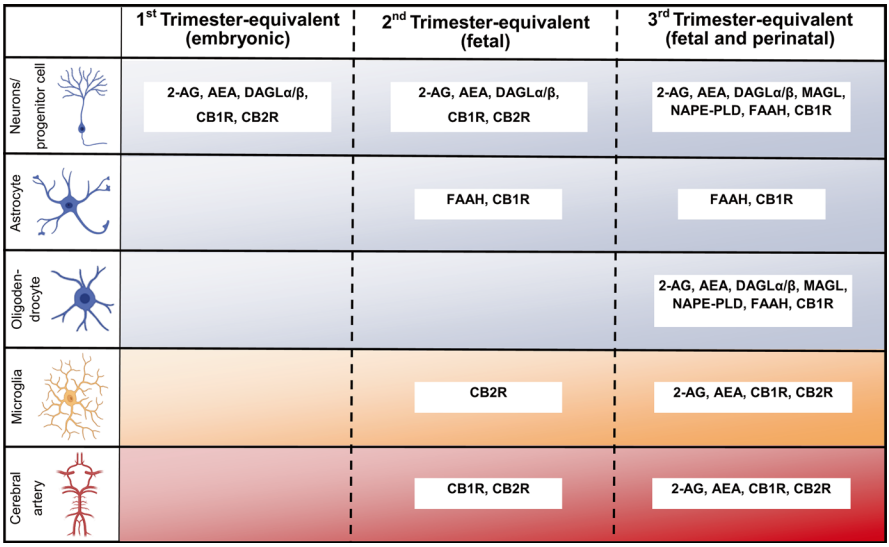


Fig. 4.1 Developmental timeline showing expression of endocannabinoid components in different brain cell types

development. We will conclude with a discussion on the importance of studying the endocannabinoid system during early development and educating the public and medical professionals about this very active signaling network that serves as a target for recreational and medical cannabis with inevitable consequences on developmental processes in the brain.

2 Endocannabinoid System's Role in Guiding Prenatal Neurodevelopment

2.1 Endocannabinoid System Components in Developing Central Nervous System

The endocannabinoid system, extensively studied for its roles in adult brain function, also plays critical roles in fetal brain development. From regulating neurogenesis to influencing synaptic connectivity, the endocannabinoid system is pivotal in shaping intricate processes that define early brain development. Major endocannabinoids (AEA and 2-AG) exhibit distinct neurodevelopmental profiles from earlier prenatal stages to later postnatal stages. Both endocannabinoids are constantly produced during the prenatal periods, but their concentrations vary (Fernández-Ruiz et al. 1999; Berrendero et al. 1999). AEA is detected in high amounts during early embryonic stages (Paria and Dey 2000) but is present in significantly lower concentrations compared to 2-AG during the fetal period in the rat brain (Fernández-Ruiz et al. 1999, 2000). At the same time, AEA levels gradually increase throughout development, eventually reaching adult levels. In contrast, 2-AG levels remain relatively constant, with a notable spike during mid-gestation in rats, stabilizing to levels similar to those found in adult brains (Berrendero et al. 1999; Fernández-Ruiz et al. 1999). The fine-tuning of endocannabinoid levels in the developing brain is achieved through a balance between their rates of production and degradation. The primary synthesizing enzyme of 2-AG, diacylglycerol lipase (DAGLα/β) shows high expression in developing subcortical and cerebellar axonal tracts during the prenatal period and transitions to dendritic regions in later stages of adulthood (Bisogno et al. 2003; Keimpema et al. 2010). Conversely, it has been shown that AEA synthesizing enzyme N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) is initially nearly undetectable in neonatal mouse brains but becomes detectable in dendritic spines of pyramidal cells towards the end of gestation (Berghuis et al. 2007). These findings suggest that 2-AG is the predominant endocannabinoid during early fetal neurodevelopment (Harkany et al. 2008; Blankman et al. 2007; Keimpema et al. 2013). Monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) are key enzymes responsible for breaking down 2-AG and AEA, respectively (Cravatt et al. 1996; Dinh et al. 2002; Muccioli et al. 2007). In the embryonic chick brain, MAGL expression is limited during early developmental stages (stage 20 in the chick) but becomes more pronounced in later

stages (stage 29 in the chick) (Watson et al. 2008). FAAH expression in neurons can be either transient or permanent. Transient expression is observed, for example, in hippocampal interneurons during late gestation (Morozov et al. 2004; Berghuis et al. 2005). This neuron type- and time-specific spike in FAAH expression suggests the role of AEA clearance in determining interneuron characteristics. The developmental pattern and expression of components within the endocannabinoid system likely reflect their specific roles in regulating neurogenesis and other critical processes essential for neuronal network emergence and maturation.

A large body of research has been focused on the role of cannabinoid receptors in driving neuronal development trajectories. Studies highlight the expression of CB1 receptors in the fetal brain, underscoring their potential significance in early neurodevelopment (Herkenham et al. 1990; Buckley et al. 1998; Fride 2004, 2008; Vitalis et al. 2008; Antypa et al. 2011). A study using chick embryos and whole mount in situ hybridization technique has identified CB1 gene expression in the earliest stage of neuronal differentiation at stage 10 of the central nervous system (specifically in the projecting interneurons of the hindbrain), followed by early neurons in the peripheral nervous system (Begbie et al. 2004). At later stages of chick development (stages 16–18), CB1 gene expression was also detected in regions of differentiating neuronal populations (vestibuloacoustic ganglia, epibranchial ganglia, mesencephalic trigeminal nucleus, and dorsal root ganglia) and nonneuronal populations (ventral forebrain and mesoderm). Subsequently, this expression of CB1 receptors was lost in later stages (stage 22) of chick development (Begbie et al. 2004). In rodents, CB1 and CB2 receptor transcripts have been observed as early as embryonic day 11, roughly equivalent to mid-gestation in humans (Buckley et al. 1998). In situ hybridization histochemistry studies in mice have shown CB1 receptor transcripts in structures such as the cerebral cortex, hippocampus, caudate-putamen, and cerebellum during later fetal stages (days 14–21), accompanied by functional activity demonstrated through agonist-stimulated [^{35}S] GTPS-binding assays (Berrendero et al. 1998). However, CB1 receptor expression is significantly downregulated in later stages after birth, along with decreased binding activity in structures like corpus callosum and white matter tracts (Romero et al. 1997). In human fetal brain development, CB1 receptors have been identified as early as the 14th week of gestation (Biegon and Kerman 2001). Autoradiography assays using radiolabeled cannabinoid ligands have revealed high concentrations of CB1 receptors across various brain regions throughout fetal development into later gestational periods, including the cerebellar cortex, hippocampus, cortical layers, and basal ganglia (Mato et al. 2003). Brain region-specific expressions of CB1 receptors using in situ hybridization histochemistry show higher concentrations in the limbic area of the hippocampus and the amygdala's basal nuclear group, with moderate concentrations in the cerebral cortex, thalamus, medial/ventral striatum, and subventricular zone by the 20th week of gestation (Wang et al. 2003; Jutras-Aswad et al. 2009). CB1 receptor expression has also been observed in murine and human fetal white matter areas, suggesting their involvement in early axonal growth (Mato et al. 2003; Mulder et al. 2008). Furthermore, CB1 receptors in fetal brains are predominantly expressed in axons of projection neurons within white matter areas contrasting with

the adult brain where GABAergic interneurons show predominant expression (Berghuis et al. 2007; Mulder et al. 2008; Vitalis et al. 2008). Overall, the expression of CB1 receptors varies across developmental stages and brain regions, indicating their transient presence during brain development (Berrendero et al. 1999; McLaughlin and Abood 1993; Fernández-Ruiz et al. 2000). Regarding CB2 receptors, expression was not detected in the chick embryonic central nervous system (Begbie et al. 2004). In neural stem cells cultured from mouse cortex at embryonic day 15, studies using western blot and immunocytochemistry point at the expression of CB2 in undifferentiated neuronal progenitor cells within the hippocampal region and low levels in differentiated neuronal cells (Molina-Holgado et al. 2007; Palazuelos et al. 2006). However, there is limited knowledge about the temporal and spatial expression of CB2 receptors during neuronal developmental processes.

2.2 Role of Endocannabinoid System in Distinct Neurodevelopmental Processes

The development of the central nervous system (CNS) is a complex and tightly regulated process. This involves several overlapping stages, beginning with neurulation, neurogenesis, and cell proliferation, followed by the specification, differentiation, and migration of neural progeny cells to their final destination within distinct brain regions to form neuronal networks (Stile & Jernigan 2010; Budday et al. 2015). The endocannabinoid system emerges as a critical regulator in these developmental processes (Galve-Roperh et al. 2006; Berghuis et al. 2004, 2005, 2007; Harkany et al. 2007; Mulder et al. 2008; Alpár et al. 2014). Endocannabinoids also oversee the delicate balance between neural progenitor cell proliferation and cell survival essential for brain growth (Aguado et al. 2006; Guzmán 2003; Guzmán et al. 2001, 2002; Mulder et al. 2008). Specifically, functional endocannabinoid signaling has been demonstrated in neural progenitor cells, with studies showing impaired hippocampal progenitor cell proliferation in cells cultured from CB1 receptor knockout and increased proliferation in FAAH knockout embryonic mice (Aguado et al. 2005). In vitro studies using DAGL and FAAH inhibitors have shown that local production of AEA and 2-AG promotes increased neural progenitor proliferation (Aguado et al. 2005; Goncalves et al. 2008; Rubio-Araiz et al. 2008). Moreover, the endocannabinoid system influences the fate determination of neural progenitors, as evidenced by its functional signaling in neurogenic zones (Aguado et al. 2005; Molina-Holgado et al. 2007; Mulder et al. 2008). In the developing neocortex of Sprague Dawley rats isolated between embryonic days 13–19, the endocannabinoid system signaling plays a crucial role in the specification of pyramidal projecting neurons and GABAergic interneurons (Lavdas et al. 1999; Harkany et al. 2007; Galve-Roperh et al. 2007). Interestingly, CB2 receptors have also been implicated in promoting neurogenesis. Studies in CB2 receptor knockdown mice have shown impaired hippocampal progenitor proliferation, whereas selective CB2 receptor

agonists increase hippocampal proliferation (Buckley et al. 2000; Goncalves et al. 2008; Palazuelos et al. 2008). Overall, these findings show the presence of tonic endocannabinoid signaling that regulates neural progenitor proliferation and survival in the fetal brain.

Endocannabinoids also influence neuronal migration and the determination of cellular identity during the final differentiation stages. Studies using chick and zebrafish models demonstrate a close correlation between CB1 receptor expression and neuronal differentiation from early embryonic stages (Begbie et al. 2004; Oltrabella et al. 2017). Experimental evidence from chemotaxis assays shows that the application of CB1 receptor agonist (WIN552122) and AEA on fetal rat cortices induces the migration of GABAergic interneurons (Berghuis et al. 2005). Furthermore, prenatal cannabis exposure in vivo has been shown to increase the density of neonatal rat hippocampal interneurons (Berghuis et al. 2005). Immunofluorescent staining of embryonic rat brain sections obtained at the mid-gestation period has revealed the presence of CB1 receptors in migrating postmitotic neurons during cerebral cortex development (Saez et al. 2014). In addition, in vivo exposure of pregnant dams to CB1 receptor agonist (WIN 55,212-2) resulted in an increased number of radial and tangential post-mitotic migrating neurons during corticogenesis (Saez et al. 2014).

Endocannabinoids facilitate cell migration via various signaling systems, including neurotrophins, growth factors, and inflammatory cytokines (Williams et al. 2003, Berghuis et al. 2005; Preet et al. 2008; Rajesh et al. 2008). Activation of CB1 receptors triggers multiple second messenger pathways (Src/Stat3, Erk1/2, and PI3K/Akt) that mediate sphingolipid signaling and cell death pathways (Galve-Roperh et al. 2000; Rueda et al. 2002; Molina-Holgado et al. 2002; Guzmán 2003; Derkinderen et al. 2003; Jordan et al. 2005; Berghuis et al. 2007). These mechanisms enable CB1 receptors to regulate crucial neurodevelopmental processes including progenitor cell proliferation, fate determination, migration, and cell specification (Galve-Roperh et al. 2007; Harkany et al. 2007).

Beyond neurogenesis and cell differentiation, endocannabinoid signaling provides axon guidance cues that are essential for neuronal maturation and the establishment of neuronal connectivity in the developing brain (Berghuis et al. 2004, 2005, 2007; Mulder et al. 2008; Watson et al. 2008). Studies in zebrafish and chick models during early embryonic stages demonstrate that disrupting CB1 receptor function via pharmacological inhibitors in vitro or CB1 gene knockdown in vivo results in defects of axonal navigation and formation of axonal bundles (Watson et al. 2008; Zuccarini et al. 2019). In vitro studies on rodent cerebrum during mid-gestation reveal high CB1 receptor expression on GABAergic interneuron axonal growth cones, with chemotropic and galvanotropic assays confirming the role of CB1 receptors in axon guidance and elongation (Berghuis et al. 2007). Endocannabinoid signaling thus connects axonal specification in the early embryonic stages to later stages of synaptogenesis in brain development (Watson et al. 2008; Berghuis et al. 2007). Similarly, studies on excitatory pyramidal cells isolated from rodent cortices during mid-gestation indicate that CB1 receptor-mediated signaling influences the elongation of long-range axons (Mulder et al. 2008). Using

CB1 receptor knockout mice models in vivo and CB1 receptor knockout within in vitro models of interneuron and pyramidal cells, indicated impaired axonal guidance and errors in postsynaptic target selection (Berghuis et al. 2007; Mulder et al. 2008; Díaz-Alonso et al. 2017). Pharmacological application of CB1 receptor antagonists also led to impaired axonal pathfinding (Berghuis et al. 2007; Mulder et al. 2008; Watson et al. 2008; Tortoriello et al. 2014). These results underscore the fundamental role of endocannabinoid signaling in axonal growth cone targeting and directionality that lead to the establishment of synapses in the developing brain. Proper distribution of CB1 receptors along developing axonal pathways in both rodent and human brains is crucial for synapse positioning on postsynaptic target cells and synaptic maturation (Berghuis et al. 2007; Harkany et al. 2007). Moreover, the co-localization of DAGLα with CB1 receptors in growth cones prevents the premature formation of synapses and thus promotes neuronal polarization and migration (Mulder et al. 2008; Maccarrone et al. 2014; Díaz-Alonso et al. 2017; Papariello et al. 2021). After initiation of synaptogenesis, DAGLα expression decreases within growth cones and redistributes to postsynaptic regions of mature neurons, while MAGL expression increases in emerging presynaptic areas (Maccarrone et al. 2014). This enzyme localization switch allows for the transition from local to paracrine 2-AG signaling, crucial for mediating synaptic strength during brain development (Keimpema et al. 2013; Maccarrone et al. 2014).

Last, but not least, is the role of the endocannabinoid system in synaptic activity and neural network development. A study using human induced pluripotent stem cells (IPSC)-derived cortical spheroids mimicking fetal brain observed synaptic localization of endocannabinoid components (CB1 receptor, DAGLα, FAAH, and MAGL), without detectable CB2 receptor expression. Treatment with CB1 receptor antagonist (SR141716A) in the cortical spheroid model increases the number of excitatory synapses and shows a biphasic response in inhibitory synapses using microelectrode array recordings (Papariello et al. 2021). This study highlights that disruption of endocannabinoid signaling during cortical synaptogenesis leads to sustained faulty synaptic activity. Cannabinoid-mediated modulation of synapses helps refine synaptic connectivity, enabling the precise wiring of neuronal circuits crucial for establishing motor coordination, cognitive functions, and emotional regulation in adult brains. Overall, the endocannabinoid system plays a multifaceted and integral role in guiding the intricate processes of neurodevelopment. From influencing early neurogenesis stages to shaping neuronal circuitry connections, the endocannabinoid system is pivotal in promoting the maturation of the nervous system effectively.

3 Endocannabinoid System's Modulation of Prenatal Glial Cell Development

While extensive research has focused on how endocannabinoids influence neuronal development in the prenatal brain, there is significant evidence suggesting their critical role in regulating the development and function of glia (Aguado et al. 2006;

Arévalo-Martín et al. 2007a, b; Sun et al. 2013). During brain development, gliogenesis follows neurogenesis (Götz and Huttner 2005) with neural progenitor cells differentiating into astrocytes and oligodendrocytes. Another major type of glial cells—microglia—is of immune macrophageal origin (Chan et al. 2007; Saijo & Glass 2011). These glial cells are essential for the development and maintenance of the developing brain, providing support, protection, and myelination to neurons (Fields & Stevens-Graham 2002; Hanisch & Kettenmann 2007; Feinberg et al. 2010; Allen & Lyons 2018). The endocannabinoid system plays a crucial role in various glial developmental processes which are essential in neurodevelopment, contributing significantly to neural circuit formation, synaptic connections, and the maintenance of neuronal homeostasis (Chotard and Salecker 2004; Rigby et al. 2020).

3.1 Astrocytes: Distribution and Role of Endocannabinoid System Components

An early study on rat midbrain isolated during embryonic day 16 revealed CB1 receptor transcript expression in nonneuronal regions, particularly in white matter and subventricular zones, where glial proliferation occurs (Berrendero et al. 1998, 1999). In vitro study using neural progenitor cells cultured from postnatal 2-day-old rat and mouse cortices (that is equivalent to the third trimester of human pregnancy as per brain developmental milestones (Oberheim et al. 2012) showed the presence of CB1 receptors and FAAH. These progenitor cells consisted of a specific class expressing glial fibrillary acidic protein (GFAP), which acts as a marker for identifying astrocytes (Oberheim et al. 2012). During early developmental stages, there is a distinct distribution pattern of CB1 and CB2 receptors among different cell types. Immunocytochemistry on human fetal cortices harvested at mid-gestation (week 17) showed predominant localization of CB1 receptors in astrocytes, while CB2 receptors were primarily expressed in microglia (Zurolo et al. 2010).

In vitro study using neural progenitor cells cultured from postnatal 2-day-old rat cortices (equivalent to the third trimester of gestation period in humans) has shown that activation of CB1 receptor through synthetic cannabinoid agonists such as WIN-55,212-2 promotes astroglial differentiation (Aguado et al. 2006). This effect is abolished in the presence of a CB1 receptor antagonist (SR141716) (Aguado et al. 2006). Similar results were observed in studies using cultured murine neural progenitor cells isolated during the late gestation period, where CB1 receptor activation also promoted astroglial differentiation (Soltys et al. 2010). In vivo studies on CB1 receptor knockout mice using immunofluorescent staining demonstrated impaired astrogliogenesis, underscoring the receptor's critical role in glial differentiation (Aguado et al. 2006). In addition, in FAAH-deficient mice, alterations in neuronal progenitor cell differentiation patterns were observed, including an increased number of differentiated astroglia cells (Aguado et al. 2006). Furthermore, CB1 receptor stimulation enhanced the survival of cultured rat astrocytes,

implicating localized signaling mechanisms involving CB1 receptor, and FAAH, that support both autocrine and paracrine signaling (Aguado et al. 2005, 2006).

3.2 Oligodendrocytes: Distribution and Role of Endocannabinoid System Components

Biochemical assays using qPCR, western blotting, and immunocytochemistry observed the expression of CB1 and CB2 receptors in progenitor oligodendrocytes and differentiating oligodendrocytes, performed on cultured cells isolated from newborn Wistar rat forebrains. It was found that cultured progenitors and differentiated oligodendrocytes expressed higher levels of CB1 receptor protein and mRNA compared to astrocytes and microglia (Molina-Holgado et al. 2002). Recent studies have further validated the transcript expression of both CB1 and CB2 receptors in oligodendrocyte precursor cells and oligodendrocytes isolated from newborn rat cortex (Sanchez-Rodriguez et al. 2018; Moreno-Luna et al. 2021). Another study utilizing cultured cells isolated from newborn Wistar rat forebrains revealed the expression of key enzymes involved in 2-AG synthesis (DAGLα/β) and degradation (MAGL) in oligodendrocytes at various developmental stages through immunofluorescent staining. Oligodendrocyte progenitor cells showed abundant expression of DAGLα/β and increased production of 2-AG, whereas differentiated oligodendrocytes exhibited higher MAGL activity (Gomez et al. 2010; Sanchez-Rodriguez et al. 2018). AEA has also been observed in oligodendrocyte precursor cells and oligodendrocytes. However, the expression of synthesizing enzyme NAPE-PLD remains low (Gomez et al. 2010). In contrast, the degradation enzyme FAAH shows higher expression levels in mature oligodendrocytes (Moreno-Luna et al. 2021). This suggests a developmental stage-dependent spike of the endocannabinoid's degradation machinery enzymes with higher expression at later stages of the oligodendrocyte maturation process. Overall, these findings indicate the influence of localized and stage-specific endocannabinoid components on the proliferative potential of progenitor cells and subsequent cellular differentiation.

Studies on *cultured cells* isolated from newborn Wistar rat cortices revealed that CB1 receptor agonists (HU210, WIN 55212–2, and ACEA) enhance the survival of oligodendrocytes progenitor cells (Molina-Holgado et al. 2002). Interestingly, while the effects of ACEA were abolished by the CB1 receptor antagonist SR141716A, the combined use of CB1 and CB2 antagonists (SR141716 and AM630) was necessary to prevent the prosurvival action induced by CB1 receptor agonists. This suggests a role for CB2 receptors in oligodendrocyte survival. Conceivably, activation of both CB1 and CB2 receptors promotes the survival of oligodendrocyte progenitor cells in culture, mediated by the ERK and PI3K–AKT signaling pathways (Aguado et al. 2006). In vitro studies on cultured cells isolated from newborn Wistar rat cortices also indicated that 2-AG plays a role in modulating the proliferation and maturation of oligodendrocytes progenitor cells (Gomez et al. 2010, 2011, 2015).

Antagonists of CB1 and CB2 receptors (SR141716 and AM630) and inhibitors of DAGL (RHC-80267 or tetrahydrolipstatin) impeded oligodendrocyte precursor cell differentiation into mature oligodendrocytes (Gomez et al. 2010). This effect is rescued by exogenous 2-AG supplementation. The ERK/MAPK pathway is implicated in the endocannabinoid-dependent differentiation process, as MEK1 inhibition with PD98059 blocked oligodendrocyte maturation (Gomez et al. 2010). In addition, 2-AG signaling is implicated in the proliferation of early oligodendrocyte precursor cells and subsequent maturation of progenitors. Inhibition of MAGL with JZL-184 or exogenous 2-AG application significantly increased oligodendrocyte progenitor cell proliferation (Gomez et al. 2015). This process is thought to be regulated by activation of the P13K/AKT/mTOR signaling pathway as evidenced by Akt and mTOR phosphorylation being required for oligodendrocyte progenitor proliferation (Gomez et al. 2011, 2015). Data using inhibitors of mTOR and P13K (rapamycin and LY294002) in the presence of cannabinoid agonists strongly inhibited oligodendrocyte proliferation. Overall, these findings suggest that the endocannabinoid system plays a novel role in oligodendrocyte proliferation and differentiation.

In vivo studies in *rodents* further support the role of endocannabinoid signaling in regulating oligodendrocyte differentiation, migration, and maturation. For instance, in vivo studies on newborn rat postnatal subventricular zone revealed that the application of CB1 receptor agonist (ACEA) increased the number of glial precursor cells (OLIG2-positive cells) through immunohistochemistry (Arévalo-Martín et al. 2007a; b). Given that the subventricular zone serves as the origin region of precursor cells giving rise to mature oligodendrocytes after migrating into the white matter (Menn et al. 2006), these findings suggest the role of CB1 receptor in oligodendrocyte proliferation. Similarly, the application of CB2 receptor agonist (JWH) increased the expression of polysialylated neural cell adhesion molecule (PSA-NCAM)-expressing migratory and oligodendrocyte precursor cells (Arévalo-Martín et al. 2007a, b). PSA-NCAM expression in the subventricular zone is known to facilitate the migration of oligodendrocyte precursor cells (Nait-Oumesmar et al. 1999; Zhang et al. 2004), suggesting that the endocannabinoid system activation enhances the migratory ability of these precursor cells. Oligodendrogenesis precedes intense myelination and occurs during the first weeks of postnatal brain development (Bjelke and Seiger 1989; Hamano et al. 1998). Research has shown that postnatal treatment (from days 1 to 14) with a cannabinoid agonist (WIN55,212–2) enhances myelin basic protein immunostaining in the external capsule of the corpus callosum, the first region in the white matter to be myelinated, and this expression is dependent on both CB1 and CB2 receptors (Arévalo-Martín et al. 2007a, b). Furthermore, a recent in vivo study administering tetrahydrocannabinol (THC) during the early postnatal period (days 6–10) in mice showed increased oligodendrocyte differentiation and myelination in the subcortical white matter (Huerga-Gómez et al. 2021). Once again, the role of the Akt/mTOR signaling pathway is implicated in this process, as the mTOR inhibitor temsirolimus abrogated the effects of THC on increased oligodendrogenesis and myelin protein levels observed via immunofluorescence (Huerga-Gómez et al. 2021). Disruptions in endocannabinoid signaling have been implicated in developmental myelination deficits, which are associated

with cognitive impairments and mental health disorders (Fields 2008; Nave and Ehrenreich 2014). Specifically, alterations in postnatal myelination have been associated with deficits in motor function (Ishii et al. 2014; Schneider et al. 2016), memory (Poggi et al. 2016), anxiety (Roy et al. 2007; Carson et al. 2015; Chen et al. 2015), and social interaction (Roy et al. 2007; Poggi et al. 2016). Overall, the endocannabinoid system emerges as an essential modulatory system in the processes of oligodendrogenesis and myelination during the postnatal period.

3.3 Microglia: Distribution and Role of Endocannabinoid System Components

Cultured microglia cells obtained from newborn Sprague Dawley rat cerebral cortices showed higher transcript and protein expression of CB2 receptors, whereas CB1 receptor expression was comparatively lower by almost ten-fold (Carlisle et al. 2002). These cultured microglial cells also contained 2-AG and AEA, as determined through liquid chromatography-mass spectroscopy (LC-MS) (Carrier et al. 2004). Immunocytochemistry on human fetal cortices harvested at mid-gestation (week 17) showed predominant expression of CB2 receptors in microglia (Zurolo et al. 2010). A study on cultured microglia cells from newborn rat cortices demonstrated increased microglial proliferation in the presence of exogenous 2-AG, but not AEA, an effect attenuated by a CB2 receptor antagonist (SR14528) (Carrier et al. 2004). In vitro studies on newborn Sprague Dawley rat cerebral cortices using Mutational Reverse Transcription-Polymerase Chain Reaction (MRT-PCR) and western blotting revealed high levels of CB2 receptor expression in inflammatory microglia, with low levels in resident microglial cells. This indicates that CB2 expression in microglia varies with their activation state (Carlisle et al. 2002). In addition, a study using human fetal cortices harvested at mid-gestation (week 17) on malformations of cortical development showed increased expression of CB2 receptors in cells of microglia lineage, suggesting the influence of endocannabinoid signaling in regulating glia-mediated inflammatory response (Zurolo et al. 2010). Increasing evidence supports the influence of the endocannabinoid system on microglial functions, particularly in response to inflammatory stimuli, highlighting its role in regulating glia-mediated inflammatory responses. The differential expression of CB2 receptors in microglia under varying activation states suggests dynamic regulatory mechanisms in response to environmental cues (Benito et al. 2008; Stella 2009).

Overall, the studies of endocannabinoid system components in developing glial cells not only underscore the diversity of cannabinoid receptor distribution among glial cell types but also suggest significant involvement in essential developmental processes. In summary, the endocannabinoid system emerges as a crucial regulatory system in glial development, affecting a wide range of processes from astroglial and oligodendroglial differentiation to microglial inflammatory responses.

4 Endocannabinoid System Control of Prenatal Cerebral Vasculature Development

4.1 Distribution of Endocannabinoid System Components in Developing Cerebral Circulation

During prenatal development, neurogenesis and gliogenesis play pivotal roles in shaping the developing brain. As neurons and glial cells undergo rapid proliferation and maturation, they create increased metabolic demands. Consequently, the development of cerebral vasculature emerges as a parallel process to ensure adequate blood flow, supplying oxygen, and nutrients critical for neuronal and glial cells' survival and function (Carmeliet and Tessier-Lavigne 2005). During the early stages of fetal development, the formation of cerebral circulation begins with the appearance of six pairs of branchial arch arteries at approximately 24 days of human gestation. The third brachial artery and the distal segments of the paired dorsal aortae fuse to give rise to the internal carotid artery (Menshawi et al. 2015; Anderson and Bamforth 2022). Following this, the internal carotid artery branches into anterior and posterior segments. Former eventually matures into the anterior and middle cerebral arteries, while latter gives rise to posterior communicating artery and posterior artery (Menshawi et al. 2015). Vertebral arteries fuse to form the basilar artery. Carotid origin of the posterior artery regresses, with rostral bifurcation of vertebral artery giving rise to posterior arteries of the vertebral–basilar system (van Raamt et al. 2006; Menshawi et al. 2015; Anderson and Bamforth 2022). Major cerebral arteries (anterior, middle, posterior) connected by Willi's circle and basilar artery with its multiple branches form cerebral circulation (Menshawi et al. 2015).

The regulation of cerebral blood flow involves signaling from neurons and glia to vascular cells like endothelial and mural cells. Mural cells, including vascular smooth muscle cells and pericytes, surround endothelial cells and manage cerebrovascular resistance and blood flow to capillaries. In adults, all major cell types involved in cerebrovascular function such as pericytes, endothelial cells, and smooth muscle cells express CB1 and CB2 receptors (Sugiura et al. 1998; Gebremedhin et al. 1999; Chen et al. 2000; Golech et al. 2004; Lu et al. 2008; Zhang et al. 2011; Benyó et al. 2016). Despite these known details of CB receptor distribution in the adult cerebral circulation, the involvement of the endocannabinoid system in regulating prenatal vascular development remains less explored in current literature. However, studies from our lab on fetal nonhuman primate (baboon) middle cerebral artery harvested during mid-gestation show the expression of both CB1 and CB2 receptors through qPCR and Western blotting (Seleverstov et al. 2017). However, we did not investigate the cellular distribution of these receptors. Furthermore, our studies using LC-MS indicate higher expression of 2-AG to AEA in baboon cerebral arteries in near-term fetuses, albeit that observation was obtained from a low number of tissue samples (Tobiasz et al. 2018). The distribution and activity of endocannabinoid synthesis and degradation enzymes in developing cerebral

circulation remain unexplored and are currently studied in our laboratory. The presence of endocannabinoid system components within cerebral veins remains to be probed.

Our investigations also reveal dynamic changes in CB1 receptor function within baboon cerebral arteries across developmental stages. Specifically, the in vitro application of CB1 receptor blocker (AM251) on middle cerebral arteries harvested from fetal baboons during mid-gestation caused artery constriction (Seleverstov et al. 2017), whereas a similar intervention resulted in artery dilation in segments from near-term fetuses (Tobiasz et al. 2018). These findings underscore the regulatory role of CB1 receptors in fetal baboon cerebral artery diameter throughout development, suggesting an active role of the endocannabinoid system in primates' fetal cerebral arteries.

4.2 Role of the Endocannabinoid System in Distinct Processes Governing Cerebral Circulation Development and Function

Cerebral vasculogenesis initiates early in the embryonic stages with the formation of primary blood vessels from angioblasts. Angiogenesis then expands and refines this vascular network through sprouting and vessel remodeling (Carmeliet et al. 1998). These processes are crucial for establishing the intricate cerebral vascular system. Despite its known role in promoting neural progenitor cell proliferation through CB1 receptors, the contribution of the endocannabinoid system to angiogenesis remains underexplored. In vitro studies on human umbilical vein endothelial cell lines using qPCR and western blotting indicate that CB1 receptor expression is induced in endothelial cells during angiogenesis, and application of anandamide stimulates proliferation at physiologically relevant concentrations (Pisanti et al. 2011). This suggests a potentially supportive role for CB1 receptor signaling in angiogenesis. While cannabinoids have been implicated in angiogenesis in other contexts, their direct impact on fetal cerebrovascular development remains unexplored. Nonetheless, understanding these mechanisms may lead to therapeutic strategies aimed at promoting healthy cerebrovascular development in fetuses exposed to adverse prenatal conditions.In the developing brain, studies have noted disruptions in cerebral blood flow regulation, such as compromised autoregulation and varying cerebral blood flow velocity, in high-risk neonates and preterm infants (Lou et al. 1979; Milligan 1980; Perlman et al. 1983). Furthermore, the immature regulation of cerebral blood flow during fetal development plays a critical role in influencing the extent of neurological impairments observed in preterm infants (Brew et al. 2014). The immature brain is particularly susceptible to hypoxia-ischemia, leading to neuronal death (Hilario et al. 2006; Leonardo and Pennypacker 2009). In vivo studies on 7-day-old Wistar rats induced with hypoxic ischemia utilized morphological staining and flow cytometry techniques to reveal recovery of brain damage

with 2-AG or AEA treatment. This was evidenced by decreased cell apoptosis, maintained mitochondrial integrity, and reduced production of reactive oxygen species (Lara-Celador et al. 2012). Furthermore, pretreatment with MAGL inhibitor URB602 demonstrated neuroprotective properties by decreasing activation of the apoptotic pathway, with effects lasting into adulthood (Carloni et al. 2012). In a premature fetal lamb model of hypoxic-ischemic brain injury, the neuroprotective effect of cannabinoids was further confirmed by in vivo administration of the synthetic cannabinoid agonist (WIN-55,212-2), which maintained mitochondrial integrity, reduced apoptotic cell death, and ameliorated inflammatory response (Alonso-Alconada et al. 2011).

Research from our lab specifically focuses on elucidating how the endocannabinoid system influences alcohol-induced changes in cerebral artery diameter, which has broader implications for neurodevelopmental processes. Studies in our laboratory and work from other groups using mouse model indicate that maternal alcohol consumption during the second trimester-equivalent of human pregnancy reduces blood velocity in the fetal middle cerebral artery (Seleverstov et al. 2017; Bake et al. 2017; Tobiasz et al. 2018). A drop in blood velocity is usually interpreted as dilation of fetal cerebral arteries (Seleverstov et al. 2017). Notably, the alcohol-induced dilation of these fetal cerebral arteries is observed ex vivo and can be prevented by in vitro application of CB1 and CB2 receptor blockers mixture (AM251 and AM630, respectively) (Seleverstov et al. 2017). Overall, documented actions of cannabinoids point to their crucial role in maintaining cellular integrity and functionality, which are essential during the developmental stages of fetal cerebrovascular networks. Although direct evidence linking endocannabinoids to fetal cerebrovascular development is limited, their neuroprotective effects and regulatory roles in cerebrovascular pharmacology suggest they may play a role in ensuring optimal development of the fetal brain. Further research is needed to elucidate specific mechanisms and potential therapeutic applications.

5 Summary, Prospects, and Concluding Remarks

In conclusion, ample evidence documents presence of highly active endocannabinoid system in the developing brain, including developing neurons, glia, and cerebral circulation (Fig. 4.1). Wide distribution of endocannabinoid system components within brain cell types during early development translates into active involvement of the endocannabinoid system into cell survival, migration, and differentiation. Endocannabinoid system plays one of the central roles during establishment of major brain structures and cell-to-cell connections.

Active role of endocannabinoid system in brain development lays a foundation for therapeutic interventions against developmental delay but also serves as a target of the cannabis misuse by prospective mothers. Indeed, prenatal recreational cannabis use is highly prevalent, affecting 3–7% of pregnancies globally and potentially as high as 23% in high-risk populations (Williamson et al. 2006; Ko et al.

2015; Volkow et al. 2019; Singh et al. 2020). Despite the increasing legalization of recreational cannabis, the safety and health effects during pregnancy remain largely inconclusive. With regards to brain development, prenatal and perinatal exposure to cannabis can disrupt endocannabinoid signaling during critical developmental periods leading to long-term neurodevelopmental consequences (Richardson et al. 1995; Huizink et al. 2003; Fried et al. 2003; Mereu et al. 2003; Antonelli et al. 2005). In the near-future, research efforts should be continued to unveil the impact of the endocannabinoid system on early brain development. Furthermore, insights into therapeutic interventions against the adverse effects associated with prenatal cannabinoid exposure are also needed.

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References

Aguado T, Monory K, Palazuelos J, et al. The endocannabinoid system drives neural progenitor proliferation. FASEB J. 2005;19(12):1704–6.

Aguado T, Palazuelos J, Monory K, et al. The endocannabinoid system promotes astroglial differentiation by acting on neural progenitor cells. J Neurosci. 2006;26(5):1551–61.

Allen NJ, Lyons DA. Glia as architects of central nervous system formation and function. Science. 2018;362(6411):181–5.

Alonso-Alconada D, Alvarez A, Hilario E. Cannabinoid as a neuroprotective strategy in perinatal hypoxic-ischemic injury. Neurosci Bull. 2011;27(4):275–85.

Alpár A, Tortoriello G, Calvigioni D, et al. Endocannabinoids modulate cortical development by configuring Slit2/Robo1 signalling. Nat Commun. 2014;5:4421.

Anderson RH, Bamforth SD. Morphogenesis of the mammalian aortic arch arteries. Front Cell Dev Biol. 2022;10:892900.

Antonelli T, Tomasini MC, Tattoli M, et al. Prenatal exposure to the CB1 receptor agonist WIN 55,212-2 causes learning disruption associated with impaired cortical NMDA receptor function and emotional reactivity changes in rat offspring. Cereb Cortex. 2005;12:2013–20.

Antypa M, Faux C, Eichele G, et al. Differential gene expression in migratory streams of cortical interneurons. Eur J Neurosci. 2011;34(10):1584–94.

Arévalo-Martín A, García-Ovejero D, Rubio-Araiz A, et al. Cannabinoids modulate Olig2 and polysialylated neural cell adhesion molecule expression in the subventricular zone of postnatal rats through cannabinoid receptor 1 and cannabinoid receptor 2. Eur J Neurosci. 2007a;26(6):1548–59.

Arévalo-Martín A, García-Ovejero D, Rubio-Araiz A, et al. Cannabinoids modulate Olig2 and polysialylated neural cell adhesion molecule expression in the subventricular zone of postnatal rats through cannabinoid receptor 1 and cannabinoid receptor 2. Eur J Neurosci. 2007b;26(6):1548–59.

Bake S, Gardner R, Tingling JD, et al. Fetal alcohol exposure alters blood flow and neurological responses to transient cerebral ischemia in adult mice. Alcohol Clin Exp Res. 2017;41(1):117–27.

Barutta F, Mastrocola R, Bellini S, et al. Cannabinoid receptors in diabetic kidney disease. Curr Diab Rep. 2018;18(2):9.

Bayer SA, Altman J, Russo RJ, Zhang X. Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. Neurotoxicology. 1993;14(1):83–144.

Begbie J, Doherty P, Graham A. Cannabinoid receptor, CB1, expression follows neuronal differentiation in the early chick embryo. J Anat. 2004;205(3):213–8.
Benito C, Tolón RM, Pazos MR, et al. Cannabinoid CB2 receptors in human brain inflammation. Br J Pharmacol. 2008;153(2):277–85.
Benyó Z, Ruisanchez É, Leszl-Ishiguro M, et al. Endocannabinoids in cerebrovascular regulation. Am J Physiol Heart Circ Physiol. 2016;310(7):H785–801.
Berghuis P, Dobszay MB, Sousa KM, et al. Brain-derived neurotrophic factor controls functional differentiation and microcircuit formation of selectively isolated fast-spiking GABAergic interneurons. Eur J Neurosci. 2004;20(5):1290–306.
Berghuis P, Dobszay MB, Wang X, et al. Endocannabinoids regulate interneuron migration and morphogenesis by transactivating the TrkB receptor. Proc Natl Acad Sci U S A. 2005;102(52):19115–20.
Berghuis P, Rajnicek AM, Morozov YM, et al. Hardwiring the brain: endocannabinoids shape neuronal connectivity. Science. 2007;316(5828):1212–6.
Berrendero F, García-Gil L, Hernández ML, et al. Localization of mRNA expression and activation of signal transduction mechanisms for cannabinoid receptor in rat brain during fetal development. Development. 1998;125(16):3179–88.
Berrendero F, Sepe N, Ramos JA, et al. Analysis of cannabinoid receptor binding and mRNA expression and endogenous cannabinoid contents in the developing rat brain during late gestation and early postnatal period. Synapse. 1999;33(3):181–91.
Biegon A, Kerman IA. Autoradiographic study of pre- and postnatal distribution of cannabinoid receptors in human brain. NeuroImage. 2001;14(6):1463–8.
Bisogno T, Howell F, Williams G, et al. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. The J Cell Biol. 2003;163(3):463–8.
Bjelke B, Seiger A. Morphological distribution of MBP-like immunoreactivity in the brain during development. Int J Dev Neurosci. 1989;7(2):145–64.
Blankman JL, Simon GM, Cravatt BF. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol. 2007;14(12):1347–56.
Bondarenko AI, Panasiuk O, Okhai I, et al. Ca^{2+}-dependent potassium channels and cannabinoid signaling in the endothelium of apolipoprotein E knockout mice before plaque formation. J Mol Cell Cardiol. 2018;115:54–63.
Booth M. (2003). Cannabis: a history.
Bouaboula M, Poinot-Chazel C, Bourrié B, et al. Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. Biochem J. 1995;312(Pt 2) (Pt 2):637–41.
Brew N, Walker D, Wong FY. Cerebral vascular regulation and brain injury in preterm infants. Am J Physiol Regul Integr Comp Physiol. 2014;306(11):R773–86.
Buckley NE, Hansson S, Harta G, Mezey E. Expression of the CB1 and CB2 receptor messenger RNAs during embryonic development in the rat. Neuroscience. 1998;82(4):1131–49.
Buckley NE, McCoy KL, Mezey E, et al. Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB(2) receptor. Eur J Pharmacol. 2000;396(2–3):141–9.
Budday S, Steinmann P, Kuhl E. Physical biology of human brain development. Front Cell Neurosci. 2015;9:257.
Bukiya AN. Physiology of the endocannabinoid system during development. Adv Exp Med Biol. 2019;1162:13–37.
Burstein SH, Rossetti RG, Yagen B, Zurier RB. Oxidative metabolism of anandamide. Prostaglandins Other Lipid Mediat. 2000;61(1–2):29–41.
Cabral GA, Raborn ES, Griffin L, et al. CB2 receptors in the brain: role in central immune function. Br J Pharmacol. 2008;153(2):240–51.
Carlisle SJ, Marciano-Cabral F, Staab A, et al. Differential expression of the CB2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. Int Immunopharmacol. 2002;2(1):69–82.

Carloni S, Alonso-Alconada D, Girelli S, et al. Pretreatment with the monoacylglycerol lipase inhibitor URB602 protects from the long-term consequences of neonatal hypoxic-ischemic brain injury in rats. Pediatr Res. 2012;72(4):400–6.
Carmeliet P, Tessier-Lavigne M. Common mechanisms of nerve and blood vessel wiring. Nature. 2005;436(7048):193–200.
Carmeliet P, Moons L, Collen D. Mouse models of angiogenesis, arterial stenosis, atherosclerosis and hemostasis. Cardiovasc Res. 1998;39(1):8–33.
Carrier EJ, Kearn CS, Barkmeier AJ, et al. Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonylglycerol, which increases proliferation via a CB2 receptor-dependent mechanism. Mol Pharmacol. 2004;65(4):999–1007.
Carson RP, Kelm ND, West KL, et al. Hypomyelination following deletion of Tsc2 in oligodendrocyte precursors. Ann Clin Transl Neurol. 2015;2(12):1041–54.
Chan WY, Kohsaka S, Rezaie P. The origin and cell lineage of microglia: new concepts. Brain Res Rev. 2007;53(2):344–54.
Chen Y, McCarron RM, Ohara Y, et al. Human brain capillary endothelium: 2-arachidonoglycerol (endocannabinoid) interacts with endothelin-1. Circ Res. 2000;87(4):323–7.
Chen X, Zhang W, Li T, et al. Impairment of oligodendroglia maturation leads to aberrantly increased cortical glutamate and anxiety-like behaviors in juvenile mice. Front Cell Neurosci. 2015;9:467.
Chotard C, Salecker I. Neurons and glia: team players in axon guidance. Trends Neurosci. 2004;27(11):655–61.
Clapper JR, Moreno-Sanz G, Russo R, et al. Anandamide suppresses pain initiation through a peripheral endocannabinoid mechanism. Nat Neurosci. 2010;13(10):1265–70.
Correa F, Wolfson ML, Valchi P, et al. Endocannabinoid system and pregnancy. Reproduction. 2016;152(6):R191–200.
Cravatt BF, Giang DK, Mayfield SP, et al. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature. 1996;384(6604):83–7.
Derkinderen P, Valjent E, Toutant M, et al. Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus. J Neurosci. 2003;23(6):2371–82.
Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol. 1988;34(5):605–13.
Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science. 1992;258(5090):1946.
Díaz-Alonso J, Sun YJ, Granger AJ, et al. Subunit-specific role for the amino-terminal domain of AMPA receptors in synaptic targeting. Proc Natl Acad Sci U S A. 2017;114(27):7136–41.
Dinh TP, Carpenter D, Leslie FM, et al. Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci U S A. 2002;99(16):10819–24.
Elphick MR, Egertová M. The neurobiology and evolution of cannabinoid signalling. Philos Trans R Soc Lond Ser B Biol Sci. 2001;356(1407):381–408.
Feinberg K, Eshed-Eisenbach Y, Frechter S, et al. A glial signal consisting of gliomedin and NrCAM clusters axonal Na+ channels during the formation of nodes of Ranvier. Neuron. 2010;65(4):490–502.
Felder CC, Joyce KE, Briley EM, et al. Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Mol Pharmacol. 1995;48(3):443–50.
Fernández-Ruiz JJ, Berrendero F, Hernández M, et al. Role of endocannabinoids in brain development. Life Sci. 1999;65(6–7):725–36.
Fernández-Ruiz J, Berrendero F, Hernández ML, Ramos JA. The endogenous cannabinoid system and brain development. Trends Neurosci. 2000;23(1):14–20.
Fields RD. White matter in learning, cognition and psychiatric disorders. Trends Neurosci. 2008;31(7):361–70.
Fields RD, Stevens-Graham B. New insights into neuron-glia communication. Science. 2002;298(5593):556–62.

Flores-Otero J, Ahn KH, Delgado-Peraza F, et al. Ligand-specific endocytic dwell times control functional selectivity of the cannabinoid receptor 1. Nat Commun. 2014;5:4589.
Fride E. The endocannabinoid-CB receptor system: importance for development and in pediatric disease. Neuro Endocrinol Lett. 2004;25(1–2):24–30.
Fride E. Multiple roles for the endocannabinoid system during the earliest stages of life: pre- and postnatal development. J Neuroendocrinol. 2008;20(Suppl 1):75–81.
Fried PA, Watkinson B, Gray R. Differential effects on cognitive functioning in 13- to 16-year-olds prenatally exposed to cigarettes and marihuana. Neurotoxicol Teratol. 2003;25(4):427–36.
Galiègue S, Mary S, Marchand J, et al. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem. 1995;232(1):54–61.
Galve-Roperh I, Sánchez C, Cortés ML, et al. Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. Nat Med. 2000;6(3):313–9.
Galve-Roperh I, Aguado T, Rueda D, et al. Endocannabinoids: a new family of lipid mediators involved in the regulation of neural cell development. Curr Pharm Des. 2006;12(18):2319–25.
Galve-Roperh I, Aguado T, Palazuelos J, Guzmán M. The endocannabinoid system and neurogenesis in health and disease. Neuroscientist. 2007;13(2):109–14.
Galve-Roperh I, Palazuelos J, Aguado T, Guzmán M. The endocannabinoid system and the regulation of neural development: potential implications in psychiatric disorders. Eur Arch Psychiatry Clin Neurosci. 2009;259(7):371–82.
Gaoni Y, Mechoulam R. Isolation, structure, and partial synthesis of an active constituent of hashish. J Am Chem Soc. 1964;86:1646–7.
Gebremedhin D, Lange AR, Campbell WB, et al. Cannabinoid CB1 receptor of cat cerebral arterial muscle functions to inhibit L-type Ca2+ channel current. Am J Phys. 1999;276(6):H2085–93.
Gerdeman G, Lovinger DM. CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. J Neurophysiol. 2001;85(1):468–71.
Godlewski G, Offertáler L, Wagner JA, Kunos G. Receptors for acylethanolamides-GPR55 and GPR119. Prostaglandins Other Lipid Mediat. 2009;89(3–4):105–11.
Golech SA, McCarron RM, Chen Y, et al. Human brain endothelium: coexpression and function of vanilloid and endocannabinoid receptors. Brain Res Mol Brain Res. 2004;132(1):87–92.
Gomez O, Arevalo-Martin A, Garcia-Ovejero D, et al. The constitutive production of the endocannabinoid 2-arachidonoylglycerol participates in oligodendrocyte differentiation. Glia. 2010;58(16):1913–27.
Gomez O, Sanchez-Rodriguez A, Le M, et al. Cannabinoid receptor agonists modulate oligodendrocyte differentiation by activating PI3K/Akt and the mammalian target of rapamycin (mTOR) pathways. Br J Pharmacol. 2011;163(7):1520–32.
Gomez O, Sanchez-Rodriguez MA, Ortega-Gutierrez S, et al. A basal tone of 2-Arachidonoylglycerol contributes to early oligodendrocyte progenitor proliferation by activating Phosphatidylinositol 3-Kinase (PI3K)/AKT and the Mammalian Target of Rapamycin (MTOR) pathways. J Neuroimmune Pharmacol. 2015;10(2):309–17.
Goncalves MB, Suetterlin P, Yip P, et al. A diacylglycerol lipase-CB2 cannabinoid pathway regulates adult subventricular zone neurogenesis in an age-dependent manner. Mol Cell Neurosci. 2008;38(4):526–36.
Götz M, Huttner WB. The cell biology of neurogenesis. Nat Rev Mol Cell Biol. 2005;6(10):777–88.
Guzmán M. Cannabinoids: potential anticancer agents. Nat Rev Cancer. 2003;3(10):745–55.
Guzmán M, Sánchez C, Galve-Roperh I. Control of the cell survival/death decision by cannabinoids. J Mol Med. 2001;78(11):613–25.
Guzmán M, Sánchez C, Galve-Roperh I. Cannabinoids and cell fate. Pharmacol Ther. 2002;95(2):175–84.
Hamano K, Takeya T, Iwasaki N, et al. A quantitative study of the progress of myelination in the rat central nervous system, using the immunohistochemical method for proteolipid protein. Brain Res Dev Brain Res. 1998;108(1–2):287–93.

Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci. 2007;10(11):1387–94.
Harkany T, Guzmán M, Galve-Roperh I, Berghuis P, Devi LA, Mackie K. The emerging functions of endocannabinoid signaling during CNS development. Trends Pharmacol Sci. 2007;28(2):83-92.
Harkany T, Keimpema E, Barabás K, Mulder J. Endocannabinoid functions controlling neuronal specification during brain development. Mol Cell Endocrinol. 2008;286(1–2 Suppl 1):S84–90.
Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC. Cannabinoid receptor localization in brain. Proc Natl Acad Sci USA. 1990;87(5):1932.
Hilario E, Alvarez A, Alvarez F, et al. Cellular mechanisms in perinatal hypoxic-ischemic brain injury. Cur Pediatr Rev. 2006;131-141(11):131.
Howlett AC, Blume LC, Dalton GD. CB(1) cannabinoid receptors and their associated proteins. Curr Med Chem. 2010;17(14):1382–93.
Huerga-Gómez A, Aguado T, Sánchez-de la Torre A, et al. Δ^9 -tetrahydrocannabinol promotes oligodendrocyte development and CNS myelination in vivo. Glia. 2021;69(3):532–45.
Huizink AC, Robles de Medina PG, Mulder EJ, et al. Stress during pregnancy is associated with developmental outcome in infancy. J Child Psychol Psychiatry. 2003;44(6):810–8.
Ishii A, Furusho M, Dupree JL, Bansal R. Role of ERK1/2 MAPK signaling in the maintenance of myelin and axonal integrity in the adult CNS. J Neurosci. 2014;34(48):16031–45.
Jordan JD, He JC, Eungdamrong NJ, et al. Cannabinoid receptor-induced neurite outgrowth is mediated by Rap1 activation through G(alpha)o/i-triggered proteasomal degradation of Rap1GAPII. J Biol Chem. 2005;280(12):11413–21.
Jutras-Aswad D, DiNieri JA, Harkany T, Hurd YL. Neurobiological consequences of maternal cannabis on human fetal development and its neuropsychiatric outcome. Eur Arch Psychiatry Clin Neurosci. 2009;259(7):395–412.
Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. Nat Med. 2008;14(9):923–30.
Katona I, Sperlágh B, Sík A, et al. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci. 1999;19(11):4544–58.
Kaur R, Ambwani SR, Singh S. Endocannabinoid system: a multi-facet therapeutic target. Curr Clin Pharmacol. 2016;11(2):110–7.
Keimpema E, Barabas K, Morozov YM, et al. Differential subcellular recruitment of monoacylglycerol lipase generates spatial specificity of 2-arachidonoyl glycerol signaling during axonal pathfinding. J Neurosci. 2010;30(42):13992–4007.
Keimpema E, Tortoriello G, Alpár A, et al. Nerve growth factor scales endocannabinoid signaling by regulating monoacylglycerol lipase turnover in developing cholinergic neurons. Proc Natl Acad Sci U S A. 2013;110(5):1935–40.
Ko JY, Farr SL, Tong VT, et al. Prevalence and patterns of marijuana use among pregnant and nonpregnant women of reproductive age. Am J Obstet Gynecol. 2015;213(2):201.e1–201.e10.
Kozak KR, Crews BC, Morrow JD, et al. Metabolism of the endocannabinoids, 2-arachidonylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides. J Biol Chem. 2002;277(47):44877–85.
Lara-Celador I, Castro-Ortega L, Alvarez A, et al. Endocannabinoids reduce cerebral damage after hypoxic-ischemic injury in perinatal rats. Brain Res. 2012;1474:91–9.
Lavdas AA, Grigoriou M, Pachnis V, Parnavelas JG. The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. J Neurosci. 1999;19(18):7881–8.
Lee TT, Hill MN, Lee FS. Developmental regulation of fear learning and anxiety behavior by endocannabinoids. Genes Brain Behav. 2016;15(1):108–24.
Leonardo CC, Pennypacker KR. Neuroinflammation and MMPs: potential therapeutic targets in neonatal hypoxic-ischemic injury. J Neuroinflammation. 2009;6:13.
Li Y, Kim J. Distinct roles of neuronal and microglial CB2 cannabinoid receptors in the mouse hippocampus. Neuroscience. 2017;363:11–25.

Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, et al. A biosynthetic pathway for anandamide. Proc Natl Acad Sci USA. 2006;103(36):13345–50.
Liu J, Wang L, Harvey-White J, Huang BX, et al. Multiple pathways involved in the biosynthesis of anandamide. Neuropharmacology. 2008;54(1):1–7.
Lou HC, Lassen NA, Friis-Hansen B. Impaired autoregulation of cerebral blood flow in the distressed newborn infant. J Pediatr. 1979;94(1):118–21.
Lu TS, Avraham HK, Seng S, et al. Cannabinoids inhibit HIV-1 Gp120-mediated insults in brain microvascular endothelial cells. J Immunol. 2008;181(9):6406–16.
Lu Y, Anderson HD. Cannabinoid signaling in health and disease. Can J Physiol Pharmacol. 2017;95(4):311–27.
Maccarrone M, van der Stelt M, Rossi A, et al. Anandamide hydrolysis by human cells in culture and brain. J Biol Chem. 1998;273(48):32332–9.
Maccarrone M, Guzmán M, Mackie K, et al. Programming of neural cells by (endo)cannabinoids: from physiological rules to emerging therapies. Nat Rev Neurosci. 2014;15(12):786–801.
Maccarrone M, Bab I, Bíró T, et al. Endocannabinoid signaling at the periphery: 50 years after THC. Trends Pharmacol Sci. 2015;36(5):277–96.
Mato S, Del Olmo E, Pazos A. Ontogenetic development of cannabinoid receptor expression and signal transduction functionality in the human brain. Eur J Neurosci. 2003;17(9):1747–54.
Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature. 1990;346(6284):561–4.
McLaughlin CR, Abood ME. Developmental expression of cannabinoid receptor mRNA. Brain Res Dev Brain Res. 1993;76(1):75–8.
Mechoulam R. Cannabinoids as therapeutic agents. CRC Press; 1986.
Mechoulam R, Ben-Shabat S, Hanus L, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol. 1995;50(1):83–90.
Menn B, Garcia-Verdugo JM, Yaschine C, et al. Origin of oligodendrocytes in the subventricular zone of the adult brain. J Neurosci. 2006;26(30):7907–18.
Menshawi K, Mohr JP, Gutierrez J. A functional perspective on the embryology and anatomy of the cerebral blood supply. J Stroke. 2015;17(2):144–58.
Mereu G, Fà M, Ferraro L, et al. Prenatal exposure to a cannabinoid agonist produces memory deficits linked to dysfunction in hippocampal long-term potentiation and glutamate release. Proc Natl Acad Sci USA. 2003;100(8):4915–20.
Milligan DW. Failure of autoregulation and intraventricular haemorrhage in preterm infants. Lancet. 1980;1(8174):896–8.
Molina-Holgado E, Vela JM, Arévalo-Martín A, et al. Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. J Neurosci. 2002;22(22):9742–53.
Molina-Holgado F, Rubio-Araiz A, García-Ovejero D, et al. CB2 cannabinoid receptors promote mouse neural stem cell proliferation. Eur J Neurosci. 2007;25(3):629–34.
Montecucco F, Di Marzo V, da Silva RF, et al. The activation of the cannabinoid receptor type 2 reduces neutrophilic protease-mediated vulnerability in atherosclerotic plaques. Eur Heart J. 2012;33(7):846–56.
Moore KL, Persaud TVN. The developing human: clinically oriented embryology. Saunders; 2003.
Moreno-Luna R, Esteban PF, Paniagua-Torija B, et al. Heterogeneity of the endocannabinoid system between cerebral cortex and spinal cord oligodendrocytes. Mol Neurobiol. 2021;58(2):689–702.
Morozov YM, Ben-Ari Y, Freund TF. The spatial and temporal pattern of fatty acid amide hydrolase expression in rat hippocampus during postnatal development. Eur J Neurosci. 2004;20(2):459–66.
Muccioli GG, Xu C, Odah E, et al. Identification of a novel endocannabinoid-hydrolyzing enzyme expressed by microglial cells. J Neurosci. 2007;27(11):2883–9.

Mukhopadhyay S, McIntosh HH, Houston DB, Howlett AC. The CB(1) cannabinoid receptor juxtamembrane C-terminal peptide confers activation to specific G proteins in brain. Mol Pharmacol. 2000;57(1):162–70.

Mulder J, Aguado T, Keimpema E, et al. Endocannabinoid signaling controls pyramidal cell specification and long-range axon patterning. Proc Natl Acad Sci U S A. 2008;105(25):8760–5.

Nait-Oumesmar B, Decker L, Lachapelle F, et al. Progenitor cells of the adult mouse subventricular zone proliferate, migrate and differentiate into oligodendrocytes after demyelination. Eur J Neurosci. 1999;11(12):4357–66.

Nave KA, Ehrenreich H. Myelination and oligodendrocyte functions in psychiatric diseases. JAMA Psychiatry. 2014;71(5):582–4.

Oberheim NA, Goldman SA, Nedergaard M. Heterogeneity of astrocytic form and function. Methods Mol Biol. 2012;814:23–45.

Oliveira da Cruz JF, Robin LM, Drago F, et al. Astroglial type-1 cannabinoid receptor (CB1): a new player in the tripartite synapse. Neuroscience. 2016;323:35–42.

Oltrabella F, Melgoza A, Nguyen B, Guo S. Role of the endocannabinoid system in vertebrates: emphasis on the zebrafish model. Develop Growth Differ. 2017;59(4):194–210.

Onaivi ES, Ishiguro H, Gong JP, et al. Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. Ann N Y Acad Sci. 2006;1074:514–36.

Palazuelos J, Aguado T, Egia A, et al. Non-psychoactive CB2 cannabinoid agonists stimulate neural progenitor proliferation. FASEB J. 2006;20(13):2405–7.

Palazuelos J, Davoust N, Julien B, et al. The CB(2) cannabinoid receptor controls myeloid progenitor trafficking: involvement in the pathogenesis of an animal model of multiple sclerosis. J Biol Chem. 2008;283(19):13320–9.

Papariello A, Taylor D, Soderstrom K, Litwa K. CB_1 antagonism increases excitatory synaptogenesis in a cortical spheroid model of fetal brain development. Sci Rep. 2021;11(1):9356.

Paria BC, Dey SK. Ligand-receptor signaling with endocannabinoids in preimplantation embryo development and implantation. Chem Phys Lipids. 2000;108(1–2):211–20.

Perlman JM, McMenamin JB, Volpe JJ. Fluctuating cerebral blood-flow velocity in respiratory-distress syndrome. Relation to the development of intraventricular hemorrhage. N Engl J Med. 1983;309(4):204–9.

Pisanti S, Picardi P, Prota L, Proto MC, Laezza C, McGuire PG, Morbidelli L, Gazzerro P, Ziche M, Das A, Bifulco M. Genetic and pharmacologic inactivation of cannabinoid CB1 receptor inhibits angiogenesis. Blood. 2011;117(20):5541–50.

Poggi G, Boretius S, Möbius W, et al. Cortical network dysfunction caused by a subtle defect of myelination. Glia. 2016;64(11):2025–40.

Preet A, Ganju RK, Groopman JE. Delta9-Tetrahydrocannabinol inhibits epithelial growth factor-induced lung cancer cell migration in vitro as well as its growth and metastasis in vivo. Oncogene. 2008;27(3):339–46.

Pressler R, Auvin S. Comparison of brain maturation among species: an example in translational research suggesting the possible use of bumetanide in newborn. Front Neurol. 2013;4:36.

Psychoyos D, Vinod KY, Cao J, et al. Cannabinoid receptor 1 signaling in embryo neurodevelopment. Birth Defects Res B Dev Reprod Toxicol. 2012;95(2):137–50.

Rajesh M, Mukhopadhyay P, Haskó G, et al. CB2 cannabinoid receptor agonists attenuate TNF-alpha-induced human vascular smooth muscle cell proliferation and migration. Br J Pharmacol. 2008;153(2):347–57.

Richardson GA, Day NL, Goldschmidt L. Prenatal alcohol, marijuana, and tobacco use: infant mental and motor development. Neurotoxicol Teratol. 1995;4:479–87.

Rigby MJ, Gomez TM, Puglielli L. Glial cell-axonal growth cone interactions in neurodevelopment and regeneration. Front Neurosci. 2020;14:203.

Rom S, Persidsky Y. Cannabinoid receptor 2: potential role in immunomodulation and neuroinflammation. J Neuroimmune Pharmacol. 2013;8(3):608–20.

Romero J, Garcia-Palomero E, Berrendero F, et al. Atypical location of cannabinoid receptors in white matter areas during rat brain development. Synapse. 1997;26(3):317–23.

Romijn HJ, Hofman MA, Gramsbergen A. At what age is the developing cerebral cortex of the rat comparable to that of the full-term newborn human baby? Early Hum Dev. 1991;26(1):61–7.
Ross RA, Craib SJ, Stevenson LA, et al. Pharmacological characterization of the anandamide cyclooxygenase metabolite: prostaglandin E2 ethanolamide. J Pharmacol Exp Ther. 2002;301(3):900–7.
Roy K, Murtie JC, El-Khodor BF, et al. Loss of erbB signaling in oligodendrocytes alters myelin and dopaminergic function, a potential mechanism for neuropsychiatric disorders. Proc Natl Acad Sci U S A. 2007;104(19):8131–6.
Rubio-Araiz A, Arévalo-Martín A, Gómez-Torres O, et al. The endocannabinoid system modulates a transient TNF pathway that induces neural stem cell proliferation. Mol Cell Neurosci. 2008;38(3):374–80.
Rueda D, Navarro B, Martinez-Serrano A, Guzman M, Galve-Roperh I. The endocannabinoid anandamide inhibits neuronal progenitor cell differentiation through attenuation of the Rap1/B-Raf/ERK pathway. J Biol Chem. 2002;277(48):46645–50.
Saez TM, Aronne MP, Caltana L, Brusco AH. Prenatal exposure to the CB1 and CB2 cannabinoid receptor agonist WIN 55,212-2 alters migration of early-born glutamatergic neurons and GABAergic interneurons in the rat cerebral cortex. J Neurochem. 2014;129(4):637–48.
Saijo K, Glass CK. Microglial cell origin and phenotypes in health and disease. Nat Rev Immunol. 2011;11(11):775–87.
Sanchez-Rodriguez MA, Gomez O, Esteban PF, et al. The endocannabinoid 2-arachidonoylglycerol regulates oligodendrocyte progenitor cell migration. Biochem Pharmacol. 2018;157:180–8.
Schneider S, Gruart A, Grade S, et al. Decrease in newly generated oligodendrocytes leads to motor dysfunctions and changed myelin structures that can be rescued by transplanted cells. Glia. 2016;64(12):2201–18.
Seleverstov O, Tobiasz A, Jackson JS, et al. Maternal alcohol exposure during mid-pregnancy dilates fetal cerebral arteries via endocannabinoid receptors. Alcohol. 2017;61:51–61.
Shire D, Calandra B, Rinaldi-Carmona M, et al. Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor. Biochim Biophys Acta. 1996;1307(2):132–6.
Simon GM, Cravatt BF. Anandamide biosynthesis catalyzed by the phosphodiesterase GDE1 and detection of glycerophospho-N-acyl ethanolamine precursors in mouse brain. J Biol Chem. 2008;283(14):9341–9.
Singh S, Filion KB, Abenhaim HA, Eisenberg MJ. Prevalence and outcomes of prenatal recreational cannabis use in high-income countries: a scoping review. BJOG. 2020;127(1):8–16.
Slipetz DM, O'Neill GP, Favreau L, et al. Activation of the human peripheral cannabinoid receptor results in inhibition of adenylyl cyclase. Mol Pharmacol. 1995;48(2):352–61.
Soltys J, Yushak M, Mao-Draayer Y. Regulation of neural progenitor cell fate by anandamide. Biochem Biophys Res Commun. 2010;400(1):21–6.
Stella N. Endocannabinoid signaling in microglial cells. Neuropharmacology. 2009;56(Suppl 1):244–53.
Stile J, Jernigan TL. The basics of brain development. Neuropsychol Rev. 2010;20(4):327–48.
Sugiura T, Kondo S, Sukagawa A, et al. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun. 1995;215(1):89–97.
Sugiura T, Kodaka T, Nakane S, et al. Detection of an endogenous cannabimimetic molecule, 2-arachidonoylglycerol, and cannabinoid CB1 receptor mRNA in human vascular cells: is 2-arachidonoylglycerol a possible vasomodulator? Biochem Biophys Res Commun. 1998;243(3):838–43.
Sun H, Jiang L, Luo X, et al. Potential tumor-suppressive role of monoglyceride lipase in human colorectal cancer. Oncogene. 2013;32(2):234–41.
Tam J, Trembovler V, Di Marzo V, et al. The cannabinoid CB1 receptor regulates bone formation by modulating adrenergic signaling. FASEB J. 2008;22(1):285–94.
Tobiasz AM, Duncan JR, Bursac Z, et al. The effect of prenatal alcohol exposure on fetal growth and cardiovascular parameters in a baboon model of pregnancy. Reprod Sci. 2018;25(7):1116–23.

Tortoriello G, Morris CV, Alpar A, et al. Miswiring the brain: Δ9-tetrahydrocannabinol disrupts cortical development by inducing an SCG10/stathmin-2 degradation pathway. EMBO J. 2014;33(7):668–85.

Turu G, Hunyady L. Signal transduction of the CB1 cannabinoid receptor. J Mol Endocrinol. 2010;44(2):75–85.

van Raamt AF, Mali WP, van Laar PJ, van der Graaf Y. The fetal variant of the circle of Willis and its influence on the cerebral collateral circulation. Cerebrovasc Dis. 2006;22(4):217–24.

Van Sickle MD, Duncan M, Kingsley PJ, et al. Identification and functional characterization of brainstem cannabinoid CB2 receptors. Science. 2005;310(5746):329–32.

Vitalis T, Lainé J, Simon A, et al. The type 1 cannabinoid receptor is highly expressed in embryonic cortical projection neurons and negatively regulates neurite growth in vitro. Eur J Neurosci. 2008;28(9):1705–18.

Volkow ND, Han B, Compton WM, McCance-Katz EF. Self-reported medical and nonmedical cannabis use among pregnant women in the United States. JAMA. 2019;322:167–9.

Wang L, Liu J, Harvey-White J, et al. Endocannabinoid signaling via cannabinoid receptor 1 is involved in ethanol preference and its age-dependent decline in mice. Proc Natl Acad Sci U S A. 2003;100(3):1393–8.

Watson S, Chambers D, Hobbs C, et al. The endocannabinoid receptor, CB1, is required for normal axonal growth and fasciculation. Mol Cell Neurosci. 2008;38(1):89–97.

Williams EJ, Walsh FS, Doherty P. The FGF receptor uses the endocannabinoid signaling system to couple to an axonal growth response. The J Cell Biol. 2003;160(4):481–6.

Williamson S, Jackson L, Skeoch C, et al. Determination of the prevalence of drug misuse by meconium analysis. Arch Dis Child Fetal Neonatal Ed. 2006;91(4):F291–2.

Zhang H, Vutskits L, Calaora V, et al. A role for the polysialic acid-neural cell adhesion molecule in PDGF-induced chemotaxis of oligodendrocyte precursor cells. J Cell Sci. 2004;117(Pt 1):93–103.

Zhang H, Hilton DA, Hanemann CO, Zajicek J. Cannabinoid receptor and N-acyl phosphatidylethanolamine phospholipase D--evidence for altered expression in multiple sclerosis. Brain Pathol. 2011;21(5):544–57.

Zuccarini G, D'Atri I, Cottone E, Mackie K, Shainer I, Gothilf Y, Provero P, Bovolin P, Merlo GR. Interference with the Cannabinoid Receptor CB1R Results in Miswiring of GnRH3 and AgRP1 Axons in Zebrafish Embryos. Int J Mol Sci. 2019;21(1):168.

Zurolo E, Iyer AM, Spliet WG, et al. CB1 and CB2 cannabinoid receptor expression during development and in epileptogenic developmental pathologies. Neuroscience. 2010;170(1):28–41.

Chapter 5
The Endocannabinoid System and Postnatal Neurodevelopment



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1 Introduction

The endocannabinoid (eCB) system plays an instrumental role in shaping neural circuitry across development. Shifts in the homeostatic balance of eCB signaling due to genetic variation or exogenous input contribute to individual differences in neurobiological development and emergent cognition and behavior (Lu and Mackie 2016; Mechoulam and Parker 2013; Meyer et al. 2018). In particular, eCB signaling is closely intertwined with patterns of stress responding and fear responding. Notably, the role of the eCB system—and how it interfaces with the developing brain and endocrine system—changes over the course of development. Since early work in the 1960s and 1970s, significant progress has been made in understanding how eCB signaling contributes to individual variation in neural and behavioral development. Adolescence in particular represents a sensitive window during which alterations to eCB signaling may lead to long-lasting impacts (Meyer et al. 2018). Together, myriad findings point to the eCB system as a promising target for the development of novel drugs for stress-related disorders.

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2 Overview of the Endocannabinoid Signaling System

The eCB system is a multipurpose neuroendocrine signaling system with high spatial and temporal precision that plays a critical role in orchestrating neurodevelopment across ontogeny. The eCB system primarily comprises two inhibitory G-protein-coupled receptors, CB1 and CB2 (Howlett 2002; Lu and Mackie 2016); two primary endogenous ligands, *N*-arachidonoylethanolamine (anandamide/AEA) and 2-arachidonoylglycerol (2-AG) (Devane et al. 1992; Sugiura et al. 1995); and two metabolic enzymes that hydrolyze AEA and 2-AG, respectively: fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) (Cravatt et al. 1996; Dinh et al. 2002; T. T.-Y. Lee et al. 2016). CB2 receptors are primarily expressed in immune cells and, neurally, in microglia (Cabral et al. 2008; Maresz et al. 2005; Núñez et al. 2004; Simone et al. 2022; Svízenská et al. 2008), although CB2 receptors have also been detected in dopaminergic neurons (Zhang et al. 2014) and appear to contribute to regulating subcortical excitatory neurotransmission (Kim and Li 2015). By contrast, CB1 receptors are primarily expressed in the central nervous system and are present in multiple neuronal populations (Egertová and Elphick 2000; Herkenham et al. 1991; Hohmann and Herkenham 1999; Hu and Mackie 2015; Lu and Mackie 2021; Simone et al. 2022; Tsou et al. 1998, 1999). In line with their role in modulating synaptic transmission, CB1 receptors show particularly dense expression on presynaptic axon terminals across both excitatory and inhibitory neurons, localized predominantly in the cerebellum and in corticolimbic regions such as the prefrontal cortex, the amygdala, and the hippocampus (Egertová and Elphick 2000; Herkenham et al. 1991; Hohmann and Herkenham 1999; Lu and Mackie 2021; Nyíri et al. 2005; Simone et al. 2022; Tsou et al. 1998, 1999). However, CB1 receptors are also expressed to a lesser degree in other neuronal regions and cell types, reflecting the multiple purposes that eCB signaling in the brain subserves (Lu and Mackie 2021). Although the synthetic and hydrolytic pathways of AEA and 2-AG are distinct, CB1 and CB2 receptors can be activated through the binding of either ligand (Lu and Mackie 2021).

3 Endocannabinoids and Synaptic Plasticity

AEA and 2-AG are synthesized on demand in the postsynaptic neuron and act in a retrograde fashion on the presynaptic neuron (Di Marzo et al. 1999; Katona and Freund 2008), where receptor binding results in suppression of neurotransmitter release (Alger 2002). eCB signaling plays an important role in governing synaptic plasticity in both the short-term and long-term (Chevaleyre et al. 2006; Diana and Marty 2004; Heifets and Castillo 2009). eCB-mediated suppression over both the short-term (e.g., depolarization-induced suppression of inhibition and depolarization-induced suppression of excitation; typically lasting tens of seconds (Diana and Marty 2004)) and long-term (e.g., long-term depression [LTD]; typically lasting

hours or longer (Bliss and Cooke 2011)) has been observed throughout the brain (Diana and Marty 2004; Heifets and Castillo 2009). LTD has been observed in the dorsal striatum (Gerdeman et al. 2002), amygdala (Azad et al. 2004; Chevaleyre et al. 2007; Marsicano et al. 2002), hippocampus (Chevaleyre and Castillo 2003; Edwards et al. 2008; Kang-Park et al. 2007; Lafourcade and Alger 2008; Yasuda et al. 2008), and prefrontal cortex (Lafourcade et al. 2007), and is thought to underlie individual variation in synaptic connectivity as well as support learning and memory (Bliss and Cooke 2011). Induction of eCB-mediated LTD involves initial activity at afferent projections, resulting in eCB release from the postsynaptic neuron (Heifets and Castillo 2009). Subsequent activation of CB1 receptors on the presynaptic neuron or nearby active neurons can then lead to inhibition of neurotransmitter release. Whether neurotransmitter release is suppressed over a relatively shorter or longer period (e.g., LTD) is determined by differential signaling processes (Chevaleyre and Castillo 2003; Diana and Marty 2004; Heifets and Castillo 2009).

4 The Development of the Endocannabinoid System

The eCB signaling system develops early in gestation and functions prenatally, serving to orchestrate neurodevelopment while itself undergoing maturational changes (Harkany et al. 2007). Following birth, the primary role of the eCB signaling system switches from informing cell fate to regulating synaptic neurotransmission and plasticity (Harkany et al. 2008; Maccarrone et al. 2014). In rodents, regulation of synaptic communication by the eCB system appears to emerge around postnatal day (PND) 10 and increases over the course of development through adulthood (Aguado et al. 2006; Berghuis et al. 2007; Berrendero et al. 1998; Fride et al. 2009; Harkany et al. 2007, 2008; Maccarrone et al. 2014; Meyer et al. 2018; Mulder et al. 2008; Rodriguez de Fonseca et al. 1993; Williams et al. 2003; Zurolo et al. 2010). However, eCB signaling appears to undergo greater change during adolescence in particular (Meyer et al. 2018; Peters and Naneix 2022). In rodents, CB1 receptor expression peaks in early adolescence (around PND 30) with the greatest expression present in the prefrontal cortex and striatum, and then declines into adulthood (~PND 70; Rodriguez de Fonseca et al. 1993). AEA levels increase after birth, peak during adolescence, and then gradually increase again into adulthood (Lee et al. 2013, 2016; Wenger et al. 2002). 2-AG levels peak after birth, then gradually increase during adolescence and decline into adulthood (Ellgren et al. 2008). Although both AEA and 2-AG levels increase during adolescence, there are differences in the respective timing of their peak expression and subsequent decline (Fig. 5.1; Ellgren et al. 2008; Heng et al. 2011; Lee et al. 2013; Meyer et al. 2018; Rubino et al. 2015). Interestingly, while less thoroughly characterized than in rodent models, eCB development in humans appears to somewhat differ. In humans, CB1 receptor expression peaks after birth, decreases gradually throughout childhood, and then decreases more sharply starting in adolescence into adulthood (Choi et al.

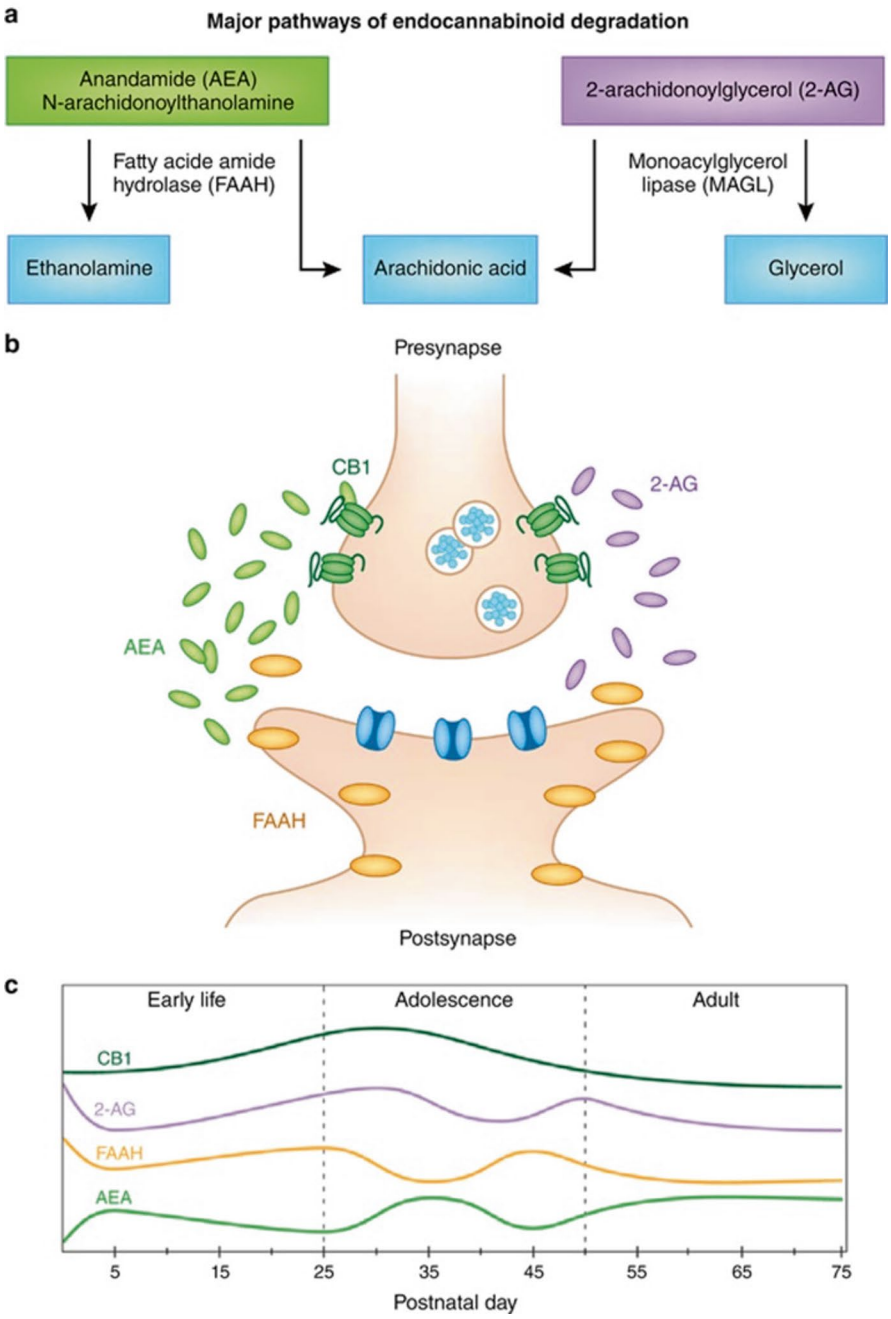


Fig. 5.1 Endocannabinoid (eCB) signaling in rodent corticolimbic regions fluctuates as function of developmental stage. (**a**) Primary hydrolytic pathways of the two primary endogenous eCB ligands, AEA and 2-AG. (**b**) Depiction of eCB signaling at an axon terminal. (**c**) Developmental fluctuation in expression and availability of eCB signaling components. Figure included with permission from Meyer and colleagues (2018)

2012; Long et al. 2012). By contrast, AEA levels appear to increase across the lifespan in conjunction with expression of both FAAH and an enzyme responsible for synthesizing AEA, N-acyl phosphatidylethanolamine phospholipase D (Long et al. 2012). Paralleling rodent findings, 2-AG levels appear to peak in early childhood, maintain tone throughout adolescence, and then gradually decrease into adulthood (Long et al. 2012). Together, dynamic changes in eCB signaling over the course of ontogeny set the stage for developmental stage-specific differences in neural functioning and behavior, and may contribute to the emergence of windows of plasticity that influence risk for psychopathology later in life (Meyer et al. 2018).

5 Sex Differences in Endocannabinoid System Development

The eCB system displays robust differences by sex over the course of development and interacts with gonadal and sex hormones to inform neurodevelopment (Lee and Gorzalka 2012; Simone et al. 2022). In both rodents and humans, the eCB system modulates activity of gonadal hormones including androgens, estrogens, progesterone, and gonadotrophins such as follicle-stimulating hormone and luteinizing hormone (Dalterio et al. 1977, 1983; Kolodny et al. 1974; Kumar and Chen 1983; Rodríguez de Fonseca et al. 1994; Tsutahara et al. 2011; Wenger et al. 2001). Across these domains, the eCB system appears to maintain optimal physiological hormonal levels by attenuating release of gonadal hormones (Gorzalka and Dang 2012), while these can in turn influence eCB signaling through feedback loops involving the hypothalamus, pituitary, and limbic regions (Gorzalka and Dang 2012; MacCarrone et al. 2000; Maccarrone et al. 2001, 2003; Nguyen and Wagner 2006). Taken together, these findings suggest that the eCB system is closely linked via bidirectional feedback loops with pubertal development and sexual maturation and may vary as a function of sex-specific behavior and hormonal differences.

Sex differences are also evident in the developmental trajectories of the eCB system. For instance, CB1 receptor levels reach their peak relatively earlier in female rodents compared to males (Rodriguez de Fonseca et al. 1993), potentially reflecting the relatively earlier onset of puberty in females. AEA expression in the hypothalamus shows a similar pattern, peaking prior to puberty in female rodents (Wenger et al. 2001). Sex differences are also evident in synaptic plasticity, with eCB-mediated LTD, or reduction in synaptic transmission efficacy, apparent in prefrontal cortical neurons in juvenile (prepubertal), adolescent, and adult female rodents, but emerging only after the onset of puberty in male rodents (Bernabeu et al. 2023). Further, eCB signaling activity in female rodents also fluctuates over the course of the estrous cycle (Bradshaw et al. 2006; El-Talatini et al. 2010; González et al. 2000), implicating gonadal hormones in potentially shaping the developmental trajectories of eCB system maturation across sexes (Murphy et al. 1991; Rodríguez de Fonseca et al. 1994).

6 The Endocannabinoid System and Neurodevelopment

The endocannabinoid system plays a crucial role in the maturation of corticolimbic circuitry, and in synaptic plasticity in particular (Heifets and Castillo 2009). Activation of presynaptic CB1 receptors can inhibit the release of neurotransmitters, thereby regulating levels of both glutamatergic (excitatory) and GABAergic (inhibitory) transmission (Bossong and Niesink 2010; Katona and Freund 2008; Lubman et al. 2015; Marsicano et al. 2003). By mediating both excitatory and inhibitory neurotransmission, eCB signaling may contribute to sculpting within- and between-network dynamics and emergent cognitive and behavioral output (Buzsáki and Draguhn 2004; Freund et al. 2003; Gerdeman et al. 2002; Raver et al. 2013; Sales-Carbonell et al. 2013). Activity-dependent synaptic plasticity is one central mechanism that sculpts structural and functional neural circuitry over the lifespan, with particular implications for circuit refinement across development. In rodents, hippocampal eCB-mediated LTD is greater in neurons obtained from adolescent (postnatal days [PND] 28–35) relative to adult (PND 75–110) rats (Kang-Park et al. 2007), and developing hippocampal glutamatergic synapses can express a distinct type of eCB-mediated LTD that peaks during infancy (PND 2–10) (Yasuda et al. 2008). By governing short- and long-term synaptic plasticity during development, eCB signaling likely shapes not only the strength of synaptic connections, but also functions such as learning, memory, emotion processing, and stress responding (Busquets-Garcia et al. 2011, 2016; Hill et al. 2018; Lee and Gorzalka 2015; Meyer et al. 2018; Morena et al. 2016).

Over the course of adolescence, AEA levels in the hippocampus, amygdala, prefrontal cortex, and hypothalamus show nonlinear trajectories, such that levels of AEA increase from PND 25 to 35, decrease at PND 45, and then increase into adulthood (Lee et al. 2013). FAAH expression shows the inverse pattern, with expression decreasing from PND 25 to 35, transiently increasing between PND 35 to 45, and decreasing into adulthood (Lee et al. 2013). Indeed, reports converge in suggesting that transient fluctuations in AEA levels during adolescence vary reciprocally with FAAH activity (Lee et al. 2013; Meyer et al. 2018). By contrast, 2-AG levels display differential trajectories relative to AEA that diverge depending on neural region (Ellgren et al. 2008).

CB1 receptor expression also follows distinct region-specific developmental trajectories. Cortical CB1 receptor expression is highest during the juvenile period, gradually decreasing over the course of adolescence into adulthood (Heng et al. 2011). Interestingly, limbic and associative cortical regions showed a larger decrease in CB1 receptors during adolescence, while sensorimotor regions maintained higher levels of CB1 receptors through adolescence, with levels converging in adulthood (Heng et al. 2011). CB1 receptor function also appears to follow a similar trajectory, where CB1 receptor-dependent inhibition of synaptic transmission in the prefrontal cortex is highest during the juvenile period and decreases over the course of adolescence into adulthood (Heng et al. 2011).

In humans, AEA levels and FAAH activity impact both neural circuit development and mental health (Gee et al. 2016; Marusak et al. 2023; Sisk et al. 2022). Individuals with the A allele of the FAAH C385A (rs324420) polymorphism—which destabilizes FAAH enzymatic activity and is associated with higher AEA levels—display higher frontolimbic tract integrity by age 12 relative to noncarriers, with concomitant decreases in anxiety (Fig. 5.2; Gee et al. 2016). As differences in neural connectivity and anxiety as a function of FAAH genotype were not observed prior to age 12, these results suggest an inflection point in early adolescence, potentially aligning with the transient adolescent-specific variation in AEA levels and FAAH activity observed in rodent samples (Ellgren et al. 2008; Lee et al. 2013). In a large sample of youth aged 9–10, FAAH genotype was associated with differences

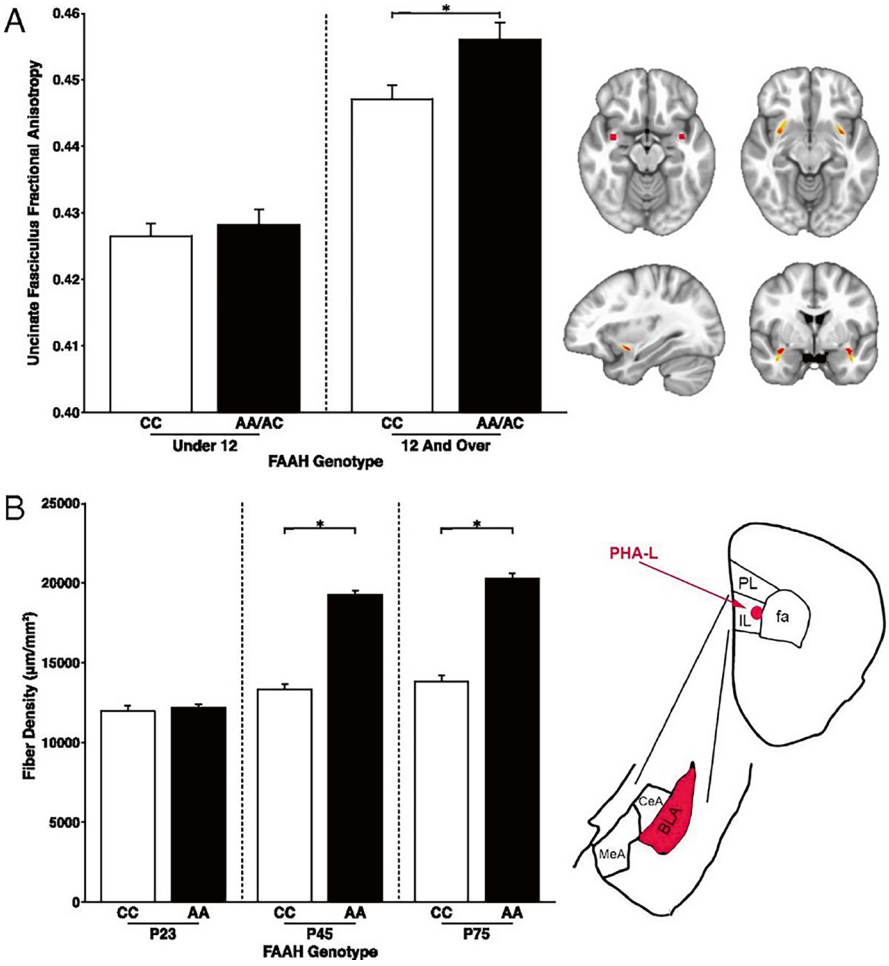


Fig. 5.2 Across species, A-allele carriers demonstrated stronger frontolimbic connectivity starting in adolescence. Figure included with permission from Gee and colleagues (2016)

in whole-brain functional connectivity (Sisk et al. 2022) and corticolimbic tract integrity (Marusak et al. 2023). Specifically, A-allele carriers demonstrated differential connectivity relative to noncarriers, such that A-allele carriers displayed stronger connectivity among some functional connections and networks while others displayed weaker connectivity (Sisk et al. 2022). Further, A-allele carriers showed lower white matter tract integrity in the fornix and parahippocampal cingulum relative to noncarriers (Marusak et al. 2023). Interestingly, while youth did not differ in anxiety levels as a function of FAAH genotype in either study, both studies identified interactions between connectivity and FAAH genotype that were associated with anxiety symptoms and were driven by noncarriers (Marusak et al. 2023; Sisk et al. 2022). These findings raise the possibility that eCB signaling-related differences in neurodevelopment are present during preadolescence, potentially preceding the emergence of protective effects of the FAAH C385A A-allele on anxiety levels in adolescence (Gee et al. 2016).

7 Endocannabinoids and the HPA Axis during Development

Development of the eCB system is closely intertwined with the neuroendocrine stress response (Dow-Edwards and Silva 2017; Lee and Gorzalka 2015). In addition to regulating synaptic plasticity, the eCB system also modulates the hypothalamic–pituitary–adrenal (HPA) axis, the primary neuroendocrine cascade governing the body's stress response (Balsevich et al. 2017; Hillard et al. 2016). The eCB system regulates HPA axis activity, maintaining homeostatic levels and attenuating HPA axis activity following stress exposure (Dow-Edwards and Silva 2017; Finn 2010; Hill et al. 2010b, c; Hill and Tasker 2012; Lee and Gorzalka 2015). This negative feedback loop between the eCB system and the HPA axis occurs via complex signaling between glucocorticoids and both AEA and 2-AG, and involves corticolimbic brain regions including the paraventricular nucleus, basolateral amygdala, hippocampus, and prefrontal cortex (Dow-Edwards and Silva 2017; Lee and Gorzalka 2015). During development, both the HPA axis and eCB signaling system undergo refinement, with changes particularly evident during adolescence (Meyer et al. 2018; Romeo 2018), implicating both systems in adolescent-specific differences in stress responding.

8 Endocannabinoids and Stress Responding

8.1 Endocannabinoid Signaling and Stress Responding

The eCB system plays an important role in regulating fear, stress, and risk for stress-related disorders such as anxiety (Higuera-Matas et al. 2015; Riebe et al. 2012; Ruehle et al. 2012). In general, enhanced eCB system signaling is associated with reductions in conditioned fear and levels of anxiety (Akirav 2011; Bluett et al. 2014;

Gray et al. 2015; Gunduz-Cinar et al. 2013). These effects may be grounded in changes in eCB system signaling within corticolimbic structures such as the prefrontal cortex, hippocampus, and amygdala (Campolongo et al. 2011; Herkenham et al. 1991; Hill et al. 2010a; Hill and Tasker 2012; Lee and Gorzalka 2015; Morena et al. 2016; Rubino and Parolaro 2008). In adult rodents, acute stress exposure decreases AEA levels in limbic regions such as the amygdala, prefrontal cortex, hippocampus, and hypothalamus (Atsak et al. 2018; Bluett et al. 2014; Gray et al. 2015; Hill et al. 2018), and these changes are thought to be mediated by corticotropin-releasing hormone (Gray et al. 2015; Hill et al. 2018; Natividad et al. 2017). By contrast, acute stress exposure increases 2-AG content in the amygdala (Bedse et al. 2017; Hill et al. 2018) and hippocampus (Atsak et al. 2018). Inhibition of 2-AG hydrolysis (via administration of an MAGL inhibitor) reduces anxiety-like behavior (Bluett et al. 2014; Morena et al. 2016; Sciolino et al. 2011; Sumislawski et al. 2011; Worley et al. 2020), as does inhibition of AEA hydrolysis via administration of an FAAH inhibitor (Morena et al. 2016), underscoring the causal link between eCB signaling and stress-related anxiety-like behavior in rodent models. However, eCB fluctuations following stress may differ between acute and chronic stress exposure. Mice exposed to chronic stress showed distinct effects—elevated 2-AG levels in the cortex and cerebellum and decreased AEA levels in the hippocampus, together with increased anxiety-like behavior (Bouter et al. 2020), as well as reduced hippocampal CB1 receptor expression (Dandi et al. 2024). Interestingly, exposure to stress may differentially stimulate hippocampal expression of the CB2 receptor gene based on whether the animal displays an anxious phenotype (Robertson et al. 2017), and enriched environments may protect against stress-related alterations to eCB signaling (Dandi et al. 2024), suggesting feedback loops between eCB signaling, experience, and behavior. In studies assessing eCB signaling following stress exposure in humans, increases in basal 2-AG levels following stress exposure were associated with reduced physiological stress responses in a male adult sample (Spohrs et al. 2022a). However, a meta-analysis examining acute stress induction in human participants did not identify a significant effect of acute psychosocial stress on levels of AEA or 2-AG (Gowatch et al. 2024). Taken together, findings suggest a mechanistic role for AEA and 2-AG signaling in specific neural regions, and that these effects may differ depending on type of stress (e.g., acute vs. chronic). Further, experiences (e.g., chronic stress, enrichment) appear to influence levels of CB1 and CB2 receptor expression (Aisenberg et al. 2017; Dandi et al. 2024; Robertson et al. 2017), emphasizing the sensitivity of eCB signaling to the environment (Goldstein Ferber et al. 2021).

During development, variation in eCB signaling during adolescence together with ongoing maturation of corticolimbic circuitry may enable an emergent sensitive period during which corticolimbic circuit development is particularly susceptible to disruption via perturbations of eCB signaling (Meyer et al. 2018). Indeed, exposure to stress during adolescence in rats increased CB1 receptor binding in the prefrontal cortex (Demaili et al. 2023; Lee and Hill 2013) and amygdala but not in the hippocampus (Lee and Hill 2013), while the same paradigm administered to adult rats resulted in increased CB1 receptor binding in the prefrontal cortex,

reduced CB1 receptor binding in the hippocampus, and no effects in the amygdala (Lee and Hill 2013). Further, juvenile male rats exposed to repeated restraint stress showed downregulation of CB1 receptor activity in the hypothalamus, impairing eCB signaling in hypothalamic but not hippocampal parvocellular neuroendocrine cells (neurons responsible for regulating HPA axis activation) (Wamsteeker et al. 2010). Emphasizing the importance of developmental stage for informing the effects of stress on eCB signaling, exogenous modification of CB1 receptor signaling during development resulted in changes that lasted into adulthood (Lee et al. 2015). In particular, CB1 receptor blockade during peri-adolescence in rats increased coping behavior during a forced swim test (a test assessing depressive-like behavior) in adulthood and altered adult levels of AEA in the amygdala and hypothalamus, as well as increased CB1 receptor expression in the prefrontal cortex (Lee et al. 2015). Rats that experienced peri-adolescent receptor blockade also showed accelerated habituation of the corticosterone response following repeated restraint stress in adulthood, suggesting that changes to developmental trajectories following manipulation of eCB signaling during development extend to HPA axis responsivity (Lee et al. 2015). In the prefrontal cortex, CB1 receptor stimulation specifically during early and mid-adolescence—but not late adolescence or adulthood—impaired typical development of inhibitory signaling in adulthood, resulting in reduced levels of GABAergic transmission (Cass et al. 2014). CB1 receptor stimulation during adolescence also altered dendritic arborization of prefrontal cortex pyramidal neurons, such that the number, length, and complexity of dendrites were reduced relative to those of control animals (Renard et al. 2016). These findings further implicate adolescence as a particularly sensitive developmental window during which disruption of eCB signaling may elicit lasting effects (Meyer et al. 2018). ECB signaling may also play an important role in synaptic pruning: adolescent stimulation of CB1 receptors prevented normative developmental reductions in receptor subunits implicated in stabilizing excitatory synapses (Rubino et al. 2015). Taken together, these results suggest that exogenous modification of eCB signaling during adolescence, but not adulthood, modifies trajectories of neurodevelopment and stress responsivity (Bambico et al. 2010; Cass et al. 2014; Zamberletti et al. 2014).

In addition to developmental stage, the effects of stress on the eCB signaling system also appear to differ as a function of sex. Male neonatal rat pups exposed to maternal deprivation showed decreased hippocampal expression of CB1 receptors (Suárez et al. 2009), decreased hippocampal MAGL expression (Suárez et al. 2010), and increased levels of hippocampal 2-AG (Llorente et al. 2008) relative to female pups. Conversely, female pups exposed to maternal deprivation demonstrated increased hippocampal mRNA expression of an enzymatic precursor to 2-AG (Suárez et al. 2010) and increased hippocampal expression of CB1 receptors specifically in the CA3 region of the hippocampus (Suárez et al. 2009). In adult rats, a reduction in AEA levels following a forced swim test was observed in the medial prefrontal cortex across sexes, but this pattern was largely driven by male rats, despite female rats mounting a stronger corticosterone response to the stressor (Vecchiarelli et al. 2022). Further, across multiple rat cohorts employed in the study, sex differences were inconsistent, highlighting the complexity of links between

eCB signaling and the stress response, as well as the need for future studies to continue examining sex differences in these associations (Vecchiarelli et al. 2022). Neonatal handling, which is associated with resilience to stress later in life, alters eCB receptor expression in a sex-specific manner (Vangopoulou et al. 2018), such that female handled rats showed decreased eCB binding in the striatum, nucleus accumbens, and CA3 and DG regions of the dorsal hippocampus and basolateral amygdala relative to male and non-handled rats (Vangopoulou et al. 2018). Together, this work demonstrates that changes in eCB signaling following stress exposure may differ by sex, brain region, and prior life experience.

8.2 Genetic Variation in Endocannabinoid Signaling and Stress Responding

Cross-species research shows that genetic variation in the FAAH gene (C385A; rs324420) reduces enzymatic breakdown of AEA by FAAH, leading to increased basal AEA levels (Cravatt et al. 2001; Dincheva et al. 2015; Sipe et al. 2002). The C385A polymorphism also confers protection against decreased AEA levels following acute stress exposure—in humans, AC heterozygotes and AA homozygotes displayed dose-dependent reductions in the negative consequences of acute stress exposure relative to CC homozygotes (Mayo et al. 2020a). The FAAH C385A polymorphism has been associated with both neural and behavioral differences, including reduced amygdala reactivity to threat (Hariri et al. 2009), quicker amygdala habituation to threat (Gunduz-Cinar et al. 2013), increased corticolimbic white matter tract integrity starting in adolescence (Gee et al. 2016), and increased frontolimbic functional connectivity (Dincheva et al. 2015; Gärtner et al. 2019). These adaptive effects may be linked with increased AEA agonism of CB1 receptors and decreased FAAH metabolism in the amygdala (Bluett et al. 2014; Gray et al. 2015; Green et al. 2021; Gunduz-Cinar et al. 2013; Hill et al. 2009; Hill and Tasker 2012), possibly in addition to greater subcortical regulation by prefrontal regions resulting from an increased number of projections from prefrontal cortical regions to the amygdala (Dincheva et al. 2015). Thus, in individuals with the A allele of the FAAH C385A polymorphism, exposure to stress may diverge from the typical rapid mobilization of FAAH to degrade AEA, and rather result in higher levels of AEA despite stressful circumstances. These protective effects have been well-established, yet in the context of acute trauma exposure, it is less clear whether the C385A polymorphism confers protection against the negative consequences of stress. Among individuals admitted to a hospital emergency department and followed longitudinally, individuals with the AA genotype displayed higher levels of circulating AEA and elevated posttraumatic stress disorder (PTSD) symptom severity (deRoon-Cassini et al. 2022), suggesting that the FAAH C385A polymorphism may not confer universally protective effects (deRoon-Cassini et al. 2022; Lazary et al. 2016, 2019).

9 Endocannabinoids and Fear Learning

Given the importance of the endocannabinoid system in regulating neurodevelopment and responding to stress, its role in informing fear learning at neural and behavioral levels has been examined across multiple studies. The FAAH C385A polymorphism, which confers enhanced AEA signaling among A-allele carriers, is associated with enhanced extinction of cued fear and extinction recall across species (Dincheva et al. 2015; Mayo et al. 2020a, b; Morena et al. 2018), as well as reductions in negative affect (Mayo et al. 2020b) and anxiety (Dincheva et al. 2015; Gee et al. 2016). Importantly, the role of AEA signaling in fear learning may differ across sexes. In one recent study, acute systemic inhibition of AEA hydrolysis had minimal effects on fear expression in male rodents, but increased fear expression in female rodents and impaired fear extinction and recall (Morena et al. 2021). Signaling via 2-AG is also critical to fear learning: reductions in MAGL activity, resulting in elevated 2-AG levels, led to enhanced hippocampal-dependent extinction learning in a context-dependent manner (Kishimoto et al. 2015) and promoted active fear coping behavior specifically in female rodents (Morena et al. 2021). Enhanced 2-AG signaling also augmented fear memory retrieval, specifically in female mice (Mizuno et al. 2022). Together, findings underscore the critical role of eCB signaling in modulating fear learning and suggest that AEA and 2-AG signaling shape fear learning differentially across sexes.

The effects of increased AEA and 2-AG signaling are mediated in part by activation of CB1 and CB2 receptors. CB1 activation in the bed nucleus of the stria terminalis (BNST), amygdala, and perirhinal cortex appear particularly significant in impacting fear learning (Gunduz-Cinar et al. 2023; Jiang et al. 2022; Lange et al. 2017). CB1 receptors localized on amygdala projections to the BNST modulate fear learning, such that CB1 receptor activation is required for animals to enter a state of sustained fear when anticipating a threat (Lange et al. 2017). CB1 receptor agonism facilitates extinction learning (Zubedat and Akirav 2017), and formation of extinction memories may be regulated in part by CB1 receptors expressed at the synapse of prefrontal cortical projections to the basolateral amygdala (Gunduz-Cinar et al. 2023). Further, inhibition of CB1 receptor activity in the basolateral amygdala during memory reconsolidation inhibits fear memory formation (Li et al. 2023). CB2 signaling also plays a critical role in the efficacy of contextual fear learning, as evidenced by the finding that knockout of CB2 receptors impaired hippocamals-dependent contextual fear memory (Li and Kim 2016).

Fear learning during development is also shaped by eCB signaling. Rats exposed to CB1 receptor agonism during adolescence in the absence of stress showed deficits in contextual fear learning in adulthood, as well as increased levels of FAAH and MAGL activity (Gleason et al. 2012). In contrast, rats exposed to chronic unpredictable stress during adolescence demonstrated enhanced hippocampal-dependent fear learning, and subsequent CB1 receptor agonism facilitated extinction learning and produced anxiolytic effects (Reich et al. 2013). Together, these findings highlight that both reduced and exacerbated CB1 receptor activation during adolescence

may disrupt typical development. Interestingly, female mice with a knock-in mutation replicating the FAAH C385A polymorphism displayed hypervigilance during adolescence, suggesting that this genotype may confer transiently heightened fear during adolescence (Gerhard et al. 2023). These findings potentially contribute to a richer understanding as to why protective effects of the FAAH C385A polymorphism may not emerge until later in development (Gee et al. 2016; Marusak et al. 2023; Sisk et al. 2022).

Among fMRI studies, enhanced eCB signaling during fear learning is associated overall with decreased activity in threat-sensitive neural regions (Gunduz-Cinar et al. 2013; Hariri et al. 2009; Paulus et al. 2021; Zabik et al. 2022). Individuals with the FAAH C385A polymorphism displayed reduced amygdala activation during extinction recall, alongside reduced dACC and increased hippocampal activation in early extinction learning (Zabik et al. 2022). Threat-related reactivity in the amygdala was reduced among A-allele carriers (Hariri et al. 2009), and A-allele carriers demonstrated quicker amygdala habituation to threat (Gunduz-Cinar et al. 2013). However, how the presence of the FAAH C385A polymorphism may impact fear learning in youth and adolescents remains unclear. Future work in this area will be valuable in determining whether neural circuitry involved in fear learning displays variation as a function of genotype prior to adolescence (Marusak et al. 2023; Sisk et al. 2022), or whether differences might emerge following a timeline more similar to those of anxiety symptoms (e.g., beginning in adolescence) (Gee et al. 2016).

10 Endocannabinoids and Longer-Term Impacts of Stress and Trauma

Exposure to adversity during development can affect cognitive, emotional, and neural development and have lasting effects on mental health (Ellis et al. 2009; Fox et al. 2017; Gee 2021; McLaughlin et al. 2019; Tottenham 2020). The eCB signaling system plays a crucial role in responding to stress and modulating emotional behavior and may mediate associations between adversity and behavioral outcomes in adulthood. Evidence from rodent models suggests that adversity exposure early in development decreases CB1 receptor expression and binding across corticolimbic brain regions (Alteba et al. 2021; Amancio-Belmont et al. 2020; Dow-Edwards et al. 2016; Hill et al. 2019), and disrupts normative stress-induced increases of 2-AG in the hippocampus (Atsak et al. 2018). However, differences emerge when considering sex and age of stress exposure. For instance, female rats exposed to prenatal stress demonstrated increased CB1 receptor binding in the hippocampus relative to males, as well as increased CB1 receptor binding in limbic regions more broadly following juvenile stress exposure relative to males (Dow-Edwards et al. 2016). One study that characterized alterations in developmental trajectories of eCB signaling across corticolimbic brain regions in male rats following juvenile maternal separation found that the effects of adversity during early development varied by

brain region and age (Hill et al. 2019). Overall, eCB levels varied in the prefrontal cortex by age only, with AEA showing an increase from the juvenile period to adulthood, whereas 2-AG showed a nonlinear increase specifically during PND 12 and 14 (Hill et al. 2019). By contrast, the amygdala and hippocampus showed effects of both age and adversity—in the amygdala, AEA levels showed a significant decrease during PND 12 and 14 in stressed rats relative to control rats, while 2-AG levels were elevated during the same days (Hill et al. 2019). Hippocampal 2-AG levels were also significantly higher at PND 12 in stressed rats relative to control rats, and then decreased at PND 40 and 70 relative to control rats (Hill et al. 2019). CB1 receptor binding was downregulated across ages as a function of adversity exposure in the prefrontal cortex and amygdala, but interestingly, CB1 receptor downregulation in the hippocampus was not evident until adulthood (PND 70) (Hill et al. 2019). While a significant focus has been placed on examining eCB signaling in canonically stress-sensitive corticolimbic neural regions, emerging evidence suggests that eCB signaling in the cerebellum is also affected by early life adversity (Moussa-Tooks et al. 2020). These findings suggest that exposure to adversity during development can exert both proximal and long-term effects on the developing brain. Further, the developmental timing of adversity exposure and the timing of measurement impact these observations, with differential effects observed across sexes.

In human research, evidence regarding the role of eCB signaling in mental health after exposure to adversity is mixed. Overall, higher levels of peripheral AEA have been linked with lower risk for psychopathology in adults with a history of adversity or trauma (Botsford et al. 2023; Perini et al. 2023). For example, in adults with a history of childhood maltreatment, higher levels of AEA at baseline and during stress exposure were associated with lower likelihood of developing substance use disorders (Perini et al. 2023). Similarly, in female adults diagnosed with PTSD, higher levels of circulating AEA were associated with reduced PTSD and depressive symptom severity (Botsford et al. 2023). Importantly, however, some studies have also suggested that the FAAH C385A A allele, the presence of which confers elevated basal AEA levels, may constitute a risk factor for individuals with childhood trauma exposure. In two studies, researchers found that in individuals with exposure to childhood trauma, there was an interaction between the FAAH C385A genotype and trauma exposure that predicted worsened mental health in A-allele carriers (Lazary et al. 2016). Similarly, a genotype conferring reduced CB2 receptor function displayed an interaction with both FAAH C385A genotype and trauma exposure to predict worsened mental health (Lazary et al. 2019). Reconciling these mixed findings will require further research that probes multiple aspects of eCB system function and examines factors that modulate eCB functioning in rodent studies, such as sex and the type and timing of adversity exposure during development.

In studies of youth, the role of eCB signaling in predicting youth mental health following exposure to trauma or adversity remains unclear. Several studies with youth found no significant links between exposure to adversity during development and eCB levels (Pantell et al. 2024) or FAAH C385A genotype (Marusak et al. 2023) in predicting psychopathology. There are several possible reasons for these

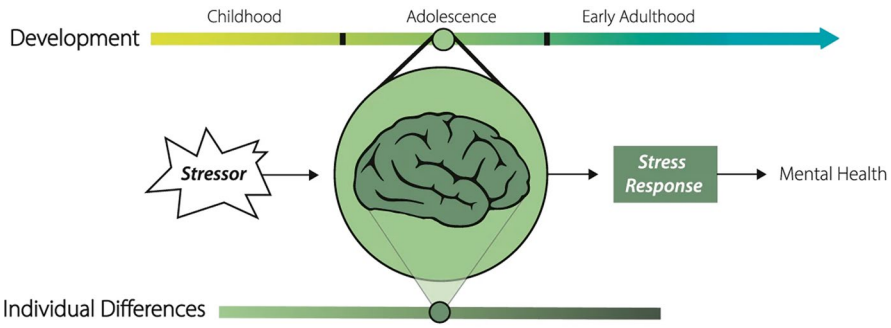


Fig. 5.3 Individual differences (such as genetic variation in eCB signaling) may contribute as a function of developmental stage to how individuals respond to a stressors, and their subsequent risk for psychopathology. Figure included with permission from Gee and colleagues (2022)

null effects when contrasted with rodent research showing stress-related differences in eCB signaling following adversity (Hill et al. 2019). First, cross-species research suggests that protective effects of genetic variation in eCB signaling may not emerge until adolescence (Gee et al. 2016; Meyer et al. 2018), and it is possible that eCB-related variation in stress responding remains latent in prepubertal youth. Second, rodent studies often examine eCB levels within specific brain regions (such as the hippocampus, amygdala, and prefrontal cortex), whereas studies in humans typically rely on circulating basal levels of AEA or genotyping information, which can be obtained noninvasively. For instance, as acute stress exposure may increase circulating AEA levels in humans (Dlugos et al. 2012; Gowatch et al. 2024), whereas acute stress exposure generally decreases neural AEA levels in rodents (Hill et al. 2018), it is challenging to make comparisons between these two measures. Future work in youth characterizing longitudinal trajectories of eCB signaling, adversity exposure, and mental health will be valuable in clarifying the role of eCB signaling in mental health following adversity exposure, and may advance the development of novel treatments and interventions that are tailored for the state of the developing brain (Fig. 5.3; Casey et al. 2015; Gee et al. 2022; Gee and Casey 2015; Sisk and Gee 2022).

11 Broader Implications of Endocannabinoid System Development for Psychopathology Risk

The eCB signaling system has been implicated in a number of psychiatric disorders, most notably in stress-related disorders such as anxiety, depression, and PTSD. A systematic review and meta-analysis found that individuals with stress-related disorders (anxiety, depression, and PTSD) demonstrated higher baseline levels of AEA and 2-AG than individuals without stress-related disorders (Gowatch et al. 2024). Given the close coupling of the HPA axis and the eCB system, these elevated levels

of eCBs may reflect dysregulated stress responding in individuals with stress-related disorders. However, there was significant heterogeneity in findings across studies (Gowatch et al. 2024), with some reporting an inverse pattern of decreased eCB levels in individuals with stress-related disorders (e.g., Walther et al. 2023). During development, some evidence suggests that the A-allele of the FAAH C385A polymorphism may confer protection against depressive symptoms during adolescence (ages 9–13), although main effects of genotype were not observed for anxiety symptoms (Desai et al. 2024). However, the A385 allele may confer protective effects against anxiety later in adolescence (Gee et al. 2016) and adulthood (Dincheva et al. 2015; Spohrs et al. 2022b). Despite mixed findings in human research, the robust characterization and therapeutic potential evidenced by rodent models of the eCB system retain it as a promising target for the development of novel therapies for stress-related disorders.

A multitude of research has established putative mechanisms involving the eCB system in anxiety. Reductions in FAAH activity, and concomitant increases in AEA levels and CB1 binding, are linked with reduced anxiety-like behavior in rodent models (Bluett et al. 2014; Kathuria et al. 2003; Manduca et al. 2015; Moreira et al. 2008; Riebe et al. 2012; Ruehle et al. 2012). Reduced anxiety-like behavior appears to be mediated via CB1 receptor activation (Aisenberg et al. 2017; Rey et al. 2012) and can be mimicked through depletion and augmentation of AEA and 2-AG levels (Bedse et al. 2017; Tevosian et al. 2023) as well as by FAAH inhibition (Gray et al. 2015). Intriguingly, depletion and augmentation of AEA and 2-AG appear to have functional redundancy in their effects on anxiety symptoms (Bedse et al. 2017). Specifically, increased anxiety-like symptoms associated with a reduction in 2-AG levels can be rescued with augmentation by AEA, while increased anxiety-like symptoms associated with AEA depletion in the amygdala can be rescued with augmentation by 2-AG. However, concurrent inhibition of both FAAH and MAGL increases anxiety-like behavior (Bedse et al. 2018). Administration of a FAAH inhibitor to rats in late adolescence, but not mid-adolescence, reduced anxiety-like and depressive-like behavior induced by exposure to early life stress (Alteba et al. 2021). Such findings suggest that modulation of CB1 receptor activity, as well as developmentally informed augmentation of AEA and/or 2-AG separately, hold potential as therapeutic targets.

Several clinical trials have begun to examine the impacts of FAAH inhibition on fear-related behavior and anxiety in humans. These studies suggest that FAAH inhibition results in elevated levels of AEA and behavioral effects that are largely consistent with those observed in rodent models (Mayo et al. 2020b; Paulus et al. 2021; Schmidt et al. 2021). One randomized controlled trial found that FAAH inhibition attenuated activation to an emotional face-processing task in corticolimbic regions including the amygdala, anterior cingulate, and insula (Paulus et al. 2021). In a separate randomized controlled trial, FAAH inhibition resulted in improvement in social anxiety symptoms for some individuals (Schmidt et al. 2021). Yet another randomized controlled trial found that FAAH inhibition elevated AEA levels, enhanced fear extinction, and attenuated responses to stress (Mayo et al. 2020b). Intriguingly, in a sample of female adults with PTSD, a randomized controlled trial

showed that exercise-induced increases in AEA were related to reduced threat responding during extinction reinstatement (Crombie et al. 2021), supporting previous evidence linking elevated AEA levels with enhanced extinction learning (Crombie et al. 2021; Mayo et al. 2020b). While these initial studies highlight the therapeutic potential of treatments that increase AEA levels and/or reduce FAAH activity in adults, how such treatments may affect youth in a therapeutic context remains unclear. Although evidence from rodent models suggests FAAH and MAGL inhibition early in development may reduce anxiety during the juvenile period (Carr et al. 2017), further research is needed to understand whether and how such pharmacological manipulation may confer benefit during development in humans.

12 Summary

The endocannabinoid system plays a central role in neurodevelopment, from the prenatal period through adulthood. Signaling via the eCB system is closely intertwined with neuroendocrine and gonadal signaling, with feedback loops among these cascades bidirectionally shaping output. The eCB system is also an important arbiter of behavioral responses to stress and fear, with dynamic changes in eCB concentrations following stress exposure and in the context of stress-related psychopathology. A rich literature using rodent models has characterized how specific components of the eCB system change over the course of development, as well as their roles in stress and fear responding. While direct comparisons between rodents and humans remain challenging, research leveraging genetic variation in eCB signaling has been a valuable source of information regarding how individual variation in eCB signaling may affect neurodevelopment and mental health across species. Together, research suggests that eCB signaling represents a promising therapeutic target, both during development and in adulthood. However, important questions remain, particularly pertaining to how eCB signaling and its modification may differ across sexes, how heterogeneity in adversity type and timing may alter eCB signaling, and how novel anxiolytic drugs targeting eCB signaling may vary in their effects depending on developmental stage.

References

Aguado T, Palazuelos J, Monory K, Stella N, Cravatt B, Lutz B, Marsicano G, Kokaia Z, Guzmán M, Galve-Roperh I. The endocannabinoid system promotes astroglial differentiation by acting on neural progenitor cells. J Neurosci Off J Soc Neurosci. 2006;26(5):1551–61. https://doi.org/10.1523/JNEUROSCI.3101-05.2006.

Aisenberg N, Serova L, Sabban EL, Akirav I. The effects of enhancing endocannabinoid signaling and blocking corticotrophin releasing factor receptor in the amygdala and hippocampus on the consolidation of a stressful event. Eur Neuropsychopharmacol. 2017;27(9):913–27. https://doi.org/10.1016/j.euroneuro.2017.06.006.

Akirav I. The role of cannabinoids in modulating emotional and non-emotional memory processes in the hippocampus. Front Behav Neurosci. 2011;5:34. https://doi.org/10.3389/fnbeh.2011.00034.

Alger BE. Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. Prog Neurobiol. 2002;68(4):247–86. https://doi.org/10.1016/S0301-0082(02)00080-1.

Alteba S, Portugalov A, Hillard CJ, Akirav I. Inhibition of Fatty Acid Amide Hydrolase (FAAH) during adolescence and exposure to early life stress may exacerbate depression-like behaviors in male and female rats. Neuroscience. 2021;455:89–106. https://doi.org/10.1016/j.neuroscience.2020.12.022.

Amancio-Belmont O, Becerril Meléndez AL, Ruiz-Contreras AE, Méndez-Díaz M, Prospéro-García O. Maternal separation plus social isolation during adolescence reprogram brain dopamine and endocannabinoid systems and facilitate alcohol intake in rats. Brain Res Bull. 2020;164:21–8. https://doi.org/10.1016/j.brainresbull.2020.08.002.

Atsak P, Morena M, Schoenmaker C, Tabak E, Oomen CA, Jamil S, Hill MN, Roozendaal B. Glucocorticoid-endocannabinoid uncoupling mediates fear suppression deficits after early—life stress. Psychoneuroendocrinology. 2018;91:41–9. https://doi.org/10.1016/j.psyneuen.2018.02.021.

Azad SC, Monory K, Marsicano G, Cravatt BF, Lutz B, Zieglgänsberger W, Rammes G. Circuitry for associative plasticity in the amygdala involves endocannabinoid signaling. J Neurosci. 2004;24(44):9953–61. https://doi.org/10.1523/JNEUROSCI.2134-04.2004.

Balsevich G, Petrie GN, Hill MN. Endocannabinoids: effectors of glucocorticoid signaling. Front Neuroendocrinol. 2017;47:86–108. https://doi.org/10.1016/j.yfrne.2017.07.005.

Bambico FR, Nguyen N-T, Katz N, Gobbi G. Chronic exposure to cannabinoids during adolescence but not during adulthood impairs emotional behaviour and monoaminergic neurotransmission. Neurobiol Dis. 2010;37(3):641–55. https://doi.org/10.1016/j.nbd.2009.11.020.

Bedse G, Hartley ND, Neale E, Gaulden AD, Patrick TA, Kingsley PJ, Uddin MJ, Plath N, Marnett LJ, Patel S. Functional redundancy between canonical endocannabinoid signaling systems in the modulation of anxiety. Biol Psychiatry. 2017;82(7):488–99. https://doi.org/10.1016/j.biopsych.2017.03.002.

Bedse G, Bluett RJ, Patrick TA, Romness NK, Gaulden AD, Kingsley PJ, Plath N, Marnett LJ, Patel S. Therapeutic endocannabinoid augmentation for mood and anxiety disorders: comparative profiling of FAAH, MAGL and dual inhibitors. Transl Psych. 2018;8(1):92. https://doi.org/10.1038/s41398-018-0141-7.

Berghuis P, Rajnicek AM, Morozov YM, Ross RA, Mulder J, Urbán GM, Monory K, Marsicano G, Matteoli M, Canty A, Irving AJ, Katona I, Yanagawa Y, Rakic P, Lutz B, Mackie K, Harkany T. Hardwiring the brain: endocannabinoids shape neuronal connectivity. Science (New York, NY). 2007;316(5828):1212–6. https://doi.org/10.1126/science.1137406.

Bernabeu A, Bara A, Murphy Green MN, Manduca A, Wager-Miller J, Borsoi M, Lassalle O, Pelissier-Alicot A-L, Chavis P, Mackie K, Manzoni OJJ. Sexually dimorphic adolescent trajectories of prefrontal endocannabinoid synaptic plasticity equalize in adulthood, reflected by endocannabinoid system gene expression. Cannabis and Cannabinoid Research. 2023;8(5):749–67. https://doi.org/10.1089/can.2022.0308.

Berrendero F, García-Gil L, Hernández ML, Romero J, Cebeira M, de Miguel R, Ramos JA, Fernández-Ruiz JJ. Localization of mRNA expression and activation of signal transduction mechanisms for cannabinoid receptor in rat brain during fetal development. Development (Cambridge, England). 1998;125(16):3179–88. https://doi.org/10.1242/dev.125.16.3179.

Bliss TVP, Cooke SF. Long-term potentiation and long-term depression: a clinical perspective. Clinics. 2011;66(Suppl 1):3–17. https://doi.org/10.1590/S1807-59322011001300002.

Bluett RJ, Gamble-George JC, Hermanson DJ, Hartley ND, Marnett LJ, Patel S. Central anandamide deficiency predicts stress-induced anxiety: behavioral reversal through endocannabinoid augmentation. Transl Psychiatry. 2014;4(7):e408. https://doi.org/10.1038/tp.2014.53.

Bossong MG, Niesink RJM. Adolescent brain maturation, the endogenous cannabinoid system and the neurobiology of cannabis-induced schizophrenia. Prog Neurobiol. 2010;92(3):370–85. https://doi.org/10.1016/j.pneurobio.2010.06.010.

Botsford C, Brellenthin AG, Cisler JM, Hillard CJ, Koltyn KF, Crombie KM. Circulating endocannabinoids and psychological outcomes in women with PTSD. J Anxiety Disord. 2023;93:102656. https://doi.org/10.1016/j.janxdis.2022.102656.

Bouter Y, Brzózka MM, Rygula R, Pahlisch F, Boost C, Leweke FM, Havemann-Reinecke U, Rohleder C. Chronic psychosocial stress causes increased anxiety-like behavior and alters endocannabinoid levels in the brain of C57Bl/6J mice. Cannabis and Cannabinoid Research. 2020;5(1):51–61. https://doi.org/10.1089/can.2019.0041.

Bradshaw HB, Rimmerman N, Krey JF, Walker JM. Sex and hormonal cycle differences in rat brain levels of pain-related cannabimimetic lipid mediators. Am J Physiol Regul Integr Comp Physiol. 2006;291(2):R349–58. https://doi.org/10.1152/ajpregu.00933.2005.

Busquets-Garcia A, Puighermanal E, Pastor A, de la Torre R, Maldonado R, Ozaita A. Differential role of anandamide and 2-arachidonoylglycerol in memory and anxiety-like responses. Biol Psychiatry. 2011;70(5):479–86. https://doi.org/10.1016/j.biopsych.2011.04.022.

Busquets-Garcia A, Gomis-González M, Srivastava RK, Cutando L, Ortega-Alvaro A, Ruehle S, Remmers F, Bindila L, Bellocchio L, Marsicano G, Lutz B, Maldonado R, Ozaita A. Peripheral and central CB1 cannabinoid receptors control stress-induced impairment of memory consolidation. Proc Natl Acad Sci. 2016;113(35):9904–9. https://doi.org/10.1073/pnas.1525066113.

Buzsáki G, Draguhn A. Neuronal oscillations in cortical networks. Science (New York, NY). 2004;304(5679):1926–9. https://doi.org/10.1126/science.1099745.

Cabral GA, Raborn ES, Griffin L, Dennis J, Marciano-Cabral F. CB2 receptors in the brain: role in central immune function. Br J Pharmacol. 2008;153(2):240–51. https://doi.org/10.1038/sj.bjp.0707584.

Campolongo P, Trezza V, Ratano P, Palmery M, Cuomo V. Developmental consequences of perinatal cannabis exposure: behavioral and neuroendocrine effects in adult rodents. Psychopharmacology. 2011;214(1):5–15. https://doi.org/10.1007/s00213-010-1892-x.

Carr RL, Armstrong NH, Buchanan AT, Eells JB, Mohammed AN, Ross MK, Nail CA. Decreased anxiety in juvenile rats following exposure to low levels of chlorpyrifos during development. Neurotoxicology. 2017;59:183–90. https://doi.org/10.1016/j.neuro.2015.11.016.

Casey BJ, Glatt CE, Lee FS. Treating the developing versus developed brain: translating preclinical mouse and human studies. Neuron. 2015;86(6):1358–68. https://doi.org/10.1016/j.neuron.2015.05.020.

Cass DK, Flores-Barrera E, Thomases DR, Vital WF, Caballero A, Tseng KY. CB1 cannabinoid receptor stimulation during adolescence impairs the maturation of GABA function in the adult rat prefrontal cortex. Mol Psychiatry. 2014;19(5):536–43. https://doi.org/10.1038/mp.2014.14.

Chevaleyre V, Castillo PE. Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. Neuron. 2003;38(3):461–72. https://doi.org/10.1016/S0896-6273(03)00235-6.

Chevaleyre V, Takahashi KA, Castillo PE. Endocannabinoid-mediated synaptic plasticity in the CNS. Ann Rev Neurosci. 2006;29:37–76. https://doi.org/10.1146/annurev.neuro.29.051605.112834.

Chevaleyre V, Heifets BD, Kaeser PS, Südhof TC, Castillo PE. Endocannabinoid-mediated long-term plasticity requires cAMP/PKA signaling and RIM1α. Neuron. 2007;54(5):801–12. https://doi.org/10.1016/j.neuron.2007.05.020.

Choi K, Le T, McGuire J, Xing G, Zhang L, Li H, Parker CC, Johnson LR, Ursano RJ. Expression pattern of the cannabinoid receptor genes in the frontal cortex of mood disorder patients and mice selectively bred for high and low fear. J Psychiatr Res. 2012;46(7):882–9. https://doi.org/10.1016/j.jpsychires.2012.03.021.

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature. 1996;384(6604):83–7. https://doi.org/10.1038/384083a0.

Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, Lichtman AH. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. Proc Natl Acad Sci USA. 2001;98(16):9371–6. https://doi.org/10.1073/pnas.161191698.

Crombie KM, Sartin-Tarm A, Sellnow K, Ahrenholtz R, Lee S, Matalamaki M, Almassi NE, Hillard CJ, Koltyn KF, Adams TG, Cisler JM. Exercise-induced increases in anandamide and BDNF during extinction consolidation contribute to reduced threat following reinstatement: preliminary evidence from a randomized controlled trial. Psychoneuroendocrinology. 2021;132:105355. https://doi.org/10.1016/j.psyneuen.2021.105355.

Dalterio SL, Bartke A, Burstein S. Cannabinoids inhibit testosterone secretion by mouse testes in vitro. Science (New York, NY). 1977;196(4297):1472–3. https://doi.org/10.1126/science.867048.

Dalterio SL, Mayfield DL, Bartke A. Effects of delta 9-THC on plasma hormone levels in female mice. Subst Alcohol Actions Misuse. 1983;4(5):339–45.

Dandi E, Kesidou E, Simeonidou C, Spandou E, Grigoriadis N, Tata DA. Sex-specific differences and the role of environmental enrichment in the expression of hippocampal CB1 receptors following chronic unpredictable stress. Brain Sci. 2024;14(4):357. https://doi.org/10.3390/brainsci14040357.

Demaili A, Portugalov A, Dudai M, Maroun M, Akirav I, Braun K, Bock J. Epigenetic (re)programming of gene expression changes of CB1R and FAAH in the medial prefrontal cortex in response to early life and adolescence stress exposure. Front Cell Neurosci. 2023;17 https://doi.org/10.3389/fncel.2023.1129946.

deRoon-Cassini TA, Bergner CL, Chesney SA, Schumann NR, Lee TS, Brasel KJ, Hillard CJ. Circulating endocannabinoids and genetic polymorphisms as predictors of posttraumatic stress disorder symptom severity: heterogeneity in a community-based cohort. Transl Psychiatry. 2022;12(1):48. https://doi.org/10.1038/s41398-022-01808-1.

Desai S, Zundel CG, Evanski JM, Gowatch LC, Bhogal A, Ely S, Carpenter C, Shampine M, O'Mara E, Rabinak CA, Marusak HA. Genetic variation in endocannabinoid signaling: anxiety, depression, and threat- and reward-related brain functioning during the transition into adolescence. Behav Brain Res. 2024;463:114925. https://doi.org/10.1016/j.bbr.2024.114925.

Devane WA, Hanuš L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science. 1992;258(5090):1946–9. https://doi.org/10.1126/science.1470919.

Di Marzo V, De Petrocellis L, Bisogno T, Melck D. Metabolism of anandamide and 2-arachidonoylglycerol: an historical overview and some recent developments. Lipids. 1999;34(S1Part3):S319–25. https://doi.org/10.1007/BF02562332.

Diana MA, Marty A. Endocannabinoid-mediated short-term synaptic plasticity: depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE). Br J Pharmacol. 2004;142(1):9–19. https://doi.org/10.1038/sj.bjp.0705726.

Dincheva I, Drysdale AT, Hartley CA, Johnson DC, Jing D, King EC, Ra S, Gray JM, Yang R, DeGruccio AM, Huang C, Cravatt BF, Glatt CE, Hill MN, Casey BJ, Lee FS. FAAH genetic variation enhances fronto-amygdala function in mouse and human. Nat Commun. 2015;6:6395. https://doi.org/10.1038/ncomms7395.

Dinh TP, Freund TF, Piomelli D. A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. Chem Phys Lipids. 2002;121(1–2):149–58. https://doi.org/10.1016/s0009-3084(02)00150-0.

Dlugos A, Childs E, Stuhr KL, Hillard CJ, de Wit H. Acute stress increases circulating anandamide and other N-acylethanolamines in healthy humans. Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology. 2012;37(11):2416–27. https://doi.org/10.1038/npp.2012.100.

Dow-Edwards D, Silva L. Endocannabinoids in brain plasticity: cortical maturation, HPA axis function and behavior. Brain Res. 2017;1654:157–64. https://doi.org/10.1016/j.brainres.2016.08.037.

Dow-Edwards D, Frank A, Wade D, Weedon J, Izenwasser S. Sexually-dimorphic alterations in cannabinoid receptor density depend upon prenatal/early postnatal history. Neurotoxicol Teratol. 2016;58:31–9. https://doi.org/10.1016/j.ntt.2016.09.004.

Edwards DA, Zhang L, Alger BE. Metaplastic control of the endocannabinoid system at inhibitory synapses in hippocampus. Proc Natl Acad Sci USA. 2008;105(23):8142–7. https://doi.org/10.1073/pnas.0803558105.

Egertová M, Elphick MR. Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB1. J Comp Neurol. 2000;422(2):159–71. https://doi.org/10.1002/(SICI)1096-9861(20000626)422:2<159::AID-CNE1>3.0.CO;2-1.

Ellgren M, Artmann A, Tkalych O, Gupta A, Hansen HS, Hansen SH, Devi LA, Hurd YL. Dynamic changes of the endogenous cannabinoid and opioid mesocorticolimbic systems during adolescence: THC effects. Eur Neuropsychopharmacol. 2008;18(11):826–34. https://doi.org/10.1016/j.euroneuro.2008.06.009.

Ellis BJ, Figueredo AJ, Brumbach BH, Schlomer GL. Fundamental dimensions of environmental risk: The impact of harsh versus unpredictable environments on the evolution and development of life history strategies. Human Nature (Hawthorne, NY). 2009;20(2):204–68. https://doi.org/10.1007/s12110-009-9063-7.

El-Talatini MR, Taylor AH, Konje JC. The relationship between plasma levels of the endocannabinoid, anandamide, sex steroids, and gonadotrophins during the menstrual cycle. Fertil Steril. 2010;93(6):1989. https://doi.org/10.1016/j.fertnstert.2008.12.033.

Finn DP. Endocannabinoid-mediated modulation of stress responses: physiological and pathophysiological significance. Immunobiology. 2010;215(8):629–46. https://doi.org/10.1016/j.imbio.2009.05.011.

Fox NA, Nelson CA, Zeanah CH. The effects of psychosocial deprivation on attachment: lessons from the Bucharest Early Intervention Project. Psychodynamic Psychiatry. 2017;45(4):441–50. https://doi.org/10.1521/pdps.2017.45.4.441.

Freund T, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev. 2003;83(3):1017. https://doi.org/10.1152/physrev.00004.2003.

Fride E, Gobshtis N, Dahan H, Weller A, Giuffrida A, Ben-Shabat S. Chapter 6 The endocannabinoid system during development: emphasis on perinatal events and delayed effects. In: Vitamins & hormones, vol. 81. Academic Press; 2009. p. 139–58. https://doi.org/10.1016/S0083-6729(09)81006-6.

Gärtner A, Dörfel D, Diers K, Witt SH, Strobel A, Brocke B. Impact of FAAH genetic variation on fronto-amygdala function during emotional processing. Eur Arch Psychiatry Clin Neurosci. 2019;269(2):209–21. https://doi.org/10.1007/s00406-018-0944-9.

Gee DG. Early adversity and development: parsing heterogeneity and identifying pathways of risk and resilience. Am J Psychiatry. 2021;178(11):998–1013. https://doi.org/10.1176/appi.ajp.2021.21090944.

Gee DG, Casey B. The impact of developmental timing for stress and recovery. Neurobiology of Stress. 2015;1:184–94. https://doi.org/10.1016/j.ynstr.2015.02.001.

Gee DG, Fetcho RN, Jing D, Li A, Glatt CE, Drysdale AT, Cohen AO, Dellarco DV, Yang RR, Dale AM, Jernigan TL, Lee FS, Casey BJ, PING Consortium. Individual differences in frontolimbic circuitry and anxiety emerge with adolescent changes in endocannabinoid signaling across species. Proc Natl Acad Sci USA. 2016;113(16):4500–5. https://doi.org/10.1073/pnas.1600013113.

Gee DG, Sisk LM, Cohodes EM, Bryce NV. Leveraging the science of stress to promote resilience and optimize mental health interventions during adolescence. Nat Commun. 2022;13(1):Article 1. https://doi.org/10.1038/s41467-022-33416-4.

Gerdeman GL, Ronesi J, Lovinger DM. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. Nat Neurosci. 2002;5(5):446–51. https://doi.org/10.1038/nn832.

Gerhard DM, Tse N, Lee FS, Meyer HC. Developmental age and fatty acid amide hydrolase genetic variation converge to mediate fear regulation in female mice. Dev Psychobiol. 2023;65(6):e22409. https://doi.org/10.1002/dev.22409.

Gleason KA, Birnbaum SG, Shukla A, Ghose S. Susceptibility of the adolescent brain to cannabinoids: long-term hippocampal effects and relevance to schizophrenia. Transl Psychiatry. 2012;2(11):e199. https://doi.org/10.1038/tp.2012.122.

Goldstein Ferber S, Trezza V, Weller A. Early life stress and development of the endocannabinoid system: a bidirectional process in programming future coping. Dev Psychobiol. 2021;63(2):143–52. https://doi.org/10.1002/dev.21944.

González S, Bisogno T, Wenger T, Manzanares J, Milone A, Berrendero F, Di Marzo V, Ramos JA, Fernández-Ruiz JJ. Sex steroid influence on cannabinoid CB(1) receptor mRNA and endocannabinoid levels in the anterior pituitary gland. Biochem Biophys Res Commun. 2000;270(1):260–6. https://doi.org/10.1006/bbrc.2000.2406.

Gorzalka BB, Dang SS. Minireview: endocannabinoids and gonadal hormones: bidirectional interactions in physiology and behavior. Endocrinology. 2012;153(3):1016–24. https://doi.org/10.1210/en.2011-1643.

Gowatch LC, Evanski JM, Ely SL, Zundel CG, Bhogal A, Carpenter C, Shampine MM, O'Mara E, Mazurka R, Barcelona J, Mayo LM, Marusak HA. Endocannabinoids and stress-related neurospsychiatric disorders: a systematic review and meta-analysis of basal concentrations and response to acute psychosocial stress. Cannabis and Cannabinoid Research. 2024;9:1217. https://doi.org/10.1089/can.2023.0246.

Gray JM, Vecchiarelli HA, Morena M, Lee TTY, Hermanson DJ, Kim AB, McLaughlin RJ, Hassan KI, Kühne C, Wotjak CT, Deussing JM, Patel S, Hill MN. Corticotropin-releasing hormone drives anandamide hydrolysis in the amygdala to promote anxiety. J Neurosci. 2015;35(9):3879–92. https://doi.org/10.1523/JNEUROSCI.2737-14.2015.

Green DGJ, Kim J, Kish SJ, Tyndale RF, Hill MN, Strafella AP, Tong J, McCluskey T, Westwood DJ, Houle S, Lobaugh NJ, Boileau I. Fatty acid amide hydrolase binding is inversely correlated with amygdalar functional connectivity: a combined positron emission tomography and magnetic resonance imaging study in healthy individuals. Journal of Psychiatry & Neuroscience: JPN. 2021;46(2):E238–46. https://doi.org/10.1503/jpn.200010.

Gunduz-Cinar O, MacPherson KP, Cinar R, Gamble-George J, Sugden K, Williams B, Godlewski G, Ramikie TS, Gorka AX, Alapafuja SO, Nikas SP, Makriyannis A, Poulton R, Patel S, Hariri AR, Caspi A, Moffitt TE, Kunos G, Holmes A. Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. Mol Psychiatry. 2013;18(7):813–23. https://doi.org/10.1038/mp.2012.72.

Gunduz-Cinar O, Castillo LI, Xia M, Van Leer E, Brockway ET, Pollack GA, Yasmin F, Bukalo O, Limoges A, Oreizi-Esfahani S, Kondev V, Báldi R, Dong A, Harvey-White J, Cinar R, Kunos G, Li Y, Zweifel LS, Patel S, Holmes A. A cortico-amygdala neural substrate for endocannabinoid modulation of fear extinction. Neuron. 2023;111(19):3053–3067.e10. https://doi.org/10.1016/j.neuron.2023.06.023.

Hariri AR, Gorka A, Hyde LW, Kimak M, Halder I, Ducci F, Ferrell RE, Goldman D, Manuck SB. Divergent effects of genetic variation in endocannabinoid signaling on human threat- and reward-related brain function. Biol Psychiatry. 2009;66(1):9–16. https://doi.org/10.1016/j.biopsych.2008.10.047.

Harkany T, Guzmán M, Galve-Roperh I, Berghuis P, Devi LA, Mackie K. The emerging functions of endocannabinoid signaling during CNS development. Trends Pharmacol Sci. 2007;28(2):83–92. https://doi.org/10.1016/j.tips.2006.12.004.

Harkany T, Keimpema E, Barabás K, Mulder J. Endocannabinoid functions controlling neuronal specification during brain development. Mol Cell Endocrinol. 2008;286(1–2 Suppl 1):S84–90. https://doi.org/10.1016/j.mce.2008.02.011.

Heifets BD, Castillo PE. Endocannabinoid signaling and long-term synaptic plasticity. Annu Rev Physiol. 2009;71(1):283–306. https://doi.org/10.1146/annurev.physiol.010908.163149.

Heng L, Beverley JA, Steiner H, Tseng KY. Differential developmental trajectories for CB1 cannabinoid receptor expression in limbic/associative and sensorimotor cortical areas. Synapse. 2011;65(4):278–86. https://doi.org/10.1002/syn.20844.

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa B, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci. 1991;11(2):563–83. https://doi.org/10.1523/JNEUROSCI.11-02-00563.1991.

Higuera-Matas A, Ucha M, Ambrosio E. Long-term consequences of perinatal and adolescent cannabinoid exposure on neural and psychological processes. Neurosci Biobehav Rev. 2015;55:119–46. https://doi.org/10.1016/j.neubiorev.2015.04.020.

Hill MN, Tasker JG. Endocannabinoid signaling, glucocorticoid-mediated negative feedback, and regulation of the hypothalamic-pituitary-adrenal axis. Neuroscience. 2012;204:5–16. https://doi.org/10.1016/j.neuroscience.2011.12.030.

Hill MN, McLaughlin RJ, Morrish AC, Viau V, Floresco SB, Hillard CJ, Gorzalka BB. Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic-pituitary-adrenal axis. Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology. 2009;34(13):2733–45. https://doi.org/10.1038/npp.2009.114.

Hill MN, Karatsoreos IN, Hillard CJ, McEwen BS. Rapid elevations in limbic endocannabinoid content by glucocorticoid hormones in vivo. Psychoneuroendocrinology. 2010a;35(9):1333–8. https://doi.org/10.1016/j.psyneuen.2010.03.005.

Hill MN, McLaughlin RJ, Bingham B, Shrestha L, Lee TTY, Gray JM, Hillard CJ, Gorzalka BB, Viau V. Endogenous cannabinoid signaling is essential for stress adaptation. Proc Natl Acad Sci. 2010b;107(20):9406–11. https://doi.org/10.1073/pnas.0914661107.

Hill MN, Patel S, Campolongo P, Tasker JG, Wotjak CT, Bains JS. Functional interactions between stress and the endocannabinoid system: from synaptic signaling to behavioral output. J Neurosci. 2010c;30(45):14980–6. https://doi.org/10.1523/JNEUROSCI.4283-10.2010.

Hill MN, Campolongo P, Yehuda R, Patel S. Integrating endocannabinoid signaling and cannabinoids into the biology and treatment of posttraumatic stress disorder. Neuropsychopharmacology. 2018;43(1):80–102. https://doi.org/10.1038/npp.2017.162.

Hill MN, Eiland L, Lee TTY, Hillard CJ, McEwen BS. Early life stress alters the developmental trajectory of corticolimbic endocannabinoid signaling in male rats. Neuropharmacology. 2019;146:154–62. https://doi.org/10.1016/j.neuropharm.2018.11.036.

Hillard CJ, Beatka M, Sarvaideo J. Endocannabinoid signaling and the hypothalamic-pituitary-adrenal axis. Compr Physiol. 2016;7(1):1–15. https://doi.org/10.1002/cphy.c160005.

Hohmann AG, Herkenham M. Localization of central cannabinoid CB1 receptor messenger RNA in neuronal subpopulations of rat dorsal root ganglia: a double-label *in situ* hybridization study. Neuroscience. 1999;90(3):923–31. https://doi.org/10.1016/S0306-4522(98)00524-7.

Howlett AC. The cannabinoid receptors. Prostaglandins Other Lipid Mediat. 2002;68–69:619–31. https://doi.org/10.1016/s0090-6980(02)00060-6.

Hu SS-J, Mackie K. Distribution of the endocannabinoid system in the central nervous system. In: Pertwee RG, editor. Endocannabinoids. Springer; 2015. p. 59–93. https://doi.org/10.1007/978-3-319-20825-1_3.

Jiang C, Wu X, Wang J, Li C, Luo G. Activation of CB1 pathway in the perirhinal cortex is necessary but not sufficient for destabilization of contextual fear memory in rats. Behav Brain Res. 2022;416:113573. https://doi.org/10.1016/j.bbr.2021.113573.

Kang-Park M-H, Wilson WA, Kuhn CM, Moore SD, Swartzwelder HS. Differential sensitivity of GABA a receptor-mediated IPSCs to cannabinoids in hippocampal slices from adolescent and adult rats. J Neurophysiol. 2007;98(3):1223–30. https://doi.org/10.1152/jn.00091.2007.

Kathuria S, Gaetani S, Fegley D, Valiño F, Duranti A, Tontini A, Mor M, Tarzia G, Rana GL, Calignano A, Giustino A, Tattoli M, Palmery M, Cuomo V, Piomelli D. Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med. 2003;9(1):76–81. https://doi.org/10.1038/nm803.

Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. Nat Med. 2008;14(9):923–30. https://doi.org/10.1038/nm.f.1869.

Kim J, Li Y. Chronic activation of CB2 cannabinoid receptors in the hippocampus increases excitatory synaptic transmission. J Physiol. 2015;593(4):871–86. https://doi.org/10.1113/jphysiol.2014.286633.

Kishimoto Y, Cagniard B, Yamazaki M, Nakayama J, Sakimura K, Kirino Y, Kano M. Task-specific enhancement of hippocampus-dependent learning in mice deficient in monoacylglycerol lipase, the major hydrolyzing enzyme of the endocannabinoid 2-arachidonoylglycerol. Front Behav Neurosci. 2015;9:134. https://doi.org/10.3389/fnbeh.2015.00134.

Kolodny RC, Masters WH, Kolodner RM, Toro G. Depression of plasma testosterone levels after chronic intensive marihuana use. N Engl J Med. 1974;290(16):872–4. https://doi.org/10.1056/NEJM197404182901602.

Kumar MS, Chen CL. Effect of an acute dose of delta 9-THC on hypothalamic luteinizing hormone releasing hormone and met-enkephalin content and serum levels of testosterone and corticosterone in rats. Subst Alcohol Actions Misuse. 1983;4(1):37–43.

Lafourcade CA, Alger BE. Distinctions among GABAA and GABAB responses revealed by calcium channel antagonists, cannabinoids, opioids, and synaptic plasticity in rat hippocampus. Psychopharmacology. 2008;198(4):539–49. https://doi.org/10.1007/s00213-007-1040-4.

Lafourcade M, Elezgarai I, Mato S, Bakiri Y, Grandes P, Manzoni OJ. Molecular components and functions of the endocannabinoid system in mouse prefrontal cortex. PLoS One. 2007;2(8):e709. https://doi.org/10.1371/journal.pone.0000709.

Lange MD, Daldrup T, Remmers F, Szkudlarek HJ, Lesting J, Guggenhuber S, Ruehle S, Jüngling K, Seidenbecher T, Lutz B, Pape HC. Cannabinoid CB1 receptors in distinct circuits of the extended amygdala determine fear responsiveness to unpredictable threat. Mol Psychiatry. 2017;22(10):1422–30. https://doi.org/10.1038/mp.2016.156.

Lazary J, Eszlari N, Juhasz G, Bagdy G. Genetically reduced FAAH activity may be a risk for the development of anxiety and depression in persons with repetitive childhood trauma. Eur Neuropsychopharmacol. 2016;26(6):1020–8. https://doi.org/10.1016/j.euroneuro.2016.03.003.

Lazary J, Eszlari N, Juhasz G, Bagdy G. A functional variant of CB2 receptor gene interacts with childhood trauma and FAAH gene on anxious and depressive phenotypes. J Affect Disord. 2019;257:716–22. https://doi.org/10.1016/j.jad.2019.07.083.

Lee TT-Y, Gorzalka BB. Timing is everything: evidence for a role of corticolimbic endocannabinoids in modulating hypothalamic–pituitary–adrenal axis activity across developmental periods. Neuroscience. 2012;204:17–30. https://doi.org/10.1016/j.neuroscience.2011.10.006.

Lee TT-Y, Gorzalka BB. Chapter two—evidence for a role of adolescent endocannabinoid signaling in regulating HPA Axis stress responsivity and emotional behavior development. In: Parsons L, Hill M, editors. International review of neurobiology, vol. 125. Academic Press; 2015. p. 49–84. https://doi.org/10.1016/bs.irn.2015.09.002.

Lee TTY, Hill MN. Age of stress exposure modulates the immediate and sustained effects of repeated stress on corticolimbic cannabinoid CB_1 receptor binding in male rats. Neuroscience. 2013;249:106–14. https://doi.org/10.1016/j.neuroscience.2012.11.017.

Lee TT-Y, Hill MN, Hillard CJ, Gorzalka BB. Temporal changes in N-acylethanolamine content and metabolism throughout the peri-adolescent period. Synapse. 2013;67(1):4–10. https://doi.org/10.1002/syn.21609.

Lee TT-Y, Hill MN, Hillard CJ, Gorzalka BB. Disruption of peri-adolescent endocannabinoid signaling modulates adult neuroendocrine and behavioral responses to stress in male rats. Neuropharmacology. 2015;99:89–97. https://doi.org/10.1016/j.neuropharm.2015.07.021.

Lee TT-Y, Hill MN, Lee FS. Developmental regulation of fear learning and anxiety behavior by endocannabinoids. Genes Brain Behav. 2016;15(1):108–24. https://doi.org/10.1111/gbb.12253.

Li Y, Kim J. CB2 cannabinoid receptor knockout in mice impairs contextual long-term memory and enhances spatial working memory. Neural Plast. 2016;2016(1):9817089. https://doi.org/10.1155/2016/9817089.

Li M, Lv X, Li T, Cui C, Yang X, Peng X, Lei J, Yang J, Ren K, Luo G, Shi Y, Yao Y, Tian B, Zhang P. Basolateral amygdala cannabinoid CB1 receptor controls formation and elimination of social fear memory. ACS Chem Neurosci. 2023;14(19):3674–85. https://doi.org/10.1021/acschemneuro.3c00297.

Llorente R, Llorente-Berzal A, Petrosino S, Marco E-M, Guaza C, Prada C, López-Gallardo M, Di Marzo V, Viveros M-P. Gender-dependent cellular and biochemical effects of maternal deprivation on the hippocampus of neonatal rats: a possible role for the endocannabinoid system. Dev Neurobiol. 2008;68(11):1334–47. https://doi.org/10.1002/dneu.20666.

Long LE, Lind J, Webster M, Weickert CS. Developmental trajectory of the endocannabinoid system in human dorsolateral prefrontal cortex. BMC Neurosci. 2012;13:87. https://doi.org/10.1186/1471-2202-13-87.

Lu H-C, Mackie K. An introduction to the endogenous cannabinoid system. Biol Psychiatry. 2016;79(7):516–25. https://doi.org/10.1016/j.biopsych.2015.07.028.

Lu H-C, Mackie K. Review of the endocannabinoid system. Biological Psychiatry Cognitive Neuroscience and Neuroimaging. 2021;6(6):607–15. https://doi.org/10.1016/j.bpsc.2020.07.016.

Lubman DI, Cheetham A, Yücel M. Cannabis and adolescent brain development. Pharmacol Ther. 2015;148:1–16. https://doi.org/10.1016/j.pharmthera.2014.11.009.

MacCarrone M, De Felici M, Bari M, Klinger F, Siracusa G, Finazzi-Agrò A. Down-regulation of anandamide hydrolase in mouse uterus by sex hormones. Eur J Biochem. 2000;267(10):2991–7. https://doi.org/10.1046/j.1432-1033.2000.01316.x.

Maccarrone M, Valensise H, Bari M, Lazzarin N, Romanini C, Finazzi-Agrò A. Progesterone up-regulates anandamide hydrolase in human lymphocytes: role of cytokines and implications for fertility. J Immunol (Baltimore, MD: 1950). 2001;166(12):7183–9. https://doi.org/10.4049/jimmunol.166.12.7183.

Maccarrone M, Bari M, Di Rienzo M, Finazzi-Agrò A, Rossi A. Progesterone activates fatty acid amide hydrolase (FAAH) promoter in human T lymphocytes through the transcription factor Ikaros. Evidence for a synergistic effect of leptin. J Biol Chem. 2003;278(35):32726. https://doi.org/10.1074/jbc.M302123200.

Maccarrone M, Guzmán M, Mackie K, Doherty P, Harkany T. Programming of neural cells by (endo)cannabinoids: from physiological rules to emerging therapies. Nat Rev Neurosci. 2014;15(12):786–801. https://doi.org/10.1038/nrn3846.

Manduca A, Morena M, Campolongo P, Servadio M, Palmery M, Trabace L, Hill MN, Vanderschuren LJMJ, Cuomo V, Trezza V. Distinct roles of the endocannabinoids anandamide and 2-arachidonoylglycerol in social behavior and emotionality at different developmental ages in rats. Eur Neuropsychopharmacol. 2015;25(8):1362–74. https://doi.org/10.1016/j.euroneuro.2015.04.005.

Maresz K, Carrier EJ, Ponomarev ED, Hillard CJ, Dittel BN. Modulation of the cannabinoid CB2 receptor in microglial cells in response to inflammatory stimuli. J Neurochem. 2005;95(2):437–45. https://doi.org/10.1111/j.1471-4159.2005.03380.x.

Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglgänsberger W, Di Marzo V, Lutz B. The endogenous cannabinoid system controls extinction of aversive memories. Nature. 2002;418(6897):530–4. https://doi.org/10.1038/nature00839.

Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, Azad SC, Cascio MG, Gutiérrez SO, van der Stelt M, López-Rodriguez ML, Casanova E, Schütz G, Zieglgänsberger W, Di Marzo V, Behl C, Lutz B. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science (New York, NY). 2003;302(5642):84–8. https://doi.org/10.1126/science.1088208.

Marusak HA, Evanski J, Desai S, Rabinak CA. Impact of childhood trauma exposure, genetic variation in endocannabinoid signaling, and anxiety on frontolimbic pathways in children. Cannabis and Cannabinoid Research. 2023;8(6):1079–89. https://doi.org/10.1089/can.2022.0144.

Mayo LM, Asratian A, Lindé J, Holm L, Nätt D, Augier G, Stensson N, Vecchiarelli HA, Balsevich G, Aukema RJ, Ghafouri B, Spagnolo PA, Lee FS, Hill MN, Heilig M. Protective effects of elevated anandamide on stress and fear-related behaviors: translational evidence from humans and mice. Mol Psychiatry. 2020a;25(5):993–1005. https://doi.org/10.1038/s41380-018-0215-1.

Mayo LM, Asratian A, Lindé J, Morena M, Haataja R, Hammar V, Augier G, Hill MN, Heilig M. Elevated anandamide, enhanced recall of fear extinction, and attenuated stress responses following inhibition of fatty acid amide hydrolase: a randomized, Controlled Experimental Medicine Trial. Biological Psychiatry. 2020b;87(6):538–47. https://doi.org/10.1016/j.biopsych.2019.07.034.

McLaughlin KA, Weissman D, Bitrán D. Childhood adversity and neural development: a systematic review. Annual Review of Developmental Psychology. 2019;1:277–312. https://doi.org/10.1146/annurev-devpsych-121318-084950.

Mechoulam R, Parker LA. The endocannabinoid system and the brain. Annual Review of Psychology. 2013;64:21–47. https://doi.org/10.1146/annurev-psych-113011-143739.

Meyer HC, Lee FS, Gee DG. The role of the endocannabinoid system and genetic variation in adolescent brain development. Neuropsychopharmacology. 2018;43(1):21–33. https://doi.org/10.1038/npp.2017.143.

Mizuno I, Matsuda S, Tohyama S, Mizutani A. The role of the cannabinoid system in fear memory and extinction in male and female mice. Psychoneuroendocrinology. 2022;138:105688. https://doi.org/10.1016/j.psyneuen.2022.105688.

Moreira FA, Kaiser N, Monory K, Lutz B. Reduced anxiety-like behaviour induced by genetic and pharmacological inhibition of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) is mediated by CB1 receptors. Neuropharmacology. 2008;54(1):141–50. https://doi.org/10.1016/j.neuropharm.2007.07.005.

Morena M, Leitl KD, Vecchiarelli HA, Gray JM, Campolongo P, Hill MN. Emotional arousal state influences the ability of amygdalar endocannabinoid signaling to modulate anxiety. Neuropharmacology. 2016;111:59–69. https://doi.org/10.1016/j.neuropharm.2016.08.020.

Morena M, Berardi A, Colucci P, Palmery M, Trezza V, Hill MN, Campolongo P. Enhancing endocannabinoid neurotransmission augments the efficacy of extinction training and ameliorates traumatic stress-induced behavioral alterations in rats. *Neuropsychopharmacology: official publication of the American college of*. Neuropsychopharmacology. 2018;43(6):1284–96. https://doi.org/10.1038/npp.2017.305.

Morena M, Nastase AS, Santori A, Cravatt BF, Shansky RM, Hill MN. Sex-dependent effects of endocannabinoid modulation of conditioned fear extinction in rats. Br J Pharmacol. 2021;178(4):983–96. https://doi.org/10.1111/bph.15341.

Moussa-Tooks AB, Larson ER, Gimeno AF, Leishman E, Bartolomeo LA, Bradshaw HB, Green JT, O'Donnell BF, Mackie K, Hetrick WP. Long-term aberrations to cerebellar endocannabinoids induced by early-life stress. Sci Rep. 2020;10(1):7236. https://doi.org/10.1038/s41598-020-64075-4.

Mulder J, Aguado T, Keimpema E, Barabás K, Ballester Rosado CJ, Nguyen L, Monory K, Marsicano G, Di Marzo V, Hurd YL, Guillemot F, Mackie K, Lutz B, Guzmán M, Lu H-C, Galve-Roperh I, Harkany T. Endocannabinoid signaling controls pyramidal cell specification and long-range axon patterning. Proc Natl Acad Sci USA. 2008;105(25):8760–5. https://doi.org/10.1073/pnas.0803545105.

Murphy LL, Rodriguez de Fonseca F, Steger RW. Delta 9-Tetrahydrocannabinol antagonism of the anterior pituitary response to estradiol in immature female rats. Steroids. 1991;56(2):97–102. https://doi.org/10.1016/0039-128x(91)90131-e.

Natividad LA, Buczynski MW, Herman MA, Kirson D, Oleata CS, Irimia C, Polis I, Ciccocioppo R, Roberto M, Parsons LH. Constitutive increases in amygdalar corticotropin-releasing factor and fatty acid amide hydrolase drive an anxious phenotype. Biol Psychiatry. 2017;82(7):500–10. https://doi.org/10.1016/j.biopsych.2017.01.005.

Nguyen QH, Wagner EJ. Estrogen differentially modulates the cannabinoid-induced presynaptic inhibition of amino acid neurotransmission in proopiomelanocortin neurons of the arcuate nucleus. Neuroendocrinology. 2006;84(2):123–37. https://doi.org/10.1159/000096996.

Núñez E, Benito C, Pazos MR, Barbachano A, Fajardo O, González S, Tolón RM, Romero J. Cannabinoid CB2 receptors are expressed by perivascular microglial cells in the human brain: an immunohistochemical study. Synapse. 2004;53(4):208–13. https://doi.org/10.1002/syn.20050.

Nyíri G, Cserép C, Szabadits E, MacKie K, Freund TF. CB1 cannabinoid receptors are enriched in the perisynaptic annulus and on preterminal segments of hippocampal GABAergic axons. Neuroscience. 2005;136(3):811–22. https://doi.org/10.1016/j.neuroscience.2005.01.026.

Pantell MS, Silveira PP, de Mendonça Filho EJ, Wing H, Brown EM, Keeton VF, Pokhvisneva I, O'Donnell KJ, Neuhaus J, Hessler D, Meaney MJ, Adler NE, Gottlieb LM. Associations between social adversity and biomarkers of inflammation, stress, and aging in children. Pediatr Res. 2024;95(6):1553–63. https://doi.org/10.1038/s41390-023-02992-6.

Paulus MP, Stein MB, Simmons AN, Risbrough VB, Halter R, Chaplan SR. The effects of FAAH inhibition on the neural basis of anxiety-related processing in healthy male subjects: a randomized clinical trial. Neuropsychopharmacology. 2021;46(5):1011–9. https://doi.org/10.1038/s41386-020-00936-w.

Perini I, Mayo LM, Capusan AJ, Paul ER, Yngve A, Kampe R, Gauffin E, Mazurka R, Ghafouri B, Stensson N, Asratian A, Hamilton JP, Kastbom Å, Gustafsson PA, Heilig M. Resilience to substance use disorder following childhood maltreatment: association with peripheral biomarkers of endocannabinoid function and neural indices of emotion regulation. Mol Psychiatry. 2023;28(6):2563–71. https://doi.org/10.1038/s41380-023-02033-y.

Peters KZ, Naneix F. The role of dopamine and endocannabinoid systems in prefrontal cortex development: adolescence as a critical period. Frontiers in Neural Circuits. 2022;16 https://doi.org/10.3389/fncir.2022.939235.

Raver SM, Haughwout SP, Keller A. Adolescent cannabinoid exposure permanently suppresses cortical oscillations in adult mice. Neuropsychopharmacology. 2013;38(12):2338–47. https://doi.org/10.1038/npp.2013.164.

Reich CG, Iskander AN, Weiss MS. Cannabinoid modulation of chronic mild stress-induced selective enhancement of trace fear conditioning in adolescent rats. Journal of Psychopharmacology (Oxford, England). 2013;27(10):947–55. https://doi.org/10.1177/0269881113499207.

Renard J, Vitalis T, Rame M, Krebs M-O, Lenkei Z, Le Pen G, Jay TM. Chronic cannabinoid exposure during adolescence leads to long-term structural and functional changes in the prefrontal cortex. Eur Neuropsychopharmacol. 2016;26(1):55–64. https://doi.org/10.1016/j.euroneuro.2015.11.005.

Rey AA, Purrio M, Viveros M-P, Lutz B. Biphasic effects of cannabinoids in anxiety responses: CB1 and GABAB receptors in the balance of GABAergic and glutamatergic neurotransmission. Neuropsychopharmacology. 2012;37(12):2624–34. https://doi.org/10.1038/npp.2012.123.

Riebe CJ, Pamplona FA, Kamprath K, Wotjak CT. Fear relief-toward a new conceptual frame work and what endocannabinoids gotta do with it. Neuroscience. 2012;204:159–85. https://doi.org/10.1016/j.neuroscience.2011.11.057.

Robertson JM, Achua JK, Smith JP, Prince MA, Staton CD, Ronan PJ, Summers TR, Summers CH. Anxious behavior induces elevated hippocampal CB2 receptor gene expression. Neuroscience. 2017;352:273–84. https://doi.org/10.1016/j.neuroscience.2017.03.061.

Rodriguez de Fonseca F, Ramos JA, Bonnin A, Fernández-Ruiz JJ. Presence of cannabinoid binding sites in the brain from early postnatal ages. Neuroreport. 1993;4(2):135.

Rodríguez de Fonseca F, Cebeira M, Ramos JA, Martín M, Fernández-Ruiz JJ. Cannabinoid receptors in rat brain areas: sexual differences, fluctuations during estrous cycle and changes after gonadectomy and sex steroid replacement. Life Sci. 1994;54(3):159–70. https://doi.org/10.1016/0024-3205(94)00585-0.

Romeo RD. The metamorphosis of adolescent hormonal stress reactivity: a focus on animal models. Front Neuroendocrinol. 2018;49:43–51. https://doi.org/10.1016/j.yfrne.2017.12.003.

Rubino T, Parolaro D. Long lasting consequences of cannabis exposure in adolescence. Mol Cell Endocrinol. 2008;286(1–2 Suppl 1):S108–13. https://doi.org/10.1016/j.mce.2008.02.003.

Rubino T, Prini P, Piscitelli F, Zamberletti E, Trusel M, Melis M, Sagheddu C, Ligresti A, Tonini R, Di Marzo V, Parolaro D. Adolescent exposure to THC in female rats disrupts developmental changes in the prefrontal cortex. Neurobiol Dis. 2015;73:60–9. https://doi.org/10.1016/j.nbd.2014.09.015.

Ruehle S, Rey AA, Remmers F, Lutz B. The endocannabinoid system in anxiety, fear memory and habituation. Journal of Psychopharmacology (Oxford, England). 2012;26(1):23–39. https://doi.org/10.1177/0269881111408958.

Sales-Carbonell C, Rueda-Orozco PE, Soria-Gómez E, Buzsáki G, Marsicano G, Robbe D. Striatal GABAergic and cortical glutamatergic neurons mediate contrasting effects of cannabinoids on cortical network synchrony. Proc Natl Acad Sci USA. 2013;110(2):719–24. https://doi.org/10.1073/pnas.1217144110.

Schmidt ME, Liebowitz MR, Stein MB, Grunfeld J, Van Hove I, Simmons WK, Van Der Ark P, Palmer JA, Saad ZS, Pemberton DJ, Van Nueten L, Drevets WC. The effects of inhibition of fatty acid amide hydrolase (FAAH) by JNJ-42165279 in social anxiety disorder: a double-blind, randomized, placebo-controlled proof-of-concept study. Neuropsychopharmacology. 2021;46(5):1004–10. https://doi.org/10.1038/s41386-020-00888-1.

Sciolino NR, Zhou W, Hohmann AG. Enhancement of endocannabinoid signaling with JZL184, an inhibitor of the 2-arachidonoylglycerol hydrolyzing enzyme monoacylglycerol lipase, produces anxiolytic effects under conditions of high environmental aversiveness in rats. Pharmacol Res. 2011;64(3):226–34. https://doi.org/10.1016/j.phrs.2011.04.010.

Simone JJ, Green MR, McCormick CM. Endocannabinoid system contributions to sex-specific adolescent neurodevelopment. Prog Neuro-Psychopharmacol Biol Psychiatry. 2022;113:110438. https://doi.org/10.1016/j.pnpbp.2021.110438.

Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc Natl Acad Sci. 2002;99(12):8394–9. https://doi.org/10.1073/pnas.082235799.

Sisk LM, Gee DG. Stress and adolescence: vulnerability and opportunity during a sensitive window of development. Curr Opin Psychol. 2022;44:286–92. https://doi.org/10.1016/j.copsyc.2021.10.005.

Sisk LM, Rapuano KM, Conley MI, Greene AS, Horien C, Rosenberg MD, Scheinost D, Constable RT, Glatt CE, Casey BJ, Gee DG. Genetic variation in endocannabinoid signaling is associated with differential network-level functional connectivity in youth. J Neurosci Res. 2022;100(3):731–43. https://doi.org/10.1002/jnr.24946.

Spohrs J, Prost M, Ulrich M, Plener PL, Bindila L, Abler B. Endocannabinoid system reactivity during stress processing in healthy humans. Biol Psychol. 2022a;169:108281. https://doi.org/10.1016/j.biopsycho.2022.108281.

Spohrs J, Ulrich M, Grön G, Plener PL, Abler B. FAAH polymorphism (rs324420) modulates extinction recall in healthy humans: an fMRI study. Eur Arch Psychiatry Clin Neurosci. 2022b;272(8):1495–504. https://doi.org/10.1007/s00406-021-01367-4.

Suárez J, Llorente R, Romero-Zerbo SY, Mateos B, Bermúdez-Silva FJ, de Fonseca FR, Viveros M-P. Early maternal deprivation induces gender-dependent changes on the expression of hippocampal CB1 and CB2 cannabinoid receptors of neonatal rats. Hippocampus. 2009;19(7):623–32. https://doi.org/10.1002/hipo.20537.

Suárez J, Rivera P, Llorente R, Romero-Zerbo SY, Bermúdez-Silva FJ, de Fonseca FR, Viveros M-P. Early maternal deprivation induces changes on the expression of 2-AG biosynthesis and degradation enzymes in neonatal rat hippocampus. Brain Res. 2010;1349:162–73. https://doi.org/10.1016/j.brainres.2010.06.042.

Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun. 1995;215(1):89–97. https://doi.org/10.1006/bbrc.1995.2437.

Sumislawski JJ, Ramikie TS, Patel S. Reversible gating of endocannabinoid plasticity in the amygdala by chronic stress: a potential role for Monoacylglycerol lipase inhibition in the prevention of stress-induced behavioral adaptation. Neuropsychopharmacology. 2011;36(13):2750–61. https://doi.org/10.1038/npp.2011.166.

Svízenská I, Dubový P, Sulcová A. Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures—a short review. Pharmacol Biochem Behav. 2008;90(4):501–11. https://doi.org/10.1016/j.pbb.2008.05.010.

Tevosian M, Todorov H, Lomazzo E, Bindila L, Ueda N, Bassetti D, Warm D, Kirischuk S, Luhmann HJ, Gerber S, Lutz B. NAPE-PLD deletion in stress-TRAPed neurons results in an anxiogenic phenotype. Transl Psychiatry. 2023;13(1):152. https://doi.org/10.1038/s41398-023-02448-9.

Tottenham N. Early adversity and the neotenous human brain. Biol Psychiatry. 2020;87(4):350–8. https://doi.org/10.1016/j.biopsych.2019.06.018.

Tsou K, Brown S, Sañudo-Peña MC, Mackie K, Walker JM. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience. 1998;83(2):393–411. https://doi.org/10.1016/S0306-4522(97)00436-3.

Tsou K, Mackie K, Sañudo-Peña MC, Walker JM. Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. Neuroscience. 1999;93(3):969–75. https://doi.org/10.1016/S0306-4522(99)00086-X.

Tsutahara NM, Weems YS, Arreguin-Arevalo JA, Nett TM, LaPorte ME, Uchida J, Pang J, McBride T, Randel RD, Weems CW. Effects of endocannabinoid 1 and 2 (CB1; CB2) receptor agonists on luteal weight, circulating progesterone, luteal mRNA for luteinizing hormone (LH) receptors, and luteal unoccupied and occupied receptors for LH in vivo in ewes. Prostaglandins Other Lipid Mediat. 2011;94(1–2):17–24. https://doi.org/10.1016/j.prostaglandins.2010.11.002.

Vangopoulou C, Bourmpoula MT, Koupourtidou C, Giompres P, Stamatakis A, Kouvelas ED, Mitsacos A. Effects of an early life experience on rat brain cannabinoid receptors in adolescence and adulthood. IBRO Reports. 2018;5:1–9. https://doi.org/10.1016/j.ibror.2018.05.002.

Vecchiarelli HA, Morena M, Lee TTY, Nastase AS, Aukema RJ, Leitl KD, Gray JM, Petrie GN, Tellez-Monnery KJ, Hill MN. Sex and stressor modality influence acute stress-induced dynamic changes in corticolimbic endocannabinoid levels in adult Sprague Dawley rats. Neurobiology of Stress. 2022;20:100470. https://doi.org/10.1016/j.ynstr.2022.100470.

Walther A, Kirschbaum C, Wehrli S, Rothe N, Penz M, Wekenborg M, Gao W. Depressive symptoms are negatively associated with hair N-arachidonoylethanolamine (anandamide) levels: a cross-lagged panel analysis of four annual assessment waves examining hair endocannabinoids and cortisol. Prog Neuro-Psychopharmacol Biol Psychiatry. 2023;121:110658. https://doi.org/10.1016/j.pnpbp.2022.110658.

Wamsteeker JI, Kuzmiski JB, Bains JS. Repeated stress impairs endocannabinoid signaling in the paraventricular nucleus of the hypothalamus. J Neurosci. 2010;30(33):11188–96. https://doi.org/10.1523/JNEUROSCI.1046-10.2010.

Wenger T, Ledent C, Csernus V, Gerendai I. The central cannabinoid receptor inactivation suppresses endocrine reproductive functions. Biochem Biophys Res Commun. 2001;284(2):363–8. https://doi.org/10.1006/bbrc.2001.4977.

Wenger T, Gerendai I, Fezza F, González S, Bisogno T, Fernandez-Ruiz J, Di Marzo V. The hypothalamic levels of the endocannabinoid, anandamide, peak immediately before the onset of puberty in female rats. Life Sci. 2002;70(12):1407. https://doi.org/10.1016/s0024-3205(01)01516-8.

Williams E-J, Walsh FS, Doherty P. The FGF receptor uses the endocannabinoid signaling system to couple to an axonal growth response. J Cell Biol. 2003;160(4):481–6. https://doi.org/10.1083/jcb.200210164.

Worley NB, Varela JA, Gaillardetz GP, Hill MN, Christianson JP. Monoacylglycerol lipase alpha inhibition alters prefrontal cortex excitability and blunts the consequences of traumatic stress in rat. Neuropharmacology. 2020;166:107964. https://doi.org/10.1016/j.neuropharm.2020.107964.

Yasuda H, Huang Y, Tsumoto T. Regulation of excitability and plasticity by endocannabinoids and PKA in developing hippocampus. Proc Natl Acad Sci. 2008;105(8):3106–11. https://doi.org/10.1073/pnas.0708349105.

Zabik NL, Iadipaolo AS, Marusak HA, Peters C, Burghardt K, Rabinak CA. A common genetic variant in fatty acid amide hydrolase is linked to alterations in fear extinction neural circuitry in a racially diverse, nonclinical sample of adults. J Neurosci Res. 2022;100(3):744–61. https://doi.org/10.1002/jnr.24860.

Zamberletti E, Beggiato S, Steardo L, Prini P, Antonelli T, Ferraro L, Rubino T, Parolaro D. Alterations of prefrontal cortex GABAergic transmission in the complex psychotic-like phenotype induced by adolescent delta-9-tetrahydrocannabinol exposure in rats. Neurobiol Dis. 2014;63:35–47. https://doi.org/10.1016/j.nbd.2013.10.028.

Zhang H-Y, Gao M, Liu Q-R, Bi G-H, Li X, Yang H-J, Gardner EL, Wu J, Xi Z-X. Cannabinoid CB2 receptors modulate midbrain dopamine neuronal activity and dopamine-related behavior in mice. Proc Natl Acad Sci USA. 2014;111(46):E5007–15. https://doi.org/10.1073/pnas.1413210111.

Zubedat S, Akirav I. The involvement of cannabinoids and mTOR in the reconsolidation of an emotional memory in the hippocampal–amygdala–insular circuit. Eur Neuropsychopharmacol. 2017;27(4):336–49. https://doi.org/10.1016/j.euroneuro.2017.01.011.

Zurolo E, Iyer AM, Spliet WGM, Van Rijen PC, Troost D, Gorter JA, Aronica E. CB1 and CB2 cannabinoid receptor expression during development and in epileptogenic developmental pathologies. Neuroscience. 2010;170(1):28–41. https://doi.org/10.1016/j.neuroscience.2010.07.004.

Part II
Prenatal and Early Life Effects

Chapter 6
Behavioral Effects of Prenatal Cannabinoid Exposure: Does Route of Administration Matter?

Halle V. Weimar, Amanda M. Brown, and Ryan J. McLaughlin

1 Prenatal Cannabis Exposure Is a Growing Public Health Concern

Cannabis is commonly used by pregnant women in the United States, with an estimated prevalence of use around 4–7% depending on the population studied (Brown et al. 2017; Ko et al. 2018; Volkow et al. 2019; Young-Wolff et al. 2017). Cannabis used among young and unmarried women during pregnancy is even higher, with estimates as high as 22% in this demographic (Young-Wolff et al. 2017). According to the US 2022 National Survey on Drug Use and Health, rates of cannabis use among pregnant women are rising at an alarming pace, with past-year cannabis use increasing from 12.6% in 2002 to 21.9% in 2022. A major concern is that these rates are likely to increase further given favorable attitudes among young people toward cannabis use for both recreational and medicinal purposes (Wen et al. 2019). Furthermore, the greater availability of cannabis following legalization also drives higher rates of use among pregnant women. In survey studies, approximately twice as many women reported using cannabis during pregnancy in states permissive of

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recreational cannabis use compared to women in states with only medical cannabis legislation (Skelton et al. 2020). In addition, the COVID-19 pandemic has likely contributed to a dramatic uptick in cannabis use during pregnancy, with cannabis use among pregnant women increasing 20.6% in the first year of the pandemic relative to the prior year (Young-Wolff et al. 2021). Finally, recreational cannabis dispensaries may be contributing to the belief that cannabis use during pregnancy poses little harm to the developing fetus. For example, among Colorado cannabis dispensaries, 36% of dispensary workers said they would recommend cannabis use for treating symptoms of pregnancy, claiming it was safe to use (Dickson et al. 2018). In addition, survey studies indicate that nearly 70% of pregnant women agreed that using cannabis once or twice a week posed little to no risk to the developing fetus (Ko et al. 2015). Thus, as the legal cannabis market continues to infiltrate the United States, the impetus for understanding the risks associated with cannabis use during pregnancy has never been greater.

Our current understanding of the long-term effects of prenatal cannabis exposure remains limited despite an extensive body of research involving both human populations and animal models. This is partly because most human studies include small sample sizes, lack replication of outcome measures across cohorts, and rely on retrospective self-report to quantify maternal cannabis use (Mulligan and Hamre 2023). In addition, the amount of Δ^9-THC in cannabis has increased significantly over the past two decades (ElSohly et al. 2016; Potter et al. 2018), and thus the effects of prenatal cannabis exposure may be different in today's offspring than in previous generations. There are also other confounding factors, such as co-use of other drugs (Crume et al. 2018; Ko et al. 2018; Metz et al. 2017), lack of prenatal care (Berenson et al. 1996; Ostrea et al. 1997), and maternal mental health issues (Goodwin et al. 2020; Young-Wolff et al. 2020), that human studies can attempt to statistically, but not always experimentally, control for. On the other hand, preclinical models of prenatal cannabis exposure provide tight internal control over these extraneous factors and offer opportunities to causally investigate associations that have been observed in human populations. However, there is a considerable degree of heterogeneity in experimental parameters across studies and until recently, most preclinical studies have employed forced injections of synthetic cannabinoids or isolated cannabis constituents that may not readily translate to human populations (McLaughlin 2018). This has created a major gap in knowledge that is only now being addressed through the application of oral and intrapulmonary routes of administration that more accurately model human cannabis consumption.

The goal of this chapter is to provide a broad overview of current models of prenatal cannabis exposure, outline their relative strengths and weaknesses, and briefly summarize our current understanding of the long-term behavioral effects of prenatal cannabis exposure according to these different routes of administration. Ultimately, it will be important for the field to reconcile disparate findings in studies using different regimens of cannabinoid exposure to better determine whether data generated with traditional approaches are consistent with data derived from more translationally relevant models.

2 Human Studies of Prenatal Cannabis Exposure: What Do we Know?

As noted above, there is an extensive body of literature examining associations between maternal cannabis use and long-term behavioral outcomes in humans, and there are already excellent reviews (Mulligan and Hamre 2023) and meta-analyses (Gunn et al. 2016; Sorkhou et al. 2023) published elsewhere on this topic. Thus, our intention is not to provide an exhaustive overview of the long-term behavioral effects of prenatal cannabis exposure in humans, but rather to briefly touch on findings from longitudinal studies that provide context for the preclinical studies described later.

The gold standard for assessing the long-term effects of prenatal cannabis exposure in humans involves the use of longitudinal designs that can distinguish the impact of many variables that affect cognitive development in children who were prenatally exposed to cannabis. Currently, there are three large-scale, comprehensive longitudinal studies in which the impacts of maternal cannabis use on the offspring have been examined: the Ottawa Prenatal Prospective Study (OPPS), the Maternal Health Practices and Child Development (MHPCD) study, and the Generation R study. The OPPS group primarily consists of low risk, middle-class white Canadians (n = 49 offspring) that were tracked annually from birth to age 6, and less frequently thereafter up to 22 years of age (Fried 2002; Fried et al. 1998; Porath and Fried 2005; Smith et al. 2006). In contrast, the higher-risk MHPCD cohort consists of a predominantly low socioeconomic status and mixed ethnicity population of individuals from Pittsburgh, PA (n = 763 mother–infant pairs) that were assessed before, during, and after pregnancy and every 2–3 years thereafter until age 22 (Day and Richardson 1991; Goldschmidt et al. 2008). Finally, the Generation R study examines a lower risk cohort from Rotterdam, the Netherlands with a higher socioeconomic status (n = 9778 pregnant women) (El Marroun et al. 2009; Jaddoe et al. 2006). There are several more recent large-scale longitudinal studies such as the Adolescent Brain Cognitive Development (ABCD) study (Cioffredi et al. 2022), the Norwegian Mother and Child Cohort (MoBa) study, and the Lifestyle and Early Achievement in Families (LEAFs) study (Klebanoff et al. 2020; Murnan et al. 2021). However, these cohorts are still in the early stages of data collection and therefore have yet to produce significant data on the topic.

Although evidence across cohorts is generally mixed, both OPPS and MHPCD cohorts have shown that at the age of 6, cannabis-exposed children scored lower on measures of memory and verbal skill (Fried et al. 1992; Leech et al. 1999). Between the ages of 9 and 12, cannabis-exposed children in the OPPS cohort displayed poorer abstract thought and visual reasoning, visual problem solving, and impulse control compared to healthy controls (Fried et al. 1998; Fried and Watkinson 2001). At age 10, children in the MHPCD group showed more instances of hyperactivity, impulsivity, and inattention, as measured by the Swanson, Noland and Pelham assessment, the Child Behavior Checklist, and the Teacher's Report form (Goldschmidt et al. 2000). Both the OPPS and MHPCD cohorts have revealed

problems with impulsivity later in life (Fried et al. 1998; Goldschmidt et al. 2000). Finally, the Generation R cohort also showed significant increases in psychotic-like behaviors in 10-year-old who were prenatally exposed to cannabis (Bolhuis et al. 2018). It is worth noting that impaired attention, increased impulsivity and psychotic-like experiences have all been linked to dysfunctional activity within the prefrontal cortex (PFC) (see reviews from Kim and Lee 2011; Smucny et al. 2022), which critically coordinates executive functioning, working memory, and decision-making processes. Accordingly, an fMRI study in young adults exposed to cannabis in utero has shown increased activity in the bilateral PFC and right premotor cortex during a response inhibition task (Smith et al. 2004). Thus, effects of prenatal cannabis exposure on developmental programming of the PFC may represent a mechanism by which enduring effects on attention and impulsivity manifest. Despite this knowledge, the correlative nature of human longitudinal data leaves many unanswered questions regarding the direct causal effects of prenatal cannabis exposure. Animal models can help to answer these questions.

3 Considerations for Modeling Prenatal Cannabis Exposure

Preclinical animal models provide valuable opportunities to systematically examine long-term effects of prenatal cannabis exposure without many of the problems inherent to human studies. The overwhelming majority of studies in the field use rats or mice as the species of choice, taking advantage of their shorter gestational period and faster developmental progression to adulthood. However, there is considerable diversity in experimental models of prenatal cannabis exposure that vary according to several factors, including the drug used (i.e., synthetic cannabinoids vs. isolated cannabis constituents like Δ^9-THC vs. whole-plant cannabis products), the exposure regimen (i.e., dose, frequency, gestational exposure period), and route of administration (i.e., injection vs. oral vs. vapor/smoke delivery). This inconsistency is often cited as a likely explanation for equivocal findings that don't necessarily align with the extant human or rodent literature.

In particular, much has been made of the importance of using translationally relevant administration routes when attempting to extrapolate data from animal studies to human cannabis users. It is well known that the most common routes of cannabis administration in humans are via intrapulmonary and oral delivery methods. However, the majority of preclinical studies administer cannabinoids via forced injections to pregnant dams, which does not accurately capture the pharmacokinetics and metabolism of Δ^9-THC following more traditional routes of administration. For instance, studies comparing the pharmacokinetics of Δ^9-THC in the blood and brain following injection vs. vapor delivery methods have shown that a dose that produces similar peak plasma concentrations of Δ^9-THC results in vastly different concentrations of Δ^9-THC and its metabolites in the brain over time (Baglot et al. 2021). Specifically, higher initial brain THC concentrations were observed following vapor delivery, whereas injection resulted in dramatically higher 11-OH-THC

concentration in blood and brain that increased over time (Baglot et al. 2021). This is important because the 11-OH-THC metabolite is a potent agonist of the type-1 cannabinoid receptor (CB1R) that has been estimated to have signaling efficacy that is 2–7× greater than Δ^9-THC (Grotenhermen 2003; Perez-Reyes et al. 1972). The increased accumulation of Δ^9-THC and 11-OH-THC in the brain following injection is thus likely to produce different physiological, psychological, and behavioral effects by providing more robust and sustained CB1R activation relative to the rapid peaks and clearance observed following vapor exposure (Baglot et al. 2021).

Studies comparing maternal–fetal transmission of Δ^9-THC and its metabolites following injection vs. inhalation exposure in pregnant rat dams have revealed similar findings. Whereas both administration routes result in accumulation of Δ^9-THC in the placenta, repeated Δ^9-THC vapor delivery resulted in fetal brain Δ^9-THC levels that were only about 30% of those seen in maternal blood (Baglot et al. 2022). Conversely, repeated Δ^9-THC injection led to roughly equivalent Δ^9-THC levels in maternal blood and fetal brain (Baglot et al. 2022). In addition, accumulation of 11-OH-THC in the fetal brain differed drastically between the two routes of administration. Whereas vapor exposure resulted in a ~155% increase in fetal brain 11-OH-THC concentration compared to maternal blood, injection resulted in a staggering ~910% increase (Baglot et al. 2022). Importantly, the doses of injected vs. vaporized Δ^9-THC delivered in this study were specifically chosen to reflect the typical concentrations used in prior prenatal studies, thus providing a means to interpret these differences in the context of the literature. Consequently, it is conceivable that data generated using models of prenatal cannabis exposure involving injection approaches may not readily translate to human populations since the most common routes of administration do not typically produce fetal brain and placental Δ^9-THC and 11-OH-THC concentrations in this range.

In addition to differences in the pharmacokinetics of Δ^9-THC following different routes of administration, the behavioral effects of Δ^9-THC also differ according to whether the drug is injected vs. vaporized. Studies from Manwell and Mallet have shown that rats receiving vaporized Δ^9-THC show facilitated extinction of conditioned aversion compared to vehicle-treated rats, whereas rats injected with Δ^9-THC showed impaired extinction (Manwell & Mallet 2015). In addition, vaporized Δ^9-THC delivery was shown to stimulate feeding to a much greater degree than parenteral administration (Manwell et al. 2014a). Importantly, vaporized Δ^9-THC elicited a conditioned place preference, whereas Δ^9-THC injections induced conditioned place avoidance (Manwell et al. 2014b). More recently, the Weerts Lab also examined the rewarding properties of vaporized Δ^9-THC and found dose- and sex-dependent effects on place preference in rats (Moore et al. 2024). Thus, route of administration can fundamentally influence cannabinoid preference, which may be an important consideration when pregnant dams are receiving daily noncontingent cannabinoid injections.

Yet another important consideration is the specific drug utilized in prenatal cannabinoid studies. While use of selective CB1R agonists or isolated cannabis constituents such as Δ^9-THC offer many advantages for probing the role of altered CB1R signaling in neurodevelopmental outcomes in a selective and controlled

manner, questions remain over whether these compounds have the same effects as whole-plant cannabis products. Importantly, synthetic cannabinoids such as WIN 55,212-2 (WIN) are potent full CB1R agonists (Pertwee 2008) that are known to recruit fundamentally different intracellular signaling pathways compared to plant-derived Δ^9-THC (Laprairie et al. 2014, 2016). Also, it has been estimated that there are >100 other phytocannabinoids present in cannabis, in addition to a host of other bioactive molecules including terpenes, flavonoids, and polyphenols that may be responsible for subtly different pharmacological and behavioral effects. Although the entourage effect hypothesis remains controversial, the interaction between these cannabinoids and other molecules may be important for determining some of its effects (or lack thereof) (Simei et al. 2024; Russo 2011).

Finally, it is common for prenatal studies to use noncontingent drug exposure models that avoid the variability introduced by individual differences in drug self-administration. However, it is well known that humans volitionally consume drugs resulting in relevant expectancy effects that can alter psychological appraisal of drug intoxication. Noncontingent drug exposure can be a repeated stressor for dams during pregnancy, particularly if the drug employed interacts with stress-related neurobiological systems like cannabinoids do (Glodosky et al. 2021). As a result, forced vs. volitional drug delivery may recruit fundamentally different neural circuits that could have important implications for offspring brain and behavior (Ahmed et al. 2020; Moolten and Kornetsky 1990). This creates a translational gap that could lead to a failure of preclinical findings to extend to human populations.

Taken together, it will be important for future studies to revisit past questions in the field and determine whether the effects observed following repeated injections are recapitulated in models that more accurately resemble human cannabis use.

4 Long-Term Behavioral Effects of Prenatal Cannabis Exposure in Rodent Models

In recent years, there has been a considerable uptick in the number of preclinical investigations into the long-term behavioral consequences of prenatal cannabis exposure in offspring. The vast majority of earlier studies employed peripheral injections or oral delivery of Δ^9-THC or synthetic CB1R agonists such as WIN55,212-2 (WIN), which allows for consistent, sustained levels of cannabinoid exposure. Intravenous delivery routes have also been used in a few prior studies, and more recently, vapor or smoke delivery methods have been adopted that may offer greater translational relevance, but often at the expense of the tight experimental control afforded by other methods. In addition, there is considerable variation with respect to the dose, timing of drug exposure, and age of behavioral testing, which limits our ability to determine which variables critically contribute to different effects across studies. Generally speaking, the behavioral domains investigated in cannabinoid-exposed offspring fall into four categories: (1) sensorimotor function,

(2) cognitive processes, (3) social and emotional behavior, and (4) drug reward. In the sections below, we summarize the main findings to date within each of these categories and attempt to compare and contrast findings according to age (i.e., adolescence vs. adulthood) and route of administration.

4.1 Sensorimotor Function

4.1.1 Injection Studies

Prenatal exposure to cannabinoids, particularly Δ^9-THC and WIN, has age- and route of administration-dependent effects on motor development and sensorimotor coordination in offspring. For instance, subcutaneous injection of WIN (0.5 mg/kg, gestational day (GD) 5-20) led to significant motor impairments in offspring, observed through delayed homing responses in early development and coordination deficits in adulthood (Antonelli et al. 2005), whereas other studies using the same exposure regimen failed to reveal differences in locomotor activity in adulthood (Bara et al. 2018). Conversely, a dose of 2 mg/kg Δ^9-THC administered subcutaneously during the same gestational period resulted in increased locomotor activity during adolescence (Brancato et al. 2020). Deficits in sensorimotor gating have been observed in male, but not female, adolescent offspring following prenatal Δ^9-THC exposure (2 mg/kg, sc, GD5-20; Traccis et al. 2021), but these deficits were only evident following a priming challenge with either Δ^9-THC (Frau et al. 2019) or stress (Sagheddu et al. 2021). Interestingly, these effects coincide with heightened sensitivity to dopamine D2 receptor agonists (Sagheddu et al. 2021), which, taken together, could indicate a potential vulnerability to psychotic-like behaviors in Δ^9-THC-exposed male offspring.

In studies that have employed intraperitoneal injection approaches, similar sensorimotor impairments have been observed in Δ^9-THC-exposed offspring. For instance, 3 mg/kg Δ^9-THC injections from GD12.5–GD16.5 led to impairments in a skilled reaching test later in life, which was not observed in Δ^9-THC-exposed CB1R knockout mice (de Salas-Quiroga et al. 2015). In a more recent study, the same dose of Δ^9-THC injected together with 30 mg/kg cannabidiol (CBD) daily from GD7-22 resulted in sensorimotor gating deficits in the prepulse inhibition test in adolescent male (but not female) offspring, but no differences in locomotor exploration (DeVuono et al. 2024). Importantly, these effects were not due to general differences in startle amplitudes since no differences were observed between prenatal treatments on this measure (DeVuono et al. 2024). Thus, intraperitoneal injection studies are largely in agreement with data using subcutaneous routes, with a potentially greater vulnerability to sensorimotor gating deficits in male Δ^9-THC-exposed offspring.

4.1.2 Oral Administration Studies

The second most common route of delivery in prenatal cannabinoid studies is via oral administration. Early studies from the 1990s have demonstrated either no change (Navarro et al. 1994a) or increased locomotor activity following oral Δ^9-THC administration that is largely dependent on timing of exposure and when during development activity is being measured (Navarro et al. 1994b). Specifically, perinatal exposure to Δ^9-THC (5 mg/kg, daily from GD5 until the last day of weaning) transiently increased both rearing and locomotor activity in both sexes at preweanling ages (postnatal day (PND) 15 and PND20), but these effects disappeared at postweanling ages when Δ^9-THC administration was stopped. Interestingly, this hyperlocomotor phenotype reemerged in familiar environments when tested in adulthood, but female adult offspring exhibited decreased locomotor activity in a socio-sexual approach test conducted in novel environmental conditions (Navarro et al. 1994b). This same group subsequently showed that the same perinatal exposure Δ^9-THC regimen produced long-term increases in rearing, grooming, and sniffing behavior specifically in female offspring (Rubio et al. 1995). Thus, female offspring seem to be more likely to display hyperlocomotion following prolonged perinatal Δ^9-THC administration.

However, a more recent study examining effects of maternal oral Δ^9-THC exposure (5 mg/kg/day) together with vaporized nicotine have shown that, similar to the injection studies described above, prenatal co-exposure results in significant sensorimotor gating deficits in the prepulse inhibition test specifically in male offspring (Lallai et al. 2022). Therefore, sex differences in the sensorimotor impacts of prenatal/perinatal cannabinoid exposure largely depend on the specific domain of sensorimotor function being evaluated, with females showing general locomotor alterations and males showing deficits in sensorimotor gating.

4.1.3 Vapor Administration Studies

In more recent years, several laboratories have adopted vaporized cannabinoid administration approaches to more accurately model the route of delivery that is most common among human cannabis users. As noted above, this is an important translational consideration given the marked differences in central accumulation of Δ^9-THC and its bioactive metabolites in injection vs. vapor delivery methods (Baglot et al. 2021). Recent studies from the Breit lab have revealed that prenatal Δ^9-THC e-cigarette exposure (100 mg/ml, six 6-s puffs over 40 min daily from GD5-20) significantly delayed sensorimotor development early in life and impaired motor coordination later in early adolescence in both sexes, whereas combined exposure to Δ^9-THC and alcohol vapor did not produce additive effects in this domain but did enhance locomotor activity specifically in male offspring (Breit et al. 2022). Moreover, combined exposure to vaporized Δ^9-THC (same exposure parameters as above) together with nicotine significantly delays sensorimotor development early in life, although this effect was not observed when either drug was

administered in isolation (Hussain et al. 2022). Notably, motor coordination later in life was impaired by either Δ^9-THC or nicotine exposure delivered alone, whereas this impairment was exacerbated in females receiving co-exposure of both drugs (Hussain et al. 2022). Finally, rats prenatally exposed to vaporized Δ^9-THC (10 mg/ml or 40 mg/ml daily from GD2 until birth) exhibited dose-dependent alterations in exploratory activity, such that offspring exposed to the lower dose of Δ^9-THC had lower dark cycle activity compared to air-exposed offspring (Roeder et al. 2024). However, both Δ^9-THC-exposed groups exhibited a general increase in locomotor activity in an open field test (Roeder et al. 2024), which aligns with the hyperlocomotor phenotype often observed in the studies described above. Altogether, these data suggest that there are sex differences in the sensorimotor effects of Δ^9-THC exposure that depend on the timing of both drug exposure and testing, the route of administration employed, and the specific sensorimotor endpoint being investigated.

4.2 Cognitive Processes

4.2.1 Injection Studies

Preclinical studies have indicated long-term impairments in different domains of cognition in offspring prenatally exposed to cannabinoids. Early studies revealed learning impairments in male WIN-exposed offspring (0.5 mg/kg daily from GD5-20) during the neonatal period such that they fail to quickly return to the nest zone on consecutive days of homing tests like their control counterparts (Antonelli et al. 2005). Learning impairments in an active avoidance task were also evident in WIN-exposed offspring at PND80, and it was argued that this is not likely due to general hyperlocomotor effects since locomotor differences were not observed between groups at this time point (Antonelli et al. 2005). Interestingly, the same prenatal WIN exposure regimen has also been shown to disrupt memory retention in a passive avoidance task in 40- and 80-day old offspring (Mereu et al. 2003). Similarly, offspring of dams that had received intravenous Δ^9-THC delivery (0.15 mg/kg from GD1 to 21) showed impaired consolidation in a passive avoidance task, while males tested in an active avoidance task made significantly more reversal errors compared to vehicle-exposed offspring (Silva et al. 2012).

There is a strong body of evidence indicating cannabinoid-induced impairments in novel object recognition task performance in male offspring following prenatal exposure to WIN (de Salas-Quiroga et al. 2020), Δ^9-THC via intraperitoneal injections (DeVuono et al. 2024), or early-life exposure to the potent CB1R agonist CP55,940 (O'Shea et al. 2006). Moreover, neonatal Δ^9-THC exposure (5 mg/kg) from PND4–14 produces similar working memory-related impairments in a delayed alternation double-Y maze task in male offspring tested in young adulthood (O'Shea and Mallet 2005). Although the studies noted above failed to examine working memory performance in female offspring, these data nonetheless suggest that male

offspring are at risk of developing working memory-related impairments following prenatal and early-life cannabinoid exposure.

Dysfunctional hippocampal-dependent contextual fear memory and spatial learning and memory have also been observed in male offspring exposed to Δ^9-THC during gestation. Specifically, Pinky et al. (2023) implanted osmotic minipumps loaded with WIN (2 mg/kg/day) into pregnant dams from GD3 until PND2. They found that male offspring exhibited a significant decrease in time spent freezing during a contextual fear retention test and showed impaired acquisition of spatial reference memory in the Morris Water Maze when compared to control offspring (Pinky et al. 2023). Although female offspring were not tested in this study either, the data nonetheless support the extant literature indicating greater vulnerability to cannabinoid-induced cognitive impairment.

It should be noted that not all injection studies consistently show cognitive deficits in cannabinoid-exposed offspring. For instance, temporal order memory and novel object recognition were spared in adult offspring exposed to this identical WIN exposure regimen (Bara et al. 2018; Manduca et al. 2020). Similarly, 2 mg/kg Δ^9-THC administered subcutaneously to pregnant dams did not result in neutral declarative memory deficits in offspring tested during adolescence (Brancato et al. 2020). These discrepancies may be due to the species used (mouse vs. rat), timing of exposure, and/or drug employed.

4.2.2 Oral Administration Studies

Studies using oral delivery methods are largely in agreement with data from injection studies. Perinatal oral administration of Δ^9-THC (5 mg/ml/kg, GD15-PND9) impaired novel object recognition in exposed male offspring and produced a phenotype that was similar to offspring that were exposed to methylazoxymethanol (MAM) during the prenatal period (Drazanova et al. 2019). Effects of perinatal Δ^9-THC on novel object recognition task performance in male rats have been replicated in a more recent study using this same exposure regimen, and interestingly, these effects were prevented by postnatal CBD administration (Di Bartolomeo et al. 2021). Given the consistency of this effect across these studies, it is perhaps not surprising that administering oral Δ^9-THC at a higher dose (10 mg/kg/12 h) and for a longer duration (GD5-PND21) also significantly impairs novel object recognition task performance in mice (Navarro et al. 2024). Perhaps more importantly however, this is one of the few studies that reliably demonstrated this effect in both male and female offspring. Finally, in the context of emotional memory, male rat offspring exposed to the 5 mg/kg oral Δ^9-THC exposure regimen from above displayed shorter latencies to exhibit avoidance in an inhibitory avoidance task when tested in adulthood, which is suggestive of impaired consolidation and/or retrieval of emotionally salient memories (Campolongo et al. 2007). Altogether, these findings using oral Δ^9-THC exposure regimens strongly align with findings from injection studies, though the early bias in the field to focus exclusively on male offspring has

led to an incomplete understanding of the domains of memory that may be negatively impacted by prenatal cannabinoid exposure in females.

4.2.3 Vapor/Smoke Administration Studies

To date, only three studies have used cannabinoid vapor or smoke delivery in pregnant dams to explore long-term cognitive impacts, but results are generally consistent with studies using more traditional routes of administration. For instance, Penman et al. (2024) have recently shown that vaporized exposure to a low dose of Δ^9-THC (10 mg daily) from GD2 until birth leads to decreased object exploration in the novel object recognition and object-based attention tests in male and female offspring during adolescence, which suggests altered attention and/or working memory performance. Interestingly, no significant effects on Morris Water Maze performance were observed, suggesting that hippocampal-dependent spatial learning and memory remained generally unaffected (Penman et al. 2024). Our laboratory has shown that prenatal cannabis vapor exposure (50 or 400 mg/ml cannabis extract, 2× daily during mating and gestation) dose-dependently increases the number of errors committed in an instrumental-based attentional set-shifting task wherein male and female rats were trained to acquire, and then subsequently shift, lever-pressing strategies to receive sucrose reinforcement (Weimar et al. 2020). Given the attentional component to this task and the task used by Penman et al. (2024), these data together would suggest that the cognitive effects of prenatal cannabinoid exposure are most evident during working memory and attention-related tasks that require PFC engagement.

Although these studies collectively tell a consistent story, there are exceptions to the conclusions drawn above. Using a model of repeated exposure to high Δ^9-THC-containing cannabis smoke during pregnancy, Sandini et al. (2023) found that cannabis-exposed offspring performed unexpectedly better on visual pairwise discrimination and reversal learning tasks conducted in touchscreen-equipped operant conditioning chambers. In this study, combustion of whole-plant cannabis (19.51% Δ^9-THC) was conducted over a 14-min period, with continuous exposure to cannabis smoke during that time, and this occurred once daily from GD6-20. Importantly, control dams were exposed to the same apparatus but did not receive any smoke exposure, and significantly more smoke-induced defecation was observed in the cannabis group, which suggests that these dams may have been under stress that could have led to different effects in the offspring (Sandini et al. 2023). Accordingly, our data from Weimar et al. (2020) also only observed significant differences in attentional set-shifting between cannabis- and air-exposed offspring; neither group performed significantly different from a vehicle group exposed to a 4:1 propylene glycol:vegetable glycerol (PG/VG) blend. Thus, these data underscore the importance of including appropriate air- and vehicle-exposed control groups to avoid the unintended effects of maternal stress due to noncontingent cannabis vapor or smoke exposure.

4.3 Social and Emotional Behavior

4.3.1 Injection Studies

Arguably the most well-studied behavioral consequences of early-life cannabinoid exposure are in the domain of social behavior. Evidence from injection studies clearly points to deleterious effects of prenatal/perinatal cannabinoid exposure on social investigation, with sex differences being observed in some (Bara et al. 2018; DeVuono et al. 2024; O'Shea et al. 2006; Vargish et al. 2017) but not other injection studies (Scheyer et al. 2020a, b). This may be due to differences in the timing of drug exposure (prenatal vs. postnatal) as well as the lack of investigation into potential sex differences in these studies, with Bara et al. (2018) and DeVuono et al. (2024) being the exceptions. Interestingly, sniffing and playing behaviors were most impaired in the study from Bara and colleagues, while the frequency of attack behaviors did not differ between groups (Bara et al. 2018). In the study by DeVuono et al. (2024), deficits in social recognition during adolescence were observed in both male and female Δ^9-THC-exposed offspring, but effects in females were only apparent when Δ^9-THC was co-administered with 30 mg/kg CBD. On the other hand, conflicting results have been observed when dams receive twice daily subcutaneous injections of 2 mg/kg Δ^9-THC from GD1-PND10. Specifically, Newsom and Kelly (2008) showed that male Δ^9-THC-exposed adult offspring unexpectedly spent more time investigating a novel conspecific compared to vehicle-injected offspring (females were not tested). The reason for this discrepancy could be related to the higher amount of Δ^9-THC exposure in dams from this study (2 mg/kg, sc, twice daily from GD1-PND10). Alternatively, postnatal Δ^9-THC (2 mg/kg/day) or WIN (0.5 mg/kg/day) exposure via breastmilk from PND1-10 similarly augments social exploration in exposed adult offspring, but this increase in social exploration comes at the expense of social discrimination (Scheyer et al. 2020a). Thus, it may be that the effects of early-life cannabinoid exposure are specific to different aspects of social behavior.

Several studies have also examined anxiety-like behavior in cannabinoid-exposed offspring across the lifespan. In early life, the frequency of ultrasonic vocalizations (USVs) emitted in response to brief separation from the litter is significantly decreased following prenatal WIN exposure in male offspring (0.5 mg/kg sc, GD5-20; Antonelli et al. 2005; Manduca et al. 2020). In the open field test, Newsom & Kelly (2008) showed that Δ^9-THC-exposed male offspring spent less time exploring the center quadrant of the open field, which is indicative of increased anxiety-like behavior (females were not tested). On the other hand, no differences in the number of center crossings in the open field were observed in offspring from Δ^9-THC-exposed dams, but significant reductions in open arm exploration in the elevated plus maze (EPM) and decreased time spent on the light side of the light/dark box were observed in males (but not females) in the study by Sarikahya et al. (2022), which strengthens the assertion that cannabinoid-exposed male offspring may be more susceptible to anxiety-like behavior later in life. However, effects on EPM

behavior are not universally observed in injection studies (Bara et al. 2018; Rubio et al. 1995; Manduca et al. 2020), and in some cases, may be transient and sex-dependent. For instance, DeVuono et al. (2024) found that coadministration of Δ^9-THC (3 mg/kg) and CBD (30 mg/kg) from GD7-22 produces opposing effects in male and female adolescent offspring, with male offspring showing an increase in open arm exploration and female offspring showing a decrease.

A few studies have examined stress coping strategies in the forced swim test (FST), which is a commonly used behavioral assay for measuring despair-like behavior. However, results of these studies are largely equivocal, with one group showing increased active coping strategies (i.e., swimming) in male (Sagheddu et al. 2021), but not female (Traccis et al. 2021) offspring, another group showing increased passive coping strategies (i.e., immobility) following intravenous Δ^9-THC delivery (0.15 mg/kg/day, GD5-PND2; Ellis et al. 2022), and a third group showing no group differences in any FST measures (Newsom and Kelly 2008). Thus, the effects of maternal cannabinoid injections on adoption of diverse stress coping strategies remains largely unknown.

4.3.2 Oral Administration Studies

Many of the early studies exploring the effects of prenatal/perinatal cannabinoid exposure on emotionality endpoints used oral gavage to administer cannabinoids to pregnant dams. With respect to social behavior, a series of studies using the same exposure regimen (5 mg/kg Δ^9-THC, GD15-PND9) have consistently indicated reductions in social interaction/exploration in Δ^9-THC-exposed male offspring during both adolescence (Trezza et al. 2008) and adulthood (Campolongo et al. 2007; Di Bartolomeo et al. 2021; Drazanova et al. 2019). Thus, data from oral studies are generally in agreement with injection studies showing impaired social behavior in cannabinoid-exposed offspring.

In some of the earliest published studies on the topic, oral Δ^9-THC administration (5 mg/kg/day from GD5-PND24) was shown to increase emergence latency in the light/dark box test in female (but not male offspring) and increase novelty-induced grooming in offspring of both sexes (Navarro et al. 1994b). The same year, a study from the same group showed that oral administration of hashish (14.5% Δ^9-THC) during the same developmental timeframe increased the frequency of water spraying-induced self-grooming in offspring of both sexes, though the increase was greater in males compared to females (Navarro et al. 1994a).

In line with results from Antonelli et al. (2005), oral Δ^9-THC administration (2.5–5 mg/kg/day) from GD15-PND9 increased the number of USVs emitted by rat pups removed from the nest (Trezza et al. 2008). When offspring were tested on the EPM in adulthood, researchers observed a decrease in open arm exploration (Trezza et al. 2008), and this apparent increase in anxiety-like behavior has recently been observed in offspring from dams receiving oral 10 mg/kg administration of Δ^9-THC twice daily from GD5-PND21 (Navarro et al. 2024). The increase in anxiety-like behavior in oral administration models is also observed with other behavioral

assays, such as the novelty-suppressed feeding test, where Δ^9-THC-exposed offspring of both sexes show an increased latency to eat, and decreased amount eaten in a novel open field (Navarro et al. 2024). Accordingly, an increased latency to approach a novel, palatable food has been observed in male (but not female) offspring following 5 mg/kg/day Δ^9-THC exposure during mating and up to GD21 (Lallai et al. 2022). Only Navarro et al. (2024) has examined adoption of stress coping strategies, showing a significant increase in immobility in offspring in the tail suspension test. Thus, there is clear consensus that oral cannabinoid delivery in pregnant dams disrupts social and emotional behavior in exposed offspring across the lifespan, with some mixed evidence for potential sex differences.

4.3.3 Vapor/Smoke Administration Studies

Only two published studies to date have used vapor/smoke delivery methods to examine long-term effects on social and emotional behavior. First, we have used noncontingent delivery of vaporized whole-plant cannabis extracts in dams during mating and gestation to explore differences in neonatal emotional reactivity using the isolation-induced USV assay, juvenile social play/investigation using the social interaction test, and adulthood anxiety-like behavior using the EPM test (Weimar et al. 2020). In this study, we showed that twice daily 400 mg/ml cannabis vapor delivery (24.8% Δ^9-THC) leads to an increase in the number of isolation-induced USVs emitted on PND6, coupled to reductions in social investigation selectively in males and a decrease in open arm exploration in the EPM when tested later in adulthood (Weimar et al. 2020). Although the finding of increased USVs is opposite of the findings from Antonelli et al. (2005) and Manduca et al. (2020), the social play and EPM data generally support the body of evidence reviewed above and collectively suggest that vaporized cannabis delivery produces effects on emotional and social behavior similar to those seen with more conventional cannabinoid delivery methods.

However, not all studies in this area show consistent results. In the one published study using cannabis smoke delivery, Sandini et al. (2023) found that cannabis-exposed offspring explored the inner zone of an open field significantly less than control offspring, but gestational cannabis smoke exposure surprisingly did not affect behavior in the EPM test or social interaction test. While there are several plausible explanations for this discrepancy, the most likely is that the quantity of drug exposure used in this study may not have been sufficient to induce a robust behavioral phenotype akin to the doses used in oral and injection studies. Future studies using this approach would benefit from quantifying circulating Δ^9-THC and its metabolites to ensure that the cannabis smoke administration method produces physiologically relevant concentrations in the dam, placenta, and fetal brain.

4.4 Drug Reward and Reinforcement

4.4.1 Injection Studies

It has been known for some time that children of drug-using parents are more than twice as likely to develop a drug or alcohol use disorder later in life (Chassin et al. 1999). In the context of prenatal cannabis exposure, the majority of evidence supporting increased drug use vulnerability in cannabinoid-exposed offspring comes from preclinical studies exploring opioid and alcohol reinforcement/reward. Studies from the Hurd lab have shown enhanced morphine place conditioning, increased responding for heroin, and more heroin-seeking behavior during mild stress and drug extinction in male offspring exposed to Δ^9-THC delivered intravenously to the dam from GD5-PND2 (DiNieri et al. 2011; Spano et al. 2007; females not tested). Accordingly, perinatal Δ^9-THC exposure (5 mg/kg/day, IP from PND4–14) enhances the rewarding properties of both low and higher doses of heroin in a place conditioning paradigm in male offspring (Singh et al. 2006), though Sarikahya et al. (2022) did not observe differences in subthreshold morphine preference when offspring were prenatally exposed to Δ^9-THC daily from GD7-22.

Interestingly, a recent preprint has provided evidence that prenatal Δ^9-THC exposure leads to increased motivation for opioid reinforcement specifically by augmenting the reinforcing efficacy of opioids independent of alterations in value-based processing (Luján et al. 2024). This coincides with increased dopamine release upon cue presentation and an overrepresentation of cue-aligned, effort-driven patterns of encoding in the nucleus accumbens (Luján et al. 2024). In line with the majority of studies described above, these effects are more pronounced in male offspring. Moreover, this could represent a common mechanism by which prenatal cannabinoid exposure impacts the motivational and rewarding properties of various other drugs, in addition to opioids.

Accordingly, two injection studies have demonstrated alterations in alcohol drinking and relapse behavior. Brancato et al. (2020) showed that 2 mg/kg Δ^9-THC injections from GD5-20 lead to increased alcohol consumption and relapse propensity in male offspring. However, females were not tested in this study, and there is reason to believe that there could be sex differences in the effects of prenatal cannabinoid exposure on alcohol drinking. Ornelas et al. (2024) administered the potent CB1R agonist CP55,940 (0.3 mg/kg, IP) to dams from GD8–11 and observed an increase in alcohol self-administration during both adolescence and adulthood in female (but not male) offspring. The lack of effect in males is incongruent with the Brancato study above, but this is likely due to a combination of several factors, such as the drug employed, the duration of drug exposure, and the strain of rat used. Regardless, there is strong evidence that pre- and perinatal injections of cannabinoids produce long-lasting effects on opioid and alcohol abuse liability, at least in animal studies.

4.4.2 Oral Administration Studies

The first studies conducted to assess the impacts of early-life cannabinoid exposure used oral gavage to deliver Δ^9-THC, typically at a dose of 5 mg/kg/day from GD5-PND24. However, results of these studies are less consistent than those from injection studies, particularly with respect to sex differences. For instance, studies from Rubio and colleagues have indicated enhanced sensitivity to the rewarding effects of a moderate dose of morphine in offspring of both sexes using place conditioning assays (Rubio et al. 1995). The same group replicated this phenomenon in male offspring in a follow-up study, but only female offspring exposed to a five-fold lower dose of Δ^9-THC (1 mg/kg) over the same time period showed greater morphine (but not food) preference compared to vehicle-exposed offspring (Rubio et al. 1998). Around the same time, Vela and colleagues showed that female (but not male) Δ^9-THC-exposed offspring exhibited a significant increase in the rate of acquisition of morphine self-administration (Vela et al. 1998). However, only males developed tolerance to the analgesic effects of morphine and exhibited opioid-like withdrawal in response to an acute injection of naloxone in a prior study from the same group (Vela et al. 1995). To further complicate matters, a study from González et al. (2003) using this same exposure regimen failed to observe effects on morphine self-administration. Thus, there is some consistency between results from oral and injection studies, but more investigation is warranted into the mechanisms underlying these potential sex-dependent effects on opioid reward/reinforcement.

One study has examined alcohol consumption following oral cannabinoid delivery. Navarro et al. (2024) administered Δ^9-THC (10 mg/kg/12 h) to pregnant dams from GD5-PND21 and observed increased self-administration of alcohol on fixed ratio schedules of reinforcement in offspring of both sexes, but only increased motivation to consume alcohol in a progressive ratio test in male adult offspring. This adds additional complexity to our understanding of the potential sex-specific effects of early-life Δ^9-THC exposure.

All the studies described to this point have used forced delivery of cannabinoids to pregnant dams. However, forced injections or installation of an oral gavage can be stressful and invasive, and there is a possibility that maternal stress could interact with cannabinoid exposure in ways that complicate interpretation of the data, especially given the well-known effects of cannabis use on the stress response and the bidirectional relationship between the endocannabinoid system and stress-related hormones (see Glodosky et al. 2021; Morena et al. 2016 for reviews of this literature). To avoid this potential confound, the Borgland lab has recently used a voluntary oral Δ^9-THC model whereby 5 mg/kg/day Δ^9-THC is consumed by pregnant mice in peanut butter from GD1-PND10 (Peterson et al. 2024). They subsequently examined the conditioned rewarding properties of cocaine and synaptic alterations in ventral tegmental area (VTA) GABA and dopamine in offspring of both sexes. Although they found, similar to Frau et al. (2019), that male offspring showed adaptations within the VTA that favor a hyperdopaminergic phenotype, they did not find evidence for enhanced preference for a cocaine-paired context in a conditioned place preference test during adolescence (Peterson et al. 2024). It remains unknown

whether changes in cocaine reward would emerge later in adulthood or whether acute stress or Δ^9-THC priming is capable of unmasking a cocaine-preferring phenotype similar to studies from Frau et al. (2019) and Sagheddu et al. (2021), respectively. As more studies begin to adopt voluntary drug delivery approaches, it will be important to directly compare effects in voluntary and forced cannabinoid delivery models in order to determine whether these approaches have different long-term impacts on the brain and behavior of offspring.

4.4.3 Vapor Administration Studies

To date, only one study involving maternal vapor exposure has been conducted to explore long-term impacts on drug reinforcement. To circumvent the potential stress associated with repeated forced vapor delivery exposure, our laboratory has recently developed a novel model of maternal cannabis use that involves response-contingent delivery of vaporized cannabis extracts in pregnant rat dams (Weimar et al. 2023). We used this model to determine whether maternal cannabis self-administration leads to long-term changes in cannabis-seeking behavior in adult offspring. Dams were trained to self-administer vaporized cannabis (150 mg/ml extract at 69.9% Δ^9-THC) in one-hour sessions twice daily throughout mating and gestation until 24–48 h. prior to birth. Dams self-administered more cannabis vapor than vehicle vapor, particularly during the first two weeks of gestation, and showed better discrimination for the vapor-associated operandum. The model produced biologically relevant changes in developing offspring, as evidenced by a reduction in body weight on PND1 compared to both air- and vehicle-exposed offspring (Weimar et al. 2023). However, when rates of cannabis vapor self-administration were assessed in male and female offspring in adulthood, we observed significant sex differences. Specifically, cannabis-exposed male (but not female) offspring unexpectedly made fewer active nosepokes and received fewer vapor deliveries compared to vehicle-exposed rats. Moreover, this relationship was seen regardless of whether they were assigned to self-administer cannabis or vehicle vapor, which may suggest a more generalized decrease in novelty-seeking behavior rather than a *bonafide* effect of cannabis exposure. On the other hand, female rats assigned to self-administer vapor in adulthood made more responses for cannabis vapor relative to vehicle vapor and earned more cannabis vapor deliveries, but this was irrespective of prenatal treatment condition (Weimar et al. 2023). These data corroborate previous studies from our group indicating greater reinforcing properties of cannabis vapor in female rats (Freels et al. 2024; Glodosky et al. 2020). Altogether, results of this study indicate that maternal cannabis vapor self-administration has complex sex-dependent effects on cannabis reinforcement in adult offspring that collectively refute the hypothesis that prenatal cannabis exposure produces a generalized increase in drug reinforcement. Future studies will be required to determine whether these effects may be dose-dependent, whether there are specific vulnerability windows (i.e., adolescence) wherein cannabis reinforcement is transiently enhanced, or whether such effects require a history of early-life or adult stress.

5 Conclusions

The behavioral impacts of maternal cannabis use on offspring are an important area of research as the legal landscape surrounding cannabis use continues to evolve. By disrupting important neurodevelopmental processes, prenatal cannabis exposure appears to result in long-term alterations that result in a plethora of maladaptive consequences. By comparing the body of evidence demonstrating behavioral deficits according to route of cannabinoid administration, a few key themes emerge.

First, despite the wide diversity of cannabinoid dosing and exposure regimens within the administration route subcategories, there is a remarkable amount of consistency in the behavioral effects observed in offspring. With respect to sensorimotor function, the majority of studies consistently show sensorimotor gating deficits predominantly in male offspring, with generalized effects on locomotion differing according to sex and timing of exposure.

Studies assessing cognitive ramifications of pre−/perinatal cannabinoid exposure have also been generally consistent across different routes of administration, with the most observed deficits in working memory during the novel object recognition test. However, studies involving maternal cannabis smoke exposure have also shown that cannabis-exposed offspring performed unexpectedly better on visual pairwise discrimination and reversal learning tasks, which suggests that drug, dose, and route of delivery may be important considerations for interrogating cognitive impacts of prenatal cannabinoid exposure.

In the context of social behavior, there is strong consensus that cannabinoid exposure leads to social discrimination and interaction deficits regardless of route of administration, and male offspring appear to be especially susceptible to this effect. Studies assessing emotionality in offspring have examined differences in ultrasonic vocalizations early in life, as well as behavior in traditional anxiety and stress assays such as the elevated plus maze, open field test, light/dark box, novelty-suppressed feeding test, and forced swim test. Despite the use of these various assays, results generally show an increase in anxiety-like behavior and increased reliance on passive stress coping strategies, though a few studies have reported no differences in some of these assays.

Finally, studies examining the effects of pre−/perinatal cannabinoid exposure on drug reward/reinforcement have consistently demonstrated increased sensitivity to opioid reward/reinforcement and increased alcohol consumption using both injection and oral administration approaches. However, there is conflicting evidence regarding sex differences, with dose and duration of maternal exposure potentially serving as key factors determining female susceptibility. In addition, though only two studies have explored effects of volitional cannabinoid consumption on drug reward/reinforcement in offspring, both failed to demonstrate significant differences in cocaine place conditioning (Peterson et al. 2024) and cannabis vapor self-administration (Weimar et al. 2023). Thus, it remains unclear whether these discrepant findings are due to the drug being examined (cocaine and cannabis vs. opioids and alcohol) or whether the volitional drug consumption, which avoids

unintended effects of stress due to forced drug delivery, produces differential effects on drug seeking compared to more traditional approaches.

Lastly, a major shortcoming identified through review of this literature is the heavy reliance on male offspring in the majority of early studies. This has led to an incomplete understanding of the effects of prenatal cannabinoid exposure on female offspring, which should hopefully be rectified as more laboratories begin to incorporate both sexes in their studies. This is critically important as sex differences exist with respect to the pharmacokinetics of cannabinoids, the distribution and function of the endocannabinoid system, and the effects of cannabinoids on the brain and behavior (Cooper and Craft 2018; Craft 2005; Craft et al. 2013; Fattore et al. 2009, 2010). By continuing to prioritize the use of sex-balanced designs and considering the dose, drug, and route of administration used in future studies, the field is poised to uncover novel long-term behavioral impacts of prenatal cannabinoid exposure and the mechanisms by which they occur. This is critically important given the diminishing social stigma of cannabis use and increasing rates of cannabis consumption among pregnant women (Brown et al. 2017).

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References

2022 National Survey on Drug Use and Health [Internet]. U.S. Department of Health and Human Services, Substance Abuse and Mental Health Services Administration, Center for Behavioral Health Statistics and Quality; 2022. Available from: https://www.samhsa.gov/data/

Ahmed SH, Badiani A, Miczek KA, Müller CP. Non-pharmacological factors that determine drug use and addiction. Neurosci Biobehav Rev. 2020;110:3–27.

Antonelli T, Tomasini MC, Tattoli M, et al. Prenatal exposure to the CB1 receptor agonist WIN 55,212-2 causes learning disruption associated with impaired cortical NMDA receptor function and emotional reactivity changes in rat offspring. Cereb Cortex. 2005;15(12):2013–20.

Baglot SL, Hume C, Petrie GN, et al. Pharmacokinetics and central accumulation of delta-9-tetrahydrocannabinol (THC) and its bioactive metabolites are influenced by route of administration and sex in rats. Sci Rep. 2021;11(1):23990.

Baglot SL, VanRyzin JW, Marquardt AE, et al. Maternal-fetal transmission of delta-9-tetrahydrocannabinol (THC) and its metabolites following inhalation and injection exposure during pregnancy in rats. J Neurosci Res. 2022;100(3):713–30.

Bara A, Manduca A, Bernabeu A, et al. Sex-dependent effects of in utero cannabinoid exposure on cortical function. elife. 2018;7:e36234.

Berenson AB, Wilkinson GS, Lopez LA. Effects of prenatal care on neonates born to drug-using women. Subst Use Misuse. 1996;31(8):1063–76.

Bolhuis K, Kushner SA, Yalniz S, et al. Maternal and paternal cannabis use during pregnancy and the risk of psychotic-like experiences in the offspring. Schizophr Res. 2018;202:322–7.

Brancato A, Castelli V, Lavanco G, et al. In utero Δ9-tetrahydrocannabinol exposure confers vulnerability towards cognitive impairments and alcohol drinking in the adolescent offspring: is there a role for neuropeptide Y? J Psychopharmacol (Oxf). 2020;34(6):663–79.

Breit KR, Rodriguez CG, Lei A. Effects of prenatal alcohol and delta-9-tetrahydrocannabinol exposure via electronic cigarettes on motor development. Alcohol Clin Exp Res. 2022;46(8):1408–22.

Brown QL, Sarvet AL, Shmulewitz D, et al. Trends in marijuana use among pregnant and nonpregnant reproductive-aged women, 2002-2014. JAMA. 2017;317(2):207.

Campolongo P, Trezza V, Cassano T, et al. Preclinical study: perinatal exposure to delta-9-tetrahydrocannabinol causes enduring cognitive deficits associated with alteration of cortical gene expression and neurotransmission in rats. Addict Biol. 2007;12(3–4):485–95.

Chassin L, Pitts SC, DeLucia C, et al. A longitudinal study of children of alcoholics: predicting young adult substance use disorders, anxiety, and depression. J Abnorm Psychol. 1999;108(1):106–19.

Cioffredi LA, Anderson H, Loso H, et al. Prenatal cannabis exposure predicts attention problems, without changes on fMRI in adolescents. Neurotoxicol Teratol. 2022;91:107089.

Cooper ZD, Craft RM. Sex-dependent effects of cannabis and cannabinoids: a translational perspective. Neuropsychopharmacology. 2018;43(1):34–51.

Craft RM. Sex differences in behavioral effects of cannabinoids. Life Sci. 2005;77(20):2471–8.

Craft RM, Marusich JA, Wiley JL. Sex differences in cannabinoid pharmacology: a reflection of differences in the endocannabinoid system? Life Sci. 2013;92(8–9):476–81.

Crume TL, Juhl AL, Brooks-Russell A, et al. Cannabis use during the perinatal period in a state with legalized recreational and medical marijuana: the association between maternal characteristics, breastfeeding patterns, and neonatal outcomes. J Pediatr. 2018;197:90–6.

Day NL, Richardson GA. Prenatal marijuana use: epidemiology, methodologie issues, and infant outcome. Clin Perinatol. 1991;18(1):77–91.

De Salas-Quiroga A, Díaz-Alonso J, García-Rincón D, et al. Prenatal exposure to cannabinoids evokes long-lasting functional alterations by targeting CB_1 receptors on developing cortical neurons. Proc Natl Acad Sci. 2015;112(44):13693–8.

De Salas-Quiroga A, García-Rincón D, Gómez-Domínguez D, et al. Long-term hippocampal interneuronopathy drives sex-dimorphic spatial memory impairment induced by prenatal THC exposure. Neuropsychopharmacology. 2020;45(5):877–86.

DeVuono MV, Nashed MG, Sarikahya MH, et al. Prenatal tetrahydrocannabinol and cannabidiol exposure produce sex-specific pathophysiological phenotypes in the adolescent prefrontal cortex and hippocampus. Neurobiol Dis. 2024;199:106588.

Di Bartolomeo M, Stark T, Maurel OM, et al. Crosstalk between the transcriptional regulation of dopamine D2 and cannabinoid CB1 receptors in schizophrenia: analyses in patients and in perinatal Δ9-tetrahydrocannabinol-exposed rats. Pharmacol Res. 2021;164:105357.

Dickson B, Mansfield C, Guiahi M, et al. Recommendations from cannabis dispensaries about first-trimester cannabis use. Obstet Gynecol. 2018;131(6):1031–8.

DiNieri JA, Wang X, Szutorisz H, et al. Maternal cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring. Biol Psychiatry. 2011;70(8):763–9.

Drazanova E, Ruda-Kucerova J, Kratka L, et al. Different effects of prenatal MAM vs. perinatal THC exposure on regional cerebral blood perfusion detected by arterial spin labelling MRI in rats. Sci Rep. 2019;9(1):6062.

El Marroun H, Tiemeier H, Steegers EAP, et al. Intrauterine cannabis exposure affects fetal growth trajectories: the generation R study. J Am Acad Child Adolesc Psychiatry. 2009;48(12):1173–81.

Ellis RJ, Bara A, Vargas CA, et al. Prenatal Δ9-tetrahydrocannabinol exposure in males leads to motivational disturbances related to striatal epigenetic dysregulation. Biol Psychiatry. 2022;92(2):127–38.

ElSohly MA, Mehmedic Z, Foster S, et al. Changes in cannabis potency over the last 2 decades (1995–2014): analysis of current data in the United States. Biol Psychiatry. 2016;79(7):613–9.

Fattore L, Fadda P, Fratta W. Sex differences in the self-administration of cannabinoids and other drugs of abuse. Psychoneuroendocrinology. 2009;34:S227–36.

Fattore L, Spano M, Altea S, et al. Drug- and cue-induced reinstatement of cannabinoid-seeking behaviour in male and female rats: influence of ovarian hormones. Br J Pharmacol. 2010;160(3):724–35.
Frau R, Miczán V, Traccis F, et al. Prenatal THC exposure produces a hyperdopaminergic phenotype rescued by pregnenolone. Nat Neurosci. 2019;22(12):1975–85.
Freels TG, Westbrook SR, Zamberletti E, et al. Sex differences in response-contingent cannabis vapor administration during adolescence mediate enduring effects on behavioral flexibility and prefrontal microglia activation in rats. Cannabis Cannabinoid Res. 2024;9(4):e1184–96.
Fried PA. Adolescents prenatally exposed to marijuana: examination of facets of complex behaviors and comparisons with the influence of in utero cigarettes. J Clin Pharmacol. 2002;42(S1):97S–102S.
Fried PA, Watkinson B. Differential effects on facets of attention in adolescents prenatally exposed to cigarettes and marihuana. Neurotoxicol Teratol. 2001;23(5):421–30.
Fried PA, Watkinson B, Gray R. A follow-up study of attentional behavior in 6-year-old children exposed prenatally to marihuana, cigarettes, and alcohol. Neurotoxicol Teratol. 1992;14(5):299–311.
Fried PA, Watkinson B, Gray R. Differential effects on cognitive functioning in 9- to 12-year olds prenatally exposed to cigarettes and marihuana. Neurotoxicol Teratol. 1998;20(3):293–306.
Glodosky NC, Cuttler C, Freels TG, et al. Cannabis vapor self-administration elicits sex- and dose-specific alterations in stress reactivity in rats. Neurobiol Stress. 2020;13:100260.
Glodosky NC, Cuttler C, McLaughlin RJ. A review of the effects of acute and chronic cannabinoid exposure on the stress response. Front Neuroendocrinol. 2021;63:100945.
Goldschmidt L, Day NL, Richardson GA. Effects of prenatal marijuana exposure on child behavior problems at age 10. Neurotoxicol Teratol. 2000;22(3):325–36.
Goldschmidt L, Richardson GA, Willford J, et al. Prenatal marijuana exposure and intelligence test performance at age 6. J Am Acad Child Adolesc Psychiatry. 2008;47(3):254–63.
González B, De Miguel R, Martín S, et al. Effects of perinatal exposure to Δ9-tetrahydrocannabinol on operant morphine-reinforced behavior. Pharmacol Biochem Behav. 2003;75(3):577–84.
Goodwin RD, Zhu J, Heisler Z, et al. Cannabis use during pregnancy in the United States: the role of depression. Drug Alcohol Depend. 2020;210:107881.
Grotenhermen F. Pharmacokinetics and pharmacodynamics of cannabinoids. Clin Pharmacokinet. 2003;42(4):327–60.
Gunn JKL, Rosales CB, Center KE, et al. Prenatal exposure to cannabis and maternal and child health outcomes: a systematic review and meta-analysis. BMJ Open. 2016;6(4):e009986.
Hussain S, Breit KR, Thomas JD. The effects of prenatal nicotine and THC E-cigarette exposure on motor development in rats. Psychopharmacology. 2022;239(5):1579–91.
Jaddoe VWV, Mackenbach JP, Moll HA, et al. The generation R study: design and cohort profile. Eur J Epidemiol. 2006;21(6):475–84.
Kim S, Lee D. Prefrontal cortex and impulsive decision making. Biol Psychiatry. 2011;69(12):1140–6.
Klebanoff MA, Fried P, Yeates KO, et al. Lifestyle and early achievement in families (LEAF) study: design of an ambidirectional cohort study of prenatal marijuana exposure and child development and behaviour. Paediatr Perinat Epidemiol. 2020;34(6):744–56.
Ko JY, Farr SL, Tong VT, et al. Prevalence and patterns of marijuana use among pregnant and nonpregnant women of reproductive age. Am J Obstet Gynecol. 2015;213(2):201.e1–201.e10.
Ko JY, Tong VT, Bombard JM, et al. Marijuana use during and after pregnancy and association of prenatal use on birth outcomes: a population-based study. Drug Alcohol Depend. 2018;187:72–8.
Lallai V, Manca L, Sherafat Y, et al. Effects of prenatal nicotine, THC, or co-exposure on cognitive behaviors in adolescent male and female rats. Nicotine Tob Res. 2022;24(8):1150–60.
Laprairie RB, Bagher AM, Kelly MEM, et al. Type 1 cannabinoid receptor ligands display functional selectivity in a cell culture model of striatal medium spiny projection neurons. J Biol Chem. 2014;289(36):24845–62.

Laprairie RB, Bagher AM, Kelly MEM, et al. Biased type 1 cannabinoid receptor signaling influences neuronal viability in a cell culture model of Huntington disease. Mol Pharmacol. 2016;89(3):364–75.

Leech SL, Richardson GA, Goldschmidt L, et al. Prenatal substance exposure: effects on attention and impulsivity of 6-year-olds. Neurotoxicol Teratol. 1999;21(2):109–18.

Luján MÁ, Young-Morrison R, Aroni S, et al. Dynamic overrepresentation of accumbal cues in food- and opioid-seeking rats after prenatal THC exposure. 2024. Preprint at http://biorxiv.org/lookup/doi/10.1101/2024.05.06.592839.

Manduca A, Servadio M, Melancia F, et al. Sex-specific behavioural deficits induced at early life by prenatal exposure to the cannabinoid receptor agonist WIN55, 212-2 depend on mGlu5 receptor signalling. Br J Pharmacol. 2020;177(2):449–63.

Manwell LA, Mallet PE. Comparative effects of pulmonary and parenteral Δ9-tetrahydrocannabinol exposure on extinction of opiate-induced conditioned aversion in rats. Psychopharmacology. 2015;232(9):1655–65.

Manwell LA, Ford B, Matthews BA, et al. A vapourized Δ9-tetrahydrocannabinol (Δ9-THC) delivery system part II: comparison of behavioural effects of pulmonary versus parenteral cannabinoid exposure in rodents. J Pharmacol Toxicol Methods. 2014a;70(1):112–9.

Manwell LA, Charchoglyan A, Brewer D, et al. A vapourized Δ9-tetrahydrocannabinol (Δ9-THC) delivery system part I: development and validation of a pulmonary cannabinoid route of exposure for experimental pharmacology studies in rodents. J Pharmacol Toxicol Methods. 2014b;70(1):120–7.

McLaughlin RJ. Toward a translationally relevant preclinical model of cannabis use. Neuropsychopharmacology. 2018;43(1):213.

Mereu G, Fà M, Ferraro L, et al. Prenatal exposure to a cannabinoid agonist produces memory deficits linked to dysfunction in hippocampal long-term potentiation and glutamate release. Proc Natl Acad Sci. 2003;100(8):4915–20.

Metz TD, Allshouse AA, Hogue CJ, et al. Maternal marijuana use, adverse pregnancy outcomes, and neonatal morbidity. Am J Obstet Gynecol. 2017;217(4):478.e1–8.

Moolten M, Kornetsky C. Oral self-administration of ethanol and not experimenter-administered ethanol facilitates rewarding electrical brain stimulation. Alcohol. 1990;7(3):221–5.

Moore CF, Davis CM, Sempio C, et al. Δ^9-tetrahydrocannabinol vapor exposure produces conditioned place preference in male and female rats. Cannabis Cannabinoid Res. 2024;9(1):111–20.

Morena M, Patel S, Bains JS, et al. Neurobiological interactions between stress and the endocannabinoid system. Neuropsychopharmacology. 2016;41(1):80–102.

Mulligan MK, Hamre KM. Influence of prenatal cannabinoid exposure on early development and beyond. Adv Drug Alcohol Res. 2023;3:10981.

Murnan AW, Keim SA, Yeates KO, et al. Behavioral and cognitive differences in early childhood related to prenatal marijuana exposure. J Appl Dev Psychol. 2021;77:101348.

Navarro M, De Fonseca FR, Hernández ML, et al. Motor behavior and nigrostriatal dopaminergic activity in adult rats perinatally exposed to cannabinoids. Pharmacol Biochem Behav. 1994a;47(1):47–58.

Navarro M, Rubio P, Rodríguez De Fonseca F. Sex-dimorphic psychomotor activation after perinatal exposure to (−)-Δ9-tetrahydrocannabinol. An ontogenic study in wistar rats. Psychopharmacology. 1994b;116(4):414–22.

Navarro D, Gasparyan A, Navarrete F, et al. Fetal cannabinoid syndrome: behavioral and brain alterations of the offspring exposed to dronabinol during gestation and lactation. Int J Mol Sci. 2024;25(13):7453.

Newsom RJ, Kelly SJ. Perinatal delta-9-tetrahydrocannabinol exposure disrupts social and open field behavior in adult male rats. Neurotoxicol Teratol. 2008;30(3):213–9.

O'Shea M, Mallet PE. Impaired learning in adulthood following neonatal ??9-THC exposure. Behav Pharmacol. 2005;16(5–6):455–61.

O'Shea M, McGregor IS, Mallet PE. Repeated cannabinoid exposure during perinatal, adolescent or early adult ages produces similar long lasting deficits in object recognition and reduced social interaction in rats. J Psychopharmacol (Oxf). 2006;20(5):611–21.

Ornelas LC, Fish EW, Dooley JC, et al. The impact of prenatal alcohol, synthetic cannabinoid and co-exposure on behavioral adaptations in adolescent offspring and alcohol self-administration in adulthood. Neurotoxicol Teratol. 2024;102:107341.

Ostrea EM, Ostrea AR, Simpson PM. Mortality within the first 2 years in infants exposed to cocaine, opiate, or cannabinoid during gestation. Pediatrics. 1997;100(1):79–83.

Penman SL, Roeder NM, Wang J, et al. Vaporized Δ9-tetrahydrocannabinol exposure in utero has negative effects on attention in a dose- and sex-dependent manner. Pharmacol Biochem Behav. 2024;242:173808.

Perez-Reyes M, Timmons MC, Lipton MA, et al. Intravenous injection in man of Δ^9 -tetrahydrocannabinol and 11-OH-Δ^9-tetrahydrocannabinol. Science. 1972;177(4049):633–5.

Pertwee RG. The diverse CB_1 and CB_2 receptor pharmacology of three plant cannabinoids: Δ^9 -tetrahydrocannabinol, cannabidiol and Δ^9 -tetrahydrocannabivarin. Br J Pharmacol. 2008;153(2):199–215.

Peterson CS, Baglot SL, Sallam NA, et al. Oral pre- and early postnatal cannabis exposure disinhibits ventral tegmental area dopamine neuron activity but does not influence cocaine preference in offspring in mice. J Neurosci Res. 2024;102(7):e25369.

Pinky PD, Bloemer J, Smith WD, et al. Prenatal cannabinoid exposure elicits memory deficits associated with reduced PSA-NCAM expression, altered glutamatergic signaling, and adaptations in hippocampal synaptic plasticity. Cells. 2023;12(21):2525.

Porath A, Fried P. Effects of prenatal cigarette and marijuana exposure on drug use among offspring. Neurotoxicol Teratol. 2005;27(2):267–77.

Potter DJ, Hammond K, Tuffnell S, et al. Potency of Δ^9 –tetrahydrocannabinol and other cannabinoids in cannabis in England in 2016: implications for public health and pharmacology. Drug Test Anal. 2018;10(4):628–35.

Roeder NM, Penman SL, Richardson BJ, et al. Vaporized Δ9-THC in utero results in reduced birthweight, increased locomotion, and altered wake-cycle activity dependent on dose, sex, and diet in the offspring. Life Sci. 2024;340:122447.

Rubio P, De Fonseca FR, Muñoz RM, et al. Long-term behavioral effects of perinatal exposure to Δ9-tetrahydrocannabinol in rats: possible role of pituitary adrenal axis. Life Sci. 1995;56(23–24):2169–76.

Rubio P, Rodríguez De Fonseca F, Martín-Calderón J, et al. Maternal exposure to low doses of Δ9-tetrahydrocannabinol facilitates morphine-induced place conditioning in adult male offspring. Pharmacol Biochem Behav. 1998;61(3):229–38.

Russo EB. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. Br J Pharmacol. 2011;163(7):1344–64.

Sagheddu C, Traccis F, Serra V, et al. Mesolimbic dopamine dysregulation as a signature of information processing deficits imposed by prenatal THC exposure. Prog Neuro-Psychopharmacol Biol Psychiatry. 2021;105:110128.

Sandini TM, Onofrychuk TJ, Roebuck AJ, et al. Repeated exposure to high-THC cannabis smoke during gestation alters sex ratio, behavior, and amygdala gene expression of Sprague Dawley rat offspring. eneuro. 2023;10(11):ENEURO.0100-23.2023.

Sarikahya MH, Cousineau S, De Felice M, et al. Prenatal THC exposure induces sex-dependent neuropsychiatric endophenotypes in offspring and long-term disruptions in fatty-acid signaling pathways directly in the mesolimbic circuitry. eneuro. 2022;9(5):ENEURO.0253-22.2022.

Scheyer AF, Borsoi M, Pelissier-Alicot AL, et al. Perinatal THC exposure via lactation induces lasting alterations to social behavior and prefrontal cortex function in rats at adulthood. Neuropsychopharmacology. 2020a;45(11):1826–33.

Scheyer AF, Borsoi M, Wager-Miller J, et al. Cannabinoid exposure via lactation in rats disrupts perinatal programming of the gamma-aminobutyric acid trajectory and select early-life behaviors. Biol Psychiatry. 2020b;87(7):666–77.

Silva L, Zhao N, Popp S, et al. Prenatal tetrahydrocannabinol (THC) alters cognitive function and amphetamine response from weaning to adulthood in the rat. Neurotoxicol Teratol. 2012;34(1):63–71.
Simei JLQ, Souza JDR, Lisboa JR, et al. Does the "entourage effect" in cannabinoids exist? A narrative scoping review. Cannabis Cannabinoid Res. 2024;9(5):1202–16.
Singh ME, McGregor IS, Mallet PE. Perinatal exposure to Δ9-tetrahydrocannabinol alters heroin-induced place conditioning and Fos-immunoreactivity. Neuropsychopharmacology. 2006;31(1):58–69.
Skelton KR, Hecht AA, Benjamin-Neelon SE. Recreational cannabis legalization in the US and maternal use during the preconception, prenatal, and postpartum periods. Int J Environ Res Public Health. 2020;17(3):909.
Smith AM, Fried PA, Hogan MJ, et al. Effects of prenatal marijuana on response inhibition: an fMRI study of young adults. Neurotoxicol Teratol. 2004;26(4):533–42.
Smith AM, Fried PA, Hogan MJ, et al. Effects of prenatal marijuana on visuospatial working memory: an fMRI study in young adults. Neurotoxicol Teratol. 2006;28(2):286–95.
Smucny J, Dienel SJ, Lewis DA, Carter CS. Mechanisms underlying dorsolateral prefrontal cortex contributions to cognitive dysfunction in schizophrenia. Neuropsychopharmacology. 2022;47(1):292–308.
Sorkhou M, Singla DR, Castle DJ, et al. Birth, cognitive and behavioral effects of intrauterine cannabis exposure in infants and children: a systematic review and meta-analysis. Addiction. 2023;119(3):411–37.
Spano MS, Ellgren M, Wang X, et al. Prenatal cannabis exposure increases heroin seeking with allostatic changes in limbic enkephalin systems in adulthood. Biol Psychiatry. 2007;61(4):554–63.
Traccis F, Serra V, Sagheddu C, et al. Prenatal THC does not affect female mesolimbic dopaminergic system in preadolescent rats. Int J Mol Sci. 2021;22(4):1666.
Trezza V, Campolongo P, Cassano T, et al. Effects of perinatal exposure to delta-9-tetrahydrocannabinol on the emotional reactivity of the offspring: a longitudinal behavioral study in Wistar rats. Psychopharmacology. 2008;198(4):529–37.
Vargish GA, Pelkey KA, Yuan X, et al. Persistent inhibitory circuit defects and disrupted social behaviour following in utero exogenous cannabinoid exposure. Mol Psychiatry. 2017;22(1):56–67.
Vela G, Fuentes J, Bonnin A, et al. Perinatal exposure to Δ9-tetrahydrocannabinol (Δ9-THC) leads to changes in opioid-related behavioral patterns in rats. Brain Res. 1995;680(1–2):142–7.
Vela G, Martín S, García-Gil L, et al. Maternal exposure to Δ9-tetrahydrocannabinol facilitates morphine self-administration behavior and changes regional binding to central μ opioid receptors in adult offspring female rats. Brain Res. 1998;807(1–2):101–9.
Volkow ND, Han B, Compton WM, et al. Self-reported medical and nonmedical cannabis use among pregnant women in the United States. JAMA. 2019;322(2):167.
Weimar HV, Wright HR, Warrick CR, et al. Long-term effects of maternal cannabis vapor exposure on emotional reactivity, social behavior, and behavioral flexibility in offspring. Neuropharmacology. 2020;179:108288.
Weimar HV, Malena AN, Ginder DE, et al. Determining impacts of prenatal cannabis exposure on cannabis vapor self-administration using a novel response-contingent vapor model in pregnant rat dams. Addict Neurosci. 2023;6:100071.
Wen H, Hockenberry JM, Druss BG. The effect of medical marijuana laws on marijuana-related attitude and perception among US adolescents and young adults. Prev Sci. 2019;20(2):215–23.
Young-Wolff KC, Tucker LY, Alexeeff S, et al. Trends in self-reported and biochemically tested marijuana use among pregnant females in California from 2009-2016. JAMA. 2017;318(24):2490.
Young-Wolff KC, Adams SR, Wi S, et al. Routes of cannabis administration among females in the year before and during pregnancy: results from a pilot project. Addict Behav. 2020;100:106125.
Young-Wolff KC, Ray GT, Alexeeff SE, et al. Rates of prenatal cannabis use among pregnant women before and during the COVID-19 pandemic. JAMA. 2021;326(17):1745.

Chapter 7
Effects of Prenatal Cannabis Exposure on Neonatal Outcomes



Iveta Kopil and Hilary Marusak

1 Introduction

Over the past 10 years, the percentage of people using cannabis has more than doubled, which may be associated with greater medical applications and the expansion of state-level legalization across the United States (US) (Vanstone et al. 2021). Despite this increase in use, the health effects of cannabis use remain poorly understood, especially for vulnerable groups such as pregnant women and neonates, who may be at elevated risk for health complications. The recent legalization and proliferation of cannabis dispensaries may contribute to perceptions that cannabis is relatively safe to use. Many expectant mothers report using cannabis to manage symptoms such as nausea, anxiety, and chronic pain (Vanstone et al. 2021; Chang et al. 2019). In parallel with trends in the general population, the prevalence and perceived safety of cannabis use during pregnancy is also increasing (Young-Wolff et al. 2022). Modern cannabis products often contain much higher concentrations of delta-9-tetrahydrocannabinol (THC)—the intoxicating component of cannabis—compared to products from decades prior. The increase in potency may lead to more severe and unpredictable effects among users, such as higher risk for substance use disorders, mental disorders including anxiety and psychosis, and impaired cognitive functions (Atakan 2012). These trends may also exacerbate outcomes for neonates with prenatal cannabis exposure (PCE). The scientific literature regarding effects of PCE on health outcomes, including neonatal adverse outcomes, is mixed, adding to

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further uncertainty about cannabis use during pregnancy. To date, there is no confirmed "safe" level of cannabis consumption for pregnant individuals. This essay aims to review the existing human and animal literature on PCE and its association with key neonatal outcomes including preterm birth (PTB), low birth weight (LBW), and neonatal intensive care unit (NICU) admission.

2 Background

2.1 What Is Cannabis and Its Constituents?

Cannabis sativa Linnaeus belongs to the family *Cannabaceae*, which is composed of flowering plants and includes hop plants, and a type of tree called hackberries (Tettey et al. 2021). This annual flowering plant is dioecious, meaning that some plants have male flowers and others have female flowers. The cannabis flower people typically smoke comes from female cannabis plants. Male cannabis plants produce pollen sacs, rather than flowers, on their nodes. Since male plants are low in cannabinoids, they are not typically consumed (Tettey et al. 2021). Palaeobotanical studies attest that cannabis was present 12,000 years ago near the Altai Mountains in Central Asia and since that time cannabis planthas migrated via nomadic peoples and their routes (Pisanti and Bifulco 2019). In conclusion, cannabis is one of humanity's oldest crops, with records of use dating to 6000 years before present and is originally indigenous to Asia.

The cannabis plant contains more than 545 different compounds, including cannabinoids and terpenoids. Cannabinoids are fatty compounds that are the most widely studied biologically active constituents of cannabis. Terpenes are volatile compounds that occur in many plants and have distinct odors. To-date, over 100 different phytocannabinoids have been isolated from the cannabis plant. The two most prevalent and commonly known cannabinoids are THC and cannabidiol (CBD), with THC as the most well-known and primary psychoactive analyte of cannabis (Freeman et al. 2021). There are four different stereoisomers of THC, but only the (−)-trans form exists naturally (Pisanti and Bifulco 2019; Baker et al. 2018).

2.2 The Endocannabinoid System (ECS)

Cannabis interacts with the body via the ECS, an extensive neuromodulatory system that plays key roles in central nervous system (CNS) development, long-term potentiation, and the response to endogenous and environmental exposures. The ECS is comprised of cannabinoid receptors, endogenous cannabinoids (endocannabinoids), and the enzymes responsible for the synthesis and degradation of endocannabinoids (Cristino et al. 2014). Cannabinoids exert their effects on the body by

binding to receptors, specifically cannabinoid receptor types 1 and 2 (Klumpers and Thacker 2019; Cristino et al. 2014). Endocannabinoids are similar to the exogenous cannabinoids (e.g., THC) present in the cannabis plant, but are naturally produced in the human body (Cristino et al. 2014). Like THC, endocannabinoids bind to and activate cannabinoid receptors on neurons and glial cells in several brain areas, including the neocortex, hippocampus, basal ganglia, amygdala, striatum, cerebellum, and hypothalamus, which may lead to altered mental and physical functions (Johnston and O'Malley 2016; Everitt 2020; Chiu et al. 2022).

2.3 Cannabis Use Is Common in the General Population

Current rates of cannabis consumption are increasing in the general population (Tettey et al. 2021). The most rapid growth in cannabis use since the 1960s has been in developed countries in North America, Western Europe, and Australia. Cannabis has been predominantly the first drug of choice for younger generations and therefore the age of initiation is usually lower than for other drugs (Kannan et al. 2024; Johnston and O'Malley 2016). An analysis of drug markets shows that the low prices of cannabis coincide with high levels of use, and vice versa (Everitt 2020). In the US, cannabis is currently the most commonly used federally illegal drug with about 18% of Americans reporting to have used it at least once in 2019 (Tettey et al. 2021).

2.4 Current Legal Status and Growing Legalization

The legal status of cannabis use, for medical or recreational purposes, varies internationally as well as across the US Support for cannabis legalization in the US increased rapidly in a relatively linear manner after the mid-1990s to around 60% by 2016 (Gallup polls: 12% to 49% between 1969 and 2011; General Social Survey: 20% to 60% between 1973 and 2016) (Chiu et al. 2022). As of April 24, 2023, cannabis has been legalized in 38 of 50 US states for medical use, and in 24 states for adult recreational use (ages 21+). At the federal level, cannabis is currently still classified as a Schedule I drug under the Controlled Substances Act, which indicates a high potential for abuse and no accepted medical use, prohibiting its use for any purpose. Similarly, under the United Nations international Single Convention on Narcotic Drugs (as amended in 1972), the cannabis plant, cannabis resin and its extracts and tinctures are classified under Schedule I, meaning use should be allowed only for "medical and scientific purposes." (Mead 2019) On August 30, 2023, the US Department of Health and Human Services recommended that cannabis be reclassified to Schedule III, which indicates low to moderate potential for abuse and some medically accepted use. This recommendation is now being considered by the Drug Enforcement Administration, which will make the decision. However, as of

this writing, cannabis remains a Schedule I controlled substance at the federal level in the US. Therefore, although cannabis remains illegal in the US at the federal level, many states are moving towards decriminalization and legalization.

2.5 Perceived Harm of Use Is Also Declining Over Time

In addition to increasing legalization, there has been a steady decline in perceived harmfulness of cannabis use. For example, an independent analysis of six research studies assessed the temporal changes in perceived harmfulness and availability of cannabis using data from the National Survey on Drug Use and Health collected between 1996 and 2018 (Chambers et al. 2023). The online survey was administered in 2017, 2020, and 2021 and completed by 5035 adults. In this study, survey participants responded to a question regarding perceived safety of daily smoking of tobacco versus cannabis (Chambers et al. 2023). When comparing the years 2021 and 2017, there were fewer participants that reported that cannabis was safer than tobacco (1213 participants [25.5%] versus 1601 participants [33.7%], respectively). Other participants reported that cannabis was somewhat safer or much safer than tobacco (2107 participants [44.3%] vs. 1742 participants [36.7%]) (Chambers et al. 2023). Therefore, since 2017, there has been a shift toward a more favorable perception of safety related to regular cannabis use.

2.6 Increases in Cannabis Potency in Recent Years

Recent research indicates that the potency of the cannabinoids in cannabis has increased dramatically since 1995. The average % THC content of the cannabis plant grew from approximately 4% in 1995 to approximately 12% in 2014 (ElSohly et al. 2016). Presently, there are THC products available such as hash oil, shatter (or budder), dab, and edibles that have been able to increase the THC concentration to more than 95%. There are currently no data indicating that high-potency THC products are beneficial for any medical condition. According to an article by Stuyt, the increased potency of these products may make them more harmful and more likely to result in cannabis use disorder compared to lower potency products (Stuyt and Stuyt 2018).

There are many different types or cultivars of cannabis, and these cultivars contain different combinations of terpenes, flavonoids, alkaloids, and cannabinoids. The relative concentration of various substances in cannabis varies across extracts and between products. This escalating potency and diverse consumption methods have raised concerns about potential harmful side effects, increased risk of cannabis use disorder, and a greater likelihood of experiencing adverse reactions.

2.7 *Effects of Cannabis in the General Population*

Smoking or ingesting cannabis slows down the responses to sight and sound stimuli, and may increase risk for dangerous driving (Volkow et al. 2019). Cannabis can make an individual sleepy, distort the sense of time and space, and impair the ability to adapt to light and dark. Cannabis use may also lower the ability to handle a quick series of tasks while driving. This poor reaction time is worsened when driving at night because cannabis may also cause a severe loss of night vision.

Among short-term effects that cannabis produces at low doses are poor memory, lower ability to learn, difficulty in thinking and solving problems, poor muscle coordination and judgment, short attention span, altered sense of time and space, and food cravings (Volkow et al. 2014). In larger doses, cannabis consumption is associated with hallucinations, delusions, poor memory, space disorientation, anxiety attacks or feelings of paranoia, and depression (Volkow et al. 2014).

Long-term effects of cannabis consumption may include increased risk of cancer, breathing problems, decreased immune system responses, memory, and learning impairment, as well as decreased fertility (Volkow et al. 2014). Smoking cannabis may expose a user to the same cancer-causing chemicals found in tobacco smoke (Volkow et al. 2014). Cannabis smoking is associated with similar breathing problems to cigarette smoking, such as coughing and wheezing (Volkow et al. 2014). In addition, reproductive hormone production may be decreased. For example, studies in men found an association between cannabis use and lower testosterone levels, which may cause decreased sperm counts and possible erectile dysfunction (Smith et al. 2010; Pizzol et al. 2019). In women, there may be irregular periods (Lo et al. 2022). Both problems would result in a decreased ability to conceive but not necessarily lead to complete infertility (Dreher et al. 1994). In sum, despite the common misconception about cannabis being safe and without any risks, existing research suggests there are risks.

2.8 *Prenatal Cannabis Exposure (PCE) and Potential Adverse Effects on Offspring*

As cannabis use has gained popularity in recent years, it has emerged as one of the most frequently used substances among pregnant women (Barbosa-Leiker et al. 2020). Emerging studies have shown that cannabis use is especially harmful for vulnerable populations such as pregnant women (Baker et al. 2018; Thompson et al. 2019). Cannabis has most often been used as an anti-emetic medicine for centuries (Russo 2007; Dreher et al. 1994). Some of the latest research has focused on understanding reasons for its use. Three principal themes appear in the literature: (1) low perception of risk and limited information among healthcare providers; (2) perceived efficacy to treat pregnancy-related nausea and vomiting; and (3)

self-medication for untreated or undertreated mental health conditions (Crume et al. 2022).

As with the general population, rates of cannabis use during pregnancy are rising, despite clinical evidence about the potential for harm and the lack of support by current obstetrical recommendation guidelines (ACOG 2017). According to a study conducted by Volkow et al. (2014), the frequency and prevalence of past-month cannabis use among pregnant individuals overall increased from 3.4% to 7.0% from 2002 to 2017. Likewise, the prevalence and frequency increased from 5.7% to 12.1% for first-trimester use from 2002 and 2017, and was higher during the first trimester compared to the second or third trimesters (Volkow et al. 2019).

In general, major congenital anomalies of the fetal body and internal organs have the highest risk of formation during the first trimester, which is typically defined as between 3 and 12 embryonic/fetal weeks. During pregnancy, each part of the fetus' body forms during a specific time. This specific time is called the "critical period of development" or "critical window" for that body part (Moore et al. 2013). Such windows represent a form of developmental phenotypic plasticity and result from the interaction between genotype and environment. During this critical time, the developing fetal body is thought to be extremely sensitive to environmental exposures, such as medications, alcohol, or cannabis. Whether these exposures lead to congenital anomalies depends on the critical window for different body parts at the time of the exposure (Moore et al. 2013). Harmful exposures during the first trimester have the greatest chance of causing major birth defects compared to exposures during later periods of pregnancy (Moore et al. 2013). This is because many important developmental changes take place during this time. The major structures of the body, such as the spine and limbs, as well as vital organs such as the heart and stomach, form in the first trimester (Moore et al. 2013). Thus, substance use among pregnant women is a critical public health issue that impacts both the mother and the fetus.

3 Objective of Literature Review

This chapter aims to review available literature on human and animal research characterizing the effects of PCE on neonatal outcomes, particularly LBW, PTB, and NICU admission. LBW is a clinical term that describes babies with newborn weight of less than 5 pounds, 8 ounces (2500 g), as defined by the World Health Organization (World Health Organization and United Nations Children's Fund (UNICEF) 2004). PTB is defined as a birth that occurs before 37 weeks of pregnancy (Quinn et al. 2016). First, we will discuss (human) clinical studies of PCE and associations with neonatal outcomes (Aim 1). Second, we will review and discuss (animal) preclinical studies which may provide mechanistic insights into human findings (Aim 2), and offer additional insights into behavioral, molecular, or neurobiological changes associated with PCE in animal models. Finally, we will summarize similarities and

differences in findings across human and nonhuman animal studies, and provide some recommendations for future research.

3.1 Search Strategy

A select literature review was conducted using PubMed and Google Scholar databases, including original articles, case reports, systematic reviews, and meta-analyses published prior to May 27, 2024. One author (IK) reviewed and selected research studies published within the past 16 years, to include more recent publications. Author HM advised on the search strategy and details. The search phrases used to conduct the review were "prenatal cannabis exposure," "neonatal outcomes," and "animal model." Search criteria were also narrowed by eliminating the studies that reported *only* on the effect of the PCE in combination with another drug such as alcohol or nicotine/tobacco.

Ultimately, 15 research articles were included for aim 1 (human studies) and 14 for aim 2 (animal studies). Search criteria were also specific to neonatal outcomes, rather than maternal and other developmental or long-term effects of PCE, which were beyond the scope of this review. Table 7.1 shows the articles using human subjects listed in chronological order from earliest published year to latest, specifying the neonatal effects. Table 7.2 shows the articles using animal models listed in chronological order from earliest published year to latest, depicting the neonatal effects of cannabis for that particular year. Neonatal outcomes circumscribed to LBW, PTB, and NICU admission for aim 1. For Aim 2, we focused on LBW, PTB, and other behavioral or molecular outcomes.

4 Results

4.1 Aim 1: Studies in Humans Characterizing Neonatal Outcomes Following PCE (Table 7.1)

For the human (clinical) studies, the focus was on the three most prevalent clinical variables studied among neonates: LBW, PTB, and NICU admission.

4.1.1 Low Birth Weight (LBW)

Out of the 15 studies listed in Table 7.1, 10 reported on LBW as an outcome. All 10 reported that PCE is associated with LBW among neonates. A study by Nguyen et al. reported that, compared with nonusers, heavy cannabis users (weekly or more) were twice as likely to deliver an LBW infant (Nguyen and Harley 2022). This

Table 7.1 Human (clinical) studies

Number	Author, research article, year	Outcomes (low birth weight, preterm birth, NICU admission)				Concurrent and under reported tobacco exposure
		Low BW	PTB	NICU	PCE effect	
1	El Marroun et al., Intrauterine cannabis exposure affects fetal growth trajectories: the Generation R Study (2009)	Y	N/A	N/A	Negative	Yes
2	van Gelder et al., Characteristics of pregnant illicit drug users and associations between cannabis use and perinatal outcome in a population-based study (2010)	Y	Y	N/A	Neutral	Yes
3	Hayatbakhsh et al., Birth outcomes associated with cannabis use before and during pregnancy (2011)	Y	Y	Y	Negative	Yes
4	Bonello et al., Mental and behavioral disorders due to substance abuse and perinatal outcomes: a study based on linked population data in New South Wales, Australia (2014)	Y	Y	Y	Negative	Yes
5	Leemaqz et al., Maternal marijuana use has independent effects on risk for spontaneous preterm birth but not other common late pregnancy complications (2015)	N/A	Y	N/A	Negative	Yes
6	Bandoli et al., Cannabis-related diagnosis in pregnancy and adverse maternal and infant outcomes (2017)	Y	Y	Y	Negative	No
7	Crume et al., Cannabis use during the perinatal period in a state with legalized recreational and medical marijuana: the association between maternal characteristics, breastfeeding patterns, and neonatal outcomes (2018)	Y	Y	Y	Negative	No
8	Corsi et al., Association between self-reported prenatal cannabis use and maternal, perinatal, and neonatal outcomes (2019)	N/A	Y	Y	Negative	Yes
9	Singh et al., Prevalence and outcomes of prenatal recreational cannabis use in high-income countries: a scoping review (2019)	N/A	Y	N/A	Neutral	Yes
10	Koto et al., Maternal cannabis use during pregnancy and maternal and neonatal outcomes: a retrospective cohort study (2022)	Y	Y	Y	Negative	Yes

(continued)

Table 7.1 (continued)

Number	Author, research article, year	Outcomes (low birth weight, preterm birth, NICU admission)				Concurrent and under reported tobacco exposure
		Low BW	PTB	NICU	PCE effect	
11	Luke et al., Cannabis use in pregnancy and maternal and infant outcomes: a Canadian cross-jurisdictional population-based cohort study	N/A	Y	N/A	Negative	Yes
15	Nguyen et al., Prenatal cannabis use and infant birth outcomes in the pregnancy risk assessment monitoring system (2022)	Y	N	N/A	Negative/ neutral	No
12	Prewitt et al., Impact of prenatal cannabis use disorder on perinatal out	N/A	N/A	Y	Negative	No
13	Brik et al., Cannabis exposure during pregnancy and perinatal outcomes: A cohort study (2024)	Y	Y	Y	Negative	No
Percent %		100	92	100		

study also noted that the odds of having an overweight (above 4000 g) infant at birth was 70% less for women who used cannabis compared to those who abstained (Nguyen and Harley 2022). Furthermore, the study by Nguyen et al. examined combined cannabis and cigarette use and found that prenatal exposure to both substances further increased the likelihood of LBW compared with no use, and the observed increased odds were greater than for either substance alone (Nguyen and Harley 2022).

4.1.2 Preterm Birth (PTB)

Thirteen studies reported on PTB. Of those 13 studies, 11 reported a higher prevalence of PTB among neonates of mothers who used cannabis during pregnancy compared to those who abstained (see Table 7.1) (Hayatbakhsh et al. 2012). The remaining two studies (Crume et al. and Nguyen et al.) did not find a significant association between PCE and PTB.

One study by Leemaqz et al. found that women who continue to use cannabis at 20 weeks' gestation were five times more likely to deliver preterm than those who do not (Leemaqz et al. 2016). In a large retrospective study of 15,321 patients diagnosed with cannabis use disorder (excluding tobacco, alcohol, or any other substance co-use) in pregnancy (Bandoli et al. 2021), risk for very PTB (<32 weeks of gestation) was the most common outcome among women with cannabis use disorder (Bandoli et al. 2021). Compared to women without cannabis use disorder, women with cannabis use disorder were more likely to identify as non-Hispanic

Table 7.2 Animal (preclinical) studies

Number	Author, research article, year	Outcomes (low birth weight, preterm birth, different variables)				Research study specifications				
		LBW	PTB	Different variables	PCE effect	Dose (of THC, WIN or CBD)	Type of drug (%THC)	Route of administration	Duration of exposure	Systemic review
1	Campolongo et al., Preclinical Study: Perinatal exposure to delta-9-tetrahydrocannabinol causes enduring cognitive deficits associated with alteration of cortical gene expression and neurotransmission in rats (2007)	No effect	No effect	Long-lasting alterations of cortical genes related to glutamatergic and noradrenergic systems, associated with a decrease in the cortical extracellular levels of both neurotransmitters (cognitive impairment)	Negative/ neutral	Daily dose (5 mg/kg, per os)	THC (Sigma, Milan, Italy) was dissolved in sesame oil (5 mg/mL)	Orally administered through a buccopharyngeal cannula	Rat dams were treated, from gestational day 15 to postnatal day 9	No
2	Trezza et al., Effects of perinatal exposure to delta-9-tetrahydrocannabinol on the emotional reactivity of the offspring: a longitudinal behavioral study in Wistar rats (2008)	No effect	No effect	Exposure to THC during pregnancy and lactation altered social play behavior in the adolescent offspring	Negative / neutral	Daily dose (2.5–5 mg/kg)	THC (Sigma, Milan, Italy) was dissolved in sesame oil (5 mg/mL)	Orally administered through a buccopharyngeal cannula	Rat dams were treated, from gestational day 15 to postnatal day 9	No

Number	Author, research article, year	Outcomes (low birth weight, preterm birth, different variables)				Research study specifications				
		LBW	PTB	Different variables	PCE effect	Dose (of THC, WIN or CBD)	Type of drug (%THC)	Route of administration	Duration of exposure	Systemic review
3	Campolongo et al., Developmental consequences of perinatal cannabis exposure: behavioral and neuroendocrine effects in adult rodents (2011)	N/A	N/A	Atypical locomotor activity, cognitive impairments, altered emotional behavior, and enhanced sensitivity to drugs of abuse in the adult rodent offspring. Some of the observed behavioral abnormalities might be related to alterations in stress hormone levels induced by maternal cannabis exposure	Negative	N/A	N/A	N/A	N/A	Yes
4	Calvigioni et al., Neuronal substrates and functional consequences of prenatal cannabis exposure (2014)	N/A	N/A	Significant molecular modifications to neurodevelopmental programs leading to neurophysiological and behavioral abnormalities	Negative	N/A	N/A	N/A	N/A	Yes

(continued)

Table 7.2 (continued)

Number	Author, research article, year	Outcomes (low birth weight, preterm birth, different variables)				Research study specifications				
		LBW	PTB	Different variables	PCE effect	Dose (of THC, WIN or CBD)	Type of drug (%THC)	Route of administration	Duration of exposure	Systemic review
5	Benevenuto et al., Recreational use of marijuana during pregnancy and negative gestational and fetal outcomes: an experimental study in mice (2017)	Reduced birth weight	N/A	The # of male pups per litter was higher and placental wet weight was increased and fetal to placental weight ratio was decreased in male fetuses, showing a sex-specific effect	Negative	5 min to marijuana smoke (0.2 g of *Cannabis*)	Cannabis sativa	Nose-only exposure (maternal inhalation)	Pregnant mice were exposed gestational day (GD) 5.5 to GD17.5	No
6	Tirado-Muñoz et al., Effects of cannabis exposure in the prenatal and adolescent periods: preclinical and clinical studies in both sexes (2020)	Reduced birth weight	N/A	Altered mesolimbic dopaminergic and hippocampal glutamatergic systems and interference with the prefrontal cortex maturation	Negative	N/A	N/A	N/A	N/A	Yes

Number	Author, research article, year	Outcomes (low birth weight, preterm birth, different variables)				Research study specifications				
		LBW	PTB	Different variables	PCE effect	Dose (of THC, WIN or CBD)	Type of drug (%THC)	Route of administration	Duration of exposure	Systemic review
7	Scheyer et al., Cannabinoid exposure via lactation in rats disrupts perinatal programming of the gamma-aminobutyric acid trajectory and select early-life behaviors (2020)	N/A	N/A	Retardation of early milestone of development, delaying the trajectory of GABA's polarity transition and altering early-life communication.	Negative	WIN (0.5 mg/kg/day) alone or with AM (0.5 mg/kg/day), or with THC (2 mg/kg/day). WIN, THC, or AM were suspended in 1:1:18 dimethyl sulfoxide, cremophor, and saline, and injected at 1 mL/kg	Synthetic cannabimimetic WIN 55,212–2 (WIN) or Δ^9-tetrahydrocannabinol	Subcutaneous injection	Rat dams were treated during the first 10 days of postnatal development	No
8	Lee et al., Exposure to Δ9-tetrahydrocannabinol during rat pregnancy leads to impaired cardiac dysfunction in postnatal life (2021)	Reduced birth weight	No effect	Smaller hearts in offspring relative to body weight	Negative	3 mg/kg of Δ^9-THC or saline	Δ^9-tetrahydrocannabinol	Intraperitoneal injection	Pregnant Wistar rats were treated on gestational days 6 to 22	No

(continued)

Table 7.2 (continued)

Number	Author, research article, year	Outcomes (low birth weight, preterm birth, different variables)				Research study specifications				
		LBW	PTB	Different variables	PCE effect	Dose (of THC, WIN or CBD)	Type of drug (%THC)	Route of administration	Duration of exposure	Systemic review
9	Pinky et al., Effects of prenatal synthetic cannabinoid exposure on the cerebellum of adolescent rat offspring (2021)	N/A	N/A	Enhancing mitochondrial function and promoting neuronal survival of cerebellum	Positive	WIN55,212–2, 2 mg/kg body weight/day at a rate of 0.25 μL/h until the pups were born	Synthetic cannabinoid agonist WIN55,212–2	Subcutaneous implantation of an osmotic mini pump (Alzet 2004). The pump delivered either vehicle (NMP) or the synthetic cannabinoid	Pregnant rats	No
10	Wanner et al., Developmental cannabidiol exposure increases anxiety and modifies genome-wide brain DNA methylation in adult female mice (2021)	No effect	N/A	Sex-specific increase in anxiety behavior affecting female offspring	Negative/ positive	20 mg/kg CBD or vehicle daily from 2 weeks prior to mating through gestation and lactation	Pharmaceutical-grade CBD	Oral administration of CBD diluted to 10 mg/mL concentration in honey	Nulliparous female wild-type Agouti viable yellow (A^{vy}) mice	No
11	Cáceres et al., Effects of prenatal cannabinoids exposure upon placenta and development of respiratory neural circuits (2023)	N/A	N/A	Influence both neural and nonneural tissues and induce embryo–fetal pathological conditions in critical processes such as neural respiratory control	Negative	N/A	N/A	N/A	N/A	Yes

Number	Author, research article, year	Outcomes (low birth weight, preterm birth, different variables)				Research study specifications				
		LBW	PTB	Different variables	PCE effect	Dose (of THC, WIN or CBD)	Type of drug (%THC)	Route of administration	Duration of exposure	Systemic review
12	Patrone et al., Sex- and age-specific respiratory alterations induced by prenatal exposure to the cannabinoid receptor agonist WIN 55,212-2 in rats (2023)	N/A	N/A	An altered hypoxic chemoreflex was observed in P0 (hyperventilation) and P6–7 (hypoventilation) males, which was absent in females	Negative / neutral	1st group vehicle (DMSO 50%, diluted in sterile water) second group treated with synthetic cannabinoid WIN dose of 0.5 mg/kg/day	Synthetic cannabinoid (WIN 55,212-2 [WIN]	Osmotic pump implanted subcutaneously	Nulliparous pregnant female Wistar rats	No

(continued)

Table 7.2 (continued)

Number	Author, research article, year	Outcomes (low birth weight, preterm birth, different variables)				Research study specifications				
		LBW	PTB	Different variables	PCE effect	Dose (of THC, WIN or CBD)	Type of drug (%THC)	Route of administration	Duration of exposure	Systemic review
13	Ginder et al., Maternal cannabis use alters excitatory inputs to corticostriatal efferent neurons in rat offspring (2024)	N/A	N/A	Increased the frequency of spontaneous glutamate release selectively in females and eliminated the sex difference in excitatory to inhibitory tone suggesting sex-specific changes in the presynaptic release mechanisms of glutamate. In contrast, PCE decreased the amplitude of glutamatergic EPSCs in both sexes suggesting a common change in postsynaptic receptor expression or trafficking.	Negative	Twice daily, hour-long self-administration sessions in a vapor chamber room	Cannabis extract: 69.81% THC, 0.89% tetrahydrocannabivarin (THCV), 0.83% cannabichromene (CBC), 2.69% cannabigerol (CBG), 1.51% cannabinol (CBN), and 0.73% Δ^8 tetrahydrocannabinol (Δ^8THC)	Vapor self-administration	Nulliparous female Sprague Dawley rats, start 10 days before mating until 24–48 h prior to giving birth prior to birth	No

Number	Author, research article, year	Outcomes (low birth weight, preterm birth, different variables)				Research study specifications				
		LBW	PTB	Different variables	PCE effect	Dose (of THC, WIN or CBD)	Type of drug (%THC)	Route of administration	Duration of exposure	Systemic review
14	Roeder et al., Vaporized Δ9-THC in utero results in reduced birthweight, increased locomotion, and altered wake- cycle activity dependent on dose, sex, and diet in the offspring (2024)	Reduced birth weight	No effect	Hyperactivity in the HDTHC groups, particularly in males treated with HFD, while the LDTHC groups displayed increased open-field activity earlier in adolescence but not later	Negative	10 mg THC, 40 mg THC, or air	Δ^9-tetrahydrocannabinol	Daily 10-min inhalations	Pregnant rats exposed from gestational day 2 through birth	No
	Percent %	57%	0%							

White, Black, or other/multiple races, be less than 34 years of age, use public insurance, have less than 12 years of education, have a mental health diagnosis, and have preexisting hypertension (Bandoli et al. 2021).

4.1.3 NICU Admission

NICU admission was reported in nine out of 15 studies. Eight of the nine studies reported greater rate of NICU admission of neonates in mothers who used cannabis during pregnancy compared to the ones who did not. One of the nine studies, by Crume et al., reported no association between PCE and NICU admission (Crume et al. 2018). A study by Hayatbakhsh et al. concluded that 2.7% of neonatal admissions could be prevented if pregnant women stopped using cannabis during pregnancy (Hayatbakhsh et al. 2012). Furthermore, the studies by Koto et al. and Nguyen et al. found that the link between PCE and NICU admission may be mediated through increased risk for neonatal respiratory depression at birth, major anomalies, fetal growth abnormalities, and preterm birth (Koto et al. 2022; Gunn et al. 2016).

4.2 Aim 2: Review of Preclinical Studies Describing Neonatal Outcomes Following Cannabis Use During Pregnancy (Table 7.2)

Aim 2 included 14 preclinical (animal model) studies. The studies used pregnant rat dams or pregnant mice. The studies varied in the route of administration, type of THC (synthetic versus Δ^9-tetrahydrocannabinol) or CBD used, and dosage and duration of drug exposure. The routes of administration included buccopharyngeal oral cannula, maternal inhalation, vapor self-administration, subcutaneous injection, intraperitoneal injection, and subcutaneous implantation of an osmotic minipump. The dosage of the drug varied from 0.5 mg/kg/day to 20 mg/kg/day. Duration of exposure also varied, but pregnant dams were treated as early as the gestational day 2, 10.5, or 15 through to postnatal day 9 or 10.

Among the 14 animal studies, the researchers used various compounds: THC dissolved in sesame oil, cannabis sativa, synthetic cannabinoid agonist WIN55,212–2, pharmaceutical-grade CBD diluted in honey, and a mixture of cannabis extract. Cannabis extract consisted of 69.81% THC, 0.89% tetrahydrocannabivarin (THCV), 0.83% cannabichromene (CBC), 2.69% cannabigerol (CBG), 1.51% cannabinol (CBN), and 0.73% Δ^8 tetrahydrocannabinol (Δ^8THC). In addition to assessing birthweight and gestational age at birth, animal studies also reported on molecular, physiological, and behavioral effects of PCE.

4.2.1 Low Birth Weight (LBW)

Seven out of the 14 studies reported on birth weight of pups. Four out of those seven studies reported reduced birth weight following PCE and three studies reported no significant effects. It is important to note that two of the three studies that reported no effect of PCEs on birth weight of pups were conducted by the same research group; the third study administered CBD.

Of the studies reporting LBW following PCE, the study by Benevenuto et al. reported that male pups from the cannabis group weighed 14.3% less (0.15 g) than male pups from the control group. In relation to female pups, no differences were observed in birth weight related to PCE (Benevenuto et al. 2017). Another study by Lee et al. reported that PCE did not affect male and female offspring birth weights differently. However, intraperitoneal injection of 3 mg/kg of Δ^9-THC led to symmetrical intrauterine growth restriction, which was reflected by a proportional decrease in birth weight (Natale et al. 2020; Lee et al. 2021). This outcome was linked with altered placental vasculature and nutrient transport, which in return attributed to the fetal growth deficits and LBW (Natale et al. 2020; Lee et al. 2021).

4.2.2 Preterm Birth (PTB)

PTB was reported as an outcome in four preclinical studies. All four studies reported no effects of PCE on PTB or gestational length of rat pups. Also, all four studies used THC, of which two (Campolongo et al. and Trezza et al.) used oral administration through a buccopharyngeal cannula (Campolongo et al. 2007; Trezza et al. 2008). One study used intraperitoneal injection (Lee et al. 2021) and one study used daily 10-min inhalations (Roeder et al. 2024). The study by Roeder et al. also tested for dose effects of PCE on gestational age. The authors found that mean gestational period was not significantly altered following a low- (10 mg) or high-dose of THC (40 mg) treatment during pregnancy (Roeder et al. 2024).

4.2.3 Cognitive and Behavioral Outcomes

Out of 14 animal studies, six mentioned cognitive impairment and behavioral abnormalities.

In a study by Campolongo and colleagues, the effects of moderate exposure to THC on offspring cognitive abilities were examined. The authors reported long-term memory impairments following PCE, as revealed by the inhibitory avoidance test (Campolongo et al. 2007). The same group also documented a disruption of short-term olfactory memory following PCE (Campolongo et al. 2007). Similarly in a study by Trezza et al., 12-day old pups from a group of rats exposed to THC during both pregnancy and lactation displayed increased anxiety-like behavior in the isolation-induced ultrasonic vocalization test, compared to unexposed rats (Trezza et al. 2008). Furthermore, when the rats with PCE reached adolescence, they were

significantly less engaged in play behavior than controls (Trezza et al. 2008). Rats exposed to THC during pregnancy and lactation were associated with modified social play behavior during adolescence as well (Trezza et al. 2008). The increased emotionality found in infant and adolescent pups in the THC exposed-group was long lasting (Trezza et al. 2008).

Another study by Wanner et al. reported that the female F1 offspring prenatally exposed to CBD showed increased anxiety-like behavior, but improved memory compared to the control group (Wanner et al. 2021). Further, young adult female F1 offspring exposed to CBD during gestation and preweaning buried nearly twice as many marbles as their unexposed peers in the control group, indicating an increase in anxiety-like behavior (Wanner et al. 2021).

In a study by Roeder, rats prenatally treated with low-dose THC generally had lower dark-cycle activity compared with controls. However, these results were not observed when increasing the THC dose (Roeder et al. 2024). Hyperactivity was observed in the high-dose THC groups, particularly in males treated with a high-fat diet. In contrast, the low-dose THC groups displayed increased anxiety-like behavior, evidenced by greater open-field activity earlier in adolescence but not later (Roeder et al. 2024). A dose-dependent effect on circadian cycle activity was also reported with the low-dose THC rats having decreased dark-cycle activity, where they normally are awake. Moreover, high-fat diet male rats in the PCE group tended to eat and weigh more than controls (Roeder et al. 2024). In summary, the effects of PCE on hyperactivity, anxiety-like behavior, circadian cycle activity were sex- and dose-specific.

4.2.4 Molecular Outcomes

The study by Ginder et al. reported that treatment of pregnant dams with vaporized cannabis resulted in an increased frequency of spontaneous glutamate release selectively in female offspring (Ginder et al. 2024). However, PCE eliminated the sex difference in excitatory to inhibitory tone, suggesting sex-specific changes in the presynaptic release mechanisms of glutamate (Ginder et al. 2024). In contrast, PCE decreased the amplitude of glutamatergic spontaneous excitatory and inhibitory postsynaptic currents in both sexes, suggesting a common change in postsynaptic receptor expression or trafficking (Ginder et al. 2024).

Another study by Campolongo et al. reported that exposure to THC at a dose of 5 mg/kg was not correlated with congenital disorders and/or overt signs of toxicity in offspring (Campolongo et al. 2007). In that study, rats received daily doses from gestational day 15 to postnatal day 9 at a dose which aimed to reflect moderate THC exposure in humans, including corrections for differences in route of administration and body surface area. PCE was associated with long-term functional alterations in rat prefrontal cortex, measured by gene expression analysis and measurement of glutamate and norepinephrine extracellular levels (Campolongo et al. 2007). Perinatal THC exposure induced long-lasting cortical gene mutations that were part of the glutamatergic and noradrenergic systems. These mutations are known to cause a further decrease in the cortical extracellular levels of these

neurotransmitters. Such alterations may account, at least in part, for the cognitive dysfunctions displayed by adult rats perinatally exposed to THC (Campolongo et al. 2007). In conclusion, PCE was not associated with gross malformations or signs of toxicity. However, the study by Campolongo suggests that PCE is associated with long-term alterations in rats' prefrontal cortex (Campolongo et al. 2007).

A study by Wanner et al. showed sex-specific genome mutations in brain DNA, including methylation, in adult mouse offspring that were exposed to human-relevant doses of CBD in utero that are equivalent to 1.5 mg/kg/day (Wanner et al. 2021). Mice F1 offspring prenatally-exposed to CBD exhibited thousands of differentially methylated loci (DMLs) that were identified in both brain regions evaluated—the cerebral cortex and hippocampus. While a mixture of hyper- and hypomethylation was identified in this study (Wanner et al. 2021), hypomethylation was predominant especially in the F1 hippocampus. Tissues of the F1 generation showed hypomethylation with 66.6% of DMLs being hypomethylated in hippocampus and 60.6% being hypomethylated in cortex.

4.2.5 Sex-Specific and Other Outcomes

Sex-specific consequences of PCE were mentioned in five out of 14 preclinical studies. It is important to note that each of those five studies used a different type of drug reported as following: cannabis sativa, THC, synthetic cannabinoid (WIN 55,212–2 [WIN]), pharmaceutical-grade CBD and cannabis extract (Benevenuto et al. 2017; Roeder et al. 2024; Wanner et al. 2021; Ginder et al. 2024; Patrone et al. 2023).

A study by Benevenuto et al. used a maternal inhalation model of PCE. The authors found a higher number of male pups per litter following PCE, as well as an increase in placental wet weight (Benevenuto et al. 2017). Furthermore, the fetal to placental weight ratio was decreased in male (but not female) fetuses following PCE, suggesting a sex-specific effect (Benevenuto et al. 2017). Patrone et al. reported that excess stimulation of the ECS with the synthetic cannabinoid WIN during pregnancy has prolonged and sex-specific outcomes for the respiratory control system (Patrone et al. 2023). For instance, altered hypoxic chemoreflex was noted in P0 (hyperventilation) and P6–7 (hypoventilation) males, which was otherwise not noted in females (Patrone et al. 2023).

One study found that the administration of the WIN to pregnant rat dams altered the cerebellum of offspring; specifically, enhanced mitochondrial function and promoted neuronal survival were noted compared to vehicle (Pinky et al. 2021).

5 Discussion

The effects of PCE on neonates is an important public health issue, given the recent rise in rates of cannabis use across many demographics, including pregnant women, as well as widespread increases in cannabis potency, legalization, and

decriminalization. While research on this topic has grown in recent years, encompassing both animal and human studies, the effects of PCE on neonatal outcomes have remained unclear. Here, we summarize the current literature and identify gaps and limitations and directions for future research.

5.1 Summary of Aim 1 Results: Human Studies

In our literature review of 15 clinical (human) studies, we focused on three common neonatal outcomes associated with PCE: LBW, PTB, and NICU admission. We found that the majority of studies reported LBW, greater risk of PTB, and greater risk of NICU admission among neonates exposed to cannabis, compared to their unexposed peers. The Surgeon General's current recommendations are that cannabis use during pregnancy can affect the developing fetus and is associated with adverse outcomes, including LBW (American Psychiatric Association 2019). Both the American College of Obstetricians and Gynecologists and the American Academy of Pediatrics recommend against using cannabis during pregnancy (ACOG 2017).

All nine clinical studies reporting on neonatal birth weight reported LBW in neonates with PCE compared to those without (Bandoli et al. 2021). The association between PCE and LBW is important given that LBW is an important determinant of child survival and development, as well as long-term consequences like the onset of noncommunicable diseases in the life course (World Health Organization and United Nations Children's Fund (UNICEF) 2004). A large amount of infant mortality and morbidity can be prevented by addressing the factors associated with LBW, especially preventable factors like PCE.

The majority of the 12 studies (92%) reporting on PTB found an increased likelihood of PTB in neonates with PCE compared to those without. This is important because premature babies are more likely to have long-term health problems compared to full-term infants. PTB (i.e., <37 weeks of gestation) is the leading cause of newborn mortality and is a risk factor for temporary or long-lasting adverse health consequences (Samuel et al. 2019). Premature infants also are at greater risk of developing long-term cognitive and developmental disabilities or even pass away from sudden infant death syndrome (Samuel et al. 2019). Two of the most serious problems related to PTB are respiratory distress and immature brains (Samuel et al. 2019). The brain is the last major organ to mature when it comes to reaching the final stages of the development before a neonate leaves the womb. The immature neurons continue to develop even after the baby is born. The more prematurely the baby is born, the greater likelihood that potential internal bleeding may impact the brain. In general, the earlier the preterm infant is born, the greater the chances for the infant intubation, which may result in a longer NICU stay.

We found only one study that reported null effects of PCE on PTB in their main analyses. In this retrospective study of 32,583 singleton pregnancies, in a crude analysis, cannabis exposure during pregnancy was associated with significantly

increased risk of PTB (Nguyen and Harley 2022). However, these associations were significantly attenuated after controlling for confounders, including prenatal tobacco exposure. In adjusted logistic regression models, no significant association was observed between PCE and PTB (Nguyen and Harley 2022). This study suggests that adjusting for confounding variables may reduce or eliminate the association between PCE and PTB.

All eight studies that reported on NICU admission demonstrated a greater risk of NICU admission among neonates with PCE compared to those without. Prolonged length of stay in the NICU may increase the incidence of neonatal complications and even mortality, and places a significant economic burden on families and strain on healthcare systems (Fu et al. 2023). The study by Hayatbakhsh et al. concluded that 2.7% of NICU admissions could be prevented if women abstained from cannabis use during pregnancy—a statistic which could have significant public health and economic implications. The study by Koto et al. suggested that the link between PCE and NICU admission may be mediated through the increased risk for neonatal respiratory depression at birth, major anomalies, fetal growth abnormalities, and PTB. This suggests that PCE may affect the length of stay in NICU, which in turn, may increase the risk of neonatal complications and financial burden on families.

5.1.1 Limitations of Human Studies

Studies in humans are limited by the presence of many confounding factors (e.g., lifestyle, socioeconomic and nutritional status, age), which may complicate our understanding of the association between PCE and neonatal outcomes. Although we excluded the research articles that directly included tobacco users, many studies did not report or control for prenatal tobacco exposure (please refer to Table 7.1 for more details). Therefore, it is difficult to disentangle the effects of prenatal cannabis from prenatal tobacco and future studies should report concurrent use of tobacco and other substances. In addition, future research should better characterize substance use in depth, e.g., onset, duration, route of administration, frequency of use, cannabis content, as well as collect and adjust for potential confounders.

Due to the vulnerable nature of pregnant women and neonates as research subjects, there are potential ethical limitations and considerations. For example, human studies primarily rely on self-report, which may be biased by underreporting or poor instrument design (Day and Richardson 1991). Many women may be reluctant to reveal their substance use that includes the illicit nature of some substances due to legal consequences, shame, guilt, and/or recall problems (Koren et al. 2002). Likewise, observed discrepancies in results might be due to the variation in the study design and evaluation of cannabis use (e.g., retrospective vs. prospective) or level of adjustment for confounding variables. In addition, the state and legal status of cannabis where the study is being conducted may also be important, given that statewide restrictions may affect the reporting of substance use.

An additional or alternative measure of cannabis use is biological verification in urine, blood, or hair. While these are often more objective than self-report, they have

limitations as they may underestimate the frequency of drug use over an extended period. Indeed, drug metabolites are measurable in bodily fluids such as blood or urine for only a few days after the drugs were last used (Chiang and Hawks 1986). Testing of meconium may address some of these limitations, by detecting maternal cannabis use during the last 20 weeks of gestation (Koren et al. 2002; Maciag et al. 2022). Meconium, the first neonatal feces, is a thick dark green mucoid material that begins to form and collect in the small intestine between the 12th and 16th week of gestation. Meconium is preferred over neonatal hair, urine, amniotic fluid, and blood as a marker of chronic in utero exposure due to the broad time window of exposure it provides that reflects the last trimester of fetal development (Moore et al. 1998). Collection of meconium represents no health risk to the mother or neonate; however, timing of collection is unpredictable due to retention for several days after birth or passage while the fetus is still in utero, or during delivery. Nonetheless, meconium may be a valuable marker of chronic in utero cannabis exposure in human studies (Ostrea et al. 1988).

Importantly, cannabinoids readily cross the placenta and appear in human breast milk, resulting in both fetal and neonatal exposure (Baker et al. 2018). Therefore, it will be important for future studies to take postnatal exposures into consideration, and to differentiate effects of cannabis exposure in utero and/or postnatal exposure through breastmilk.

In sum, human studies revealed that PCE may have adverse effects on neonatal outcomes. The burgeoning cannabis movement in the US and the momentum behind cannabis law reform both contribute to the critical need to adequately understand the risk of cannabis use on neonatal outcomes.

5.2 Summary of Aim 2 Results: Animal Studies

Our literature review of 14 preclinical (animal) studies revealed important similarities and differences to the human studies. LBW results of the animal studies did not completely replicate the results of the human studies; in particular, 100% of human studies included here reported that PCE is associated with LBW compared to only 57% of animal studies. The effects of PCE on PTB observed in human studies were not replicated in the animal studies. While 85% of human studies reported greater risk of PTB following PCE, no animal studies (0%) reported this association. Together, these findings suggest that human and animal studies are relatively mixed regarding effects of PCE on neonatal outcomes.

Animal studies have played a crucial role in elucidating the physiological and developmental effects of PCE. The present review of preclinical studies indicates that there is inconsistency regarding the effects of PCE on neonatal outcomes. Out of seven studies that reported LBW, four reported LBW following PCE; three reported null effects. Of note, two of the three studies reporting null effects administered THC via buccopharyngeal canula, which may cause stress, which also has

several biological effects. The third study reporting null findings administered CBD, which may have distinct effects from THC.

Of the four studies that reported LBW following PCE, two studies delivered the THC via inhalation of the smoke, which may better mimic common human routes of administration. The third study reporting that reported significant effects of PCE on LBW used peritoneal injections of THC. Therefore, preclinical studies that used smoked cannabis routes of administration, which are the most common route of administration in humans, may be more likely to replicate the human studies of LBW and PCE compared to other routes of administration. Interestingly, future studies should investigate whether combustion of any plant material is associated with LBW of neonates, given that prenatal tobacco exposure has also been associated with LBW (Maciag et al. 2022).

All four studies reporting on gestational length reported null effects of PCE. The gestational period in rodents is approximately 21 days long. Many studies fail to measure length differences in hours, which may be more precise than days. This limitation is likely due to the fact that conception and birth time is often unknown, and mating is reported to happen "overnight." Therefore, the complexity of animal gestation, breeding, and handling may contribute to the lack of direct replication of human studies.

The effects of PCE on litter size also were mixed, with some studies reporting null effects while other reports suggested sex-dependent effects. More specifically, in one study, PCE was associated with an increase in the number of males per litter (Benevenuto et al. 2017). These sex-specific effects of PCE were also documented for alterations in the respiratory control system, with males exhibiting greater sensitivity to cannabinoid exposure than females (Patrone et al. 2023). On the other hand, female embryos were more susceptible to the effects of cannabis smoke early in pregnancy, during the period of implantation, compared to males. It is known that male and female embryos differ not only in sex chromosomes but also in the way they convey specific metabolic processes. Differences in sex and autosomal-related gene expression may further conditions the sex-specific susceptibility (Kobayashi et al. 2006; Donjacour et al. 2014). In conclusion, some of the effects of PCE may be sex-dependent—a hypothesis which should be better examined in human studies.

Several animal studies also examined behavioral and molecular changes and effects on different brain areas associated with the PCE. One behavioral study (Wanner et al.) reported null effects of PCE on anxiety-like behavior, but positive effects on memory (Wanner et al. 2021). However, the study used oral CBD and not THC which suggests that CBD might have mixed effects compared to THC.

Wanner et al. also measured methylation and examined molecular changes following PCE. The authors identified thousands of differentially methylated loci in the cortex of F0 females who were directly exposed to CBD in utero, in the absence of anxiety or memory changes (Wanner et al. 2021). Site-specific methylation changes were observed, suggesting that prenatal CBD exposure may have positive or negative effects on gene expression. The magnitude and the direction of these changes strongly depended on the genic region, binding of methylation readers, and other factors. Hypomethylation usually resulted in chromosomal instability,

activation of transposons, and reduced neuronal survival in the CNS. These findings demonstrate that developmental CBD exposure is linked with mixed behavioral outcomes and perturbation of the brain epigenome. Cannabinoid exposure during critical windows of methylation pattern setting may result in permanently altered gene expressions in the brain.

While there is an agreement that PCE results in various neurobehavioral alterations among offspring, the degree of these effects varies across studies. Trezza et al., concluded that PCE induces subtle but long-lasting effects on offspring behavior (Trezza et al. 2008) whereas Campolongo, Calvigioni, and Wanner reported more conspicuous behavioral and neurophysiological abnormalities after PCE, even at low doses (Trezza et al. 2008; Wanner et al. 2021; Campolongo et al. 2011; Calvigioni et al. 2014). Together, these findings suggest that the magnitude of the effect of the PCE may vary based on the specifications of the study drug and route of administration.

Studies focusing on neurological alterations following PCE have centered mostly on the mesolimbic system. A notable exception is a study by Pinky et al., which demonstrated significant effects of PCE on the cerebellum, a brain region involved in motor coordination, associative learning, and processing of temporal operations (Stella 2013). The authors found enhanced mitochondrial function and promotion of neuronal survival following PCE (Pinky et al. 2021). These results contrast with previous studies focusing on other brain regions, particularly the medial prefrontal cortex, mesolimbic dopaminergic and hippocampal glutamatergic systems, where cannabis exposure was associated with adverse effects (Ginder et al. 2024; Tirado-Muñoz et al. 2020). A possible explanation for these discrepancies is that the effects of PCE may vary depending on the brain region involved. Differences may also be attributed to route of administration and drug. For example, unlike other studies, the study Pinky et al. used the synthetic cannabimimetic WIN 55,212–2 (WIN) delivered via subcutaneous implantation of an osmotic mini pump, with the aim of mimicking chronic cannabinoid exposure (Pinky et al. 2021). The mini pump combines the benefits of extended release with high bioavailability. Other studies relied on buccopharyngeal canula, intraperitoneal and subcutaneous injection, as well as self-administration of vapor or daily 10-minute inhalations. Different routes of administration affect the absorption and metabolism of the drug and should therefore be taken into consideration.

Besides the CNS, the cardiovascular and respiratory systems were also reported to be altered by PCE. A study by Lee et al. documented a decreased heart to body ratio at birth following PCE (Lee et al. 2021). This finding is significant considering that LBW is also associated with elevated risk of long-term cardiovascular disease (Barker et al. 1989). Likewise, two additional studies (Caceres and Patrone) showed a lower ability to regulate the proper respiratory system functions following PCE (Patrone et al. 2023; Cáceres et al. 2023). Finally, rats prenatally treated with low-dose THC had lowered dark-cycle activity as compared to controls, suggesting circadian effects. Intriguingly, the group exposed to a high-dose THC did not show this

effect (Roeder et al. 2024). These results suggest that the effects of PCE on respiratory function and circadian cycle activity may be dose-dependent.

5.2.1 Limitations of Animal Studies

Unlike human studies which showed greater risk of PTB among offspring with PCE, animal studies did not consistently report pregnancy length alterations following PCE. One possible explanation for this discrepancy could be that the gestation period in rodents is not a complete reflection of human pregnancy. In particular, the gestational period of rats is much shorter in duration than humans, and many studies often do not measure pregnancy length in hours, which may be more precise than days.

Another potential limitation of animal studies is the route of administration. While smoking and edibles are the most common routes used in humans, animal studies commonly use subcutaneous or intraperitoneal injections. To better mimic human cannabis use, some researchers have incorporated nasal inhalation to mimic smoking, vapor self-administration to mimic vaping and a subcutaneous pump to mimic chronic exposure. The results of these routes of administration more closely resemble the results of human research studies with respect to LBW. However, more research is needed to determine effects of dose, timing, frequency, and chronicity. In addition, it is unclear whether the effects are specific to combustion of cannabis plant material or any plant material (e.g., tobacco).

Animal studies also varied greatly in the type of research drug used. There were five types of "cannabis" used in the animal research studies, which are included in Table 7.2. These include Δ^9-tetrahydrocannabinol (THC), "cannabis sativa," pharmaceutical grade CBD, synthetic cannabinoid (WIN 55,212–2 [WIN]), and cannabis extract. Cannabis extract consisted of: 69.81% THC, 0.89% tetrahydrocannabivarin (THCV), 0.83% cannabichromene (CBC), 2.69% cannabigerol (CBG), 1.51% cannabinol (CBN), and 0.73% Δ^8 tetrahydrocannabinol (Δ^8THC). This variation may substantially affects the outcomes and the drug's effect on animal behavior, as well as the drug's absorption and metabolism.

Another limitation is that cannabis contains many different compounds, which may have differential or synergistic effects on outcomes. Combustion of the female cannabis plant flower and subsequent inhalation of cannabis smoke into the airway delivers 2575 different compounds that are detected, of which 536 are tentatively identified (Graves et al. 2020). Of these identified compounds, 110 different compounds are known to cause negative health effects through carcinogenic, mutagenic, teratogenic, or other toxic mechanisms (Graves et al. 2020). Likewise, the current literature reviews of the effects of synthetic and natural cannabinoid-based drugs further highlights the disparities between them. Synthetic drugs contain a mixture of psychoactive compounds that mostly bind cannabinoid receptors with high potency (Cohen and Weinstein 2018). These synthetic drugs are capable of

replicating the effects of natural cannabis and THC. In addition, these drugs can induce more severe adverse effects (such as respiratory difficulties, hypertension, tachycardia, chest pain, muscle twitches, acute renal failure, anxiety, agitation, psychosis, suicidal ideation, and cognitive impairment) (Cohen and Weinstein 2018). Furthermore, the effects of ingesting pure Δ^9-THC versus CBD (without THC) also differ in their effects on humans, as well as on animals.

Moreover, mice and rats have been used for biomedical animal research for years. They have been the preferred species due to their anatomical and physiological similarity to humans, as well as shorter gestational age. Mice also share common genetic features with humans (Lubs et al. 2012). As such, scientists can manipulate the mouse genome, model specific diseases, test new drugs, and investigate the genetics of diseases before trying them on human models. Rats are commonly used for behavioral studies because they are much more social than mice and their behavior better mimics behavior seen in humans (Lubs et al. 2012). But neither rats nor mice can fully suffice when it comes to mimicking human research studies and their implications on vulnerable populations such as neonates. Future studies could investigate the possibility of using monkeys (primates) to better observe the effects of PCE on animal behavior and other effects. Overall animal models still serve as a significant and essential tool allowing researchers to gain insights into physiological and behavioral effects of PCE.

6 Conclusion

The current review addressed both human and animal studies on the effects of PCE on neonatal outcomes. Human studies generally reported higher rates of PTB, LBW, and NICU admissions following PCE. While animal models largely confirmed the effects on LBW, the findings on PTB were inconsistent, likely due to differences in drug administration, type, and length of exposure. Animal studies also showed that PCE led to neurotransmitter alterations in dopamine, GABA, and the ECS, as well as impaired executive function. However, a positive effect on neuronal survival was observed in preclinical studies using CBD.

Despite efforts to exclude confounding factors, many human studies did not report, screen, or control for significant confounds, such as maternal tobacco exposure. Future longitudinal research carefully identifying and controlling for potential confounds is needed to identify effects that may be specific to PCE. This distinction is crucial for developing therapeutic strategies and informing pregnant women about the risks of cannabis use.

As perinatal cannabis use rises, pregnant and lactating women may struggle to make informed decisions due to mixed perceptions of its benefits and potential harms. In addition, the impact of paternal cannabis exposure before conception remains understudied. Given the prevalence of cannabis use, more research is needed to address its safety and effects on reproductive health, pregnancy, and lactation.

References

ACOG. Marijuana use during pregnancy and lactation [Internet]. 2017. www.acog.org/About-ACOG/ACOG-Departments/.

American Psychiatric Association. Psychiatry.org—U.S. Surgeon General's Warning on Marijuana use and the developing brain. 2019.

Atakan Z. Cannabis, a complex plant: different compounds and different effects on individuals. Ther Adv Psychopharmacol. 2012;2(6):241–54.

Baker T, Datta P, Rewers-Felkins K, Thompson H, Kallem RR, Hale TW. Transfer of inhaled cannabis into human breast milk. Obstet Gynecol. 2018;131(5):783–8.

Bandoli G, Jelliffe-Pawlowski L, Schumacher B, Baer RJ, Felder JN, Fuchs JD, et al. Cannabis-related diagnosis in pregnancy and adverse maternal and infant outcomes. Drug Alcohol Depend. 2021;225:108757.

Barbosa-Leiker C, Burduli E, Smith CL, Brooks O, Orr M, Gartstein M. Daily cannabis use during pregnancy and postpartum in a state with legalized recreational cannabis. J Addict Med. 2020;14(6):467–74.

Barker DJ, Osmond C, Law CM. The intrauterine and early postnatal origins of cardiovascular disease and chronic bronchitis. J Epidemiol Community Health. 1989;43(3):237–40.

Benevenuto SG, Domenico MD, Martins MAG, Costa NS, de Souza ARL, Costa JL, et al. Recreational use of marijuana during pregnancy and negative gestational and fetal outcomes: an experimental study in mice. Toxicology. 2017;376:94–101.

Cáceres D, Ochoa M, González-Ortiz M, Bravo K, Eugenín J. Effects of prenatal cannabinoids exposure upon placenta and development of respiratory neural circuits. Adv Exp Med Biol. 2023;1428:199–232.

Calvigioni D, Hurd YL, Harkany T, Keimpema E. Neuronal substrates and functional consequences of prenatal cannabis exposure. Eur Child Adolesc Psychiatry. 2014;23(10):931–41.

Campolongo P, Trezza V, Cassano T, Gaetani S, Morgese MG, Ubaldi M, et al. Preclinical Study: perinatal exposure to delta-9-tetrahydrocannabinol causes enduring cognitive deficits associated with alteration of cortical gene expression and neurotransmission in rats. Addict Biol. 2007;12(3–4):485–95.

Campolongo P, Trezza V, Ratano P, Palmery M, Cuomo V. Developmental consequences of perinatal cannabis exposure: behavioral and neuroendocrine effects in adult rodents. Psychopharmacology (Berl). 2011;214(1):5–15.

Chambers J, Keyhani S, Ling PM, Hoggatt KJ, Hasin D, Nguyen N, et al. Perceptions of safety of daily cannabis vs tobacco smoking and secondhand smoke exposure, 2017–2021. JAMA Netw Open. 2023;6(8):E2328691.

Chang JC, Tarr JA, Holland CL, De Genna NM, Richardson GA, Rodriguez KL, et al. Beliefs and attitudes regarding prenatal marijuana use: perspectives of pregnant women who report use. Drug Alcohol Depend. 2019;196:14–20.

Chiang CN, Hawks RL. Implications of drug levels in body fluids: basic concepts. NIDA Res Monogr. 1986;73:62–83.

Chiu V, Hall W, Chan G, Hides L, Leung J. A systematic review of trends in US attitudes toward cannabis legalization. Subst Use Misuse. 2022;57(7):1052–61.

Cohen K, Weinstein AM. Synthetic and non-synthetic cannabinoid drugs and their adverse effects—a review from public health prospective. Front Public Health. 2018;6:162.

Cristino L, Becker T, Di Marzo V. Endocannabinoids and energy homeostasis: an update. Biofactors. 2014;40:389–97.

Crume TL, Juhl AL, Brooks-Russell A, Hall KE, Wymore E, Borgelt LM. Cannabis use during the perinatal period in a state with legalized recreational and medical marijuana: the association between maternal characteristics, breastfeeding patterns, and neonatal outcomes. J Pediatr. 2018;197:90–6.

Crume TL, Powers S, Dufford AJ, Kim P. Cannabis and pregnancy: factors associated with cannabis use among pregnant women and the consequences for offspring neurodevelopment and early postpartum parenting behavior. Curr Addict Rep. 2022;9:195–202.
Day NL, Richardson GA. Prenatal marijuana use: epidemiology, methodology issues, and infant outcome. Clin Perinatol. 1991;18(1):77–91.
Donjacour A, Liu X, Lin W, Simbulan R, Rinaudo PF. In vitro fertilization affects growth and glucose metabolism in a sex-specific manner in an outbred mouse model. Biol Reprod. 2014;90(4):80.
Dreher MC, Nugent K, Hudgins R. Prenatal Marijuana exposure and neonatal outcomes in Jamaica: an ethnographic study. Pediatrics. 1994;93:254–60. http://publications.aap.org/pediatrics/article-pdf/93/2/254/984109/254.pdf.
ElSohly MA, Mehmedic Z, Foster S, Gon C, Chandra S, Church JC. Changes in cannabis potency over the last 2 decades (1995-2014): analysis of current data in the United States. Biol Psychiatry. 2016;79(7):613–9.
Everitt W. Global presence of your research at Cannabis 2020 scheduled during April 8–9, 2020 at Sydney, Australia. 2020.
Freeman TP, Craft S, Wilson J, Stylianou S, ElSohly M, Di Forti M, et al. Changes in delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) concentrations in cannabis over time: systematic review and meta-analysis. Addiction. 2021;116:1000–10.
Fu M, Song W, Yu G, Yu Y, Yang Q. Risk factors for length of NICU stay of newborns: a systematic review. Front Pediatr. 2023;11:1121406.
Ginder DE, Weimar H V, Lindberg JEM, Fisher ZDG, Lim MM, Peters JH, et al. Prenatal cannabis effects on corticostriatal neurons maternal cannabis use alters excitatory inputs to corticostriatal efferent neurons in rat offspring. 2024. https://doi.org/10.1101/2024.03.03.583210.
Graves BM, Johnson TJ, Nishida RT, Dias RP, Savareear B, Harynuk JJ, et al. Comprehensive characterization of mainstream marijuana and tobacco smoke. Sci Rep. 2020;10(1):7160.
Gunn JKL, Rosales CB, Center KE, Nuñez A, Gibson SJ, Christ C, et al. Prenatal exposure to cannabis and maternal and child health outcomes: a systematic review and meta-analysis. BMJ Open. 2016;6(4):e009986.
Hayatbakhsh MR, Flenady VJ, Gibbons KS, Kingsbury AM, Hurrion E, Mamun AA, et al. Birth outcomes associated with cannabis use before and during pregnancy. Pediatr Res. 2012;71(2):215–9.
Johnston LD, O'Malley PM. Monitoring the future national survey on drug use, 1975–2021: overview, key findings on adolescent drug use. Ann Arbor: Institute for Social Research, University of Michigan; 2016.
Kannan K, Nattala P, Mahadevan J, Meena KS. Knowledge, attitude, and expectancies related to cannabis use: a cross-sectional community study among college students from Bangalore, Karnataka. Indian J Psychol Med. 2024;46(3):221–7.
Klumpers LE, Thacker DL. A brief background on cannabis: from plant to medical indications. J AOAC Int. 2019;102(2):412–20.
Kobayashi S, Isotani A, Mise N, Yamamoto M, Fujihara Y, Kaseda K, et al. Comparison of gene expression in male and female mouse blastocysts revealed imprinting of the X-linked gene, Rhox5/Pem, at preimplantation stages. Curr Biol. 2006;16(2):166–72.
Koren G, Chan D, Klein J, Karaskov T. Estimation of fetal exposure to drugs of abuse, environmental tobacco smoke, and ethanol. Ther Drug Monit. 2002;24(1):23–5.
Koto P, Allen VM, Fahey J, Kuhle S. Maternal cannabis use during pregnancy and maternal and neonatal outcomes: a retrospective cohort study. BJOG. 2022;129(10):1687–94.
Lee K, Laviolette SR, Hardy DB. Exposure to Δ9-tetrahydrocannabinol during rat pregnancy leads to impaired cardiac dysfunction in postnatal life. Pediatr Res. 2021;90(3):532–9.
Leemaqz SY, Dekker GA, McCowan LM, Kenny LC, Myers JE, Simpson NAB, et al. Maternal marijuana use has independent effects on risk for spontaneous preterm birth but not other common late pregnancy complications. Reprod Toxicol. 2016;62:77–86.
Lo JO, Hedges JC, Girardi G. Impact of cannabinoids on pregnancy, reproductive health, and offspring outcomes. Am J Obstet Gynecol. 2022;227:571–81.

Lubs HA, Stevenson RE, Schwartz CE. Fragile X and X-linked intellectual disability: four decades of discovery. Am J Hum Genet. 2012;90(4):579–90.
Maciag MC, Yousuf A, Hauptman M. Impact of prenatal exposure to smoking on child health. Clin Obstet Gynecol. 2022;65(2):388–96.
Mead A. Legal and regulatory issues governing cannabis and cannabis-derived products in the United States. Front Plant Sci. 2019;10:697.
Moore C, Negrusz A, Lewis D. Determination of drugs of abuse in meconium. J Chromatogr B Biomed Sci Appl. 1998;713(1):137–46.
Moore KL et al. The Developing Human: Clinically Oriented Embryology, 9th ed. Philadelphia, PA: Saunders, an imprint of Elsevier, Inc. 2013.
Natale BV, Gustin KN, Lee K, Holloway AC, Laviolette SR, Natale DRC, et al. Δ9-tetrahydrocannabinol exposure during rat pregnancy leads to symmetrical fetal growth restriction and labyrinth-specific vascular defects in the placenta. Sci Rep. 2020;10(1):544.
Nguyen VH, Harley KG. Prenatal cannabis use and infant birth outcomes in the pregnancy risk assessment monitoring system. J Pediatr. 2022;240:87–93.
Ostrea EM, Parks PM, Brady MJ. Rapid isolation and detection of drugs in meconium of infants of drug-dependent mothers. Clin Chem. 1988;34(11):2372–3.
Patrone LGA, Ferrari GD, da Silva RM, Alberici LC, Lopes NP, Stabile AM, et al. Sex- and age-specific respiratory alterations induced by prenatal exposure to the cannabinoid receptor agonist WIN 55,212-2 in rats. Br J Pharmacol. 2023;180(13):1766–89.
Pinky PD, Majrashi M, Fujihashi A, Bloemer J, Govindarajulu M, Ramesh S, et al. Effects of prenatal synthetic cannabinoid exposure on the cerebellum of adolescent rat offspring. Heliyon. 2021;7(4):e06730.
Pisanti S, Bifulco M. Medical cannabis: a plurimillennial history of an evergreen. J Cell Physiol. 2019;234(6):8342–51.
Pizzol D, Demurtas J, Stubbs B, Soysal P, Mason C, Isik AT, et al. Relationship between cannabis use and erectile dysfunction: a systematic review and meta-analysis. Am J Mens Health. 2019;13(6):1557988319892464.
Quinn JA, Munoz FM, Gonik B, Frau L, Cutland C, Mallett-Moore T, et al. Preterm birth: case definition & guidelines for data collection, analysis, and presentation of immunization safety data. Vaccine. 2016;34(49):6047–56.
Roeder NM, Penman SL, Richardson BJ, Wang J, Freeman-Striegel L, Khan A, et al. Vaporized Δ9-THC in utero results in reduced birthweight, increased locomotion, and altered wake-cycle activity dependent on dose, sex, and diet in the offspring. Life Sci. 2024;340:122447.
Russo EB. History of cannabis and its preparations in saga, science, and sobriquet. Chem Biodivers. 2007;4:1614–48.
Samuel TM, Sakwinska O, Makinen K, Burdge GC, Godfrey KM, Silva-Zolezzi I. Preterm birth: a narrative review of the current evidence on nutritional and bioactive solutions for risk reduction. Nutrients. 2019;11(8):1811.
Scheyer AF, Borsoi M, Wager-Miller J, Pelissier-Alicot AL, Murphy MN, Mackie K, Manzoni OJJ. Cannabinoid Exposure via Lactation in Rats Disrupts Perinatal Programming of the Gamma-Aminobutyric Acid Trajectory and Select Early-Life Behaviors. Biol Psychiatry. 2020;87(7):666–677. https://doi.org/10.1016/j.biopsych.2019.08.023. Epub 2019 Sep 5. PMID: 31653479; PMCID: PMC7056509.
Smith AMA, Ferris JA, Simpson JM, Shelley J, Pitts MK, Richters J. Cannabis use and sexual health. J Sex Med. 2010;7(2 Pt 1):787–93.
Stella N. Chronic THC intake modifies fundamental cerebellar functions. J Clin Invest. 2013;123(8):3208–10.
Stuyt EM, Stuyt E. The problem with the current high potency THC marijuana from the perspective of an addiction psychiatrist. Mo Med. 2018;115:482–6.
Tettey JNA, Crean C, Rodrigues J, Angeline Yap TW, Lee Wendy Lim J, Shirley Lee HZ, et al. United Nations Office on drugs and crime: recommended methods for the identification and analysis of synthetic cannabinoid receptor agonists in seized materials. Forensic Sci Int. 2021;3:100129.

Thompson R, Dejong K, Lo J. Marijuana use in pregnancy: a review. Obstet Gynecol Surv. 2019;74:415–28.

Tirado-Muñoz J, Lopez-Rodriguez AB, Fonseca F, Farré M, Torrens M, Viveros MP. Effects of cannabis exposure in the prenatal and adolescent periods: preclinical and clinical studies in both sexes. Front Neuroendocrinol. 2020;57:100841.

Trezza V, Campolongo P, Cassano T, Macheda T, Dipasquale P, Carratù MR, et al. Effects of perinatal exposure to delta-9-tetrahydrocannabinol on the emotional reactivity of the offspring: a longitudinal behavioral study in Wistar rats. Psychopharmacology (Berl). 2008;198(4):529–37.

Vanstone M, Taneja S, Popoola A, Panday J, Greyson D, Lennox R, et al. Reasons for cannabis use during pregnancy and lactation: a qualitative study. CMAJ. 2021;193(50):E1906–14.

Volkow ND, Baler RD, Compton WM, Weiss SRB. Adverse health effects of marijuana use. N Engl J Med. 2014;370(23):2219–27.

Volkow ND, Han B, Compton WM, McCance-Katz EF. Self-reported medical and nonmedical cannabis use among pregnant women in the United States. JAMA. 2019;322(2):167.

Wanner NM, Colwell M, Drown C, Faulk C. Developmental cannabidiol exposure increases anxiety and modifies genome-wide brain DNA methylation in adult female mice. Clin Epigenetics. 2021;13(1):4.

World Health Organization & United Nations Children's Fund (UNICEF). Low birthweight: country, regional and global estimates. New York and Geneva: UNICEF and the World Health Organization; 2004. p. 1–31.

Young-Wolff KC, Foti TR, Green A, Altschuler A, Does MB, Jackson-Morris M, et al. Perceptions about cannabis following legalization among pregnant individuals with prenatal cannabis use in California. JAMA Netw Open. 2022;5(12):e2246912.

Chapter 8
From In Utero to Adolescence: Determining the Impact of Prenatal Cannabinoid Exposure on Neurodevelopment Using Animal Models

Jacqueline-Marie N. Ferland

1 Introduction

The essential neurodevelopmental period from infancy to adolescence marks a critical period that sets the foundation for adult cognitive function and affective control. The development of social attachments, affect regulation, and cognition emerge across this period, and it is well established that environmental insults like maternal drug exposure can alter neurodevelopment, which can set the stage for the emergence of psychopathologies (Oei 2018). These behavioral phenotypes are underpinned by the rapid maturation of limbic and reward systems followed by adolescent refinement of these mechanisms and the development of the prefrontal cortex (Cosio-Guirado et al. 2024; Wainberg et al. 2022). Synaptogenesis and the development of synaptic transmission during this period are influenced by prenatal programming, which can alter the behavior presentation of the offspring with protracted effects into adulthood (Bara et al. 2021). The endocannabinoid system plays a critical role in the development of these circuits (Harkany et al. 2008), and clinical literature indicates that prenatal cannabis exposure is associated with molecular and morphological alterations in the placenta and fetal brain which may set the stage for future psychopathologies (Hurd et al. 2019; Rompala et al. 2021; Young-Wolff et al. 2024). Despite the growing evidence that cannabis promotes these phenotypes, debates still remain as to whether cannabis is the cause of these outcomes.

Preclinical animal models have been essential in establishing the causal impact of prenatal cannabinoid exposure on brain and behavioral development early in life. The majority of animal models of in utero cannabis exposure utilize parenteral administration of either the main psychoactive constituent in cannabis,

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delta-9-tetrahydrocannabinol (THC), a partial agonist of the CB1 receptor, or full cannabinoid receptor agonist WIN 55,212-2 (WIN). Although nonhuman primate models have the greatest translational potential, the majority of preclinical studies are completed in rodents. In these models, cannabinoids are administered to the pregnant dam between gestational days 5–20, which corresponds to the first and second trimester in humans, or during lactation, which represents the third trimester (Bara et al. 2021). This approach allows the researcher to determine the direct impact of cannabinoids on gestational outcomes, biological substrates in placenta and progeny, as well as the behavioral outcomes in the offspring. Cannabinoid-induced molecular, morphological, and physiological alterations are observed in the fetus or neonate and have been associated with the emergence of behavioral deficits in rodent infancy (postnatal day (PND) 0–21, the juvenile period (PND 21–31), and adolescence (~PND 32–52). These models have demonstrated causal impacts of prenatal cannabinoid exposure on the placenta, brain, and neurobehavioral outcomes in offspring. This chapter describes the known effects of in utero cannabinoid exposure on the placenta and fetal development as well as neurobiological changes observed in the infant, juvenile, and adolescent offspring brain and associated behavioral phenotypes observed in these early developmental periods.

2 Impacts of Cannabinoid Exposure on the Placenta

Alongside the early development of the fetus is the necessary formation of the placenta, a temporary organ which acts as the crucial link between mother and fetus. The placenta expresses cannabinoid CB1 and CB2 receptors which mediate placental trophoblast differentiation, migration, and invasion, and mediate immune responsivity (Rokeby et al. 2023). These diverse trophoblast cells are essential for fetal development and orchestrate the transport of biomolecules, gas and nutrient exchange (Brett et al. 2014; Burton and Fowden 2015; Jansson 2016; Lewis et al. 2018; Shallie and Naicker 2019), synthesis and release of neurotransmitters (Rosenfeld 2021), and immune interactions between the mother and the fetus (Tong and Abrahams 2020). Cannabinoids can cross the placental barrier where they can directly act upon fetal CB receptors but may also act on placenta CB receptors (Bara et al. 2021; Hutchings et al. 1989). Therefore, cannabinoid modulation of the placenta can influence the transmission of key nutrients and molecules or influence the maternal immune response to the developing fetus or exogenous pathogens.

Despite emerging evidence showing adverse impact of prenatal cannabis exposure on placental function and outcomes and early childhood behavior (Young-Wolff et al. 2024; Sorkhou et al. 2024; Keim et al. 2024), few clinical or preclinical studies have investigated the relationship between cannabinoid associated perturbations in the placenta and these outcomes. Most studies on the impact of cannabinoids have leveraged in vitro models of trophoblast cell lines which have demonstrated that THC influences trophoblast invasion, differentiation, and secretion of placental hormones (Costa et al. 2015). These alterations may contribute to

cannabis-induced pregnancy complications (Young-Wolff et al. 2024), though how these alterations impact fetal development or behavior is less understood. A recent clinical study revealed that in utero cannabis exposure was associated with reduced placental CB1 mRNA expression and downregulation of immune genes (Rompala et al. 2021). The offspring in this study were studied longitudinally, and in early childhood those with prenatal cannabis exposure exhibited higher levels of anxiety, aggression, and hyperactivity. Anxiety in these children correlated with placental immune dysregulation, suggesting a key relationship between placental immune function and outcomes in the offspring, particularly interferon and the immune response. A recent preclinical study in rats recapitulated these findings and demonstrated that exposure to vaped THC during pregnancy reduced placental cytokines like IL-1 beta, IL-17A, MIP-1a (Black et al. 2023). Similar alterations were observed in the corresponding fetal brain with reductions in multiple cytokines and chemokines (Black et al. 2023). These data suggest that immune dysregulation in the placenta induced by THC exposure may translate to alterations in immune function in the fetal brain. Interestingly, this study also demonstrated that route of administration produced distinct alterations in placental cytokines, as intraperitoneal injections of THC increased several immune markers (Black et al. 2023). These data emphasize that animal models of prenatal THC exposure must consider route of administration as the distinct pharmacodynamics of inhaled vs. injected THC and metabolism can influence these outcomes and the ability to compare these results to effects observed in humans (Baglot et al. 2021, 2022). However, much more research is needed as to how the placental immune perturbations induced by cannabinoid exposure directly impact offspring behavior as well as the effect of cannabinoid exposure on other placental functions such as production of neurotransmitters or other nutrients essential for fetal development.

3 Impact of Prenatal Cannabinoid Exposure on the Fetal and Neonate Brain

Unlike the placenta, substantially more research has focused on the impact of prenatal cannabinoid exposure on the fetal brain. CB1 signaling influences the development of corticofugal connectivity and axon outgrowth (Keimpema et al. 2011; Mulder et al. 2008; Berghuis et al. 2007), and exogenous modulation of this system alters development and function of these circuits. Indeed, prenatal exposure to THC or WIN is associated with reduced CB1 expression and function in the cerebrum (Tortoriello et al. 2014; De Salas-Quiroga et al. 2015), abrogated neurite branching (Ferraro et al. 2009), and altered differentiation of GABAergic and glutamatergic neurons (Saez et al. 2014). Perturbations in morphology have been linked to changes in expression of microtubule proteins such as SCG10/stathmin-2, a microtubule-binding protein in axons, which is reduced in the striatum and hippocampus of cannabinoid exposed pups, an alteration also detected in cannabis-exposed human fetal

brain tissue (Tortoriello et al. 2014). Reduced SCG10 is also associated with altered localization of CB1Rs and reorganization of cortical circuitry and impaired long-term depression in the hippocampus (Tortoriello et al. 2014). These data demonstrate a causal link between these morphological changes and functional outcomes which may influence behavior.

Preclinical studies have also demonstrated that beyond these molecular and morphological changes gestational cannabinoid alters the function of GABAergic and glutamatergic cells in the cortex, hippocampus, and ventral tegmental area. Cannabinoid exposure during lactation delays the switch from excitation to inhibition in GABAergic cells (Scheyer et al. 2020). In the hippocampus and cortex, prenatal THC or WIN blunt evoked glutamate release in neonates and adolescents (Castaldo et al. 2010; Ferraro et al. 2009; Antonelli et al. 2005), which may impair signaling in the cortex and hippocampus. However, in contrast to these regions, the opposite is observed in the ventral tegmental area (VTA), a key dopamine-producing nucleus which mediates affect and reward related behaviors. In the VTA, gestational THC exposure increases molecular crowding at the presynaptic active zone of GABAergic terminals, impairing GABAergic transmission, and increases excitability of dopaminergic neurons, leading to increased dopamine release mesocorticolimbic structures (Frau et al. 2019). Interestingly, basal dopamine release is not impacted by drug exposure, but rather drug-induced release is significantly increased in THC exposed pups, indicating increased sensitivity to drug which predisposes the rat to drug-induced behavioral deficits (Frau et al. 2019). Fetal brains with gestational cannabinoid exposure also show significantly reduced dopamine D2 receptor expression in the nucleus accumbens and amygdala (Dinieri et al. 2011; Wang et al. 2004) which may represent a compensatory downregulation of these receptors in response to elevated dopamine. However, reduced striatal D2 receptor expression is a phenotype observed in individuals with substance use disorder (Volkow and Morales 2015). These findings emphasize that several cannabis-associated effects evident on the human developing brain (Bara et al. 2021; Morris et al. 2011) can be causally ascribed to THC or cannabinoid exposure despite the many confounds of human studies. Such alterations to GABAergic, glutamatergic, and dopaminergic signaling shape the expression of social, affective, and cognitive phenotypes as they emerge in offspring.

4 The Impact of Prenatal Cannabinoid Exposure on Infant Social Orientation and Communication

Similar to humans, rodents are born altricial and require continued nurturing in early development. Therefore, neonate rodents present age-appropriate behaviors including social orientation and communication to build bonds with the mother.

This early social development is commonly measured using the nest homing test in which the pup is briefly removed from the dam and tested for the latency to return to bedding from the nest. Due to the strong need for maternal care and safety, following separation, neonates rapidly return to the nest. Disruptions in this early social behavior are an early indicator of social cognitive deficits (Bignami 1996). The endocannabinoid system is positioned to modulate regions such as the amygdala and nucleus accumbens, which contribute to these behaviors, which can be disrupted by prenatal cannabis or cannabinoid exposure (Bara et al. 2021). Some studies show that neither prenatal THC nor WIN exposure impacts homing at PND 13. However, Antonelli and colleagues demonstrated that although WIN rats showed similar homing at postnatal day 6, after repeated homing tests control animals showed faster latencies to enter the nest whereas WIN animals did not (Antonelli et al. 2005). These data suggest that although initial social recognition may be intact after cannabinoid exposure, social memory or social reward may be impaired after repeated separations. The discrepancy between WIN and THC preexposed animals may be the result of the full agonism of CB1/CB2 of WIN, causing more substantial disturbances. However, given the rise in use of potent cannabis products, more research is needed to determine whether higher THC doses during the prenatal period produce similar deficits in the offspring.

Rodents communicate through ultrasonic vocalizations (USVs). In rats, these USV calls are diverse and span ~40 kHz range and have been known to drive social interactions, maternal retrieval, and communication. In recent years, more research has focused on the impact of in utero cannabinoid exposure on USV call frequency, type, and structure. These studies have shown that prenatal cannabinoid exposure impacts pup USV communication, but at distinct developmental timepoints. At PND 9–10, pups exposed to either WIN and THC exposure during lactation produced higher kHz frequency calls compared to control and fewer calls, but the opposite was observed at PND15 (Scheyer et al. 2020). This phenotype tracked the delayed GABA excitatory/inhibitory switch observed in the cortex, suggesting a causal relationship between alterations in cortical GABA function and social communication (Scheyer et al. 2020). A newer study using a model of THC vapor self-administration showed that at PND 6, THC exposed pups made more USV calls while separated from the nest, particularly frequency modulated calls. Such calls may be recruited during maternal-pup communication (Seffer et al. 2014; Simola and Granon 2019). Interestingly, the opposite is observed after gestational WIN exposure, with pups exhibiting fewer USV calls (Manduca et al. 2020; Antonelli et al. 2005). These data indicate that prenatal cannabinoid exposure alters this social dynamic. Despite the importance of USV communication for social development, little is known about the role nuanced call types, structure, and frequencies play in these relationships. With new deep learning methods of both USVs and social behavior (Coffey et al. 2019; Goodwin et al. 2024; Pereira et al. 2022), future studies may further disentangle the types of calls impacted by gestational cannabinoid exposure on social cognitive development.

5 Impact of Prenatal Cannabinoid Exposure on Juvenile and Adolescent Behavior

After weaning (typically PND 21), rodents start to develop more complex social hierarchies which are initially established using play (Pellis et al. 2023). Social play robustly increases after weaning and continues into adolescence and has been shown to be critical for normal social development, and disruption of social dynamics or play during this time can cause a myriad of aberrant behaviors later in life (Achterberg and Vanderschuren 2023; Vanderschuren et al. 2016). Therefore, these dynamics are an essential part of behavioral development. In rats, these behaviors are diverse but typically begin with one rodent initiating play by "pouncing" onto another rat, and the other rat responding by "pinning" the initiator to continue the interaction. These actions recruit endocannabinoids and are disrupted by CB1 antagonists (Trezza et al. 2012). Although no changes in play were observed after WIN exposure, juvenile pups from dams that underwent maternal exposure to vaporized THC engaged in fewer play bouts and social investigations (Weimar et al. 2020), revealing early deficits in social dynamics. Importantly, reduced social play was not simply the result of increased anxiety-like behavior, as prenatal THC exposure did not increase anxiety in the elevated plus maze (Weimar et al. 2020). These new data indicate that THC in utero disrupts mechanisms involved in play development, which can have protracted impacts on this critical behavior even early in life.

In addition to the maturation of the endocannabinoid system, the dopaminergic system also undergoes significant changes from the juvenile period through adolescence (Reynolds and Flores 2021). Given the marked alterations in dopaminergic signaling described previously, Frau and colleagues investigated whether prenatal THC exposure influenced dopamine-dependent including prepulse inhibition and locomotor activity in juvenile offspring (Frau et al. 2019). Interestingly, although no differences were observed in these metrics under control conditions, re-exposure to THC impaired prepulse inhibition (a phenotype associated with psychosis) and induced hyperlocomotion. Even more striking, these effects were uniquely observed in the male offspring, suggesting that biological sex may mediate the long-term effects of prenatal cannabinoid exposure (Frau et al. 2019; Traccis et al. 2021). Male WIN exposed rats also exhibited elevated locomotor activity at PND 13 and 40 (infancy and adolescence, respectively) (Mereu et al. 2003). These data collectively indicate that prenatal cannabinoid exposure increases dopamine signaling linked behavioral phenotypes relevant to emotional dysregulation, addiction, and psychosis, particularly in male offspring.

6 Conclusions and Future Directions

Existing preclinical data show that exposure to cannabinoids in utero has a causal impact on offspring brain development, physiology, and behavior. These impacts are observed from the placenta to behavior early in life, which may shape the

developmental trajectory of the individual, increasing vulnerability to psychopathology like phenotypes in adulthood. Despite these seminal findings, great gaps in our understanding of the impact of prenatal cannabis use remain. These are already evident in the animal literature, with sometimes distinct results observed following WIN and THC exposure such as opposite effects observed (USV communication) or null effects in one condition but significant effects in the other (social play). These differences are likely due to the difference in CB signal transduction between WIN and THC. Furthermore, the majority of these studies used subcutaneous or intravenous administered cannabinoids, which have distinct pharmacodynamics compared to commonly used routes of cannabis administration such as smoking, vaping, or consuming edibles. Ongoing work using these routes of administration and using concentrations of cannabinoids that mimic modern potent cannabis products will improve our understanding of the impact of prenatal cannabis use from the womb through adulthood.

References

Achterberg EJM, Vanderschuren L. The neurobiology of social play behaviour: past, present and future. Neurosci Biobehav Rev. 2023;152:105319.

Antonelli T, Tomasini MC, Tattoli M, Cassano T, Tanganelli S, Finetti S, Mazzoni E, Trabace L, Steardo L, Cuomo V, Ferraro L. Prenatal exposure to the CB1 receptor agonist WIN 55,212-2 causes learning disruption associated with impaired cortical NMDA receptor function and emotional reactivity changes in rat offspring. Cereb Cortex. 2005;15:2013–20.

Baglot SL, Hume C, Petrie GN, Aukema RJ, Lightfoot SHM, Grace LM, Zhou R, Parker L, Rho JM, Borgland SL, McLaughlin RJ, Brechenmacher L, Hill MN. Pharmacokinetics and central accumulation of delta-9-tetrahydrocannabinol (THC) and its bioactive metabolites are influenced by route of administration and sex in rats. Sci Rep. 2021;11:23990.

Baglot SL, Vanryzin JW, Marquardt AE, Aukema RJ, Petrie GN, Hume C, Reinl EL, Bieber JB, McLaughlin RJ, McCarthy MM, Hill MN. Maternal-fetal transmission of delta-9-tetrahydrocannabinol (THC) and its metabolites following inhalation and injection exposure during pregnancy in rats. J Neurosci Res. 2022;100:713–30.

Bara A, Ferland JN, Rompala G, Szutorisz H, Hurd YL. Cannabis and synaptic reprogramming of the developing brain. Nat Rev Neurosci. 2021;22:423–38.

Berghuis P, Rajnicek AM, Morozov YM, Ross RA, Mulder J, Urban GM, Monory K, Marsicano G, Matteoli M, Canty A, Irving AJ, Katona I, Yanagawa Y, Rakic P, Lutz B, Mackie K, Harkany T. Hardwiring the brain: endocannabinoids shape neuronal connectivity. Science. 2007;316:1212–6.

Bignami G. Economical test methods for developmental neurobehavioral toxicity. Environ Health Perspect. 1996;104(Suppl 2):285–98.

Black T, Baccetto SL, Barnard IL, Finch E, Mcelroy DL, Austin-Scott FVL, Greba Q, Michel D, Zagzoog A, Howland JG, Laprairie RB. Characterization of cannabinoid plasma concentration, maternal health, and cytokine levels in a rat model of prenatal cannabis smoke exposure. Sci Rep. 2023;13:21070.

Brett KE, Ferraro ZM, Yockell-Lelievre J, Gruslin A, Adamo KB. Maternal-fetal nutrient transport in pregnancy pathologies: the role of the placenta. Int J Mol Sci. 2014;15:16153–85.

Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. Philos Trans R Soc Lond B Biol Sci. 2015;370:20140066.

Castaldo P, Magi S, Cataldi M, Arcangeli S, Lariccia V, Nasti AA, Ferraro L, Tomasini MC, Antonelli T, Cassano T, Cuomo V, Amoroso S. Altered regulation of glutamate release and decreased functional activity and expression of GLT1 and GLAST glutamate transporters in the hippocampus of adolescent rats perinatally exposed to Delta(9)-THC. Pharmacol Res. 2010;61:334–41.

Coffey KR, Marx RE, Neumaier JF. DeepSqueak: a deep learning-based system for detection and analysis of ultrasonic vocalizations. Neuropsychopharmacology. 2019;44:859–68.

Cosio-Guirado R, Tapia-Medina MG, Kaya C, Pero-Cebollero M, Villuendas-Gonzalez ER, Guardia-Olmos J. A comprehensive systematic review of fMRI studies on brain connectivity in healthy children and adolescents: current insights and future directions. Dev Cogn Neurosci. 2024;69:101438.

Costa MA, Fonseca BM, Marques F, Teixeira NA, Correia-da-Silva G. The psychoactive compound of Cannabis sativa, Delta(9)-tetrahydrocannabinol (THC) inhibits the human trophoblast cell turnover. Toxicology. 2015;334:94–103.

De Salas-Quiroga A, Diaz-Alonso J, Garcia-Rincon D, Remmers F, Vega D, Gomez-Canas M, Lutz B, Guzman M, Galve-Roperh I. Prenatal exposure to cannabinoids evokes long-lasting functional alterations by targeting CB1 receptors on developing cortical neurons. Proc Natl Acad Sci U S A. 2015;112:13693–8.

Dinieri JA, Wang X, Szutorisz H, Spano SM, Kaur J, Casaccia P, Dow-Edwards D, Hurd YL. Maternal cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring. Biol Psychiatry. 2011;70:763–9.

Ferraro L, Tomasini MC, Beggiato S, Gaetani S, Cassano T, Cuomo V, Amoroso S, Tanganelli S, Antonelli T. Short- and long-term consequences of prenatal exposure to the cannabinoid agonist WIN55,212-2 on rat glutamate transmission and cognitive functions. J Neural Transm (Vienna). 2009;116:1017–27.

Frau R, Miczan V, Traccis F, Aroni S, Pongor CI, Saba P, Serra V, Sagheddu C, Fanni S, Congiu M, Devoto P, Cheer JF, Katona I, Melis M. Prenatal THC exposure produces a hyperdopaminergic phenotype rescued by pregnenolone. Nat Neurosci. 2019;22:1975–85.

Goodwin NL, Choong JJ, Hwang S, Pitts K, Bloom L, Islam A, Zhang YY, Szelenyi ER, Tong X, Newman EL, Miczek K, Wright HR, McLaughlin RJ, Norville ZC, Eshel N, Heshmati M, Nilsson SRO, Golden SA. Simple behavioral analysis (SimBA) as a platform for explainable machine learning in behavioral neuroscience. Nat Neurosci. 2024;27:1411–24.

Harkany T, Mackie K, Doherty P. Wiring and firing neuronal networks: endocannabinoids take center stage. Curr Opin Neurobiol. 2008;18:338–45.

Hurd YL, Manzoni OJ, Pletnikov MV, Lee FS, Bhattacharyya S, Melis M. Cannabis and the developing brain: insights into its long-lasting effects. J Neurosci. 2019;39:8250–8.

Hutchings DE, Martin BR, Gamagaris Z, Miller N, Fico T. Plasma concentrations of delta-9-tetrahydrocannabinol in dams and fetuses following acute or multiple prenatal dosing in rats. Life Sci. 1989;44:697–701.

Jansson T. Placenta plays a critical role in maternal-fetal resource allocation. Proc Natl Acad Sci U S A. 2016;113:11066–8.

Keim SA, Fried P, Yeates KO, Boone KM, Vrantsidis DM, Dean A, Murnan AW, Rausch J, Klebanoff MA. Prenatal cannabis exposure and executive function and aggressive behavior at age 5 years. JAMA Pediatr. 2024;178:1316.

Keimpema E, Mackie K, Harkany T. Molecular model of cannabis sensitivity in developing neuronal circuits. Trends Pharmacol Sci. 2011;32:551–61.

Lewis RM, Childs CE, Calder PC. New perspectives on placental fatty acid transfer. Prostaglandins Leukot Essent Fatty Acids. 2018;138:24–9.

Manduca A, Servadio M, Melancia F, Schiavi S, Manzoni OJ, Trezza V. Sex-specific behavioural deficits induced at early life by prenatal exposure to the cannabinoid receptor agonist WIN55, 212-2 depend on mGlu5 receptor signalling. Br J Pharmacol. 2020;177:449–63.

Mereu G, Mauro FA, Ferraro L, Cagiano R, Antonelli T, Tattoli M, Ghiglieri V, Tanganelli S, Gessa GL, Cuomo V. Prenatal exposure to a cannabinoid agonist produces memory deficits linked to

dysfunction in hippocampal long-term potentiation and glutamate release. Proc Natl Acad Sci U S A. 2003;100:4915–20.
Morris CV, Dinieri JA, Szutorisz H, Hurd YL. Molecular mechanisms of maternal cannabis and cigarette use on human neurodevelopment. Eur J Neurosci. 2011;34:1574–83.
Mulder J, Aguado T, Keimpema E, Barabas K, Ballester Rosado CJ, Nguyen L, Monory K, Marsicano G, Di Marzo V, Hurd YL, Guillemot F, Mackie K, Lutz B, Guzman M, Lu HC, Galve-Roperh I, Harkany T. Endocannabinoid signaling controls pyramidal cell specification and long-range axon patterning. Proc Natl Acad Sci U S A. 2008;105:8760–5.
Oei JL. Adult consequences of prenatal drug exposure. Intern Med J. 2018;48:25–31.
Pellis SM, Pellis VC, Ham JR, Stark RA. Play fighting and the development of the social brain: the rat's tale. Neurosci Biobehav Rev. 2023;145:105037.
Pereira TD, Tabris N, Matsliah A, Turner DM, Li J, Ravindranath S, Papadoyannis ES, Normand E, Deutsch DS, Wang ZY, McKenzie-Smith GC, Mitelut CC, Castro MD, D'uva J, Kislin M, Sanes DH, Kocher SD, Wang SS, Falkner AL, Shaevitz JW, Murthy M. SLEAP: a deep learning system for multi-animal pose tracking. Nat Methods. 2022;19:486–95.
Reynolds LM, Flores C. Mesocorticolimbic dopamine pathways across adolescence: diversity in development. Front Neural Circuits. 2021;15:735625.
Rokeby ACE, Natale BV, Natale DRC. Cannabinoids and the placenta: receptors, signaling and outcomes. Placenta. 2023;135:51–61.
Rompala G, Nomura Y, Hurd YL. Maternal cannabis use is associated with suppression of immune gene networks in placenta and increased anxiety phenotypes in offspring. Proc Natl Acad Sci U S A. 2021;118:e2106115118.
Rosenfeld CS. The placenta-brain-axis. J Neurosci Res. 2021;99:271–83.
Saez TM, Aronne MP, Caltana L, Brusco AH. Prenatal exposure to the CB1 and CB2 cannabinoid receptor agonist WIN 55,212-2 alters migration of early-born glutamatergic neurons and GABAergic interneurons in the rat cerebral cortex. J Neurochem. 2014;129:637–48.
Scheyer AF, Borsoi M, Wager-Miller J, Pelissier-Alicot AL, Murphy MN, Mackie K, Manzoni OJJ. Cannabinoid exposure via lactation in rats disrupts perinatal programming of the gamma-aminobutyric acid trajectory and select early-life behaviors. Biol Psychiatry. 2020;87:666–77.
Seffer D, Schwarting RK, Wohr M. Pro-social ultrasonic communication in rats: insights from playback studies. J Neurosci Methods. 2014;234:73–81.
Shallie PD, Naicker T. The placenta as a window to the brain: a review on the role of placental markers in prenatal programming of neurodevelopment. Int J Dev Neurosci. 2019;73:41–9.
Simola N, Granon S. Ultrasonic vocalizations as a tool in studying emotional states in rodent models of social behavior and brain disease. Neuropharmacology. 2019;159:107420.
Sorkhou M, Singla DR, Castle DJ, George TP. Birth, cognitive and behavioral effects of intrauterine cannabis exposure in infants and children: a systematic review and meta-analysis. Addiction. 2024;119:411–37.
Tong M, Abrahams VM. Immunology of the placenta. Obstet Gynecol Clin North Am. 2020;47:49–63.
Tortoriello G, Morris CV, Alpar A, Fuzik J, Shirran SL, Calvigioni D, Keimpema E, Botting CH, Reinecke K, Herdegen T, Courtney M, Hurd YL, Harkany T. Miswiring the brain: Δ9-tetrahydrocannabinol disrupts cortical development by inducing an SCG10/stathmin-2 degradation pathway. EMBO J. 2014;33:668–85.
Traccis F, Serra V, Sagheddu C, Congiu M, Saba P, Giua G, Devoto P, Frau R, Cheer JF, Melis M. Prenatal THC does not affect female mesolimbic dopaminergic system in preadolescent rats. Int J Mol Sci. 2021;22:1666.
Trezza V, Damsteegt R, Manduca A, Petrosino S, Van Kerkhof LW, Pasterkamp RJ, Zhou Y, Campolongo P, Cuomo V, Di Marzo V, Vanderschuren LJ. Endocannabinoids in amygdala and nucleus accumbens mediate social play reward in adolescent rats. J Neurosci. 2012;32:14899–908.
Vanderschuren LJ, Achterberg EJ, Trezza V. The neurobiology of social play and its rewarding value in rats. Neurosci Biobehav Rev. 2016;70:86–105.

Volkow ND, Morales M. The brain on drugs: from reward to addiction. Cell. 2015;162:712–25.
Wainberg M, Jacobs GR, Voineskos AN, Tripathy SJ. Neurobiological, familial and genetic risk factors for dimensional psychopathology in the adolescent brain cognitive development study. Mol Psychiatry. 2022;27:2731–41.
Wang X, Dow-Edwards D, Anderson V, Minkoff H, Hurd YL. In utero marijuana exposure associated with abnormal amygdala dopamine D2 gene expression in the human fetus. Biol Psychiatry. 2004;56:909–15.
Weimar HV, Wright HR, Warrick CR, Brown AM, Lugo JM, Freels TG, McLaughlin RJ. Long-term effects of maternal cannabis vapor exposure on emotional reactivity, social behavior, and behavioral flexibility in offspring. Neuropharmacology. 2020;179:108288.
Young-Wolff KC, Adams SR, Alexeeff SE, Zhu Y, Chojolan E, Slama NE, Does MB, Silver LD, Ansley D, Castellanos CL, Avalos LA. Prenatal cannabis use and maternal pregnancy outcomes. JAMA Intern Med. 2024;184:1083–93.

Chapter 9
Modulation of the Cannabinoid System for Neonatal Pain Management

Brian M. Timmerman and Susanne Brummelte

1 Painful Procedures: A Distinct Source of Neonatal Stress

Infants that are born preterm (i.e., before 37 weeks of gestation) or with other medical complications often spend a significant amount of time in the neonatal intensive care unit (NICU) after parturition. Many of the medical procedures that infants undergo during this time are necessary for their survival but are also stressful or painful. Preterm infants in particular often receive over a dozen painful procedures (such as injections, blood draws, intubation, etc.) per day in NICUs (Carbajal et al. 2008). The stress from these procedures is likely exacerbated in preterm infants because they are particularly susceptible to pain sensation due to the immaturity of their nociceptive system (Fitzgerald 2005; Ranger and Grunau 2014). Though basic nociceptive signaling is active in preterm infants, as ascending nociceptive pathways are functional by 20 weeks gestational age and the peripheral nervous system is mature by 24 weeks gestational age (Hatfield 2014), the descending inhibitory nociceptive pathways are not mature until later (Fitzgerald 2005; Ranger and Grunau 2014). In fact, descending nociceptive pathways from the periaqueductal gray, which modulate nociceptive signaling, do not mature until an infant is beyond term-equivalent age (McPherson et al. 2020). This uninhibited nociceptive activity is further exacerbated in preterm infants because dopaminergic and norepinephrinergic activity cannot modulate nociceptive signals until 36–40 weeks gestational age (Hatfield 2014). The skin of neonates is also hyperinnervated by nociceptive high-threshold Aδ fibers and low-threshold Aß fibers which likely increases the

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intensity of nociceptive signaling (Hatfield 2014). As a result, in the nervous system of preterm neonates nociceptive signaling is easily provoked but there is limited ability to mitigate its intensity (for a more thorough review of nociception in preterm infants, please see Hatfield 2014).

Not surprisingly, neonatal pain has multiple documented effects on neurodevelopment, especially in preterm infants. Very preterm infants (born 28–32 weeks gestational age) who received more painful procedures in the NICU have decreased white and gray matter maturation at term-equivalent age (Brummelte et al. 2012; Duerden et al. 2018) as well as impaired motor and cognitive development at eight and 18 months of age (Grunau et al. 2009). Even at school-age, the number of painful procedures that ex-preterm children received in the NICU continues to predict impaired white matter maturation (Vinall et al. 2014) as well as decreased cortical thickness (Ranger et al. 2013), decreased cerebellar volume (Ranger et al. 2015), and altered cortical activity rhythms associated with impaired visuoperceptual ability (Doesburg et al. 2013a, b). Animal studies similarly provide evidence that neonatal pain creates multidimensional changes in neurodevelopment, including impaired long-term spatial memory (Anand et al. 2007; Nuseir et al. 2017), altered neurogenesis rates in juveniles (Lee et al. 2016; Malheiros et al. 2014), and adult hypoalgesia (Laprairie and Murphy 2009). As a result of the ample evidence that neonatal pain is indeed "felt" by even very immature neonates (Hall and Anand 2005) and has a significant impact on neurodevelopment, research in recent decades has focused on ways to mitigate the stress caused by neonatal pain exposure.

Current pain management methods for neonates in the NICU can be categorized as either pharmacological methods or non-pharmacological methods, which can be categorized more broadly as environmental or behavioral, which in recent decades are increasingly utilized in NICUs (Lago et al. 2009). The two most prominent comfort methods are maintaining skin-to-skin contact with infants (also called kangaroo care) and the oral administration of sucrose, although there is some disagreement as to whether sucrose should be considered a pharmacological or non-pharmacological method (McPherson et al. 2020). Both methods successfully reduce behavioral responsiveness during stressful procedures, and kangaroo care additionally reduces hormonal stress responses (Campbell-Yeo et al. 2015; Walker 2014). Impressively, longitudinal human studies have shown that even 30 minutes of skin-to-skin contact has beneficial effects a decade later (Feldman et al. 2014). However, these methods have several limitations. Medical equipment such as incubators and ventilators can limit opportunities for caregivers to establish physical contact for kangaroo care, and it often seems more stressful to remove an infant from their incubator or bed to only briefly hold them during a quick procedure. Oral sucrose is more accessible than kangaroo care and has been repeatedly documented to reduce behavioral pain responses (i.e., less crying, frowning, fussing, etc.; Stevens et al. 2016). However, oral sucrose is unable to mitigate cortical activity, metabolic activity, CORT responses, or hyperalgesia induced by neonatal stress exposure (Gao et al. 2016; McPherson and Grunau 2014; Ranger et al. 2018; Tremblay et al. 2017). Further, studies indicate that repeated sucrose exposure may cause persistent alterations in the neurodevelopment of preterm infants, in part by altering development of

the dopaminergic and cholinergic systems (Gao et al. 2016; McPherson and Grunau 2014; Ranger et al. 2018; Tremblay et al. 2017). Overall, non-pharmacological comfort measures in NICUs greatly reduce neonates' immediate reactions to pain, and skin-to-skin care has multiple long-term benefits, but there is room to improve the care and treatment of this vulnerable population with more effective and feasible relief from pain-related stress.

Pharmacological pain management primarily involves opioids such as morphine and fentanyl, or nonsteroidal anti-inflammatory drugs like acetaminophen. However, opioids are often underutilized for neonatal pain management because of concerns about adverse effects from neonatal opioid exposure (Walker 2014), although they are commonly used for sedation. The consequences of inadequate opioid administration are also worsened by altered metabolism in preterm infants, which may cause morphine to be less effective for their pain management (Kaneyasu 2012). Additionally, most nonsteroidal anti-inflammatory (NSAID) drugs are also not recommended for preterm infants (Hall and Anand 2014), which leaves acetaminophen as one of the few nonopioid drugs used in the NICU. Acetaminophen has been shown to reduce the need for opioids after major surgery in neonates (Walker 2014), but unfortunately, it is observed to be ineffective for the treatment of procedural pain for preterm infants (Lago et al. 2009; Ohlsson and Shah 2020).

Even when infants receive pharmacological pain management for nonsurgical painful procedures, which is reportedly only done 30–60% of the time (Carbajal et al. 2008), the pharmacological methods that are currently available have serious limitations. As a result, there is room for a great deal of improvement in the pharmacological management of pain for preterm infants. Therefore, this chapter will review the potential for cannabinoids in the treatment of pain-related stress in neonates. We will first discuss the involvement of the HPA axis and the endocannabinoid system in pain processing in neonates and then review the current evidence that supports the idea that cannabinoids may be a target for future investigation and pharmacological approaches for pain management in this unique population.

2 Hypothalamic–Pituitary–Adrenal Axis (HPA Axis) Activity After Pain-Related Stress Exposure in the Neonatal Period

The HPA axis is the primary neuroendocrine network that coordinates biological stress responses, and it is already functional in preterm infants. In brief, activity in the HPA axis begins with activation of the paraventricular nucleus of the hypothalamus, which then triggers the secretion of corticotropin-releasing hormone into the bloodstream (Micale and Drago 2018). Corticotropin-releasing hormone then binds to receptors on the pituitary and causes the secretion of adrenocorticotrophic hormone, which further signals the adrenal cortices to release steroid hormones known as glucocorticoids. Glucocorticoids, primarily cortisol in humans and corticosterone in rodents (CORTs), bind with glucocorticoid receptors throughout the body to inhibit a wide range of biological processes including immune function,

inflammation, digestion, metabolism, and cell proliferation via genomic regulation. Suppression of the axis is mostly regulated through negative feedback signaling back to the hypothalamus (for a thorough overview of the HPA axis please see the review by Papadimitriou and Priftis (2009).

Early-life brain development depends on low CORT levels (Daskalakis et al. 2015; McEwen 1987). During gestation, elevated levels of the enzyme 11β-hydroxysteroid dehydrogenase type 2 keep CORT levels low (Brown et al. 1996). However, preterm birth prematurely removes infants from the protective fetal environment and exposes them to external stressors. Interestingly, preterm infants often display inappropriately low levels of cortisol considering the stress of illness and the number of stressful procedures that they undergo (Brummelte et al. 2011; Fernandez et al. 2008; Grunau et al. 2007). However, preterm infants do display CORT responses to painful procedures (Herrington et al. 2004). Further, low basal cortisol levels observed at 3 months of gestational age in a preterm sample (compared to full-term controls) disappeared at 8 months of age, when preterm infants revealed higher basal cortisol levels compared to their full-term peers (Grunau et al. 2007). Consistent with this, older ex-preterm children and adults often show HPA axis hyperreactivity in response to stressors and waking up in the morning (Buske-Kirschbaum et al. 2007). The observed switch in HPA reactivity suggests that the early adverse environment caused reprogramming of the HPA axis and permanent changes to neuroendocrine stress regulation (Matthews 2002; Meaney et al. 2007).

Painful procedures appear to be driving the observed change in the HPA axis, as the number of painful procedures that preterm infants receive in the NICU predicts higher CORT levels at baseline and in response to novelty at 8 months of age (Grunau et al. 2004). Further, increased HPA axis activity from repeated or chronic stress exposure results in persistently elevated CORT levels which in turn can inhibit neural stem/progenitor cell proliferation (Carson et al. 2016) and neuronal migration (Fukumoto et al. 2009). This increased HPA axis activity in response to neonatal painful procedures likely alters neurodevelopment, as supported by evidence that more painful procedures in the NICU predict slower thalamic growth in very preterm infants (Duerden et al. 2018). Therefore, preterm birth leaves infants particularly vulnerable to altered or impaired neurodevelopment from HPA axis activity induced by neonatal stress (Anand et al. 2007). Better pain management for preterm infants that can help avoid excessive HPA activation for these infants will likely benefit their long-term neurodevelopmental trajectories and may help prevent reprogramming of the HPA activity.

3 The Endocannabinoid System (ECS) in Pain-Related Stress Modulation

Although a lot of research has focused on the relationship between the HPA axis and consequences of stress-related pain exposure, it is important to also consider the influence of the endocannabinoid system (ECS). As described in detail in previous

chapters, the ECS is a retrograde neurotransmitter system that regulates HPA axis activity as well as synaptic signaling, nociception, the proliferation and differentiation of neural progenitor/stem cells, axon migration, and synaptogenesis (Corcoran et al. 2015; Fride 2008; Harkany et al. 2008; Hill and Tasker 2012).

The ECS is present and functional within the nervous system for the entirety of the lifespan and much of gestational development. The two primary neurotransmitter receptors in the ECS are cannabinoid receptor 1 (CB1 receptor) and cannabinoid receptor 2 (CB2 receptor). They are observed to be present early in gestation, with CB1 receptors present in human embryonic brain tissue as young as 14 weeks gestational age and CB1 receptor mRNA present in rodent embryonic brain tissue as young as 11 days gestational age (Fride 2008). Despite the ECS's presence from such an early age it continues to develop gradually, as the concentration of CB receptors throughout most of the central nervous system increases from gestation through adulthood (Belue et al. 1995; Mato et al. 2003; Rodriguez de Fonseca et al. 1993; Romero et al. 1997). Interestingly, CB1 receptors are highly concentrated in neonatal white matter regions that do not express CB1 receptors at adulthood (Fride 2008). The transient expression of CB receptors in these regions during early development likely occurs because CB1 receptors are involved in the guidance of axon growth cones (as reviewed by Micale and Drago 2018) and thus axon migration. This supports the theory that the ECS is important for proper neurodevelopment, but less is known about whether these transient receptors can influence pain perception.

The ECS has been identified as a regulator of nociception through various pathways. As discussed in Chap. 3, the ECS utilizes multiple lipid-based neurotransmitters known as endocannabinoids, but the two most-studied endocannabinoids are 2-arachadonoylglycerol (2-AG) and anandamide (AEA), which are full or partial agonists at CB receptors, respectively. Notably, AEA is also an agonist of the vanilloid receptor 1 (TRPV1) receptor, a nonselective cation channel involved in thermal sensation, temperature regulation, and pain perception (Ross 2003). AEA activity is antinociceptive in part through AEA's binding with TRPV1 receptors in the spinal cord (Argaw et al. 2011; Watson et al. 2008) although TRPV1 activity is observed to modulate nociception in a dose-dependent manner, with TRPV1 agonists promoting hypoalgesia at low doses and hyperalgesia at high doses (Corcoran et al. 2015). AEA is also a partial agonist of the orphan G-protein coupled receptor GPR55 (Sharir and Abood 2010). Although the total effects of GPR55 activation are still unclear, studies indicate that it is involved in the regulation of axon growth cone migration (Castillo et al. 2010; Pazos et al. 2013) and potentially nociception (Zou and Kumar 2018). CB1 receptors in regions such as the periaqueductal gray, amygdala, rostral ventromedial medulla, and prefrontal cortex are also observed to modulate pain-related behavior and activation of these receptors may help alleviate pain-induced stress (as reviewed by Corcoran et al. 2015).

It is well established that the HPA axis and ECS interact, and thus modulating the ECS could help regulate neonatal stress responses. For instance, studies have found that elevated corticotrophin-releasing hormone levels decrease AEA levels in the hippocampus and amygdala via the upregulation of fatty acid-amide hydroxylase (FAAH), an enzyme that catabolizes AEA in postsynaptic terminals (Hill and Tasker

2012; Morena et al. 2016). Importantly, AEA levels in the basolateral amygdala mediate the intensity of HPA axis activity, and CB1 receptor activity there is responsible for tonic inhibition of the HPA axis (Hill and Tasker 2012; Morena et al. 2016). The basolateral amygdala is thus a key brain region for the regulation of HPA axis activity by the ECS.

While decreased AEA levels in the amygdala promotes higher intensity HPA axis activity and the downstream secretion of more CORT, CORT in turn causes elevated 2-AG levels in the hypothalamus, hippocampus, medial prefrontal cortex, pituitary, and hypothalamus (Micale and Drago 2018). The CORT-induced increase of 2-AG in these regions acts as a form of glucocorticoid-mediated fast negative feedback for the HPA axis, suppressing further activity in the HPA axis and promoting its return to baseline activity levels. As such, the ECS is critical to the regulation of basal and stress-induced signaling in the HPA. Taken together, multiple studies have found that activity in the ECS is involved in the modulation of nociception (Horvath et al. 2008) and stress regulation, and the ECS may therefore be a promising target for pain management in neonates. Interestingly, the antinociceptive effects of nonsteroidal anti-inflammatory drugs also involve the ECS to varying degrees (Sharir and Abood 2010; Sharir et al. 2012) further supporting the idea that manipulations of this system could be relatively safe. The combination of potential effectiveness and relative safety make cannabinoid compounds strong candidates for new methods of pharmacological pain management.

4 The ECS as a Potential Target for Pain Management

4.1 Important Exogenous Cannabinoids

As discussed in detail in previous chapters, there are over 100 different cannabinoid compounds found in the cannabis plant (phytocannabinoids), but the two most-studied compounds are tetrahydrocannabinol (THC) and cannabidiol (CBD). These two compounds are also the most concentrated phytocannabinoids in cannabis, and they are both implicated in cannabis's antinociceptive effects. THC is an especially potent partial agonist of CB1 and CB2 receptors and credited with the principal psychoactive effects from cannabis use (McPartland et al. 2007). CBD is similarly a partial agonist of the CB1 and CB2 receptors, albeit with a far weaker affinity for either receptor (Mlost et al. 2020), no intoxicating properties, and fewer side effects than THC. Although CBD is additionally a negative allosteric modulator of both CB receptors, its effect on the ECS is not primarily a result of direct interaction with CB receptors. Instead, CBD influences ECS activity mostly by increasing the concentration of AEA. CBD has been theorized to increase AEA by inhibiting FAAH, an enzyme located in postsynaptic terminals that is responsible for AEA catabolism. While CBD does have modest binding affinity for FAAH in rodents, its affinity for human FAAH is calculated to be too weak for physiological relevance (Elmes et al.

2015; Mlost et al. 2020). Recent research instead suggests that CBD primarily increases AEA levels in humans by inhibiting fatty acid-binding proteins, which are responsible for cellular uptake and intracellular transportation of AEA to FAAH (Bisogno et al. 2001; Elmes et al. 2015; Huang et al. 2016). However, the precise mechanisms of intracellular endocannabinoid transportation are not well known. In addition, CBD may also increase AEA levels by promoting the activity of N-acyl phosphatidyl ethanolamine-specific phospholipase D, the enzyme primarily responsible for the synthesis of AEA and related compounds known as N-acyl ethanolamines (Leishman et al. 2018; Rimmerman et al. 2012). Unfortunately, not much is known about whether these mechanisms are different or already fully functional in neonates.

Like AEA, CBD also binds to TRPV1 receptors (Bisogno et al. 2001; Ross 2003). Activation of TRPV1 receptors by CBD and AEA appears to dose-dependently desensitize TRPV1 receptors and reduce later nociceptive signaling, and CBD's influence on TRPV1 receptors is responsible for a significant portion of CBD's antinociceptive effects (Mlost et al. 2020). TRPV1 receptor activity is also necessary for AEA uptake in endothelial tissue (Hofmann et al. 2014) which suggests that CBD's previously mentioned effects on AEA levels may be modulated by TRPV1 receptors.

CBD also interacts with several other neurotransmitter receptors that appear to contribute to its antinociceptive effects. CBD acts as a partial agonist with a strong affinity for dopamine D2 receptors, which are found on presynaptic terminals and inhibit adenylyl cyclase activity similar to CB receptors (Mlost et al. 2020). Notably, D2 receptors promote antinociception in descending inhibitory pain pathways (Leweke et al. 2012; Li et al. 2019). CBD also acts as an agonist with a strong affinity for peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear membrane-bound receptor which regulates gene transcription and is partially responsible for the anti-inflammatory effects of CBD (O'Sullivan 2016). Further, CBD is also a strong inhibitor of adenosine reuptake and a positive allosteric modulator of serotonin 1A ($5HT_{1A}$) receptors, and CBD's interaction with PPARγ and $5HT_{1A}$ receptors may further contribute to CBD's ability to reduce hyperalgesia and allodynia in models of inflammatory or chronic pain (Mlost et al. 2020).

Although studies suggest that CBD does not meaningfully inhibit FAAH in humans (Elmes et al. 2015; Mlost et al. 2020), FAAH modulation is still a viable avenue of increasing ECS activity, and pharmaceutical companies have researched several compounds that are FAAH inhibitors. Since both FAAH inhibitors and CBD increase AEA levels, they are likely to have similar effects via the ECS, as is supported by ample evidence that they are both anxiolytic, antinociceptive, and anti-inflammatory (Ahn et al. 2009; Campos et al. 2016; Hohmann et al. 2005; Mlost et al. 2020). Further, any differences between the effects of FAAH inhibitors and CBD are likely due to activity that is independent of AEA (for a review of FAAH inhibitors' potential interactions with off-target proteins, please see the review by Dider et al. 2016; for a review of CBD's relevant pharmacology, please see the review by Mlost et al. 2020).

5 The Management of Pain-Related Stress with Cannabinoids in Adults

Most research on pain management with cannabinoids has focused on cannabis use or THC-containing compounds to treat chronic pain, inflammatory pain, or neuropathic pain, and found them to be quite effective in this regard. Particularly, evidence suggests that concomitant cannabinoid and opioid treatment can reduce the opioid dosage required for chronic pain management (Hill et al. 2017). Further, De Vita et al. (2018) report that cannabinoids significantly improve pain thresholds, pain tolerance, and pain unpleasantness ratings from experimentally induced pain without significantly altering ratings of pain intensity. Similarly, a meta-analysis and a Cochrane review found that oral cannabinoids were not effective for reducing ratings of pain intensity after acute pain (De Vita et al. 2018; Gazendam et al. 2020). However, Gazendam et al. (2020) found that if cannabinoids were administered intramuscularly, they were moderately effective for reducing acute pain ratings. This discrepancy between studies suggests that the nociceptive effects of cannabinoids are either dose-dependent and/or that the first pass metabolism after oral consumption significantly diminishes cannabinoids' effectiveness. Taken together, research suggests that cannabinoids can modulate the affective aspect of pain at lower doses but at higher doses can also reduce nociception.

Similar to THC, research on pain management with CBD has mostly focused on managing chronic and inflammatory pain and found it to be highly effective for this purpose (Costa et al. 2007; Hammell et al. 2016; Wong and Cairns 2019; Xiong et al. 2012). For instance, De Vita et al. (2021) found that 50 mg sublingual CBD reduced pain unpleasantness ratings from experimentally induced thermal pain in humans. Animal studies likewise indicate that CBD may influence affective processing of acute pain and pain intensity at different doses. Genaro et al. (2017) found that 1–3 mg/kg (i.p.) CBD induced conditioned place preference and 3–10 mg/kg (i.p.) CBD increased nociceptive thresholds in rats who received paw incisions, but not in sham-operated rats. Further, both the conditioned place preference and decreased thermal pain sensitivity from CBD depended on its activity in the medial prefrontal cortex, a brain region that modulates the emotional aspect of pain Genaro et al. (2017). Goncalves et al. (2014) found increased paw withdrawal thresholds during hot-plate testing in rats that received 3 or 6 mg/kg (i.p.) CBD, but not those that received 0.75, 1.5, 4.5, or 12 mg/kg, indicating a dose-dependent effect of CBD on pain thresholds. These findings suggest that CBD can modulate the emotional aspect of pain but at the right dosage may additionally alleviate nociception, consistent with observations from studies that used THC-containing cannabinoid compounds (Duerden et al. 2018). Further, CBD decreases rat pups' ultrasonic vocalizations immediately after pain exposure (Timmerman et al. 2025), which suggests that CBD decreases pups' behavioral pain responses. Importantly, CBD has considerable antinociceptive effects but far fewer adverse effects than THC-containing compounds (Carlini 2004; Huestis et al. 2019).

6 Potential for the Management of Pain-Related Stress with Cannabinoids in Neonates

There is presently little published research on the use of cannabinoids for the management of pain-related stress in neonates, but evidence indicates that the same systems by which cannabinoids are believed to be effective in adults are also present and potentially functional in neonates.

As previously mentioned, CB receptors are present in the nervous system as early as 14 weeks gestational age in humans and 11 days gestational age in rodents, and CB receptor concentrations in most brain regions gradually increase through adulthood (Fride 2008). Evidence additionally suggests that the ECS functions similarly in neonatal and adult stress responses. Acute stress exposure transiently reduces AEA levels in the hippocampus of PD12 rats (Marco et al. 2013) and CB1 receptors regulate HPA axis activity in PD8 rats (Buwembo et al. 2013), consistent with what is seen in adult rats (Morena et al. 2016).

Similar to the adult ECS (Rubino and Parolaro 2011) sex differences also appear to be present in the neonatal ECS. Rodriguez de Fonseca et al. (1993) found that the forebrain of female rats compared to males had more CB receptors at PD2 and less CB receptors at PD5, as well as less CB receptors in the striatum and midbrain at PD10. Suarez et al. (2009) also found at PD13 that females had higher CB1 receptor density in the hippocampal CA3 subregion but lower CB2 receptor density in the dentate gyrus when compared to males. It is difficult to predict the precise effects of these sex differences in CB receptor distribution, but they may modulate the effects of cannabinoids via the neonatal ECS.

Regarding TRPV1 receptors, there is some controversy about when and where they first appear in various regions of the central nervous system. However, TRPV1 receptor expression on neural stem cells and their association with neurogenesis suggests they are present in proliferative zones by early embryonic development (Ramirez-Barrantes et al. 2016). In addition, neonatal rats display behavioral responses when exposed to the TRPV1 receptor agonist capsaicin, which indicates that TRPV1 receptors are present and functional in nociceptive pathways of rats at birth, at the latest (Holzer et al. 1979; Jeong and Seong 2014; Newson et al. 2014).

GPR55 receptors are observed to be present in the nervous system and able to guide axon migration as early as gestational day 14 in mice (Cherif et al. 2015), which suggests they can also modulate nociception during the neonatal period. Further, GPR55 receptors appear to be uniquely suited for pain management in neurologically immature subjects, as activation of GPR55 receptors in the rostroventral medulla and periaqueductal gray is antinociceptive for neonatal and adolescent rats but not adults (Kwok et al. 2017). This suggests that ECS modulation may in some ways be more effective for pain management in neonates than it is in adults.

GPR55 also interacts with CBD, where CBD acts as an antagonist (Cherif et al. 2015; Sharir and Abood 2010). This leads to a paradoxical effect of CBD directly inhibiting GPR55 activity while simultaneously increasing concentrations of the GPR55 partial agonist AEA. However, the pharmacodynamics of CBD's interaction

with GPR55 are not well known, which makes it difficult to predict CBD's net effect on GPR55 activity in vivo.

Outside of the ECS, CBD is also an effective inhibitor of adenosine uptake (Mlost et al. 2020), and acute pain is modulated by adenosine activity in the periphery and the central nervous system (Adebiyi et al. 2019). Importantly, adenosine receptors are present and functional from early gestation (Weaver 1996), which suggests that CBD's influence on adenosine activity may be able to modulate acute pain in neonates. CBD is also a strong partial agonist of dopamine 2 (D2) receptors (Mlost et al. 2020), which are believed to be responsible for the effect of oral sucrose on behavioral pain responses in preterm infants. CBD likely at least partially reduces behavioral pain responses in the same manner and may concomitantly modulate the observed effects of oral sucrose on neonatal dopaminergic neurodevelopment (McPherson and Grunau 2014). CBD may also reduce nociception in adults partially via D2 receptor activity in descending inhibitory pathways, although they cannot modulate pain perception before 36 weeks gestational age (McPherson and Grunau 2014). CBD is also a positive allosteric modulator of $5HT_{1A}$ receptors and an agonist at PPARγ receptors (Hatfield 2014; McPherson and Grunau 2014). Modulation of either receptor likely contributes to CBD's capacity for alleviating inflammatory pain, although there is no evidence that they contribute to acute pain management and not much is known whether their role or contribution is different during early development or the neonatal phase.

7 Potential for Preventing the Long-Term Effects of Neonatal Pain-Related Stress with Cannabinoids

The greatest potential benefit from treating neonatal pain-related stress with cannabinoids may be preventing the effects of pain on long-term neurodevelopment. Preterm birth leaves infants especially vulnerable to neonatal stress during a period of profound neurodevelopment. As previously noted, preterm infants in NICUs receive numerous painful procedures per day, and these painful procedures are a common source of stress. Recurrent stress causes persistently high levels of CORT which can suppress neurogenesis and neuronal migration, as well as alter later-life HPA axis activity (Jellyman et al. 2012; McCormick et al. 2001). Like the HPA axis, development of the ECS is also altered by neonatal stress and Vangopoulou et al. (2018) suggest these changes in the ECS are driven by changes in HPA axis function. Preventing neonatal pain from elevating CORT levels may therefore alleviate multiple long-term effects of neonatal pain on neurodevelopment, the HPA axis, and the ECS. Cannabinoids are likely to be effective in this regard, as studies have found that CB1 receptor agonist CP55940 (Patel et al. 2004), FAAH inhibitor URB597 (Bedse et al. 2014; McLaughlin et al. 2016; Patel et al. 2004), and CBD administration one-time or daily for 7 days (Appiah-Kusi et al. 2020; Hurd et al. 2019) both mitigate the elevation of plasma CORT levels that typically occurs after stress

exposure in adult rats. Further, preliminary data indicates that CBD may prevent neonatal pain from slowing adult CORT recovery, but it might additionally alter baseline CORT levels (Timmerman et al. 2025).

FAAH activity promotes CORT secretion (Morena et al. 2016) and FAAH inhibitors may therefore be especially effective at keeping CORT levels low during pain exposure. Interestingly, McLaughlin et al. (2016) found that treating 10-day-old rat pups with an FAAH inhibitor decreased pups' CORT response to restraint stress if they had been exposed to the limited nest/bedding model of neonatal stress, but not in normally reared pups. Although CORT reactivity in rat pups during the stress hyporesponsive period has limited translational validity, these results suggest that FAAH inhibitors may be effective for managing later-life stress responses in organisms that did not get adequate neonatal pain management.

Cannabinoids may be able to mitigate the effects of neonatal pain-related stress on oligodendrocytes and white matter development (Brummelte et al. 2012; Ilyasov et al. 2018). CB receptor agonists promote the proliferation and maturation of oligodendrocytes, and also protect preOLs from inflammation and excitotoxic damage (Ilyasov et al. 2018). This effect may be even more pronounced in preterm infants, as CB receptors are transiently expressed on white matter tracts as late as 30 weeks of gestational age in humans and up to the neonatal period in rats (Mato et al. 2003; Romero et al. 1997). Further, CBD acts as an antioxidant (Booz 2011) and is neuroprotective in animal models of adult and neonatal hypoxic ischemia via activation of $5HT_{1A}$ receptors, increased adenosine signaling, and increased endocannabinoid activity at CB2 receptors (Castillo et al. 2010; Hayakawa et al. 2004; Mishima et al. 2005; Pazos et al. 2012, 2013). These findings suggest that cannabinoids can promote neonatal white matter development while simultaneously mitigating the multiple ways that it can be impaired by neonatal pain. Further, CBD can be additionally neuroprotective because of its interaction with neurotransmitter systems outside the ECS and because it's particularly anti-inflammatory (Burstein 2015). Decreasing inflammation could help prevent long-term adverse effects of increased inflammatory markers due to pain-related stress early in life (Pittman et al. 2019).

Neonatal pain in some paradigms is observed to increase adult anxiety-like behavior in rodents (Timmerman et al. 2025; Page et al. 2005; Schellinck et al. 2003). Surprisingly, CBD is unable to stop neonatal pain from increasing anxiety-like behavior in adult rats (Timmerman et al. 2025; Rego et al. 2024). These results suggest that neonatal pain likely alters adult limbic system function, and that CBD is ineffective at preventing it despite acutely modulating the affective aspect of pain in adult humans (De Vita et al. 2018). Future research into these findings is warranted to determine the mechanisms by which neonatal pain alters later-life anxiety-like behavior, as well as if another pharmacological compound could prevent the long-term effects of neonatal pain on behavior if administered concomitantly with CBD.

Pharmacological modulation of the ECS may decrease neonatal pain-related stress by reducing nociception and HPA axis activity, but it may also reduce stress via other indirect mechanisms such as improving food intake. Activity in the ECS is important for the initiation of suckling/nursing behavior (Fride 2008), and suckling/

nursing reduces stress responses in neonates (Blass and Watt 1999; Cong et al. 2017). Increased suckling behavior would likely also increase neonates' nutritional intake, which additionally influences neurodevelopment (Walker 2005). Therefore, cannabinoids could in part mitigate stress responses and long-term effects in animal models of neonatal pain by increasing neonates' suckling and nursing. Future research that investigates the potential for cannabinoids to mitigate neonatal pain and prevent long-term developmental outcomes in animal models should therefore ensure to account for changes in suckling behavior and food intake in cannabinoid-treated animals.

8 Potential Concerns About Cannabinoids for Neonates

While cannabinoids have potential for mitigating the effects of neonatal pain-related stress, there is also good reason to be cautious about implementing them in neonatal care. Studies indicate that early-life exposure to cannabis or THC-containing compounds is associated with reproductive and developmental toxicity (as reviewed by Akingbasote et al. 2022). However, the consequences exposure to most individual cannabinoid compounds, especially in early-life, are largely unknown.

Notably, a clinical trial for the FAAH inhibitor BIA 10–2474 was stopped after the death of 1 participant and neurological injury to 4 out of the 5 other participants in the same dosage group (Mallet et al. 2016). Although the FDA declared in 2016 that other FAAH inhibitors did not share similar risks (U.S. Food and Drug Administration 2016), an in silico structural proteomic analysis found that many FAAH inhibitors may bind with off-target proteins that could explain the severe adverse effects of BIA 10–2474 (Dider et al. 2016). Therefore, FAAH inhibitors may not be the best target to explore for alleviating neonatal stress, but more research in this area will help illuminate exact mechanisms, potential uses, and risk factors.

Understanding the potential for pharmacological manipulation of the ECS to alleviate the consequences of neonatal pain-related stress also requires investigation into the full effects of cannabinoids. More specifically, GPR55 is not well understood, but it is observed to bind with AEA and CBD (Ryberg et al. 2007; Sharir and Abood 2010; Sharir et al. 2012), and the effects of cannabinoids via GPR55 may be important for determining treatment options. Future studies should investigate the effects of GPR55 agonists and antagonists, alone and/or in combination with CBD or other methods of increasing AEA concentration, to discover how GPR55 activity contributes to the effects of cannabinoids. In addition, the long-term effects of neonatal exposure to cannabinoids are largely unknown and this is especially true for CBD. Most of the research on early-life cannabinoid exposure has focused on gestational exposure and/or exposure to THC-containing compounds to elucidate the consequences of offspring cannabinoid exposure that is secondary to maternal cannabinoid use (Grant et al. 2018; Scheyer et al. 2020; Schneider 2009; Trezza et al. 2012; Wanner et al. 2021). However, the effects of THC can be drastically different

from the effects of discrete CBD exposure, and prenatal exposure to stress or psychoactive substances can have the inverse effect of neonatal exposure (Benoit et al. 2015; Catalani et al. 1993; McCormick et al. 2001; Modir et al. 2014).

Cherif et al. (2015) report that CBD repels neuronal growth cones in vitro, which understandably raises concerns that CBD may interfere with early-life neurodevelopment. CBD likely repels neuronal growth cones in vitro because it is an antagonist of GPR55, a G-protein coupled receptor involved in growth cone migration (Ryberg et al. 2007). However, in vivo CBD also increases the concentration of AEA, a partial agonist of GPR55 (Sharir et al. 2012) which is far more potent than CBD at GPR55 (Ryberg et al. 2007). This collectively suggests that although CBD is an antagonist of GPR55, direct interaction between CBD and GPR55 might have minimal effect on growth cone migration.

There is also concern that because cannabinoids impair cell proliferation and promote apoptosis in cancer cells (Daris et al. 2019) they may do the same to neural and glial cells. CBD's influence in this regard appears to be dose dependent. Schiavon et al. (2016) found that repeated CBD administration at low doses increases cell proliferation in the dentate gyrus and subventricular zone, but repeated administration of CBD at high doses decreases cell proliferation. CBD also dose-dependently promotes apoptosis of mature oligodendrocytes and oligodendrocyte progenitors in vitro (Mato et al. 2010; Mecha et al. 2012). However, low doses of CBD are neuroprotective for oligodendrocyte progenitors (Mecha et al. 2012) which make up the majority of white matter in humans between 24- and 40-weeks gestational age (Gopagondanahalli et al. 2016). This suggests that CBD at low-to-moderate doses will primarily protect oligodendrocytes and promote their maturation in infants up to term-equivalent age, and is supported by evidence that low doses of CBD in animal models of hypoxic ischemia prevent hypomyelination, protect mature oligodendrocyte levels, and reduce white matter injury (Ceprian et al. 2019; Mori et al. 2017).

The action of CBD as a partial agonist at dopamine D2 receptors warrants attention, as the dopamine system is implicated in multiple neurodevelopmental and neurodegenerative disorders, and the neurodevelopmental consequences of oral sucrose are attributed to altered dopaminergic activity (McPherson and Grunau 2014). This review was unable to find any published research on the effects of neonatal exposure to a selective D2 receptor partial agonist. However, gestational exposure to antipsychotic medication, which typically act on dopamine receptors, appears to have only limited and transient effects on neurodevelopmental behavior (Galbally et al. 2014). This suggests that neonatal exposure to a dopamine D2 receptor partial agonist such as cannabidiol may not have significant long-term detrimental effects on neurodevelopment, although further research is necessary to confirm this.

Recently, research has raised concerns about the effects of cannabinoids on the development and function of the reproductive system. Several studies have documented cannabinoids to be reproductive toxins (Akingbasote et al. 2022; Carvalho et al. 2020; Corsi et al. 2021). This is likely to be at least partially a result of cannabinoids' modulation of the ECS, which is vital to reproductive function. Although most studies about cannabinoids and the reproductive system have focused on

cannabinoid consumption at adulthood, there is evidence that exposure to cannabinoids during the neonatal period can also alter adult reproductive function. Dalterio et al. (1984) and Dalterio and deRooij (1986) report that administering 50 mg/kg of CBD p.o. to rat dams within 12 h after giving birth causes male offspring to have decreased testicular weight, spermatozoa count, and testicular testosterone concentrations at adulthood, presumably because they were exposed to the CBD while nursing. Importantly, cannabinoids accumulate in milk during lactation (Moss et al. 2021; Wymore et al. 2021) and concentrated levels of CBD in dams' milk could have led to offspring in these studies receiving unexpectedly large doses. Further, Dalterio et al. (1983) found that cannabinoids have a dose-dependent effect on testicular testosterone levels in vitro, with micromolar concentrations of CBD increasing testosterone production in mouse testes but nanomolar concentrations having no effect on testicular testosterone production. The endocannabinoid system is also particularly important for female reproductive function (as reviewed by Walker et al. 2019). There is evidence that cannabis consumption can influence female reproductive function, in part by altering the length of some menstrual phases and potentially decreasing fertility (as reviewed by Corsi et al. 2021). However, research on this topic is limited and there are especially few studies about the ways cannabidiol exposure in early-life or adulthood affects the female reproductive system.

Taken together, more research is needed on the long-term effects of neonatal exposure to CBD or other compounds that target the ECS to fully understand their potential detrimental effects. This is increasingly important for CBD as its popularity grows and thus the risk of prenatal or neonatal CBD exposure increases as well. Therefore, research on neonatal CBD exposure is needed if not just to investigate how CBD may help alleviate pain-related stress, but also to discover how CBD can influence early-life neurodevelopment for better or worse.

9 Conclusion

The ECS modulates nociception, inflammation and the HPA axis, which are all implicated in the effects of neonatal pain-related stress (Llorente et al. 2007, 2008; Lopez-Gallardo et al. 2008). Of the cannabinoid compounds, CBD may be particularly effective for preventing the short- and long-term effects of neonatal pain-related stress. In addition to CBD being nonpsychoactive and having relatively few adverse effects, research suggests that it is effective for modulating nociception because it reduces inflammation, acts as an agonist of TRPV1 receptors, and increases synaptic concentrations of AEA and adenosine which both modulate nociception. CBD's effect on the GPR55 is also of interest because of CBD's potential to modulate pain as GPR55 appears to guide axon migration and modulate neonatal nociception, but the pharmacodynamics of this receptor are not well understood. Further, neonates have elevated CB receptor and GPR55 concentrations that may make CBD even more effective than it is in adults. However, CBD has pharmacological action at many different neurotransmitters, receptors, and enzymes, and the

consequences of each interaction are not well understood. More research is necessary to characterize the ability of CBD to modulate pain in neonates, the effects of CBD via GPR55 receptors, and if CBD's effects via GPR55 are at least partially capable of reducing neonatal pain-related stress.

CBD may be able to alleviate some of the long-term effects of neonatal pain-related stress because it protects immature oligodendrocytes, promotes oligodendrogenesis and neurogenesis, and decreases stress reactivity by moderating the affective aspect of pain and reducing CORT responses to stress. However, it seems ineffective in preventing negative effects of neonatal pain on long-term anxiety-like behavior in animals (Timmerman et al. 2025; Grunau 2013; Llorente et al. 2007, 2008; Lopez-Gallardo et al. 2008; McLaughlin et al. 2016), suggesting that the consequences of neonatal pain-related stress are complex and may go beyond simple pain management.

Like CBD, other ECS-targeted compounds such as FAAH inhibitors which prevent the catabolism of AEA, or fatty acid-binding protein inhibitors which prevent AEA's uptake and transportation to FAAH, may be beneficial for treating neonatal pain-related stress. However, there exist several caveats. FAAH inhibitors are observed to have potentially serious adverse effects (Mallet et al. 2016), and preclinical research suggests they may only be effective for reducing stress responses if there are prior sources of stress that have gone unaddressed (McLaughlin et al. 2016). Inhibitors of fatty acid-binding proteins may influence the ECS in a similar manner to CBD and they appear to be antinociceptive (Peng et al. 2017). However, very little research has been done on fatty acid-binding proteins or compounds that inhibit them, and the totality of their actions is not known.

Although there is greater risk and uncertainty associated with the use of ECS-targeted compounds beyond CBD, future research with CBD may benefit from investigating it alongside compounds that share similar mechanisms of action. For example, the administration of fatty acid binding protein inhibitors or FAAH inhibitors in comparison to CBD may help determine what role AEA concentrations and the ECS have in CBD's beneficial effects. In addition, CBD has many pharmacological actions, and its effects are notably dose dependent. It may be that CBD's dose-dependent effects are due to the different affinities and potencies across CBD's mechanisms of action. Determining which of CBD's actions are beneficial or detrimental by comparing CBD's effects with similar compounds may therefore help refine the pharmacological targets and improve scientific understanding of treatment options for neonatal pain-related stress.

Taken together, pharmacological manipulation of the ECS with cannabinoids may have potential for mitigating some short- and long-term consequences of neonatal pain-related stress. Specifically, cannabidiol reduces the affective dimension of pain, reduces part of the HPA axis response to stress, promotes neuron and glia proliferation, protects immature neuron and glia from oxidative stress and excitotoxicity, and dose-dependently modulates nociception.

CBD may decrease neonatal pain-related stress by reducing nociception and HPA axis activity, but it may also reduce stress via other indirect mechanisms such as improving food intake. Though most research studies thus far have focused on

the potential for CBD to modulate nociception in adults, it is tempting to suggest that neonates may benefit from cannabinoid treatments as well, given that most receptors and endocannabinoids are expressed early during development. Future research should investigate how manipulation of the ECS may prevent neonatal stress-induced changes in the ECS, HPA axis, and nociceptive functioning as well as how neonatal pain-related stress alters the ECS itself.

References

Adebiyi MG, Manalo J, Kellems RE, Xia Y. Differential role of adenosine signaling cascade in acute and chronic pain. Neurosci Lett. 2019;712:134483. https://doi.org/10.1016/j.neulet.2019.134483.

Ahn K, Johnson DS, Cravatt BF. Fatty acid amide hydrolase as a potential therapeutic target for the treatment of pain and CNS disorders. Expert Opin Drug Discovery. 2009;4(7):763–84. https://doi.org/10.1517/17460440903018857.

Akingbasote J, Szlapinski S, Charrette A, Hilmas CJ, Guthrie N. Safety of cannabis- and hemp-derived constituents in reproduction and development. In: Reproductive and developmental toxicology; 2022. p. 455–87. https://doi.org/10.1016/b978-0-323-89773-0.00024-2.

Anand KJ, Garg S, Rovnaghi CR, Narsinghani U, Bhutta AT, Hall RW. Ketamine reduces the cell death following inflammatory pain in newborn rat brain. Pediatr Res. 2007;62(3):283–90. https://doi.org/10.1203/PDR.0b013e3180986d2f.

Appiah-Kusi E, Petros N, Wilson R, Colizzi M, Bossong MG, Valmaggia L, Mondelli V, McGuire P, Bhattacharyya S. Effects of short-term cannabidiol treatment on response to social stress in subjects at clinical high risk of developing psychosis. Psychopharmacology (Berl). 2020;237(4):1121–30. https://doi.org/10.1007/s00213-019-05442-6.

Argaw A, Duff G, Zabouri N, Cecyre B, Chaine N, Cherif H, Tea N, Lutz B, Ptito M, Bouchard JF. Concerted action of CB1 cannabinoid receptor and deleted in colorectal cancer in axon guidance. J Neurosci. 2011;31(4):1489–99. https://doi.org/10.1523/JNEUROSCI.4134-09.2011.

Bedse G, Colangeli R, Lavecchia AM, Romano A, Altieri F, Cifani C, Cassano T, Gaetani S. Role of the basolateral amygdala in mediating the effects of the fatty acid amide hydrolase inhibitor URB597 on HPA axis response to stress. Eur Neuropsychopharmacol. 2014;24(9):1511–23. https://doi.org/10.1016/j.euroneuro.2014.07.005.

Belue RC, Howlett AC, Westlake TM, Hutchings DE. The ontogeny of cannabinoid receptors in the brain of postnatal and aging rats. Neurotoxicol Teratol. 1995;17(1):25–30. https://doi.org/10.1016/0892-0362(94)00053-g.

Benoit JD, Rakic P, Frick KM. Prenatal stress induces spatial memory deficits and epigenetic changes in the hippocampus indicative of heterochromatin formation and reduced gene expression. Behav Brain Res. 2015;281:1–8. https://doi.org/10.1016/j.bbr.2014.12.001.

Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I, Moriello AS, Davis JB, Mechoulam R, Di Marzo V. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br J Pharmacol. 2001;134(4):845–52. https://doi.org/10.1038/sj.bjp.0704327.

Blass EM, Watt LB. Suckling- and sucrose-induced analgesia in human newborns. Pain. 1999;83(3):611–23. https://doi.org/10.1016/S0304-3959(99)00166-9.

Booz GW. Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress. Free Radic Biol Med. 2011;51(5):1054–61. https://doi.org/10.1016/j.freeradbiomed.2011.01.007.

Brown RW, Diaz R, Robson AC, Kotelevtsev YV, Mullins JJ, Kaufman MH, Seckl JR. The ontogeny of 11 beta-hydroxysteroid dehydrogenase type 2 and mineralocorticoid receptor gene

expression reveal intricate control of glucocorticoid action in development. Endocrinology. 1996;137(2):794–7. https://doi.org/10.1210/endo.137.2.8593833.

Brummelte S, Grunau RE, Zaidman-Zait A, Weinberg J, Nordstokke D, Cepeda IL. Cortisol levels in relation to maternal interaction and child internalizing behavior in preterm and full-term children at 18 months corrected age. Dev Psychobiol. 2011;53(2):184–95. https://doi.org/10.1002/dev.20511.

Brummelte S, Grunau RE, Chau V, Poskitt KJ, Brant R, Vinall J, Gover A, Synnes AR, Miller SP. Procedural pain and brain development in premature newborns. Ann Neurol. 2012;71(3):385–96. https://doi.org/10.1002/ana.22267.

Burstein S. Cannabidiol (CBD) and its analogs: a review of their effects on inflammation. Bioorg Med Chem. 2015;23(7):1377–85. https://doi.org/10.1016/j.bmc.2015.01.059.

Buske-Kirschbaum A, Krieger S, Wilkes C, Rauh W, Weiss S, Hellhammer DH. Hypothalamic-pituitary-adrenal axis function and the cellular immune response in former preterm children. J Clin Endocrinol Metab. 2007;92(9):3429–35. https://doi.org/10.1210/jc.2006-2223.

Buwembo A, Long H, Walker CD. Participation of endocannabinoids in rapid suppression of stress responses by glucocorticoids in neonates. Neuroscience. 2013;249:154–61. https://doi.org/10.1016/j.neuroscience.2012.10.057.

Campbell-Yeo ML, Disher TC, Benoit BL, Johnston CC. Understanding kangaroo care and its benefits to preterm infants. Pediatr Health Med Ther. 2015;6:15–32. https://doi.org/10.2147/PHMT.S51869.

Campos AC, Fogaca MV, Sonego AB, Guimaraes FS. Cannabidiol, neuroprotection and neuropsychiatric disorders. Pharmacol Res. 2016;112:119–27. https://doi.org/10.1016/j.phrs.2016.01.033.

Carbajal R, Rousset A, Danan C, Coquery S, Nolent P, Ducrocq S, Saizou C, Lapillonne A, Granier M, Durand P, Lenclen R, Coursol A, Hubert P, de Saint Blanquat L, Boelle PY, Annequin D, Cimerman P, Anand KJ, Breart G. Epidemiology and treatment of painful procedures in neonates in intensive care units. JAMA. 2008;300(1):60–70. https://doi.org/10.1001/jama.300.1.60.

Carlini EA. The good and the bad effects of (−) trans-delta-9-tetrahydrocannabinol (Δ9-THC) on humans. Toxicon. 2004;44(4):461–7. https://doi.org/10.1016/j.toxicon.2004.05.009.

Carson R, Monaghan-Nichols AP, DeFranco DB, Rudine AC. Effects of antenatal glucocorticoids on the developing brain. Steroids. 2016;114:25–32. https://doi.org/10.1016/j.steroids.2016.05.012.

Carvalho RK, Andersen ML, Mazaro-Costa R. The effects of cannabidiol on male reproductive system: a literature review. J Appl Toxicol. 2020;40(1):132–50. https://doi.org/10.1002/jat.3831.

Castillo A, Tolon MR, Fernandez-Ruiz J, Romero J, Martinez-Orgado J. The neuroprotective effect of cannabidiol in an in vitro model of newborn hypoxic-ischemic brain damage in mice is mediated by CB(2) and adenosine receptors. Neurobiol Dis. 2010;37(2):434–40. https://doi.org/10.1016/j.nbd.2009.10.023.

Catalani A, Marinelli M, Scaccianoce S, Nicolai R, Muscolo LAA, Porcu A, Koran'yi L, Piazza PV, Angelucci L. Progeny of mothers drinking corticosterone during lactation has lower stress-induced corticosterone secretion and better cognitive performance. Brain Res. 1993;624(1–2):209–15. https://doi.org/10.1016/0006-8993(93)90079-3.

Ceprian M, Vargas C, Garcia-Toscano L, Penna F, Jimenez-Sanchez L, Achicallende S, Elezgarai I, Grandes P, Hind W, Pazos MR, Martinez-Orgado J. Cannabidiol administration prevents hypoxia-ischemia-induced hypomyelination in newborn rats. Front Pharmacol. 2019;10:1131. https://doi.org/10.3389/fphar.2019.01131.

Cherif H, Argaw A, Cecyre B, Bouchard A, Gagnon J, Javadi P, Desgent S, Mackie K, Bouchard JF. Role of GPR55 during axon growth and target innervation. eNeuro. 2015;2(5):ENEURO.0011-15.2015. https://doi.org/10.1523/ENEURO.0011-15.2015.

Cong X, Wu J, Vittner D, Xu W, Hussain N, Galvin S, Fitzsimons M, McGrath JM, Henderson WA. The impact of cumulative pain/stress on neurobehavioral development of preterm infants in the nicu. Early Hum Dev. 2017;108:9–16. https://doi.org/10.1016/j.earlhumdev.2017.03.003.

Corcoran L, Roche M, Finn DP. The role of the brain's endocannabinoid system in pain and its modulation by stress. Int Rev Neurobiol. 2015;125:203–55. https://doi.org/10.1016/bs.irn.2015.10.003.

Corsi DJ, Murphy MSQ, Cook J. The effects of cannabis on female reproductive health across the life course. Cannabis Cannabinoid Res. 2021;6(4):275–87. https://doi.org/10.1089/can.2020.0065.

Costa B, Trovato AE, Comelli F, Giagnoni G, Colleoni M. The non-psychoactive cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. Eur J Pharmacol. 2007;556(1–3):75–83. https://doi.org/10.1016/j.ejphar.2006.11.006.

Dalterio SL, deRooij DG. Maternal cannabinoid exposure. Effects on spermatogenesis in male offspring. Int J Androl. 1986;9(4):250–8. https://doi.org/10.1111/j.1365-2605.1986.tb00888.x.

Dalterio SL, Bartke A, Mayfield D. Cannabinoids stimulate and inhibit testosterone production in vitro and in vivo. Life Sci. 1983;32(6):605–12. https://doi.org/10.1016/0024-3205(83)90205-9.

Dalterio S, Steger R, Mayfield D, Bartke A. Early cannabinoid exposure influences neuroendocrine and reproductive functions in mice: II. Postnatal effects. Pharmacol Biochem Behav. 1984;20(1):115–23. https://doi.org/10.1016/0091-3057(84)90111-4.

Daris B, Tancer Verboten M, Knez Z, Ferk P. Cannabinoids in cancer treatment: therapeutic potential and legislation. Bosn J Basic Med Sci. 2019;19(1):14–23. https://doi.org/10.17305/bjbms.2018.3532.

Daskalakis NP, De Kloet ER, Yehuda R, Malaspina D, Kranz TM. Early life stress effects on glucocorticoid-BDNF interplay in the hippocampus. Front Mol Neurosci. 2015;8:68. https://doi.org/10.3389/fnmol.2015.00068.

De Vita MJ, Moskal D, Maisto SA, Ansell EB. Association of cannabinoid administration with experimental pain in healthy adults: a systematic review and meta-analysis. JAMA Psychiatry. 2018;75(11):1118–27. https://doi.org/10.1001/jamapsychiatry.2018.2503.

De Vita MJ, Maisto SA, Gilmour CE, McGuire L, Tarvin E, Moskal D. The effects of cannabidiol and analgesic expectancies on experimental pain reactivity in healthy adults: a balanced placebo design trial. Exp Clin Psychopharmacol. 2021. https://doi.org/10.1037/pha0000465.

Dider S, Ji J, Zhao Z, Xie L. Molecular mechanisms involved in the side effects of fatty acid amide hydrolase inhibitors: a structural phenomics approach to proteome-wide cellular off-target deconvolution and disease association. NPJ Syst Biol Appl. 2016;2:16023. https://doi.org/10.1038/npjsba.2016.23.

Doesburg SM, Chau CM, Cheung TPL, Moiseev A, Ribary U, Herdman AT, Miller SP, Cepeda IL, Synnes A, Grunau RE. Neonatal pain-related stress, functional cortical activity and visual-perceptual abilities in school-age children born at extremely low gestational age. Pain. 2013a;154(10):1946–52. https://doi.org/10.1016/j.pain.2013.04.009.

Doesburg SM, Moiseev A, Herdman AT, Ribary U, Grunau RE. Region-specific slowing of alpha oscillations is associated with visual-perceptual abilities in children born very preterm. Front Hum Neurosci. 2013b;7:791. https://doi.org/10.3389/fnhum.2013.00791.

Duerden EG, Grunau RE, Guo T, Foong J, Pearson A, Au-Young S, Lavoie R, Chakravarty MM, Chau V, Synnes A, Miller SP. Early procedural pain is associated with regionally-specific alterations in thalamic development in preterm neonates. J Neurosci. 2018;38(4):878–86. https://doi.org/10.1523/JNEUROSCI.0867-17.2017.

Elmes MW, Kaczocha M, Berger WT, Leung K, Ralph BP, Wang L, Sweeney JM, Miyauchi JT, Tsirka SE, Ojima I, Deutsch DG. Fatty acid-binding proteins (FABPs) are intracellular carriers for Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). J Biol Chem. 2015;290(14):8711–21. https://doi.org/10.1074/jbc.M114.618447.

Feldman R, Rosenthal Z, Eidelman AI. Maternal-preterm skin-to-skin contact enhances child physiologic organization and cognitive control across the first 10 years of life. Biol Psychiatry. 2014;75(1):56–64. https://doi.org/10.1016/j.biopsych.2013.08.012.

Fernandez EF, Montman R, Watterberg KL. ACTH and cortisol response to critical illness in term and late preterm newborns. J Perinatol. 2008;28(12):797–802. https://doi.org/10.1038/jp.2008.190.

Fitzgerald M. The development of nociceptive circuits. Nat Rev Neurosci. 2005;6(7):507–20. https://doi.org/10.1038/nrn1701.

Fride E. Multiple roles for the endocannabinoid system during the earliest stages of life: pre- and postnatal development. J Neuroendocrinol. 2008;20 Suppl 1:75–81. https://doi.org/10.1111/j.1365-2826.2008.01670.x.

Fukumoto K, Morita T, Mayanagi T, Tanokashira D, Yoshida T, Sakai A, Sobue K. Detrimental effects of glucocorticoids on neuronal migration during brain development. Mol Psychiatry. 2009;14(12):1119–31. https://doi.org/10.1038/mp.2009.60.

Galbally M, Snellen M, Power J. Antipsychotic drugs in pregnancy: a review of their maternal and fetal effects. Ther Adv Drug Saf. 2014;5(2):100–9. https://doi.org/10.1177/2042098614522682.

Gao H, Gao H, Xu G, Li M, Du S, Li F, Zhang H, Wang D. Efficacy and safety of repeated oral sucrose for repeated procedural pain in neonates: a systematic review. Int J Nurs Stud. 2016;62:118–25. https://doi.org/10.1016/j.ijnurstu.2016.07.015.

Gazendam A, Nucci N, Gouveia K, Abdel Khalik H, Rubinger L, Johal H. Cannabinoids in the management of acute pain: a systematic review and meta-analysis. Cannabis Cannabinoid Res. 2020;5(4):290–7. https://doi.org/10.1089/can.2019.0079.

Genaro K, Fabris D, Arantes ALF, Zuardi AW, Crippa JAS, Prado WA. Cannabidiol is a potential therapeutic for the affective-motivational dimension of incision pain in rats. Front Pharmacol. 2017;8:391. https://doi.org/10.3389/fphar.2017.00391.

Goncalves TC, Londe AK, Albano RI, de Araujo Junior AT, de Aguiar Azeredo M, Biagioni AF, Vasconcellos TH, Dos Reis Ferreira CM, Teixeira DG, de Souza Crippa JA, Vieira D, Coimbra NC. Cannabidiol and endogenous opioid peptide-mediated mechanisms modulate antinociception induced by transcutaneous electrostimulation of the peripheral nervous system. J Neurol Sci. 2014;347(1–2):82–9. https://doi.org/10.1016/j.jns.2014.09.024.

Gopagondanahalli KR, Li J, Fahey MC, Hunt RW, Jenkin G, Miller SL, Malhotra A. Preterm hypoxic-ischemic encephalopathy. Front Pediatr. 2016;4:114. https://doi.org/10.3389/fped.2016.00114.

Grant KS, Petroff R, Isoherranen N, Stella N, Burbacher TM. Cannabis use during pregnancy: pharmacokinetics and effects on child development. Pharmacol Ther. 2018;182:133–51. https://doi.org/10.1016/j.pharmthera.2017.08.014.

Grunau RE. Neonatal pain in very preterm infants: long-term effects on brain, neurodevelopment and pain reactivity. Rambam Maimonides Med J. 2013;4(4):e0025. https://doi.org/10.5041/RMMJ.10132.

Grunau RE, Weinberg J, Whitfield MF. Neonatal procedural pain and preterm infant cortisol response to novelty at 8 months. Pediatrics. 2004;114(1):e77–84. https://doi.org/10.1542/peds.114.1.e77.

Grunau RE, Haley DW, Whitfield MF, Weinberg J, Yu W, Thiessen P. Altered basal cortisol levels at 3, 6, 8 and 18 months in infants born at extremely low gestational age. J Pediatr. 2007;150(2):151–6. https://doi.org/10.1016/j.jpeds.2006.10.053.

Grunau RE, Whitfield MF, Petrie-Thomas J, Synnes AR, Cepeda IL, Keidar A, Rogers M, Mackay M, Hubber-Richard P, Johannesen D. Neonatal pain, parenting stress and interaction, in relation to cognitive and motor development at 8 and 18 months in preterm infants. Pain. 2009;143(1–2):138–46. https://doi.org/10.1016/j.pain.2009.02.014.

Hall RW, Anand KJS. Physiology of pain and stress in the newborn. NeoReviews. 2005;6(2):e61–8. https://doi.org/10.1542/neo.6-2-e61.

Hall RW, Anand KJ. Pain management in newborns. Clin Perinatol. 2014;41(4):895–924. https://doi.org/10.1016/j.clp.2014.08.010.

Hammell DC, Zhang LP, Ma F, Abshire SM, McIlwrath SL, Stinchcomb AL, Westlund KN. Transdermal cannabidiol reduces inflammation and pain-related behaviours in a rat model of arthritis. Eur J Pain. 2016;20(6):936–48. https://doi.org/10.1002/ejp.818.

Harkany T, Keimpema E, Barabas K, Mulder J. Endocannabinoid functions controlling neuronal specification during brain development. Mol Cell Endocrinol. 2008;286(1–2 Suppl 1):S84–90. https://doi.org/10.1016/j.mce.2008.02.011.

Hatfield LA. Neonatal pain: what's age got to do with it? Surg Neurol Int. 2014;5(Suppl 13):S479–89. https://doi.org/10.4103/2152-7806.144630.

Hayakawa K, Mishima K, Abe K, Hasebe N, Takamatsu F, Yasuda H, Ikeda T, Inui K, Egashira N, Iwasaki K, Fujiwara M. Cannabidiol prevents infarction via the non-CB1 cannabinoid receptor mechanism. Neuroreport. 2004;15(15):2381–5. https://doi.org/10.1097/00001756-200410250-00016.

Herrington CJ, Olomu IN, Geller SM. Salivary cortisol as indicators of pain in preterm infants: a pilot study. Clin Nurs Res. 2004;13(1):53–68. https://doi.org/10.1177/1054773803259665.

Hill MN, Tasker JG. Endocannabinoid signaling, glucocorticoid-mediated negative feedback, and regulation of the hypothalamic-pituitary-adrenal axis. Neuroscience. 2012;204:5–16. https://doi.org/10.1016/j.neuroscience.2011.12.030.

Hill KP, Palastro MD, Johnson B, Ditre JW. Cannabis and pain: a clinical review. Cannabis Cannabinoid Res. 2017;2(1):96–104. https://doi.org/10.1089/can.2017.0017.

Hofmann NA, Barth S, Waldeck-Weiermair M, Klec C, Strunk D, Malli R, Graier WF. Trpv1 mediates cellular uptake of anandamide and thus promotes endothelial cell proliferation and network-formation. Biol Open. 2014;3(12):1164–72. https://doi.org/10.1242/bio.20149571.

Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R, Krey JF, Walker JM, Holmes PV, Crystal JD, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D. An endocannabinoid mechanism for stress-induced analgesia. Nature. 2005;435(7045):1108–12. https://doi.org/10.1038/nature03658.

Holzer P, Jurna I, Gamse R, Lembeck F. Nociceptive threshold after neonatal capsaicin treatment. Eur J Pharmacol. 1979;58(4):511–4. https://doi.org/10.1016/0014-2999(79)90327-3.

Horvath G, Kekesi G, Nagy E, Benedek G. The role of TRPV1 receptors in the antinociceptive effect of anandamide at spinal level. Pain. 2008;134(3):277–84. https://doi.org/10.1016/j.pain.2007.04.032.

Huang H, McIntosh AL, Martin GG, Landrock D, Chung S, Landrock KK, Dangott LJ, Li S, Kier AB, Schroeder F. FABP1: a novel hepatic endocannabinoid and cannabinoid binding protein. Biochemistry. 2016;55(37):5243–55. https://doi.org/10.1021/acs.biochem.6b00446.

Huestis MA, Solimini R, Pichini S, Pacifici R, Carlier J, Busardo FP. Cannabidiol adverse effects and toxicity. Curr Neuropharmacol. 2019;17(10):974–89. https://doi.org/10.2174/1570159X17666190603171901.

Hurd YL, Spriggs S, Alishayev J, Winkel G, Gurgov K, Kudrich C, Oprescu AM, Salsitz E. Cannabidiol for the reduction of cue-induced craving and anxiety in drug-abstinent individuals with heroin use disorder: a double-blind randomized placebo-controlled trial. Am J Psychiatry. 2019;176(11):911–22. https://doi.org/10.1176/appi.ajp.2019.18101191.

Ilyasov AA, Milligan CE, Pharr EP, Howlett AC. The endocannabinoid system and oligodendrocytes in health and disease. Front Neurosci. 2018;12:733. https://doi.org/10.3389/fnins.2018.00733.

Jellyman JK, Allen VL, Forhead AJ, Holdstock NB, Fowden AL. Hypothalamic-pituitary-adrenal axis function in pony foals after neonatal ACTH-induced glucocorticoid overexposure. Equine Vet J Suppl. 2012;41:38–42. https://doi.org/10.1111/j.2042-3306.2011.00448.x.

Jeong KY, Seong J. Neonatal capsaicin treatment in rats affects TRPV1-related noxious heat sensation and circadian body temperature rhythm. J Neurol Sci. 2014;341(1–2):58–63. https://doi.org/10.1016/j.jns.2014.03.054.

Kaneyasu M. Pain management, morphine administration, and outcomes in preterm infants: a review of the literature. Neonatal Netw. 2012;31(1):21–30. https://doi.org/10.1891/0730-0832.31.1.21.

Kwok CH, Devonshire IM, Imraish A, Greenspon CM, Lockwood S, Fielden C, Cooper A, Woodhams S, Sarmad S, Ortori CA, Barrett DA, Kendall D, Bennett AJ, Chapman V, Hathway GJ. Age-dependent plasticity in endocannabinoid modulation of pain processing through postnatal development. Pain. 2017;158(11):2222–32. https://doi.org/10.1097/j.pain.0000000000001027.

Lago P, Garetti E, Merazzi D, Pieragostini L, Ancora G, Pirelli A, Bellieni CV, Pain Study Group of the Italian Society of Neonatology. Guidelines for procedural pain in the newborn. Acta Paediatr. 2009;98(6):932–9. https://doi.org/10.1111/j.1651-2227.2009.01291.x.

Laprairie JL, Murphy AZ. Neonatal injury alters adult pain sensitivity by increasing opioid tone in the periaqueductal gray. Front Behav Neurosci. 2009;3:31. https://doi.org/10.3389/neuro.08.031.2009.

Lee JH, Espinera AR, Chen D, Choi KE, Caslin AY, Won S, Pecoraro V, Xu GY, Wei L, Yu SP. Neonatal inflammatory pain and systemic inflammatory responses as possible environmental factors in the development of autism spectrum disorder of juvenile rats. J Neuroinflammation. 2016;13(1):109. https://doi.org/10.1186/s12974-016-0575-x.

Leishman E, Manchanda M, Thelen R, Miller S, Mackie K, Bradshaw HB. Cannabidiol's upregulation of n-acyl ethanolamines in the central nervous system requires n-acyl phosphatidyl ethanolamine-specific phospholipase D. Cannabis Cannabinoid Res. 2018;3(1):228–41. https://doi.org/10.1089/can.2018.0031.

Leweke FM, Piomelli D, Pahlisch F, Muhl D, Gerth CW, Hoyer C, Klosterkotter J, Hellmich M, Koethe D. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl Psychiatry. 2012;2:e94. https://doi.org/10.1038/tp.2012.15.

Li C, Liu S, Lu X, Tao F. Role of descending dopaminergic pathways in pain modulation. Curr Neuropharmacol. 2019;17(12):1176–82. https://doi.org/10.2174/1570159X17666190430102531.

Llorente R, Arranz L, Marco EM, Moreno E, Puerto M, Guaza C, De la Fuente M, Viveros MP. Early maternal deprivation and neonatal single administration with a cannabinoid agonist induce long-term sex-dependent psychoimmunoendocrine effects in adolescent rats. Psychoneuroendocrinology. 2007;32(6):636–50. https://doi.org/10.1016/j.psyneuen.2007.04.002.

Llorente R, Llorente-Berzal A, Petrosino S, Marco EM, Guaza C, Prada C, Lopez-Gallardo M, Di Marzo V, Viveros MP. Gender-dependent cellular and biochemical effects of maternal deprivation on the hippocampus of neonatal rats: a possible role for the endocannabinoid system. Dev Neurobiol. 2008;68(11):1334–47. https://doi.org/10.1002/dneu.20666.

Lopez-Gallardo M, Llorente R, Llorente-Berzal A, Marco EM, Prada C, Di Marzo V, Viveros MP. Neuronal and glial alterations in the cerebellar cortex of maternally deprived rats: gender differences and modulatory effects of two inhibitors of endocannabinoid inactivation. Dev Neurobiol. 2008;68(12):1429–40. https://doi.org/10.1002/dneu.20672.

Malheiros JM, Lima M, Avanzi RD, Gomes da Silva S, Suchecki D, Guinsburg R, Covolan L. Repetitive noxious neonatal stimuli increases dentate gyrus cell proliferation and hippocampal brain-derived neurotrophic factor levels. Hippocampus. 2014;24(4):415–23. https://doi.org/10.1002/hipo.22235.

Mallet C, Dubray C, Duale C. Faah inhibitors in the limelight, but regrettably. Int J Clin Pharmacol Ther. 2016;54(7):498–501. https://doi.org/10.5414/CP202687.

Marco EM, Scattoni ML, Rapino C, Ceci C, Chaves N, Macri S, Maccarrone M, Laviola G. Emotional, endocrine and brain anandamide response to social challenge in infant male rats. Psychoneuroendocrinology. 2013;38(10):2152–62. https://doi.org/10.1016/j.psyneuen.2013.04.004.

Mato S, Del Olmo E, Pazos A. Ontogenetic development of cannabinoid receptor expression and signal transduction functionality in the human brain. Eur J Neurosci. 2003;17(9):1747–54. https://doi.org/10.1046/j.1460-9568.2003.02599.x.

Mato S, Victoria Sanchez-Gomez M, Matute C. Cannabidiol induces intracellular calcium elevation and cytotoxicity in oligodendrocytes. Glia. 2010;58(14):1739–47. https://doi.org/10.1002/glia.21044.

Matthews SG. Early programming of the hypothalamo-pituitary-adrenal axis. Trends Endocrinol Metab. 2002;13(9):373–80. https://doi.org/10.1016/s1043-2760(02)00690-2.

McCormick CM, Rioux T, Fisher R, Lang K, MacLaury K, Teillon SM. Effects of neonatal corticosterone treatment on maze performance and HPA axis in juvenile rats. Physiol Behav. 2001;74(3):371–9. https://doi.org/10.1016/s0031-9384(01)00574-1.

McEwen BS. Steroid hormones and brain development: some guidelines for understanding actions of pseudohormones and other toxic agents. Environ Health Perspect. 1987;74:177–84. https://doi.org/10.1289/ehp.8774177.

McLaughlin RJ, Verlezza S, Gray JM, Hill MN, Walker CD. Inhibition of anandamide hydrolysis dampens the neuroendocrine response to stress in neonatal rats subjected to suboptimal rearing conditions. Stress. 2016;19(1):114–24. https://doi.org/10.3109/10253890.2015.1117448.

McPartland JM, Glass M, Pertwee RG. Meta-analysis of cannabinoid ligand binding affinity and receptor distribution: interspecies differences. Br J Pharmacol. 2007;152(5):583–93. https://doi.org/10.1038/sj.bjp.0707399.

McPherson C, Grunau RE. Neonatal pain control and neurologic effects of anesthetics and sedatives in preterm infants. Clin Perinatol. 2014;41(1):209–27. https://doi.org/10.1016/j.clp.2013.10.002.

McPherson C, Miller SP, El-Dib M, Massaro AN, Inder TE. The influence of pain, agitation, and their management on the immature brain. Pediatr Res. 2020;88(2):168–75. https://doi.org/10.1038/s41390-019-0744-6.

Meaney MJ, Szyf M, Seckl JR. Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. Trends Mol Med. 2007;13(7):269–77. https://doi.org/10.1016/j.molmed.2007.05.003.

Mecha M, Torrao AS, Mestre L, Carrillo-Salinas FJ, Mechoulam R, Guaza C. Cannabidiol protects oligodendrocyte progenitor cells from inflammation-induced apoptosis by attenuating endoplasmic reticulum stress. Cell Death Dis. 2012;3:e331. https://doi.org/10.1038/cddis.2012.71.

Micale V, Drago F. Endocannabinoid system, stress and HPA axis. Eur J Pharmacol. 2018;834:230–9. https://doi.org/10.1016/j.ejphar.2018.07.039.

Mishima K, Hayakawa K, Abe K, Ikeda T, Egashira N, Iwasaki K, Fujiwara M. Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine1a receptor-dependent mechanism. Stroke. 2005;36(5):1077–82. https://doi.org/10.1161/01.STR.0000163083.59201.34.

Mlost J, Bryk M, Starowicz K. Cannabidiol for pain treatment: focus on pharmacology and mechanism of action. Int J Mol Sci. 2020;21(22). https://doi.org/10.3390/ijms21228870.

Modir F, Elahdadi Salmani M, Goudarzi I, Lashkarboluki T, Abrari K. Prenatal stress decreases spatial learning and memory retrieval of the adult male offspring of rats. Physiol Behav. 2014;129:104–9. https://doi.org/10.1016/j.physbeh.2014.02.040.

Morena M, Patel S, Bains JS, Hill MN. Neurobiological interactions between stress and the endocannabinoid system. Neuropsychopharmacology. 2016;41(1):80–102. https://doi.org/10.1038/npp.2015.166.

Mori MA, Meyer E, Soares LM, Milani H, Guimaraes FS, de Oliveira RMW. Cannabidiol reduces neuroinflammation and promotes neuroplasticity and functional recovery after brain ischemia. Prog Neuropsychopharmacol Biol Psychiatry. 2017;75:94–105. https://doi.org/10.1016/j.pnpbp.2016.11.005.

Moss MJ, Bushlin I, Kazmierczak S, Koop D, Hendrickson RG, Zuckerman KE, Grigsby TM. Cannabis use and measurement of cannabinoids in plasma and breast milk of breastfeeding mothers. Pediatr Res. 2021;90(4):861–8. https://doi.org/10.1038/s41390-020-01332-2.

Newson PN, van den Buuse M, Martin S, Lynch-Frame A, Chahl LA. Effects of neonatal treatment with the TRPV1 agonist, capsaicin, on adult rat brain and behaviour. Behav Brain Res. 2014;272:55–65. https://doi.org/10.1016/j.bbr.2014.06.036.

Nuseir KQ, Alzoubi KH, Alhusban A, Bawaane A, Al-Azzani M, Khabour OF. Sucrose and naltrexone prevent increased pain sensitivity and impaired long-term memory induced by repetitive neonatal noxious stimulation: role of BDNF and beta-endorphin. Physiol Behav. 2017;179:213–9. https://doi.org/10.1016/j.physbeh.2017.06.015.

Ohlsson A, Shah PS. Paracetamol (acetaminophen) for prevention or treatment of pain in newborns. Cochrane Database Syst Rev. 2020;1:CD011219. https://doi.org/10.1002/14651858.CD011219.pub4.

O'Sullivan SE. An update on ppar activation by cannabinoids. Br J Pharmacol. 2016;173(12):1899–910. https://doi.org/10.1111/bph.13497.

Page GG, Blakely WP, Kim M. The impact of early repeated pain experiences on stress responsiveness and emotionality at maturity in rats. Brain Behav Immun. 2005;19(1):78–87. https://doi.org/10.1016/j.bbi.2004.05.002.

Papadimitriou A, Priftis KN. Regulation of the hypothalamic-pituitary-adrenal axis. Neuroimmunomodulation. 2009;16(5):265–71. https://doi.org/10.1159/000216184.

Patel S, Roelke CT, Rademacher DJ, Cullinan WE, Hillard CJ. Endocannabinoid signaling negatively modulates stress-induced activation of the hypothalamic-pituitary-adrenal axis. Endocrinology. 2004;145(12):5431–8. https://doi.org/10.1210/en.2004-0638.

Pazos MR, Cinquina V, Gomez A, Layunta R, Santos M, Fernandez-Ruiz J, Martinez-Orgado J. Cannabidiol administration after hypoxia-ischemia to newborn rats reduces long-term brain injury and restores neurobehavioral function. Neuropharmacology. 2012;63(5):776–83. https://doi.org/10.1016/j.neuropharm.2012.05.034.

Pazos MR, Mohammed N, Lafuente H, Santos M, Martinez-Pinilla E, Moreno E, Valdizan E, Romero J, Pazos A, Franco R, Hillard CJ, Alvarez FJ, Martinez-Orgado J. Mechanisms of cannabidiol neuroprotection in hypoxic-ischemic newborn pigs: role of 5HT(1A) and CB2 receptors. Neuropharmacology. 2013;71:282–91. https://doi.org/10.1016/j.neuropharm.2013.03.027.

Peng X, Studholme K, Kanjiya MP, Luk J, Bogdan D, Elmes MW, Carbonetti G, Tong S, Gary Teng YH, Rizzo RC, Li H, Deutsch DG, Ojima I, Rebecchi MJ, Puopolo M, Kaczocha M. Fatty-acid-binding protein inhibition produces analgesic effects through peripheral and central mechanisms. Mol Pain. 2017;13:1744806917697007. https://doi.org/10.1177/1744806917697007.

Pittman QJ, Gómez CD, Read J, Lewis ML, Acharjee S. Early life inflammation—it sticks to the brain. Curr Opin Behav Sci. 2019;28:136–41. https://doi.org/10.1016/j.cobeha.2019.02.008.

Ramirez-Barrantes R, Cordova C, Poblete H, Munoz P, Marchant I, Wianny F, Olivero P. Perspectives of TRPV1 function on the neurogenesis and neural plasticity. Neural Plast. 2016;2016:1568145. https://doi.org/10.1155/2016/1568145.

Ranger M, Grunau RE. Early repetitive pain in preterm infants in relation to the developing brain. Pain Manag. 2014;4(1):57–67. https://doi.org/10.2217/pmt.13.61.

Ranger M, Chau CM, Garg A, Woodward TS, Beg MF, Bjornson B, Poskitt K, Fitzpatrick K, Synnes AR, Miller SP, Grunau RE. Neonatal pain-related stress predicts cortical thickness at age 7 years in children born very preterm. PLoS One. 2013;8(10):e76702. https://doi.org/10.1371/journal.pone.0076702.

Ranger M, Zwicker JG, Chau CM, Park MT, Chakravarthy MM, Poskitt K, Miller SP, Bjornson BH, Tam EW, Chau V, Synnes AR, Grunau RE. Neonatal pain and infection relate to smaller cerebellum in very preterm children at school age. J Pediatr. 2015;167(2):292–298e291. https://doi.org/10.1016/j.jpeds.2015.04.055.

Ranger M, Tremblay S, Chau CMY, Holsti L, Grunau RE, Goldowitz D. Adverse behavioral changes in adult mice following neonatal repeated exposure to pain and sucrose. Front Psychol. 2018;9:2394. https://doi.org/10.3389/fpsyg.2018.02394.

Rego DSB, Calio ML, Filev R, Mello LE, Leslie A. Long-term effects of cannabidiol and/or fentanyl exposure in rats submitted to neonatal pain. J Pain. 2024;25(3):715–29. https://doi.org/10.1016/j.jpain.2023.10.001.

Rimmerman N, Bradshaw HB, Kozela E, Levy R, Juknat A, Vogel Z. Compartmentalization of endocannabinoids into lipid rafts in a microglial cell line devoid of Caveolin-1. Br J Pharmacol. 2012;165(8):2436–49. https://doi.org/10.1111/j.1476-5381.2011.01380.x.

Rodriguez de Fonseca F, Ramos JA, Bonnin A, Fernandez-Ruiz JJ. Presence of cannabinoid binding sites in the brain from early postnatal ages. Neuroreport. 1993;4(2):135–8. https://doi.org/10.1097/00001756-199302000-00005.

Romero J, Garcia-Palomero E, Berrendero F, Garcia-Gil L, Hernandez ML, Ramos JA, Fernandez-Ruiz JJ. Atypical location of cannabinoid receptors in white matter areas during rat brain development. Synapse. 1997;26(3):317–23. https://doi.org/10.1002/(SICI)1098-2396(199707)26:3<317::AID-SYN12>3.0.CO;2-S.

Ross RA. Anandamide and vanilloid TRPV1 receptors. Br J Pharmacol. 2003;140(5):790–801. https://doi.org/10.1038/sj.bjp.0705467.

Rubino T, Parolaro D. Sexually dimorphic effects of cannabinoid compounds on emotion and cognition. Front Behav Neurosci. 2011;5:64. https://doi.org/10.3389/fnbeh.2011.00064.

Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J, Elebring T, Nilsson K, Drmota T, Greasley PJ. The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol. 2007;152(7):1092–101. https://doi.org/10.1038/sj.bjp.0707460.

Schellinck HM, Stanford L, Darrah M. Repetitive acute pain in infancy increases anxiety but does not alter spatial learning ability in juvenile mice. Behav Brain Res. 2003;142(1–2):157–65. https://doi.org/10.1016/s0166-4328(02)00406-0.

Scheyer AF, Borsoi M, Wager-Miller J, Pelissier-Alicot AL, Murphy MN, Mackie K, Manzoni OJJ. Cannabinoid exposure via lactation in rats disrupts perinatal programming of the gamma-aminobutyric acid trajectory and select early-life behaviors. Biol Psychiatry. 2020;87(7):666–77. https://doi.org/10.1016/j.biopsych.2019.08.023.

Schiavon AP, Bonato JM, Milani H, Guimaraes FS, Weffort de Oliveira RM. Influence of single and repeated cannabidiol administration on emotional behavior and markers of cell proliferation and neurogenesis in non-stressed mice. Prog Neuropsychopharmacol Biol Psychiatry. 2016;64:27–34. https://doi.org/10.1016/j.pnpbp.2015.06.017.

Schneider M. Cannabis use in pregnancy and early life and its consequences: animal models. Eur Arch Psychiatry Clin Neurosci. 2009;259(7):383–93. https://doi.org/10.1007/s00406-009-0026-0.

Sharir H, Abood ME. Pharmacological characterization of GPR55, a putative cannabinoid receptor. Pharmacol Ther. 2010;126(3):301–13. https://doi.org/10.1016/j.pharmthera.2010.02.004.

Sharir H, Console-Bram L, Mundy C, Popoff SN, Kapur A, Abood ME. The endocannabinoids anandamide and virodhamine modulate the activity of the candidate cannabinoid receptor GPR55. J Neuroimmune Pharmacol. 2012;7(4):856–65. https://doi.org/10.1007/s11481-012-9351-6.

Stevens B, Yamada J, Ohlsson A, Haliburton S, Shorkey A. Sucrose for analgesia in newborn infants undergoing painful procedures. Cochrane Database Syst Rev. 2016;7:CD001069. https://doi.org/10.1002/14651858.CD001069.pub5.

Suarez J, Llorente R, Romero-Zerbo SY, Mateos B, Bermudez-Silva FJ, de Fonseca FR, Viveros MP. Early maternal deprivation induces gender-dependent changes on the expression of hippocampal CB(1) and CB(2) cannabinoid receptors of neonatal rats. Hippocampus. 2009;19(7):623–32. https://doi.org/10.1002/hipo.20537.

Timmerman B, Honeycutt JA, Skully J, Patel D, Khan W, Neagu A, et al. Investigating the effectiveness of cannabidiol to mitigate the adverse consequences of exposure to neonatal procedural pain. bioRxiv. 2025;2002.2021.639541. https://doi.org/10.1101/2025.02.21.639541.

Tremblay S, Ranger M, Chau CMY, Ellegood J, Lerch JP, Holsti L, Goldowitz D, Grunau RE. Repeated exposure to sucrose for procedural pain in mouse pups leads to long-term widespread brain alterations. Pain. 2017;158(8):1586–98. https://doi.org/10.1097/j.pain.0000000000000961.

Trezza V, Campolongo P, Manduca A, Morena M, Palmery M, Vanderschuren LJ, Cuomo V. Altering endocannabinoid neurotransmission at critical developmental ages: impact on rodent emotionality and cognitive performance. Front Behav Neurosci. 2012;6:2. https://doi.org/10.3389/fnbeh.2012.00002.

U.S. Food & Drug Administration. FDA finds drugs under investigation in the U.S. related to French BIA 10–2474 drug do not pose similar safety risks. 2016. https://www.fda.gov/drugs/drug-safety-and-availability/fda-finds-drugs-under-investigation-us-related-french-bia-10-2474-drug-do-not-pose-similar-safety.

Vangopoulou C, Bourmpoula MT, Koupourtidou C, Giompres P, Stamatakis A, Kouvelas ED, Mitsacos A. Effects of an early life experience on rat brain cannabinoid receptors in adolescence and adulthood. IBRO Rep. 2018;5:1–9. https://doi.org/10.1016/j.ibror.2018.05.002.

Vinall J, Miller SP, Bjornson BH, Fitzpatrick KP, Poskitt KJ, Brant R, Synnes AR, Cepeda IL, Grunau RE. Invasive procedures in preterm children: brain and cognitive development at school age. Pediatrics. 2014;133(3):412–21. https://doi.org/10.1542/peds.2013-1863.

Walker CD. Nutritional aspects modulating brain development and the responses to stress in early neonatal life. Prog Neuropsychopharmacol Biol Psychiatry. 2005;29(8):1249–63. https://doi.org/10.1016/j.pnpbp.2005.08.010.

Walker SM. Neonatal pain. Paediatr Anaesth. 2014;24(1):39–48. https://doi.org/10.1111/pan.12293.

Walker OS, Holloway AC, Raha S. The role of the endocannabinoid system in female reproductive tissues. J Ovarian Res. 2019;12(1):3. https://doi.org/10.1186/s13048-018-0478-9.

Wanner NM, Colwell M, Drown C, Faulk C. Developmental cannabidiol exposure increases anxiety and modifies genome-wide brain DNA methylation in adult female mice. Clin Epigenetics. 2021;13(1):4. https://doi.org/10.1186/s13148-020-00993-4.

Watson S, Chambers D, Hobbs C, Doherty P, Graham A. The endocannabinoid receptor, CB1, is required for normal axonal growth and fasciculation. Mol Cell Neurosci. 2008;38(1):89–97. https://doi.org/10.1016/j.mcn.2008.02.001.

Weaver DR. A1-adenosine receptor gene expression in fetal rat brain. Dev Brain Res. 1996;94(2):205–23. https://doi.org/10.1016/s0165-3806(96)80013-7.

Wong H, Cairns BE. Cannabidiol, cannabinol and their combinations act as peripheral analgesics in a rat model of myofascial pain. Arch Oral Biol. 2019;104:33–9. https://doi.org/10.1016/j.archoralbio.2019.05.028.

Wymore EM, Palmer C, Wang GS, Metz TD, Bourne DWA, Sempio C, Bunik M. Persistence of delta-9-tetrahydrocannabinol in human breast milk. JAMA Pediatr. 2021;175(6):632–4. https://doi.org/10.1001/jamapediatrics.2020.6098.

Xiong W, Cui T, Cheng K, Yang F, Chen SR, Willenbring D, Guan Y, Pan HL, Ren K, Xu Y, Zhang L. Cannabinoids suppress inflammatory and neuropathic pain by targeting alpha3 glycine receptors. J Exp Med. 2012;209(6):1121–34. https://doi.org/10.1084/jem.20120242.

Zou S, Kumar U. Cannabinoid receptors and the endocannabinoid system: signaling and function in the central nervous system. Int J Mol Sci. 2018;19(3):833. https://doi.org/10.3390/ijms19030833.

Chapter 10
Pharmaceutical-Grade Cannabidiol in Pediatric Epilepsy



Aimee F. Luat and Kayla A. Hetrick

1 Introduction

is defined by the International League Against Epilepsy (ILAE) as a disease of the brain characterized by: (1) at least two unprovoked (or reflex) seizures occurring more than 24 hours apart; (2) one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk after two unprovoked seizures, a probability of at least 60% over the next 10 years; (3) diagnosis of an epilepsy syndrome (Fisher et al. 2014). Epilepsy is a common neurological disorder affecting more than two million people in the United States (US), and one in 26 people will develop epilepsy in their lifetime (Hesdorffer et al. 2011). Approximately 1 in 150 children are diagnosed with epilepsy during the first 10 years of life with peak incidence during infancy (Aaberg et al. 2017).

Although the majority of seizures associated with epilepsy can be controlled with one or two anti-seizure medications (ASMs), uncontrolled seizures persist in approximately 30% of individuals (Kwan and Brodie, 2000). Drug-resistant epilepsy (DRE) is defined by the International League Against Epilepsy (ILAE) as failure of adequate trials of two tolerated and appropriately chosen and utilized ASMs, whether used as monotherapy or in combination, to achieve sustained seizure freedom (Kwan et al. 2010). Despite the introduction of newer ASMs in the last 20 years, the number of individuals with DRE remains largely unchanged (Chen

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et al. 2018). There is a clear unmet need for the development of additional safe and effective epilepsy therapies.

In the past few decades, there has been a growing interest in natural remedies and complementary approaches for the treatment of various neurological disorders (Parvez 2018). Similarly, with the legalization of cannabis for medical and recreational purposes in the US in the past two decades (Perlman et al. 2021), the role of the endocannabinoid system (ECS) as a therapeutic target for various neurological conditions, including epilepsy, has become an active field of research. Class I evidence from four randomized, placebo-controlled trials led to Food and Drug Administration (FDA) approval of cannabidiol (CBD), Epidiolex®—herein referred to as pharmaceutical-grade CBD unless otherwise noted or referenced—as adjunctive therapy for the treatment of seizures associated with Dravet syndrome (DS), Lennox-Gastaut syndrome (LGS), and tuberous sclerosis complex (TSC) in individuals two years of age or older in 2018 (Epidiolex® (CBD) [package insert] 2024; Devinsky et al. 2017, 2018a; Miller et al. 2020; Thiele et al. 2018). In 2021, based on the results of an additional randomized, placebo-controlled trial (Thiele et al. 2021), the label was further updated to include use for the treatment of seizures associated with tuberous sclerosis complex and the minimum age was expanded to include use in individuals one year of age or older in all three populations studied (Epidiolex® (CBD) [package insert] 2024). While head-to-head trials have not been conducted, evidence suggests that pharmaceutical-grade CBD efficacy is comparable to that of other ASMs and provides an additional option for the treatment of epilepsy (Arzimanoglou et al. 2020).

In this chapter, we provide an overview on the history of cannabis use for epilepsy treatment and the clinical use of pharmaceutical-grade CBD for the treatment of epilepsy. In addition, we provide a comprehensive review of the relevant pharmacokinetics (PK) and pharmacodynamics (PD) of pharmaceutical-grade CBD. The data discussed herein specifically refers to Epidiolex® (CBD) unless otherwise referenced, and the information cannot be extrapolated to non-FDA-approved CBD products.

2 Counseling Patients on Unregulated Cannabidiol Products

Counseling patients regarding the differences between pharmaceutical-grade CBD and non-FDA-approved, unregulated CBD products is important, but various barriers can exist (Wechsler et al. 2024). Some practitioners may feel uncomfortable navigating these conversations due to knowledge gaps or lack of awareness regarding concerns associated with unregulated CBD products. In addition, patients and caregivers may be steadfast in their desire to use these products, especially if pharmaceutical-grade CBD is not covered by insurance; they may also view these products as "natural" and therefore perceive them to be devoid of the risks they associate with pharmaceutical medications. Given the stigma that can still be associated with use of cannabis and cannabis-derived products, some patients or

caregivers may not disclose use of unregulated CBD products to their healthcare providers. Finally, there is a significant amount of misinformation in the cannabis industry (Hang et al. 2016) due, at least in part, to the highly unregulated and highly profitable nature of this market.

Patients and caregivers should be made aware that use of unregulated CBD products may pose certain risks to consumers, some of which are directly related to properties of the cannabis plant. For instance, cannabis is an extremely efficient phytoremediator, which can lead to accumulation of various hazardous substances (Placido and Lee 2022; Husain et al. 2019). Risks with the use or consumption of unregulated cannabis-derived products may include, but are not limited to, deviation from label claims of CBD content, presence of Δ-9-tetrahydrocannabinol (THC) even when not labeled or advertised to contain THC, and ambiguity regarding other cannabinoids or molecules that may be present; uncertainty regarding how to dose products, especially given the prevalence of mislabeling, batch-to-batch variability, and the likelihood of drug–drug interactions (DDIs); and inadequate quality control and testing measures to ensure lack of hazardous substances in the final product (e.g., fungal or bacterial pathogens, heavy metals, phthalates, pesticides, residual solvents, etc.) (Gardener et al. 2022; Gidal et al. 2024).

3 History of Cannabis Use for Epilepsy

Cannabis has been used as a medical treatment since ancient times with anecdotal evidence of its use for recreation and various medicinal purposes. Historical evidence suggests that cannabis was already present about 11,700 years ago in Central Asia (Russo 2007; Crocq 2020). The discovery of 3800-year-old ancient papyri and clay tablets indicate that cannabis was used as a remedy for various conditions in Ancient Egypt and Mesopotamia (Russo 2007). As early as the 1840s, medical reports suggested the potential anticonvulsant effect of cannabis extracts (O'Shaughnessy 1843; Reynolds 1890). Cannabidiol was first isolated from the cannabis plant in 1940 by Adams and colleagues (1940), while THC was first extracted in 1942 by Wollner, Matchett, Levine, and Loewe (Pertwee 2006). The structure and stereochemistry of these molecules were not elucidated until several decades later in the 1960s by Mechoulam, who later synthesized CBD and Δ-9-THC in his laboratory (Pertwee 2006). Several other investigators—including Mechoulam— demonstrated the effectiveness of CBD and its derivatives in protecting experimental animals from convulsions induced by various techniques (Carlini et al. 1973; Consroe et al. 1973; Chescher and Jackson 1974; Consroe and Wolkin 1977).

In 1980, a small clinical trial indicated the potential anticonvulsant properties of CBD in human epilepsy, demonstrated through reduction in seizure frequency in seven of eight patients with epilepsy taking CBD compared to only one of eight taking placebo (Cunha et al. 1980). Anticonvulsant effect of smoking marijuana was also reported by Consroe et al. (1975) in one patient. Research was primarily focused on THC, though, given its well-known psychoactive and euphoric effects.

For more than 30 years, the anti-seizure properties of CBD remained almost unnoticed. In 2009, Professor Mechoulam even commented *"Nobody has done any work on CBD in the clinic in epilepsy, and I just wonder why."* (Crippa et al. 2023). Years later, the story of Charlotte Figi, a girl with DS treated with a high CBD:THC strain of cannabis, attracted media attention as her convulsions significantly decreased from about 50 per day to only two-to-three per month with use of this product, later marketed as *Charlotte's Web* (Maa and Figi 2014). The success story of Charlotte even motivated many families who had family members affected by epilepsy to move to Colorado—where medical marijuana was both legal and accessible at the time—in hopes of accesing these products for epilepsy treatment (Detyniecki and Hirsch 2015). Thereafter, uncontrolled studies on the efficacy of a CBD extract and of pharmaceutical-grade CBD in the treatment of DRE emerged (Hussain et al. 2015; Devinsky et al. 2016). Subsequently, the rapidly expanding body of evidence supporting the role of CBD in epilepsy was evident given the notable rise in the number of publications on this topic.

4 Proposed Mechanisms of Cannabidiol Anti-Seizure Effects

The molecular targets of most conventional ASMs are restricted to modulation of voltage-gated ion channels; enhancement of γ-aminobutyric acid (GABA) inhibition via $GABA_A$ receptors, GABA transporters, or GABA transaminase (the enzyme that breaks down GABA); modulation of synaptic proteins involved in neurotransmitter release, such as synaptic vesicle protein 2A (SV2A) and the α2δ subunit of voltage-gated calcium (Ca^{2+}) channels; and inhibition of synaptic excitation by ionotropic glutamate receptors (GluR), including α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors (Rogawski et al. 2016).

Cannabidiol has been shown to have a wide variety of molecular targets, including activity on enzymes, transporters, receptors, and ion channels (Zavala-Tecuapetla et al. 2022). Unlike THC, CBD acts minimally on the orthosteric sites of the cannabinoid 1 (CB1) and CB2 receptors and thus, lacks detectable euphoric effects and has limited propensity for abuse (McPartland et al. 2015; Schoedel et al. 2018). There is considerable ubiquity in the proposed mechanisms of action (MOAs) of CBD, and the specific targets to be reviewed here include modulation of transient receptor potential vanilloid type 1 (TRPV1), G protein-coupled receptor 55 (GPR55), and adenosine via activity at equilibrative nucleoside transporter 1 (ENT1) (Gray and Whalley 2020).

4.1 Transient Receptor Potential Vanilloid 1 (TRPV1)

Transient receptor vanilloid 1, also known as the capsaicin receptor and the vanilloid receptor 1, is a ligand-gated, nonselective cation channel with Ca^{2+} preference that is well-expressed in the peripheral sensory neurons where it plays a role in pain

perception, typically through activation by capsaicin (Brederson et al. 2013). In the central nervous system (CNS), TRPV1 is also widely expressed in various brain regions (e.g., prefrontal cortex entorhinal cortex, hippocampus, hypothalamus, striatum, substantia nigra, dorsolateral periaqueductal gray) in both the excitatory and inhibitory presynaptic and postsynaptic nerve terminals. Activation of TRPV1 by certain agonists—including endovanilloids, endocannabinoids, and phytocannabinoids—leads to increased intracellular Ca^{2+}, which results in enhanced excitatory synaptic transmission (Meza et al. 2022).

Upregulation of TRPV1 expression has been demonstrated in human temporal lobe epilepsy (Sun et al. 2013) and in cortical lesions in TSC and other type IIb focal cortical dysplasias (Shu et al. 2013). In a febrile seizure rat model, TRPV1 overexpression resulted in significant elevation of pro-inflammatory cytokines whereas TRPV1-deficient mice showed tolerance to hyperthermia and reduced susceptibility to febrile seizures (Huang et al. 2015). In an in vitro rat hippocampal slice model, CBD and its analog, cannabidivarin (CBDV), desensitized TRPV1 in a dose-dependent manner, reducing neuronal hyperexcitability (Iannotti et al. 2014). In agreement with these data, the anticonvulsive effects of CBD 50 mg/kg and 100 mg/kg significantly increased seizure threshold in wild-type mice ompared with TRPV1 knockout and vehicle controls (Gray et al. 2020). In this same study, this effect was only observed at 100 mg/kg in TRPV1 knockout mice compared with knockout vehicle mice in which CBD-increased seizure threshold was partially attenuated; which may indicate the involvement of targets other than TRPV1 with regard to anti-seizure effects of CBD (Gray et al. 2020).

4.2 G Protein-Coupled Receptor 55 (GPR55)

GPR55 is another CBD target that is highly expressed in dorsal root ganglion and periaqueductal gray matter where it modulates the pain pathways (Boleti et al. 2022). In the CNS, GPR55 is expressed in the hippocampus, hypothalamus, frontal cortex, striatum, and cerebellum (Marichal-Cancino et al. 2017). Activation of GPR55 by its endogenous ligand, lysophosphatidylinositol (LPI), evokes intracellular Ca^{2+} influx in the hippocampal neuronal bodies and presynpatic excitatory nerve terminals, contributing to a pro-excitatory state (Rosenberg et al. 2023). Indeed, in chronic epileptic rats, increased hippocampal GPR55-LPI expression was shown to enhance presynaptic glutamate release and attenuate inhibitory synaptic strength by reducing GABA clustering at inhibitory postsynaptic sites; antagonism of GPR55 by CBD modulates the GPR55-LPI pathway to inhibit glutamate release and restore the epileptic hippocampal excitatory and inhibitory imbalance (Rosenberg et al. 2023). In a mouse model of DS, CBD-induced inhibition of the LPI-activated GPR55 receptor caused reduction of both seizures and autism-like behavior, attenuating social deficits (Kaplan et al. 2017).

4.3 Equilibrative Nucleoside Transporter 1 (ENT1) and Adenosine

Adenosine is an endogenous molecule present ubiquitously in mammalian organisms, and it affects several physiological functions via four cell surface G protein-coupled receptors (A_1, A_{2A}, A_{2B}, and A_3) (Borea et al. 2018). In the brain, adenosine acts as an inhibitory modulator of neuronal excitability, and disrupted adenosine homeostasis can be seen in both animal and human epilepsies (Boison 2016). Adenosine plays a role in seizure termination through presynaptic inhibition and postsynaptic membrane potential stabilization, hence called a "seizure terminator" (During and Spencer 1992; Lado and Moshe 2008; Boison 2016; Weltha et al. 2019). Cannabidiol acts as a competitive inhibitor at the ENT1 receptor, which mediates adenosine reuptake; hence, CBD-induced ENT1 inhibition leads to an increase in extracellular adenosine levels (Pandolfo et al. 2011; Mijangos-Moreno et al. 2014; Wright and Lee 2019), thus promoting seizure termination. Recent studies also suggest that CBD could be a negative allosteric modulator of adenosine receptors (Sanchez-Fernandez et al. 2023; Raich et al. 2023).

4.4 Efficacy and Safety of Pharmaceutical-Grade Cannabidiol in Epilepsy

The efficacy and safety of pharmaceutical-grade CBD for the treatment of seizures associated with DS, LGS, and TSC was demonstrated in five randomized, placebo-controlled trials—two in DS (Devinsky et al. 2017; Miller et al. 2020), two in LGS (Devinsky et al. 2018a; Thiele et al. 2018), and one in TSC (Thiele et al. 2021). In all five trials, pharmaceutical-grade CBD significantly reduced the primary endpoint seizure types compared to placebo (Fig. 10.1a). The proportion of patients who achieved a 50% responder rate (50RR) (i.e., ≥50% reduction in primary endpoint seizure types frequency from baseline) was statistically significant compared to placebo for all dosing arms in all five trials except for Devinsky et al. (2017) and the CBD 25 mg/kg/day dosing arm in Thiele et al. (2021) (Fig. 10.1b). Further information regarding each of the randomzied, placebo-controlled trials is summarized in the sections that follow.

While not placebo-controlled, long-term safety and effectiveness data have also been captured in the corresponding open-label extension (OLE) studies for DS (Scheffer et al. 2021), LGS (Patel et al. 2021), and TSC (Thiele et al. 2023). Safety and effectivness data are also available from various real-world studies, most notably for almost 1000 patients with DRE who enrolled in a large expanded access program (EAP) (i.e., compassionate use program) which began in 2014 prior to FDA approval of pharmaceutical-grade CBD (Szaflarski et al. 2023; Flamini et al. 2023). Data for the OLEs and the EAP are also summarized below.

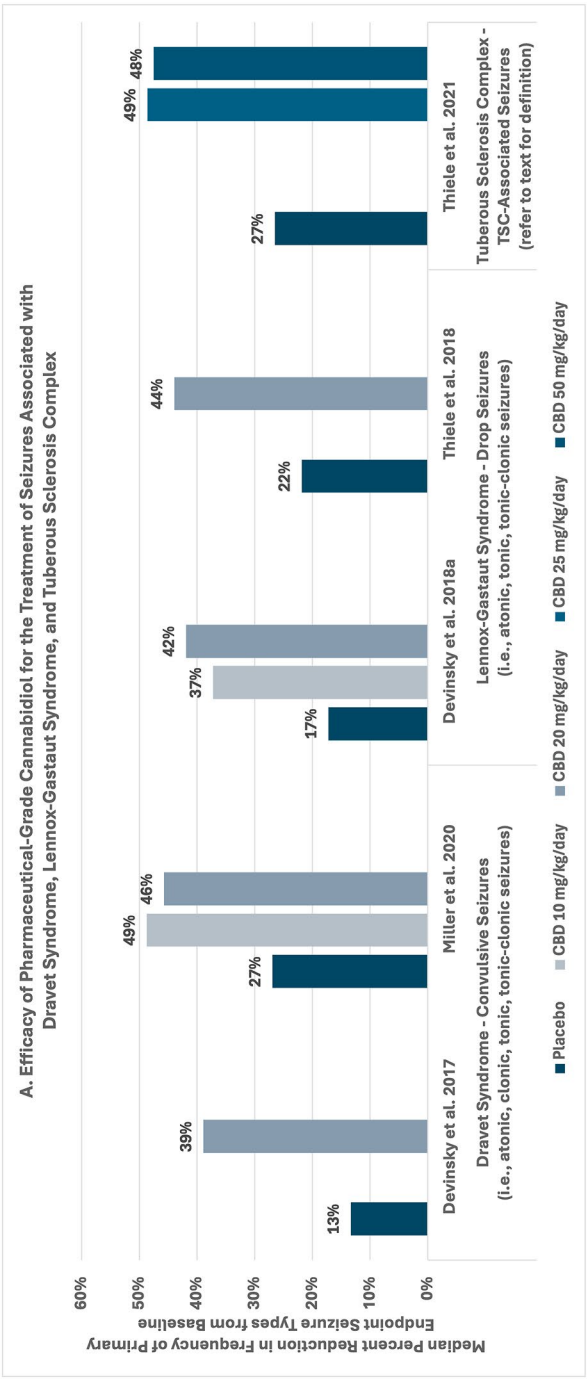


Fig. 10.1 Efficacy of pharmaceutical-grade cannabidiol versus placebo in seizures associated with Lennox-Gastaut syndrome, Dravet syndrome, and tuberous sclerosis complex in five randomized, placebo-controlled trials. (**a**) Primary efficacy outcome: median percent reduction in primary endpoint seizure types frequency from baseline during the treatment period. (**b**) Key secondary efficacy outcome: proportion of patients who achieved ≥50% reduction in primary endpoint seizure types frequency from baseline during the treatment period. Data derived from Devinsky et al. (2017, 2018a), Miller et al. (2020), and Thiele et al. (2018, 2021). Reduction from baseline in primary endpoint seizure types frequency compared to placebo was statistically significant for all dosing arms in all five trials (p values not shown). The proportion of patients who achieved a 50% responder rate (i.e., ≥50% reduction in primary endpoint seizure types frequency from baseline) compared to placebo was statistically significant for all dosing arms in all five trials except for Devinsky et al. (2017) and the CBD 25 mg/kg/day dosing arm in Thiele et al. (2021). *CBD* cannabidiol, *TSC* tuberous sclerosis complex, *mg* milligrams, *kg* kilograms

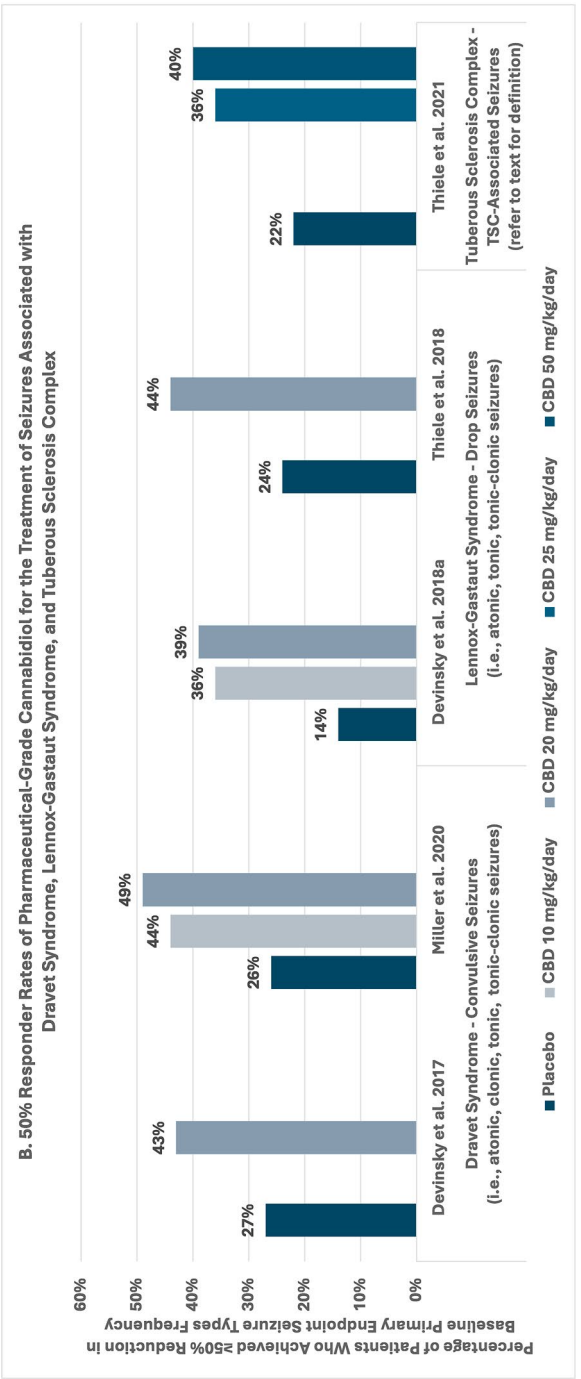


Fig. 10.1 (continued)

4.5 *Efficacy and Safety of Pharmaceutical-Grade Cannabidiol in Dravet Syndrome*

Dravet syndrome is a rare, infantile-onset, developmental epileptic encephalopathy (DEE) associated with drug-resistant seizures and multiple comorbidites; DS is commonly associated with pathogenic *SCN1A* genetic variants resulting in haploinsufficiency of the α-1 subunit of the sodium channel, $Na_v1.1$. (Wirrell et al. 2022). Patients aged 2 to 18 years with a confirmed diagnosis of DS were eligible to participate in the two randomized, placebo-controlled trials if they met the following key inclusion criteria: uncontrolled seizures despite current ASMs; taking at least one ASM; at least four convulsive seizures during the 4-week baseline period (Devinsky et al. 2017; Miller et al. 2020). Use of medications and non-pharmacologic interventions, including ketogenic diet (KD) and vagus nerve stimulation (VNS), had to be stable for four weeks before screening and throughout the trials. Key exclusion criteria included: clinically significant unstable illness (other than epilepsy) in the four weeks before screening; alcohol or substance abuse; use of recreational or medical cannabis in the previous three months; current use of felbamate for less than one year.

The primary efficacy outcome for both DS trials was change from baseline in convulsive seizure frequency during the 14-week treatment period (Devinsky et al. 2017; Miller et al. 2020). The 14-week treatment period consisted of a 2-week titration period and a 12-week maintenance period. A key secondary efficacy outcome for both trials was the proportion of patients who achieved 50 RR in convulsive seizure activity from baseline. These efficacy outcome results are illustrated in Fig. 10.1a, b. Note that patients in the Devinsky et al. (2017) trial were randomized to CBD 20 mg/kg/day or matching placebo whereas patients in the Miller et al. (2020) trial were randomized to CBD 10 mg/kg/day, CBD 20 mg/kg/day, or matching placebo. A pooled post hoc analysis of the DS trials demonstrated that differences in reduction of primary endpoint seizure types between CBD and placebo emerged during the titration period and became nominally significant by day 13 for CBD 10 mg/kg/day and day 12 for CBD 20 mg/kg/day (Cohen et al. 2021). Differences in 50RR between CBD and placebo also emerged during titration (Cohen et al. 2021).

Adverse events (AEs) occurring in ≥10% of patients for each of the DS trials (Devinsky et al. 2017; Miller et al. 2020) are shown in Table 10.1. In Devinsky et al. (2017), 93% of patients randomized to pharmaceutical-grade CBD reported AEs compared to 75% randomized to placebo. Of these, 84% were rated as mild or moderate severity in the CBD group compared to 95% in the placebo group. In this trial, AEs reported in ≥10% of patients included diarrhea, vomiting, fatigue, pyrexia, upper respiratory tract infection (URTI), decreased appetite, convulsion, lethargy, and somnolence. Discontinuation due to AEs occurred in eight patients randomized to CBD and one patient randomized to placebo. In Miller et al. (2020), 87.5% of patients randomized to CBD reported AEs compared to 89.2% on placebo; of these, 92% were judged as mild or moderate in severity. In this trial, AEs reported in ≥10% of patients in any treatment arm included decreased appetite, diarrhea,

Table 10.1 Adverse effects reported in ≥10% of patients enrolled in the pharmaceutical-grade cannabidiol randomized, placebo-controlled trials

Trial population	Dravet syndrome (Miller et al. 2020)			Dravet syndrome (Devinsky et al. 2017) and Lennox-Gastaut Syndrome (Devinsky et al. 2018a; Thiele et al. 2018)			Tuberous sclerosis complex (Thiele et al. 2021)		
Randomized dose (mg/kg/day)	PBO	CBD 10	CBD 20	PBO	CBD 10	CBD 20	PBO	CBD 25	CBD 50
Adverse effect	*N* = 65 (%)	*N* = 64 (%)	*N* = 69 (%)	*N* = 227 (%)	*N* = 75 (%)	*N* = 238 (%)	*N* = 76 (%)	*N* = 75 (%)	*N* = 73 (%)
Hepatic									
Transaminase elevation	ALT—0; AST—0	ALT—5; AST—5	ALT—13; AST—12	3	8	16	ALT—0; AST—0	ALT—9; AST—11	ALT—22; AST—19
GGT	<10	<10	<10	<10	<10	<10	0	16	14
Gastrointestinal									
Decreased appetite	17	17	29	5	16	22	12	20	23
Diarrhea	12	17	26	9	9	20	25	31	56
Vomiting	6	6	16	<10	< 10	<10	9	17	18
Constipation	<10	<10	<10	<10	<10	<10	8	11	7
Nervous system									
Somnolence	14	25	23	8	23	25	9	13	26
Fatigue, malaise, asthenia	11	8	22	4	11	12	<10	<10	<10
Insomnia, sleep disorder, poor quality sleep	<10	<10	<10	4	11	5	<10	<10	<10
Seizure	<10	<10	<10	<10	<10	<10	7	7	11
Status epilepticus	14	8	10	<10	<10	<10	<10	<10	<10
Infections									

Infection, all	<10	<10	<10	31	41	40	<10	<10	<10
Infection, other	<10	<10	<10	24	25	21	<10	<10	<10
Infection, viral	<10	<10	<10	6	7	11	<10	<10	<10
Infection, upper respiratory tract	<10	<10	<10	<10	<10	<10	13	9	10
Other									
Rash	<10	<10	<10	3	7	13	<10	<10	<10
Pyrexia	17	23	22	< 10	< 10	< 10	8	19	16
Nasopharyngitis	8	6	12	< 10	< 10	< 10	16	15	15
Cough	<10	<10	<10	<10	<10	<10	7	11	4

mg milligrams, *kg* kilograms, *PBO* placebo, *CBD* cannabidiol, *N* number, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* gamma-glutamyltransferase

somnolence, pyrexia, fatigue, vomiting, nasopharyngitis, status epilepticus, and transaminitis. Discontinuation due to AEs occurred in 7% of patients randomized to CBD 20 mg/kg/day. In a pooled post hoc analysis of the DS trials, onset of the first reported AE occurred during the titration phase in approximately half of patients randomized to placebo or CBD and during the first four weeks of the maintenance phase in approximately 25% (Cohen et al. 2021). Of those who experienced AEs, resolution occurred within four weeks of onset in approximately 47% of patients randomized to placebo compared to 36% randomized to CBD and after four weeks of onset but before study end in approximately 18% of patients randomized to placebo versus 20% in those randomized to CBD (Cohen et al. 2021).

4.6 Efficacy and Safety of Pharmaceutical-Grade Cannabidiol in Lennox-Gastaut Syndrome

Lennox-Gastaut syndrome (LGS) is a severe, drug-resistant DEE associated with multiple seizure types—one of which must include tonic seizure—with onset prior to 18 years of age, cognitive and behavioral impairments, and diffuse slow spike-and-wave (SSW) and generalized paroxysmal fast activity (PFA) on electroencephalography (EEG) (Nelson and Knupp 2023). The etiologies of LGS are varied and many. Falls from atonic and/or tonic seizures—often referred to as "drop seizures"—are a hallmark feature of LGS and can lead to significant injuries and morbidity.

Similar to DS, CBD was studied in two randomized, placebo-controlled trials in individuals with LGS (Devinsky et al. 2018a; Thiele et al. 2018). To be eligible for the trials, patients with LGS 2 to 55 years of age had to meet the following key inclusion critiera: EEG with SSW complexes (<3 Hz); at least two types of generalized seizures, including drop seizures, for at least six months; taking between one and four ASMs; at least two drop seizures each week during the 4-week baseline period. In addition, all medication doses and non-pharmacologic interventions for epilepsy (including KD and VNS) had to be stable in the four weeks before screening and throughout the trial. Key exclusion criteria generally mirror those of the DS trials. The primary efficacy outcome for both LGS trials was change from baseline in drop seizure frequency during the 14-week treatment period. In both studies, drop seizures were defined as an epileptic atonic, tonic, or tonic–clonic seizure involving the entire body, trunk, or head that led or could have led to a fall, injury, or slumping in a chair. The 14-week treatment period consisted of a 2-week titration period and a 12-week maintenance period. A key secondary efficacy outcome for both trials was the proportion of patients who achieved 50RR. These efficacy outcome results are illustrated in Fig. 10.1a, b. Note that patients in the Thiele et al. (2018) trial were randomized to CBD 20 mg/kg/day or matching placebo whereas patients in the Devinsky et al. (2018a) trial were randomized to CBD 10 mg/kg/day, CBD 20 mg/kg/day, or matching placebo.

Pooled AEs occurring in ≥10% of patients for both LGS trials are shown in Table 10.1 (Devinsky et al. 2018a, b; Thiele et al. 2018). In Thiele et al. (2018), 86% of patients randomized to pharmaceutical-grade CBD reported AEs compared to 69% randomized to placebo. Of these, 78% were rated as mild or moderate severity in the CBD group compared to 97% in the placebo group. In this trial, AEs reported in ≥10% of patients included diarrhea, somnolence, pyrexia, decreased appetite, and vomiting. Discontinuation due to AEs occurred in 14% of patients randomized to CBD and 1% of patients randomized to placebo. In Devinsky et al., 84% and 94% of patients randomized to CBD 10 mg/kg/day and 20 mg/kg/day, respectively, reported AEs compared to 72% in the placebo group; of these, 89% were judged as mild or moderate in severity. In this trial, AEs reported in ≥10% of patients in any treatment arm included somnolence, decreased appetite, diarrhea, URTI, pyrexia, vomiting, nasopharyngitis, and status epilepticus. Discontinuation due to AEs occurred in 1% and 8% of patients randomized to CBD 10 mg/kg/day and 20 mg/kg/day, respectively, and in 1% of patients randomized to placebo. In a pooled post hoc analysis of the LGS trials, onset of the first reported AE occurred during the titration phase in approximately 46% of patients and during the first four weeks of the maintenance period in 24% of patients (Privitera et al. 2021). Of those who experienced AEs, resolution occurred within four weeks of onset in more than 40% of patients and by the end of the study in more than 60% of patients (Privitera et al. 2021).

4.7 Efficacy and Safety of Pharmaceutical-Grade Cannabidiol in Tuberous Sclerosis Complex

Tuberous sclerosis complex is a rare, multisystem, genetic disorder caused by pathogenic variants in the TSC1 or TSC2 genes resulting in increased mechanistic/mammalian target of rapamycin (mTOR) activity. This can result in excessive cellular growth and proliferation with occurrence of benign hamartomas in multiple organs, including brain, skin, kidney, lungs, heart, and eyes. Seizures (focal seizures and epileptic spasms) occur in approximately 85% of patients with TSC (Northrup et al. 2021), and—in contrast to the general epilepsy population—DRE occurs in ≥60% of patients with TSC (Chu-Shore et al. 2010).

To be eligible for participation in the randomized, placebo-controlled trial (Thiele et al. 2021), patients 1 to 65 years of age had to have a definite clinical diagnosis of TSC and meet the following key inclusion criteria: medication-resistant epilepsy; at least 8 TSC-associated seizures during the 4-week baseline period with at least one seizure occurring in at least 3 of the 4 weeks; taking at least one ASM. Key exclusion criteria included the following: history of non-epileptic seizures; clinically significant illness other than epilepsy; epilepsy surgery in the six months before screening; felbamate use for less than one year before screening; use of oral mTOR inhibitors.

The primary efficacy outcome for this trial was change from baseline in TSC-associated seizure frequency during the 16-week treatment period, which included 4 weeks of titration and 12 weeks of maintenance. The titration period for this study was longer than for the DS and LGS trials because higher doses were utilized compared to the DS and LGS trials; this was driven by clinician dosing experience in patients with TSC who had enrolled in the EAP. Patients were randomized to CBD 25 mg/kg/day, CBD 50 mg/kg/day, or matching placebo. In this trial, TSC-associated seizures were defined as countable focal motor seizures without impairment of awareness, focal seizures with impairment of awareness, focal seizures evolving to bilateral motor seizures, and generalized seizures (atonic, clonic, tonic, or tonic–clonic). A key secondary efficacy outcome for both trials was the 50RR. These efficacy outcome results are demonstrated in Fig. 10.1a, b. In a post hoc analysis of this trial, differences in primary endpoint seizure types frequency reductions from baseline between CBD and placebo emerged on day six and became nominally significant by day 10 (Wu et al. 2022). Separation in the 50RR between CBD and placebo also emerged by day 10 (Wu et al. 2022).

Adverse events occurring in ≥10% of patients during the TSC trial are shown in Table 10.1 (Thiele et al. 2021). Adverse events were reported by 93% of patients randomized to CBD 25 mg/kg/day, 100% of patients randomized to CBD 50 mg/kg/day, and 95% of those randomized to placebo; of these, 88% were categorized as mild or moderate severity (Thiele et al. 2020). In this trial, AEs reported in ≥10% of patients in any treatment arm included diarrhea, somnolence, decreased appetite, transaminitis, vomiting, pyrexia, nasopharyngitis, seizure, URTI, constipation, and cough. Discontinuation due to AEs occurred in 11% of patients randomized to CBD 25 mg/kg/day, 14% of patients randomized to 50 mg/kg/day, and 11% of patients randomized to placebo. The most common AEs leading to withdraw from the trial were rash, transaminitis, somnolence, and urticaria. Permanent dose reductions secondary to an AE were made in 12% of patients randomized to CBD 25 mg/kg/day, 29% of patients randomized to CBD 50 mg/kg/day, and 5% of patients randomized to placebo. In a post hoc analysis, AEs occurred during the first two weeks in 54%, 61%, and 67% of patients randomized to placebo, CBD 25 mg/kg/day, and CBD 50 mg/kg/day, respectively (Wu et al. 2022). In patients who experienced an AE, resolution occurred within four weeks of onset in 42% of those randomized to placebo and in 27% of those randomized to CBD; by the end of the trial, resolution occurred in 78% of patients randomized to placebo and 51% of patients randomized to CBD (Wu et al. 2022).

5 Safety and Effectiveness of Pharmaceutical-Grade Cannabidiol in Open-Label Extension Studies

Individuals who successfully completed one of the five randomized, placebo-controlled trials were eligible to enroll in a corresponding OLE (Scheffer et al. 2021; Patel et al. 2021; Thiele et al. 2023). For the DS (Scheffer et al. 2021) and

LGS (Patel et al. 2021) OLEs, patients were titrated from CBD 2.5 mg/kg/day to 20 mg/kg/day over a 2-week period. The dose could then be reduced or increased to 30 mg/kg/day based on response and tolerability. For the TSC OLE (Thiele et al. 2023), the initial target dose was 25 mg/kg/day, which could then be reduced or increased up to 50 mg/kg/day based on response and tolerability. The primary endpoint for each of the OLEs was safety up to 156 weeks. Key secondary endpoints included percent reduction in the primary endpoint seizure types frequency from baseline and primary endpoint seizure types 50RR, as defined in the corresponding randomized, placebo-controlled trials.

Of 330 individuals who completed the original DS trials (Devinsky et al. 2017; Miller et al. 2020), 315 (95%) enrolled in the DS OLE (Scheffer et al. 2021). Median treatment duration was 444 days (range, 18–1535). Patients received a mean modal dose of 22 mg/kg/day. Adverse events occurred in 97% of patients, most commonly diarrhea (43%), pyrexia (39%), decreased appetite (31%), and somnolence (28%). Discontinuation due to AEs occurred in 28 (9%) patients. In the 69 (22%) patients who developed transaminase elevation greater than 3 times the upper limit of normal (>3x ULN), 84% were on concomitant valproic acid. Median reduction from baseline in monthly seizure frequency for convulsive seizures—assessed in 12-week intervals up to week 156—ranged from 45% to 74%.

Of 368 patients who completed the LGS trials (Devinsky et al. 2018a; Thiele et al. 2018), 366 (99.5%) enrolled in the LGS OLE (Patel et al. 2021). Median treatment duration was 1090 days (range, 3–1421). Patients received a mean modal dose of 24 mg/kg/day. Adverse events occurred in 96% of patients, most commonly convulsion (39%), diarrhea (38%), pyrexia (34%), and somnolence (29%). Discontinuation due to AEs occurred in 12% of patients. In the 55 (15%) patients who developed transaminase elevation >3x ULN, 40 (73%) were on concomitant valproic acid. Median reduction from baseline in drop seizure frequency—assessed in 12-week intervals up to week 156—ranged from 48% to 71%.

Of 201 individuals who completed the TSC trial (Thiele et al. 2021), 199 (99%) entered the TSC OLE (Thiele et al. 2023). Median treatment duration was 631 days (range, 18–1462). Patients received a mean modal dose of 28 mg/kg/day. Adverse events were reported in 96% of patients, most commonly diarrhea (47%), seizures (30%), pyrexia (24%), and decreased appetite (24%). Discontinuation due to AEs occurred in 9% of patients, while the most common reason for withdrawal from the OLE was for transition to commercial product in 56% of patients. Transaminitis occurred in 7% of patients overall. Median reduction in TSC-associated seizures—assessed in 12-week intervals up to 156 weeks—ranged from 53% to 90%.

Taken together, the DS, LGS, and TSC OLE trials (Scheffer et al. 2021; Patel et al. 2021; Thiele et al. 2023) indicate that CBD is well-tolerated with a comparable safety profile to that demonstrated in the corresponding randomized, placebo-controlled trials (Devinsky et al. 2017, 2018a; Miller et al. 2020; Thiele et al. 2018, 2021). In addition, the OLE data demonstrate that sustained seizure reduction is possible with long-term use of CBD. Key limitations of OLE data must be considered, however, including the non-randomized, uncontrolled, open-label nature wherein the effect of changes in other therapies may play a role in rates of both AEs

and seizure reduction. In addition, effectiveness data obtained at later timepoints may be more reflective of continued use in individuals with greater success with and/or tolerability to CBD treatment, whereas individuals with less seizure reduction benefits and/or lower tolerability may have discontinued the OLE earlier. To control for potential enrichment of effectiveness over time due to withdrawal from the OLEs, sensitivity analyses using last observation carried forward (LOCF) methodology were performed. Median seizure reduction and 50RR using LOCF showed similar-to-less pronounced rates for these outcomes.

5.1 Real-World Effectiveness and Safety of Pharmaceutical-Grade Cannabidiol in Drug-Resistant Epilepsies

Beyond the pharmaceutical-grade CBD randomized, placebo-controlled trial and OLE data, safety and effectiveness data are available for more than 800 patients who enrolled in the EAP across 35 U.S.-based epilepsy centers (Szaflarski et al. 2023; Flamini et al. 2023). To enroll, patients were required to be receiving stable doses of ASMs for at least four weeks before enrollment and taking a median of three ASMs at baseline. Patients received pharmaceutical-grade CBD 2 to 10 mg/kg/day titrated to effectiveness and tolerability to a maximum of 25 to 50 mg/kg/day, depending on the site. Patient visits occurred every 2 to 4 weeks through week 16, then every two 2 to 12 weeks thereafter. Efficacy endpoints were evaluated across 12-week visit windows for up to 192 weeks, and AEs were documented at each visit.

The effectiveness and AE profile of pharmaceutical-grade CBD in the EAP (Szaflarski et al. 2023; Flamini et al. 2023), and in other real-world datasets, was consistent with the overall efficacy and safety profile demonstrated in the randomized, placebo-controlled trials and corresponding OLEs. The most commonly utilized ASMs at baseline were clobazam (47%), levetiracetam (34%), and valproate (28%). Median reduction from baseline seizure frequency at 24-week visit intervals for convulsive seizure types ranged from 58% to 100% for clonic seizures, 54% to 73% for tonic seizures, 47% to 72% for tonic–clonic seizures, 77% to 96% for atonic seizures, and 61% to 77% for focal to bilateral tonic–clonic seizures (Flamini et al. 2023). Reduction for nonconvulsive seizure types and epileptic spasms during these same intervals ranged from 60% to 76% for focal seizures, 95% to 100% for absence seizures (typical and atypical), 63% to 89% for myoclonic seizures, 50% to 100% for myoclonic absence seizures, and 80% to 95% for epileptic spasms. At least 49% of patients achieved 50RR in reduction of convulsive and nonconvulsive seizure types at almost all 24-week intervals (Flamini et al. 2023).

Safety was assessed for up to 252 weeks in the EAP (Szaflarski et al. 2023). While AEs were reported in 88% of patients, only 7% withdrew due to AEs. The most common AEs occurring in ≥20% of patients in the EAP were diarrhea (33%), seizure (24%), and somnolence (23%). Serious AEs were reported in 41% of

patients, most commonly seizure (14%) and status epilepticus (7%). Transaminitis occurred in 4% of patients.

6 Clinical Considerations Regarding the Safety and Tolerability Profile of Pharmaceutical-Grade Cannabidiol

As described, the safety and tolerability profile of pharmaceutical-grade CBD has been demonstrated across five randomized, placebo-controlled trials as well as longitudinally in the respective OLEs and in the EAP. For a full list of warnings, precautions, and AE rates, the reader is referred to the United States Prescribing Information (USPI), while AEs reported in at least 10% of patients are summarized in prior sections and in Table 10.1. In general, the proportion of individuals who experience AEs with pharmaceutical-grade CBD seems to be dose-related, though other factors may also contribute. Selected AEs—including hypersensitivity, suicidal ideation and behavior, and corrected QT interval (QTc) prolongation—are briefly reviewed as is use in pregnancy. Specific factors that may be associated with higher rates of transaminitis and somnolence and sedation are reviewed in greater detail.

6.1 Hypersensitivity to Pharmaceutical-Grade Cannabidiol or Any Ingredients in the Product

Cannabidiol is contraindicated in patients with a history of hypersensitivity to the molecule or any ingredients in the product; it should be noted that sesame oil is the vehicle utilized in pharmaceutical-grade CBD (Epidiolex® (CBD) [package insert] 2024). Rash was the most common reason for discontinuation in the TSC trial (Thiele et al. 2023), occurring in 3% of patients in the CBD 25 mg/kg/day arm, 3% of patients in the CBD 50 mg/kg/day arm, and in 0% of patients randomized to placebo; urticaria was a reason for discontinuation in 3% of patients in the CBD 50 mg/kg/day arm. Additionally, there are post-marketing reports of various skin rash manifestations (Singh and Antimisiaris 2020; Souza et al. 2022) as well as a case report of eosinophilic esophagitis (Patel et al. 2022) associated with use of pharmaceutical-grade CBD.

6.2 Suicidal Behavior and Ideation

All ASMs—including FDA-approved CBD—are labeled with a risk of suicidality as mandated by the FDA in 2008 based on a meta-analysis of 11 ASMs in which patients randomized to one of the ASMs had approximately twice the risk (adjusted

relative risk 1.8, 95% CI: 1.2–2.7) of suicidal thinking or behavior compared to patients randomized to placebo (Busco 2008; Christensen et al. 2007). A more recent meta-analysis evaluated suicidality risk for ASMs approved after 2008 (Klein et al. 2021), and two of the pharmaceutical-grade CBD randomized, placebo-controlled trials (Devinsky et al. 2017; Thiele et al. 2018) were included. While no suicidalities were reported for CBD, it should be noted that the Columbia-Classification Algorithm of Suicide Assessment—which has been used since 2011 to prospectively evaluate suicidality in ASM trials—is not validated in patients with severe cognitive impairment or in young children. As such, this scale may not have adequately captured such risk for the DS and LGS trial populations.

6.3 QTc Prolongation

While concentration-dependent prolongation of the QTc has been observed, pharmaceutical-grade CBD did not prolong the QTc more than 10 ms when administered as monotherapy at recommended doses with a high-fat meal (Epidiolex® (CBD) [package insert] 2024). The potential effect of CBD DDIs or concurrent use of other offending agents is not known.

6.4 Hepatic Injury: Rates, Risk Factors, and Monitoring

Potential risk factors for transaminitis associated with pharmaceutical-grade CBD include concurrent use of valproic acid derivatives and/or other medications known to impact the liver, higher doses of CBD, and/or baseline hepatic dysfunction (Epidiolex® (CBD) [package insert] 2024). Regarding the potential dose-related effect, ALT elevations >3x ULN were reported in 17% of patients taking CBD 20 mg/kg/day and 12% of patients taking CBD 25 mg/kg/day versus only 1% in patients taking CBD 10 mg/kg/day. Transaminitis was the most common reason for discontinuation in the DS (Devinsky et al. 2017) and LGS (Devinsky et al. 2018a; Thiele et al. 2018) trials, occurring in 1.3% of patients randomized to CBD 10 mg/kg/day and 5.9% of patients randomized to CBD 20 mg/kg/day versus 0.4% of patients on placebo (Epidiolex® (CBD) [package insert] 2024). In the TSC trial (Thiele et al. 2021), discontinuation due to ALT elevation occurred only in the CBD 50 mg/kg/day arm in 3% of patients. In approximately two-thirds of cases, resolution of transaminitis occurred with discontinuation of CBD or reduction of CBD dose and/or concomitant valproic acid derivatives. Resolution was spontaneous without dose reduction or discontinuation of CBD in the remaining approximate one-third of cases. The majority of transaminitis cases occurred within the first two months of treatment in the randomized, placeob-controlled trials, though transaminase elevations can occur at any time on therapy (Epidiolex® (CBD) [package insert] 2024). In the post-market setting, cases of cholestatic or mixed patterns of liver injury have

been reported. Cases of elevated ammonia levels have also been reported in individuals who also had transaminase elevations; most of these cases reported concomitant use of valproic acid derivatives, clobazam, or both.

Regarding increased rates of transaminitis with concurrent use of valproic acid derivatives, incidence of ALT elevation >3x ULN was 21% in patients taking both CBD and a valproic acid derivative versus 3% in patients taking CBD without a valproic acid derivative in the DS (Devinsky et al. 2017) and LGS (Devinsky et al. 2018a; Thiele et al. 2018) trials (Epidiolex® (CBD) [package insert] 2024). In patients with TSC receiving CBD (25 mg/kg/day) and concurrent valproic acid derivatives, ALT elevations >3x ULN occurred in 25% versus 6% in those on CBD without valproic acid derivatives (Epidiolex® (CBD) [package insert] 2024).

Serum transaminase and total bilirubin levels should be obtained at baseline and at one, three, and six months after initiation of CBD as well as periodically thereafter or as clinically indicated (e.g., within one month of dose changes and/or addition of or changes to other medications known to impact the liver). Dose adjustment and slower titration are recommended in patients with moderate (Child–Pugh B) or severe (Child–Pugh C) hepatic impairment.

6.5 Somnolence and Sedation

Somnolence and sedation with pharmaceutical-grade CBD use may be more common early in treatment and improve over time (Epidiolex® (CBD) [package insert] 2024). Potential risk factors for somnolence and sedation include use of higher doses of CBD as well as PK- and/or PD-related drug-drug interactions (DDIs), most notabaly between pharmaceutical-grade CBD and clobazam as discussed in more detail in the corresponding section. In the DS (Devinsky et al. 2017) and LGS (Devinsky et al. 2018a; Thiele et al. 2018) randomized, placebo-controlled trials, rates of somnolence were 27% in patients randomized to CBD 10 mg/kg/day and 34% in patients randomized to CBD 20 mg/kg/day versus 11% in those randomized to placebo (Epidiolex® (CBD) [package insert] 2024). The rates were higher in those receiving concurrent clobazam (46% versus 16% in those not on clobazam). Discontinuation due to somnolence, sedation, and lethargy across the DS (Devinsky et al. 2017) and LGS (Devinsky et al. 2018; Thiele et al. 2018) clinical trials occurred in 3% of patients taking CBD 20 mg/kg/day compared to 0% of patients taking CBD 10 mg/kg/day or placebo. In the TSC trial (Thiele et al. 2021), somnolence was reported in 13% of patients randomized to CBD 25 mg/kg/day and 26% of patients randomized to CBD 50 mg/kg/day versus 9% in those who received placebo; somnolence was a reason for discontinuation in 3% of patients in the CBD 50 mg/kg/day arm (Epidiolex® (CBD) [package insert] 2024). Rates of somnolence in this trial (Thiele et al. 2021) were higher in those receiving concurrent clobazam (33% versus 14% in those not on clobazam) (Epidiolex® (CBD) [package insert] 2024).

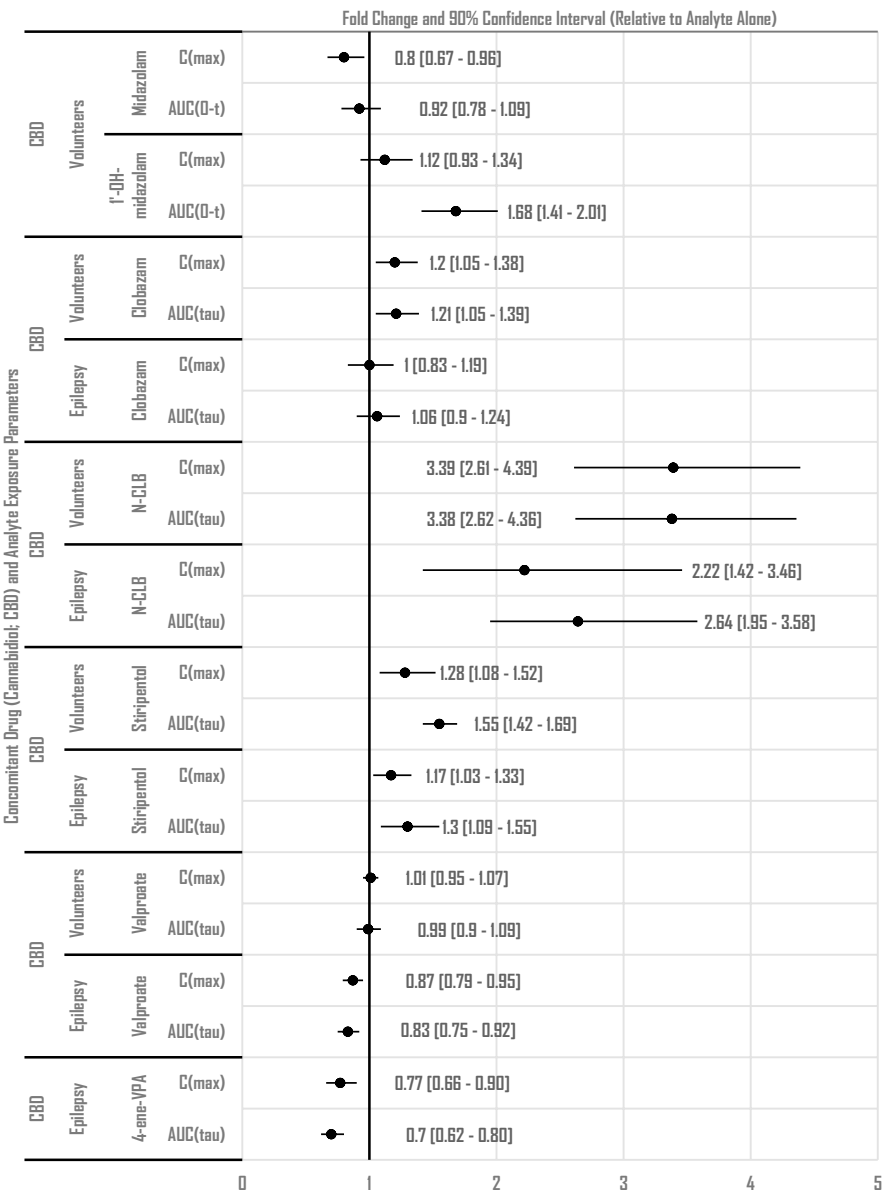


Fig. 10.2 Figure adapted from Patsalos et al. (2020) showing treatment ratios with 90% CIs for (**a**) the effect of standard probes and other ASMs on exposure of CBD and active metabolite, 7-OH-CBD and (**b**) the effect of CBD on exposure of standard probes and other ASMs. A fold-change (i.e., treatment ratio) of 1 indicates no change in exposure with the concomitant drug relative to the analyte alone. (*CI* confidence interval, *ASM* anti-seizure medication, *CBD* cannabidiol, *7-OH-CBD* 7-hydroxy-CBD, volunteers, healthy volunteers; epilepsy, patients with epilepsy, *1′-OH-midazolam* 1′-hydroxymidazolam, *C(max)* maximum observed plasma concentration, *AUC(0-t)* area under the concentration-time curve up to time *t*, where *t* is the last time point with a concentration above lower limit of quantification, *AUC(tau)* AUC over the dosing interval, *N-CLB* N-desmethylclobazam)

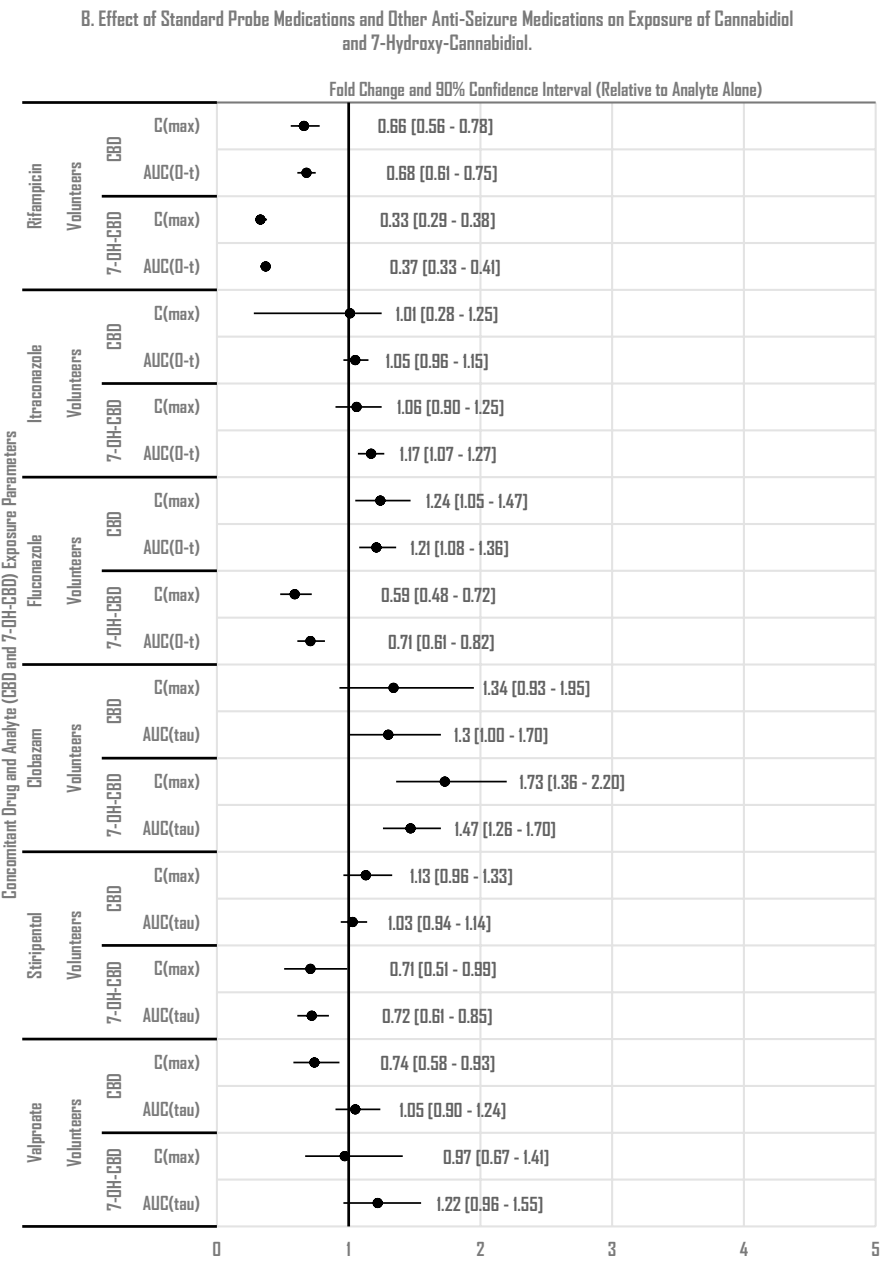


Fig. 10.2 (continued)

As demonstrated, when not used with clobazam and regardless of dose, the rates of somnolence and sedation associated with use of pharmaceutical-grade CBD appear to be similar to rates reported with placebo. In one real-world data set, half of patients who discontinued CBD due to sleepiness, drowsiness, and sedation were taking clobazam (Georgieva et al. 2023). Likewise, a consensus panel recommends empiric reduction of clobazam be considered when CBD is added, and that this may be especially prudent in individuals who are already experiencing sedation at baseline or in those who are already receiving the maximum tolerated dose of clobazam (Wechsler et al. 2024).

Importantly, if somnolence and sedation can be managed effectively when CBD and clobazam are used together, there may be benefits in terms of seizure reduction effectiveness (Wechsler et al. 2024). Though there has been question regarding whether CBD has anti-seizure effects independent of the PK interaction with clobazam (i.e., increase in exposure to the active clobazam metabolite, N-desmethylclobazam; N-CLB) (Perucca 2017; Bialer and Perucca 2020), results from several meta-analyses and other studies CBD does have anti-seizure effect independent of clobazam,and reduction in seizure frequency is generally more robust when the two are used together (Lattanzi et al. 2020; Devinsky et al. 2020; Bialer and Perucca 2020; Savage et al. 2020; Gunning et al. 2021). Aside from the PK interaction in which the active metabolite of each agent is increased by the other (described below and demonstrated in Fig. 10.2a, b), preclinical data suggests pharmacodynamic synergy between CBD and clobazam (Anderson et al. 2019; Rana et al. 2023).

6.5.1 Pregnancy

Adequate data on the developmental risks associated with pharmaceutical-grade CBD use in humans are not available, though preclinical animal data suggests it may cause fetal harm (Epidiolex® (CBD) [package insert] 2024). Patients should be advised to notify their healthcare provider if they become pregnant or intend to become pregnant while receiving CBD. Individuals who are pregnant should be encouraged to enroll in both the Epidiolex®.

Pregnancy Surveillance Program and the North American Antiepileptic Drug (NAAED) Pregnancy Registry. The purpose of the surveillance program and exposure registry is to collect information about the safety of Epidiolex® and other ASMs drugs during pregnancy.

7 Pharmacokinetics of Pharmaceutical-Grade Cannabidiol

Pharmaceutical-grade CBD is well-known to exhibit significant inter- and intra-patient PK variability (Taylor et al. 2018; Devinsky et al. 2018b; Wheless et al. 2019; Contin et al. 2021; Zhang et al. 2024; Johannessen Landmark et al. 2025).

There are many patient- and condition-specific factors that may contribute to this variability, such as age, fasting versus fed status, genetic polymorphisms of metabolic enzymes, DDIs, end-organ function, and others. While these factors will not be discussed comprehensively, acknowledgement of their impact on the clinical interpretation and application of available pharmaceutical-grade CBD PK information is extremely important and should be considered in the management and optimization of therapeutic regimens in clinical practice. The benefits of therapeutic drug monitoring of pharmaceutical-grade CBD and/or concomitant medications has been proposed to help ensure effective, safe, and individualized treatment given the complexity of CBD PK parameters and variability (Johannessen Landmark et al. 2025).

7.1 Absorption and Bioavailability

After single oral doses, CBD appears rapidly in plasma and maximum plasma concentrations are achieved in approximately four to five hours (Taylor et al. 2018). The absolute bioavailability of CBD is low (approximately 6%) under fasted conditions, and the reasons for this are likely multifactorial, including poor aqueous solubility and extensive first-pass metabolism in the gastrointestinal tract and liver. Administration with a high-fat, high-caloric meal results in an approximate five-fold increase in CBD maximum plasma concentration (C_{max}) and an approximate four-fold increase in area under the plasma concentration-time curve (AUC) compared to administration in the fasted state (Taylor et al. 2018; Crockett et al. 2019). Following single-dose administration, there was a trend to a less-than-dose proportional increase in exposure to CBD and its metabolites, postulated to be due to a reduction in bioavailability as doses are increased (Taylor et al. 2018). With multiple doses, however, exposure to CBD increased in an almost dose-proportional manner at steady-state (Taylor et al. 2018). Despite low oral bioavailability, CBD should be titrated to effect in clinical practice and therapeutic dosing ranges have been established, as described, in five randomized, placebo-controlled trials and in real-world practice.

7.2 Distribution

Pharmaceutical-grade CBD has a large apparent volume of distribution (V_d), ranging from 20,963 to 42,849 liters (Taylor et al. 2018), suggesting wide tissue distribution. Tissue distribution, including to the brain, was observed in animal studies, and higher levels of CBD were detected in jejunum, liver, and adipose tissues than serum following intraperitoneal administration in rats (Lust et al. 2022). Formal studies have not been conducted to determine the impact of obesity or differences in body composition, such as between pediatric and adult patients, on the distribution

or other of CBD. Given the highly lipophilic nature of CBD, this information could be clinically useful to inform optimal body weight that should be used to determine weight-based doses based on these factors.

7.3 Metabolism

Cannabidiol demonstrates high oral clearance, likely because of extensive hepatic metabolism via cytochrome P450 isoenzymes (CYP450) and 5′-diphosphoglucuronosyltransferases (UGTs) (Patsalos et al. 2020). Formation of its active metabolite—7-hydroxy-cannabidiol (7-OH-CBD)—is primarily catalyzed by CYP2C19 along with contributions from other CYP enzymes, including CYP3A4 (Jiang et al. 2011; Ujvary and Hanus 2016; review in Patsalos et al. 2020). More recently, it has been suggested that CYP2C9 may be a major contributor to 7-OH-CBD formation, especially for individuals with lower CYP2C19 activity (Beers et al. 2021). Formation of the most abundant circulating CBD metabolite—7-carboxy-cannabidiol (7-COOH-CBD)—has generally been attributed to metabolism by CYP3A4 (Taylor et al. 2018; Patsalos et al. 2020), but its formation may also be dependent on cytosolic nicotinamide adenine dinucleotide (NAD^+)-dependent enzymes with lesser contributions from nicotinamide adenine dinucleotide phosphate (NADPH)-dependent microsomal enzymes (Beers et al. 2023). Both CBD and 7-OH-CBD can also undergo direct conjugation by UGT1A9, UGT2B7, and UGT2B17 (Epidiolex® (CBD) [package insert] 2024). Drug-drug interactions related to some of these metabolic pathways are described in a later section.

While first-pass metabolism and its potential contribution to low bioavailability is often touted as a negative characteristic of pharmaceutical-grade CBD, hepatic CYP-mediated hydroxylation results in the formation of 7-OH-CBD. This active metabolite was shown to be five times more potent in its anti-seizure effect than CBD in the 6 Hz psychomotor seizure model based on brain exposure (Rana et al. 2023), and 7-OH-CBD also showed anti-seizure activity in the maximal electroshock seizure threshold test model (Whalley et al. 2017). Bypassing liver metabolism through alternate routes of administration (e.g., topical, transdermal, inhalation, etc.) with use of non-oral formulations (e.g., topical gel, transdermal patch, dry powder inhaler, etc.) can result in significantly lower levels of 7-OH-CBD (Zamarripa et al. 2024) and presumably, reduced anti-seizure effect. Despite this, CBD metabolite concentrations and exposures are not consistently reported in PK studies of these other products and formulations. On the other hand, the contribution of CBD metabolites to the AE profile of CBD has not been well-described, and it is possible that reduction in their exposure could improve tolerability and allow for higher CBD doses to be utilized. Devinsky et al. (2021) reported a reduction and/or total absence of AEs when a dry-powder inhaler CBD formulation with 10 mg of powder containing 2.1 mg CBD was compared to Epidiolex® 50 mg.

Notably, these doses are much lower than those utilized, and proven effective, in the randomized, plaebo-controlled trials of pharmaceutical-grade CBD, and complete absence of AEs may indicate lack of systemic and or brain exposure rather than an improved tolerability profile. Additionally, it is important to acknowledge that alternate routes of administration and formulations may result in significant alterations with regard to PK parameters, including time-course of the overall exposure profiles, when compared to pharmaceutical-grade CBD and it is unknown how this may impact efficacy and tolerability until these alternate formulations are systematically studied in randomized, placebo-controlled trials (Millar et al. 2018; Lucas et al. 2018; Zamarripa et al. 2024).

As previously mentioned, CYP2C19 may be predominantly responsible for the metabolism of CBD to 7-OH-CBD. The impact of CYP2C19 polymorphisms on CBD and metabolite disposition, clinical effectiveness, and tolerability has not been fully elucidated yet may be considerable. For instance, the implications of such polymorphisms—which may include improved effectiveness and/or reduced tolerability—is well-described for some of the other ASMs, including impact on clearance of the active metabolite of clobazam, N-CLB, via CYP2C19-mediated biotransformation to an inactive metabolite (Giraud et al. 2004; Seo et al. 2008; Yamamoto et al. 2013; Klein et al. 2019; Dean 2019). Plasma concentrations of N-CLB are five times higher for CYP2C19 poor metabolizers versus extensive metabolizers, placing individuals at risk of increased adverse effects (Dean 2019) but also improved effectiveness (Seo et al. 2008). Based on this, it is plausible that clinically relevant changes in CBD and/or 7-OH-CBD exposure may occur in individuals with CYP2C19 polymorphisms, but the clinical impact of these changes on effectiveness and safety need to be studied (Klein et al. 2019; Beers et al. 2023). In addition, CYP2C19 polymorphism status is also likely to result in alterations to expected DDIs when substrates, inducers, and/or inhibitors of this enzyme are used concurrently with pharmaceutical-grade CBD (Beers et al. 2023; Klein et al. 2019).

7.4 Elimination Processes and Half-Life

Many individuals use the terms elimination and excretion synonymously, but this is not appropriate as each term has a distinct PK meaning. Drug elimination refers to the sum of processes responsible for removing an administered drug from the body; drug elimination is generally considered to encompass both metabolism and excretion (Garza et al. 2023). Drug excretion specifically refers to the processes or mechanisms by which the active drug and/or its metabolites are physically removed from the body, such as through the urine, bile, and feces (Talevi and Bellera 2018). Drug elimination can also occur via metabolic biotransformation to active or inactive metabolites, primarily by the liver, followed by excretion via the kidneys or other organs (Garza et al. 2023).

Elimination of CBD and its metabolites is multiphasic (Taylor et al. 2018; review in Patsalos et al. 2020) and occurs via both metabolism and excretion. Excretion occurs primarily in the feces with minor renal clearance (Epidiolex® (CBD) [package insert] 2024). Worth mentioning are post-mortem studies which indicate CBD is detectable in bile. While this may be a result of biliary excretion processes, it is also possible that CBD may undergo enterohepatic recycling, which could have important clinical implications with regard to its PK properties (e.g., multiple peaks), especially potential DDIs with medications that interrupt enterohepatic recycling that could decrease exposure to CBD (Gronewald and Skopp 2011; Fabritius et al. 2012). Drug elimination half-life ($t_{½}$)—defined as the time required for the serum concentration of a drug to decrease by 50%—is the oldest pharmacokinetic parameter, yet its clinical application is commonly misunderstood by clinicians, leading to inappropriate assumptions when individualizing and optimizing medication regimens (Sahin and Bennet 2008; Gidal et al. 2017; Hallare and Gerriets 2023). While commonly misconceived to be synonymous with drug clearance, drug elimination $t_{½}$ is actually a parameter that reflects the sum of all pharmacokinetic properties of a given drug, including absorption, distribution, elimination—whether by biotransformation and/or excretion—and excretion (Gidal et al. 2017). Terminal elimination $t_{½}$ is defined as drug elimination during the final phase of elimination after the last dose has been administered (i.e., the medication discontinued) and once subsequent absorption and distribution of this dose are entirely complete. Even though a given drug can exhibit multiple half-lives as a result of its inherent PK characteristics (e.g., rate of absorption, multi-compartment distribution, etc.) and other patient- and condition-specific factors, terminal elimination $t_{½}$ is the most commonly published $t_{½}$ value. For drugs with simple linear PK, terminal elimination $t_{½}$ may be an accurate measure to inform drug dosing. For drugs with more complex PK characteristics (e.g., slow absorption, multi-compartment distribution, significant DDIs, etc.), however, it may be a poor predictor of drug accumulation and fluctuation and thus, not useful to inform drug dosing at steady state. For all these reasons and more, alternate $t_{½}$ parameters—such as "effective $t_{½}$" and "apparent multiple-dosing $t_{½}$"—have been proposed in order to more accurately characterize drug accumulation and loss over an entire dosing interval at steady state to better inform dose optimization (Sahin and Bennet 2008; Gidal et al. 2017). With regard to pharmaceutical-grade CBD, the terminal elimination $t_{½}$ for 750 mg and 1500 mg administered twice daily were shown to be approximately 56 and 61 hours, respectively. The corresponding effective $t_{½}$ estimates for these doses—which may be a more informative $t_{½}$ parameter to inform dosing regimens, as described—are 10 and 17 hours, respectively, hence the rationale for twice daily dosing of pharmaceutical-grade CBD despite the long terminal elimination $t_{½}$ (Taylor et al. 2018).

7.5 *Pharmacokinetic Considerations for Use of Pharmaceutical-Grade Cannabidiol in Individuals with End-Organ Dysfunction*

7.5.1 Hepatic Dysfunction

A randomized, placebo-controlled, Phase I PK study compared changes in exposure to CBD and its major metabolites (6-OH-CBD, 7-OH-CBD, 7-COOH-CBD) in individuals with mild, moderate, and severe hepatic impairment relative to individuals with normal hepatic function (Taylor et al. 2019). Exposure to CBD and all metabolites except 7-COOH-CBD was increased in individuals with mild, moderate, and severe hepatic impairment compared to individuals with normal hepatic function. The authors describe the changes in exposure as clinically relevant only for moderate or severe hepatic dysfunction. Based on the results of this study, the pharmaceutical-grade CBD USPI recommends reduction in starting and maintenance doses as well as slower titration in individuals with moderate (Child–Pugh B) and severe (Child–Pugh C) hepatic impairment (Epidiolex® (CBD) [package insert] 2024). Importantly—in individuals in this study with mild hepatic impairment—the upper end of the 90% confidence intervals (CIs) for the C_{max} and AUC geometric least-squares mean ratios for CBD and each metabolite demonstrated increases in the range of approximately 2-to-7-fold (Taylor et al. 2019), and many clinicians would consider this to be clinically relevant even if statistical significance thresholds were not met. Additionally, the single-dose nature of the study for a molecule with significant inter- and intra-patient PK variability and large V_d combined with the low dose utilized (200 mg, equivalent to approximately 2.5 mg/kg in a 70-kg patient) and the limited number of individuals studied in the trial may have limited sensitivity and specificity to measure statistically significant changes in plasma exposure across all three hepatic impairment severities.

7.5.2 Renal Dysfunction

The authors of a randomized, placebo-controlled Phase 1 PK study report no statistically significant changes in exposure to CBD or its major metabolites (6-OH-CBD, 7-OH-CBD, 7-COOH-CBD) in individuals with mild, moderate, or severe renal impairment compared to those with normal renal function (Tayo et al. 2020). Based on this study (Tayo et al. 2020), the labeling does not suggest dose modification of pharmaceutical-grade CBD for individuals with renal impairment (Epidiolex® (CBD) [package insert] 2024). Clinically, the results of this study should be interpreted with caution, however, given the single-dose nature for a molecule with significant inter- and intra-patient variability and large V_d, combined with the low dose utilized (200 mg, equivalent to approximately 2.5 mg/kg in a 70-kg patient) and the limited number of individuals sampled. Taken together, these factors may have limited sensitivity and specificity to measure statistically significant changes in plasma

exposure in the presence of renal dysfunction. In addition, some of the numeric differences in exposure parameters warrant determination of clinical relevance, especially considering that some of the 90% CIs for the geometric least-squares mean ratios for C_{max} and AUC approach increases in the range of 2.5-to-3.5-fold. Finally, these results are not representative of changes in exposure that might occur with continued dosing in individuals with renal impairment, such as the potential for drug accumulation. Additionally, there are no controlled studies to inform whether CBD and/or any of its metabolites are removed by dialysis.

7.6 Review of Clinically Relevant Drug–Drug Interactions between Pharmaceutical-Grade CBD and Selected Anti-Seizure Medications

Aside from significant hepatic metabolism, CBD potently inhibits CYP3A4 and CYP2C19 in human liver microsomes, and UGT1A9 and UGT2B7 in vitro (Patsalos et al. 2020). These in vitro data necessitated subsequent human pharmacokinetic DDI studies to characterize in vivo DDIs associated with use of pharmaceutical-grade CBD. These studies have been conducted with standard index substrate, inhibitor, and inducer probes to evaluate the effect of CBD on other drugs and vice versa. For example, effects of CBD induction and inhibition on CYP3A4 were evaluated using midazolam as the index substrate for CYP3A4. Effects of a strong CYP3A4 and CYP2C19 inducer on CBD exposure were assessed using rifampicin. Likewise, the effects of a strong CYP3A4 inhibitor and a strong CYP2C19 inhibitor on CBD exposure were characterized using itraconazole and fluconazole, respectively. Changes in plasma exposure (C_{max} and/or AUC) of CBD and other ASMs/drugs from several DDI studies are summarized in Fig. 10.2a, b, adapted from Patsalos et al. (2020). In this review of several CBD DDI studies, an effect was not considered to be clinically meaningful if the 90% CI fell within 0.5 to 2. Clinical context regarding relevance of the magnitude of changes in plasma exposure must always be considered, however, even if statistical significance thresholds are not met.

Finally, commentary on the potential contribution of selected DDIs for selected AEs associated with pharmaceutical-grade CBD were previously reviewed in Section 6.

7.6.1 Effects of Other Drugs on Pharmaceutical-Grade Cannabidiol Exposure

The effects of rifampicin, itraconazole, and fluconazole on CBD and 7-OH-CBD are shown in Fig. 10.2b, adapted from Patsalos et al. (2020); the reader is referred to this publication and the original PK studies for details regarding study

methodology as well as changes in exposure to 7-COOH-CBD and any other metabolites. As demonstrated, co-administration of CBD with a potent CYP3A4 and/or CYP2C19 inducer can result in a significant reduction in CBD and 7-OH-CBD exposure. A twofold or greater increase in CBD dose may be warranted when co-administered with potent CYP3A4 and/or CYP2C19 inducers (Epidiolex® (CBD) [package insert] 2024). Relatively small changes occur when CBD is coadministered with a CYP3A4 inhibitor. Administration of CBD with CYP2C19 inhibitors resulted in slightly increased exposure to the parent molecule while exposure to 7-OH-CBD—the active metabolite of CBD—was decreased. While reduction in 7-OH-CBD could theoretically lead to decreased effectiveness for seizure reduction, this may be offset by the relatively similar increase in the parent molecule; thus, it is difficult to determine if CBD dose adjustment is needed when combined with a CYP2C19 inhibitor. Additionally, the CYP2C19 inhibitor utilized in the referenced study, fluconazole—though less potent than itraconazole—is also a moderate CYP3A4 inhibitor (Yamaguchi et al. 2021) and thus, it is possible that this further influenced the changes in exposure seen for CBD and/or 7-OH-CBD (e.g., contributed to increased CBD exposure).

7.6.2 Effects of Pharmaceutical-Grade Cannabidiol on Exposure of Other Anti-Seizure Medications and Metabolites

The effect of CBD on exposure to other ASMs and clinically relevant metabolites is also illustrated in Fig. 10.2a, adapted from Patsalos et al. (2020). The reader is referred to this publication and the original PK studies for details regarding study methodology. Comments regarding real or theoretical clinical implications are further explained below where not previously discussed.

7.6.3 Clobazam and N-Desmethylclobazam (Multiple Overlapping Substrate and Induction/Inhibition Pathways)

Clobazam is metabolized to its active metabolite, N-CLB, via CYP3A4, and N-CLB is a weak UGT inhibitor (Patsalos et al. 2020; Klein et al. 2019). Preclinical data suggests that N-CLB is approximately 20% as potent in its anti-seizure effect as the parent molecule, and it is subsequently metabolized to an inactive metabolite—thus metabolically inactivated/cleared—by CYP2C19 (Klein et al. 2019). The impact of pharmaceutical-grade CBD on clobazam and N-CLB exposures is demonstrated in Fig. 10.2a, adapted from Patsalos et al. (2020); notably, concurrent use of clobazam with pharmaceutical-grade CBD also increases 7-OH-CBD, the active metabolite of CBD. This may be due to weak UGT inhibition by N-CLB versus competition for overlapping metabolic enzymes (Patsalos et al. 2020). General considerations when using clobazam and pharmaceutical-grade CBD together, such as the increased association with somnolence and sedation, were previously reviewed.

7.6.4 Stiripentol (Potent CYP2C19 Inhibitor)

Stiripentol is metabolized to an inactive metabolite via CYP2C19, CYP3A4, and CYP1A2 (Diacomit® (stiripentol) [package insert] 2022). Stiripentol is both a substrate for and a potent inhibitor of CYP3A4 and CYP2C19, respectively. Changes in plasma exposure for concurrent use of pharmaceutical-grade CBD and stiripentol are illustrated in Fig. 10.2a, b, adapted from Patsalos et al. (2020). Clinicians frequently inquire what is expected with regard to N-CLB exposure if a patient is receiving two CYP2C19 inhibitors, such as CBD and stiripentol or CBD and cenobamate, at the same time. Notably, Devinsky et al. (2018b) demonstrated that plasma N-CLB concentrations were not further increased when CBD was added to a treatment regimen already containing clobazam and stiripentol at baseline. This indicates that maximal CYP2C19 inhibition threshold may be achieved by stiripentol and/or that addition of other less potent CYP2C19 inhibitors (e.g., CBD) will not result in further increases in N-CLB levels. As previously discussed, the impact of CYP2C19 polymorphisms in this setting is unknown.

7.6.5 Valproate and 2-Propyl-4-Pentenoic Acid (Multiple Overlapping Substrate and Induction/Inhibition Pathways)

The metabolic pathways of valproic acid derivatives are complex and beyond the scope of this chapter, but major routes include glucuronidation and β-oxidation (Ghodke-Puranik et al. 2013). As demonstrated in Fig. 10.2a, b, adapted from Patsalos et al. (2020), there is a lack of a significant increase in exposure to valproate and one of its major hepatotoxic metabolites when coadministered with CBD. This may indicate that increased rates of transaminitis when CBD and valproic acid derivatives are used together, as previously described, is due to PD rather than PK factors (Patsalos et al. 2020).

7.6.6 Cenobamate (Multiple Overlapping Substrate and Induction/Inhibition Pathways)

Formal DDI studies have not been conducted for cenobamate and pharmaceutical-grade CBD when used together. Overlap in substrate, induction, and inhibition pathways for these agents is multiple—with opposing effects on some enzymes—and thus, changes in acute and/or net steady-state plasma exposure for each agent are difficult to predict with existing data. Based on the phenomenon of maximal CYP2C19 inhibition threshold as described above (Devinsky et al. 2018b), it is unlikely that addition of CBD to an individual already receiving concurrent cenobamate and clobazam will result in further increase in N-CLB exposure. Because cenobamate seems to be a more potent inhibitor of CYP2C19 than CBD based on clinical reports, however, it is possible that further increases in N-CLB levels may occur if cenobamate is added to a baseline regimen containing CBD and clobazam.

Potential for further increase in N-CLB exposure in such cases may not be clinically relevant, however, especially in individuals who are tolerating clobazam at baseline.

7.6.7 Everolimus (P-Glycoprotein and CYP3A4 Substrate)

Everolimus is an oral mTOR inhibitor that may be used for patients with TSC, either as an ASM and/or for the treatment of other clinical manifestations of TSC (Northrup et al. 2021; Krueger et al. 2013; Franz et al. 2018; Overwater et al. 2019; Previtali et al. 2023). Case reports and retrospective studies have demonstrated an increase in systemic exposure to oral mTOR inhibitors, including everolimus, when used concurrently with CBD (Wiemer-Kruel et al. 2019; Sabus et al. 2020). A randomized, placebo-controlled Phase 1 PK DDI study demonstrated that everolimus peak levels and total exposure were increased in the range of 2.5-fold when used with pharmaceutical-grade CBD (Wray et al. 2023). Potential mechanisms for this observed DDI may occur through P-glycoprotein (P-gp) and/or CYP-mediated pathways (Wiemer-Kruel et al. 2019; Sabus et al. 2020; Wray et al. 2023). Close monitoring of everolimus levels is suggested when used concurrently with CBD to guide everolimus dose adjustment and prevent everolimus-related AEs (Wray et al. 2023; Epidiolex® (CBD) [package insert] 2024). For patients already on CBD in whom everolimus is to be initiated, lower everolimus starting doses should be used along with close monitoring of levels to guide subsequent dose adjustments (Wechsler et al. 2024; Epidiolex® (CBD) [package insert] 2024).

8 Drug–Drug Interaction Summary

Commentary on the potential contribution of selected DDIs to specific AEs associated with pharmaceutical-grade CBD were previously addressed. While this chapter provides data and commentary for known clinically meaningful CBD-associated DDIs, the information contained herein is not reflective of all possible DDIs that may be clinically relevant. Importantly, DDIs for CBD and non-ASMs outside of standard PK study probes have not been reviewed, though clinically significant interactions between CBD and other drugs, whether proven or theoretical, do exist and have been reported (Balachandran et al. 2021; Brown et al. 2023–nonregulated CBD product; Karazniewicz-Lada et al. 2021; Epidiolex® (CBD) [package insert] 2024). Finally, information regarding DDIs along with their implications and management is often oversimplified. The reasons for this are multifactorial and may include the considerable complexity of metabolic substrate, induction, and inhibition pathways; overlap in such pathways, including concurrent opposition in induction or inhibition for shared metabolic enzymes; lack of additive nature to predict net effects when metabolic pathways overlap; patient-specific variation in DDIs in the setting of polypharmacy with ASMs and medications for other conditions; and genetic polymorphisms that may alter inherent function of metabolic enzymes

and transporters. Furthermore, many other patient- and condition-specific factors can alter drug exposure beyond what might be expected from DDIs alone.

9 Conclusion

Epilepsy is a common neurological disorder, and it is estimated that one in 26 individuals will develop epilepsy over the course of a lifetime. Despite the availability of many ASMs, including newer ASMs in the last two decades, the rate of drug-refractory cases remains generally unchanged. In the last decade, several randomized, placebo-controlled clinical trials and OLE studies have demonstrated pharmaceutical-grade CBD to be a generally well-tolerated and effective option for the treatment of seizures associated with rare, pediatric-onset epilepsy syndromes in both children and adults. The PK and PD of CBD, including clinically relevant DDIs, are incredibly complex, but basic knowledge and awareness of this information can be used to help optimize the effectiveness and tolerability of pharmaceutical-grade CBD in real-world practice. Further studies are warranted to better characterize potential differences in PK in selected populations, such as in pregnancy and in the elderly. Additionally, additional randomized, placebo-controlled studies to assess the efficacy and safety of pharmaceutical-grade CBD in other epilepsies and epilepsy syndromes may be warranted.

References

Aaberg KM, Gunnes N, Bakken IJ, et al. Incidence and prevalence of childhood epilepsy: a nationwide cohort study. Pediatrics. 2017;139(5):e20163908. https://doi.org/10.1542/peds.2016-3908.

Adams R, Hunt M, Clark JH. Structure of cannabidiol, a product isolated from maihuana extract of Minnesota wild hemp. J Am Chem Soc. 1940;62:196–200.

Anderson LL, Absalom NL, Abelev SV, et al. Coadministered cannabidiol and clobazam: preclinical evidence for both pharmacodynamic and pharmacokinetic interactions. Epilepsia. 2019;60(11):2224–34.

Arzimanoglou A, Brandi U, Cross HJ, et al. Epilepsy and cannabidiol: a guide to treatment. Epileptic Disord. 2020;22:1–14.

Balachandran P, Elsohly M, Hill KP. Cannabidiol interactions with medications, illicit substances, and alcohol: a comprehensive review. J Gen Intern Med. 2021;36(7):2074–84.

Beers J, Fu D, Jackson KD. Cytochrome P450–catalyzed metabolism of Cannabidiol to the active metabolite 7-Hydroxy-Cannabidiol. Drug Metab Dispos. 2021;49(10):882–91.

Beers J, Authement A, Isoherranen N, et al. Cytosolic enzymes generate cannabinoid metabolites 7-Carboxycannabidiol and 11-Nor-9-carboxytetrahydrocannabinol. ACS Med Chem Lett. 2023;14(5):614–20.

Bialer M, Perucca E. Does cannabidiol have antiseizure activity independent of its interactions with clobazam? An appraisal of the evidence from randomized controlled trials. Epilepsia. 2020;61(6):1082–9. https://doi.org/10.1111/epi.16542.

Boison D. Adenosinergic signaling in epilepsy. Neuropharmacology. 2016;104:131–9.

Boleti AP, Frihling BEF, Silva PSE, et al. Biochemical aspects and therapeutic mechanisms of cannabidiol in epilepsy. Neurosci Biobehav Rev. 2022;132:1214–28.
Borea PA, Gessi S, Merighi S, et al. Pharmacology of adenosine receptors: the state of the art. Physiol Rev. 2018;98:1591–625.
Brederson JD, Kym PR, Szallasi A. Targeting TRP channels for pain relief. Eur J Pharmacol. 2013;716:61–76.
Brown SA, Boyd K, Thomason AR. Geriatric pharmacotherapy case series: potential drug interactions with antiplatelet medications and Cannabidiol—a focus on P2Y12 inhibitors. Sr Pharm Care. 2023;38:141–7.
Busco M. FDA advisory members agree antiepileptics pose suicidality risk, nix need for black-box warning. Medscape Medical News; 2008.
Case Report, Cannabidiol-Induced Skin Rash. A Case Series and Key Recommendations. Frontiers in Pharmacology 2022. https://doi.org/10.3389/fphar.2022.881617
Carlini EA, Leite JR, Tannhauser M, et al. Letter: Cannabidiol and Cannabis sativa extract protect mice and rats against convulsive agents. J Pharm Pharmacol. 1973;25:664–5.
Chen Z, Brodie MJ, Liew D, et al. Treatment outcomes in patients with newly diagnosed epilepsy treated with established and new antiepileptic drugs: a 30-year longitudinal cohort study. JAMA Neurol. 2018;75:279–86.
Chescher GB, Jackson DM. Anticonvulsant effects of cannabinoids in mice: drug interactions within cannabinoids and cannabinoid interactions with phenytoin. Pyschopharmcologia. 1974;37:255–64.
Christensen J, Vestergaard M, Mortensen PB, et al. Epilepsy and risk of suicide: a population-based case–control study. Lancet Neurol. 2007;6(8):693–8. https://doi.org/10.1016/S1474-4422(07)70175-8.
Chu-Shore CJ, Major P, Camposano S, et al. The natural history of epilepsy in tuberous sclerosis complex. Epilepsia. 2010;51:1236–41.
Cohen MJ, Checketts D, Dunayevich E, et al. Time to onset of cannabidiol treatment effects in Dravet syndrome: analysis from two randomized controlled trials. Epilepsia. 2021;62(9):2218–27. https://doi.org/10.1111/epi.16974.
Consroe PF, Wolkin A. Cannabidiol-antiepileptic drug comparisions and interactions in experimentally induced seizures in rats. J Pharmacol Exp Ther. 1977;201:26–32.
Consroe PF, Man DP, Chin L, et al. Letter: reduction of audiogenic seizure by delta8 and delta9-tetrahydrocannabinols. J Pharm Pharmacol. 1973;25:764–5.
Consroe PF, Wood GC, Buchsbaum H. Anticonvulsive nature of marihuana smoking. JAMA. 1975;234:306–7.
Contin M, Mohamed S, Santucci M, et al. Cannabidiol in pharmacoresistant epilepsy: Clinical pharmacokinetic data from an expanded access program. Frontiers in Pharmacology 2021;12:367801. https://doi.org/10.3389/fphar.2021.637801.
Crippa JA, Guimaraes FS, Zuardi AW, et al. Dr. Raphael Mechoulam, cannabis and cannabinoids pioneer (November 5, 1930–March 9, 2023) and his legacy for Brazilian pharmacology. Braz J Psychiatry. 2023;45:201–2.
Crockett J, Critchley D, Tayo B, et al. A phase I, randomized, pharmacokinetic trial of the effect of different meal compositions, whole milk, and alcohol on cannabidiol exposure and safety in healthy subjects. Epilepsia. 2019;61:267–77.
Crocq MA. History of cannabis and the endocannabinoid system. Dialogues Clin Neurosci. 2020;22:223–8.
Cunha JM, Carlini EA, Pereira AE, et al. Chronic administration of cannabidiol to healthy volunteers and epileptic patients. Pharmacology. 1980;21:175–85.
De Petrocellis L, Ligresti A, Moriello S, et al. Effects of cannabinoids and cannabinoid-enriched cannabis extract on TRP channels and endocannabinoid metabolic enzymes. Br J Pharmacol. 2011;163:1479–94.
Dean L. Clobazam therapy and CYP2C19 genotype. In: Pratt VM, Scott SA, Pirmohamed M, et al., editors. Medical genetics summaries [internet]. Bethesda, MD: National Center for biotechnology information (US); 2019. https://www.ncbi.nlm.nih.gov/books/NBK546789/.

Detyniecki K, Hirsch L. Marijuana use in epilepsy: the myth and the reality. Curr Neurol Neurosci Rep. 2015;15:65. https://doi.org/10.1007/s11910-015-0586-5.
Devinsky O, Marsh E, Friedman D, et al. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. Lancet Neurol. 2016;15:270–8.
Devinsky O, Cross HJ, Laux L, et al. Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. N Engl J Med. 2017;376:2011–20.
Devinsky O, Patel AD, Cross JH, et al. Effect of Cannabidiol on drop seizures in the Lennox-Gastaut syndrome. N Engl J Med. 2018a;378:1888–97.
Devinsky O, Patel AD, Thiele EA, et al. Randomized, dose-ranging safety trial of cannabidiol in Dravet syndrome. Neurology. 2018b;90:e1204–11.
Devinsky O, Thiele EA, Wright S, et al. Cannabidiol efficacy independent of clobazam: meta-analysis of four randomized controlled trials. Acta Neurol Scand. 2020;2020:531. https://doi.org/10.1111/ane.13305.
Devinsky O, Kraft K, Rusch L, et al. Improved bioavailability with dry powder Cannabidiol inhalation: a phase one clinical study. J Pharm Sci. 2021;110(12):3946–52.
Diacomit® (stiripentol) [package insert]. Paris: Biocodex; 2022.
During MJ, Spencer DD. Adenosine: a potential mediator of seizure arrest and postictal refractoriness. Ann Neurol. 1992;32:618–24.
Epidiolex® (cannabidiol) [package insert]. Palo Alto, CA: Jazz Pharmaceuticals; 2024.
Fabritius M, Staub C, Mangin P, et al. Distribution of free and conjugated cannabinoids in human bile samples. Forensic Sci Int. 2012;223:114–8.
Fisher RS, Acevedo C, Arzimanoglou A, et al. ILAE official report: a practical clinical definition of epilepsy. Epilepsia. 2014;55:475–82.
Flamini RJ, Comi AM, Bebin EM, et al. Efficacy of cannabidiol in convulsive and nonconvulsive seizure types associated with treatment-resistant epilepsies in the expanded access program. Epilepsia. 2023;64:e156–63.
Franz DN, Lawson JA, Yapici Z, et al. Everolimus for treatment-refractory seizures in tuberous sclerosis complex. Neurol Clin Pract. 2018;8(5):412–20.
Gardener H, Wallin C, Bowen J. Heavy metal and phthalate contamination and labeling integrity in a large sample of US commercially available cannabidiol (CBD) products. Sci Total Environ. 2022;851:158110. https://doi.org/10.1016/j.scitotenv.2022.158110.
Garza AZ, Park SB, Kocz R. Drug elimination. In: StatPearls. Treasure Island, FL: StatPearls; 2023. PMID: 31613442.
Georgieva D, Langley J, Hartkopf K, et al. Real-world, long-term evaluation of the tolerability and therapy retention of Epidiolex® (cannabidiol) in patients with refractory epilepsy. Epilepsy Behav. 2023;141:109159.
Ghodke-Puranik Y, Thorn CF, Lamba JK, et al. Valproic acid pathway: pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics. 2013;23:236. https://doi.org/10.1097/FPC.0b013e32835ea0b2.
Gidal BE, Clark AM, Anders B, Gilliam F. The application of half-life in clinical decision making: comparison of the pharmacokinetics of extended-release topiramate (USL255) and immediate-release topiramate. Epilepsy Res. 2017;129:26–32.
Gidal BE, Vandrey R, Wallin C, et al. Product labeling accuracy and contamination analysis of commercially available cannabidiol product samples. Front Pharmacol. 2024;15:1335441. https://doi.org/10.3389/fphar.2024.1335441.
Gidal B, Clark A, Anders B, et al. The application of half-life in clinical decision making: Comparison of the pharmacokinetics of extended-release topiramate (USL255) and immediate-release topiramate. Epilepsy Research 2017;129:26–32.
Giraud C, Tran A, Rey E, et al. In vitro characterization of clobazam metabolism by recombinant cytochrome P450 enzymes: importance of CYP2C19. Drug Metab Dispos. 2004;32(11):1279–86.
Gray RA, Whalley BJ. The proposed mechanisms of action of CBD in epilepsy. Epileptic Disord. 2020;22:10–5.
Gray RA, Stott CG, Jones NA, et al. Anticonvulsive properties of cannabidiol in a model of generalized seizure are transient receptor potential vanilloid$_1$ dependent. Cannabis Cannabinoid Res. 2020;5:145–9.

Gronewald A, Skopp G. A preliminary investigation on the distribution of cannabinoids in man. Forensic Sci Int. 2011;210:e7–e11.
Gunning B, Mazurkiewicz-Bełdzińska M, Chin RFM, et al. Cannabidiol in conjunction with clobazam: analysis of four randomized controlled trials. Acta Neurol Scand. 2021;143(2):154–63. https://doi.org/10.1111/ane.13351.
Hang NA, Kieschnick D, Sottile JE, et al. Training and practices of cannabis dispensary staff. Cannabis Cannabinoid Res. 2016;1(1):244–51. https://doi.org/10.1089/can.2016.0024.
Hallare J, Gerriets V. Half Life. [Updated 2023 Jun 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554498/.
Hesdorffer DC, Logroscino G, Benn EK, et al. Estimating risk for developing epilepsy: a population-based study in Rochester, Minnesota. Neurology. 2011;76:23–7.
Holford N, Rim D-S. Clearance. Transl Clin Pharmacol. 2015;23(2):42–5.
Huang WX, Yu F, Sanchez RM, et al. TRPV1 promotes repetitive febrile seizures by proinflammatory cytokines in immature brain. Brain Behav Immun. 2015;48:68–77.
Husain R, Weeden H, Bogush D, et al. Enhanced tolerance of industrial hemp (*Cannabis sativa* L.) plants on abandoned mind land soil leads to overexpression of cannabinoids. PLoS One. 2019;14(8):e0221570. https://doi.org/10.1371/journal.pone.0221570.
Hussain SA, Zhou R, Jacobson C, et al. Perceived efficacy of cannabidiol-enriched cannabis extracts for treatment of pediatric epilepsy: a potential role for infantile spasms and Lennox-Gastaut syndrome. Epilepsy Behav. 2015;47:138–41.
Iannotti FA, Hill CL, Leo A, et al. Nonpsychotropic plant cannabinoids, cannabidivarin (CBDV) and cannabidiol (CBD), activate and desensitize transient receptor potential vanilloid 1 (TRPV1) channels in vitro: potential for the treatment of neuronal hyperexcitability. ACS Chem Nerosci. 2014;5:1131–41.
Jiang R, Yamaori S, Takeda S, et al. Identification of cytochrome P450 enzymes responsible for metabolism of cannabidiol by human liver microsomes. Life Sci. 2011;89(5–6):165–70. https://doi.org/10.1016/j.lfs.2011.05.018.
Johannessen Landmark C, Saetre J, Gottas A, et al. Pharmacokinetic variability and use of therapeutic drug monitoring of cannabidiol in patients with refractory epilepsy. Epilepsia 2025;00:1–15. https://doi.org/10.1111/epi.18284.
Kaplan JS, Stella N, Catterall WA, et al. Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. Proc Natl Acad Sci. 2017;114:11229–34.
Karazniewicz-Lada M, Glowka AK, Mikulska AA, et al. Pharmacokinetic drug-drug interactions among antiepileptic drugs, including CBD, drugs used to treat COVID-19, and nutrients. Int J Mol Sci. 2021;22:9582. https://doi.org/10.3390/ijms22179582.
Klein P, Tolber D, Gidal B. Drug–drug interactions and pharmacodynamics of concomitant clobazam and cannabidiol or stiripentol in refractory seizures. Epilepsy Behav. 2019;99:106459. https://doi.org/10.1016/j.yebeh.2019.106459.
Klein P, Devinsky O, French J, et al. Suicidality risk of newer antiseizure medications: a meta-analysis. JAMA Neurol. 2021;78(9):1118–27. https://doi.org/10.1001/jamaneurol.2021.2480.
Krueger DA, Wilfong AA, Holland-Bouley K, et al. Everolimus treatment of refractory epilepsy in tuberous sclerosis complex. Ann Neurol. 2013;74:679–87.
Kwan P, Brodie MJ. Early identification of refractory epilepsy. N Engl J Med. 2000;342:314–9.
Kwan P, Arzimanoglou A, Berg AT, et al. Definition of drug resistant epilepsy: consensus proposal by the ad hoc task force of the ILAE commission on therapeutic strategies. Epilepsia. 2010;51:1069–77.
Lado FA, Moshe SL. How do seizures stop? Epilepsia. 2008;49:1651–4.
Lattanzi S, Trinka E, Striano P, et al. Cannabidiol efficacy and clobazam status: a systematic review and meta-analysis. Epilepsia. 2020;61(6):1090–8.
Lucas CJ, Galettis P, Schneider J. The pharmacokinetics and the pharmacodynamics of cannabinoids. Br J Clin Pharmacol. 2018;84:2477–82. https://doi.org/10.1111/bcp.13710.
Lust CAC, Lin X, Rock EM, et al. Short communication: Tissue distribution of major cannabinoids following intraperitoneal injection in male rats. PLOS One. 2022;17(1):e0262633. https://doi.org/10.1371/journal.pone.0262633.

Maa E, Figi P. The case for medical marijuana in epilepsy. Epilepsia. 2014;55:783–6.
Marichal-Cancino BA, Fajardo-Valdez A, Ruiz-Contreras AE, et al. Advances in the physiology of GPR55 in the central nervous system. Curr Neuropharmacol. 2017;15:771–8.
McPartland JM, Duncan M, Di Marzo V, et al. Are cannabidiol and Δ(9)-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. Br J Pharmacol. 2015;172:737–53.
Mechoulam R, Shvo Y. Hashish. I The structure of cannabidiol. Tetrahedron. 1963;19:2073–8.
Meza RC, Ancaten-Gonzalez C, Chiu CQ, et al. Transient receptor potential vanilloid 1 function at central synapses in health and disease. Front Cell Neurosci. 2022;16:864828. https://doi.org/10.3389/fncel.2022.864828.
Mijangos-Moreno S, Poot-Ake A, Arankowsky-Sandoval G, et al. Intrahypothalamic injection of cannabidiol increases the extracellular levels of adenosine in nucleus accumbens in rats. Neurosci Res. 2014;84:60–3.
Millar SA, Stone NL, Yates AS, et al. A systematic review on the pharmacokinetics of Cannabidiol in humans. Front Pharmacol. 2018;9:1365. https://doi.org/10.3389/fphar.2018.01365.
Miller I, Scheffer IE, Gunning B, et al. Dose-ranging effect of adjunctive oral cannabidiol vs placebo on convulsive seizure frequency in Dravet syndrome: a randomized clinical trial. JAMA Neurol. 2020;77(5):613–21. https://doi.org/10.1001/jamaneurol.2020.0073.
Nelson JA, Knupp KG. Lennox-Gastaut syndrome: current treatments, novel therapeutics, and future directions. Neurotherapeutics. 2023;20:1255–62.
Northrup H, Aronow ME, Bebin EM, et al. Updated international tuberous sclerosis complex diagnostic criteria and surveillance and management recommendations. Pediatr Neurol. 2021;123:50–66.
O'Shaughnessy WB. On the preparations of indian hemp, or Gunjah. Prov Med J Retrosp Med Sci. 1843;5:363–9.
Overwater IE, Rietman AB, van Eeghen AM, et al. Everolimus for the treatment of refractory seizures associated with tuberous sclerosis complex (TSC): current perspectives. Ther Clin Risk Manag. 2019;15:951–5.
Pandolfo P, Silveirinha V, dos Santos-Rodrigues A, et al. Cannabinoids inhibit the synpatic uptake of adenosine and dopamine in the rat and mouse striatum. Eur J Pharmacol. 2011;655:38–45.
Parvez MK. Natural or plant products for the treatment of neurological disorders: current knowledge. Curr Drug Metab. 2018;19:424–8.
Patel AD, Mazurkiewicz-Bełdzińska M, Chin RF, et al. Long-term safety and efficacy of add-on cannabidiol in patients with Lennox–Gastaut syndrome: results of a long-term open-label extension trial. Epilepsia. 2021;62:2228–39.
Patel M, Middleton JP, Goodkin HP, et al. Persistent diarrhea and eosinophilic esophagitis resulting from chronic cannabidiol usage for refractory epilepsy. Journal of Pediatric Gastroenterology and Nutrition Reports 2022;3:4(e253). https://doi.org/10.1097/PG9.0000000000000253.
Patsalos PN, Szaflarksi JP, Gidal B, et al. Clinical implications of trials investigating drug-drug interactions between cannabidiol and enzyme inducers or inhibitors or common anti-seizure drugs. Epilepsia. 2020;61:1854–68.
Perlman AI, McLeod HM, Ventresca EC, et al. Medical cannabis state and federal regulations: implications for United States health care entities. Mayo Clin Proc. 2021;96:2671–81.
Perucca E. Cannabinoids in the treatment of epilepsy: hard evidence at last? J Epilepsy Res. 2017;7(2):61–76.
Placido DF, Lee CC. Potential of industrial hemp for phytoremediation of heavy metals. Plan Theory. 2022;11(5):595. https://doi.org/10.3390/plants11050595.
Privitera M, Bhathal H, Wong M, et al. Time to onset of cannabidiol (CBD) treatment effect in Lennox–Gastaut syndrome: analysis from two randomized controlled trials. Epilepsia. 2021;62(5):1130–40.
Raich I, Lillo J, Ferreiro-Vera C, et al. Cannabidiol at nanomolar concentrations negatively affects signaling through adenosine A2A receptor. Int J Mol Sci. 2023;24:17500. https://doi.org/10.3390/ijms242417500.

Rana R, Rajasekaran K, Knappertz V, et al. Pharmacodynamic synergism contributes to the antiseizure action of cannabidiol and clobazam. Exp Neurol. 2023;360:114286. https://doi.org/10.1016/j.expneurol.2022.114286.

Reynolds J. On the therapeutic uses and toxic effects of Cannabis indica. Lancet. 1890;135:637–8.

Rogawski MA, Loscher W, Rho JM. Mechanisms of action of Antiseizure drugs and the ketogenic diet. Cold Spring Harb Perspect Med. 2016;6(5):a022780. https://doi.org/10.1101/cshperspect.a022780.

Rosenberg EC, Chamberland S, Bazelot M, et al. Cannabidiol modulates excitatory-inhibitory ratio to counter hippocampal hyperactivity. Neuron. 2023;111:1282–300.

Russo EB. History of cannabis and its preparation in saga, science, and sobrequet. Chem Biodivers. 2007;4:1614–48.

Sabus A, Winzent S, Hemenway M, et al. Drug interaction between everolimus and cannabidiol in pediatric patients with subependymal giant cell astrocytomas: a single institution experience. Neuro Oncol. 2020;22:iii284.

Sahin S, Benet LZ. The operational multiple dosing half-life: a key to defining drug accumulation in patients and to designing extended release dosage forms. Pharm Res. 2008;25(12):2869–77.

Sanchez-Fernandez N, Gomez-Acero L, Sarasola LI, et al. Cannabidiol negatively modulates adenosine A_{2A} receptor functioning in living cells. Acta Neuropsychiatr. 2023;22:1–5.

Savage TE, Sourbron J, Bruno PL, et al. Efficacy of cannabidiol in subjects with refractory epilepsy relative to concomitant use of clobazam. Epilepsy Res. 2020;160:106263. https://doi.org/10.1016/j.eplepsyres.2019.106263.

Scheffer IE, Halford JJ, Miller I, et al. Add-on cannabidiol in patients with Dravet syndrome: results of a long-term open label extension trial. Epilepsia. 2021;62:2505–17.

Schoedel KA, Szeto I, Setnik B, et al. Abuse potential assessment of cannabidiol (CBD) in recreational polydrug users: a randomized, double-blind, controlled trial. Epilepsy Behav. 2018;88:162–71.

Seo T, Nagata R, Ishitsu T, et al. Impact of CYP2C19 polymorphisms on the efficacy of clobazam therapy. Pharmacogenomics. 2008;9(5):527–37. https://doi.org/10.2217/14622416.9.5.527.

Shu HF, Yu SX, Zhang CQ, et al. Expression of $TRPV_1$ in cortical lesions from patients with tuberous sclerosis complex and focal cortical dysplasia type IIb. Brain Dev. 2013;35:252–60.

Singh J, Antimisiaris MF. Epidiolex-induced skin rash. Epileptic Disorders 2020;22(4):511–514.

Souza JDS, Fassoni-Ribeiro M, Batista RM, et al. Case report: Cannabidiol-induced skin rash: A case series and key recommendations. Frontier in Pharmacology 2022;13:881617. https://doi.org/10.3389/fphar.2022.881617.

Sun FJ, Guo W, Zheng DH, et al. Increased expression of $TRPV_1$ in the cortex and hippocampus from patients with mesial temporal lobe epilepsy. J Mol Neurosci. 2013;49:182–93.

Szaflarski JP, Devinsky O, Lopez M, et al. Long-term efficacy and safety of cannabidiol in patients with treatment-resistant epilepsies: four-year results from the expanded access program. Epilepsia. 2023;64(3):619–29.

Talevi A, Bellera CL. Drug excretion. In: Talevi A, Quiroga P, editors. ADME processes in pharmaceutical sciences. Cham: Springer; 2018. https://doi.org/10.1007/978-3-319-99593-9_5.

Taylor L, Gidal B, Blakey G, et al. A phase I, randomized, double-blind, placebo-controlled, single ascending dose, multiple dose, and food effect trial of the safety, tolerability and pharmacokinetics of highly purified Cannabidiol in healthy subjects. CNS Drugs. 2018;32:1053–67.

Taylor L, Crockett J, Tayo B, et al. A phase one, open-label, parallel-group, single-dose trial of the pharmacokinetics and safety of Cannabidiol (CBD) in subjects with mild to severe hepatic impairment. J Clin Pharmacol. 2019;59(8):1110–9. https://doi.org/10.1002/jcph.1412.

Tayo B, Taylor L, Sahebkar F, et al. A phase I, open-label, parallel-group, single-dose trial of the pharmacokinetics, safety, and tolerability of Cannabidiol in subjects with mild to severe renal impairment. Clin Pharmacokinet. 2020;59(6):747–55. https://doi.org/10.1007/s40262-019-00841-6.

Thiele EA, Marsh ED, French JA, et al. Cannabidiol in patients with seizures associated with Lennox-Gastaut syndrome (GWPCARE4): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2018;391:1085–96.

Thiele EA, Bebin ME, Bhathal H, et al. Add-on cannabidiol treatment for drug-resistant seizures in tuberous sclerosis complex: a placebo-controlled randomized clinical trial. JAMA Neurol. 2021;78:285–92.

Thiele E, Bebin ME, Filloux F, et al. Long-term safety and efficacy of add-on Cannabidiol (CBD) for seizures associated with tuberous sclerosis complex (TSC): 3-year results from GWPCARE6 open-label extension (OLE). Neurology. 2023;100(17 supp. 2). https://doi.org/10.1212/WNL.0000000000020260.

Ujvary I, Hanus L. Human metabolites of Cannabidiol: a review on their formation, biological activity, and relevance in therapy. Cannabis Cannabinoid Res. 2016;1(1):90–101.

Wechsler R, Burdette DE, Gidal B, et al. Consensus panel recommendations for the optimization of EPIDIOLEX® treatment for seizures associated with Lennox–Gastaut syndrome, Dravet syndrome, and tuberous sclerosis complex. Epilepsia Open. 2024;9:1632–42. https://doi.org/10.1002/epi4.12956.

Weltha L, Reemmer J, Boison D. The role of adenosine in epilepsy. Brain Res Bull. 2019;151:46–54.

Whalley B, Stott C, Gray R, et al. The human metabolite of cannabidiol, 7-hydroxyl cannabidiol, but not 7-carboxy cannabidiol, is anticonvulsant in the maximal electroshock seizure threshold test (MEST) in mouse. Am Epilepsy Soc Meeting Abstr. 2017;1:435.

Wheless JW, Dlugos D, Miller I, et al. Pharmacokinetics and tolerability of multiple doses of pharmaceutical-grade synthetic Cannabidiol in pediatric patients with treatment-resistant epilepsy. CNS Drugs. 2019;33:593–604. https://doi.org/10.1007/s40263-019-00624-4.

Wiemer-Kruel A, Stiller B, Bast T. Cannabidiol interacts significantly with everolimus-report of a patient with tuberous sclerosis complex. Neuropediatrics. 2019;50(6):400–3.

Wirrell EC, Hood V, Knupp KG, et al. International consensus on diagnosis and management of Dravet syndrome. Epilepsia. 2022;63:1761–77.

Wray L, Berwaertz J, Critchley D, et al. Pharmacokinetic drug-drug interaction with coadministration of Cannabidiol and Everolimus in a phase one healthy volunteer trial. Clin Pharmacol Drug Dev. 2023;12(9):911–9.

Wright NJ, Lee SY. Structures of human ENT in complex with adenosine reuptake inhibitors. Nat Struct Mol Biol. 2019;26:599–606.

Wu J, Cock H, Devinsky O, et al. Time to onset of Cannabidiol (CBD) treatment effect and resolution of adverse events (AEs) in the Tuberous Sclerosis Complex (TSC) Phase 3 randomized controlled trial (GWPCARE6) (674). Neurology. 2020;94(15_supplement). https://doi.org/10.1212/WNL.94.15_supplement.67.

Yamaguchi Y, Akiyoshi T, Kawamura G, et al. Comparison of the inhibitory effects of azole antifungals on cytochrome P450 3A4 genetic variants. Drug Metab Pharmacokinet. 2021;38:100384. https://doi.org/10.1016/j.dmpk.2021.100384.

Yamamoto Y, Takahashi Y, Imai K, et al. Influence of CYP2C19 polymorphism and concomitant antiepileptic drugs on serum clobazam and N-Desmethyl clobazam concentrations in patients with epilepsy. Ther Drug Monitor. 2013;35:305–12.

Zamarripa CA, Tilton HE, Lin S, et al. Pharmacokinetics and pharmacodynamics of five distinct commercially available hemp-derived topical cannabidiol products. J Anal Toxicol. 2024;48:81–98.

Zavala-Tecuapetla C, Luna-Munguia H, Lopez-Meraz ML, et al. Advances and challenges of cannabidiol as an anti-seizure strategy: preclinical evidence. Int J Mol Sci. 2022;23:16181. https://doi.org/10.3390/ijms232416181.

Zhang Q, Melchert PW, Markowitz JS. Pharmacokinetic variability of oral cannabidiol and its major metabolites after short-term high-dose exposure in healthy subjects. Med Cannabis Cannabinoids. 2024;7(1):1–9. https://doi.org/10.1159/000535726.

Part III
Adolescence as a Sensitive Period

Chapter 11
Adolescence as a Window of Vulnerability to Cannabis Use: Insights from Animal Studies



Kwang-Mook Jung, Hye-Lim Lee, and Daniele Piomelli

1 Introduction

Human adolescence is marked by sweeping physical, emotional, and cognitive changes (Sisk et al. 2020). One of its most recognizable features is a heightened propensity for risk-taking behaviors, which can manifest in forms as varied as engaging in dangerous driving, participating in extreme sports, or experimenting with mind-altering substances (Crone and van Duijvenvoorde 2021). Among these substances, cannabis arguably captivates the curiosity of teenagers more than any other. For example, data from the 2023 Monitoring the Future survey show that almost one-third of 12th-grade students in the United States used the drug at least once in the previous year, with about 12% reporting daily use for a month or longer (Miech et al. 2023). While teenage cannabis use raises concern among parents, educators, and policymakers, understanding its risks against the backdrop of adolescent development is crucial.

During this period of sexual maturation and rapid physical growth, forebrain structures involved in higher-order mental functions—cognition, decision-making, social behavior, etc.—also undergo pronounced structural changes (Sisk et al.

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2020). Well into young adulthood, between 20- and 25-year-old in humans, neuronal networks and glial cell populations within these structures retain part of their earlier developmental plasticity (Blakemore 2012a, b; Blakemore and Robbins 2012; Masuda et al. 2020) making them exquisitely sensitive to environmental influences that interfere with brain neurotransmission, including trauma, stress, and neuroactive substance use (Sisk et al. 2020). Cannabis belongs in this category because its mind-altering (psychotropic) constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC), produces its effects by engaging the endocannabinoid system (ECS) (Fig. 11.1), a signaling complex that contributes in unique ways to the postnatal development of the central nervous system (CNS) (Maccarrone et al. 2014).

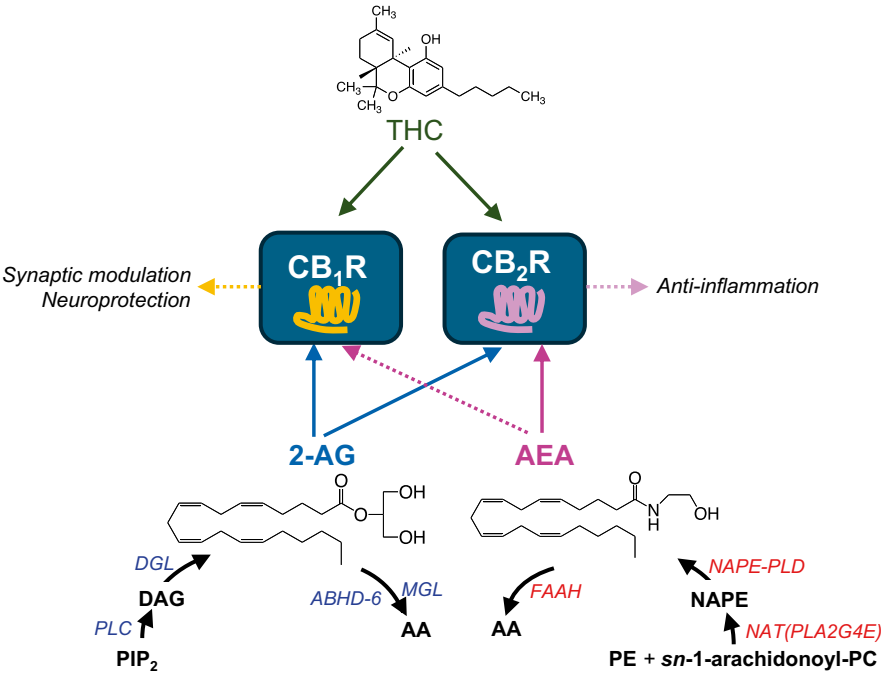


Fig. 11.1 Overview of the ECS. This signaling complex includes the cannabinoid receptors CB_1 and CB_2 (CB_1R and CB_2R), the endogenous ligands anandamide (AEA) and 2-arachidonyl-*sn*-glycerol (2-AG), as well as proteins responsible for their synthesis and breakdown. Phospholipase C (PLC), activated by occupation of certain receptors, converts phosphatidylinositol-4,5-bisphosphate (PIP_2) into 1,2-diacylglycerol (DAG). DAG is broken down by diacylglycerol lipase (DGL) to form 2-AG, which is further hydrolyzed by either monoglyceride lipase (MGL) or, to a lesser extent, α,β-hydrolase domain-containing protein 6 (ABHD-6). AEA formation begins with the transfer of the *sn*-1 arachidonoyl chain of 1,2-diarachidonoyl-PC to the free amino group of phosphatidylethanolamine (PE), forming *N*-arachidonoyl-phosphatidylethanolamine (*N*-arachidonoyl-PE, NAPE), catalyzed by *N*-acyl transferase (NAT)/PLA2G4E. NAPE is converted to anandamide by a NAPE-specific phospholipase D (PLD). After transport inside cells, anandamide is degraded by the serine amidase fatty acid amide hydrolase (FAAH)

Exposure to Δ^9-THC during the teenage years—particularly when frequent and at high doses—can interfere with activity of the ECS wherever this system is present, including the brain, potentially leading to lasting effects on mental and physical health. Over the past 30 years, epidemiological surveys have consistently found strong associations between adolescent cannabis use and long-term impairments in cognition, mood, and psychosocial functioning, which can extend into adulthood even after cessation (Albaugh et al. 2021; Schweinsburg et al. 2008). In addition, persistent alterations in body mass, core metabolism, and immunity have been reported (Meier et al. 2016, 2019; Hayatbakhsh et al. 2010; Barre et al. 2022; Alshaarawy and Anthony 2019; Le Strat and Le Foll 2011; Maggirwar and Khalsa 2021).

Despite their reproducibility, these associations may be influenced by reverse causation (cannabis use as an attempt at self-medication) or other factors such as shared genetic, epigenetic, and socioeconomic vulnerabilities (Rogeberg 2013; Schaefer et al. 2021a, b, 2023; Zellers et al. 2024). Establishing a causal link between cannabis use and its potential health consequences is crucial for clarifying these relationships and guiding the design and implementation of effective preventive measures. When investigating the effects of cannabis in human populations, causality can be assessed using a variety of approaches, including discordant twin analysis, Mendelian randomization, and quasi-experimental interventions (for review, see Schaefer et al. 2023). Human and animal laboratory experiments occupy a unique place in this context as they allow researchers to test the biological plausibility of observational findings, explore causal links under controlled conditions, investigate underlying mechanisms, and point to new directions for human research.

This chapter is focused on a series of results obtained by our laboratory—in collaboration with other members of the Center of Excellence *Impact of Cannabinoids Across the Lifespan* (ICAL), funded by the National Institute on Drug Abuse—using a rodent model of frequent but moderate exposure to Δ^9-THC during adolescence. Contextualized within the current literature, the findings reviewed here confirm the hypothesis that adolescence is a period of unique vulnerability to the effects of Δ^9-THC. Exposure to the drug during this critical developmental window—even at doses that are only moderately psychoactive—has lasting impact on multiple components of the adult ECS, which are associated with neurobehavioral abnormalities and impairments in the response to microbial infection and psychosocial stress. The studies also identified unexpected modifications in adipose organ function, which provide a plausible molecular basis for the persistent metabolic alterations observed in people who regularly use cannabis.

2 The Endocannabinoid System in the Adolescent Brain

Δ^9-THC is responsible for the pharmacological effects of most cannabis-derived products currently available. This terpenoid molecule, one of over 140 synthesized in the plant's female inflorescence (Hanus et al. 2016), acts by engaging G protein-coupled CB_1 and CB_2 cannabinoid receptors, an integral part of the ECS (Piomelli

and Mabou Tagne 2022) (Fig. 11.1). Other ECS components are the lipid-derived mediators, anandamide and 2-arachidonoyl-*sn*-glycerol (2-AG), and the enzymes and transporters responsible for the formation and deactivation of these mediators. In the CNS, the ECS is involved in various aspects of prenatal and postnatal development, including neuronal migration, axonal guidance, and synapse generation (Harkany and Cinquina 2021). Furthermore, it serves as a framework for retrograde synaptic transmission—a form of neuronal communication where signals travel from the postsynaptic spine back to the presynaptic terminal—fine tuning synaptic activity and plasticity throughout the brain and spinal cord (Piomelli 2003; Piomelli 2014). The ECS is also present outside the CNS, where it regulates adipogenesis, lipogenesis, and immunity, among other processes (for review, see Marinelli et al. 2023; Pandey et al. 2009; Jung et al. 2022).

The brain ECS undergoes significant changes during adolescence. Studies in rodents have shown that anandamide mobilization (the balance of its on-demand production and rapid elimination) and expression of CB_1 receptors peak during mid-adolescence and decline into adulthood (Laprairie et al. 2012; Wenger et al. 2002; Casey et al. 2015). This profile may reflect the influence exerted by endocannabinoid signals on two key aspects of adolescent behavior: heightened sensitivity to natural and drug rewards, and increased inclination toward novelty-seeking and risk-taking (Balogh et al. 2013). By inappropriately activating CB_1 receptors in CNS neurons, Δ^9-THC disrupts this regulation, leading to structural and functional changes that can continue until adulthood. Consuming the drug during adolescence could also persistently alter microglia function. These cells express CB_1 and CB_2 receptors (Sharma et al. 2021) and undergo profound modifications during their transition to an adult phenotype (Masuda et al. 2020; Borst et al. 2021). Similar to the effects on neural circuits, Δ^9-THC may disturb this transition, causing lasting abnormalities in the transcriptional and functional profiles of adult microglial subpopulations (Masuda et al. 2020).

3 Modeling Cannabis Exposure in Rodents

Preclinical investigations are yielding valuable insights into the consequences of early-life exposure to Δ^9-THC. In a 2013 study (Cutando et al. 2013), young-adult (postnatal day 49–70) mice were treated twice daily with doses of Δ^9-THC ranging from 1 mg/kg to 20 mg/kg (intraperitoneal, IP) for 5 consecutive days and then immediately sacrificed. Molecular and immunochemical analyses revealed enhanced expression in cerebellum of the microglial activation marker, Iba-1, along with increased production of interleukin 1β (IL1β) and other indicators of neuroinflammation. Deficits in cerebellar conditioned learning and fine motor coordination paralleled those effects. Another study, published in 2015 (Zamberletti et al. 2015), examined the impact of escalating doses of Δ^9-THC (2.5 to 10 mg/kg, IP) administered twice-daily to female rats on postnatal days 35 to 45, when rodents are

considered adolescent (Brust et al. 2015). Investigations carried out after the rats had reached young adulthood (postnatal day 75) brought to light multiple signs of inflammation in the prefrontal cortex—but not hippocampus, nucleus accumbens, or amygdala—including heightened expression of the cytokine, tumor necrosis factor-α (TNF-α). Similar results were obtained in a follow-up study (Gabaglio et al. 2021), also in female rats, in which the same ascending-dose protocol was used to compare the effects of Δ^9-THC alone with those of Δ^9-THC combined with the non-psychotropic cannabis constituent, cannabidiol. Increased expression of Iba-1 and arborization of microglial processes (also an indicator of microglia activation) were observed in rats immediately after administration of five daily doses of 10 mg/kg Δ^9-THC (IP) (Mohammadpanah et al. 2023). Lastly, two reports focused on the synthetic cannabinoid agonists, Win-55,212-2 and JWH-018, are noteworthy. The dosing regimens used were particularly aggressive, given the high potency and efficacy of those agents compared to Δ^9-THC: Win-55,212-2 was administered at 2 mg/kg (IP) once daily from postnatal day 35 to 45; and JWH-018 was administered at doses escalating from 0.5 mg/kg to 1.5 mg/kg (IP) from postnatal day 25 to 39 (Wan et al. 2022; Izquierdo-Luengo et al. 2023). Treatment with Win-55,212-2 increased Iba-1 expression in medial prefrontal cortex but, unlike in the Δ^9-THC studies mentioned above, this effect was observed only after adult male mice had been challenged with the bacterial endotoxin, lipopolysaccharide (LPS) (Wan et al. 2022). JWH-018 also caused microglia activation in the prefrontal cortex, accompanied by neurobehavioral abnormalities (increased anxiety-like behavior, diminished prepulse inhibition) and reduced number of perineuronal nets, which were observed only in male animals (Izquierdo-Luengo et al. 2023).

Despite minor discrepancies, the studies outlined above concord in indicating that Δ^9-THC activates microglia and triggers neuroinflammation in the rodent brain. This is a significant finding that aligns Δ^9-THC with opioids and most psychostimulants (with the exception of nicotine) (Soares and Picciotto 2024), all of which are known to induce an activated microglia phenotype (for review, see da Silva et al. 2023; Green et al. 2022). However, the studies suffer from two limitations that limit their real-world (ecological) significance. The first is their focus on high and/or escalating doses of Δ^9-THC, which are primarily relevant to adolescents who consume high-potency cannabis products multiple times a day. While this type of users exists and might become more common due to the increased availability of products with high Δ^9-THC content (Chan et al. 2017; Meier 2017), most teenagers do not have the financial means and/or practical opportunity to develop such an intense use pattern, even when consuming cannabis daily. Another significant limitation of the studies is the lack of consideration for how species-, age-, and sex-dependent differences in the pharmacokinetics and distribution of Δ^9-THC could impact the drug's pharmacodynamics. It is implausible to assume that Δ^9-THC is absorbed, metabolized, and distributed equally across species, ages, and sexes. But if these key properties differ, so too will pharmacodynamics, which is dictated by the drug's concentration in the relevant biophase—the site within the body where Δ^9-THC exerts a given effect.

Researchers are now working to overcome these limitations by exploring ecologically relevant strategies that place greater emphasis on the interactions between the administered dose of Δ^9-THC, the route of administration, and the biophase concentrations attained by the drug. This approach contextualizes the actions of Δ^9-THC within its pharmacokinetic and distribution properties—which indeed depend, as discussed below, on animal species, biological sex, and age—and takes into account the varied patterns of cannabis use adopted by teenagers.

3.1 Pharmacokinetic and Pharmacodynamic Properties of Δ⁹-THC in Adolescent Mice and Rats

To be ecologically relevant, animal models must build on human data. These show that smoking a cannabis cigarette with a Δ^9-THC content of 1.75–3.55%—the amount most commonly found in cannabis available from 1970 to 1995 (Freeman et al. 2021)—results in peak blood concentrations ranging from approximately 50 to 250 ng/mL (for review, see Huestis and Cone 2004). Since these exposure levels are associated with clear psychotropic activity (Huestis and Cone 2004; Cooper and Haney 2009), we examined what dosages of Δ^9-THC would produce similar exposures in adolescent and young-adult (postnatal day 33 and 70, respectively) mice and rats of both sexes. The exact temporal boundaries of adolescence in rodents are hard to define, but postnatal days 30 and 43 are often thought to mark its beginning and end, respectively (Brust et al. 2015). We evaluated Δ^9-THC doses of 0.5, 1.6, and 5 mg/kg and, to remove systemic absorption as a source of variability, administered the drug by IP injection. Other options, such as inhalation or oral ingestion, were also evaluated but were deprioritized due to inter-sample variability and other considerations (Ruiz et al. 2021a; Torrens et al. 2023; English et al. 2024). In addition to Δ^9-THC, the first-pass metabolites 11-hydroxy-Δ^9-THC (11-OH-THC, bioactive) and 11-nor-9-carboxy-Δ^9-THC (11-COOH-THC, inactive) were quantified in blood (plasma), brain, and white adipose tissue, using a validated liquid chromatography/mass spectrometry method (Vozella et al. 2019).

In a series of reports published between 2020 and 2024 (Torrens et al. 2020; Torrens et al. 2022; Torrens et al. 2024; Ruiz et al. 2021b), we identified a number of species-, sex-, and age-dependent differences in Δ^9-THC pharmacokinetics and distribution, the most significant of which are summarized here. First, regardless of sex or age, mice and rats differ in the maximum circulating concentration (C_{max}) of Δ^9-THC, with mice showing substantially higher C_{max} compared to rats. For this reason, C_{max} values comparable to those measured in intoxicated humans (50–250 ng/mL) can be achieved with a lower dose of Δ^9-THC in mice (1.6 mg/kg) than in rats (5 mg/kg). This higher systemic exposure does not translate, however, into greater psychoactive impact. In fact, we found that the adolescent mouse brain is partially protected from Δ^9-THC, possibly due to heightened expression of the blood–brain

barrier proteins Abcg2 (an efflux transporter) and claudin-5 (a tight-junction component) (Torrens et al. 2020; Torrens et al. 2024). This protection is observed in both males and females and likely explains the limited responses of adolescent mice to a 5 mg/kg dose of Δ^9-THC, which at this age does not affect locomotor activity or food intake and causes only a moderate decrease in core body temperature (Torrens et al. 2020; Lee et al. 2022) (three prototypical responses to CB_1 receptor activation in mice) (Fride et al. 2006; Kirkham 2005; Wenger and Moldrich 2002). Thus, regardless of sex, 5 mg/kg Δ^9-THC represents a moderately active dose when administered to adolescent mice but a fully effective dose when administered to adult mice.

Significant sex differences also exist. In both mice and rats, the conversion of Δ^9-THC into the highly bioactive metabolite 11-OH-THC is greater in females than males, mirroring findings reported in humans (Zamarripa et al. 2023). Relatedly, irrespective of sex, adolescent mice and rats metabolize Δ^9-THC into 11-OH-THC more effectively than adults do. This is intriguing as it suggests that adolescent mice and rats of both sexes resemble adult females in their exposure to 11-OH-THC, whose pharmacological properties are still imprecisely characterized. Lastly, confirming previous research (Kreuz and Axelrod 1973; Gunasekaran et al. 2009), we found across all experimental groups that white adipose tissue serves as a primary site of Δ^9-THC accumulation and can store the drug for weeks after its complete removal from bloodstream and brain. This finding, together with the quasi-ubiquitous distribution of the ECS in the mammalian body, highlights the importance of gauging the central effects of Δ^9-THC within the context of its systemic impact.

The experiments summarized thus far outline a model for investigating the effects of Δ^9-THC exposure in adolescent and adult mice and rats of both sexes. The ecological validity of this model is supported by pharmacokinetic and pharmacodynamic data, which give grounds for its use as a proxy for daily consumption of moderate psychoactive doses of cannabis in humans. As noted above, this use pattern is observed in approximately 12% of 12th-grade students in the United States (Kelleghan et al. 2022). The model can also be adapted to evaluate other use patterns, such as intermittent or high-intensity consumption, but cannot be utilized to study voluntary intake, for which operant approaches are more suitable (English et al. 2024; Freels et al. 2023; Freels et al. 2024). When we applied this protocol to examine the consequences of adolescent exposure to Δ^9-THC, we found that the drug produced, in both mice and rats, sex-dependent neurobehavioral changes that lasted well into adulthood (postnatal day 70 and beyond) and were linked to a transient disruption of microglial homeostasis (Ruiz et al. 2021b; Lee et al. 2022; Lee et al. 2024; Schoch et al. 2018; Le et al. 2022). In addition, we identified striking alterations in systemic metabolism and adipose organ function (Lin et al. 2023), which are reminiscent of those seen in people who regularly use cannabis.

4 Effects of Adolescent Δ^9-THC Exposure on Adult Brain Function

4.1 Brain Response to Viral Infection

In a 2024 study (Lee et al. 2024), we used the experimental paradigm outlined above to examine whether adolescent exposure to a moderately psychoactive dose of Δ^9-THC (5 mg/kg, IP) has enduring effects on the brain's neuroimmune response to viral infection. For this investigation, male and female mice were given the drug once daily from postnatal day 30 to 43. When the mice reached young adulthood (postnatal day 70), they received injections of polyinosinic acid:polycytidylic acid [Poly(I:C)], a Toll-like receptor-3 agonist and a potent activator of the response to RNA viruses (Bartok and Hartmann 2020). As expected (Cunningham et al. 2007), Poly(I:C) administration was quickly followed by an acute sickness reaction with elevated body temperature and attenuated locomotor activity. The increase in body temperature was lessened by adolescent Δ^9-THC treatment in female but not male mice, while the decrease in motor activity was unaffected in both sexes (Lee et al. 2024).

RNA sequencing analyses showed that the brains of Poly(I:C)-treated mice exhibited a substantial upregulation of immune-related genes, which was reduced by adolescent Δ^9-THC treatment in females but not in males. By contrast, the viral mimic produced a male-selective downregulation in the transcription of genes involved in neurodevelopment and synaptic transmission, which was prevented by Δ^9-THC. No such effects were seen when Δ^9-THC was administered in adulthood (postnatal days 70–83). In sum, viral infection causes complex transcriptional alterations in the mouse brain, which are sexually dimorphic and differentially affected by early-life Δ^9-THC exposure. Most notably, in female mice the exposure dampens the neuroimmune response to Poly(I:C) whereas in male mice it forestalls the transcriptional downregulation of neuron-related genes evoked by this agent.

4.2 Cannabis Impact on Microglial Function

4.2.1 Role of Microglia in Health and Disease

Microglia are resident macrophages of the brain and play crucial roles in its perinatal development and adult homeostasis (Masuda et al. 2020; Borst et al. 2021). After birth, they are responsible for synaptic pruning. This process is critical for neocortical maturation as it removes redundant or weak synaptic connections, strengthens essential ones, and facilitates the maturation of neural circuits (Faust et al. 2021). Once this developmental stage is complete, microglia lose some of their postnatal heterogeneity (Masuda et al. 2020) and switch to a homeostatic phenotype, surveilling the CNS for infectious agents, obsolete synapses, and damaged neurons (Borst

et al. 2021). In healthy brains, microglia are long-lived compared to other brain cell lineages. However, after sensing a harmful stimulus, they can undergo clonal proliferation and release humoral signals that attract monocytes and other circulating immune cells to the brain. While these responses may have evolved as a protective mechanism against infectious agents, they can also contribute to pathogenesis. Indeed, microglia dysfunction and the appearance of disease-associated microglia phenotypes (Deczkowska et al. 2018) have been implicated not only in neuroinflammatory or neurodegenerative pathologies (e.g., multiple sclerosis and Alzheimer's), but also in schizophrenia (Rodrigues-Neves et al. 2022), depression (Wang et al. 2022), and the maladaptive response to psychosocial stress (Schramm and Waisman 2022).

4.2.2 Effects of Adolescent Δ^9-THC Exposure on Microglial Defense Genes

Microglia express CB_1 and CB_2 receptors (Stella 2010), yet the impact of acute or prolonged exposure to Δ^9-THC on these cells remains incompletely understood. The microglial response to the drug is less consistent than that seen with opioids and most psychostimulants, which clearly enhance microglia's pro-inflammatory activity (for review, see da Silva et al. 2023; Green et al. 2022). While dosing regimens involving high and/or escalating doses of Δ^9-THC do induce both an activated microglia phenotype and neuroinflammation (Cutando et al. 2013; Zamberletti et al. 2015; Gabaglio et al. 2021; Mohammadpanah et al. 2023; Wan et al. 2022; Izquierdo-Luengo et al. 2023), a strikingly opposite effect is observed with a more moderate level of adolescent exposure (Fig. 11.2).

Using the protocol outlined above—a single daily injection of Δ^9-THC (5 mg/kg, IP) from postnatal day 30 to 43—in a 2022 report (Lee et al. 2022) we showed that adolescent Δ^9-THC administration leads to a lasting reduction in the expression of microglial defense genes, indicative of a blunted reactivity to external or internal danger signals. Transcriptome-wide analyses were conducted on microglia isolated by fluorescence-activated cell sorting (FACS) on postnatal days 49 and 70. At the earliest time point—6 days after the last Δ^9-THC injection, when the drug had been completely cleared from the brain but was still present in fat depots—the transcription of many genes involved in microglia homeostasis, innate immunity, and "sensome" (the collection of microglial proteins that detect and respond to changes in the environment) (Hickman et al. 2013) was attenuated. Moreover, genes typically upregulated in disease-associated microglia were either unaltered or suppressed. This transcriptional profile aligns with a blunting, rather than a stimulation, of microglia's inflammatory activity. Importantly, Δ^9-THC's ability to promote this hypoactive microglia phenotype was restricted to adolescence: when we administered the drug to young-adult/adult mice (on postnatal days 70 to 83) and examined the microglia 27 days later, we found that only 22 genes were differentially regulated between the control and Δ^9-THC group—less than 1% of those affected when

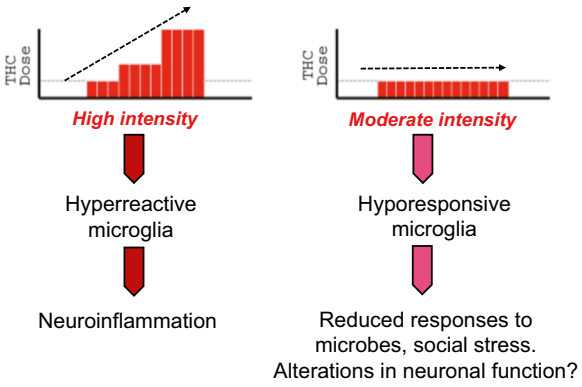


Fig. 11.2 Dose-selective effects of adolescent Δ^9-THC exposure to microglia phenotypes. Experiments in rodents indicate that while exposure to high and escalating doses of THC during adolescence leads to an activated microglia phenotype and neuroinflammation, an opposite effect is seen with exposure to a moderately psychoactive dose of Δ^9-THC

the drug was administered in adolescence (a total of 2359 genes, approximately 10% of the entire mouse genome). This striking difference underscores the unique sensitivity of adolescence to agents, such as Δ^9-THC, that interfere with the normal functioning of the ECS.

4.2.3 Effects of Adolescent Δ^9-THC on the ECS in Microglia

In the same 2022 study (Lee et al. 2022), we identified several modifications in the expression of ECS-related genes (Fig. 11.2). Genes encoding three anandamide-metabolizing enzymes were affected: cytosolic phospholipase A_2 (*Pla2g4e*) and fatty acid amide hydrolase (*Faah*) were upregulated, whereas N-acylphosphatidylethanolamine-specific phospholipase D (*Napepld*) was downregulated. In addition, transcription of the 2-AG-hydrolyzing enzyme monoglyceride lipase (*Mgll*) was dampened. Microglial expression of the CB_1 receptor gene (*Cnr1*) increased whereas that of the CB_2 receptor gene (*Cnr2*) decreased. By postnatal day 70, only three of these changes remained detectable: upregulation of *Faah* and downregulation of *Napepld*, suggestive of an attenuation of anandamide-mediated signaling, and downregulation of *Mgll*, suggestive of a heightened 2-AG-mediated signaling. The increase in *Faah* expression is particularly notable, as elevated levels of this anandamide-hydrolyzing enzyme were observed across all five microglial subpopulations identified in Δ^9-THC-treated mice using high-resolution cytometry by time-of-flight (Lee et al. 2022). By postnatal day 120, the transcription of ECS-related genes had returned to normal.

4.2.4 Effects of Adolescent Δ^9-THC Exposure on Microglial Responses to Bacterial Infection

As noted above, the transcriptional landscape of microglia after adolescent Δ^9-THC exposure suggests a diminished capacity to respond to danger signals. To investigate this further, we treated male and female mice with Δ^9-THC (5 mg/kg, IP) on postnatal days 30–43 and then challenged them with the gram-negative endotoxin LPS on postnatal day 70 (Lee et al. 2022). In vehicle-treated mice, LPS administration was followed by the expected upregulation of microglial immune-related genes, increased Iba-1 expression, altered microglia morphology, and monocyte migration into the bloodstream and brain. These responses were suppressed in the Δ^9-THC-treated group, where most enriched gene categories were not related to immunity, and Iba-1 expression, microglia morphology, and monocyte migration were all but normalized (Fig. 11.2). In addition, these effects were dependent on CB_1 receptor activation as they were prevented by co-administering Δ^9-THC with the CB_1 inverse agonist AM251 but not the CB_2 inverse agonist AM630 (Fig. 11.3). Finally, the effects were comparable in male and female mice, with sexual dimorphisms transpiring only in Iba-1 expression and microglia morphology (which in females were less marked than males). These findings corroborate those described above for viral infection (Lee et al. 2024), indicating that daily adolescent exposure to Δ^9-THC, even at doses that are moderately psychoactive, produces an adult microglial phenotype unresponsive to microbial attack.

4.2.5 Effects of Adolescent Δ^9-THC Exposure on Microglia-Dependent Responses to Social Stress

Microglia play a pivotal role in the reaction to psychosocial stress (Ramirez et al. 2017) and have been implicated in the pathogenesis of stress-related pathologies such as major depressive disorder (Mondelli et al. 2017; Gritti et al. 2021). The discovery that adolescent Δ^9-THC treatment causes a persistent impairment in microglia-mediated immune responses suggests that the reaction to social stress could also be affected. To test this, we exposed young-adult male mice that had received Δ^9-THC (5 mg/kg, IP) or vehicle during adolescence to a repeated social defeat protocol. This variation of the resident-intruder paradigm (Golden et al. 2011) replicates multiple facets of the human response to social stress and relies on microglial activation (Golden et al. 2011; Powell et al. 2013; Wohleb et al. 2013).

Vehicle-treated mice reacted to social defeat with a series of neurohumoral changes that included elevated circulating corticosterone and IL-6 levels, along with various indicators of neuroinflammation, such as upregulation of *Il1b*, *Il6*, and *Tnfa* gene expression in brain tissue. In striking contrast, no such changes were observed in mice that had been exposed to Δ^9-THC in adolescence. In another experiment, using the social interaction test (Golden et al. 2011; Krishnan et al. 2007; Kinsey et al. 2007), we found that vehicle-treated mice exposed to social defeat spent more

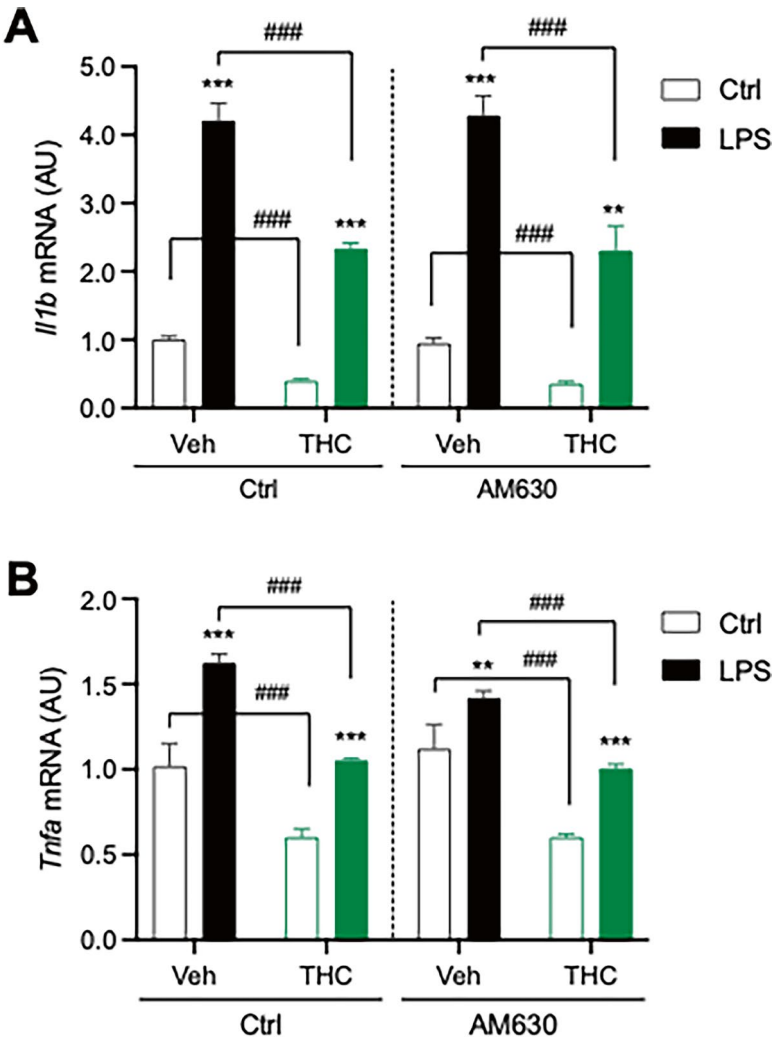


Fig. 11.3 The effects of adolescent Δ^9-THC exposure on microglia do not require CB_2R. Levels of (**a**) *Il1b* and (**b**) *Tnfa* mRNA in mice treated with vehicle or Δ^9-THC alone (left), or vehicle or Δ^9-THC plus the CB_2R-selective inverse agonist AM630 (3 mg/kg, i.p.) (right) during postnatal days 30–43. Gene expression was measured in purified microglia on postnatal day 70 by qRT-PCR ($n = 3$/group). $^{\#}P < 0.05$, $^{**}P < 0.01$, and $^{***/\#\#\#}P < 0.001$, two-way ANOVA followed by Tukey's multiple comparison test. Unpublished results

time in the avoidance zone of the test arena and less time in the social interaction zone, indicating social withdrawal. Again, the Δ^9-THC-exposed group exhibited no such changes. Finally, we assessed the animals' innate fear for aviary predators using the elevated plus maze test. As expected (Krishnan et al. 2007), vehicle-treated mice who experienced social defeat were more anxious than non-defeated

mice, as evidenced by reduced time in and fewer entries into the open arms of the maze. By contrast, no increased fear behavior was observed in mice that had undergone Δ^9-THC treatment in adolescence. In conclusion, male mice exposed to Δ^9-THC during adolescence were unable to mount a normative neurobehavioral response to social stressors in young adulthood. If such a deficit occurred also in people who regularly consumed cannabis as teenagers, it could have significant, as-yet-uncharacterized repercussions on their familial, social, and professional lives.

4.3 Cannabis Impact on Synaptic Plasticity and Memory

4.3.1 Cannabis Use, Hippocampal Plasticity, and Episodic Memory

Epidemiological surveys have linked adolescent cannabis use to a higher incidence of memory and cognitive issues in later life, and similar effects have been reported in animal models (for review, see Rubino and Parolaro 2011; Blest-Hopley et al. 2020). Nevertheless, our understanding of how cannabis exposure impacts synaptic function in forebrain regions essential for memory encoding remains scattered. In rats, early-life administration of escalating doses of Δ^9-THC (2.5 mg/kg to 10 mg/kg, IP, twice daily, from postnatal day 35 to 45) reduces GABAergic neurotransmission and ECS-dependent long-term depression (LTD) in the frontal cortex without impacting long-term potentiation (LTP) in the same regions (Zamberletti et al. 2014; Rubino et al. 2015). The same treatment protocol was also shown to decrease synaptic spine density in the hippocampal dentate gyrus (Rubino et al. 2009). Given the crucial role of the hippocampus in the acquisition of spatial and episodic memory (for review, see Dede et al. 2016; Dickerson and Eichenbaum 2010), determining whether these changes are associated with functional alterations in hippocampal synaptic plasticity is an important step toward understanding how adolescent cannabis use may affect everyday adult life. Focus on episodic memory—the encoding, storage, and retrieval of information about personal events (Tulving 2002)—is particularly important as this form of long-term memory is impaired in people who use cannabis (Smith et al. 2015; Lawn et al. 2022), who also have more false memories than people who don't use it (Kloft et al. 2019; Kloft et al. 2020). False memories are recollections of events that did not actually occur or are remembered incorrectly, a phenomenon closely connected with episodic memory (Straube 2012).

4.3.2 Effects of Adolescent Δ^9-THC Exposure on Hippocampal LTP and Episodic Memory

The impact of Δ^9-THC on hippocampal synaptic plasticity and episodic memory was evaluated in a systematic series of experiments, published in 2022 (Le et al. 2022), in adult mice and rats of both sexes which had been subjected to the adolescent Δ^9-THC exposure protocol described above. The treatment did not alter basic

properties of synaptic transmission in hippocampus but strongly depressed—in male mice (not rats) and females of both species—a unique endocannabinoid-dependent form of LTP that occurs at terminals of the lateral perforant path (LPP) onto dentate gyrus granule cells (Wang et al. 2018). Disruption of plasticity was associated with impaired acquisition of a component of episodic memory that relies on LPP function. Adolescent Δ^9-THC treatment also attenuated LTP in field CA1 pyramidal neurons along with the associated ability to retain spatial information. Of note, the latter effect only occurred in female mice and rats, where this classical form of LTP also depends on locally produced estrogen. The results identify projection system-specific and sexually dimorphic consequences of adolescent Δ^9-THC exposure that could underlie discrete effects of early-life Δ^9-THC use on adult memory and cognition.

5 Effects of Adolescent Δ^9-THC Exposure on Systemic Metabolism and Adipose Organ Function

5.1 Cannabis and Energy Balance

Cannabis stimulates hedonic eating, the craving for sweet and fatty foods, commonly known in American pop culture as "the munchies." One would expect, therefore, that people who regularly use cannabis eat more and consequently have a higher body mass index (BMI) than the general population. However, the opposite appears to be the case. Cross-sectional and longitudinal studies, including the National Epidemiologic Survey on Alcohol and Related Conditions (Alshaarawy and Anthony 2019), have consistently linked cannabis use with lower BMI, waist circumference, and other cardiometabolic risk factors (Meier et al. 2016; Meier et al. 2019; Hayatbakhsh et al. 2010; Barre et al. 2022; Alshaarawy and Anthony 2019; Le Strat and Le Foll 2011; Maggirwar and Khalsa 2021). This finding suggests, paradoxically, that consumption of cannabis during adolescence leads to a lasting improvement in adult metabolic health.

Several observations support this possibility. First, habitual cannabis use often begins in adolescence. For instance, in a cohort of adults who successfully recovered from cannabis use disorder (a medical condition characterized by a problematic pattern of cannabis use leading to clinically significant impairments) (Yap et al. 2015), continuous drug use started between the ages of 15 and 17 (Kelly et al. 2017). This suggests that the metabolic profile of adults who regularly use cannabis may, at least in part, reflect lasting molecular changes initiated by aberrant cannabinoid receptor activation during their teenage years. Second, all components of the ECS are present in white and brown adipose tissues—the parenchymal components of the adipose organ (Cinti 2022)—where endocannabinoid substances may serve as "thrifty" autocrine or paracrine signals to boost lipogenesis and reduce non-shivering thermogenesis (DiPatrizio and Piomelli 2012). Lastly, preclinical and clinical studies have demonstrated significant anti-obesity effects of CB_1 inverse

agonists and neutral antagonists, either acting globally or peripherally. These agents are effective primarily because they enhance lipolysis, increase energy expenditure, and boost fatty acid oxidation by counteracting the energy-conserving signals of the ECS in adipose, liver, and other organs (for review, see Quarta and Cota 2020).

5.2 *Effects of Adolescent Δ⁹-THC Exposure on Systemic Metabolism*

A report published by our lab in 2024 demonstrated that Δ^9-THC exposure during adolescence causes sweeping changes in adult energy metabolism (Lin et al. 2023). The study was prompted by the unexpected observation that adolescent male and female mice treated with Δ^9-THC gained significantly less weight than control mice treated with only vehicle. This effect could not be attributed to changes in growth rate, food intake, locomotor activity, or alterations in the gut microbiome. It could be entirely prevented, however, by co-administering Δ^9-THC with a global CB_1 inverse agonist or a peripherally restricted CB_1 neutral antagonist. Additionally, Δ^9-THC administration failed to reduce weight gain in adolescent mice in which the CB_1 receptor had been deleted postnatally in adipocytes. Lastly, and significantly, the same Δ^9-THC regimen that reduced weight gain during adolescence was ineffective when tested on adult mice. The findings indicate that daily exposure to a moderately psychoactive dose of Δ^9-THC decreases body weight gain in adolescent mice through a developmentally regulated mechanism that requires CB_1 receptor activation in differentiated adipocytes.

Metabolic analyses conducted after the mice had reached young adulthood revealed persistent alterations in their metabolic profile. In addition to reduced fat mass and increased lean mass, these alterations included attenuation of respiratory exchange ratio (RER), indicating a greater reliance on fat for energy. Mice that had been exposed to Δ^9-THC also exhibited lower energy expenditure, partial resistance to diet-induced obesity and dyslipidemia, enhanced thermogenesis, and impaired lipolysis in response to cold or β-adrenergic receptor stimulation.

A combination of transcriptomics, proteomics, and metabolomics analyses revealed numerous molecular changes in the adipose organ of Δ^9-THC-treated mice, which were likely implicated in the development of this complex metabolic phenotype. Arguably, the most striking of these changes was the ectopic expression of sarcomere proteins—such as titin, troponin, and myosin—which are normally restricted to muscle. In brown adipose tissue, this anomalous expression was associated with metabolomics alterations (e.g., elevated concentrations of essential and nonessential amino acids, decreased NADPH/$NADP^+$ ratio) that were indicative of enhanced anabolic processing. The findings indicate that exposure to Δ^9-THC during adolescence, but not adulthood, induces an abnormal "pseudo-lean" state—one that outwardly appears similar to healthy leanness but is actually linked to long-term deficits in two key functions served by the adipose organ: thermogenesis and lipolysis. In addition to affecting general health, these deficits could compromise

brain energetics during prolonged physical activity, stress, and other conditions—including breastfeeding, diabetes, and alcohol use disorder—where this organ relies on the release of fatty acids from adipose tissue and their conversion to ketone bodies in the liver (Lopez-Ojeda and Hurley 2023; Wiers et al. 2021).

6 Summary and Conclusions

While no single experimental model can fully capture the diversity of human cannabis use, specific aspects of this phenomenon can be replicated by thoughtfully incorporating real-world data into a series of complementary focused models. Adopting this approach, preclinical researchers are now evaluating treatment regimens that explore varying use intensities, age and duration of use, routes of administration, and cannabis compositions. They are also, to the extent possible, establishing systematic correlations between the pharmacological actions of Δ^9-THC and its concentrations in functionally relevant biophases, such as the brain and adipose. This multifaceted strategy inevitably yields protocol-specific results but their variation, rather than reflecting experimental inconsistency, is crucial for understanding the complex impact of cannabis on human health.

The studies reviewed in this chapter serve as a prime example as they underscore multiple differences in the pharmacological properties of Δ^9-THC across mice versus rats, adolescents versus adults, and females versus males, which correlate with distinct neurocognitive and behavioral outcomes. They also demonstrate that different intensities of adolescent exposure to Δ^9-THC can influence key physiological processes—such as brain reactivity to internal and external danger signals—in opposing ways. Additionally, the studies reveal unexpected effects of adolescent Δ^9-THC exposure on the function of peripheral organs such as the adipose, and thus offer new insights into the potential health risks associated with cannabis use. Integrating the causal and mechanistic knowledge generated from this research with the datasets issuing from large-scale human projects such as the ABCD study® (Adolescent Brain Cognitive Development) is, despite its challenges, critical to advance our understanding of the consequences of early-life cannabis use.

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References

Albaugh MD, et al. Association of cannabis use during adolescence with neurodevelopment. JAMA Psychiatry. 2021;78(9):1–11.

Alshaarawy O, Anthony JC. Are cannabis users less likely to gain weight? Results from a national 3-year prospective study. Int J Epidemiol. 2019;48(5):1695–700.

Balogh KN, Mayes LC, Potenza MN. Risk-taking and decision-making in youth: relationships to addiction vulnerability. J Behav Addict. 2013;2(1):1.

Barre T, et al. Cannabis use is inversely associated with overweight and obesity in hepatitis B virus-infected patients (ANRS CO22 Hepather cohort). Cannabis Cannabinoid Res. 2022;7(5):677–89.
Bartok E, Hartmann G. Immune sensing mechanisms that discriminate self from altered self and foreign nucleic acids. Immunity. 2020;53(1):54–77.
Blakemore SJ. Imaging brain development: the adolescent brain. Neuroimage. 2012a;61(2):397–406.
Blakemore SJ. Development of the social brain in adolescence. J R Soc Med. 2012b;105(3):111–6.
Blakemore SJ, Robbins TW. Decision-making in the adolescent brain. Nat Neurosci. 2012;15(9):1184–91.
Blest-Hopley G, et al. Is the adolescent brain at greater vulnerability to the effects of cannabis? A narrative review of the evidence. Front Psych. 2020;11:859.
Borst K, Dumas AA, Prinz M. Microglia: immune and non-immune functions. Immunity. 2021;54(10):2194–208.
Brust V, Schindler PM, Lewejohann L. Lifetime development of behavioural phenotype in the house mouse (*Mus musculus*). Front Zool. 2015;12 Suppl 1(Suppl 1):S17.
Casey BJ, Glatt CE, Lee FS. Treating the developing versus developed brain: translating preclinical mouse and human studies. Neuron. 2015;86(6):1358–68.
Chan GCK, et al. User characteristics and effect profile of butane hash oil: an extremely high-potency cannabis concentrate. Drug Alcohol Depend. 2017;178:32–8.
Cinti S. The endocrine adipose organ. Rev Endocr Metab Disord. 2022;23(1):1–4.
Cooper ZD, Haney M. Actions of delta-9-tetrahydrocannabinol in cannabis: relation to use, abuse, dependence. Int Rev Psychiatry. 2009;21(2):104–12.
Crone EA, van Duijvenvoorde ACK. Multiple pathways of risk taking in adolescence. Dev Rev. 2021;62:100996.
Cunningham C, et al. The sickness behaviour and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (poly I:C). Brain Behav Immun. 2007;21(4):490–502.
Cutando L, et al. Microglial activation underlies cerebellar deficits produced by repeated cannabis exposure. J Clin Invest. 2013;123(7):2816–31.
da Silva MCM, et al. Role of microglia in psychostimulant addiction. Curr Neuropharmacol. 2023;21(2):235–59.
Deczkowska A, et al. Disease-associated microglia: a universal immune sensor of neurodegeneration. Cell. 2018;173(5):1073–81.
Dede AJ, et al. Learning and remembering real-world events after medial temporal lobe damage. Proc Natl Acad Sci U S A. 2016;113(47):13480–5.
Dickerson BC, Eichenbaum H. The episodic memory system: neurocircuitry and disorders. Neuropsychopharmacology. 2010;35(1):86–104.
DiPatrizio NV, Piomelli D. The thrifty lipids: endocannabinoids and the neural control of energy conservation. Trends Neurosci. 2012;35(7):403–11.
English A, et al. A preclinical model of THC edibles that produces high-dose cannabimimetic responses. Elife. 2024;12:12.
Faust TE, Gunner G, Schafer DP. Mechanisms governing activity-dependent synaptic pruning in the developing mammalian CNS. Nat Rev Neurosci. 2021;22(11):657–73.
Freels TG, et al. Sex differences in adolescent cannabis vapor self-administration mediate enduring effects on behavioral flexibility and prefrontal microglia activation in rats. bioRxiv; 2023.
Freels TG, et al. Sex differences in response-contingent cannabis vapor administration during adolescence mediate enduring effects on behavioral flexibility and prefrontal microglia activation in rats. Cannabis Cannabinoid Res. 2024;9(4):e1184–96.
Freeman TP, et al. Changes in delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) concentrations in cannabis over time: systematic review and meta-analysis. Addiction. 2021;116(5):1000–10.
Fride E, et al. Behavioral methods in cannabinoid research. Methods Mol Med. 2006;123:269–90.

Gabaglio M, et al. Long-term consequences of adolescent exposure to THC-rich/CBD-poor and CBD-rich/THC-poor combinations: a comparison with pure THC treatment in female rats. Int J Mol Sci. 2021;22(16):8899.
Golden SA, et al. A standardized protocol for repeated social defeat stress in mice. Nat Protoc. 2011;6(8):1183–91.
Green JM, Sundman MH, Chou YH. Opioid-induced microglia reactivity modulates opioid reward, analgesia, and behavior. Neurosci Biobehav Rev. 2022;135:104544.
Gritti D, et al. Neuroinflammation in major depressive disorder: a review of PET imaging studies examining the 18-kDa translocator protein. J Affect Disord. 2021;292:642–51.
Gunasekaran N, et al. Reintoxication: the release of fat-stored delta(9)-tetrahydrocannabinol (THC) into blood is enhanced by food deprivation or ACTH exposure. Br J Pharmacol. 2009;158(5):1330–7.
Hanus LO, et al. Phytocannabinoids: a unified critical inventory. Nat Prod Rep. 2016;33(12):1357–92.
Harkany T, Cinquina V. Physiological rules of endocannabinoid action during fetal and neonatal brain development. Cannabis Cannabinoid Res. 2021;6(5):381–8.
Hayatbakhsh MR, et al. Cannabis use and obesity and young adults. Am J Drug Alcohol Abuse. 2010;36(6):350–6.
Hickman SE, et al. The microglial sensome revealed by direct RNA sequencing. Nat Neurosci. 2013;16(12):1896–905.
Huestis MA, Cone EJ. Relationship of Delta 9-tetrahydrocannabinol concentrations in oral fluid and plasma after controlled administration of smoked cannabis. J Anal Toxicol. 2004;28(6):394–9.
Izquierdo-Luengo C, et al. Adolescent exposure to the spice/K2 cannabinoid JWH-018 impairs sensorimotor gating and alters cortical perineuronal nets in a sex-dependent manner. Transl Psychiatry. 2023;13(1):176.
Jung KM, Lin L, Piomelli D. The endocannabinoid system in the adipose organ. Rev Endocr Metab Disord. 2022;23(1):51–60.
Kelleghan AR, et al. Associations of cannabis product source and subsequent cannabis use among adolescents. Drug Alcohol Depend. 2022;233:109374.
Kelly JF, et al. Prevalence and pathways of recovery from drug and alcohol problems in the United States population: implications for practice, research, and policy. Drug Alcohol Depend. 2017;181:162–9.
Kinsey SG, et al. Repeated social defeat causes increased anxiety-like behavior and alters splenocyte function in C57BL/6 and CD-1 mice. Brain Behav Immun. 2007;21(4):458–66.
Kirkham TC. Endocannabinoids in the regulation of appetite and body weight. Behav Pharmacol. 2005;16(5–6):297–313.
Kloft L, et al. False memory formation in cannabis users: a field study. Psychopharmacology (Berl). 2019;236(12):3439–50.
Kloft L, et al. Cannabis increases susceptibility to false memory. Proc Natl Acad Sci U S A. 2020;117(9):4585–9.
Kreuz DS, Axelrod J. Delta-9-tetrahydrocannabinol: localization in body fat. Science. 1973;179(4071):391–3.
Krishnan V, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell. 2007;131(2):391–404.
Laprairie RB, Kelly ME, Denovan-Wright EM. The dynamic nature of type 1 cannabinoid receptor (CB(1)) gene transcription. Br J Pharmacol. 2012;167(8):1583–95.
Lawn W, et al. The CannTeen study: verbal episodic memory, spatial working memory, and response inhibition in adolescent and adult cannabis users and age-matched controls. Psychopharmacology (Berl). 2022;239(5):1629–41.
Le Strat Y, Le Foll B. Obesity and cannabis use: results from 2 representative national surveys. Am J Epidemiol. 2011;174(8):929–33.
Le AA, et al. Persistent sexually dimorphic effects of adolescent THC exposure on hippocampal synaptic plasticity and episodic memory in rodents. Neurobiol Dis. 2022;162:105565.

Lee HL, et al. Frequent low-Dose Delta(9)-tetrahydrocannabinol in adolescence disrupts microglia homeostasis and disables responses to microbial infection and social stress in young adulthood. Biol Psychiatry. 2022;92(11):845–60.
Lee HL, et al. Frequent low-impact exposure to THC during adolescence causes persistent sexually dimorphic alterations in the response to viral infection in mice. Pharmacol Res. 2024;199:107049.
Lin L, et al. Adolescent exposure to low-dose THC disrupts energy balance and adipose organ homeostasis in adulthood. Cell Metab. 2023;35(7):1227–1241 e7.
Lopez-Ojeda W, Hurley RA. Ketone bodies and brain metabolism: new insights and perspectives for neurological diseases. J Neuropsychiatry Clin Neurosci. 2023;35(2):104–9.
Maccarrone M, et al. Programming of neural cells by (endo)cannabinoids: from physiological rules to emerging therapies. Nat Rev Neurosci. 2014;15(12):786–801.
Maggirwar SB, Khalsa JH. The link between cannabis use, immune system, and viral infections. Viruses. 2021;13(6):1099.
Marinelli S, et al. Endocannabinoid signaling in microglia. Glia. 2023;71(1):71–90.
Masuda T, et al. Microglia heterogeneity in the single-cell era. Cell Rep. 2020;30(5):1271–81.
Meier MH. Associations between butane hash oil use and cannabis-related problems. Drug Alcohol Depend. 2017;179:25–31.
Meier MH, et al. Associations between cannabis use and physical health problems in early midlife: a longitudinal comparison of persistent cannabis vs tobacco users. JAMA Psychiatry. 2016;73(7):731–40.
Meier MH, et al. Associations between cannabis use and cardiometabolic risk factors: a longitudinal study of men. Psychosom Med. 2019;81(3):281–8.
Miech RA, Johnston LD, Patrick ME, O'Malley PM, Bachman JG. Monitoring the future national survey results on drug use, 1975–2023: secondary school students. In: Monitoring the future monograph series. Ann Arbor, MI: Institute for Social Research, University of Michigan; 2023.
Mohammadpanah M, et al. Exposure to Delta9-tetrahydrocannabinol leads to a rise in caspase-3, morphological changes in microglial, and astrocyte reactivity in the cerebellum of rats. Toxicol Res (Camb). 2023;12(6):1077–94.
Mondelli V, et al. Brain microglia in psychiatric disorders. Lancet Psychiatry. 2017;4(7):563–72.
Pandey R, et al. Endocannabinoids and immune regulation. Pharmacol Res. 2009;60(2):85–92.
Piomelli D. The molecular logic of endocannabinoid signalling. Nat Rev Neurosci. 2003;4(11):873–84.
Piomelli D. More surprises lying ahead. The endocannabinoids keep us guessing. Neuropharmacology. 2014;76(Pt B):228–34.
Piomelli D, Mabou Tagne A. Endocannabinoid-based therapies. Annu Rev Pharmacol Toxicol. 2022;62:483–507.
Powell ND, et al. Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via beta-adrenergic induction of myelopoiesis. Proc Natl Acad Sci U S A. 2013;110(41):16574–9.
Quarta C, Cota D. Anti-obesity therapy with peripheral CB1 blockers: from promise to safe(?) practice. Int J Obes (Lond). 2020;44(11):2179–93.
Ramirez K, Fornaguera-Trias J, Sheridan JF. Stress-induced microglia activation and monocyte trafficking to the brain underlie the development of anxiety and depression. Curr Top Behav Neurosci. 2017;31:155–72.
Rodrigues-Neves AC, Ambrosio AF, Gomes CA. Microglia sequelae: brain signature of innate immunity in schizophrenia. Transl Psychiatry. 2022;12(1):493.
Rogeberg O. Correlations between cannabis use and IQ change in the Dunedin cohort are consistent with confounding from socioeconomic status. Proc Natl Acad Sci U S A. 2013;110(11):4251–4.
Rubino T, Parolaro D. Sexually dimorphic effects of cannabinoid compounds on emotion and cognition. Front Behav Neurosci. 2011;5:64.

Rubino T, et al. Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. Hippocampus. 2009;19(8):763–72.
Rubino T, et al. Adolescent exposure to THC in female rats disrupts developmental changes in the prefrontal cortex. Neurobiol Dis. 2015;73:60–9.
Ruiz CM, et al. Pharmacokinetic and pharmacodynamic properties of aerosolized ("vaped") THC in adolescent male and female rats. Psychopharmacology (Berl). 2021a;238(12):3595–605.
Ruiz CM, et al. Pharmacokinetic, behavioral, and brain activity effects of Delta(9)-tetrahydrocannabinol in adolescent male and female rats. Neuropsychopharmacology. 2021b;46(5):959–69.
Schaefer JD, et al. Associations between adolescent cannabis use and young-adult functioning in three longitudinal twin studies. Proc Natl Acad Sci U S A. 2021a;118(14):e2013180118.
Schaefer JD, et al. Adolescent cannabis use and adult psychoticism: a longitudinal co-twin control analysis using data from two cohorts. J Abnorm Psychol. 2021b;130(7):691–701.
Schaefer JD, Nelson KM, Wilson S. The effects of adolescent cannabis use on psychosocial functioning: a critical review of the evidence. Psychiatr Clin North Am. 2023;46(4):677–89.
Schoch H, et al. Adolescent cannabinoid exposure effects on natural reward seeking and learning in rats. Psychopharmacology (Berl). 2018;235(1):121–34.
Schramm E, Waisman A. Microglia as central protagonists in the chronic stress response. Neurol Neuroimmunol Neuroinflamm. 2022;9(6):e200023.
Schweinsburg AD, Brown SA, Tapert SF. The influence of marijuana use on neurocognitive functioning in adolescents. Curr Drug Abuse Rev. 2008;1(1):99–111.
Sharma DS, et al. Endocannabinoid system: role in blood cell development, neuroimmune interactions and associated disorders. J Neuroimmunol. 2021;353:577501.
Sisk CL, Romeo RD, O. University Press Scholarship. Coming of age : the neurobiology and psychobiology of puberty and adolescence. New York, NY: Oxford University Press; 2020. Oxford scholarship online.
Smith MJ, et al. Cannabis-related episodic memory deficits and hippocampal morphological differences in healthy individuals and schizophrenia subjects. Hippocampus. 2015;25(9):1042–51.
Soares AR, Picciotto MR. Nicotinic regulation of microglia: potential contributions to addiction. J Neural Transm (Vienna). 2024;131(5):425–35.
Stella N. Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. Glia. 2010;58(9):1017–30.
Straube B. An overview of the neuro-cognitive processes involved in the encoding, consolidation, and retrieval of true and false memories. Behav Brain Funct. 2012;8:35.
Torrens A, et al. Comparative pharmacokinetics of Delta(9)-tetrahydrocannabinol in adolescent and adult male mice. J Pharmacol Exp Ther. 2020;374(1):151–60.
Torrens A, et al. Comparative pharmacokinetics of Delta(9)-tetrahydrocannabinol in adolescent and adult male and female rats. Cannabis Cannabinoid Res. 2022;7(6):814–26.
Torrens A, et al. Nasal accumulation and metabolism of Delta(9)-tetrahydrocannabinol following aerosol ('vaping') administration in an adolescent rat model. Pharmacol Res. 2023;187:106600.
Torrens A, et al. Comparative pharmacokinetics of Delta9-tetrahydrocannabinol in adolescent and adult female mice. Cannabis Cannabinoid Res. 2024;9:1537.
Tulving E. Episodic memory: from mind to brain. Annu Rev Psychol. 2002;53:1–25.
Vozella V, et al. Fast and sensitive quantification of Delta(9)-tetrahydrocannabinol and its main oxidative metabolites by liquid chromatography/tandem mass spectrometry. Cannabis Cannabinoid Res. 2019;4(2):110–23.
Wan X, et al. Gut-microbiota-brain axis in the vulnerability to psychosis in adulthood after repeated cannabis exposure during adolescence. Eur Arch Psychiatry Clin Neurosci. 2022;272(7):1297–309.
Wang W, et al. Atypical endocannabinoid signaling initiates a new form of memory-related plasticity at a cortical input to hippocampus. Cereb Cortex. 2018;28(7):2253–66.

Wang H, et al. Microglia in depression: an overview of microglia in the pathogenesis and treatment of depression. J Neuroinflammation. 2022;19(1):132.
Wenger T, Moldrich G. The role of endocannabinoids in the hypothalamic regulation of visceral function. Prostaglandins Leukot Essent Fatty Acids. 2002;66(2–3):301–7.
Wenger T, et al. The hypothalamic levels of the endocannabinoid, anandamide, peak immediately before the onset of puberty in female rats. Life Sci. 2002;70(12):1407–14.
Wiers CE, et al. Ketogenic diet reduces alcohol withdrawal symptoms in humans and alcohol intake in rodents. Sci Adv. 2021;7(15):eabf6780.
Wohleb ES, et al. Stress-induced recruitment of bone marrow-derived monocytes to the brain promotes anxiety-like behavior. J Neurosci. 2013;33(34):13820–33.
Yap M, et al. Use of cannabis in severe childhood epilepsy and child protection considerations. J Paediatr Child Health. 2015;51(5):491–6.
Zamarripa CA, et al. Assessment of orally administered Delta9-tetrahydrocannabinol when coadministered with Cannabidiol on Delta9-tetrahydrocannabinol pharmacokinetics and pharmacodynamics in healthy adults: a randomized clinical trial. JAMA Netw Open. 2023;6(2):e2254752.
Zamberletti E, et al. Alterations of prefrontal cortex GABAergic transmission in the complex psychotic-like phenotype induced by adolescent delta-9-tetrahydrocannabinol exposure in rats. Neurobiol Dis. 2014;63:35–47.
Zamberletti E, et al. Cortical neuroinflammation contributes to long-term cognitive dysfunctions following adolescent delta-9-tetrahydrocannabinol treatment in female rats. Eur Neuropsychopharmacol. 2015;25(12):2404–15.
Zellers S, et al. Limited psychological and social effects of lifetime cannabis use frequency: evidence from a 30-year community study of 4,078 twins. J Psychopathol Clin Sci. 2024;133(1):115–28.

Chapter 12
Cognitive Effects of Adolescent and Young Adult Regular Cannabis Use



Krista M. Lisdahl

1 Introduction

Cannabis continues to be the second most widely used psychoactive substance among adolescents, with around 29% of high school seniors using in the past year (Miech et al. 2023). Cannabis contains numerous cannabinoids, including delta-9-tetrahydrocannabinol (delta-9-THC) and cannabidiol (CBD), that interact with the endogenous endocannabinoid system (eCB) (see Chaps. 1–5) (Di Marzo et al. 2005; Eggan and Lewis 2007; Herkenham et al. 1990). The eCB system includes the cannabinoid receptor 1 (CB1R), CB2R, and two endogenous ligands (N-arachidonoylethanolamine or anandamide and 2-arachidonoylglycerol or 2-AG), enzymes related to their biosynthesis and degradation, and related lipid mediators that share biochemical properties (Di Marzo et al. 2005; Eggan and Lewis 2007; Herkenham et al. 1990) (see Chap. 3). Critical for cognitive development, the eCB system plays a role in cell differentiation, neurogenesis, myelination of axons, activity-dependent refinement of neuronal connections, analgesia, and anti-inflammatory processes (Diaz-Alonso et al. 2012; Katona and Freund 2008; Lee and Gorzalka 2012; Mackie 2005; Benarroch 2014; Kano et al. 2009; Manterola et al. 2018). As outlined in Chaps. 3–5, animal and human research has shown high density of CB1R in the prefrontal cortex (PFC), parietal cortex, cerebellum, limbic system, and ventral striatum and CB1R expression is highest during adolescence (Eggan and Lewis 2007; Heng et al. 2011; Herkenham et al. 1990; Viveros et al. 2007); thus, the eCB system is thought to play a vital role during adolescent neurodevelopment (Oltrabella et al. 2017).

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Animal models show that during adolescence, anandamide and 2-AG significantly increase and CB1R expression peaks and these changes may underlie fine tuning of functional coupling, dendritic complexity, pruning of weak connections (especially in frontal and parietal regions), and improved white matter integrity by modulating oligodendrocytes (Bernal-Chico et al. 2023; Ellgren et al. 2008; Heng et al. 2011; Mato et al. 2003; Terry et al. 2009). Consistent with these structural findings, combined preclinical and adult evidence suggests that eCB system signaling modulates executive functioning (e.g., cognitive control, working memory, planning), reward response, and affective processing (Hillard 2018; Oltrabella et al. 2017; Volkow et al. 2017). Thus, the eCB system plays a significant role in the dynamic changes that occur during the critical developmental period of adolescence (Brown et al. 2012; Casey et al. 2008; Gee et al. 2016; Hare et al. 2008; Mills et al. 2014a, b; Pfefferbaum et al. 2016; Raznahan et al. 2014; Schmitt et al. 2014; Sisk et al. 2022; Squeglia et al. 2013), which is also characterized by a normative increase in risk-taking behaviors including the initiation of cannabis use (Lisdahl et al. 2018; Sullivan et al. 2022b).

The potential mechanisms underlying cannabis neurocognitive effects are likely multifactorial. Animal findings have found that repeated THC exposure is linked with alterations in white matter integrity, functional coupling, gene expression, glucose uptake, microglial apoptosis, altered dopamine, glutamate and eCB signaling, and reduced neuroplasticity in the hippocampus and PFC (Coleman et al. 2022; Zuo et al. 2022c; Miederer et al. 2017; Hasegawa et al. 2023; Ginder et al. 2022; Poulia et al. 2021; Stringfield and Torregrossa 2021). On balance, preclinical studies demonstrated that repeated exposure to THC during adolescence is linked with associative memory and executive functioning deficits, with more mixed findings in attentional set-shifting, spatial working memory, and object memory; findings generally suggest that adolescent exposure is more detrimental than adult exposure (see Zamberletti and Rubino 2022 and Chap. 11 for review), although some studies utilizing more ecologically valid cannabis dosing (e.g., smoked cannabis) have not found cognitive deficits associated with adolescent administration (Hernandez et al. 2021). Human research has also shown that repeated exposure to exogenous cannabinoids during adolescent development—particularly delta-9-THC, the principal psychoactive compound responsible for the euphoric and rewarding effects of cannabis—may disrupt or downregulate the eCB system (Hirvonen et al. 2012; Spindle et al. 2021; Sloan et al. 2019; Martin et al. 2023; Morgan et al. 2013; Thieme et al. 2014), alter glutamate signaling and synaptic refinement (Chowdhury et al. 2024; Zuo et al. 2022a, b; Zuo and Lukas 2023), and cause epigenetic changes in genes related to cellular survival and neurodevelopment (Machado et al. 2024). Collectively, these alterations may lead to brain structure, function and subtle downstream cognitive changes during the vulnerable period of adolescent brain development (see Lisdahl et al. 2013, 2014, 2021a; Sagar and Gruber 2018; Fischer et al. 2020; Murray et al. 2022; Zehra et al. 2018).

1.1 Overview: Impact of Regular Cannabis Use on Cognition in Adolescents and Young Adults

Historically, the effects of repeated cannabis use on cognitive functions in youth have been predominantly examined through cross-sectional comparisons. Although some studies have found no cognitive differences (Takagi et al. 2011), overall, the existing body of research has generally observed subtly poorer performance in regular cannabis users compared to controls, particularly in cognitive domains such as memory, processing speed, complex attention, and executive function (for reviews see (Lisdahl et al. 2014; Sagar and Gruber 2018; Bourque and Potvin 2021; Gonzalez et al. 2017; Lees et al. 2021; Scott 2023; Blest-Hopley et al. 2020; Ellingson et al. 2021a; Zehra et al. 2018)). Several meta-analyses examining combined cohorts have now been conducted, primarily pulling from cross-sectional studies. One of the first of these meta-analyses found a small effect size for cannabis effects on learning and memory, but minimal findings for other cognitive domains (Grant et al. 2003). Follow-up meta-analyses were relatively consistent in reporting the largest observed effects for learning and memory, followed by processing speed, complex attention, and executive functioning (Schreiner and Dunn 2012; Scott et al. 2018). In a novel meta-analysis focused specifically on the prospective *longitudinal* impact of cannabis on IQ, the authors concluded that frequent or dependent cannabis use was linked with an average decline of two IQ points following adolescent cannabis exposure (Power et al. 2021). More recent meta-analyses generally agreed with prior findings and reported that adolescent and young adult regular cannabis users demonstrated the most consistent reductions in learning and memory (small to moderate effects), as well as small deficits in processing speed, complex attention, and executive function (inhibitory control, working memory, decision-making), (Dellazizzo et al. 2022; Duperrouzel et al. 2020; Lovell et al. 2020).

While adolescent and young adult cross-sectional studies have often demonstrated associations between cannabis use and reduced cognitive performance, the temporal ordering of these effects remains uncertain. Longitudinal research designs are considered the gold standard for evaluating the sequential impact of cannabis on cognition and developing evidence for causation. Gonzalez et al. (2017) reviewed seven longitudinal studies and generally concluded that cannabis use during adolescence leads to modest neuropsychological decline over time, especially in IQ and verbal learning and memory. In a follow-up review focused on the longitudinal predictors of and impact of regular cannabis use, Debenham et al. (2021) concluded that regular cannabis use results in learning and memory, attention, and planning declines, cannabis consequences are dose-dependent, and may remain relatively stable for up to 3–4 year follow-up periods (Debenham et al. 2021). Thus, longitudinal research has suggested that the persistence of heavy cannabis use during late adolescence is associated with greater cognitive decline, especially in the domains of IQ, learning and short-term memory, processing speed, and executive function (i.e., inhibitory control, working memory, planning) after controlling for baseline function and comorbid substance use (Infante et al. 2020; Jacobus et al. 2015;

Becker et al. 2018; Camchong et al. 2017; Gonzalez et al. 2017), even after 4 weeks of abstinence (Jacobus et al. 2015). Notably, the vast majority of both cross-sectional and longitudinal studies combined users into a singular group, although reviews have pointed out that effect sizes are largest in the heaviest users and some studies have demonstrated dose-dependent decline (Camchong et al. 2017; Gonzalez et al. 2017; Infante et al. 2020; Becker et al. 2018; Lees et al. 2021; Debenham et al. 2021).

Still, one major criticism of the majority of longitudinal studies to date is that they may not have fully accounted for potential premorbid factors that *predict* cannabis use initiation (Debenham et al. 2021). Large-scale, prospective, longitudinal studies that measured neurocognitive and psychosocial phenotyping prior to the onset of cannabis experimentation are relatively rare. One of the first studies to prospectively follow youth from childhood into young adulthood found that after controlling for pre-cannabis initiation factors, adolescent and young adult current cannabis users had lower IQ, immediate and delayed memory, and psychomotor processing speed (Fried et al. 2005). Those who were considered former users (abstinent greater than 3 months) no longer demonstrated decrements, suggesting preliminary evidence of recovery of function. This is consistent with Meier and colleagues (Meier et al. 2012) who found that persistent cannabis dependence during ages 13 to 38 was linked with reduced trajectories in IQ, complex attention, verbal learning, psychomotor speed, and executive functioning. The largest decrements in IQ were observed in adolescent cannabis use onset, although the sample size of this subgroup at follow-up was relatively small. In addition, a potential weakness of the initial analyses is that childhood socioeconomic status (SES) was not fully considered, although follow-up analyses ruled out the influence of SES (Moffitt et al. 2013). In a prospective longitudinal study in adolescent boys from ages 13 to 20, Castellanos-Ryan et al. (2017) found that the relationships between cognition and cannabis use were bidirectional; poorer short-term and working memory and high verbal IQ were linked with early onset cannabis use (Castellanos-Ryan et al. 2017). In turn, increased cannabis use predicted decline in verbal IQ, learning, and perseverative reward responding. However, the decline in verbal IQ was mediated by low-educational achievement, calling causality into question. Pardini et al. (2015) found that cannabis use from ages 11 to 16 was not linked with academic function after controlling for potential confounds (i.e., other substance use, peer group delinquency); however, cannabis use prospectively predicted parent-reported attention problems in girls. Similarly, Paige and Colder (2020) found that early cannabis use at ages 12–14 significantly predicted parent-reported adolescent attention control at ages 18–21, above and beyond premorbid attentional control, comorbid substance use, and antisocial symptoms (Paige and Colder 2020). Two additional longitudinal studies found that increased cannabis use was *prospectively* linked with poorer verbal learning and memory in teens (Ajmera et al. 2021; Barthelemy et al. 2019). Finally, a recent longitudinal study examining the impact of *light* cannabis exposure over an 8-year follow-up period found no declines in cognitive functioning, although cannabis users did have elevated conduct problems and inattention/hyperactivity

symptoms (Macedo et al. 2024). This finding suggests that relatively occasional adolescent cannabis use was not linked with cognitive decline.

Notably, several of the above prospective longitudinal studies generally started baseline assessments in the early adolescent years, after potential substance initiation and often neglected to fully account for environmental and genetic familial risk factors. More recent prospective *twin- or sibling*-informed longitudinal studies have suggested that observed differences in IQ between cannabis users and nonusers may be due to shared familial risk factors predicting onset of substance use (Jackson et al. 2016; Meier et al. 2018; Schaefer et al. 2021). In contrast, in a longitudinal family-controlled analysis, Ellingson et al. (2021b) found that earlier cannabis use onset and greater recent and lifetime use were linked with poorer verbal memory, after adjusting for familial factors (Ellingson et al. 2021b). In summary, overall the longitudinal research aligns with earlier cross-sectional findings, suggesting a prospective relationship between increased cannabis use and subtle declines in IQ, verbal memory, complex attention, processing speed, and facets of executive functioning. However, additional longitudinal studies utilizing twin or sibling designs are needed to fully account for potential familial and genetic factors that may confound the relationship between cannabis use and cognition. Such methodologies can provide more robust evidence of causality by ruling out preexisting differences that may predispose individuals to initiate cannabis use (Brumback et al. 2021; Tapert et al. 2002a; Weng et al. 2024; Boer et al. 2022; Castellanos-Ryan et al. 2017; Kardan et al. 2024; Squeglia et al. 2014; Wade et al. 2019a).

1.2 Cannabis and Cognition: Sex Differences

Another crucial factor to consider in examining the impact of cannabis on adolescent cognition is the potential for unique sex-based effects (Crane et al. 2013a). The developing adolescent brain exhibits significant sex-at-birth differences, with girls typically reaching structural maturity in limbic, striatal, and frontoparietal regions earlier than boys and demonstrating distinct patterns of functional connectivity maturation (Giedd et al. 2012; Lenroot and Giedd 2010; Raznahan et al. 2014; Zuo et al. 2010). On balance, preclinical studies have reported significant sex-differences in the eCB system, which plays a role in sexual dimorphism, including differences in CB1R sensitivity, hormone-linked fluctuations in eCB signaling, and cannabis-related effects such as gene expression, brain morphometry, and neuroinflammation (Burston et al. 2010; Farquhar et al. 2019; Laurikainen et al. 2019; Cooper and Craft 2018; Zuo et al. 2022c; Coleman et al. 2022; Ginder et al. 2022; Rubino et al. 2008, 2015; Zamberletti et al. 2015). In a narrative review, Ginder et al. (2022) found that adolescent cannabinoid administration in animals was associated with sex-dependent effects on the morphometry of the PFC and hippocampus, as well as on synaptic transmission and glial function (Ginder et al. 2022). In addition, they reported that cognitive effects, especially in working memory and object recognition, were more consistently found in male animals (Ginder et al. 2022).

The existing human literature on the cognitive effects of adolescent cannabis use has been predominantly male-centric, with many studies either having disproportionately male samples or failed to thoroughly investigate potential sex differences (Crane et al. 2013a). This is a significant limitation, as the neurodevelopmental impacts of cannabis may differentially influence male and female adolescents, potentially obscuring unique sex-based trajectories when combining these groups in the analysis. Indeed, several studies to date that have examined sex as a potential moderator have reported differential effects; male cannabis users have demonstrated increased vulnerability to differences in IQ (Schnakenberg Martin et al. 2021), processing speed (Lisdahl and Price 2012; Schnakenberg Martin et al. 2021), decision-making (Crane et al. 2013b), verbal learning (Schnakenberg Martin et al. 2021), and visual recognition (Savulich et al. 2021) effects. Female cannabis users demonstrate higher rates of comorbid mental and physical health problems (Brabete et al. 2020), have worse withdrawal symptoms (Schlienz et al. 2017), and cognitive studies have found increased vulnerability for cannabis-related deficits in verbal memory (Crane et al. 2013b), attention (Savulich et al. 2021), self-reported disinhibition symptoms (Wright et al. 2016), and executive functioning (Banks et al. 2022; Savulich et al. 2021). Collectively, these studies emphasize the necessity of recruiting representative samples of male and female cannabis users in research in order to more effectively elucidate potential sex-based disparities in neurocognitive outcomes.

1.3 Cannabis and Cognition: Findings by Cognitive Domain

1.3.1 IQ

Cross-sectional studies have linked lower IQ estimates with heavy cannabis use during adolescence (Wade et al. 2024b; Pope et al. 2003). Sex differences are somewhat conflicted, one study found female users to be more vulnerable (Crane et al. 2015) and another found male users were more vulnerable (Schnakenberg Martin et al. 2021) to IQ deficits. In a novel study examining unique cannabinoid predictors of cognition in young adolescents (13–14 years old), hair toxicology evidence of greater delta-9-THC exposure was linked with poorer IQ estimate in a dose-dependent manner in young adolescents (Wade et al. 2024b). Longitudinal studies have found that persistance of heavy cannabis use during adolescence (Camchong et al. 2017) and early onset cannabis use (Fried et al. 2005; Meier et al. 2012) predicted lower IQ, even after three months of abstinence (Fried et al. 2005); although some evidence suggests this may be partially due to comorbid nicotine use (Mokrysz et al. 2016). Meta-analytic evidence has reported that prospective, longitudinal studies have provided evidence of an average decline of two IQ points following adolescent cannabis exposure (Power et al. 2021). Still, prospective, *twin-informed* longitudinal studies have found that twin-discordant pairs do not demonstrate cannabis-related IQ decline (Jackson et al. 2016; Meier et al. 2018; Schaefer et al. 2021). Further, Castellanos-Ryan et al. (2017) found that the relationships between

cognition and cannabis use were bidirectional, *higher* verbal IQ was unexpectedly linked with earlier onset cannabis use (Castellanos-Ryan et al. 2017). These studies demonstrate that regular cannabis use is bidirectionally linked with IQ, though larger-scale, prospective twin-informed studies are needed to determine causality.

1.3.2 Memory

Verbal and visual memory abilities continue to develop throughout adolescence, as these cognitive functions are closely tied to the maturation of the prefrontal cortex and hippocampus. One of the most consistent cross-sectional findings is that adolescent and young adult regular cannabis users demonstrate reduced verbal learning and memory compared to matched nonusers (Crane et al. 2013b; Becker et al. 2014; Dougherty et al. 2013; Hanson et al. 2010; Winward et al. 2014; Harvey et al. 2007; McHale and Hunt 2008; Solowij et al. 2011; Tait et al. 2011), (Thoma et al. 2011; Winward et al. 2014; Lawn et al. 2022; Medina et al. 2007a; Wiedmann et al. 2022). Meta-analyses have reported that cannabis-related verbal memory decrements are in the small to medium effect size range (Grant et al. 2003; Scott et al. 2018; Schreiner and Dunn 2012; Duperrouzel et al. 2020; Dellazizzo et al. 2022). Visual memory decrements have also been reported, though less commonly, in relation to cannabis use (Wade et al. 2019a, 2024b; Selamoglu et al. 2021). Dose-dependent relationships have been reported between cannabis markers and verbal memory (Block et al. 2022; Wade et al. 2021a, 2024b; Medina et al. 2007a; Solowij et al. 2011; Thoma et al. 2011; Cuttler et al. 2023; Wittemann et al. 2021). Wade et al. (2021b) found that THC metabolite (11-nor-9-carboxy-Δ9-tetrahydrocannabinol or THCCOOH) concentration was significantly linked with poorer overall learning and delayed recall, while self-reported cannabis use was associated with poorer initial learning and short delay recall (Wade et al. 2021a). Sex differences have also been observed, although findings are mixed, with vulnerability seen in both female (Crane et al. 2015; Crane et al. 2013b) and male users (Hirst et al. 2021; Schnakenberg Martin et al. 2021). In contrast, Schnakenberg Martin et al. (2021) found that female cannabis users actually demonstrated better immediate memory performance compared to female nonusers, though their delayed recall did not significantly differ (Schnakenberg Martin et al. 2021). Continued cannabis use during adolescence has also been longitudinally linked with verbal learning and memory decrements (Jacobus et al. 2015; Tait et al. 2011), with particular risk in early onset users (Becker et al. 2018; Ellingson et al. 2021b; Willford et al. 2021). Finally, prospective longitudinal studies have revealed that cannabis use onset and escalation is prospectively linked with poorer verbal learning and memory (Ajmera et al. 2021; Barthelemy et al. 2019; Fried et al. 2005). In a large prospective study (Meier et al. 2012), after controlling for several potential confounding variables, persistent cannabis dependence was linked with poorer verbal list learning, although follow-up twin-informed analyses did not find differences (Meier et al. 2018). In contrast, in a longitudinal family-controlled analysis, Ellingson et al. (2021b) found that earlier cannabis use onset and greater recent and lifetime use were linked with poorer

verbal memory, after adjusting for shared familial factors (Ellingson et al. 2021b). Still, there is evidence that poorer short-term memory is linked with greater risk of early onset cannabis use (Castellanos-Ryan et al. 2017), so additional large-scale prospective studies are needed to provide causal evidence.

1.3.3 Complex Attention

Complex visuospatial attention continues to develop during adolescence and includes processes such as sustained attention, selective or divided attention, attentional control, and attentional lapses; these functions are primarily linked with dorsal and ventral attention networks that mature into early adulthood (Harris et al. 2022; Meehan et al. 2017). Meta-analyses (Scott et al. 2018; Dellazizzo et al. 2022; Schreiner and Dunn 2012) and cross-sectional studies have generally found a modest impact of repeated cannabis use on complex attention, including divided attention, sustained attention, and attentional control (Dougherty et al. 2013; Hanson et al. 2010; Winward et al. 2014; Harvey et al. 2007; Lisdahl and Price 2012; Mathias et al. 2011; Medina et al. 2007a; Bechtel et al. 2022; Fontes et al. 2011; McHale and Hunt 2008), and evidence suggests a dose-dependent relationship (Mathias et al. 2011; Lisdahl and Price 2012; Medina et al. 2007a; Thames et al. 2014). Longitudinal investigations have also linked combined cannabis and alcohol use during adolescence with decrements in complex attention (Jacobus et al. 2015; Tapert et al. 2002b). Paige and Colder (2020) found that early adolescent cannabis use was predicted increased parent-report of attentional control problems during late adolescence, above and beyond baseline attention, antisocial symptoms, and comorbid substance use. Similar findings were seen in female adolescent cannabis users (Pardini et al. 2015). Notably, Wallace et al. (2019) found that cannabis-linked attention deficits were not due to subclinical attention-deficient hyperactivity disorder symptoms (Wallace et al. 2019) and in an overlapping sample, two weeks of monitored cannabis abstinence was linked with improvements in sustained attention function (Wallace et al. 2020). One prospective study (Meier et al. 2012) found links with persistent adolescent cannabis dependence and poorer sustained attention, though this was not replicated in a twin-informed design (Meier et al. 2018) and prior work has demonstrated attention problems may precede substance use risk (Tapert et al. 2002a; Weng et al. 2024). Thus, prospective longitudinal evidence is needed to determine causality.

1.3.4 Psychomotor Speed

Psychomotor processing speed is linked with white matter maturation, which continues to develop during adolescence (Li et al. 2024). Meta-analyses (Dellazizzo et al. 2022; Schreiner and Dunn 2012) and cross-sectional adolescent and young adult studies have reported reduced processing speed in regular cannabis users

(Gruber et al. 2012; Medina et al. 2007a; Winward et al. 2014; Lisdahl and Price 2012; Thames et al. 2014; Wade et al. 2019b), and poorer function has been associated with greater cannabis exposure (Lisdahl and Price 2012; Medina et al. 2007a; Schnakenberg Martin et al. 2021; Wade et al. 2019b), co-use of cannabis and alcohol (Jacobus et al. 2015), and earlier age of cannabis use onset (Gruber et al. 2012; Jacobus et al. 2015). Preliminary findings have shown that male users may have increased vulnerability to cannabis-related psychomotor slowing (Lisdahl and Price 2012; Schnakenberg Martin et al. 2021). Longitudinal (Jacobus et al. 2015; Camchong et al. 2017) and prospective (Meier et al. 2012; Fried et al. 2005) evidence has demonstrated reduced processing speed with sustained cannabis during adolescence. However, to date there is no prospective, longitudinal evidence controlling for shared genetic or familial risk factors.

1.3.5 Executive Functioning

Executive functions, which are higher-order cognitive processes subserved by the prefrontal cortex, undergo substantial development during adolescence. These functions include planning, decision-making, working memory, cognitive control, and cognitive flexibility. Meta-analyses have found mixed findings regarding the impact of cannabis on executive function, but generally conclude modest impacts (Duperrouzel et al. 2020; Dellazizzo et al. 2022; Lovell et al. 2020; Schreiner and Dunn 2012; Scott et al. 2018). Cross-sectional evidence exists for regular adolescent and young adult cannabis use being linked with modest decrements in executive function domains such as planning and sequencing ability (Lisdahl and Price 2012; Becker et al. 2014; Grant et al. 2012; Medina et al. 2007a), working memory (Becker et al. 2014), (Winward et al. 2014; Wade et al. 2019b, 2024a, b; Selamoglu et al. 2021; Harvey et al. 2007), decision-making (Crane et al. 2013b; Dougherty et al. 2013; Becker et al. 2014; Casey and Cservenka 2020; Dougherty et al. 2013; Grant et al. 2012; Solowij et al. 2012; Dougherty et al. 2013; Mathias et al. 2011; Selamoglu et al. 2021; Fontes et al. 2011; Francis et al. 2023; Gonzalez et al. 2012; Hanson et al. 2010, 2014), cognitive control (Lisdahl and Price 2012; Dougherty et al. 2013; Cousijn et al. 2013; Moreno et al. 2012; Mathias et al. 2011; Fontes et al. 2011; Sagar et al. 2015; Wade et al. 2024a, b), impulsivity (Gruber et al. 2014; Wade et al. 2021b; Moreno et al. 2012; Solowij et al. 2012; Wright et al. 2016), and verbal fluency(McHale and Hunt 2008). Findings have supported dose-dependent and earlier age of onset effects (Wade et al. 2024a, b; Lisdahl and Price 2012; Medina et al. 2007a; Sagar et al. 2015; Gruber et al. 2012; Tamm et al. 2013; Hanson et al. 2014; Solowij et al. 2012; Pope et al. 2003; Fontes et al. 2011; Selamoglu et al. 2021; Jacobus et al. 2015; Dali et al. 2023). Sex differences have also emerged, with female cannabis users demonstrating greater behavioral symptoms of disinhibition (Wright et al. 2016), reduced response flexibility during a decision-making task (Banks et al. 2022), and greater impact on working memory over time (Noorbakhsh

et al. 2020). In a longitudinal study examining the impact of cannabis and alcohol on cognition over a three-year follow-up period, Jacobus et al. (2015) reported that earlier age of cannabis onset was linked with poorer performance on working memory and sequencing ability, although deficits in executive functioning were not predicted by group status. Becker et al. (2018) found that continued adolescent heavy cannabis use during a 2-year follow-up period was linked with reduced performance in working memory and planning, but not motivated decision-making, and earlier age of onset was linked with increased longitudinal decline in planning (Becker et al. 2018). Paige and Colder (2020) did not find that early adolescent cannabis use predicted parent-reported impulsivity in late adolescence. Prospective longitudinal data has found associations between persistent cannabis dependence and poorer executive functioning (Meier et al. 2012), and working memory deficits were greater in twin-matched cannabis using youth, though familial factors explained other executive function tasks (Meier et al. 2018; Ellingson et al. 2021b). In addition, executive functioning deficits may not be detectable in former users with at least three months of abstinence (Fried et al. 2005), suggesting potential recovery of function with abstinence. Finally, studies have found that poor executive functioning, impulsivity, and prefrontal function *predict* adolescent cannabis use onset (Boer et al. 2022; Green et al. 2024; Felton et al. 2015; Prince van Leeuwen et al. 2011). For example, one study demonstrated that poorer inhibitory functioning at ages 12–14 was predictive of increased cannabis use by later adolescence (Squeglia et al. 2014). Therefore, additional work is needed to clarify sex differences and confirm causal relationships between cannabis exposure and executive functioning in adolescents.

1.3.6 Visuospatial Function

Generally speaking, cross-sectional studies have not reported visuospatial deficits associated with cannabis exposure (Duperrouzel et al. 2020; Dellazizzo et al. 2022; Lovell et al. 2020; Schreiner and Dunn 2012; Scott et al. 2018). In a longitudinal analysis, cannabis use was linked with visuospatial functioning during a 3-year follow-up period (Jacobus et al. 2015), though this may be primarily due to comorbid alcohol exposure (Infante et al. 2020; Jacobus et al. 2015).

1.3.7 Language Function

Cross-sectional and longitudinal studies have not reported general language decrements in adolescent and young adult cannabis users (Duperrouzel et al. 2020; Dellazizzo et al. 2022; Lovell et al. 2020; Schreiner and Dunn 2012; Scott et al. 2018).

1.3.8 Affective and Socioemotional Skills

Potential impact of repeated cannabis use during adolescence on affective and socioemotional constructs such as empathy (Olalde-Mathieu et al. 2024), apathy or motivation (Aloi et al. 2018; Britton et al. 2024; Shollenbarger et al. 2015, 2019; Skumlien et al. 2023; Taylor and Filbey 2021), and affective processing skills (Wade et al. 2021b; Sullivan et al. 2022a; Spechler et al. 2015; Wesley et al. 2016) have only been rarely investigated, and findings to date have been mixed (Winters et al. 2021). Empathy is thought to be linked with increased understanding of drug-related consequences during adolescence; a recent meta-review found that across five studies, cross-sectionally there was a general negative association between adolescent substance use and empathy (Winters et al. 2021) and longitudinal studies found increases in empathy with decreases in substance use over time during adolescence (Winters et al. 2020, 2024). Few studies have specifically examined these constructs in adolescent cannabis users; Olalde-Mathieu et al. (2024) found that cannabis users demonstrated elevated emotional comprehension, an empathy trait, that was linked with greater anterior cingulate and left somatomotor cortex connectivity patterns.

Although increased apathy and reduced motivation in cannabis users has been described clinically for decades, research results regarding apathy, motivation or effort effects in cannabis users are mixed. Acute cannabis intoxication has been linked with decreased effort allocation during decision-making tasks (Lawn et al. 2016; Silveira et al. 2016) and effort mediated the relationship between cannabis use and verbal memory (Hirst et al. 2017). In contrast, Taylor and Filbey found that young adult cannabis users had *greater* effort allocation on a decision-making task compared to controls following 24 h of abstinence and did not report elevated symptoms of low motivation (Taylor and Filbey 2021). Similarly, Skumlien et al. (2023) found no differences in apathy, anhedonia or effortful decision-making in adolescent or adult cannabis users (Skumlien et al. 2023). Although cross-sectional links between adolescent cannabis use and greater disengagement, lower planning and lower valuing of school, longitudinal analyses did not reveal a significant prospective link between increased cannabis use and changes in motivation over time in teens (Pacheco-Colón et al. 2021). It is also possible that there are vulnerable subgroups within cannabis users that may be more vulnerable to affective processing consequences. Our group has previously reported increased current symptoms of depression and apathy were linked with poorer white matter volume and frontolimbic white matter integrity in regular adolescent cannabis users (Shollenbarger et al. 2015; Medina et al. 2007b), suggesting that there may be a subset of cannabis users with increased white matter damage that are at greater risk for apathy, depressive or anhedonic symptoms.

Even fewer studies have examined other socioemotional or affective processing skills in adolescent cannabis users. One of the first studies to address this question found that greater social cognitive distortions during early adolescence mediated the links between disinhibition and had greater odds of cannabis use by age 19 (Kirisci et al. 2004). In a prospective longitudinal design, Fluharty et al. (2018) found a bidirectional relationship between social cognition and cannabis use during

adolescence; poorer nonverbal communication skills were associated with lower risk of cannabis use, while escalation of adolescent cannabis use was associated with poorer social reciprocity (Fluharty et al. 2018). Adolescent and young adult cannabis users have demonstrated slower affective recognition (Wade et al. 2021b), which was correlated with smaller anterior cingulate brain volume (Maple et al. 2019) and is consistent with prior research demonstrating abnormal brain response to facial affective cues in adolescent and young adult cannabis users (Sullivan et al. 2022a; Gruber et al. 2009; Spechler et al. 2015, 2020; Wesley et al. 2016). In a longitudinal study, Heitzeg et al. (2015) found that increased cannabis use during adolescence was associated with increased negative emotionality and decreased resiliency over time, and this was linked with neuronal affective processing response (Heitzeg et al. 2015). It is important to consider that many of these studies did not measure preadolescent socioemotional functioning prior to any substance experimentation, and emotional regulation and affective processing have been robustly linked with early cannabis initiation (Debenham et al. 2021; Brumback et al. 2021; Yan et al. 2024).

1.3.9 Educational/Occupational Impact

There are relatively few studies that have examined the ecological validity of cannabis-related reduced cognitive functioning, though family members have reported increased everyday problems in attention and memory in heavy cannabis users (Meier et al. 2012; Paige and Colder 2020) and cannabis users self-report significant cognitive concerns (Rubin-Kahana et al. 2024). One potential impact could be academic performance, as cannabis initiation and escalation occur during late adolescence into young adulthood, when many young people are heavily engaged in their academic or vocational training. Several longitudinal studies have examined the influence of cannabis use on academic achievement, generally finding that continued cannabis use was linked with reduced college academic achievement (Meda et al. 2017; Suerken et al. 2016; Mullin and Cservenka 2024; Meier et al. 2015) and reduced graduate school degree achievement (Allen et al. 2018), although some did not find links after controlling for confounding factors such as comorbid substance use (Mokrysz et al. 2016; Pardini et al. 2015). Meier and colleagues (Meier 2021) conducted a selective review of prospective longitudinal studies conducted from 2015 to 2020 and found that after adjusting for comorbid substance use and baseline grades, cannabis use did not prospectively predict academic grades. In contrast, several studies supported a prospective link between adolescent cannabis use and overall degree attainment, adult income, and lower adult prestigious occupational achievement (Boden et al. 2020; Thompson et al. 2019; Zhang et al. 2016; Schaefer et al. 2021) and some evidence has suggested girls may be at particular risk for cannabis-related reduced academic achievement (Crane et al. 2015). Still, it is important to note that prospective relationships between these outcomes may not be due to cognitive functioning, but other cannabis-related factors (e.g., affiliation with substance using peer group, antisocial behavior, sociocultural factors and

inequity, criminal justice involvement) (Feldstein Ewing et al. 2015, 2022; Green et al. 2017; Meier 2021; Schaefer et al. 2021). Notably, after accounting for depression and co-substance use, adolescent cannabis users still demonstrated reduced perceived value of school, academic motivation, academic problem behaviors, and school discipline, which may mediate reduced academic performance observed in some studies (Pacheco-Colón et al. 2021; Schaefer et al. 2021).

1.4 Resilient/Vulnerable Subgroups and Consideration of Confounds

The vast majority of the studies examining cannabis effects to date excluded those with psychiatric and medical comorbidities. Thus, these findings may not generalize to samples that include potentially resilient or vulnerable subgroups, such as individuals with comorbid psychiatric conditions (e.g., Schizophrenia and Attention Deficient Hyperactivity Disorder) (Bogaty et al. 2018; Dellazizzo et al. 2023; Francisco et al. 2023) (also see Chaps. 15–19), differential levels of aerobic fitness (Brellenthin et al. 2017; Crombie et al. 2021a, b; Ferreira-Vieira et al. 2014; Sirotiak et al. 2023; Sullivan et al. 2020, 2021; Wade et al. 2019b), or have medical conditions that may impact the relationship between cannabis use and neurocognition (Sagar et al. 2021; Ayoub et al. 2024; Meza et al. 2024b). Further, the literature to date oftentimes did not consider additional potential confounding or moderating factors (beyond demographics, psychopathology, and family history of SUD) that may impact cannabis-related cognitive function, such as prenatal cannabis exposure (see Chaps. 6–8), second-hand cannabis exposure (see Chap. 21; Wade et al. 2023a), and current sleep quality (Maple et al. 2016; Brown et al. 2024; Cohen-Zion et al. 2009; Hser et al. 2017; Sullivan et al. 2022c). Additional research utilizing larger samples with more diverse variation in psychopathology, prenatal exposure, second-hand drug exposure, and other health and physical wellness factors are needed to examine novel moderators of cannabis effects.

Sociocultural and social determinants of health factors can also impact adolescent development, neurocognition, and substance use risk (Amaro et al. 2006; Gonzalez et al. 2021; Zucker et al. 2018; Feldstein Ewing et al. 2022) and recent evidence suggest cannabis-related cultural factors may moderate the impact of cannabis use on neurocognition (Cousijn et al. 2024; Prashad et al. 2017; Block et al. 2022; Kroon et al. 2023, 2024). For example, Kroon and colleagues found that across two cultural groups (Texas and Netherlands), regional-related cannabis attitudes moderated the relationship between cannabis exposure and brain response to a working memory task and resting state connectivity patterns (Kroon et al. 2023, 2024). The literature examining the potential influence of complex sociocultural factors, including factors related to social inequity (Feldstein Ewing et al. 2022), on the impact of cannabis use on cognition in teens is sparse and warrants further investigation.

2 Current Research Needs

2.1 Complexities of Cannabis Use

Scientists have also emphasized the need for a more nuanced analysis of cannabis effects, including examining dose-dependent relationships, the influence of age of initiation, specific cannabinoid composition, modes of use, potency, concurrent use of other substances, and individual genetics related to eCB signaling (Lisdahl et al. 2014, 2021a; Bourque and Potvin 2021; Gonzalez et al. 2017; Lees et al. 2021; Scott 2023; Budney et al. 2015; Debenham et al. 2021; Streck et al. 2019; Meier 2017; Schacht et al. 2012; Filbey et al. 2010; Taurisano et al. 2021; Budney et al. 2024; Cuttler et al. 2021, 2023; Meehan-Atrash and Rahman 2021). Although dose-dependent and age of onset relationships have been reported in the literature for over a decade (Lisdahl et al. 2014, 2021a), very few studies have examined the impact of differential modes of use (e.g., vaping vs. smoking flower or concentrate, edibles), specific cannabinoid content, or potency effects on cognition in youth (Lisdahl et al. 2021a). This is particularly concerning given that delta-9-THC potency is at all-time high and other cannabinoids such as CBD and delta-8-THC are increasingly popular (Budney et al. 2024; Chye et al. 2021; Cuttler et al. 2021, 2023; Kruger and Kruger 2022, 2023; Meehan-Atrash and Rahman 2021). Notably, CBD may disrupt CB1R activity and mitigate the impact of other cannabinoids such as delta-9-THC (Chye et al. 2021). Preclinical studies have demonstrated some neuroprotective effects of CBD when combined with THC (Gabaglio et al. 2021), although acute human studies have provided mixed evidence, with some demonstrating mitigating effects of cognition, while others did not (Chye et al. 2021; Englund et al. 2023; Lawn et al. 2023). Human CBD administration studies have also provided some preliminary evidence of protective effects for cognition in healthy adults (Colizzi and Bhattacharyya 2017; Colizzi et al. 2020) and heavy cannabis users (Lorenzetti et al. 2024; Solowij et al. 2018). Though self-report of specific cannabinoid content may be challenging in youth, emerging methods such as hair toxicology to assess the concentration of specific cannabinoids hold promise for enhancing our understanding of the unique effects of cannabinoids on cognition (Wade et al. 2023b). Nevertheless, to date, there have been very limited adolescent studies examining the impact of specific cannabis modes of use, potency, or cannabinoid content on cognition. Cuttler et al. (2023) compared primarily cannabis flower users, concentrate users, and nonusers on memory tests and found all users demonstrated worse verbal free recall and episodic prospective memory, while only the concentrate users demonstrated impairments in source memory compared to non-users. Studies harnessing toxicology data have found that increased presence of THC metabolite (THCCOOH) was linked with poorer overall learning and delayed recall, while self-reported cannabis use was uniquely associated with poorer initial learning and short delay recall (Wade et al. 2021a). In a subsample of youth aged 13–14 enrolled in the large-scale Adolescent Brain Cognitive Development (ABCD) Study®, Wade et al. (2024b) found

significant relationships between THCCOOH and visual IQ estimate and inhibitory control, while CBD levels did not significantly predict cognition (Wade et al. 2024b). No large-scale, prospective, twin-informed, longitudinal findings examining the impact of cannabis on cognition during adolescence that utilize these methods have been published to date, though data is being collected as part of the ABCD Study® (Lisdahl et al. 2018).

2.2 Substance Co-Use

Cannabis use often co-occurs with alcohol and nicotine use during adolescence (Kolp et al. 2024; Lisdahl et al. 2021b; Sullivan et al. 2022b; Wade et al. 2022; Miech et al. 2023; Jacobus et al. 2015; Bedillion et al. 2021; Hernandez Mejia et al. 2021; Parlar et al. 2021). Adolescent cannabis and alcohol combined use has been associated with poorer working memory, delay discounting, math ability, cognitive flexibility, and visuospatial performance (Winward et al. 2014; Jacobus et al. 2015; Deniel et al. 2021), although two studies found potential *buffering* effects of cannabis on alcohol use in relation to memory performance (Mahmood et al. 2010; Vinader-Caerols and Monleón 2021). The impact of both cannabis and alcohol on the same day, or co-use days, has rarely been examined in adolescent users; one study to date found that increased past month co-use days were significantly related to poorer sustained attention, above and beyond overall alcohol and cannabis exposure (Wade et al. 2020). Studies examining the co-use or simultaneous use of combined cannabis and nicotine on cognition are even more limited (Hernandez Mejia et al. 2021); one study found that cannabis use was significantly associated with episodic memory only in light cigarette users, but not regular smokers, suggesting that recent cigarette use may mask memory decrements (Schuster et al. 2015). Thus, additional studies examining the unique impact of co- or simultaneous use of cannabis and other drugs of abuse on cognition during development are needed.

2.3 Recovery of Cognitive Function?

Meta-analyses have revealed that cross-sectional studies requiring minimal abstinence (<72 h) demonstrate the most robust cognitive decrements (Scott et al. 2018) and studies comparing recent to past users suggest potential recovery of cognitive function with sustained abstinence (Burke et al. 2024; Lorenzetti et al. 2021; Fried et al. 2005), although one meta-analysis found an impact of abstinence only on executive function measures (Lovell et al. 2020). Cannabis users have demonstrated improved verbal memory, especially female users, following approximately one month of abstinence (Meza et al. 2024a; Schuster et al. 2018), though these studies did not include a non-using control group and results may be due to practice effects. Improved memory, processing speed, sustained attention and verbal fluency

performance improvements have been reported following 28 days of monitored cannabis abstinence in individuals with multiple sclerosis (Meza et al. 2024b) while visual search, selective attention and working memory functioning were improved with abstinence in individuals with major depressive disorder (Sorkhou et al. 2022). However, these findings may not generalize to younger cohorts without comorbid medical or psychiatric disorders. Only two studies to date have conducted repeated cognitive testing in regular cannabis-using youth and non-users during a period of monitored abstinence (2–4 weeks); they demonstrated that users had significant recovery in verbal learning and memory (Hanson et al. 2010) and sustained attention (Wallace et al. 2020) performance. These data suggest that the subtle cognitive decrements observed in young cannabis users may start to normalize after several weeks of abstinence. Ultimately, this research offers useful clinical recommendations; healthcare professionals should advise their clients to refrain from cannabis use for a period of two weeks prior to neuropsychological testing in order to minimize the potential impact of recent withdrawal symptoms and acute effects.

3 Conclusions

Adolescent cannabis use has been cross-sectionally and longitudinally linked with subtle decrements in cognition, with overall patterns suggesting a prospective relationship between increased cannabis use and modest declines in IQ, verbal memory, complex attention, processing speed, and facets of executive functioning. Additional research is needed to examine the impact of chronic cannabis use on more novel outcomes, such as socioemotional and affective processing skills. Notably, the relationship between adolescent and young adult cannabis use and cognition is rather complex and may be influenced by various factors, such as the amount and frequency of use, the type and potency of the cannabis product, cannabinoid content, the age at which cannabis use was initiated, the presence of cannabis use disorder symptoms, the user's sex, the duration of cannabis abstinence, and the presence of any comorbid medical or psychiatric conditions. Given the rising delta-9-THC potency and popularity of concentrate use, as well as the more recent introduction of cannabinoids such as delta-8-THC, additional research is needed to examine the influence of more detailed cannabinoid use patterns on cognition in youth. Research examining the potential impact of very occasional or light cannabis (less than weekly) use on cognition in youth is extremely limited, and it remains unknown whether sustained abstinence during sensitive developmental periods can lead to a return to one's original predrug use cognitive trajectory. Finally, prospective, longitudinal studies employing twin or sibling comparison designs have called into question whether cannabis *causes* these cognitive decrements, or if they are better accounted for by shared familial and genetic risk factors associated with substance use onset risk. These potential limitations emphasize the need for large-scale, diverse, twin- and sibling-informed prospective longitudinal studies, such as the ABCD Study®, that simultaneously evaluate demographics, mental health (Barch

et al. 2018), sociocultural, family, environmental (Zucker et al. 2018), and physical health/genetic (Iacono et al. 2018; Uban et al. 2018) factors, as well as detailed substance use patterns and drug toxicology (Lisdahl et al. 2018, 2021b; Sullivan et al. 2022b) and neurocognitive outcomes (Casey et al. 2018; Luciana et al. 2018). These methods are crucial to determine whether the previously reported neurocognitive deficits are precursors to cannabis use trajectories or consequences of cannabis use, to identify potentially vulnerable or resilient subgroups, characterize potential moderators, and examine the impact of sustained abstinence on cognitive recovery.

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References

Ajmera N, Collins PF, Weiss H, Luciana M. Initiation of moderately frequent cannabis use in adolescence and young adulthood is associated with declines in verbal learning and memory: a longitudinal comparison of pre- versus post-initiation cognitive performance. J Int Neuropsychol Soc. 2021;27(6):621–36. https://doi.org/10.1017/S1355617721000631.

Allen HK, Lilly F, Beck KH, Vincent KB, Arria AM. Graduate degree completion: Associations with alcohol and marijuana use before and after enrollment. Addict Behav Rep. 2018;9:100156. https://doi.org/10.1016/j.abrep.2018.100156. PMID: 30627627; PMCID: PMC6322074.

Aloi J, Blair KS, Crum KI, Meffert H, White SF, Tyler PM, et al. Adolescents show differential dysfunctions related to alcohol and cannabis use disorder severity in emotion and executive attention neuro-circuitries. Neuroimage Clin. 2018;19:782–92. https://doi.org/10.1016/j.nicl.2018.06.005.

Amaro H, Arévalo S, Gonzalez G, Szapocznik J, Iguchi MY. Needs and scientific opportunities for research on substance abuse treatment among hispanic adults. Drug Alcohol Depend. 2006;84(Suppl 1):S64–75. https://doi.org/10.1016/j.drugalcdep.2006.05.008.

Ayoub SM, Holloway BM, Miranda AH, Roberts BZ, Young JW, Minassian A, Ellis RJ. The impact of cannabis use on cognition in people with HIV: evidence of function-dependent effects and mechanisms from clinical and preclinical studies. Curr HIV/AIDS Rep. 2024;21(3):87–115. https://doi.org/10.1007/s11904-024-00698-w.

Banks PJ, Bennett PJ, Sekuler AB, Gruber AJ. Cannabis use is associated with sexually dimorphic changes in executive control of visuospatial decision-making. Front Integr Neurosci. 2022;16:884080. https://doi.org/10.3389/fnint.2022.884080.

Barch DM, Albaugh MD, Avenevoli S, Chang L, Clark DB, Glantz M, et al. Demographic, physical and mental health assessments in the adolescent brain and cognitive development study: rationale and description. Dev Cogn Neurosci. 2018;32:55–66. https://doi.org/10.1016/j.dcn.2017.10.010.

Barthelemy OJ, Richardson MA, Heeren TC, Chen CA, Liebschutz JM, Forman L, et al. Do differences in learning performance precede or follow initiation of marijuana use? J Stud Alcohol Drugs. 2019;80(1):5–14. https://doi.org/10.15288/jsad.2019.80.5.

Bechtel S, Lazar V, Albuisson E, Schwan R, Laprévote V, Bernardin F, Schwitzer T. Assessment of neuropsychological impairments in regular cannabis users. Encéphale. 2022;48(2):132–8. https://doi.org/10.1016/j.encep.2021.02.013.

Becker MP, Collins PF, Luciana M. Neurocognition in college-aged daily marijuana users. J Clin Exp Neuropsychol. 2014;36(4):379–98. https://doi.org/10.1080/13803395.2014.893996.

Becker MP, Collins PF, Schultz A, Urošević S, Schmaling B, Luciana M. Longitudinal changes in cognition in young adult cannabis users. J Clin Exp Neuropsychol. 2018;40(6):529–43. https://doi.org/10.1080/13803395.2017.1385729.

Bedillion MF, Blaine SK, Claus ED, Ansell EB. The effects of alcohol and cannabis co-use on neurocognitive function, brain structure, and brain function. Curr Behav Neurosci Rep. 2021;8(4):134–49. https://doi.org/10.1007/s40473-021-00243-8.

Benarroch EE. Synaptic effects of cannabinoids: complexity, behavioral effects, and potential clinical implications. Neurology. 2014;83(21):1958–67. https://doi.org/10.1212/WNL.0000000000001013.

Bernal-Chico A, Tepavcevic V, Manterola A, Utrilla C, Matute C, Mato S. Endocannabinoid signaling in brain diseases: emerging relevance of glial cells. Glia. 2023;71(1):103–26. https://doi.org/10.1002/glia.24172.

Blest-Hopley G, Colizzi M, Giampietro V, Bhattacharyya S. Is the adolescent brain at greater vulnerability to the effects of cannabis? A narrative review of the evidence. Front Psych. 2020;11:859. https://doi.org/10.3389/fpsyt.2020.00859.

Block RI, Jager G, Luijten M, Ramsey NF. Associations of regular marijuana use by adolescent boys with verbal memory and perseveration. Psychol Rep. 2022;125(2):839–61. https://doi.org/10.1177/0033294121988992.

Boden JM, Dhakal B, Foulds JA, Horwood LJ. Life-course trajectories of cannabis use: a latent class analysis of a New Zealand birth cohort. Addiction. 2020;115(2):279–90. https://doi.org/10.1111/add.14814.

Boer OD, El Marroun H, Franken IHA. Brain morphology predictors of alcohol, tobacco, and cannabis use in adolescence: a systematic review. Brain Res. 2022;1795:148020. https://doi.org/10.1016/j.brainres.2022.148020.

Bogaty SER, Lee RSC, Hickie IB, Hermens DF. Meta-analysis of neurocognition in young psychosis patients with current cannabis use. J Psychiatr Res. 2018;99:22–32. https://doi.org/10.1016/j.jpsychires.2018.01.010.

Bourque J, Potvin S. Cannabis and cognitive functioning: from acute to residual effects, from randomized controlled trials to prospective designs. Front Psych. 2021;12:596601. https://doi.org/10.3389/fpsyt.2021.596601.

Brabete AC, Greaves L, Hemsing N, Stinson J. Sex- and gender-based analysis in cannabis treatment outcomes: a systematic review. Int J Environ Res Public Health. 2020;17(3):872. https://doi.org/10.3390/ijerph17030872.

Brellenthin AG, Crombie KM, Hillard CJ, Koltyn KF. Endocannabinoid and mood responses to exercise in adults with varying activity levels. Med Sci Sports Exerc. 2017;49(8):1688–96. https://doi.org/10.1249/MSS.0000000000001276.

Britton MK, DeFelice J, Porges EC, Cohen R, Li Y, et al. Association between cannabis use disorder and greater apathy in adults with HIV. Drug Alcohol Depend. 2024;261:111354. https://doi.org/10.1016/j.drugalcdep.2024.111354.

Brown TT, Kuperman JM, Chung Y, Erhart M, McCabe C, Hagler D, et al. Neuroanatomical assessment of biological maturity. Curr Biol. 2012;22(18):1693–8. https://doi.org/10.1016/j.cub.2012.07.002.

Brown T, Ackerman RA, Kroon E, Kuhns L, Cousijn J, Filbey FM. The role of sleep in the link between cannabis use and memory function: evidence from a cross-sectional study. Am J Drug Alcohol Abuse. 2024;50(4):547–56. https://doi.org/10.1080/00952990.2024.2362832.

Brumback T, Thompson W, Cummins K, Brown S, Tapert S. Psychosocial predictors of substance use in adolescents and young adults: longitudinal risk and protective factors. Addict Behav. 2021;121:106985. https://doi.org/10.1016/j.addbeh.2021.106985.

Budney AJ, Sargent JD, Lee DC. Vaping cannabis (marijuana): parallel concerns to e-cigs? Addiction. 2015;110(11):1699–704. https://doi.org/10.1111/add.13036.

Budney AJ, Borodovsky JT, Struble CA, Habib MI, Shmulewitz D, et al. Estimating THC consumption from smoked and vaped cannabis products in an online survey of adults who use cannabis. Cannabis Cannabinoid Res. 2024;9(2):688–98. https://doi.org/10.1089/can.2022.0238.

Burke CO, Boutouis S, Spence JS, Filbey FM. Residual and enduring effects of cannabis use on cognitive and psychomotor function: a study of adults during unrestricted cannabis use, short-term abstinence, and protracted abstinence. Exp Clin Psychopharmacol. 2024;33:53. https://doi.org/10.1037/pha0000732.

Burston JJ, Wiley JL, Craig AA, Selley DE, Sim-Selley LJ. Regional enhancement of cannabinoid CB_1 receptor desensitization in female adolescent rats following repeated Delta-tetrahydrocannabinol exposure. Br J Pharmacol. 2010;161(1):103–12. https://doi.org/10.1111/j.1476-5381.2010.00870.x.

Camchong J, Lim KO, Kumra S. Adverse effects of cannabis on adolescent brain development: a longitudinal study. Cereb Cortex. 2017;27(3):1922–30. https://doi.org/10.1093/cercor/bhw015.

Casey JL, Cservenka A. Effects of frequent marijuana use on risky decision-making in young adult college students. Addict Behav Rep. 2020;11:100253. https://doi.org/10.1016/j.abrep.2020.100253.

Casey BJ, Getz S, Galvan A. The adolescent brain. Dev Rev. 2008;28(1):62–77. https://doi.org/10.1016/j.dr.2007.08.003.

Casey BJ, Cannonier T, Conley MI, Cohen AO, Barch DM, Heitzeg M, et al. The adolescent brain cognitive development (ABCD) study: imaging acquisition across 21 sites. Dev Cogn Neurosci. 2018;32:43–54. https://doi.org/10.1016/j.dcn.2018.03.001.

Castellanos-Ryan N, Pingault JB, Parent S, Vitaro F, Tremblay RE, Séguin JR. Adolescent cannabis use, change in neurocognitive function, and high-school graduation: a longitudinal study from early adolescence to young adulthood. Dev Psychopathol. 2017;29(4):1253–66. https://doi.org/10.1017/S0954579416001280.

Chowdhury KU, Holden ME, Wiley MT, Suppiramaniam V, Reed MN. Effects of cannabis on glutamatergic neurotransmission: the interplay between cannabinoids and glutamate. Cells. 2024;13(13):1130. https://doi.org/10.3390/cells13131130.

Chye Y, Kirkham R, Lorenzetti V, McTavish E, Solowij N, Yücel M. Cannabis, cannabinoids, and brain morphology: a review of the evidence. Biol Psychiatry Cogn Neurosci Neuroimaging. 2021;6(6):627–35. https://doi.org/10.1016/j.bpsc.2020.07.009.

Cohen-Zion M, Drummond SP, Padula CB, Winward J, Kanady J, Medina KL, Tapert SF. Sleep architecture in adolescent marijuana and alcohol users during acute and extended abstinence. Addict Behav. 2009;34(11):976–9. https://doi.org/10.1016/j.addbeh.2009.05.011.

Coleman JR, Madularu D, Ortiz RJ, Athanassiou M, Knudsen A, et al. Changes in brain structure and function following chronic exposure to inhaled vaporised cannabis during periadolescence in female and male mice: a multimodal MRI study. Addict Biol. 2022;27(3):e13169. https://doi.org/10.1111/adb.13169.

Colizzi M, Bhattacharyya S. Does cannabis composition matter? Differential effects of Delta-9-tetrahydrocannabinol and Cannabidiol on human cognition. Curr Addict Rep. 2017;4(2):62–74. https://doi.org/10.1007/s40429-017-0142-2.

Colizzi M, Ruggeri M, Bhattacharyya S. Unraveling the intoxicating and therapeutic effects of cannabis ingredients on psychosis and cognition. Front Psychol. 2020;11:833. https://doi.org/10.3389/fpsyg.2020.00833.

Cooper ZD, Craft RM. Sex-dependent effects of cannabis and cannabinoids: a translational perspective. Neuropsychopharmacology. 2018;43(1):34–51. https://doi.org/10.1038/npp.2017.140.

Cousijn J, Watson P, Koenders L, Vingerhoets WA, Goudriaan AE, Wiers RW. Cannabis dependence, cognitive control and attentional bias for cannabis words. Addict Behav. 2013;38(12):2825–32. https://doi.org/10.1016/j.addbeh.2013.08.011.

Cousijn J, Kuhns L, Filbey F, Freeman TP, Kroon E. Cannabis research in context: the case for measuring and embracing regional similarities and differences. Addiction. 2024;119(9):1502–4. https://doi.org/10.1111/add.16460.

Crane NA, Schuster RM, Fusar-Poli P, Gonzalez R. Effects of cannabis on neurocognitive functioning: recent advances, neurodevelopmental influences, and sex differences. Neuropsychol Rev. 2013a;23(2):117–37. https://doi.org/10.1007/s11065-012-9222-1.

Crane NA, Schuster RM, Gonzalez R. Preliminary evidence for a sex-specific relationship between amount of cannabis use and neurocognitive performance in young adult cannabis users. J Int Neuropsychol Soc. 2013b;19(9):1009–15. https://doi.org/10.1017/S135561771300088X.

Crane NA, Schuster RM, Mermelstein RJ, Gonzalez R. Neuropsychological sex differences associated with age of initiated use among young adult cannabis users. J Clin Exp Neuropsychol. 2015;37(4):389–401. https://doi.org/10.1080/13803395.2015.1020770.

Crombie KM, Cisler JM, Hillard CJ, Koltyn KF. Aerobic exercise reduces anxiety and fear ratings to threat and increases circulating endocannabinoids in women with and without PTSD. Ment Health Phys Act. 2021a;20:100366. https://doi.org/10.1016/j.mhpa.2020.100366.

Crombie KM, Sartin-Tarm A, Sellnow K, Ahrenholtz R, Lee S, et al. Exercise-induced increases in anandamide and BDNF during extinction consolidation contribute to reduced threat following reinstatement: preliminary evidence from a randomized controlled trial. Psychoneuroendocrinology. 2021b;132:105355. https://doi.org/10.1016/j.psyneuen.2021.105355.

Cuttler C, LaFrance EM, Stueber A. Acute effects of high-potency cannabis flower and cannabis concentrates on everyday life memory and decision making. Sci Rep. 2021;11(1):13784. https://doi.org/10.1038/s41598-021-93198-5.

Cuttler C, Petrucci AS, LaFrance EM. Cognitive test performance in chronic cannabis flower users, concentrate users, and non-users. Sci Rep. 2023;13(1):8068. https://doi.org/10.1038/s41598-023-35217-1.

Dali G, Poulton A, Hester R. Chronic cannabis use and error awareness: the effect on learning from errors. PLoS One. 2023;18(3):e0283158. https://doi.org/10.1371/journal.pone.0283158.

Debenham J, Birrell L, Champion K, Lees B, Yücel M, Newton N. Neuropsychological and neurophysiological predictors and consequences of cannabis and illicit substance use during neurodevelopment: a systematic review of longitudinal studies. Lancet Child Adolesc Health. 2021;5(8):589–604. https://doi.org/10.1016/S2352-4642(21)00051-1.

Dellazizzo L, Potvin S, Giguère S, Dumais A. Evidence on the acute and residual neurocognitive effects of cannabis use in adolescents and adults: a systematic meta-review of meta-analyses. Addiction. 2022;117(7):1857–70. https://doi.org/10.1111/add.15764.

Dellazizzo L, Potvin S, Giguère S, Dumais A. The potential paradoxical neurocognitive effects of cannabis use in patients with psychotic disorders: a critical meta-review of meta-analytical evidence. Cannabis Cannabinoid Res. 2023;8(3):464–75. https://doi.org/10.1089/can.2021.0163.

Deniel S, Mauduy M, Cheam-Bernière C, Mauny N, Montcharmont C, et al. Why should we ask binge drinkers if they smoke cannabis? Additive effect of alcohol and cannabis use on college students' neuropsychological performance. Addict Behav Rep. 2021;14:100362. https://doi.org/10.1016/j.abrep.2021.100362.

Di Marzo V, De Petrocellis L, Bisogno T. The biosynthesis, fate and pharmacological properties of endocannabinoids. Handb Exp Pharmacol. 2005;(168):147–185.

Diaz-Alonso J, Guzman M, Galve-Roperh I. Endocannabinoids via CB(1) receptors act as neurogenic niche cues during cortical development. Philos Trans R Soc Lond B Biol Sci. 2012;367(1607):3229–41. https://doi.org/10.1098/rstb.2011.0385.

Dougherty DM, Mathias CW, Dawes MA, Furr RM, Charles NE, et al. Impulsivity, attention, memory, and decision-making among adolescent marijuana users. Psychopharmacology (Berl). 2013;226(2):307–19. https://doi.org/10.1007/s00213-012-2908-5.

Duperrouzel JC, Granja K, Pacheco-Colón I, Gonzalez R. Adverse effects of cannabis use on neurocognitive functioning: a systematic review of meta- analytic studies. J Dual Diagn. 2020;16(1):43–57. https://doi.org/10.1080/15504263.2019.1626030.

Eggan SM, Lewis DA. Immunocytochemical distribution of the cannabinoid CB1 receptor in the primate neocortex: a regional and laminar analysis. Cereb Cortex. 2007;17(1):175–91. https://doi.org/10.1093/cercor/bhj136.

Ellgren M, Artmann A, Tkalych O, Gupta A, Hansen HS, Hansen S, et al. Dynamic changes of the endogenous cannabinoid and opioid mesocorticolimbic systems during adolescence:

THC effects. Eur Neuropsychopharmacol. 2008;18(11):826–34. https://doi.org/10.1016/j.euroneuro.2008.06.009.

Ellingson JM, Hinckley JD, Ross JM, Schacht JP, Bidwell LC, Bryan A, et al. The neurocognitive effects of cannabis across the lifespan. Curr Behav Neurosci Rep. 2021a;8(4):124–33. https://doi.org/10.1007/s40473-021-00244-7.

Ellingson JM, Ross JM, Winiger E, Stallings MC, Corley RP, Friedman NP, et al. Familial factors may not explain the effect of moderate-to-heavy cannabis use on cognitive functioning in adolescents: a sibling-comparison study. Addiction. 2021b;116(4):833–44. https://doi.org/10.1111/add.15207.

Englund A, Oliver D, Chesney E, Chester L, Wilson J, Sovi S, et al. Does cannabidiol make cannabis safer? A randomised, double-blind, cross-over trial of cannabis with four different CBD:THC ratios. Neuropsychopharmacology. 2023;48(6):869–76. https://doi.org/10.1038/s41386-022-01478-z.

Farquhar CE, Breivogel CS, Gamage TF, Gay EA, Thomas BF, Craft RM, Wiley JL. Sex, THC, and hormones: effects on density and sensitivity of CB. Drug Alcohol Depend. 2019;194:20–7. https://doi.org/10.1016/j.drugalcdep.2018.09.018.

Feldstein Ewing SW, Filbey FM, Loughran TA, Chassin L, Piquero AR. Which matters most? Demographic, neuropsychological, personality, and situational factors in long-term marijuana and alcohol trajectories for justice-involved male youth. Psychol Addict Behav. 2015;29(3):603–12. https://doi.org/10.1037/adb0000076.

Feldstein Ewing SW, Karalunas SL, Kenyon EA, Yang M, Hudson KA, Filbey FM. Intersection between social inequality and emotion regulation on emerging adult cannabis use. Drug Alcohol Depend Rep. 2022;3:100050. https://doi.org/10.1016/j.dadr.2022.100050.

Felton JW, Collado A, Shadur JM, Lejuez CW, MacPherson L. Sex differences in self-report and behavioral measures of disinhibition predicting marijuana use across adolescence. Exp Clin Psychopharmacol. 2015;23(4):265–74. https://doi.org/10.1037/pha0000031.

Ferreira-Vieira TH, Bastos CP, Pereira GS, Moreira FA, Massensini AR. A role for the endocannabinoid system in exercise-induced spatial memory enhancement in mice. Hippocampus. 2014;24(1):79–88. https://doi.org/10.1002/hipo.22206.

Filbey FM, Schacht JP, Myers US, Chavez RS, Hutchison KE. Individual and additive effects of the CNR1 and FAAH genes on brain response to marijuana cues. Neuropsychopharmacology. 2010;35(4):967–75. https://doi.org/10.1038/npp.2009.200. Epub 2009 Dec 9. PMID: 20010552; PMCID: PMC2820137.

Fischer AS, Tapert SF, Lee Louie D, Schatzberg AF, Singh MK. Cannabis and the developing adolescent brain. Curr Treat Options Psychiatry. 2020;7(2):144–61. https://doi.org/10.1007/s40501-020-00202-2.

Fluharty ME, Heron J, Munafò MR. Longitudinal associations of social cognition and substance use in childhood and early adolescence: findings from the Avon longitudinal study of parents and children. Eur Child Adolesc Psychiatry. 2018;27(6):739–52. https://doi.org/10.1007/s00787-017-1068-x.

Fontes MA, Bolla KI, Cunha PJ, Almeida PP, Jungerman F, Laranjeira RR, et al. Cannabis use before age 15 and subsequent executive functioning. Br J Psychiatry. 2011;198(6):442–7. https://doi.org/10.1192/bjp.bp.110.077479.

Francis AN, Camprodon JA, Filbey F. Functional hyperconnectivity between corticocerebellar networks and altered decision making in young adult cannabis users: evidence from 7T and multivariate pattern analysis. Psychiatr Res Neuroimaging. 2023;331:111613. https://doi.org/10.1016/j.pscychresns.2023.111613.

Francisco AP, Lethbridge G, Patterson B, Goldman Bergmann C, Van Ameringen M. Cannabis use in attention—deficit/hyperactivity disorder (ADHD): a scoping review. J Psychiatr Res. 2023;157:239–56. https://doi.org/10.1016/j.jpsychires.2022.11.029.

Fried PA, Watkinson B, Gray R. Neurocognitive consequences of marihuana—a comparison with pre-drug performance. Neurotoxicol Teratol. 2005;27(2):231–9. https://doi.org/10.1016/j.ntt.2004.11.003.

Gabaglio M, Zamberletti E, Manenti C, Parolaro D, Rubino T. Long-term consequences of adolescent exposure to THC-rich/CBD-poor and CBD-rich/THC-poor combinations: a comparison with pure THC treatment in female rats. Int J Mol Sci. 2021;22(16):8899. https://doi.org/10.3390/ijms22168899.

Gee DG, Fetcho RN, Jing D, Li A, Glatt CE, Drysdale AT, et al. Individual differences in frontolimbic circuitry and anxiety emerge with adolescent changes in endocannabinoid signaling across species. Proc Natl Acad Sci U S A. 2016;113(16):4500–5. https://doi.org/10.1073/pnas.1600013113.

Giedd JN, Raznahan A, Mills KL, Lenroot RK. Review: magnetic resonance imaging of male/female differences in human adolescent brain anatomy. Biol Sex Differ. 2012;3(1):19. https://doi.org/10.1186/2042-6410-3-19.

Ginder DE, Wright HR, McLaughlin RJ. The stoned age: sex differences in the effects of adolescent cannabinoid exposure on prefrontal cortex structure and function in animal models. Int Rev Neurobiol. 2022;161:121–45. https://doi.org/10.1016/bs.irn.2021.07.005.

Gonzalez R, Schuster RM, Mermelstein RJ, Vassileva J, Martin EM, Diviak KR. Performance of young adult cannabis users on neurocognitive measures of impulsive behavior and their relationship to symptoms of cannabis use disorders. J Clin Exp Neuropsychol. 2012;34(9):962–76. https://doi.org/10.1080/13803395.2012.703642.

Gonzalez R, Pacheco-Colón I, Duperrouzel JC, Hawes SW. Does cannabis use cause declines in neuropsychological functioning? A review of longitudinal studies. J Int Neuropsychol Soc. 2017;23(9–10):893–902. https://doi.org/10.1017/S1355617717000789.

Gonzalez R, Thompson EL, Sanchez M, Morris A, Gonzalez MR, Feldstein Ewing SW, et al. An update on the assessment of culture and environment in the ABCD study®: emerging literature and protocol updates over three measurement waves. Dev Cogn Neurosci. 2021;52:101021. https://doi.org/10.1016/j.dcn.2021.101021.

Grant I, Gonzalez R, Carey CL, Natarajan L, Wolfson T. Non-acute (residual) neurocognitive effects of cannabis use: a meta-analytic study. J Int Neuropsychol Soc. 2003;9(5):679–89. https://doi.org/10.1017/S1355617703950016.

Grant JE, Chamberlain SR, Schreiber L, Odlaug BL. Neuropsychological deficits associated with cannabis use in young adults. Drug Alcohol Depend. 2012;121(1–2):159–62. https://doi.org/10.1016/j.drugalcdep.2011.08.015.

Green KM, Doherty EE, Ensminger ME. Long-term consequences of adolescent cannabis use: examining intermediary processes. Am J Drug Alcohol Abuse. 2017;43(5):567–75. https://doi.org/10.1080/00952990.2016.1258706.

Green R, Wolf BJ, Chen A, Kirkland AE, Ferguson PL, Browning BD, et al. Predictors of substance use initiation by early adolescence. Am J Psychiatry. 2024;181(5):423–33. https://doi.org/10.1176/appi.ajp.20230882.

Gruber SA, Rogowska J, Yurgelun-Todd DA. Altered affective response in marijuana smokers: an FMRI study. Drug Alcohol Depend. 2009;105(1–2):139–53. https://doi.org/10.1016/j.drugalcdep.2009.06.019.

Gruber SA, Dahlgren MK, Sagar KA, Gönenc A, Killgore WD. Age of onset of marijuana use impacts inhibitory processing. Neurosci Lett. 2012;511(2):89–94. https://doi.org/10.1016/j.neulet.2012.01.039.

Gruber SA, Dahlgren MK, Sagar KA, Gönenç A, Lukas SE. Worth the wait: effects of age of onset of marijuana use on white matter and impulsivity. Psychopharmacology (Berl). 2014;231(8):1455–65. https://doi.org/10.1007/s00213-013-3326-z.

Hanson KL, Winward JL, Schweinsburg AD, Medina KL, Brown SA, Tapert SF. Longitudinal study of cognition among adolescent marijuana users over three weeks of abstinence. Addict Behav. 2010;35(11):970–6. https://doi.org/10.1016/j.addbeh.2010.06.012.

Hanson KL, Thayer RE, Tapert SF. Adolescent marijuana users have elevated risk-taking on the balloon analog risk task. J Psychopharmacol. 2014;28(11):1080–7. https://doi.org/10.1177/0269881114550352.

Hare TA, Tottenham N, Galvan A, Voss HU, Glover GH, Casey BJ. Biological substrates of emotional reactivity and regulation in adolescence during an emotional go-nogo task. Biol Psychiatry. 2008;63(10):927–34. https://doi.org/10.1016/j.biopsych.2008.03.015.

Harris JC, Wallace AL, Thomas AM, Wirtz HG, Kaiver CM, Lisdahl KM. Disrupted resting state attentional network connectivity in adolescent and young adult cannabis users following two-weeks of monitored abstinence. Brain Sci. 2022;12(2):287. https://doi.org/10.3390/brainsci12020287.

Harvey MA, Sellman JD, Porter RJ, Frampton CM. The relationship between non-acute adolescent cannabis use and cognition. Drug Alcohol Rev. 2007;26(3):309–19. https://doi.org/10.1080/09595230701247772.

Hasegawa Y, Kim J, Ursini G, Jouroukhin Y, Zhu X, Miyahara, Y.,…Kamiya, A. Microglial cannabinoid receptor type 1 mediates social memory deficits in mice produced by adolescent THC exposure and 16p11.2 duplication. Nat Commun. 2023;14(1):6559. https://doi.org/10.1038/s41467-023-42276-5.

Heitzeg MM, Cope LM, Martz ME, Hardee JE, Zucker RA. Brain activation to negative stimuli mediates a relationship between adolescent marijuana use and later emotional functioning. Dev Cogn Neurosci. 2015;16:71–83. https://doi.org/10.1016/j.dcn.2015.09.003.

Heng L, Beverley JA, Steiner H, Tseng KY. Differential developmental trajectories for CB1 cannabinoid receptor expression in limbic/associative and sensorimotor cortical areas. Synapse. 2011;65(4):278–86. https://doi.org/10.1002/syn.20844.

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC. Cannabinoid receptor localization in brain. Proc Natl Acad Sci U S A. 1990;87(5):1932–6.

Hernandez Mejia M, Wade NE, Baca R, Diaz VG, Jacobus J. The influence of cannabis and nicotine co-use on neuromaturation: a systematic review of adolescent and young adult studies. Biol Psychiatry. 2021;89(2):162–71. https://doi.org/10.1016/j.biopsych.2020.09.021.

Hernandez CM, Orsini CA, Blaes SL, Bizon JL, Febo M, Bruijnzeel AW, Setlow B. Effects of repeated adolescent exposure to cannabis smoke on cognitive outcomes in adulthood. J Psychopharmacol. 2021;35(7):848–63. https://doi.org/10.1177/0269881120965931.

Hillard CJ. Circulating endocannabinoids: from whence do they come and where are they going? Neuropsychopharmacology. 2018;43(1):155–72. https://doi.org/10.1038/npp.2017.130.

Hirst RB, Young KR, Sodos LM, Wickham RE, Earleywine M. Trying to remember: effort mediates the relationship between frequency of cannabis use and memory performance. J Clin Exp Neuropsychol. 2017;39(5):502–12. https://doi.org/10.1080/13803395.2016.1237617.

Hirst R, Vaughn D, Arastu S, Deneen A, Pilavjian H. Female sex as a protective factor in the effects of chronic cannabis use on verbal learning and memory. J Int Neuropsychol Soc. 2021;27(6):581–91. https://doi.org/10.1017/S1355617721000217.

Hirvonen J, Goodwin RS, Li CT, Terry GE, Zoghbi SS, Morse C, et al. Reversible and regionally selective downregulation of brain cannabinoid CB1 receptors in chronic daily cannabis smokers. Mol Psychiatry. 2012;17(6):642–9. https://doi.org/10.1038/mp.2011.82.

Hser YI, Mooney LJ, Huang D, Zhu Y, Tomko RL, McClure E, et al. Reductions in cannabis use are associated with improvements in anxiety, depression, and sleep quality, but not quality of life. J Subst Abuse Treat. 2017;81:53–8. https://doi.org/10.1016/j.jsat.2017.07.012.

Iacono WG, Heath AC, Hewitt JK, Neale MC, Banich MT, Luciana MM, et al. The utility of twins in developmental cognitive neuroscience research: how twins strengthen the ABCD research design. Dev Cogn Neurosci. 2018;32:30–42. https://doi.org/10.1016/j.dcn.2017.09.001.

Infante MA, Nguyen-Louie TT, Worley M, Courtney KE, Coronado C, Jacobus J. Neuropsychological trajectories associated with adolescent alcohol and cannabis use: a prospective 14-year study. J Int Neuropsychol Soc. 2020;26(5):480–91. https://doi.org/10.1017/S1355617719001395.

Jacobus J, Squeglia LM, Infante MA, Castro N, Brumback T, Meruelo AD, Tapert SF. Neuropsychological performance in adolescent marijuana users with co-occurring alcohol use: a three-year longitudinal study. Neuropsychology. 2015;29:829.

Jackson NJ, Isen JD, Khoddam R, Irons D, Tuvblad C, Iacono WG, McGue M, Raine A, Baker LA. Impact of adolescent marijuana use on intelligence: Results from two longitudinal twin studies. Proc Natl Acad Sci U S A. 2016;113(5):E500–8. https://doi.org/10.1073/pnas.1516648113. Epub 2016 Jan 19. PMID: 26787878; PMCID: PMC4747759.

Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. Physiol Rev. 2009;89(1):309–80. https://doi.org/10.1152/physrev.00019.2008.

Kardan O, Weigard A, Cope L, Martz M, Angstadt M, McCurry KL, et al. Functional brain connectivity predictors of prospective substance use initiation and their environmental correlates. medRxiv. 2024. https://doi.org/10.1101/2024.05.29.24308134.

Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. Nat Med. 2008;14(9):923–30. https://doi.org/10.1038/nm.f.1869.

Kirisci L, Tarter RE, Vanyukov M, Reynolds M, Habeych M. Relation between cognitive distortions and neurobehavior disinhibition on the development of substance use during adolescence and substance use disorder by young adulthood: a prospective study. Drug Alcohol Depend. 2004;76(2):125–33. https://doi.org/10.1016/j.drugalcdep.2004.04.015.

Kolp H, Horvath S, Fite PJ, Metrik J, Stuart GL, Lisdahl KM, Shorey RC. Development of the alcohol and cannabis simultaneous use scale (ACSUS) in college students. J Subst Use. 2024;29(4):509–16. https://doi.org/10.1080/14659891.2023.2183149.

Kroon E, Kuhns L, Colyer-Patel K, Filbey F, Cousijn J. Working memory-related brain activity in cannabis use disorder: the role of cross-cultural differences in cannabis attitudes. Addict Biol. 2023;28(6):e13283. https://doi.org/10.1111/adb.13283.

Kroon E, Toenders YJ, Kuhns LN, Cousijn J, Filbey F. Resting state functional connectivity in dependent cannabis users: the moderating role of cannabis attitudes. Drug Alcohol Depend. 2024;256:111090. https://doi.org/10.1016/j.drugalcdep.2024.111090.

Kruger JS, Kruger DJ. Delta-8-THC: Delta-9-THC's nicer younger sibling? J Cannabis Res. 2022;4(1):4. https://doi.org/10.1186/s42238-021-00115-8.

Kruger DJ, Kruger JS. Consumer experiences with Delta-8-THC: medical use, pharmaceutical substitution, and comparisons with Delta-9-THC. Cannabis Cannabinoid Res. 2023;8(1):166–73. https://doi.org/10.1089/can.2021.0124.

Laurikainen H, Tuominen L, Tikka M, Merisaari H, Armio RL, Sormunen E, et al. Sex difference in brain CB1 receptor availability in man. Neuroimage. 2019;184:834–42. https://doi.org/10.1016/j.neuroimage.2018.10.013.

Lawn W, Freeman TP, Pope RA, Joye A, Harvey L, Hindocha C, et al. Acute and chronic effects of cannabinoids on effort-related decision-making and reward learning: an evaluation of the cannabis 'amotivational' hypotheses. Psychopharmacology (Berl). 2016;233(19–20):3537–52. https://doi.org/10.1007/s00213-016-4383-x.

Lawn W, Fernandez-Vinson N, Mokrysz C, Hogg G, Lees R, Trinci K, et al. The CannTeen study: verbal episodic memory, spatial working memory, and response inhibition in adolescent and adult cannabis users and age-matched controls. Psychopharmacology (Berl). 2022;239(5):1629–41. https://doi.org/10.1007/s00213-022-06143-3.

Lawn W, Trinci K, Mokrysz C, Borissova A, Ofori S, Petrilli K, et al. The acute effects of cannabis with and without cannabidiol in adults and adolescents: a randomised, double-blind, placebo-controlled, crossover experiment. Addiction. 2023;118(7):1282–94. https://doi.org/10.1111/add.16154.

Lee TT, Gorzalka BB. Timing is everything: evidence for a role of corticolimbic endocannabinoids in modulating hypothalamic-pituitary-adrenal axis activity across developmental periods. Neuroscience. 2012;204:17–30. https://doi.org/10.1016/j.neuroscience.2011.10.006.

Lees B, Debenham J, Squeglia LM. Alcohol and cannabis use and the developing brain. Alcohol Res. 2021;41(1):11. https://doi.org/10.35946/arcr.v41.1.11.

Lenroot RK, Giedd JN. Sex differences in the adolescent brain. Brain Cogn. 2010;72(1):46–55. https://doi.org/10.1016/j.bandc.2009.10.008.

Li M, Dang X, Chen Y, Chen Z, Xu X, Zhao Z, Wu D. Cognitive processing speed and accuracy are intrinsically different in genetic architecture and brain phenotypes. Nat Commun. 2024;15(1):7786. https://doi.org/10.1038/s41467-024-52222-8.

Lisdahl KM, Price JS. Increased marijuana use and gender predict poorer cognitive functioning in adolescents and emerging adults. J Int Neuropsychol Soc. 2012;18(4):678–88. https://doi.org/10.1017/S1355617712000276.

Lisdahl KM, Gilbart ER, Wright NE, Shollenbarger S. Dare to delay? The impacts of adolescent alcohol and marijuana use onset on cognition, brain structure, and function. Front Psych. 2013;4:53. https://doi.org/10.3389/fpsyt.2013.00053.

Lisdahl KM, Wright NE, Kirchner-Medina C, Maple KE, Shollenbarger S. Considering cannabis: the effects of regular cannabis use on neurocognition in adolescents and young adults. Curr Addict Rep. 2014;1(2):144–56. https://doi.org/10.1007/s40429-014-0019-6.

Lisdahl KM, Sher KJ, Conway KP, Gonzalez R, Feldstein Ewing SW, Nixon S, J.,…Heitzeg, M. Adolescent brain cognitive development (ABCD) study: overview of substance use assessment methods. Dev Cogn Neurosci. 2018;32:80–96. https://doi.org/10.1016/j.dcn.2018.02.007.

Lisdahl K, Filbey F, Gruber S. Introduction to JINS special issue: clarifying the complexities of cannabis and cognition. J Int Neuropsychol Soc. 2021a;27(6):515–9. https://doi.org/10.1017/S1355617721000874.

Lisdahl KM, Tapert S, Sher KJ, Gonzalez R, Nixon SJ, Feldstein Ewing SW, et al. Substance use patterns in 9-10 year olds: baseline findings from the adolescent brain cognitive development (ABCD) study. Drug Alcohol Depend. 2021b;227:108946. https://doi.org/10.1016/j.drugalcdep.2021.108946.

Lorenzetti V, Takagi M, van Dalen Y, Yücel M, Solowij N. Investigating the residual effects of chronic cannabis use and abstinence on verbal and visuospatial learning. Front Psych. 2021;12:663701. https://doi.org/10.3389/fpsyt.2021.663701.

Lorenzetti V, McTavish E, Broyd S, van Hell H, Thomson D, Ganella E, et al. Daily Cannabidiol administration for 10 weeks modulates hippocampal and Amygdalar resting-state functional connectivity in cannabis users: a functional magnetic resonance imaging open-label clinical trial. Cannabis Cannabinoid Res. 2024;9(4):e1108–21. https://doi.org/10.1089/can.2022.0336.

Lovell ME, Akhurst J, Padgett C, Garry MI, Matthews A. Cognitive outcomes associated with long-term, regular, recreational cannabis use in adults: a meta-analysis. Exp Clin Psychopharmacol. 2020;28(4):471–94. https://doi.org/10.1037/pha0000326.

Luciana M, Bjork JM, Nagel BJ, Barch DM, Gonzalez R, Nixon SJ, Banich MT. Adolescent neurocognitive development and impacts of substance use: overview of the adolescent brain cognitive development (ABCD) baseline neurocognition battery. Dev Cogn Neurosci. 2018;32:67–79. https://doi.org/10.1016/j.dcn.2018.02.006.

Macedo I, Paiva TO, Pasion R, Daedelow L, Heinz A, Magalhães A, et al. Light cannabis use and the adolescent brain: an 8-years longitudinal assessment of mental health, cognition, and reward processing. Psychopharmacology (Berl). 2024;241(7):1447–61. https://doi.org/10.1007/s00213-024-06575-z.

Machado AS, Bragança M, Vieira-Coelho M. Epigenetic effects of cannabis: a systematic scoping review of behavioral and emotional symptoms associated with cannabis use and exocannabinoid exposure. Drug Alcohol Depend. 2024;263:111401. https://doi.org/10.1016/j.drugalcdep.2024.111401.

Mackie K. Distribution of cannabinoid receptors in the central and peripheral nervous system. Handb Exp Pharmacol. 2005;(168):299–325.

Mahmood OM, Jacobus J, Bava S, Scarlett A, Tapert SF. Learning and memory performances in adolescent users of alcohol and marijuana: interactive effects. J Stud Alcohol Drugs. 2010;71(6):885–94. https://doi.org/10.15288/jsad.2010.71.885. PMID: 20946746; PMCID: PMC2965487.

Manterola A, Bernal-Chico A, Cipriani R, Canedo-Antelo M, Moreno-García Á, Martín-Fontecha M, et al. Deregulation of the endocannabinoid system and therapeutic potential of ABHD6 blockade in the cuprizone model of demyelination. Biochem Pharmacol. 2018;157:189–201. https://doi.org/10.1016/j.bcp.2018.07.042.

Maple KE, McDaniel KA, Shollenbarger SG, Lisdahl KM. Dose-dependent cannabis use, depressive symptoms, and FAAH genotype predict sleep quality in emerging adults: a pilot study. Am J Drug Alcohol Abuse. 2016;42(4):431–40. https://doi.org/10.3109/00952990.2016.1141913.

Maple KE, Thomas AM, Kangiser MM, Lisdahl KM. Anterior cingulate volume reductions in abstinent adolescent and young adult cannabis users: association with affective processing deficits. Psychiatr Res Neuroimaging. 2019;288:51–9. https://doi.org/10.1016/j.pscychresns.2019.04.011.

Martin EL, Baker NL, Sempio C, Christians U, Klawitter J, McRae-Clark AL. Sex differences in endocannabinoid tone in a pilot study of cannabis use disorder and acute cannabis abstinence. Addict Biol. 2023;28(10):e13337. https://doi.org/10.1111/adb.13337.

Mathias CW, Blumenthal TD, Dawes MA, Liguori A, Richard DM, Bray B, et al. Failure to sustain prepulse inhibition in adolescent marijuana users. Drug Alcohol Depend. 2011;116(1–3):110–6. https://doi.org/10.1016/j.drugalcdep.2010.11.020.

Mato S, Del Olmo E, Pazos A. Ontogenetic development of cannabinoid receptor expression and signal transduction functionality in the human brain. Eur J Neurosci. 2003;17(9):1747–54.

McHale S, Hunt N. Executive function deficits in short-term abstinent cannabis users. Hum Psychopharmacol. 2008;23(5):409–15. https://doi.org/10.1002/hup.941.

Meda SA, Gueorguieva RV, Pittman B, Rosen RR, Aslanzadeh F, Tennen H, Leen S, Hawkins K, Raskin S, Wood RM, Austad CS, Dager A, Fallahi C, Pearlson GD. Longitudinal influence of alcohol and marijuana use on academic performance in college students. PLoS One. 2017;12(3):e0172213. https://doi.org/10.1371/journal.pone.0172213. PMID: 28273162; PMCID: PMC5342177.

Medina KL, Hanson KL, Schweinsburg AD, Cohen-Zion M, Nagel BJ, Tapert SF. Neuropsychological functioning in adolescent marijuana users: subtle deficits detectable after a month of abstinence. J Int Neuropsychol Soc. 2007a;13(5):807–20. https://doi.org/10.1017/S1355617707071032.

Medina KL, Nagel BJ, Park A, McQueeny T, Tapert SF. Depressive symptoms in adolescents: associations with white matter volume and marijuana use. J Child Psychol Psychiatry. 2007b;48(6):592–600. https://doi.org/10.1111/j.1469-7610.2007.01728.x.

Meehan TP, Bressler SL, Tang W, Astafiev SV, Sylvester CM, Shulman GL, Corbetta M. Top-down cortical interactions in visuospatial attention. Brain Struct Funct. 2017;222(7):3127–45. https://doi.org/10.1007/s00429-017-1390-6.

Meehan-Atrash J, Rahman I. Cannabis vaping: existing and emerging modalities, chemistry, and pulmonary toxicology. Chem Res Toxicol. 2021;34(10):2169–79. https://doi.org/10.1021/acs.chemrestox.1c00290.

Meier MH. Associations between butane hash oil use and cannabis-related problems. Drug Alcohol Depend. 2017;179:25–31. https://doi.org/10.1016/j.drugalcdep.2017.06.015.

Meier MH. Cannabis use and psychosocial functioning: evidence from prospective longitudinal studies. Curr Opin Psychol. 2021;38:19–24. https://doi.org/10.1016/j.copsyc.2020.07.001.

Meier MH, Caspi A, Ambler A, Harrington H, Houts R, Keefe RS, et al. Persistent cannabis users show neuropsychological decline from childhood to midlife. Proc Natl Acad Sci U S A. 2012;109(40):E2657–64. https://doi.org/10.1073/pnas.1206820109.

Meier MH, Hill ML, Small PJ, Luthar SS. Associations of adolescent cannabis use with academic performance and mental health: a longitudinal study of upper middle class youth. Drug Alcohol Depend. 2015;156:207–12. https://doi.org/10.1016/j.drugalcdep.2015.09.010.

Meier MH, Caspi A, Danese A, Fisher HL, Houts R, Arseneault L, Moffitt TE. Associations between adolescent cannabis use and neuropsychological decline: a longitudinal co-twin control study. Addiction. 2018;113(2):257–65. https://doi.org/10.1111/add.13946.

Meza C, Stefan C, Staines WR, Feinstein A. A preliminary investigation of sex differences in cognitive and fMRI changes following 28 days of cannabis abstinence. Mult Scler Relat Disord. 2024a;89:105759. https://doi.org/10.1016/j.msard.2024.105759.

Meza C, Stefan C, Staines WR, Feinstein A. The effects of cannabis abstinence on cognition and resting state network activity in people with multiple sclerosis: a preliminary study. Neuroimage Clin. 2024b;43:103622. https://doi.org/10.1016/j.nicl.2024.103622.

Miech RA, Johnston LD, Patrick ME, et al. Monitoring the future national survey results on drug use, 1975–2023: secondary school students. In: Monitoring the future monograph series. Ann Arbor, MI: Institute for Social Research, University of Michigan; 2023.

Miederer I, Uebbing K, Röhrich J, Maus S, Bausbacher N, Krauter K, et al. Effects of tetrahydrocannabinol on glucose uptake in the rat brain. Neuropharmacology. 2017;117:273–81. https://doi.org/10.1016/j.neuropharm.2017.02.011.

Mills KL, Goddings AL, Clasen LS, Giedd JN, Blakemore SJ. The developmental mismatch in structural brain maturation during adolescence. Dev Neurosci. 2014a;36(3–4):147–60. https://doi.org/10.1159/000362328.

Mills KL, Lalonde F, Clasen LS, Giedd JN, Blakemore SJ. Developmental changes in the structure of the social brain in late childhood and adolescence. Soc Cogn Affect Neurosci. 2014b;9(1):123–31. https://doi.org/10.1093/scan/nss113.

Moffitt TE, Meier MH, Caspi A, Poulton R. Reply to Rogeberg and Daly: no evidence that socioeconomic status or personality differences confound the association between cannabis use and IQ decline. Proc Natl Acad Sci U S A. 2013;110(11):E980–2. https://doi.org/10.1073/pnas.1300618110.

Mokrysz C, Landy R, Gage SH, Munafò MR, Roiser JP, Curran HV. Are IQ and educational outcomes in teenagers related to their cannabis use? A prospective cohort study. J Psychopharmacol. 2016;30(2):159–68. https://doi.org/10.1177/0269881115622241.

Moreno M, Estevez AF, Zaldivar F, Montes JM, Gutiérrez-Ferre VE, Esteban L, et al. Impulsivity differences in recreational cannabis users and binge drinkers in a university population. Drug Alcohol Depend. 2012;124(3):355–62. https://doi.org/10.1016/j.drugalcdep.2012.02.011.

Morgan CJ, Page E, Schaefer C, Chatten K, Manocha A, Gulati S, et al. Cerebrospinal fluid anandamide levels, cannabis use and psychotic-like symptoms. Br J Psychiatry. 2013;202(5):381–2. https://doi.org/10.1192/bjp.bp.112.121178.

Mullin CJ, Cservenka A. Cannabis Use and Academic Performance in College Students: The Role of Procrastination. Cannabis. 2024;7(2):108–122. https://doi.org/10.26828/cannabis/2024/000215. PMID: 38975598; PMCID: PMC11225980.

Murray CH, Huang Z, Lee R, de Wit H. Adolescents are more sensitive than adults to acute behavioral and cognitive effects of THC. Neuropsychopharmacology. 2022;47(7):1331–8. https://doi.org/10.1038/s41386-022-01281-w.

Noorbakhsh S, Afzali MH, Boers E, Conrod PJ. Cognitive function impairments linked to alcohol and cannabis use during adolescence: a study of gender differences. Front Hum Neurosci. 2020;14:95. https://doi.org/10.3389/fnhum.2020.00095.

Olalde-Mathieu VE, Atilano-Barbosa D, Angulo-Perkins A, Licea-Haquet GL, Dominguez-Frausto CA, Barrios FA, Alcauter S. Empathy-related differences in the anterior cingulate functional connectivity of regular cannabis users when compared to controls. J Neurosci Res. 2024;102(1):e25252. https://doi.org/10.1002/jnr.25252.

Oltrabella F, Melgoza A, Nguyen B, Guo S. Role of the endocannabinoid system in vertebrates: emphasis on the zebrafish model. Dev Growth Differ. 2017;59(4):194–210. https://doi.org/10.1111/dgd.12351.

Pacheco-Colón I, Hawes SW, Duperrouzel JC, Gonzalez R. Evidence lacking for cannabis users slacking: a longitudinal analysis of escalating cannabis use and motivation among adolescents. J Int Neuropsychol Soc. 2021;27(6):637–47. https://doi.org/10.1017/S135561772000096X.

Paige KJ, Colder CR. Long-term effects of early adolescent marijuana use on attentional and inhibitory control. J Stud Alcohol Drugs. 2020;81(2):164–72. https://doi.org/10.15288/jsad.2020.81.164.

Pardini D, White HR, Xiong S, Bechtold J, Chung T, Loeber R, Hipwell A. Unfazed or dazed and confused: does early adolescent marijuana use cause sustained impairments in attention and

academic functioning? J Abnorm Child Psychol. 2015;43(7):1203–17. https://doi.org/10.1007/s10802-015-0012-0.

Parlar M, MacKillop E, Petker T, Murphy J, MacKillop J. Cannabis use, age of initiation, and neurocognitive performance: findings from a large sample of heavy drinking emerging adults. J Int Neuropsychol Soc. 2021;27(6):533–45. https://doi.org/10.1017/S1355617721000618.

Pfefferbaum A, Rohlfing T, Pohl KM, Lane B, Chu W, Kwon D, et al. Adolescent development of cortical and White matter structure in the NCANDA sample: role of sex, ethnicity, puberty, and alcohol drinking. Cereb Cortex. 2016;26(10):4101–21. https://doi.org/10.1093/cercor/bhv205.

Pope HG Jr, Gruber AJ, Hudson JI, Cohane G, Huestis MA, Yurgelun-Todd D. Early-onset cannabis use and cognitive deficits: what is the nature of the association? Drug Alcohol Depend. 2003;69(3):303–10. https://doi.org/10.1016/s0376-8716(02)00334-4. PMID: 12633916.

Poulia N, Delis F, Brakatselos C, Polissidis A, Koutmani Y, Kokras N, et al. Detrimental effects of adolescent escalating low-dose Δ. Br J Pharmacol. 2021;178(7):1722–36. https://doi.org/10.1111/bph.15394.

Power E, Sabherwal S, Healy C, et al. Intelligence quotient decline following frequent or dependent cannabis use in youth: a systematic review and meta-analysis of longitudinal studies. Psychol Med. 2021;51(2):194–200. https://doi.org/10.1017/S0033291720005036.

Prashad S, Milligan AL, Cousijn J, Filbey FM. Cross-cultural effects of cannabis use disorder: evidence to support a cultural neuroscience approach. Curr Addict Rep. 2017;4(2):100–9. https://doi.org/10.1007/s40429-017-0145-z.

Prince van Leeuwen A, Creemers HE, Verhulst FC, Ormel J, Huizink AC. Are adolescents gambling with cannabis use? A longitudinal study of impulsivity measures and adolescent substance use: the TRAILS study. J Stud Alcohol Drugs. 2011;72(1):70–8. https://doi.org/10.15288/jsad.2011.72.70.

Raznahan A, Shaw PW, Lerch JP, Clasen LS, Greenstein D, Berman R, et al. Longitudinal four-dimensional mapping of subcortical anatomy in human development. Proc Natl Acad Sci U S A. 2014;111(4):1592–7. https://doi.org/10.1073/pnas.1316911111.

Rubin-Kahana DS, Butler K, Hassan AN, Sanches M, Le Foll B. Cannabis use characteristics associated with self-reported cognitive function in a nationally representative U.S. sample. Subst Use Misuse. 2024;59(9):1303–12. https://doi.org/10.1080/10826084.2024.2340975.

Rubino T, Vigano' D, Realini N, Guidali C, Braida D, Capurro V, et al. Chronic delta 9-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. Neuropsychopharmacology. 2008;33(11):2760–71. https://doi.org/10.1038/sj.npp.1301664.

Rubino T, Prini P, Piscitelli F, Zamberletti E, Trusel M, Melis M, et al. Adolescent exposure to THC in female rats disrupts developmental changes in the prefrontal cortex. Neurobiol Dis. 2015;73:60–9. https://doi.org/10.1016/j.nbd.2014.09.015.

Sagar KA, Gruber SA. Marijuana matters: reviewing the impact of marijuana on cognition, brain structure and function, & exploring policy implications and barriers to research. Int Rev Psychiatry. 2018;1-17:251. https://doi.org/10.1080/09540261.2018.1460334.

Sagar KA, Dahlgren MK, Gönenç A, Racine MT, Dreman MW, Gruber SA. The impact of initiation: early onset marijuana smokers demonstrate altered stroop performance and brain activation. Dev Cogn Neurosci. 2015;16:84–92. https://doi.org/10.1016/j.dcn.2015.03.003.

Sagar KA, Dahlgren MK, Lambros AM, Smith RT, El-Abboud C, Gruber SA. An observational, longitudinal study of cognition in medical cannabis patients over the course of 12 months of treatment: preliminary results. J Int Neuropsychol Soc. 2021;27(6):648–60. https://doi.org/10.1017/S1355617721000114.

Savulich G, Rychik N, Lamberth E, Hareli M, Evins AE, Sahakian BJ, Schuster RM. Sex differences in neuropsychological functioning are domain-specific in adolescent and young adult regular cannabis users. J Int Neuropsychol Soc. 2021;27(6):592–606. https://doi.org/10.1017/S1355617720001435.

Schaefer JD, Hamdi NR, Malone SM, Vrieze S, Wilson S, McGue M, Iacono WG. Associations between adolescent cannabis use and young-adult functioning in three longitudinal twin

studies. Proc Natl Acad Sci U S A. 2021;118(14):e2013180118. https://doi.org/10.1073/pnas.2013180118.

Schacht JP, Hutchison KE, Filbey FM. Associations between cannabinoid receptor-1 (CNR1) variation and hippocampus and amygdala volumes in heavy cannabis users. Neuropsychopharmacology. 2012;37(11):2368–76. https://doi.org/10.1038/npp.2012.92. Epub 2012 Jun 6. PMID: 22669173; PMCID: PMC3442352.

Schlienz NJ, Budney AJ, Lee DC, Vandrey R. Cannabis withdrawal: a review of neurobiological mechanisms and sex differences. Curr Addict Rep. 2017;4(2):75–81. https://doi.org/10.1007/s40429-017-0143-1.

Schmitt JE, Neale MC, Fassassi B, Perez J, Lenroot RK, Wells EM, Giedd JN. The dynamic role of genetics on cortical patterning during childhood and adolescence. Proc Natl Acad Sci U S A. 2014;111(18):6774–9. https://doi.org/10.1073/pnas.1311630111.

Schnakenberg Martin AM, D'Souza DC, Newman SD, Hetrick WP, O'Donnell BF. Differential cognitive performance in females and males with regular cannabis use. J Int Neuropsychol Soc. 2021;27(6):570–80. https://doi.org/10.1017/S1355617721000606.

Schreiner AM, Dunn ME. Residual effects of cannabis use on neurocognitive performance after prolonged abstinence: a meta-analysis. Exp Clin Psychopharmacol. 2012;20(5):420–9. https://doi.org/10.1037/a0029117.

Schuster RM, Crane NA, Mermelstein R, Gonzalez R. Tobacco may mask poorer episodic memory among young adult cannabis users. Neuropsychology. 2015;29(5):759–66. https://doi.org/10.1037/neu0000173.

Schuster RM, Gilman J, Schoenfeld D, Evenden J, Hareli M, Ulysse C, et al. One month of cannabis abstinence in adolescents and young adults is associated with improved memory. J Clin Psychiatry. 2018;79(6):17m11977. https://doi.org/10.4088/JCP.17m11977.

Scott JC. Impact of adolescent cannabis use on neurocognitive and brain development. Child Adolesc Psychiatr Clin N Am. 2023;32(1):21–42. https://doi.org/10.1016/j.chc.2022.06.002.

Scott JC, Slomiak ST, Jones JD, Rosen AFG, Moore TM, Gur RC. Association of cannabis with cognitive functioning in adolescents and young adults: a systematic review and meta-analysis. JAMA Psychiatry. 2018;75(6):585–95. https://doi.org/10.1001/jamapsychiatry.2018.0335.

Selamoglu A, Langley C, Crean R, Savulich G, Cormack F, Sahakian BJ, Mason B. Neuropsychological performance in young adults with cannabis use disorder. J Psychopharmacol. 2021;35(11):1349–1355. https://doi.org/10.1177/02698811211050548. Epub 2021 Oct 25. PMID: 34694178; PMCID: PMC8600580.

Shollenbarger SG, Price J, Wieser J, Lisdahl K. Poorer frontolimbic white matter integrity is associated with chronic cannabis use, FAAH genotype, and increased depressive and apathy symptoms in adolescents and young adults. Neuroimage Clin. 2015;8:117–25. https://doi.org/10.1016/j.nicl.2015.03.024.

Shollenbarger S, Thomas AM, Wade NE, Gruber SA, Tapert SF, Filbey FM, Lisdahl KM. Intrinsic frontolimbic connectivity and mood symptoms in young adult cannabis users. Front Public Health. 2019;7:311. https://doi.org/10.3389/fpubh.2019.00311.

Silveira MM, Adams WK, Morena M, Hill MN, Winstanley CA. Tetrahydrocannabinol decreases willingness to exert cognitive effort in male rats. J Psychiatr Neurosci. 2016;41(6):150363.

Sirotiak Z, Gallagher BT, Smith-Hernandez CA, Showman LJ, Hillard CJ, Brellenthin AG. Endocannabinoid and psychological responses to acute resistance exercise in trained and untrained adults. PLoS One. 2023;18(12):e0291845. https://doi.org/10.1371/journal.pone.0291845.

Sisk LM, Rapuano KM, Conley MI, Greene AS, Horien C, Rosenberg MD, et al. Genetic variation in endocannabinoid signaling is associated with differential network-level functional connectivity in youth. J Neurosci Res. 2022;100(3):731–43. https://doi.org/10.1002/jnr.24946.

Skumlien M, Mokrysz C, Freeman TP, Valton V, Wall MB, Bloomfield M, et al. Anhedonia, apathy, pleasure, and effort-based decision-making in adult and adolescent cannabis users and controls. Int J Neuropsychopharmacol. 2023;26(1):9–19. https://doi.org/10.1093/ijnp/pyac056.

Sloan ME, Grant CW, Gowin JL, Ramchandani VA, Le Foll B. Endocannabinoid signaling in psychiatric disorders: a review of positron emission tomography studies. Acta Pharmacol Sin. 2019;40(3):342–50. https://doi.org/10.1038/s41401-018-0081-z.

Solowij N, Jones KA, Rozman ME, Davis SM, Ciarrochi J, Heaven PC, et al. Verbal learning and memory in adolescent cannabis users, alcohol users and non-users. Psychopharmacology (Berl). 2011;216(1):131–44. https://doi.org/10.1007/s00213-011-2203-x.

Solowij N, Jones KA, Rozman ME, Davis SM, Ciarrochi J, Heaven PC, et al. Reflection impulsivity in adolescent cannabis users: a comparison with alcohol-using and non-substance-using adolescents. Psychopharmacology (Berl). 2012;219(2):575–86. https://doi.org/10.1007/s00213-011-2486-y.

Solowij N, Broyd SJ, Beale C, Prick JA, Greenwood LM, van Hell H, et al. Therapeutic effects of prolonged Cannabidiol treatment on psychological symptoms and cognitive function in regular cannabis users: a pragmatic open-label clinical trial. Cannabis Cannabinoid Res. 2018;3(1):21–34. https://doi.org/10.1089/can.2017.0043.

Sorkhou M, Rabin RA, Rabin JS, Kloiber S, McIntyre RS, George TP. Effects of 28 days of cannabis abstinence on cognition in major depressive disorder: a pilot study. Am J Addict. 2022;31(5):454–62. https://doi.org/10.1111/ajad.13305.

Spechler PA, Orr CA, Chaarani B, Kan KJ, Mackey S, Morton A, et al. Cannabis use in early adolescence: evidence of amygdala hypersensitivity to signals of threat. Dev Cogn Neurosci. 2015;16:63–70. https://doi.org/10.1016/j.dcn.2015.08.007.

Spechler PA, Chaarani B, Orr C, Albaugh MD, Fontaine NR, Higgins ST, et al. Longitudinal associations between amygdala reactivity and cannabis use in a large sample of adolescents. Psychopharmacology (Berl). 2020;237(11):3447–58. https://doi.org/10.1007/s00213-020-05624-7.

Spindle TR, Kuwabara H, Eversole A, Nandi A, Vandrey R, Antoine DG, et al. Brain imaging of cannabinoid type I (CB). Addict Biol. 2021;26(6):e13061. https://doi.org/10.1111/adb.13061.

Squeglia LM, Jacobus J, Sorg SF, Jernigan TL, Tapert SF. Early adolescent cortical thinning is related to better neuropsychological performance. J Int Neuropsychol Soc. 2013;19(9):962–70. https://doi.org/10.1017/S1355617713000878.

Squeglia LM, Jacobus J, Nguyen-Louie TT, Tapert SF. Inhibition during early adolescence predicts alcohol and marijuana use by late adolescence. Neuropsychology. 2014;28(5):782–90. https://doi.org/10.1037/neu0000083.

Streck JM, Hughes JR, Klemperer EM, Howard AB, Budney AJ. Modes of cannabis use: a secondary analysis of an intensive longitudinal natural history study. Addict Behav. 2019;98:106033. https://doi.org/10.1016/j.addbeh.2019.106033.

Stringfield SJ, Torregrossa MM. Intravenous self-administration of delta-9-THC in adolescent rats produces long-lasting alterations in behavior and receptor protein expression. Psychopharmacology (Berl). 2021;238(1):305–19. https://doi.org/10.1007/s00213-020-05684-9.

Sullivan RM, Wallace AL, Wade NE, Swartz AM, Lisdahl KM. Assessing the role of cannabis use on cortical surface structure in adolescents and young adults: exploring gender and aerobic fitness as potential moderators. Brain Sci. 2020;10(2):117. https://doi.org/10.3390/brainsci10020117.

Sullivan RM, Wallace AL, Wade NE, Swartz AM, Lisdahl KM. Cannabis use and brain volume in adolescent and Young adult cannabis users: effects moderated by sex and aerobic fitness. J Int Neuropsychol Soc. 2021;27(6):607–20. https://doi.org/10.1017/S135561772100062X.

Sullivan RM, Maple KE, Wallace AL, Thomas AM, Lisdahl KM. Examining inhibitory affective processing within the rostral anterior cingulate cortex among abstinent cannabis-using adolescents and Young adults. Front Psych. 2022a;13:851118. https://doi.org/10.3389/fpsyt.2022.851118.

Sullivan RM, Wade NE, Wallace AL, Tapert SF, Pelham WE, Brown SA, et al. Substance use patterns in 9 to 13-year-olds: longitudinal findings from the adolescent brain cognitive develop-

ment (ABCD) study. Drug Alcohol Depend Rep. 2022b;5:100120. https://doi.org/10.1016/j.dadr.2022.100120.

Sullivan RM, Wallace AL, Stinson EA, Montoto KV, Kaiver CM, Wade NE, Lisdahl KM. Assessment of withdrawal, mood, and sleep inventories after monitored 3-week abstinence in cannabis-using adolescents and Young adults. Cannabis Cannabinoid Res. 2022c;7(5):690–9. https://doi.org/10.1089/can.2021.0074.

Suerken CK, Reboussin BA, Egan KL, Sutfin EL, Wagoner KG, Spangler J, Wolfson M. Marijuana use trajectories and academic outcomes among college students. Drug Alcohol Depend. 2016;162:137–45. https://doi.org/10.1016/j.drugalcdep.2016.02.041. Epub 2016 Mar 19. PMID: 27020322; PMCID: PMC4835174.

Tait RJ, Mackinnon A, Christensen H. Cannabis use and cognitive function: 8-year trajectory in a young adult cohort. Addiction. 2011;106(12):2195–203. https://doi.org/10.1111/j.1360-0443.2011.03574.x.

Takagi M, Lubman DI, Cotton S, Fornito A, Baliz Y, Tucker A, Yücel M. Executive control among adolescent inhalant and cannabis users. Drug Alcohol Rev. 2011;30(6):629–37. https://doi.org/10.1111/j.1465-3362.2010.00256.x.

Tamm L, Epstein JN, Lisdahl KM, Molina B, Tapert S, Hinshaw SP, et al. Impact of ADHD and cannabis use on executive functioning in young adults. Drug Alcohol Depend. 2013;133(2):607–14. https://doi.org/10.1016/j.drugalcdep.2013.08.001.

Tapert SF, Baratta MV, Abrantes AM, Brown SA. Attention dysfunction predicts substance involvement in community youths. J Am Acad Child Adolesc Psychiatry. 2002a;41(6):680–6. https://doi.org/10.1097/00004583-200206000-00007.

Tapert SF, Granholm E, Leedy NG, Brown SA. Substance use and withdrawal: neuropsychological functioning over 8 years in youth. J Int Neuropsychol Soc. 2002b;8(7):873–83.

Taurisano P, Pergola G, Monda A, Antonucci LA, Di Carlo P, Piarulli F, Passiatore R, Papalino M, Romano R, Monaco A, Rampino A, Bonvino A, Porcelli A, Popolizio T, Bellotti R, Bertolino A, Blasi G. The interaction between cannabis use and a CB1-related polygenic co-expression index modulates dorsolateral prefrontal activity during working memory processing. Brain Imaging Behav. 2021;15(1):288–99. https://doi.org/10.1007/s11682-020-00256-z.

Taylor MB, Filbey FM. Residual effects of cannabis use on effort-based decision-making. J Int Neuropsychol Soc. 2021;27(6):559–69. https://doi.org/10.1017/S1355617721000473.

Terry GE, Liow JS, Zoghbi SS, Hirvonen J, Farris AG, Lerner A, et al. Quantitation of cannabinoid CB1 receptors in healthy human brain using positron emission tomography and an inverse agonist radioligand. Neuroimage. 2009;48(2):362–70. https://doi.org/10.1016/j.neuroimage.2009.06.059.

Thames AD, Arbid N, Sayegh P. Cannabis use and neurocognitive functioning in a non-clinical sample of users. Addict Behav. 2014;39(5):994–9. https://doi.org/10.1016/j.addbeh.2014.01.019.

Thieme U, Schelling G, Hauer D, Greif R, Dame T, Laubender RP, et al. Quantification of anandamide and 2-arachidonoylglycerol plasma levels to examine potential influences of tetrahydrocannabinol application on the endocannabinoid system in humans. Drug Test Anal. 2014;6(1–2):17–23. https://doi.org/10.1002/dta.1561.

Thoma RJ, Monnig MA, Lysne PA, Ruhl DA, Pommy JA, Bogenschutz M, et al. Adolescent substance abuse: the effects of alcohol and marijuana on neuropsychological performance. Alcohol Clin Exp Res. 2011;35(1):39–46. https://doi.org/10.1111/j.1530-0277.2010.01320.x.

Thompson K, Leadbeater B, Ames M, Merrin GJ. Associations between marijuana use trajectories and educational and occupational success in young adulthood. Prev Sci. 2019;20(2):257–69. https://doi.org/10.1007/s11121-018-0904-7.

Uban KA, Horton MK, Jacobus J, Heyser C, Thompson WK, Tapert SF, et al. Biospecimens and the ABCD study: rationale, methods of collection, measurement and early data. Dev Cogn Neurosci. 2018;32:97–106. https://doi.org/10.1016/j.dcn.2018.03.005.

Vinader-Caerols C, Monleón S. Binge drinking, alone or with cannabis, during adolescence triggers different effects on immediate visual memory in men and women. Front Psych. 2021;12:797221. https://doi.org/10.3389/fpsyt.2021.797221.

Viveros MP, Marco EM, Llorente R, Lopez-Gallardo M. Endocannabinoid system and synaptic plasticity: implications for emotional responses. Neural Plast. 2007;2007:52908. https://doi.org/10.1155/2007/52908.

Volkow ND, Hampson AJ, Baler RD. Don't worry, be happy: endocannabinoids and cannabis at the intersection of stress and reward. Annu Rev Pharmacol Toxicol. 2017;57:285–308. https://doi.org/10.1146/annurev-pharmtox-010716-104615.

Wade NE, Bagot KS, Cota CI, Fotros A, Squeglia LM, Meredith LR, Jacobus J. Orbitofrontal cortex volume prospectively predicts cannabis and other substance use onset in adolescents. J Psychopharmacol. 2019a;33(9):1124–31. https://doi.org/10.1177/0269881119855971.

Wade NE, Wallace AL, Swartz AM, Lisdahl KM. Aerobic fitness level moderates the association between cannabis use and executive functioning and psychomotor speed following abstinence in adolescents and Young adults. J Int Neuropsychol Soc. 2019b;25(2):134–45. https://doi.org/10.1017/S1355617718000966.

Wade NE, Bagot KS, Tapert SF, Gruber SA, Filbey FM, Lisdahl KM. Cognitive functioning related to binge alcohol and cannabis co-use in abstinent adolescents and Young adults. J Stud Alcohol Drugs. 2020;81(4):479–83.

Wade NE, Baca R, Courtney KE, McCabe CJ, Infante MA, Huestis MA, Jacobus J. Preliminary evidence for cannabis and nicotine urinary metabolites as predictors of verbal memory performance and learning among young adults. J Int Neuropsychol Soc. 2021a;27(6):546–58. https://doi.org/10.1017/S1355617721000205.

Wade NE, Gilbart E, Swartz AM, Lisdahl KM. Assessing aerobic fitness level in relation to affective and behavioral functioning in emerging adult cannabis users. Int J Ment Health Addict. 2021b;19(2):546–59. https://doi.org/10.1007/s11469-019-00091-4.

Wade NE, Tapert SF, Lisdahl KM, Huestis MA, Haist F. Substance use onset in high-risk 9-13 year-olds in the ABCD study. Neurotoxicol Teratol. 2022;91:107090. https://doi.org/10.1016/j.ntt.2022.107090.

Wade NE, McCabe CJ, Wallace AL, Gonzalez MR, Hoh E, Infante MA, et al. Clouding up cognition? Secondhand cannabis and tobacco exposure related to cognitive functioning in youth. Biol Psychiatry Glob Open Sci. 2023a;3(2):233–42. https://doi.org/10.1016/j.bpsgos.2022.01.010.

Wade NE, Sullivan RM, Tapert SF, Pelham WE, Huestis MA, Lisdahl KM, Haist F. Concordance between substance use self-report and hair analysis in community-based adolescents. Am J Drug Alcohol Abuse. 2023b;49(1):76–84. https://doi.org/10.1080/00952990.2023.2164931.

Wade NE, Courtney KE, Wallace AL, Hatz L, Jacobus J. Investigating sex differences and age of onset in emotion regulation, executive functioning, and cannabis use in adolescents and young adults. J Cannabis Res. 2024a;6(1):20. https://doi.org/10.1186/s42238-024-00225-z.

Wade NE, Wallace AL, Huestis MA, Lisdahl KM, Sullivan RM, Tapert SF. Cannabis use and neurocognitive performance at 13-14 years-old: optimizing assessment with hair toxicology in the adolescent brain cognitive development (ABCD) study. Addict Behav. 2024b;150:107930. https://doi.org/10.1016/j.addbeh.2023.107930.

Wallace AL, Wade NE, Hatcher KF, Lisdahl KM. Effects of cannabis use and subclinical ADHD symptomology on attention based tasks in adolescents and young adults. Arch Clin Neuropsychol. 2019;34(5):700–5. https://doi.org/10.1093/arclin/acy080. PMID: 30295694; PMCID: PMC6702590.

Wallace AL, Wade NE, Lisdahl KM. Impact of 2 weeks of monitored abstinence on cognition in adolescent and young adult cannabis users. J Int Neuropsychol Soc. 2020;26(8):776–84. https://doi.org/10.1017/S1355617720000260.

Weng Y, Kruschwitz J, Rueda-Delgado LM, Ruddy KL, Boyle R, Franzen L, et al. A robust brain network for sustained attention from adolescence to adulthood that predicts later substance use. Elife. 2024;13:RP97150. https://doi.org/10.7554/eLife.97150.

Wesley MJ, Lile JA, Hanlon CA, Porrino LJ. Abnormal medial prefrontal cortex activity in heavy cannabis users during conscious emotional evaluation. Psychopharmacology (Berl). 2016;233(6):1035–44. https://doi.org/10.1007/s00213-015-4180-y.

Wiedmann M, Kuitunen-Paul S, Basedow LA, Wolff M, DiDonato N, Franzen J, et al. DNA methylation changes associated with cannabis use and verbal learning performance in adolescents: an exploratory whole genome methylation study. Transl Psychiatry. 2022;12(1):317. https://doi.org/10.1038/s41398-022-02025-6.

Willford JA, Goldschmidt L, De Genna NM, Day NL, Richardson GA. A longitudinal study of the impact of marijuana on adult memory function: prenatal, adolescent, and young adult exposures. Neurotoxicol Teratol. 2021;84:106958. https://doi.org/10.1016/j.ntt.2021.106958.

Winters DE, Wu W, Fukui S. Longitudinal effects of cognitive and affective empathy on adolescent substance use. Subst Use Misuse. 2020;55(6):983–9. https://doi.org/10.1080/10826084.2020.1717537.

Winters DE, Brandon-Friedman R, Yepes G, Hinckley JD. Systematic review and meta-analysis of socio-cognitive and socio-affective processes association with adolescent substance use. Drug Alcohol Depend. 2021;219:108479. https://doi.org/10.1016/j.drugalcdep.2020.108479.

Winters DE, Massey SH, Sakai JT. Adolescent substance use outcomes in response to social consequences of use: the role of empathy. J Drug Issues. 2024;54(2):202–17. https://doi.org/10.1177/00220426231159303.

Winward JL, Hanson KL, Tapert SF, Brown SA. Heavy alcohol use, marijuana use, and concomitant use by adolescents are associated with unique and shared cognitive decrements. J Int Neuropsychol Soc. 2014;20(8):784–95. https://doi.org/10.1017/S1355617714000666.

Wittemann M, Brielmaier J, Rubly M, Kennel J, Werler F, Schmitgen MM, et al. Cognition and cortical thickness in heavy cannabis users. Eur Addict Res. 2021;27(2):115–22. https://doi.org/10.1159/000509987.

Wright NE, Scerpella D, Lisdahl KM. Marijuana use is associated with behavioral approach and depressive symptoms in adolescents and emerging adults. PLoS One. 2016;11(11):e0166005. https://doi.org/10.1371/journal.pone.0166005.

Yan X, Bedillion MF, Claus ED, Huang-Pollock C, Ansell EB. Sex differences in the prospective association of excessively long reaction times and hazardous cannabis use at six months. Addict Behav Rep. 2024;20:100558. https://doi.org/10.1016/j.abrep.2024.100558.

Zamberletti E, Rubino T. Dos(e)age: role of dose and age in the long-term effect of cannabinoids on cognition. Molecules. 2022;27(4):1411. https://doi.org/10.3390/molecules27041411.

Zamberletti E, Gabaglio M, Prini P, Rubino T, Parolaro D. Cortical neuroinflammation contributes to long-term cognitive dysfunctions following adolescent delta-9-tetrahydrocannabinol treatment in female rats. Eur Neuropsychopharmacol. 2015;25(12):2404–15. https://doi.org/10.1016/j.euroneuro.2015.09.021.

Zehra A, Burns J, Liu CK, Manza P, Wiers CE, Volkow ND, Wang GJ. Cannabis addiction and the brain: a review. J Neuroimmune Pharmacol. 2018;13(4):438–52. https://doi.org/10.1007/s11481-018-9782-9.

Zhang C, Brook JS, Leukefeld CG, Brook DW. Trajectories of marijuana use from adolescence to adulthood as predictors of unemployment status in the early forties. Am J Addict. 2016;25(3):203–9. https://doi.org/10.1111/ajad.12361.

Zucker RA, Gonzalez R, Feldstein Ewing SW, Paulus MP, Arroyo J, Fuligni A, et al. Assessment of culture and environment in the adolescent brain and cognitive development study: rationale, description of measures, and early data. Dev Cogn Neurosci. 2018;32:107–20. https://doi.org/10.1016/j.dcn.2018.03.004.

Zuo CS, Lukas SE. Chronic cannabis use alters dACC-striatal glutamatergic balance. Pharmacol Biochem Behav. 2023;225:173544. https://doi.org/10.1016/j.pbb.2023.173544.

Zuo XN, Kelly C, Di Martino A, Mennes M, Margulies DS, Bangaru S, et al. Growing together and growing apart: regional and sex differences in the lifespan developmental trajectories of functional homotopy. J Neurosci. 2010;30(45):15034–43. https://doi.org/10.1523/JNEUROSCI.2612-10.2010.

Zuo CS, Davis KA, Kuppe MK, Dahlgren MK, Gruber S, Fitzmaurice GM, Lukas SE. Elevated striatal glutamate + glutamine in recreational cannabis users during abstinence. J Psychiatr Res. 2022a;146:192–200. https://doi.org/10.1016/j.jpsychires.2021.12.041.

Zuo CS, Davis KA, Lukas SE. Lower dACC glutamate in cannabis users during early phase abstinence. Neuropsychopharmacology. 2022b;47(11):1969–75. https://doi.org/10.1038/s41386-022-01321-5.

Zuo Y, Iemolo A, Montilla-Perez P, Li HR, Yang X, Telese F. Chronic adolescent exposure to cannabis in mice leads to sex-biased changes in gene expression networks across brain regions. Neuropsychopharmacology. 2022c;47(12):2071–80. https://doi.org/10.1038/s41386-022-01413-2.

Chapter 13
Adolescent Cannabis Use Disorder



Leslie H. Lundahl and David M. Ledgerwood

1 Introduction

Adolescence is a critical time of marked physical, psychological, and social development that coincides with initiation of substance use, including cannabis (Gray and Squeglia 2018; Patton et al. 2004). About 1.8 million youth aged 12–20 years tried cannabis for the first time in 2023 (Substance Abuse and Mental Health Services Administration [SAMHSA], 2024), a statistic made even more alarming by the fact that approximately 30% of adolescents who use cannabis will develop cannabis use disorder (CUD; Hasin et al. 2015). In fact, about 5% of adolescents met DSM 5 criteria for CUD in 2023, and the potential for negative cannabis-related health outcomes is greatly increased when use escalates to the level of a use disorder.

Accompanying the drastic changes in the legal landscape of cannabis over the last 20 years is an ever-expanding array of cannabis products available for consumption coupled with a significant increase in THC potency in these products. One study found that among adolescents who report cannabis use, 72% also endorsed having used high potency concentrates (e.g., products containing up to 90% THC) at least once in their lifetime (Meier et al. 2019). Anecdotal reports and emerging data show that use of high potency THC concentrates is associated with CUD that develops more rapidly, presents with more severe symptomatology, and is more difficult to treat than disorders associated with lower potency cannabis products.

Given that adolescence is a uniquely vulnerable developmental stage during which chronic cannabis exposure can alter neurodevelopment, disrupt emotional, social and academic functioning, and lead to significant adverse mental and

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physical health consequences, it is essential that mental health and other clinicians understand the impacts of CUD on adolescents. In this chapter we provide an overview of the effects of CUD on adolescent health and behavior. We also discuss screening for and diagnosis of CUD in adolescents, and review evidence-based treatment options and efficacy.

2 Prevalence and Trends

The 2023 Monitoring the Future survey found that rates of 12th grade cannabis use in the United States were stable across 2021 and 2022 at 31% each year, but these levels were significantly lower than during the pre-COVID 19 pandemic years (2020 and 2019 rates 35% and 36%, respectively; Miech et al. 2023). The same survey found increases in cannabis vaping among eighth, tenth and 12th grade students between 2021 and 2022, showing vaping may be emerging as a substitute to traditional cannabis smoking. In total, 6.3% of 12th graders reported using cannabis at least 20 of the past 30 days.

Updates published in 2024 reveal that contrary to past years of the survey, female respondents reported slightly higher use than males at all three grade levels for lifetime and past 12-month use, and higher among eighth and tenth grade girls for past 30-day use (Miech et al. 2024). Twelfth grade annual prevalence of use was higher among African American teens than among White or Hispanic 12th grade students, but rates showed little difference at younger grades based on race/ethnicity. Additional data from the Monitoring the Future survey found that 11.4% of 12th-grade students self-reported use of hemp-derived but psychoactive Delta-8-THC in the past 12 months (Harlow et al. 2024).

Some studies show legalization of cannabis does not appear to have significantly changed the probability of frequency of use among youth (Bailey et al. 2023). However, the overall findings in the literature appear to be somewhat mixed, and more studies show increased use of cannabis among young adults since legalization (O'Grady et al. 2024). Further, cannabis use prevalence may increase among individuals who perceive its use as less harmful following legalization (Mennis et al. 2023).

3 Diagnostic Criteria

Diagnostic criteria for cannabis use disorder (CUD) are the same for adolescents as they are for adults. The Diagnostic and Statistical Manual for Mental Disorders, fifth edition, text revised (American Psychiatric Association 2022) defines CUD as a "problematic pattern of cannabis use leading to clinically significant impairment or distress, as manifested by at least two [symptoms] occurring within a 12-month period. Symptoms include (American Psychiatric Association 2022):

1. Cannabis is often used in larger amounts or over a longer period than was intended.
2. There is a persistent desire or unsuccessful efforts to cut down or control cannabis use.
3. A great deal of time is spent in activities necessary to obtain cannabis, use cannabis, or recover from its effects.
4. Craving, or a strong desire or urge to use cannabis.
5. Recurrent cannabis use resulting in a failure to fulfill major role obligations at work, school, or home.
6. Continued cannabis use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of cannabis.
7. Important social, occupational, or recreational activities are given up or reduced because of cannabis use.
8. Recurrent cannabis use in situations in which it is physically hazardous.
9. Cannabis use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by cannabis.
10. Tolerance, as defined by either of the following:
    (a) A need for markedly increased amounts of cannabis to achieve intoxication or desired effect.
    (b) Markedly diminished effect with continued use of the same amount of cannabis.
11. Withdrawal, as manifested by either of the following:
    (a) The characteristic withdrawal syndrome for cannabis.
    (b) Cannabis (or a closely related substance) is taken to relieve or avoid withdrawal symptoms.

Severity specifiers include mild (2–3 symptoms present), moderate (4–5 symptoms present), or severe (6 or more symptoms present). Additional specifiers are available to denote whether the disorder is in remission (sustained remission, early remission), and whether the individual is in a controlled environment where access to cannabis is limited. Additional DSM 5 TR diagnoses include Cannabis Intoxication, Cannabis Withdrawal, and Unspecified Cannabis-Related Disorder. Cannabis withdrawal consisting primarily of affective and behavioral symptoms (e.g., craving, depressed mood, irritability, restlessness), and also some physical symptoms (e.g., headache, nausea, sweating), has been identified among adolescents in treatment for CUD, but the prevalence and magnitude of withdrawal symptoms may be lower than among adults (Vandrey et al. 2005).

Broadly speaking, the DSM-5 TR CUD symptoms are applicable to adolescents, but there are some developmental considerations that may be applied. Cannabis use is most likely to begin during adolescence or early adulthood (Farmer et al. 2015; Hasin et al. 2016). Further, adolescents are thought to be at particularly high risk of developing substance use disorders because this is a period of rapid brain development which makes young people particularly vulnerable to developing addictions

(Casey and Jones 2010). Childhood adverse events and homelessness prior to the 12th grade are also significantly associated with CUD in early adulthood (Moss et al. 2020). Further, there is evidence that cannabis exposure through peer influences can have an important impact on the development of CUD (Kuntsche and Jordan 2006).

4 Screening

Several screening tools are available to assess adolescent substance use and identify those who may be at risk of developing a cannabis (or other substance) use disorder. Information from assessments can also be used to guide the appropriate level of intervention. Some of the more commonly used, validated evidence-based screening tools for adolescents include: (1) The **Brief Screening Instrument for Adolescent Tobacco, Alcohol, and Drug Use (BSTAD)** is a frequency of use measure that can be used to characterize alcohol, tobacco, and cannabis use by adolescents into different risk categories (no reported use, low risk, high risk). Further assessment is prompted if any substance use is reported. Levy et al. (2023) reported high agreement between the BSTAD and a diagnostic interview. (2) The **CRAFFT** (Car, Relax, Alone, Family, Forget, Trouble) is a well-validated screening instrument (Knight et al. 2002) that can be self-administered as a questionnaire or by clinician interview and is intended for youth aged 12–21 years. It is designed to identify substance use, substance-related riding/driving risk and potential substance use disorder. The updated CRAFFT 2.1 (2018) includes vaping and edibles as methods of cannabis administration. **(3) The Global Appraisal of Individual Needs Short Screener (GAIN-SS)** screens for mental health conditions and substance use (Dennis et al. 2006). It can be self-administered by both adults and adolescents. Alternatively, health care providers may conduct unstructured interviews to explore substance-related consequences and identify potential targets for additional assessment and treatment.

5 CUD, and Adolescent Development and Health Outcomes

5.1 Mental Health

Cannabis use during adolescence is associated with other co-occurring mental health conditions and adolescence appears to be a critical period of brain development where teens are particularly vulnerable to the harmful effects of cannabis on mental health (Lubman et al. 2015). One large-scale epidemiological study using National Survey on Drug Use and Health data for adolescents aged 12–17 revealed cannabis use to be associated with major depression, suicidal ideation, cognitive difficulties, truancy, low GPA, arrest, fighting and aggression, with additional

increased prevalence of these difficulties for youth CUD (Sultan et al. 2023). Female participants with non-disordered and disordered cannabis use experienced significantly greater depression and suicidal ideation than did male participants in those groupings.

Patton et al. (2002) found that daily cannabis use among Australian young women was related to a five-fold increase in depression and anxiety symptoms in a 6-year longitudinal study. Greater frequency of cannabis use also predicted a two-fold increase in later anxiety and depression symptoms. Another Australian longitudinal study revealed that teens with early onset of cannabis use (by age 12) experienced more antisocial behavior, violence, cannabis- and alcohol-related harms, and nicotine/tobacco use at age 19 (Scholes-Balog et al. 2016). Another study found that cannabis and alcohol use during early adolescence was associated with poorer school performance, lower self-esteem, poorer family relationships, lower resistance to peer pressure, greater sensation seeking, positive cannabis expectancies, and conduct disorder (Flory et al. 2004). Crane et al. (2015) found a longitudinal relationship between adolescent cannabis use and later development of depression among males but not females. Others have also found relationships between early cannabis use and later development of depression and/or anxiety (e.g., Capaldi et al. 2022; Schaefer et al. 2021). In addition, there is evidence that mental health problems related to cannabis use during adolescence are still present in mid-adulthood (Brook et al. 2016; Brook et al. 2011a; Capaldi et al. 2022).

Early cannabis use may also increase risk for the development of psychotic disorders such as schizophrenia (Capaldi et al. 2022; Moore et al. 2007). Recent reviews of the literature have reported that studies consistently find early cannabis use to predict later development of psychotic disorders, earlier onset of psychosis, and a biological link between long-term and heavy cannabis use and psychosis (see Hasan et al. 2020; Volkow et al. 2016). Nevertheless, others have reported that the mechanism of the association between psychosis and cannabis use is not clear (e.g., see Ksir and Hart 2016).

Not all studies find clear evidence of the association between cannabis and development of mental health difficulties. Butler et al. (2019) revealed that cannabis use is associated with depression and anxiety among a sample of Canadian youth, although this relationship was no longer significant after controlling for a measure of overall psychological health. Womack et al. (2016) found only modest support that cannabis use during adolescence was associated with depressive symptoms during young adulthood among men from a lower socioeconomic background. Similarly, a study of upper middle class high school students revealed a relationship between persistent cannabis use and delinquency, but this association was no longer significant after controlling for alcohol and tobacco use, and cannabis use was not related to internalizing symptoms (Meier et al. 2015).

Despite the lack of universality, the findings that cannabis use during youth can lead to later mental health difficulties have largely been borne out in meta-analyses of longitudinal studies. One large meta-analysis of 14 longitudinal studies with 76,058 youth found later depression was associated with early cannabis use, especially when heavy cannabis use was considered (Lev-Ran et al. 2014). Another

recent meta-analysis (11 studies with 23,317 participants) revealed that cannabis use during adolescence was related to later depression and suicidal ideation, but not anxiety disorder (Gobbi et al. 2019).

Studies have also found greater development of additional mental health problems related to adolescent cannabis use. Cannabis use at a young age is associated with later suicidality (Fergusson et al. 2002). Several additional mental health problems are associated with early cannabis use including attention problems (Capaldi et al. 2022; Foster et al. 2018), antisocial and oppositional behaviors (Brook et al. 2011b; Fergusson et al. 2002; Foster et al. 2018; Schaefer et al. 2021), and substance misuse (Fergusson et al. 2002; Foster et al. 2018; Schaefer et al. 2021).

Animal and human studies have attempted to examine mechanisms underlying the interrelationship between cannabis use and mental health difficulties. Studies with CB1 knockout mice have demonstrated that CB1 receptor availability is associated with depressive and aggressive responses during stress (Martin et al. 2002). Additional studies have shown the role of CB1 receptor antagonists for modulating anxiety and depression (Griebel et al. 2005). Together these studies provide support for the role of cannabinoid receptors in emotional expression.

Human studies provide additional evidence for mechanisms underlying the relationship between cannabis and mental health problems. One study found that greater brain white matter volume was associated with lower depression scores among adolescents who use cannabis, but not those who do not use cannabis (Medina et al. 2007). Additional studies have shown, consistent with the animal studies described above, that CB1 receptors play a role in brain regions involved in the regulation of mood (Hungund et al. 2004; Vinod et al. 2005). A rare longitudinal mechanistic study examined brain function during emotionally arousing tasks among participants followed from early adolescence into young adulthood (Heitzeg et al. 2015). Participants who used cannabis heavily in adolescence did not differ from comparison participants (who used cannabis minimally) during adolescence but experienced significantly greater negative emotionality and lower resiliency in early adulthood. Neuroimaging findings showed that early heavy cannabis use was associated with lower activation in brain regions involved in emotional processing. Specifically, these participants demonstrated lowered activation in the right insula, prefrontal cortex, and occipital cortex during a negatively valanced word task, and lower activation in the right inferior parietal lobe during a positively valanced word task, but amygdala activation reduced in both tasks. Taken together, the studies described here provide evidence that cannabis use (and particularly heavy use) during adolescence may negatively impact later emotional function.

5.2 Physical Health

Because smoking is the most common route of cannabis administration for teens (Knapp et al. 2019) and the pulmonary effects of tobacco smoking are well documented, much of the data on cannabis exposure and lung function come from

comparisons of cannabis to tobacco smoking (e.g., Taylor et al. 2002; Hancox et al. 2010). Respiratory issues found to be associated with chronic cannabis exposure in adults include higher rates of coughing, wheezing, shortness of breath, and sputum production (Taylor et al. 2000). Compelling evidence links chronic cannabis use with increased risk for chronic bronchitis (Tashkin et al. 2012) and asthma (Bramness and von Soest 2019), but associations between cannabis use and COPD (Callaghan et al. 2013) or lung cancer (Gracie and Hancox 2021) are currently less clear.

Of particular concern among adolescents is potential lung damage caused by vaping THC, specifically e-cigarette or vaping product use-associated lung injury (EVALI). EVALI is characterized by shortness of breath, cough, chest pain, fever, and nausea. Severe cases have resulted in hospitalization, intubation and mechanical ventilation, and death. Of the 2668 hospitalized cases of EVALI reported to the CDC as of January 14, 2020, all reported using vaping products and most often these products contained THC. About 16% of these EVALI cases involved patients under the age of 18 years. Importantly, most EVALI patients reported obtaining their THC vaping products from friends, family, online dealers, or had made them themselves. Vitamin E acetate is a thickening agent often used in noncommercial THC-containing vape products and is strongly linked to EVALI (Blount et al. 2019). A study of 51 EVALI patients found that THC was found in most bronchoalveolar-lavage (BAL) samples and vitamin E acetate was found in all samples; these substances were not detected in BAL fluid samples from a control group (King et al. 2020). The number of EVALI cases has declined sharply since 2020, which may be due to removal of vitamin E acetate from THC-containing vape products (FDA 2019a) and/or reduction in the supply of informally sourced products (FDA 2019b).

The effects of cannabis use on cardiovascular health, particularly heart rate and blood pressure, remain unclear due to mixed research findings and limited sample sizes. Probably the most consistent cardiovascular effect of acute cannabis intoxication is an increase in heart rate (Menkes et al. 1991), but the long-term effects of cannabis use on heart rate are less clear. Research on adolescents in this area is lacking.

Cannabis use has been linked with higher rates of impaired sleep and sleep disturbance. Cross-sectional studies involving adolescents indicate that although many adolescents report using cannabis to improve their sleep (Tuvel et al. 2023), any short-term benefit is offset by potential negative long-term effects especially in frequent or heavy users. For example, cannabis use is associated with difficulty falling or staying asleep (Roane and Taylor 2008), going to bed later and experiencing shorter sleep time (Troxel et al. 2015), and insufficient sleep on school nights (Kwan et al. 2020). Pasch et al. (2012) found that among youth aged 11–18 years cannabis use predicted increased weekend sleep and weekend oversleep, and 2 years later cannabis use was associated with lower weekend sleep duration. Sleep in adolescence is a vital public health issue, as sleep has been cited to be particularly important during periods of brain maturation (Dahl and Lewin 2002). The associations between cannabis use and sleep in adolescence are complex and poorly understood.

A rare but potentially serious side effect of chronic use is cannabinoid hyperemesis syndrome (CHS) which is characterized by recurrent nausea, repeated cyclic

vomiting, and abdominal pain that can occur due to prolonged use of high-dose cannabis (Sorensen et al. 2017; DeVuono and Parker 2019). Complications include kidney failure due to persistent vomiting and dehydration (Nourbakhsh et al. 2019). Prevalence rates in adults may be as high as 32% in those with CUD (Habboushe et al. 2018) but rates in adolescents are more difficult to estimate (Lonsdale and Wilsey 2022). One study reported on 34 adolescent cases in a single institution over a 10-year period. The authors reported that CHS was more common in girls and found in patients as young as 13 years old (Lonsdale et al. 2020). Criteria for diagnosing CHS in adolescents include regular (i.e., weekly) use of cannabis for 3 months or longer, onset or worsening of episodic nausea or vomiting after onset of regular cannabis use, and absence of other underlying medical conditions which could explain the symptoms. Additional criteria include symptomatic relief with hot showers/baths, weight loss, abdominal pain, and change in bowel habit (Lonsdale et al. 2020). The most effective treatment appears to be complete cessation of cannabis use, although hot baths or showers have been reported anecdotally to confer some relief (e.g., Richards et al. 2017). CHS appears to be increasing in prevalence and the greater availability of higher potency formulations and products is likely to contribute to these rates. Currently, little is known about the pathophysiology and treatment of this disorder, especially for adolescents.

In summary, the available literature supports an association between long-term and frequent cannabis use and negative effects on pulmonary function and sleep quality. Some evidence suggests that long-term cannabis use may be associated with serious side effects like EVALI and CHS. Further research is needed to better characterize the effects of CUD on the physical health outcomes in adolescents.

5.3 Psychosocial Functioning

A robust literature indicates that frequent or chronic adolescent cannabis use is linked to negative outcomes in multiple important psychosocial domains. Because these studies are largely observational in nature, potential mechanisms underlying these associations are unclear. However, the few existing longitudinal studies lend consistent evidence that adolescent cannabis use is associated with impaired parent–child, peer, and romantic relationships, lower academic functioning and socioeconomic attainment, and increased risk of legal system involvement.

5.4 Parent-Child Relationship

The few studies to examine the potential impact of chronic cannabis use on the quality of parent–child relationships have yielded some evidence for a correlation between adolescent cannabis use and negative relationships with others. For example, high school seniors who reported regular cannabis use were more likely to

report impaired relationships with parents, although it must be noted that alcohol-related negative social outcomes were worse compared to those reported with cannabis (Palamar et al. 2014). Similarly, Foster et al. (2018) found that adolescents who reported weekly cannabis use were more likely to experience parent–child conflict compared to their non-using peers.

5.5 Peer and Romantic Relationships

Peer and romantic relationships are important for social well-being (Mitic et al. 2020), particularly in adolescence when a shift from the family to peers occurs, reflecting a move toward independence. At this time, peer approval, rejection, and pressure become central determinants of adolescent behavior (Steinberg and Morris 2001) as teens learn to relate to peers, to adjust their behavior in response to social feedback, and to resolve conflict.

Higher frequency cannabis use during adolescence has been associated with increased difficulty with partner and peer relationships (Brook et al. 2002; Foster et al. 2018). For example, Foster et al. (2018) found that adolescents who reported weekly cannabis use had fewer prosocial and more antisocial peers compared to nonusers. Adolescents who use cannabis frequently are less likely to be in a romantic relationship (Chan et al. 2021), and when they are, tend to report lower levels of relationship satisfaction (Fergusson and Horwood 1997). Interestingly, whether certain peer relationships increase the likelihood of cannabis use or early-onset cannabis use leads to association with substance-using peers remains unclear.

Taken together, support for a direct link between cannabis use and impaired social functioning is fairly limited. Although some studies have found a significant link between cannabis use and negative interpersonal relationships, the lack of rigorous research makes it difficult to draw definitive conclusions. Some evidence indicates that more frequent and persistent cannabis use during adolescence may be associated with some negative social consequences, but whether cannabis use directly contributes to impaired social functioning is unknown.

5.6 Cannabis and Academic Achievement

Because cannabis can have negative effects on attention, learning, and memory that can persist after the acute effects of the drug have dissipated (Schweinsburg et al. 2008) and even up to 1 year after cessation of cannabis use (Meier et al. 2012), a number of prospective longitudinal studies have examined the links between cannabis use and academic achievement. Many of these studies demonstrate that teens who smoke cannabis have poorer academic outcomes compared to those who do not. For example, in their study of youth followed from birth to age 16 years, Fergusson et al. (1996) found that using cannabis prior to the age of 15 years was

associated with a three-fold greater likelihood of dropping out of school at age 15–16. Silins et al. (2014) similarly reported that teens who started cannabis use prior to the age of 17 years were significantly more likely to develop CUD and less likely to graduate from high school or complete undergraduate degrees compared to nonusers. Finally, data from the Minnesota Twin Study indicated that heavier cannabis use during adolescence was consistently associated with lower grade point average (GPA) and motivation to perform academically, and with greater likelihood of academic and school discipline problems (Schaefer et al. 2021).

Some studies have reported only indirect links between cannabis use and educational attainment. In a study using longitudinal growth curve modeling to examine cannabis use and GPA over 4 years of college, Arria et al. (2013) found an indirect path in which cannabis use led to increased absences from class, which resulted in lower GPA. A direct path from cannabis use to lower GPA was not observed.

Greater frequency and persistence of cannabis use have been linked with lower academic performance. In a sample of Australian students, Degenhardt et al. (2013) found poorer post-school outcomes at age 24 years in those who reported weekly or near-weekly cannabis use during adolescence compared to those who never used cannabis, although adjustment for cigarette use and other demographic variables reduced the strength of this association. Conversely, Foster et al. (2018) reported that weekly cannabis users with and without CUD experienced more academic problems (e.g., lower GPA, negative attitudes about school) and lower educational attainment than nonusers. Finally, young adults who reported early onset and persistent high or increasingly frequent cannabis use during adolescence also experienced poorer academic achievement (Thompson et al. 2019).

Similar to social and emotional functioning, the link between cannabis use and negative educational outcomes is correlational and it is likely other factors, like tobacco and alcohol use, play a shared role in both cannabis use and academic achievement. Other substance use, especially tobacco and alcohol, is probably a confounding variable in studies of cannabis and educational outcomes. For example, in their systematic review MacLeod et al. (2004) noted a significant reduction in the strength of association between cannabis use and negative educational outcomes after controlling for alcohol and tobacco use. Similarly, data from the Avon Longitudinal Study of Parents and Children revealed that heavy cannabis use at age 15 predicted lower academic performance at age 16, but this effect disappeared when controlling for tobacco use (Mokrysz et al. 2016). These results underscore the importance of considering other factors, particularly concurrent tobacco and alcohol use, when interpreting the effects of cannabis on academic performance.

5.7 Cannabis and Future Socioeconomic Status

Several studies have shown longer-term associations between heavy cannabis use in adolescence and occupational difficulties in adulthood. For example, lower occupational prestige and lower income were observed in young adults who reported early

onset and persistent high or increasingly frequent cannabis use during the transition from adolescence to young adulthood (Thompson et al. 2019). Hyggen (2012) observed lower levels of work commitment among cannabis users that were evident during late adolescence (17–20 years) through middle age. The link between frequent cannabis use and lack of motivation to achieve occupational goals may be a developmental continuation of the association between adolescent cannabis use and poor educational achievement (Brook et al. 1999).

Prospective longitudinal studies also demonstrate that high levels of cannabis use in adolescence predict higher levels of unemployment and reduced income at age 25 (Fergusson and Boden 2008; Brook et al. 2011a), and greater likelihood of being unemployed at age 43 compared to nonusers or light users (Zhang et al. 2016). Green et al. (2017) found in their sample of urban Black youth followed from ages 6–42 that heavy cannabis use in adolescence was associated with being poor in midlife. Finally, a study of men across three developmental stages—adolescence (13–20 years), early adulthood (20–30 years), and mid-adulthood (30–38 years)—revealed that cannabis use in prior developmental periods was associated with lower income levels in adulthood, even after controlling for potential confounds (Capaldi et al. 2022).

In summary, correlational and longitudinal evidence support a link between adolescent cannabis use and future socioeconomic status. As with other areas of functioning, establishing causality is a problem (Lee et al. 2015); it is unclear whether cannabis use contributes to unemployment or whether other shared risk factors related to academic or vocational achievement might account for both cannabis use and cannabis-related problems. Regardless of the mechanism, it appears that more chronic patterns of cannabis use in adolescence are associated with lower occupational prestige, lower income, and greater risk of unemployment (Thompson et al. 2019).

The findings presented above demonstrate the lack of consensus as to whether cannabis use limits academic achievement or psychosocial development as controlling for shared familial factors and other substance use often reduces associations between cannabis use and psychosocial outcomes (Schaefer et al. 2021). It may be that some effects of cannabis use on educational performance and other psychosocial domains may depend more on the social context in which cannabis use occurs rather than on cannabis-specific neurophysiological effects (Fergusson and Horwood 1997). That is, teens who engage in drug purchasing and use likely affiliate with a peer group that eschews normative behavior such as academic achievement, gaining employment, or placing value on interpersonal relationships. Thus, it may be associating with delinquent or substance-using peers that accounts for at least some of the apparent cannabis-related effects on academic performance (Brook et al. 2016) and other psychosocial domains. Stronger evidence is clearly needed to characterize the role of chronic cannabis use in these outcomes (Macleod et al. 2004; Volkow et al. 2014).

5.8 Cannabis and Cognitive Development

Adolescence is a "critical period" of neurodevelopment characterized by substantial changes in cognitive capacities, particularly in working memory, abstraction, self-regulation, and cognitive control (Gur et al. 2012). An extensive literature has focused on whether cannabis use during this vulnerable period can alter normal trajectories of cognitive development. Frequent and heavy cannabis use in adolescence has been shown to impact cognitive function (Lorenzetti et al. 2020), including declining IQ from childhood to middle age (Meier et al. 2012) and impaired performance on specific cognitive tasks (e.g., Scott et al. 2018; Broyd et al. 2016). However, not all studies have demonstrated cannabis-related impairments in neurocognitive functioning (e.g., Lawn et al. 2022) and evidence shows that cannabis abstinence can reverse some of these observed deficits (Scott 2023). A comprehensive review of this important topic is found in Chap. 12

6 Treatment for CUD

About 5% of adolescents who use cannabis are estimated to meet DSM-5 criteria for a CUD. In 2023, approximately 19% of 12–17 year olds with CUD received treatment, which means that more than 80% did not receive any treatment (SAMHSA 2024). According to the Treatment Episode Data Set (SAMHSA 2023), during 2021 a total of 20,646 youth aged 12–17 years were admitted to a publicly funded substance use disorder treatment program for CUD. The majority reported being referred to treatment by the criminal justice system (43%). The remaining were self-referred (19%) or referred by a health care provider (15%), community agency (13%), or school (10%).

Early intervention is essential for lessening or preventing negative impacts of CUD on academic, cognitive, social, and health functioning in adolescence and adulthood. The majority of adolescents in treatment for CUD receive behavioral therapy. Below we review evidence-based treatments designed to reduce use and severity of CUD symptoms in youth. We also explore potential pharmacotherapies, which are limited but show some promise for reducing withdrawal symptoms like craving.

6.1 Behavioral Therapies and Counseling

6.1.1 Cognitive Behavioral and Motivational Treatments

Cognitive behavioral (CBT) approaches to treatment are generally short term, focused interventions designed to have patients recognize situations in which they are likely to use substances, avoid these situations when appropriate, and cope more effectively with problematic thoughts, behaviors and situations that are associated

with use (Carroll 1996). CBT approaches have demonstrated broad efficacy in the treatment of substance use (Boness et al. 2023). However, relatively few randomized controlled trials (RCTs) have examined the efficacy of CBT treatments alone (i.e., not in combination with other approaches) for the treatment of CUD among adolescents. One of these found no significant changes in cannabis and alcohol use for an intensive CBT condition or a comparison usual care condition, although there were small/medium effect sizes favoring the CBT condition on days of cannabis use (Wolff et al. 2020). There were also small but nonsignificant effects for CBT on measures of externalizing symptoms, depressed mood and anxiety, and significantly fewer juvenile justice contacts. Another study found significant reductions in heavy drinking days and days of cannabis use for CBT (compared with usual care) among teens with co-occurring SUD and suicidality (Esposito-Smythers et al. 2011). Shifts away from spending time with cannabis-using friends during CBT is also associated with greater reductions in cannabis craving and use (Meisel et al. 2021).

Most treatments that use CBT also include motivational interviewing (MI) or motivational enhancement therapy (MET). MI/MET is a collaborative, goal-oriented approach that emphasizes change language and bolstering an individual's intrinsic motivation for change (Miller and Rollnick 2012). Adding a motivational check-in to a brief CBT intervention for cannabis use has demonstrated reductions in use at 6-month follow-up assessments relative to an assessment only condition, although not at other follow-ups, and the brief check-ins did not increase engagement to the brief intervention (Walker et al. 2016). A multisite German clinical trial compared a manualized 10-session MET/CBT to a delayed treatment control condition among youth who regularly used cannabis. That study revealed an increase in negative drug screens throughout treatment compared to the delayed treatment condition, and abstinence rates remained higher than baseline throughout posttreatment and follow-up assessments for the CBT/MET condition (Hoch et al. 2014). MET+CBT, both in 5-and 12-session protocols, has also demonstrated efficacy in reducing cannabis use among youth in a large-scale trial (Dennis et al. 2004). Another RCT examining combined MET/CBT found significant within treatment reductions in frequency of cannabis use, but these reductions were significantly greater when individual therapy was augmented by family therapy (Barrett et al. 2001).

6.1.2 Behavioral Treatments

Contingency management (CM) treatments are behavioral interventions in which behavior change occurs via extrinsic reinforcement of target behaviors, such as providing a negative drug test. CM has demonstrated broad efficacy for encouraging abstinence across several substances of abuse among adults (see Pfund et al. 2024), and among adolescents for tobacco (e.g., Krishnan-Sarin et al. 2006) and alcohol use (Godley et al. 2014). Several studies have also examined the efficacy of CM for CUD in adolescents. A 14-week intervention consisting of abstinence-based voucher reinforcement, family management and combined MET/CBT resulted in more weeks of continuous cannabis abstinence during treatment, compared to a

matched intervention consisting of MET/CBT, CM for attendance only and parent psychoeducation (Stanger et al. 2009). There were no significant time by treatment condition interactions for point-prevalence abstinence at the follow-up timepoints, and no significant interactions for self-reported cannabis use. These authors investigated a similar set of interventions but also introduced a home-based parent-delivered CM and found CM to be associated with greater cannabis continuous abstinence during treatment, and greater abstinence at follow-up (Stanger et al. 2015). Combined clinic-and parent-directed CM interventions have also demonstrated initial evidence for reducing cannabis use and improving adolescent externalizing behaviors and negative parenting behaviors (Kamon et al. 2005). In a study of adolescents admitted to residential treatment, CM resulted in significant reductions in both alcohol and cannabis use compared with usual care (Godley et al. 2014). Not all studies have demonstrated efficacy of CM for cannabis use. Kaminer et al. (2014) found no differences between voucher-based reinforcement compared with attendance-based reward when added to CBT. An additional feasibility trial failed to show benefits of CM for cannabis abstinence among adolescents recruited from outpatient treatment (Killeen et al. 2012).

The Adolescent Community Reinforcement Approach (ACRA) is another behavioral treatment that focuses on the restructuring of the client's environment to encourage prosocial activities that are inconsistent with substance use and teaches more adaptive coping and communication skills (Azrin et al. 1982). One preliminary study comparing usual residential care to usual care plus an assertive continuing care protocol that consisted of ACRA and case management found the assertive care condition led to greater cannabis abstinence at 3-months post residential discharge, as well as fewer days of alcohol use and greater use of continuing care services (Godley et al. 2002). ACRA also resulted in decreased days of use and increased percentage of adolescents in recovery from use in the large scale Cannabis Youth Treatment (CYT) study (Dennis et al. 2004).

6.1.3 Family Therapies

Several forms of family therapy have been studied to examine their effectiveness in reducing youth cannabis use problems. For example, multisystemic treatment is an intervention for youth with delinquency issues that focuses on the youth's fit within environmental systems (the family, school, peer groups) that can influence the development and maintenance of behaviors such as substance use (Henggeler 1999). MST is effective at reducing cannabis use among youth with delinquency issues who are involved in the juvenile justice system (Henggeler et al. 1999). Long-term follow up assessment of the effectiveness of MST has revealed higher abstinence rates for MST treated youth (compared with standard community services), as well as long-term benefits for reducing aggressive criminal activity (Henggeler et al. 2002).

Another form of family involved treatment that has been examined in relation to cannabis use is multidimensional family therapy (MDFT). This intervention is a

family-based treatment that is based on research on adolescent and family development, and developmental factors leading to youth substance use (Liddle et al. 2001). An early clinical trial comparing MDFT to adolescent group therapy and a multifamily educational intervention for youth referred for combination cannabis and alcohol use problems revealed that MDFT demonstrated superior efficacy regarding substance use reduction, although cannabis use was not examined separately. The large-scale Cannabis Youth Treatment (CYT) study specifically examined cannabis and showed that MDFT is effective at reducing cannabis use and increasing recovery among youth (Dennis et al. 2004).

6.1.4 Head-to-Head Comparisons

Relatively few large-scale trials have compared results across multiple forms of therapy for adolescent cannabis use. One of the largest to date, the CYT study, examined data from two trials at four sites that examined the efficacy of four interventions in a combined sample of 600 adolescents and their parents (Trial 1: five sessions of MET+CBT; 12 sessions of MET+CBT; Family education and therapy. Trial 2: five sessions of MET+CBT; Adolescent Community Reinforcement Approach (ACRA); Multidimensional Family Therapy (MDFT); Dennis et al. 2004). All treatment conditions led to significant increases in days of cannabis abstinence and percent of adolescents in recovery from use, and outcomes were similar across treatment sites and treatment assignments. But there were some differences found for cost effectiveness, with the five session MET/CBT proving highly cost effective in both trials, the twelve session MET/CBT and ACRA demonstrating high-cost cost-effectiveness in trials 1 and 2, respectively.

Additional studies have also conducted head-to-head comparisons of evidence-based treatments. Comparison of combined MI and CBT to a combined relaxation training plus substance-education/12-step (RT/SET) intervention demonstrated weak support for MI + CBT in reducing predatory aggression, but RT/SET had more favorable outcomes related to alcohol use, and neither intervention seemed to reduce cannabis use (Stein et al. 2020). Hendriks et al. (2012) revealed that CBT and MDFT may have differential efficacy based on participant characteristics. In their secondary analysis of 109 youth, they found that CBT tended to work better for older adolescents and those without a history of co-occurring disorders, whereas MDFT worked better for younger adolescents and those who experienced conduct disorder or internalizing psychopathology. An additional study that explored individual CBT/MET and functional family therapy, alone and in combination, revealed that family therapy alone and combined family and individual approaches led to the greatest reductions in cannabis use frequency from pretreatment to 4 months (Barrett et al. 2001). Combined therapy and psychoeducational groups led to the greatest reductions at the seven-month assessment. These studies demonstrate the importance of comparing evidence-based therapy, particularly as they may be differentially effective for groups with different presenting problems or with differing access to treatment resources.

6.2 Pharmacotherapy for CUD

Currently there is no FDA-approved medication for treating CUD. The majority of studies in this area involved adults, focused on decreasing cannabis use or reducing withdrawal symptoms, and have yielded mixed results. For example, Mason et al. (2012) found that gabapentin, an anticonvulsant, reduced cannabis use over a 12-week treatment period and also decreased some symptoms of withdrawal, including sleep disturbance, craving, and irritability, but did not increase likelihood of abstinence and led to high drop out rates. Quetiapine, an atypical antipsychotic, reduced cannabis use in daily users (Mariani et al. 2021) but at lower doses was associated with increased cannabis craving and self-administration during relapse (Cooper et al. 2013). The synthetic THC derivative nabilone attenuated some withdrawal symptoms like irritability, sleep disturbance, and reduced relapse risk, but did not increase abstinence (Haney et al. 2013). Dronabinol, another synthetic oral THC preparation, reduced withdrawal-related anxiety, irritability and cannabis craving, but did not reduce use and increased drug liking (Levin et al. 2011). Nabiximols, an oral spray containing a 1:1 ratio of THC to cannabidiol (CBD) extracted from the *Cannabis sativa* plant decreased withdrawal severity and use and increased treatment retention, but relapse rates were high and the potential for drug diversion was a concern (Allsop et al. 2014). Results of studies on noradrenergic (e.g., bupropion, venlafaxine, and atomoxetine) and GABAergic agents (e.g., baclofen and depakote) indicated these potential medications were poorly tolerated and in some cases increased cannabis craving and worsened withdrawal symptoms (Brezing and Levin 2018; Kondo et al. 2020).

Few studies have been conducted with adolescents. Gray and colleagues (2010) tested the effects of N-Acetylcysteine (NAC), an antioxidant and glutamate modulator, in youth aged 18–21 years with CUD and found significant reductions in the number of THC-positive urine samples and cannabis craving over the 4-week treatment period. In a subsequent study of adolescents and youth aged 15–21 years, Gray et al. (2012) demonstrated that NAC combined with contingency management and brief physician-delivered abstinence counseling resulted in more participants submitting THC-negative urine screens compared to placebo. Interestingly, NAC did not show similar effects in adults (Gray et al. 2017), which shows that investigations of potential compounds to treat CUD in adolescents should not be limited to medications that appear to be efficacious in adults. Some putative medications may confer adolescent-specific benefit, especially as adolescents may respond differently from adults due to developmental and neurobiological factors. Finally, topiramate, an anticonvulsant used to treat migraine and seizure disorders, combined with Motivational Enhancement Therapy reduced the amount of cannabis smoked per day in treatment-seeking cannabis users (15–24 years) over a 4-week titration and 2-week steady treatment period (Miranda et al. 2017). However, less than 50% of the topiramate group completed the study due to unpleasant side effects (e.g., memory difficulties, confusion, slowed thinking) which may limit the clinical utility of topiramate for CUD (Gray et al. 2018).

6.3 Summary of Treatment Approaches

In summary, multiple evidence-based treatment options exist for adolescents with Cannabis Use Disorders (CUDs), although many adolescents do not achieve abstinence and those who do often relapse after treatment. Complicating this picture is that the studies referenced above were completed prior to changes in cannabis legislation, the increased availability of high-potency THC products (e.g., cannabis flower containing as much as 30% THC, concentrates and oils containing as much as 90% THC, etc.), and evolving routes of administration (e.g., edibles and vaporizers). All of these changes likely contribute to an increased risk of developing CUD and existing treatment approaches may need to be adapted to accommodate these shifting cannabis use parameters. The use of digital platforms is growing in popularity, particularly among this technology-savvy group, and apps that deliver components of Cognitive Behavior Therapy and Motivational Enhancement Therapy may be ideal for adolescents. Further research is needed to establish the acceptability and efficacy of these technology-driven approaches to CUD treatment in adolescents.

Finally, results from pharmacotherapy studies in adults are mixed and only NAC has shown potential clinical benefit in treatment of adolescents with CUD. Whereas low-dose cannabinoid agonists appear to mitigate some withdrawal symptoms in adults, research on safety and efficacy in adolescents is lacking.

7 Conclusion

Cannabis use during adolescence is clearly associated with negative outcomes in early and mid-adulthood in numerous psychosocial domains, as reported in the majority of the studies cited above. Many of these studies are rigorous, longitudinal investigations, providing further support to this relationship. Despite this strong correlational support, we also acknowledge that the current literature largely cannot answer questions as to whether early cannabis use *causes* these psychosocial problems. To examine this question, more research is needed using longitudinal methods that may demonstrate these causal relationships (e.g., twin and adoption studies).

Additional considerations should be taken into account when evaluating the current literature on CUD and psychosocial problems among youth. For example, some of the early studies did not adequately address confounding factors that may account for the relationships between cannabis use and psychosocial functioning such as use of alcohol or tobacco, or other environmental challenges. Further, the studies reported here recruited youth from among a wide-range of samples, including those from less or more privileged backgrounds, narrow versus diverse populations, and from a variety of different countries, meaning that we cannot necessarily directly compare findings from different studies. Based on these observations, it is important that studies include analyses of such factors as gender/sex, family socioeconomic status, and psychological factors such as depression or anxiety (Macleod et al. 2004).

Nevertheless, correlational evidence for a link between early cannabis use and psychosocial problems in social, academic, vocational, and mental and physical health, is strong. Further, the questions addressed in this chapter concerning the potential harmful impact of cannabis on youth grow in importance with the rapidly changing landscape of cannabis potency, availability and legality; for example, THC concentrations have increased substantially over just a few decades (ElSohly et al. 2016), and high-potency THC concentrates are widely available at cannabis dispensaries. Although the science on these higher potency products and the implications of increased legalization are at a nascent stage, there is potential for the negative psychosocial consequences of cannabis use among youth to be amplified in the near future. To meet this challenge, it is vital that future research continue to address the role of cannabis in the development of psychosocial difficulties, and to use these findings to tailor interventions to prevent and treat these problems.

References

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed., text rev. ed. American Psychiatric Association; 2022. https://doi.org/10.1176/appi.books.9780890425787.

Allsop DJ, Copeland J, Lintzeris N, Dunlop AJ, Montebello M, Sadler C, Rivas GR, Holland RM, Muhleisen P, Norberg MM, Booth J, McGregor IS. Nabiximols as an agonist replacement therapy during cannabis withdrawal: a randomized clinical trial. JAMA Psychiatry. 2014;71(3):281–91. https://doi.org/10.1001/jamapsychiatry.2013.3947. PMID: 24430917.

Arria AM, Caldeira KM, Vincent KB, Winick ER, Baron RA, O'Grady KE. Discontinuous college enrollment: associations with substance use and mental health. Psychiatr Serv. 2013;64(2):165–72. https://doi.org/10.1176/appi.ps.201200106.

Azrin NH, Sisson RW, Meyers R, Godley M. Alcoholism treatment by disulfiram and community reinforcement therapy. J Behav Ther Exp Psychiatry. 1982;13(2):105–12.

Bailey JA, Tiberio SS, Kerr DC, Epstein M, Henry KL, Capaldi DM. Effects of cannabis legalization on adolescent cannabis use across 3 studies. Am J Prev Med. 2023;64(3):361–7.

Barrett H, Slesnick N, Brody JL, Turner CW, Peterson TR. Treatment outcomes for adolescent substance abuse at 4-and 7-month assessments. J Consult Clin Psychol. 2001;69(5):802.

Blount BC, Karwowski MP, Shields PG, et al. Lung injury response laboratory working group. Vitamin E acetate in bronchoalveolar-lavage fluid associated with EVALI. N Engl J Med. 2019;382:NEJMoa1916433.

Boness CL, Votaw VR, Schwebel FJ, Moniz-Lewis DI, McHugh RK, Witkiewitz K. An evaluation of cognitive behavioral therapy for substance use disorders: a systematic review and application of the society of clinical psychology criteria for empirically supported treatments. Clinical Psychol. 2023;30:129.

Bramness JG, von Soest T. A longitudinal study of cannabis use increasing the use of asthma medication in young Norwegian adults. BMC Pulm Med. 2019;19(1):52. Published 2019 Feb 26. https://doi.org/10.1186/s12890-019-0814-x.

Brezing CA, Levin FR. The Current State of Pharmacological Treatments for Cannabis Use Disorder and Withdrawal. Neuropsychopharmacology. 2018;43(1):173–94. https://doi.org/10.1038/npp.2017.212. Epub 2017 Sep 6. PMID: 28875989; PMCID: PMC5719115.

Brook JS, Balka EB, Whiteman M. The risks for late adolescence of early adolescent marijuana use. Am J Public Health. 1999;89(10):1549–54. https://doi.org/10.2105/ajph.89.10.1549.

Brook DW, Brook JS, Zhang C, Cohen P, Whiteman M. Drug use and the risk of major depressive disorder, alcohol dependence, and substance use disorders. Arch Gen Psychiatry. 2002;59(11):1039–44. https://doi.org/10.1001/archpsyc.59.11.1039.

Brook JS, Lee JY, Brown EN, Finch SJ, Brook DW. Developmental trajectories of marijuana use from adolescence to adulthood: personality and social role outcomes. Psychol Rep. 2011a;108(2):339–57. https://doi.org/10.2466/10.18.PR0.108.2.339-357.

Brook JS, Zhang C, Brook DW. Developmental trajectories of marijuana use from adolescence to adulthood: personal predictors. Arch Pediatr Adolesc Med. 2011b;165(1):55–60. https://doi.org/10.1001/archpediatrics.2010.248.

Brook JS, Zhang C, Leukefeld CG, Brook DW. Marijuana use from adolescence to adulthood: developmental trajectories and their outcomes. Soc Psychiatry Psychiatr Epidemiol. 2016;51(10):1405–15. https://doi.org/10.1007/s00127-016-1229-0.

Broyd SJ, van Hell HH, Beale C, Yücel M, Solowij N. Acute and chronic effects of cannabinoids on human cognition-a systematic review. Biol Psychiatry. 2016;79(7):557–67. https://doi.org/10.1016/j.biopsych.2015.12.002.

Butler A, Patte KA, Ferro MA, Leatherdale ST. Interrelationships among depression, anxiety, flourishing, and cannabis use in youth. Addict Behav. 2019;89:206–15. https://doi.org/10.1016/j.addbeh.2018.10.007.

Callaghan RC, Allebeck P, Sidorchuk A. Marijuana use and risk of lung cancer: a 40-year cohort study. Cancer Causes Control. 2013;24(10):1811–20. https://doi.org/10.1007/s10552-013-0259-0.

Capaldi DM, Tiberio SS, Kerr DC, Owen LD. Associations of cannabis use across adolescence and early adulthood with health and psychosocial adjustment in early adulthood and Midadulthood in men. Subst Abuse. 2022;16:11782218221096154. https://doi.org/10.1177/11782218221096154.

Carroll KM. Relapse prevention as a psychosocial treatment: a review of controlled clinical trials. Exp Clin Psychopharmacol. 1996;4(1):46–54. https://doi.org/10.1037/1064-1297.4.1.46.

Casey BJ, Jones RM. Neurobiology of the adolescent brain and behavior: implications for substance use disorders. J Am Acad Child Adolesc Psychiatry. 2010;49(12):1189–201.

Chan GCK, Becker D, Butterworth P, et al. Young-adult compared to adolescent onset of regular cannabis use: a 20-year prospective cohort study of later consequences. Drug Alcohol Rev. 2021;40(4):627–36. https://doi.org/10.1111/dar.13239.

Cooper ZD, Foltin RW, Hart CL, Vosburg SK, Comer SD, Haney M. A human laboratory study investigating the effects of quetiapine on marijuana withdrawal and relapse in daily marijuana smokers. Addict Biol. 2013;18(6):993–1002. https://doi.org/10.1111/j.1369-1600.2012.00461.x. Epub 2012 Jun 28. PMID: 22741619; PMCID: PMC3465638.

Crane NA, Langenecker SA, Mermelstein RJ. Gender differences in the associations among marijuana use, cigarette use, and symptoms of depression during adolescence and young adulthood. Addict Behav. 2015;49:33–9. https://doi.org/10.1016/j.addbeh.2015.05.014.

Dahl RE, Lewin DS. Pathways to adolescent health sleep regulation and behavior. J Adolesc Health. 2002;31(6 Suppl):175–84. https://doi.org/10.1016/s1054-139x(02)00506-2.

Degenhardt L, Ferrari AJ, Calabria B, et al. The global epidemiology and contribution of cannabis use and dependence to the global burden of disease: results from the GBD 2010 study. PLoS One. 2013;8(10):e76635. Published 2013 Oct 24. https://doi.org/10.1371/journal.pone.0076635.

Dennis M, Godley SH, Diamond G, Tims FM, Babor T, Donaldson J, Funk R. The cannabis youth treatment (CYT) study: main findings from two randomized trials. J Subst Abus Treat. 2004;27(3):197–213.

Dennis ML, Chan Y, Funk R. Development and validation of the GAIN short screener (GSS) for internalizing, externalizing and substance use disorders and crime/violence problems among adolescents and adults. Am J Addict. 2006;15:80–91.

DeVuono MV, Parker LA. Cannabisi hyperemesis syndrome: a review of potential mechanisms. Cannabis Cannabinoid Res. 2019;5(2):132–44. https://doi.org/10.1089/can.2019.0059.

ElSohly MA, Mehmedic Z, Foster S, Gon C, Chandra S, Church JC. Changes in Cannabis Potency Over the Last 2 Decades (1995-2014): Analysis of Current Data in the United States. Biol Psychiatry. 2016;79(7):613–9. https://doi.org/10.1016/j.biopsych.2016.01.004. Epub 2016 Jan 19. PMID: 26903403; PMCID: PMC4987131.

Esposito-Smythers C, Spirito A, Kahler CW, Hunt J, Monti P. Treatment of co-occurring substance abuse and suicidality among adolescents: a randomized trial. J Consult Clin Psychol. 2011;79(6):728–39. https://doi.org/10.1037/a0026074. Epub 2011 Oct 17. PMID: 22004303; PMCID: PMC3226923.

Farmer RF, Kosty DB, Seeley JR, Duncan SC, Lynskey MT, Rohde P, Lewinsohn PM. Natural course of cannabis use disorders. Psychol Med. 2015;45(1):63–72.

Fergusson DM, Boden JM. Cannabis use and later life outcomes. Addiction. 2008;103(6):969–78. https://doi.org/10.1111/j.1360-0443.2008.02221.x.

Fergusson DM, Horwood LJ. Early onset cannabis use and psychosocial adjustment in young adults. Addiction. 1997;92(3):279–96.

Fergusson DM, Horwood LJ, Lynskey MT. Childhood sexual abuse and psychiatric disorder in young adulthood: II. Psychiatric outcomes of childhood sexual abuse. J Am Acad Child Adolesc Psychiatry. 1996;35(10):1365–74. https://doi.org/10.1097/00004583-199610000-00024.

Fergusson DM, Horwood LJ, Swain-Campbell N. Cannabis use and psychosocial adjustment in adolescence and young adulthood. Addiction. 2002;97(9):1123–35. https://doi.org/10.1046/j.1360-0443.2002.00103.x.

Flory K, Lynam D, Milich R, Leukefeld C, Clayton R. Early adolescent through young adult alcohol and marijuana use trajectories: early predictors, young adult outcomes, and predictive utility. Dev Psychopathol. 2004;16(1):193–213.

Food and Drug Administration FDA. Lung illnesses associated with use of vaping products. Silver Spring, MD: US Department of Health and Human Services, Food and Drug Administration; 2019a. https://www.fda.gov/news-events/public-health-focus/lung-illnesses-associated-use-vaping-products.

Food and Drug Administration FDA. DEA seize 44 websites advertising sale of illicit THC vaping cartridges to US consumers as part of operation vapor lock. Silver Spring, MD: US Department of Health and Human services, Food and Drug Administration; 2019b. https://www.fda.gov/news-events/press-announcements/fda-dea-seize-44-websites-advertising-sale-illicit-thc-vaping-cartridges-us-consumers-part-operation.

Foster NE, Anema JR, Cherkin D, et al. Prevention and treatment of low back pain: evidence, challenges, and promising directions. Lancet. 2018;391(10137):2368–83. https://doi.org/10.1016/S0140-6736(18)30489-6.

Gobbi G, Atkin T, Zytynski T, et al. Association of Cannabis use in adolescence and risk of depression, anxiety, and suicidality in young adulthood: a systematic review and meta-analysis. JAMA Psychiatry. 2019;76(4):426–34. https://doi.org/10.1001/jamapsychiatry.2018.4500.

Godley MD, Godley SH, Dennis ML, Funk R, Passetti LL. Preliminary outcomes from the assertive continuing care experiment for adolescents discharged from residential treatment. J Subst Abus Treat. 2002;23(1):21–32.

Godley MD, Godley SH, Dennis ML, Funk RR, Passetti LL, Petry NM. A randomized trial of assertive continuing care and contingency management for adolescents with substance use disorders. J Consult Clin Psychol. 2014;82(1):40.

Gracie K, Hancox RJ. Cannabis use disorder and the lungs. Addiction. 2021;116(1):182–90. https://doi.org/10.1111/add.15075.

Gray KM, Squeglia LM. Research review: what have we learned about adolescent substance use? J Child Psychol Psychiatry. 2018;59(6):618–27. https://doi.org/10.1111/jcpp.12783.

Gray JC, Treloar Padovano H, Wemm SE, Miranda R Jr. Predictors of Topiramate Tolerability in Heavy Cannabis-Using Adolescents and Young Adults: A Secondary Analysis of a Randomized, Double-Blind, Placebo-Controlled Trial. J Clin Psychopharmacol. 2018;38(2):134–37. https://doi.org/10.1097/JCP.0000000000000843. PMID: 29424802; PMCID: PMC5825284.

Gray KM, Carpenter MJ, Baker NL, DeSantis SM, Kryway E, Hartwell KJ, McRae-Clark AL, Brady KT. A double-blind randomized controlled trial of N-acetylcysteine in cannabis-dependent adolescents. Am J Psychiatry. 2012;169(8):805–12. https://doi.org/10.1176/appi.ajp.2012.12010055. Erratum in: Am J Psychiatry. 2012 Aug 1;169(8):869. PMID: 22706327; PMCID: PMC3410961.

Gray KM, Sonne SC, McClure EA, Ghitza UE, Matthews AG, McRae-Clark AL, Carroll KM, Potter JS, Wiest K, Mooney LJ, Hasson A, Walsh SL, Lofwall MR, Babalonis S, Lindblad RW, Sparenborg S, Wahle A, King JS, Baker NL, Tomko RL, Haynes LF, Vandrey RG, Levin FR. A randomized placebo-controlled trial of N-acetylcysteine for cannabis use disorder in adults. Drug Alcohol Depend. 2017;177:249–57. https://doi.org/10.1016/j.drugalcdep.2017.04.020. Epub 2017 Jun 10. PMID: 28623823; PMCID: PMC5535813.

Green KM, Doherty EE, Ensminger ME. Long-term consequences of adolescent cannabis use: examining intermediary processes. Am J Drug Alcohol Abuse. 2017;43(5):567–75. https://doi.org/10.1080/00952990.2016.1258706.

Griebel G, Stemmelin J, Scatton B. Effects of the cannabinoid CB1 receptor antagonist rimonabant in models of emotional reactivity in rodents. Biol Psychiatry. 2005;57(3):261–7. https://doi.org/10.1016/j.biopsych.2004.10.032.

Gur RC, Richard J, Calkins ME, et al. Age group and sex differences in performance on a computerized neurocognitive battery in children age 8-21. Neuropsychology. 2012;26(2):251–65. https://doi.org/10.1037/a0026712.

Habboushe J, Rubin A, Liu H, Hoffman RS. The prevalence of cannabinoid hyperemesis syndrome among regular marijuana smokers in an urban public hospital. Basic Clin Pharmacol Toxicol. 2018;122(6):660–2. https://doi.org/10.1111/bcpt.12962.

Hancox RJ, Poulton R, Ely M, et al. Effects of cannabis on lung function: a population-based cohort study. Eur Respir J. 2010;35(1):42–7. https://doi.org/10.1183/09031936.00065009.

Haney M, Cooper ZD, Bedi G, Vosburg SK, Comer SD, Foltin RW. Nabilone decreases marijuana withdrawal and a laboratory measure of marijuana relapse. Neuropsychopharmacology. 2013;38(8):1557–65. https://doi.org/10.1038/npp.2013.54. Epub 2013 Feb 26. PMID: 23443718; PMCID: PMC3682150.

Harlow AF, Miech RA, Leventhal AM. Adolescent Δ8-THC and marijuana use in the US. JAMA. 2024;331(10):861–5.

Hasan A, von Keller R, Friemel CM, et al. Cannabis use and psychosis: a review of reviews. Eur Arch Psychiatry Clin Neurosci. 2020;270(4):403–12. https://doi.org/10.1007/s00406-019-01068-z.

Hasin DS, Saha TD, Kerridge BT, Goldstein RB, Chou SP, Zhang H, Jung J, Pickering RP, Ruan WJ, Smith SM, Huang B, Grant BF. Prevalence of marijuana use disorders in the United States between 2001-2002 and 2012-2013. JAMA Psychiatry. 2015;72(12):1235–42. https://doi.org/10.1001/jamapsychiatry.2015.1858.

Hasin DS, Kerridge BT, Saha TD, Huang B, Pickering R, Smith SM, Grant BF. Prevalence and correlates of DSM-5 cannabis use disorder, 2012-2013: findings from the National Epidemiologic Survey on alcohol and related conditions–III. Am J Psychiatry. 2016;173(6):588–99.

Heitzeg MM, Cope LM, Martz ME, Hardee JE. Neuroimaging risk markers for substance abuse: recent findings on inhibitory control and reward system functioning. Curr Addict Rep. 2015;2(2):91–103. https://doi.org/10.1007/s40429-015-0048-9.

Hendriks V, van der Schee E, Blanken P. Matching adolescents with a cannabis use disorder to multidimensional family therapy or cognitive behavioral therapy: treatment effect moderators in a randomized controlled trial. Drug Alcohol Depend. 2012;125(1–2):119–26.

Henggeler SW. Multisystemic therapy: an overview of clinical procedures, outcomes, and policy implications. Child Psychol Psychiatry Rev. 1999;4(1):2–10.

Henggeler SW, Pickrel SG, Brondino MJ. Multisystemic treatment of substance-abusing and-dependent delinquents: outcomes, treatment fidelity, and transportability. Ment Health Serv Res. 1999;1:171–84.

Henggeler SW, Clingempeel WG, Brondino MJ, Pickrel SG. Four-year follow-up of multisystemic therapy with substance-abusing and substance-dependent juvenile offenders. J Am Acad Child Adolesc Psychiatry. 2002;41(7):868–74.

Hoch E, Bühringer G, Pixa A, Dittmer K, Henker J, Seifert A, Wittchen HU. CANDIS treatment program for cannabis use disorders: findings from a randomized multi-site translational trial. Drug Alcohol Depend. 2014;134:185–93.

Hungund BL, Vinod KY, Kassir SA, et al. Upregulation of CB1 receptors and agonist-stimulated [35S]GTPgammaS binding in the prefrontal cortex of depressed suicide victims. Mol Psychiatry. 2004;9(2):184–90. https://doi.org/10.1038/sj.mp.4001376.

Hyggen C. Does smoking cannabis affect work commitment? Addiction. 2012;107(7):1309–15. https://doi.org/10.1111/j.1360-0443.2012.03796.x.

Kaminer Y, Burleson JA, Burke R, Litt MD. The efficacy of contingency management for adolescent cannabis use disorder: a controlled study. Subst Abus. 2014;35(4):391–8.

Kamon J, Budney A, Stanger C. A contingency management intervention for adolescent marijuana abuse and conduct problems. J Am Acad Child Adolesc Psychiatry. 2005;44(6):513–21.

Killeen TK, McRae-Clark AL, Waldrop AE, Upadhyaya H, Brady KT. Contingency management in community programs treating adolescent substance abuse: a feasibility study. J Child Adolesc Psychiatr Nurs. 2012;25(1):33–41.

King BA, Jones CM, Baldwin GT, Briss PA. The evali and youth vaping epidemics—implications for public health. N Engl J Med. 2020;382(8):689–91. https://doi.org/10.1056/NEJMp1916171. Epub 2020 Jan 17. PMID: 31951683; PMCID: PMC7122126.

Knapp AA, Lee DC, Borodovsky JT, Auty SG, Gabrielli J, Budney AJ. Emerging trends in Cannabis Administration among adolescent cannabis users. J Adolesc Health. 2019;64(4):487–93. https://doi.org/10.1016/j.jadohealth.2018.07.012. Epub 2018 Sep 8. PMID: 30205931; PMCID: PMC6408312.

Knight JR, Sherritt L, Shrier LA, Harris SK, Chang G. Validity of the CRAFFT substance abuse screening test among adolescent clinic patients. Arch Pediatr Adolesc Med. 2002;156(6):607–14.

Kondo KK, Morasco BJ, Nugent SM, Ayers CK, O'Neil ME, Freeman M, Kansagara D. Pharmacotherapy for the Treatment of Cannabis Use Disorder: A Systematic Review. Ann Intern Med. 2020;172(6):398–412. https://doi.org/10.7326/M19-1105. Epub 2020 Mar 3. PMID: 32120384.

Krishnan-Sarin S, Duhig AM, McKee SA, McMahon TJ, Liss T, McFetridge A, Cavallo DA. Contingency management for smoking cessation in adolescent smokers. Exp Clin Psychopharmacol. 2006;14(3):306.

Ksir C, Hart CL. Cannabis and psychosis: a critical overview of the relationship. Curr Psychiatry Rep. 2016;18(2):12. https://doi.org/10.1007/s11920-015-0657-y.

Kuntsche E, Jordan MD. Adolescent alcohol and cannabis use in relation to peer and school factors: results of multilevel analyses. Drug Alcohol Depend. 2006;84(2):167–74. https://doi.org/10.1016/j.drugalcdep.2006.01.014.

Kwan LY, Eaton DL, Andersen SL, et al. This is your teen brain on drugs: in search of biological factors unique to dependence toxicity in adolescence. Neurotoxicol Teratol. 2020;81:106916. https://doi.org/10.1016/j.ntt.2020.106916.

Lawn W, Fernandez-Vinson N, Mokrysz C, Hogg G, Lees R, Trinci K, Petrilli K, Borissova A, Ofori S, Waters S, Michór P, Wall MB, Freeman TP, Curran HV. The CannTeen study: verbal episodic memory, spatial working memory, and response inhibition in adolescent and adult cannabis users and age-matched controls. Psychopharmacology (Berl). 2022;239(5):1629–41. https://doi.org/10.1007/s00213-022-06143-3. Epub 2022 Apr 29. Erratum in: Psychopharmacology (Berl). 2022;239(7):2371. https://doi.org/10.1007/s00213-022-06169-7. PMID: 35486121; PMCID: PMC9110435.

Lee JO, Hill KG, Hartigan LA, Boden JM, Guttmannova K, Kosterman R, Bailey JA, Catalano RF. Unemployment and substance use problems among young adults: does childhood low socioeconomic status exacerbate the effect? Soc Sci Med. 2015;143:36–44.

Lev-Ran S, Roerecke M, Le Foll B, George TP, McKenzie K, Rehm J. The association between cannabis use and depression: a systematic review and meta-analysis of longitudinal studies. Psychol Med. 2014;44(4):797–810. https://doi.org/10.1017/S0033291713001438.

Levin FR, Mariani JJ, Brooks DJ, Pavlicova M, Cheng W, Nunes EV. Dronabinol for the treatment of cannabis dependence: a randomized, double-blind, placebo-controlled trial. Drug Alcohol Depend. 2011;116(1-3):142–50. https://doi.org/10.1016/j.drugalcdep.2010.12.010. Epub 2011 Feb 18. PMID: 21310551; PMCID: PMC3154755.

Levy S, Brogna M, Minegishi M, et al. Assessment of screening tools to identify substance use disorders among adolescents. JAMA Netw Open. 2023;6(5):e2314422. https://doi.org/10.1001/jamanetworkopen.2023.14422.

Liddle HA, Dakof GA, Parker K, Diamond GS, Barrett K, Tejeda M. Multidimensional family therapy for adolescent drug abuse: Results of a randomized clinical trial. Am J Drug Alcohol Abuse. 2001;27(4):651–88.

Lonsdale H, Wilsey MJ. Paediatric cannabinoid hyperemesis. Curr Opin Pediatr. 2022;34(5):510–5. https://doi.org/10.1097/MOP.0000000000001157. Epub 2022 Aug 10. PMID: 35946907.

Lonsdale H, Kimsey KM, Brown JM, et al. Pediatric cannabinoid hyperemesis: asingle institution 10-year case series. J Adolesc Health. 2020;68:255–61.

Lorenzetti V, Hoch E, Hall W. Adolescent cannabis use, cognition, brain health and educational outcomes: a review of the evidence. Eur Neuropsychopharmacol. 2020;36:169–80. https://doi.org/10.1016/j.euroneuro.2020.03.012.

Lubman DI, Cheetham A, Yü Cel M. Cannabis and adolescent brain development. Pharmacol Ther. 2015;148:1–16. https://doi.org/10.1016/j.pharmthera.2014.11.009.

Macleod J, Oakes R, Copello A, et al. Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. Lancet. 2004;363(9421):1579–88. https://doi.org/10.1016/S0140-6736(04)16200-4.

Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O. Involvement of CB1 cannabinoid receptors in emotional behaviour. Psychopharmacology. 2002;159(4):379–87. https://doi.org/10.1007/s00213-001-0946-5.

Mariani JJ, Pavlicova M, Jean Choi C, Basaraba C, Carpenter KM, Mahony AL, Brooks DJ, Bisaga A, Naqvi N, Nunes EV, Levin FR. Quetiapine treatment for cannabis use disorder. Drug Alcohol Depend. 2021;218:108366. https://doi.org/10.1016/j.drugalcdep.2020.108366. Epub 2020 Oct 20. PMID: 33153828.

Mason BJ, Crean R, Goodell V, Light JM, Quello S, Shadan F, Buffkins K, Kyle M, Adusumalli M, Begovic A, Rao S. A proof-of-concept randomized controlled study of gabapentin: effects on cannabis use, withdrawal and executive function deficits in cannabis-dependent adults. Neuropsychopharmacology. 2012;37(7):1689–98. https://doi.org/10.1038/npp.2012.14. Epub 2012 Feb 29. PMID: 22373942; PMCID: PMC3358737.

Medina KL, Nagel BJ, Park A, McQueeny T, Tapert SF. Depressive symptoms in adolescents: associations with white matter volume and marijuana use. J Child Psychol Psychiatry. 2007;48(6):592–600. https://doi.org/10.1111/j.1469-7610.2007.01728.x.

Meier MH, Caspi A, Ambler A, et al. Persistent cannabis users show neuropsychological decline from childhood to midlife. Proc Natl Acad Sci USA. 2012;109(40):E2657–64. https://doi.org/10.1073/pnas.1206820109.

Meier MH, Hill ML, Small PJ, Luthar SS. Associations of adolescent cannabis use with academic performance and mental health: a longitudinal study of upper middle class youth. Drug Alcohol Depend. 2015;156:207–12. https://doi.org/10.1016/j.drugalcdep.2015.09.010.

Meier MH, Docherty M, Leischow SJ, Grimm KJ, Pardini D. Cannabis concentrate use in adolescents. Pediatrics. 2019;144(3):e20190338. https://doi.org/10.1542/peds.2019-0338.

Meisel SN, Carpenter RW, Treloar Padovano H, Miranda R Jr. Day-level shifts in social contexts during youth cannabis use treatment. J Consult Clin Psychol. 2021;89(4):251.

Menkes DB, Howard RC, Spears GF, Cairns ER. Salivary THC following cannabis smoking correlates with subjective intoxication and heart rate. Psychopharmacology. 1991;103(2):277–9. https://doi.org/10.1007/BF02244217.

Mennis J, McKeon TP, Stahler GJ. Recreational cannabis legalization alters associations among cannabis use, perception of risk, and cannabis use disorder treatment for adolescents and young adults. Addict Behav. 2023;138:107552.

Miech RA, Johnston LD, Patrick ME, et al. Monitoring the future national survey results on drug use, 1975–2022: secondary school students. Monitoring the future monograph series. Ann Arbor, MI: Institute for Social Research, University of Michigan; 2023.

Miech RA, Johnston LD, Patrick ME, O'Malley PM. Monitoring the future national survey results on drug use, 1975–2023: overview and detailed results for secondary school students. *Monitoring the future monograph series*. Ann Arbor, MI: Institute for Social Research, University of Michigan; 2024. https://monitoringthefuture.org/results/annual-reports/.

Miller WR, Rollnick S. Motivational interviewing. Helping people change. Guilford Press; 2012.

Mitic M, Woodcock K, Amering M, et al. Toward an integrated model of supportive peer relationships in early adolescence: a systematic review and exploratory meta-analysis. Front Psychol. 2020;12:589403.

Miranda R Jr, Treloar H, Blanchard A, Justus A, Monti PM, Chun T, Swift R, Tidey JW, Gwaltney CJ. Topiramate and motivational enhancement therapy for cannabis use among youth: a randomized placebo-controlled pilot study. Addict Biol. 2017;22(3):779–90. https://doi.org/10.1111/adb.12350. Epub 2016 Jan 11. PMID: 26752416; PMCID: PMC4940362.

Mokrysz C, Landy R, Gage SH, Munafò MR, Roiser JP, Curran HV. Are IQ and educational outcomes in teenagers related to their cannabis use? A prospective cohort study. J Psychopharmacol. 2016;30(2):159–68. https://doi.org/10.1177/0269881115622241.

Moore TH, Zammit S, Lingford-Hughes A, et al. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 2007;370(9584):319–28. https://doi.org/10.1016/S0140-6736(07)61162-3.

Moss HB, Ge S, Trager E, Saavedra M, Yau M, Ijeaku I, Deas D. Risk for substance use disorders in young adulthood: associations with developmental experiences of homelessness, foster care, and adverse childhood experiences. Compr Psychiatry. 2020;100:152175.

Nourbakhsh M, Miller A, Gofton J, Jones G, Adeagbo B. Cannabis hyperemesis syndrome: reports of fatal cases. J Forensic Sci. 2019;64(1):270–4. https://doi.org/10.1111/1556-4029.13819.

O'Grady MA, Iverson MG, Suleiman AO, Rhee TG. Is legalization of recreational cannabis associated with levels of use and cannabis use disorder among youth in the United States? A rapid systematic review. Eur Child Adolesc Psychiatry. 2024;33(3):701–23. https://doi.org/10.1007/s00787-022-01994-9.

Palamar JJ, Fenstermaker M, Kamboukos D, Ompad DC, Cleland CM, Weitzman M. Adverse psychosocial outcomes associated with drug use among US high school seniors: a comparison of alcohol and marijuana. Am J Drug Alcohol Abuse. 2014;40(6):438–46. https://doi.org/10.3109/00952990.2014.943371.

Pasch KE, Latimer LA, Cance JD, Moe SG, Lytle LA. Longitudinal bi-directional relationships between sleep and youth substance use. J Youth Adolesc. 2012;41(9):1184–96. https://doi.org/10.1007/s10964-012-9784-5. Epub 2012 Jul 1. PMID: 22752878; PMCID: PMC3431186.

Patton GC, Coffey C, Carlin JB, Degenhardt L, Lynskey M, Hall W. Cannabis use and mental health in young people: cohort study. BMJ. 2002;325(7374):1195–8. https://doi.org/10.1136/bmj.325.7374.1195.

Patton GC, McMorris BJ, Toumbourou JW, Hemphill SA, Donath S, Catalano RF. Puberty and the onset of substance use and abuse. Pediatrics. 2004;114(3):e300–6. https://doi.org/10.1542/peds.2003-0626-F.

Pfund RA, Ginley MK, Boness CL, Rash CJ, Zajac K, Witkiewitz K. Contingency management for drug use disorders: meta-analysis and application of Tolin's criteria. Clin Psychol Sci Pract. 2024;31(2):136–50. https://doi.org/10.1037/cps0000121.

Richards JR, Gordon BK, Danielson AR, Moulin AK. Pharmacologic treatment of cannabinoid hyperemesis syndrome: a systematic review. Pharmacotherapy. 2017;37(6):725–34. https://doi.org/10.1002/phar.1931.

Roane BM, Taylor DJ. Adolescent insomnia as a risk factor for early adult depression and substance abuse. Sleep. 2008;31(10):1351–6.

Schaefer JD, Hamdi NR, Malone SM, et al. Associations between adolescent cannabis use and young-adult functioning in three longitudinal twin studies. Proc Natl Acad Sci USA. 2021;118(14):e2013180118. https://doi.org/10.1073/pnas.2013180118.

Scholes-Balog KE, Hemphill SA, Evans-Whipp TJ, Toumbourou JW, Patton GC. Developmental trajectories of adolescent cannabis use and their relationship to young adult social and behavioural adjustment: a longitudinal study of Australian youth. Addict Behav. 2016;53:11–8. https://doi.org/10.1016/j.addbeh.2015.09.008.

Schweinsburg AD, Brown SA, Tapert SF. The influence of marijuana use on neurocognitive functioning in adolescents. Curr Drug Abuse Rev. 2008;1(1):99–111. https://doi.org/10.2174/1874473710801010099.

Scott JC, Slomiak ST, Jones JD, Rosen AFG, Moore TM, Gur RC. Association of Cannabis with Cognitive Functioning in adolescents and young adults: a systematic review and meta-analysis. JAMA Psychiatry. 2018;75(6):585–95. https://doi.org/10.1001/jamapsychiatry.2018.0335.

Scott JC. Impact of Adolescent Cannabis Use on Neurocognitive and Brain Development. Psychiatr Clin North Am. 2023;46(4):655–76. https://doi.org/10.1016/j.psc.2023.03.012. PMID: 37879830.

Silins E, Horwood LJ, Patton GC, et al. Young adult sequelae of adolescent cannabis use: an integrative analysis. Lancet Psychiatry. 2014;1(4):286–93. https://doi.org/10.1016/S2215-0366(14)70307-4.

Sorensen CJ, DeSanto K, Borgelt L, Phillips KT, Monte AA. Cannabinoid hyperemesis syndrome: diagnosis, pathophysiology, and treatment—a systematic review. J Med Toxicol. 2017;13(1):71–87. https://doi.org/10.1007/s13181-016-0595-z.

Stanger C, Budney AJ, Kamon JL, Thostensen J. A randomized trial of contingency management for adolescent marijuana abuse and dependence. Drug Alcohol Depend. 2009;105(3):240–7.

Stanger C, Ryan SR, Scherer EA, Norton GE, Budney AJ. Clinic-and home-based contingency management plus parent training for adolescent cannabis use disorders. J Am Acad Child Adolesc Psychiatry. 2015;54(6):445–53.

Stein LAR, Martin R, Clair-Michaud M, Lebeau R, Hurlbut W, Kahler CW, Rohsenow D. A randomized clinical trial of motivational interviewing plus skills training vs. relaxation plus education and 12-steps for substance using incarcerated youth: effects on alcohol, marijuana and crimes of aggression. Drug Alcohol Depend. 2020;207:107774.

Steinberg L, Morris AS. Adolescent development. Annu Rev Psychol. 2001;52:83–110. https://doi.org/10.1146/annurev.psych.52.1.83.

Substance Abuse and Mental Health Services Administration. (2023). Treatment episode data set 2021: annual detailed tables. Rockville, MD: Center for Behavioral Health Statistics and Quality, Substsance Abuse and Mental Health Services Administration. https://www.samhsa.gov/data/.

Substance Abuse and Mental Health Services Administration. (2024). Key substance use and mental health indicators in the United States: Results from the 2023 National Survey on Drug Use and Health (HHS Publication No. PEP24-07-021, NSDUH Series H-59). Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration. https://www.samhsa.gov/data/report/2023-nsduh-annual-national-report

Sultan RS, Zhang AW, Olfson M, Kwizera MH, Levin FR. Nondisordered cannabis use among US adolescents. JAMA Netw Open. 2023;6(5):e2311294–4.

Tashkin DP, Simmons MS, Tseng CH. Impact of changes in regular use of marijuana and/or tobacco on chronic bronchitis. COPD. 2012;9(4):367–74. https://doi.org/10.3109/15412555.2012.671868.

Taylor DR, Poulton R, Moffitt TE, Ramankutty P, Sears MR. The respiratory effects of cannabis dependence in young adults. Addiction. 2000;95(11):1669–77. https://doi.org/10.1046/j.1360-0443.2000.951116697.x.

Taylor DR, Fergusson DM, Milne BJ, et al. A longitudinal study of the effects of tobacco and cannabis exposure on lung function in young adults. Addiction. 2002;97(8):1055–61. https://doi.org/10.1046/j.1360-0443.2002.00169.x.

Thompson K, Leadbeater B, Ames M, Merrin GJ. Associations between marijuana use trajectories and educational and occupational success in young adulthood. Prev Sci. 2019;20(2):257–69. https://doi.org/10.1007/s11121-018-0904-7.

Troxel WM, Ewing B, D'Amico EJ. Erratum to examining racial/ethnic disparities in the association between adolescent sleep and alcohol or marijuana use [sleep health 1 (2015) 104-108]. Sleep Health. 2015;1(3):221. https://doi.org/10.1016/j.sleh.2015.07.008.

Tuvel AL, Winiger EA, Ross JM. A Review of the effects of adolescent Cannabis use on physical health. Child Adolesc Psychiatr Clin N Am. 2023;32(1):85–105. https://doi.org/10.1016/j.chc.2022.07.005. Epub 2022 Oct 22. PMID: 36410908; PMCID: PMC10165991.

Vandrey R, Budney AJ, Kamon JL, Stanger C. Cannabis withdrawal in adolescent treatment seekers. Drug Alcohol Depend. 2005;78(2):205–10.

Vinod KY, Arango V, Xie S, et al. Elevated levels of endocannabinoids and CB1 receptor-mediated G-protein signaling in the prefrontal cortex of alcoholic suicide victims. Biol Psychiatry. 2005;57(5):480–6. https://doi.org/10.1016/j.biopsych.2004.11.033.

Volkow ND, Baler RD, Compton WM, Weiss SR. Adverse health effects of marijuana use. N Engl J Med. 2014;370(23):2219–27. https://doi.org/10.1056/NEJMra1402309.

Volkow ND, Swanson JM, Evins AE, et al. Effects of cannabis use on human behavior, including cognition, motivation, and psychosis: a review. JAMA Psychiatry. 2016;73(3):292–7. https://doi.org/10.1001/jamapsychiatry.2015.3278.

Walker DD, Stephens RS, Blevins CE, Banes KE, Matthews L, Roffman RA. Augmenting brief interventions for adolescent marijuana users: the impact of motivational check-ins. J Consult Clin Psychol. 2016;84(11):983–92. https://doi.org/10.1037/ccp0000094. PMID: 27762569; PMCID: PMC5113812.

Wolff J, Esposito-Smythers C, Frazier E, Stout R, Gomez J, Massing-Schaffer M, Spirito A. A randomized trial of an integrated cognitive behavioral treatment protocol for adolescents receiving home-based services for co-occurring disorders. J Subst Abus Treat. 2020;116:108055.

Womack SR, Shaw DS, Weaver CM, Forbes EE. Bidirectional associations between cannabis use and depressive symptoms from adolescence through early adulthood among at-risk young men. J Stud Alcohol Drugs. 2016;77(2):287–97. https://doi.org/10.15288/jsad.2016.77.287.

Zhang C, Brook JS, Leukefeld CG, Brook DW. Trajectories of marijuana use from adolescence to adulthood as predictors of unemployment status in the early forties. Am J Addict. 2016;25(3):203–9. https://doi.org/10.1111/ajad.12361.

Chapter 14
Early Cannabis Use in the Context of Developmental Brain Disorders: A Particular Focus on Schizophrenia and Autism Spectrum Disorder

Georgia Watt and Tim Karl

1 Introduction

Cannabis use can have significant effects on mental health, particularly in relation to conditions like schizophrenia and autism spectrum disorder (ASD), but the relationships are complex and at times conflicting evidence has been published. Importantly, this area of research is still developing and the potential therapeutic benefits of (medicinal) cannabis use need to be balanced with the potential risks involved in using medicinal cannabis during adolescence.

Cannabis use during adolescence has been associated with an increased risk of developing schizophrenia. This effect appears to be most prominent in individuals with a genetic predisposition for the disorder or in combination with other environmental risk factors. The consumption of cannabis during this critical period of brain development can not only affect schizophrenia risk but also contribute to worsening symptoms and increased risk of psychotic relapse once the disease has established itself.

Based on this knowledge, it is an interesting development that the therapeutic use of (medicinal) cannabis, in particular of cannabis strains rich in cannabidiol (CBD), has received increasing attention in the context of ASD therapy (Silva Junior et al. 2021). This is even though insights gained into the validity of this therapeutic approach are currently mostly anecdotal and not always consistent. It appears that mostly CBD-rich cannabis formulations with low Δ-9-tetrahydrocannabinol (THC) content have been evaluated with some success in treating disease symptoms in children (Bilge and Ekici 2021) but the effects of adolescent cannabis use have also been considered (Hacohen et al. 2022).

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Adolescence is a particularly sensitive period for the effects of cannabis (or any other compound) on ongoing brain development; thus, adolescent cannabis use may have more pronounced effects on mental health compared to adult use. The role of adolescent cannabis use in the context of mental health (considering both detrimental as well as potentially therapeutic effects) has not been well researched to date. The following sections will provide some insights into the effects of cannabis use on the adolescent brain and its longer-term effects in the context of mental health considerations.

1.1 The Adolescent Brain

1.1.1 Physiological Changes during Adolescence

During adolescence, the brain undergoes a period of maturation and experiences a number of large changes (Rubino and Parolaro 2016), which makes adolescence a developmental period particularly vulnerable to exogenous interventions or impacts. The brain experiences changes in gross morphology, characterised by the decrease of grey matter and increase in white matter (Rubino and Parolaro 2016). These changes initially occur in more primitive brain regions, with the anterior frontal and temporal lobes being the last to fully mature (Rubino and Parolaro 2016). Literature suggests that the decrease in grey matter during adolescence may be a result of synaptic pruning, a developmental advancement which results in more efficient neural communication (Rubino and Parolaro 2016). Similarly, the white matter increase is thought to be due to increased myelination and axon calibre, an indication that axons are becoming more organised and therefore more efficient (Rubino and Parolaro 2016). Importantly, circuitry connectivity refinement is reported to occur mostly in the prefrontal cortex (PFC), amygdala, striatum, and thalamus (Rubino and Parolaro 2016), regions relevant for cognition, reward, and emotion (Bara et al. 2021).

Additionally, preclinical research has demonstrated that a number of neurotransmitter systems undergo developmental changes during adolescence, most notably the dopaminergic, gamma-aminobutyric acid (GABA)-ergic and glutamatergic systems (Rubino and Parolaro 2016). Studies have found that during adolescence there is a peak in dopamine receptor expression and neuron activity in cortical and subcortical areas (Andersen et al. 2000; Tarazi et al. 1998; McCutcheon and Marinelli 2009). The development of the GABAergic system during adolescence is specific to individual brain regions, with the neocortex experiencing refinement until the end of adolescence, while the GABAergic system in the hippocampus achieves maturity before the onset of puberty (Hedner et al. 1984; Kilb 2012; Zamberletti et al. 2014). Finally, from adolescence to adulthood there are changes in the subunit expression of N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors evident in the PFC (Rubino and Parolaro 2016).

1.1.2 The Endocannabinoid System in the Adolescent Brain

The endocannabinoid system (eCBS) plays an important regulatory role in all stages of development as it is broadly involved in determination of cell fate, neuronal migration as well as the regulation of signalling pathways and synaptic transmission (Bara et al. 2021). More specifically, the eCBS plays part in various development processes, including neurogenesis, glial formation, neuronal migration, axonal elongation, axonal bundling, synaptogenesis and synaptic pruning (Berghuis et al. 2007; Maccarrone et al. 2014; Mulder et al. 2008). For example, the endocannabinoid tone is thought to be crucial to the maturation of the glutamatergic system in the PFC (Rubino et al. 2015). Interestingly, studies have indicated that the dynamic nature of the eCBS during adolescence occurs mainly in regions involved in cognition, reward and motivation (i.e. PFC, nucleus accumbens and caudate putamen) (Rubino and Parolaro 2016). Indeed, the eCBS has a powerful regulatory control of synaptic function in these regions thereby impacting a wide range of behaviours in adulthood, including cognition, motor function, emotions, reward and food intake (Meyer et al. 2018). It should also be noted that the eCBS continues to play an important role in neurotransmission modulation and synaptic plasticity in the mature brain (Castillo et al. 2012).

The expression of the cannabinoid receptor 1 (CB1) varies across developmental periods, notably studies have found that CB1 density increases from adolescence to adulthood, when CB1 becomes the most abundant G-protein coupled receptor (Rubino et al. 2015; Belue et al. 1995; Verdurand et al. 2011; Ellgren et al. 2008; Eggan et al. 2010; Mackie 2005; Herkenham et al. 1990; Wang et al. 2003). In adulthood, CB1 receptors are mostly found in the cerebral cortex, basal ganglia, hippocampus, and cerebellum (Mackie 2005) in the presynaptic terminals of glutamatergic and GABAergic cells (Freund et al. 2003; Marsicano and Lutz 1999). In vivo studies have found that CB1 receptor expression and endocannabinoid signalling in neurons play a vital role in neuronal migration and differentiation of GABAergic and glutamatergic cortical cells, cholinergic basal forebrain neurons, hypothalamic neurons and GABAergic cerebellar cells (Keimpema et al. 2013). One study indicated that CB1 receptors could also play a role in the elimination of synaptic pruning of excitatory synapses as the administration of a CB1 receptor antagonist, AM251, from early to late adolescence inhibited the decrease in PSD95, gluN2A and gluA2, which all play fundamental roles in the synaptic organisation of the PFC during adolescence (Rubino et al. 2015).

The endocannabinoids, anandamide (AEA) and 2-Arachidonoylglycerol (2-AG), both have distinct expression profiles throughout adolescence (Rubino and Parolaro 2016). 2-AG levels have been found to fluctuate throughout adolescence—peaking in early and late adolescence—then stabilising in adulthood (Ellgren et al. 2008; Berrendero et al. 1999). However, anandamide levels increase throughout adolescence, with levels being three times greater in later adolescence in the PFC compared to the early stages (Ellgren et al. 2008). The dynamic nature of the eCBS, including its receptors, endocannabinoids and enzymes, during adolescence and their involvement in crucial developmental and maturational brain processes,

emphasises that exogenous factors which interfere with this fine-balanced system could lead to altered brain maturation and changed behavioural responses (Rubino and Parolaro 2016).

1.2 The Effect of Cannabis Use on the Adolescent Brain

1.2.1 Physiological Changes of Cannabis Use on Brain Development

Cannabis abuse during adolescence has been found to disrupt normal neuromaturation processes, including grey and white matter development and synaptic pruning (Lubman et al. 2015a). Interestingly, studies have found that adolescent cannabis use leads to altered neurodevelopment in cortices which are rich in CB1 receptors (Cousijn et al. 2012; Albaugh et al. 2021). Albaugh and colleagues (Albaugh et al. 2021) found that cannabis use dose-dependently led to increased cortical thinning in the PFC, while Cousijn and colleagues found that increased cannabis use resulted in reduced grey matter volume in the hippocampus and amygdala (Cousijn et al. 2012). Further, adolescent cannabis exposure can lead to alterations in dendritic spine density (Rubino and Parolaro 2016), with cannabinoids causing reduced dendritic spine density in rodent studies. A study in male rats treated with the synthetic cannabinoid WIN55212–2 (WIN: potent CB1 and CB2 agonist) during adolescence revealed an immediate reduction in spine density in the nucleus accumbens (Carvalho et al. 2016). Similarly, adolescent THC administration caused a significant reduction in spine density in pyramidal neurons in the PFC of adult female rats (Rubino et al. 2015). Furthermore, adolescent THC exposure was found to decrease spine density, dendrite length and number in the hippocampus in adulthood (Rubino et al. 2009). Importantly, reduction of dendritic spines has also been detected after low-dose adolescent cannabinoid challenge (Rubino et al. 2015; Miller et al. 2019) and was accompanied by a reorganisation of genes relevant for neuronal development and synaptic plasticity (Batool et al. 2019).

As noted above, the key neurotransmitter systems that are altered during adolescence are the GABAergic, glutamatergic and dopaminergic systems. Notably, preclinical studies have found that adolescent cannabis use results in developmental impairments in the GABAergic and glutamatergic systems (Rubino and Parolaro 2016). For example, adolescent THC exposure has profound effects on excitatory and inhibitory mechanisms in adulthood, with those being mainly observed in the PFC (Bara et al. 2021). A preclinical study found that WIN administration during early or mid-adolescence resulted in the downregulation of GABAergic transmission in the PFC (Cass et al. 2014). Similarly, adolescent THC challenge caused a reduction in glutamic acid decarboxylase 67 (GAD67) and basal GABA levels in the PFC (Zamberletti et al. 2014), potentially leading to a reduction in the GABAergic tone later in life (Lee et al. 2019).

Looking at effects on the glutamatergic system, adolescent THC exposure increases postsynaptic glutamatergic markers in the adult PFC (Zamberletti et al. 2016; Prini et al. 2017) and alters the expression of glutamate receptor subunits,

gluN2A and gluN2B, which play a role in synaptic plasticity (Zamberletti et al. 2016; Prini et al. 2017). Indeed, studies have found that after adolescent THC exposure, levels of gluN2A peak during adolescence rather than increasing into adulthood, conversely, gluN2B levels remain elevated after adolescent THC exposure rather than declining from mid-adolescence into adulthood (Zamberletti et al. 2016; Prini et al. 2017). These findings indicate that adolescent THC causes key markers for synaptic plasticity to mature earlier, in line with the premature pruning of dendritic spines (Batool et al. 2019). Thus, the reduction of GABAergic markers in combination with the beforementioned elevation in glutamatergic effects, indicates that adolescent cannabis exposure increases excitatory output later in life (Bara et al. 2021). Other examples for long-lasting effects of adolescent cannabis exposure include a study where administration of the synthetic CB1 agonist CP-55,940 (CP) during adolescence reduced potassium-evoked glutamate release in the hippocampus of adult female mice (Higuera-Matas et al. 2012).

It is important to note that the effect of adolescent cannabis use on neurotransmitter systems may be mediated not only directly, but at least in part, by modulating the eCBS as a natural endogenous target of adolescent cannabis use (Rubino et al. 2015). For example, chronic cannabis use in adolescence is widely reported to downregulate and desensitise CB1 receptors throughout the brain (Wiley and Burston 2014; Rubino et al. 2008). This effect on CB1 receptors appears to be dose-dependent as well as sex-dependent (Wiley and Burston 2014; Rubino et al. 2008). One study found that a moderate dose of THC increased CB1 receptors in the amygdala (Silva et al. 2016), while other studies reported that escalating high doses of THC reduced CB1 receptor levels in various brain areas, including the amygdala, PFC, striatum, hippocampus, thalamus, substantia nigra, ventral tegmental area (VTA) and cerebellum (Rubino et al. 2015; Rubino et al. 2008; Kruse et al. 2019). A study in female rodents exposed to THC during adolescence found that CB1 receptor binding decreases immediately after the last THC injection and not only persists into adulthood but is also paralleled by a reduction in anandamide levels in the PFC as well (Rubino et al. 2015). However, this relationship appears complex as Rubino and colleagues did not find changes to 2-AG levels despite another study reporting that 2-AG levels are discordant in animals exposed to THC (Ellgren et al. 2008). Finally, a study described that adolescent THC exposure prevents endocannabinoid-mediated long-term depression (LTD) in the PFC, which could be reversed by increasing anandamide availability (Cuccurazzu et al. 2018).

More broadly, adolescent cannabis use has been associated with an increased risk of developing psychiatric conditions, including psychosis disorders, anxiety, depression and addiction (Renard et al. 2014; Rubino et al. 2012). One of the most concerning potential consequences of early cannabis abuse is its impact on the risk to develop schizophrenia, as adolescent cannabis use is seen as a component/cumulative cause for schizophrenia (Arseneault et al. 2004). Conversely, adolescent cannabis use has recently been suggested as a form of self-medication for individuals with ASD (Silva Junior et al. 2021). Thus, in the next sections, the dichotomous effect of adolescent cannabis use in schizophrenia and autism spectrum disorder (ASD) will be outlined in more detail.

2 Adolescent Cannabis Use and the Development of Schizophrenia

Cannabis use is a well-known risk factor for schizophrenia, with adolescence being a heightened period of susceptibility (Lubman et al. 2015b). Chronic cannabis use has been associated with changes to the eCBS, most notably CB1 receptor changes, changes to neurotransmitter systems (i.e. dopaminergic, glutamatergic, GABAergic, serotonergic and noradrenergic systems) as well as changes to dendritic spine morphology and neuroinflammation (Chesworth and Karl 2017), all of which align with changes observed in post-mortem tissue from patients with schizophrenia. Importantly, chronic cannabis use has also been found to induce behavioural changes relevant to the positive, negative, cognitive and sensorimotor symptoms present in patients with schizophrenia (Chesworth and Karl 2017). It is important to note that there are a range of factors which influence the risk associated with cannabis use leading to schizophrenia, including genetic predisposition, amount and frequency of cannabis use, cannabinoid composition, poly-drug use, early-life trauma and comorbid mental disorders. Thus, adolescent cannabis use is seen as a component/cumulative cause for schizophrenia (Arseneault et al. 2004).

2.1 Physiological Changes Induced by Adolescent Cannabis Use which Are Associated with the Development of Schizophrenia

2.1.1 Changes to the Endocannabinoid System

As discussed previously, various aspects of the eCBS are impacted by adolescent cannabis use. The most notable changes in relevance to schizophrenia appear to be changes to CB1 receptor protein and messenger RNA expression (Chesworth and Karl 2017). In rodent models, chronic adolescent cannabinoid treatment reduced CB1 receptor mRNA and protein expression in several brain regions, including the PFC, hippocampus, striatum, substantia nigra, hypothalamus, amygdala and cerebellum (reviewed in (Chesworth and Karl 2017)). Indeed, adolescent treatment with THC, WIN or CP reduces CB1 receptor binding in the PFC (Rubino et al. 2015; Klein et al. 2011; Zamberletti et al. 2015; Lovelace et al. 2015; Thibault et al. 2013; Long et al. 2013). Importantly, these effects are affected by dose and treatment regime, as low doses do not alter CB1 receptor expression in the PFC (Ginovart et al. 2012) and shorter, sub-chronic regimes do not affect cortical CB1 receptor levels either (Tai et al. 2015)–see also (Long et al. 2013). Studies have shown that adolescent cannabinoid treatment reduces cortical long-term depression (Rubino et al. 2015) and impairs short-term recognition and spatial memory in adulthood (Rubino et al. 2015; Lovelace et al. 2015), suggesting that reduced CB1 receptor function may be involved in associated cognitive impairments. In line with this, in patients with schizophrenia, cannabis use causes cortical thinning in areas critical

for cognitive function and these areas are rich in CB1 receptors (i.e. dorsal lateral PFC and anterior cingulate cortex) (Rais et al. 2010; Rapp et al. 2013).

In the hippocampus, preclinical studies have found that chronic cannabinoid treatment causes sex-specific effects on CB1 receptor expression and binding with several reporting reductions (Rubino et al. 2008; Thibault et al. 2013; Long et al. 2013; Puighermanal et al. 2013; Cha et al. 2007; Lopez-Gallardo et al. 2012; Silva et al. 2015), although other studies could not find an impact on hippocampal expression levels (Gleason et al. 2012; Weed et al. 2016; Behan et al. 2012) or even revealed elevated hippocampal CB1 receptor protein and mRNA levels (Lopez-Gallardo et al. 2012; Winsauer et al. 2011). Interestingly, reductions in CB1 receptor expression appear to occur on hippocampal GABAergic interneurons (Thibault et al. 2013; Puighermanal et al. 2013). In the striatum and substantia nigra chronic adolescent cannabinoid treatment also appears to have an impact on CB1 receptors. A number of preclinical studies provided evidence that chronic THC during adolescence decreases CB1 receptor binding and mRNA expression in the striatum (Klein et al. 2011; Long et al. 2013; Ginovart et al. 2012) and reduces CB1 receptor mRNA and protein expression in the substantia nigra (Winsauer et al. 2011; Klug and Van Den Buuse 2013; Realini et al. 2011). It is currently unclear whether these CB1 receptor changes in the striatum and substantia nigra are associated with any behavioural changes. Finally, chronic adolescent THC treatment has also been found to reduce CB1 receptor binding and expression in the hypothalamus, amygdala and cerebellum (Rubino et al. 2008; Silva et al. 2016; Long et al. 2013; Tai et al. 2015; Cutando et al. 2013).

2.1.2 Dendritic Spines and Density

It has been proposed that changes to dendritic spine density and morphology contribute to schizophrenia aetiology, as developmental synaptic pruning and remodelling and onset of schizophrenia occur in the same developmental period (Selemon and Zecevic 2015). Indeed, reduced dendritic spine density and arborisation have been documented in the PFC of brain tissue from patients with schizophrenia (Moyer et al. 2015). Importantly, preclinical studies have found that chronic THC treatment during adolescence reduces dendritic spine length and number in the hippocampus (Rubino et al. 2009) and adolescence CP treatment reduces the number, length and complexity of dendritic spines in the PFC as well (Renard et al. 2016). The reduced hippocampal dendritic density was associated with cognitive impairments in spatial memory in the radial arm maze (Rubino et al. 2009).

2.1.3 Neurotransmitter Systems

As mentioned previously, adolescent cannabis use alters neurotransmitter systems, including the dopaminergic, glutamatergic, GABAergic, serotonergic and noradrenergic systems. Importantly, preclinical and clinical studies have demonstrated that

these neurotransmitter systems are also altered in patients with schizophrenia and some play key roles in the disease progression (Chesworth and Karl 2017).

The dopaminergic system has been reported to be dysregulated in patients with schizophrenia in a region and receptor specific manner. There is an elevation in dopamine receptor 2 (D2) expression in the striatum and PFC, but a reduction of the D2 receptor in other regions (i.e. thalamus) (Bolkan et al. 2016; Brisch et al. 2014; Howes et al. 2012). Similarly, D1 receptor expression is elevated in the parieto-temporal cortex but reduced in the PFC (Brisch et al. 2014). Importantly, adolescent cannabinoid exposure has been found to cause region and receptor specific alterations to the dopaminergic system. Preclinical studies have found that D1 receptor binding and mRNA expression is elevated in the nucleus accumbens following adolescent CP treatment (Fanarioti et al. 2015; Higuera-Matas et al. 2010) and D2/3 receptor binding is increased in the dorsal striatum following THC exposure (Ginovart et al. 2012). However, other studies find reduced D2 receptor binding levels in the hippocampus following adolescent CP treatment (Higuera-Matas et al. 2010), decreased D2 receptor mRNA and protein expression after WIN administration (Ginovart et al. 2012; Fanarioti et al. 2015; Gomes et al. 2014) and adolescent THC results in a downregulation of dopaminergic TH+ cell density and mRNA expression in the VTA (Ginovart et al. 2012; Behan et al. 2012). The downregulation of VTA dopaminergic function following cannabinoid exposure has been linked to behavioural deficits of schizophrenia rodent models in domains relevant to positive and cognitive symptoms (Gomes et al. 2014; Abboussi et al. 2016).

Increased glutamate levels have been reported in the PFC, basal ganglia and hippocampus in patients with schizophrenia (Moghaddam and Javitt 2012; Poels et al. 2014) and NMDA receptor dysfunction is also evident (Moghaddam and Javitt 2012; Balu and Coyle 2015). Importantly, chronic adolescent THC exposure increases glutamate release in the PFC and striatum (Zamberletti et al. 2014) and elevates cortical levels of NMDA and AMPA receptor subunits (gluN2, gluN2B and gluA1) (Rubino et al. 2015; Long et al. 2013). Similarly, chronic WIN treatment increases phosphorylation of the NMDAR subunit (N2RB) in the infralimbic cortex of the PFC (Kirschmann et al. 2017). However, there have been some conflicting reports which have found that adolescent cannabinoid treatment decreases metabotropic glutamate receptor expression In the PFC (Lovelace et al. 2015).

2.2 Behavioural Changes Induced by Adolescent Cannabis Use which Are Associated with the Development of Schizophrenia

2.2.1 Behaviours Relevant to Positive Symptoms

Positive symptoms of schizophrenia include paranoia and grandiose delusions, perceptual alteration, suspiciousness, conceptual disorganisation and fragmented thinking (Chesworth and Karl 2017), which are often modelled in rodent models by

elevated locomotor activity as an indicator of e.g. increased dopaminergic tone (Powell and Miyakawa 2006). Preclinical studies have found that while adult rats develop locomotor tolerance to chronic or sub-chronic treatment with THC or CP (Puighermanal et al. 2013; O'Brien et al. 2013; Wiley et al. 2008), adolescent rats do not (Wiley and Burston 2014; Klug and Van Den Buuse 2013; Wiley et al. 2008; Harte and Dow-Edwards 2010). Importantly, this altered tolerance during adolescence appears to be modulated by genetic susceptibility to schizophrenia (i.e. mutations in the *neuregulin 1* and *catechol-O-methyltransferase* genes) (Long et al. 2013; O'Tuathaigh et al. 2010).

2.2.2 Behaviours Relevant to Negative Symptoms

Negative symptoms of schizophrenia include social withdrawal, emotional blunting, anhedonia, avolition and alogia (Chesworth and Karl 2017), which are often modelled in rodents with social interaction, social preference and sucrose preference tests (Powell and Miyakawa 2006). Preclinical studies have found that chronic adolescent treatment with THC or WIN impairs social interaction when tested during the treatment period (Klein et al. 2011; Long et al. 2013; Quinn et al. 2008) and when tested drug-free during adulthood (Zamberletti et al. 2014; Zamberletti et al. 2015; Realini et al. 2011; O'Tuathaigh et al. 2010; Quinn et al. 2008; Leweke and Schneider 2011; O'Tuathaigh et al. 2012) . Chronic treatment with THC and WIN during adolescence was also able to reduce sucrose preference when animals were testing drug free in adulthood (Rubino et al. 2008; Realini et al. 2011; Bambico et al. 2010). Interestingly, preclinical studies have suggested that CB1 receptor expression in the amygdala may contribute to social impairments in schizophrenia as chronic adolescent THC treatment reduces social interaction and CB1 receptor expression in the amygdala of adult female rats (Realini et al. 2011). Importantly, these social impairments are reversible with URB597 treatment (an inhibitor of endocannabinoid degradation).

2.2.3 Cognitive Impairments

Cognitive impairments in schizophrenia include deficits in attention, working memory, long-term memory and executive function (Chesworth and Karl 2017). Preclinical studies have demonstrated that chronic exposure to THC during adolescence impairs spatial working memory (Zamberletti et al. 2014; Rubino et al. 2009; Rubino et al. 2008; Zamberletti et al. 2015; O'Tuathaigh et al. 2010; Steel et al. 2011) and short-term recognition memory (Puighermanal et al. 2013; Kirschmann et al. 2017). Further, sub-chronic THC in late adolescence was found to slow acquisition of cerebellar-dependent delayed eye-blink conditioning (Cutando et al. 2013). It is important to note that some cannabinoid-induced cognitive impairments appear to be dependent on the type of cannabinoid administered as they differ in potency and cannabinoid receptor binding specifics. For example, chronic adolescent WIN

treatment impairs spatial acquisition in the Morris Water Maze (Abboussi et al. 2016; Abush and Akirav 2012), recall of associative fear learning (Gleason et al. 2012) and reversal learning in adult rats (Gomes et al. 2014). However, these domains do not appear to be affected by adolescent THC treatment (Rubino et al. 2015, 2009; Weed et al. 2016; Winsauer et al. 2011; Winsauer and Sutton 2014). It has been suggested that THC only affects performance in operant tasks where there is a high cognitive load (Verrico et al. 2014; Irimia et al. 2015).

2.2.4 Sensorimotor Gating Disruption

Pre-pulse inhibition (PPI) is a measure of sensorimotor gating and an endophenotype of schizophrenia in both rodent models as well as humans (Braff and Geyer 1990). PPI is associated with aspects of cognition and global function (Powell et al. 2012). Preclinical studies have shown that chronic adolescent treatment with THC, CP or WIN impairs PPI in adulthood in rats but not in mice (Long et al. 2013; Gleason et al. 2012; Klug and Van Den Buuse 2013; Llorente-Berzal et al. 2011; Marusich et al. 2014; Wegener and Koch 2009)—but see also Izquierdo-Luengo et al. (2023). Further, chronic cannabinoid treatment in late adolescence did not impair PPI when animals were tested drug free (Silva et al. 2016; Gleason et al. 2012; Tournier and Ginovart 2014). In humans, previous cannabis use has been found to impair PPI, but the specific detrimental role of adolescent cannabis use on this behavioural domain is less well explored (Chadwick et al. 2013).

2.3 Summary

Cannabis is a well-established component/cumulative risk factor for schizophrenia, with adolescence being a heightened period of susceptibility. Indeed, chronic adolescent cannabis use causes numerous changes in the brain, which are associated with the development of schizophrenia including alterations to the eCBS and in particular CB1 receptors, other disease-relevant neurotransmitter systems and dendritic spine morphology. Further, adolescent cannabis use has been found to induce behavioural changes relevant to the positive, negative, and cognitive symptoms of schizophrenia and can increase the risk for psychotic relapse.

3 Adolescent Cannabis Use in the Context of Autism Spectrum Disorder (ASD)

ASD is a neurodevelopmental disorder characterised by deficits in social interaction, verbal and nonverbal communication and stereotyped and repetitive behaviours (Assoc 2013). It is also frequently associated with generalised anxiety and cognitive deficits (Zamberletti et al. 2017; de Bruin et al. 2007; Gillott and Standen

2007; Simonoff et al. 2008). The eCBS is involved in modulating emotional responses, mood, behavioural reactions and social interactions, thus, it is thought that individuals with ASD exhibit changes to the eCBS (Chakrabarti et al. 2015). Interestingly, it has been reported that people with ASD try to manage their symptoms by self-medicating with cannabis and its derivatives (Hua et al. 2021), as there appears to be growing evidence that cannabinoids can alleviate symptoms associated with ASD (Aran et al. 2019a; Barchel et al. 2018), however, there is limited evidence for its efficacy, safety and tolerability (Silva Junior et al. 2021) and highly standardised, double-blind, placebo-controlled studies are needed to substantiate these initial findings (Hacohen et al. 2022).

3.1 Physiological Changes in ASD and the Effect of Cannabis Exposure Early in Life (During Childhood or Adolescence)

Several alterations to the eCBS have been reported in individuals with ASD, including altered endocannabinoid tone, cannabinoid receptor expression and genetic variations in core eCBS genes (Silva Junior et al. 2021). A clinical trial in children with ASD revealed that AEA plasma concentrations were significantly lower in ASD patients than in controls (Karhson et al. 2018), and another trial revealed reduced serum levels of AEA, palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) compared to controls (Aran et al. 2019b). Interestingly, the latter trial found no change in serum levels of 2-AG or arachidonic acid (Aran et al. 2019b). These findings are supported by preclinical data, which suggest that AEA signalling plays a modulating role on rodent behaviours associated with ASD (Trezza et al. 2012). Current data suggests that reduced endocannabinoid tone may be the primary cause of neuronal hyper-excitability often associated with ASD (Bozzi et al. 2018; Sohal and Rubenstein 2019) and lower levels of endocannabinoids may play a central role in linking some environmental risk factors to ASD, for example exposure to pharmaceutical agents (Schultz et al. 2021).

Post-mortem studies have revealed that CB1 receptor expression is reduced in several brain areas in ASD patients (Purcell et al. 2001). Endocannabinoid signalling at CB1 receptors is vital in regulating synaptic plasticity at excitatory and inhibitory synapses and CB1 receptors play a regulatory role in the maturation of excitatory and inhibitory neurons. Thus, altered CB1 receptor expression may be associated with ASD phenotypes as a result of an imbalance in excitatory and inhibitory neurotransmission (Hashimotodani et al. 2007; Fernandez-Ruiz et al. 1999; Galve-Roperh et al. 2009). Additionally, evidence suggests that the eCBS plays a role in immunological dysfunctions associated with ASD as a result of increased CB2 expression in peripheral blood mononuclear cells and blood monocyte-derived macrophage cells present in ASD patients (Siniscalco et al. 2014; Siniscalco et al. 2013).

A study of rare genetic variants in genes coding the eCBS from a large sample of patients with a broad range of neurological disorders found that heterozygous variants in DAGL-α were associated with seizures and ASD (Smith et al. 2017). Neuroimaging research has also found that the CB1 receptor gene, *CNR1*, is associated with social reward sensitivity in humans (Jana et al. 2024), indicating that variations in CB1 receptors may contribute to social reward processing in individuals with ASD.

As a result of changes to the eCBS being associated with ASD, several studies have investigated the therapeutic potential of cannabinoids in ASD, although this research has particularly focused on the phytocannabinoid cannabidiol (CBD) and treatment often starts already in childhood prior to adolescence (Poleg et al. 2019). fMRI studies in ASD patients treated with a single dose of 600 mg oral CBD revealed that CBD decreased GABA+ in the dorsomedial PFC of ASD patients but increased GABA+ in neurotypical participants, indicating that the GABAergic system in ASD individuals is distinct to that of neurotypical individuals (Pretzsch et al. 2019a). CBD also elevated subcortical levels of glutamate and glutamine in both ASD and neurotypical participants (Pretzsch et al. 2019a). Further, these studies demonstrated that CBD increased fractional amplitude of low-frequency fluctuations in the cerebellar vermis and right fusiform gyrus in ASD individuals, which was not seen in neurotypical participants (Pretzsch et al. 2019b). The cerebellar vermis and the associated cerebellar-subcortical-cortical circuitry are critical for movement, language and social processing (D'Mello and Stoodley 2015) and the fusiform gyrus is involved in the visual processing of words and facial recognition (McCandliss et al. 2003; Morris et al. 2007), all of which can be altered in ASD.

3.2 Behavioural Changes with Relevance to ASD Induced by Cannabis Use in Early Life (During Childhood or Adolescence)

Several studies have found that cannabis and its derivatives are able to alleviate not only molecular changes in the brain of ASD patients (refer 3.1) but also symptoms associated with the disorder, including bouts of self-mutilation and anger, hyperactivity, sleep disorders and restlessness, anxiety, psychomotor agitation, irritability, aggression, sensory sensitivity, cognition, attention, social interaction, language change and depression (Aran et al. 2019a; Barchel et al. 2018).

3.2.1 Pre-Clinical Studies

Pre-clinical studies have demonstrated that modulation of the eCBS is able to alleviate several symptoms associated with ASD (Zamberletti et al. 2017), for example when manipulating cannabinoid receptors in genetic mouse models with relevance

for ASD. Treatment of a *fragile X mental retardation* (*Fmr1*) knockout model with the CB1 receptor antagonist, rimonabant, restored cognitive deficits, seizure susceptibility and nociceptive desensitisation (Busquets-Garcia et al. 2013). This beneficial effect was thought to be at least partly mediated by rimonabant correcting the overactivation of mTOR signalling and dendritic spine morphology in the hippocampus of knockout mice (Busquets-Garcia et al. 2013). Further, blocking CB2 receptors with the CB2 antagonist AM630 reduced anxiety-like behaviours and audiogenic seizure susceptibility in the same model (Busquets-Garcia et al. 2013), demonstrating the distinct involvement of the CB1 and CB2 receptors in the behavioural phenotype of this mouse model for Fragile X syndrome (FXS). It should be noted here that individuals affected by FXS are frequently co-diagnosed with ASD (Kaufmann et al. 2017). Additionally, another study reported low doses of rimonabant and the CB1 receptor neutral antagonist NESS0327 preventing the development of cognitive deficits in *Fmr1* knockout mice (Gomis-González et al. 2016). This therapeutic-like effect was associated with restoring mGlu-LTD in the hippocampus of knockout mice.

Another therapeutic target appears to be the manipulation of AEA signalling as it too alleviated the behavioural phenotype of genetic models with relevance to ASD. For example, the acute treatment with an inhibitor of fatty acid amide hydrolase (FAAH), URB597, improved anxiety-like behaviours and aversive memory although it did not affect social behaviours of *Fmr1* knockout mice (Qin et al. 2015). Conversely, another study found that acute administration of URB597 completely reversed social impairment in this mouse model (Wei et al. 2016), suggesting that AEA activity may have a prosocial effect. The discrepancy in these results may result from different treatment protocols and/or background strain differences between the two studies (Wei et al. 2016; Qin et al. 2015). Interestingly, increasing AEA levels through acute administration of URB597 completely reversed social deficits in a different mouse model for ASD, i.e. the *BTBR* $T^{+}Itpr3^{tf}/J$ (*BTBR*) knockout mouse model (Wei et al. 2016). Importantly, another study evaluated the effects of chronic CBD on BTBR mice and found that CBD was able to dose-dependently attenuate elevated repetitive self-grooming behaviours, hyperlocomotion, as well as social deficits typically seen in these mice (Shrader et al. 2024).

Finally, studies utilising environmental models for ASD, i.e. the valproic acid (VPA) and lipopolysaccharide (LPS)-induced rat models of ASD revealed that FAAH inhibition was associated with restoring social deficits. In particular, the administration of the FAAH inhibitor, PF3845, attenuated social deficits in male but not female rats exposed to VPA (Kerr et al. 2016), suggesting this may be a sex-specific effect. Similarly, the FAAH inhibitor, PF-04457845, prevented the development of social deficits in rats exposed to LPS (Doenni et al. 2016).

3.2.2 Clinical Trials

Several clinical trials have demonstrated the therapeutic potential of cannabinoids, namely CBD, in alleviating symptoms associated with ASD (Silva Junior et al. 2021). A retrospective study in children with ASD and severe behavioural problems (mean age 11.8 years +/− 3.5 years) revealed that treatment with CBD-rich cannabis (plant containing 20:1 CBD/THC ratio) for 7–13 months resulted in significant improvement in several behavioural domains as scored by parents on the caregiver global impression of change (CGIC) scale (Aran et al. 2019a). Significant improvements were reported for general behavioural problems (61% of children), for anxiety (39%) and for communication (47%) (Aran et al. 2019a). Further, improvement in disruptive behaviour was improved by 29% and 33%, respectively, after cannabis treatment when using the home situation questionnaire-ASD (HSQ-ASD) and the autism parenting stress index (APSI) (Aran et al. 2019a). A clinical trial in 53 children with ASD (mean age 11 years) who were treated with CBD/THC (20:1) oil for a median duration of 66 days (range: 30–588 days) revealed that cannabinoid treatment reduced self-harm and anger bouts (67.6% of children), hyperactivity (68.4%), sleep problems (71.4%) and anxiety (47.1%) (Barchel et al. 2018). Similarly, a study in 188 patients with ASD (mean age 12 years +/− 7 years) treated with a CBD-rich cannabis oil (containing 30% CBD and 1.5% THC) for 6 months reported improvements in restlessness, anger bouts, agitation and sleep problems in 91%, 90.3%, 85.2% and 78.1% of cases respectively (Bar-Lev Schleider et al. 2019). Furthermore, over 80% of parents reported a significant global improvement in their children's behaviour (Bar-Lev Schleider et al. 2019). An observational study in 18 patients treated with cannabis extract enriched with CBD (75:1 CBD/THC ratio) revealed improvements in sleep disorders, epileptic seizures, motor development, communication and cognitive performance (Fleury-Teixeira et al. 2019). Two national surveys reviewed 156 participants using cannabis and its derivatives for ASD (i.e. cannabis, THC/CBD combinations, as well as CBD in a mix of forms including flower, edibles, vaporised, gums and tinctures) and reported improved anxiety, irritability, anger bouts, agitation, hyperactivity and sleep problems (Adams et al. 2019). Finally, a single case study of a 6-year-old boy with ASD treated with dronabinol (THC) for 6 months found reductions in hyperactivity, lethargy, irritability, stereotypy and inappropriate speech (Kurz and Blaas 2010). All of these studies suggest that cannabis derivatives given during childhood /adolescence are able to alleviate at least some symptoms of ASD, in particular sleep problems, anger bouts, agitation and anxiety. It is important to note that reported adverse effects were mild and often transient, with the most common side effects being sleep difficulties, restlessness and change in appetite (Aran et al. 2019a; Barchel et al. 2018; Bar-Lev Schleider et al. 2019; Adams et al. 2019).

3.3 Summary

The eCBS plays a vital role in modulating several behavioural processes relevant to ASD, including emotional processing, mood, sociability and anxiety. Furthermore, the eCBS is altered in individuals with ASD, most prominent are impaired AEA signalling and CB receptor activation. Finally, preclinical and clinical studies indicate that the use of cannabis (in particular CBD-rich and THC-low cannabis strains) or its derivatives (in particular CBD) in early life may be beneficial and alleviate key symptoms of ASD without causing major side effects. However, the long-term effects of such treatments in early life have not been considered in great detail.

4 Conclusion

There is convincing evidence linking adolescent cannabis use with increased risks for mental health issues, most significantly, the increased risk to develop schizophrenia. This is due to the multitude of effects adolescent cannabis use has on disease-relevant brain processes and behavioural domains. However, more research is needed to fully understand the nature and extent of these effects and to clarify contributing factors including genetic candidates and environmental risk factors. This research is even more important as limited evidence suggests that cannabis exposure early in life (childhood or adolescence), in the context of emerging medicinal cannabis therapies, may be beneficial for the treatment of at least some imbalances in brain processes and behaviours relevant to ASD. In this context, it is important to note that individual responses to cannabis can vary (e.g. based on genetic heterogeneity and having been exposed to different environments during development), and what constitute a risk for one person may not have the same effect in another thereby opening up the possibility that cannabis use early in life may have some therapeutic relevance. However, it is important for individuals, especially those with a predisposition to or a diagnosis of mental health conditions, to approach cannabis use with caution and to consult healthcare professionals for personalised advice.

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References

Abboussi O, et al. Behavioral effects of D3 receptor inhibition and 5-HT4 receptor activation on animals undergoing chronic cannabinoid exposure during adolescence. Metab Brain Dis. 2016;31(2):321–7.

Abush H, Akirav I. Short-and long-term cognitive effects of chronic cannabinoids administration in late-adolescence rats. PLoS One. 2012;7(2):e31731.

Adams J, et al. Rating of the safety and effectiveness of marijuana, thc/cbd, and cbd for autism spectrum disorders: results of two national surveys. Autism Open Access. 2019;9:1–5.

Albaugh MD, et al. Association of Cannabis use during Adolescence with Neurodevelopment. JAMA Psychiatry. 2021;78(9):1–11.

Andersen SL, et al. Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. Synapse. 2000;37(2):167–9.

Aran A, et al. Brief report: Cannabidiol-rich cannabis in children with autism Spectrum disorder and severe behavioral problems-a retrospective feasibility study. J Autism Dev Disord. 2019a;49(3):1284–8.

Aran A, et al. Lower circulating endocannabinoid levels in children with autism spectrum disorder. Mol Autism. 2019b;10:2.

Arseneault L, et al. Causal association between cannabis and psychosis: examination of the evidence. Br J Psychiatry. 2004;184(2):110–7.

Assoc AP. Diagnostic and statistical manual of mental disorders, fifth edition (DSM-5). Am Psychiatric Assoc. 2013;21(21):591–643.

Balu DT, Coyle JT. The NMDA receptor 'glycine modulatory site' in schizophrenia: D-serine, glycine, and beyond. Curr Opin Pharmacol. 2015;20:109–15.

Bambico FR, et al. Chronic exposure to cannabinoids during adolescence but not during adulthood impairs emotional behaviour and monoaminergic neurotransmission. Neurobiol Dis. 2010;37(3):641–55.

Bara A, et al. Cannabis and synaptic reprogramming of the developing brain. Nat Rev Neurosci. 2021;22(7):423–38.

Barchel D, et al. Oral Cannabidiol use in children with autism Spectrum disorder to treat related symptoms and co-morbidities. Front Pharmacol. 2018;9:1521.

Bar-Lev Schleider L, et al. Real life experience of medical cannabis treatment in autism: analysis of safety and efficacy. Sci Rep. 2019;9(1):200.

Batool S, et al. Synapse formation: from cellular and molecular mechanisms to neurodevelopmental and neurodegenerative disorders. J Neurophysiol. 2019;121(4):1381–97.

Behan AT, et al. Chronic adolescent exposure to delta-9-tetrahydrocannabinol in COMT mutant mice: impact on indices of dopaminergic, endocannabinoid and GABAergic pathways. Neuropsychopharmacology. 2012;37(7):1773–83.

Belue RC, et al. The ontogeny of cannabinoid receptors in the brain of postnatal and aging rats. Neurotoxicol Teratol. 1995;17(1):25–30.

Berghuis P, et al. Hardwiring the brain: endocannabinoids shape neuronal connectivity. Science. 2007;316(5828):1212–6.

Berrendero F, et al. Analysis of cannabinoid receptor binding and mRNA expression and endogenous cannabinoid contents in the developing rat brain during late gestation and early postnatal period. Synapse. 1999;33(3):181–91.

Bilge S, Ekici B. CBD-enriched cannabis for autism spectrum disorder: an experience of a single center in Turkey and reviews of the literature. J Cannabis Res. 2021;3(1):53.

Bolkan SS, Carvalho Poyraz F, Kellendonk C. Using human brain imaging studies as a guide toward animal models of schizophrenia. Neuroscience. 2016;321:77–98.

Bozzi Y, Provenzano G, Casarosa S. Neurobiological bases of autism–epilepsy comorbidity: a focus on excitation/inhibition imbalance. Eur J Neurosci. 2018;47(6):534–48.

Braff DL, Geyer MA. Sensorimotor gating and schizophrenia. Human and animal model studies. Arch Gen Psychiatry. 1990;47(2):181–8.

Brisch R, et al. The role of dopamine in schizophrenia from a neurobiological and evolutionary perspective: old fashioned, but still in vogue. Front Psych. 2014;5:47.
Busquets-Garcia A, et al. Targeting the endocannabinoid system in the treatment of fragile X syndrome. Nat Med. 2013;19(5):603–7.
Carvalho AF, et al. Repeated administration of a synthetic cannabinoid receptor agonist differentially affects cortical and accumbal neuronal morphology in adolescent and adult rats. Brain Struct Funct. 2016;221(1):407–19.
Cass DK, et al. CB1 cannabinoid receptor stimulation during adolescence impairs the maturation of GABA function in the adult rat prefrontal cortex. Mol Psychiatry. 2014;19(5):536–43.
Castillo PE, et al. Endocannabinoid signaling and synaptic function. Neuron. 2012;76(1):70–81.
Cha YM, et al. Sex differences in the effects of delta9-tetrahydrocannabinol on spatial learning in adolescent and adult rats. Behav Pharmacol. 2007;18(5–6):563–9.
Chadwick B, Miller ML, Hurd YL. Cannabis use during adolescent development: susceptibility to psychiatric illness. Front Psychiatry. 2013;**4**:4.
Chakrabarti B, et al. Endocannabinoid signaling in Autism. Neurotherapeutics. 2015;12(4):837–47.
Chesworth R, Karl T. Molecular basis of cannabis-induced schizophrenia-relevant behaviours: insights from animal models. Curr Behav Neurosci Rep. 2017;4:254–79.
Cousijn J, et al. Grey matter alterations associated with cannabis use: results of a VBM study in heavy cannabis users and healthy controls. NeuroImage. 2012;59(4):3845–51.
Cuccurazzu B, et al. Adult cellular neuroadaptations induced by adolescent THC exposure in female rats are rescued by enhancing anandamide signaling. Int J Neuropsychopharmacol. 2018;21(11):1014–24.
Cutando L, et al. Microglial activation underlies cerebellar deficits produced by repeated cannabis exposure. J Clin Invest. 2013;123(7):2816–31.
de Bruin EI, et al. High rates of psychiatric co-morbidity in PDD-NOS. J Autism Dev Disord. 2007;37(5):877–86.
D'Mello AM, Stoodley CJ. Cerebro-cerebellar circuits in autism spectrum disorder. Front Neurosci. 2015;9:408.
Doenni V, et al. Deficient adolescent social behavior following early-life inflammation is ameliorated by augmentation of anandamide signaling. Brain Behav Immun. 2016;58:237–47.
Eggan SM, et al. Development of cannabinoid 1 receptor protein and messenger RNA in monkey dorsolateral prefrontal cortex. Cereb Cortex. 2010;20(5):1164–74.
Ellgren M, et al. Dynamic changes of the endogenous cannabinoid and opioid mesocorticolimbic systems during adolescence: THC effects. Eur Neuropsychopharmacol. 2008;18(11):826–34.
Fanarioti E, et al. Behavioral and neurochemical changes in mesostriatal dopaminergic regions of the rat after chronic administration of the cannabinoid receptor agonist WIN55, 212-2. Int J Neuropsychopharmacol. 2015;18(6):pyu097.
Fernandez-Ruiz JJ, et al. Role of endocannabinoids in brain development. Life Sci. 1999;65(6–7):725–36.
Fleury-Teixeira P, et al. Effects of CBD-enriched Cannabis sativa extract on autism Spectrum disorder symptoms: an observational study of 18 participants undergoing compassionate use. Front Neurol. 2019;10:1145.
Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev. 2003;83(3):1017–66.
Galve-Roperh I, et al. The endocannabinoid system and the regulation of neural development: potential implications in psychiatric disorders. Eur Arch Psychiatry Clin Neurosci. 2009;259(7):371–82.
Gillott A, Standen PJ. Levels of anxiety and sources of stress in adults with autism. J Intellect Disabil. 2007;11(4):359–70.
Ginovart N, et al. Chronic Δ9-tetrahydrocannabinol exposure induces a sensitization of dopamine D2/3 receptors in the mesoaccumbens and nigrostriatal systems. Neuropsychopharmacology. 2012;37(11):2355–67.

Gleason KA, et al. Susceptibility of the adolescent brain to cannabinoids: long-term hippocampal effects and relevance to schizophrenia. Transl Psychiatry. 2012;2(11):e199.
Gomes FV, Guimaraes FS, Grace AA. Effects of pubertal cannabinoid administration on attentional set-shifting and dopaminergic hyper-responsivity in a developmental disruption model of schizophrenia. Int J Neuropsychopharmacol. 2014;18(2):pyu018.
Gomis-González M, et al. Possible therapeutic doses of cannabinoid type 1 receptor antagonist reverses key alterations in fragile X syndrome mouse model. Genes. 2016;7(9):56.
Hacohen M, et al. Children and adolescents with ASD treated with CBD-rich cannabis exhibit significant improvements particularly in social symptoms: an open label study. Transl Psychiatry. 2022;12(1):375.
Harte LC, Dow-Edwards D. Sexually dimorphic alterations in locomotion and reversal learning after adolescent tetrahydrocannabinol exposure in the rat. Neurotoxicol Teratol. 2010;32(5):515–24.
Hashimotodani Y, Ohno-Shosaku T, Kano M. Endocannabinoids and synaptic function in the CNS. Neuroscientist. 2007;13(2):127–37.
Hedner T, Iversen K, Lundborg P. Central GABA mechanisms during postnatal development in the rat: neurochemical characteristics. J Neural Transm. 1984;59(2):105–18.
Herkenham M, et al. Cannabinoid receptor localization in brain. Proc Natl Acad Sci. 1990;87(5):1932–6.
Higuera-Matas A, et al. Periadolescent exposure to cannabinoids alters the striatal and hippocampal dopaminergic system in the adult rat brain. Eur Neuropsychopharmacol. 2010;20(12):895–906.
Higuera-Matas A, et al. Sex-specific disturbances of the glutamate/GABA balance in the hippocampus of adult rats subjected to adolescent cannabinoid exposure. Neuropharmacology. 2012;62(5–6):1975–84.
Howes OD, et al. The nature of dopamine dysfunction in schizophrenia and what this means for treatment. Arch Gen Psychiatry. 2012;69(8):776–86.
Hua DY-H, et al. Cannabis and cannabidiol use among autistic and non-autistic adults in the UK: a propensity score-matched analysis. BMJ Open. 2021;11(12):e053814.
Irimia C, et al. Persistent effects of chronic Δ9-THC exposure on motor impulsivity in rats. Psychopharmacology. 2015;232:3033–43.
Izquierdo-Luengo C, et al. Adolescent exposure to the spice/K2 cannabinoid JWH-018 impairs sensorimotor gating and alters cortical perineuronal nets in a sex-dependent manner. Transl Psychiatry. 2023;13(1):176.
Jana A, et al. Unraveling the endocannabinoid system: exploring its therapeutic potential in autism Spectrum disorder. NeuroMolecular Med. 2024;26(1):20.
Karhson DS, et al. Plasma anandamide concentrations are lower in children with autism spectrum disorder. Mol Autism. 2018;9:1–6.
Kaufmann WE, et al. Autism Spectrum disorder in fragile X syndrome: Cooccurring conditions and current treatment. Pediatrics. 2017;139(Suppl 3):S194–s206.
Keimpema E, Calvigioni D, Harkany T. Endocannabinoid signals in the developmental programming of delayed-onset neuropsychiatric and metabolic illnesses. Biochem Soc Trans. 2013;41(6):1569–76.
Kerr DM, Gilmartin A, Roche M. Pharmacological inhibition of fatty acid amide hydrolase attenuates social behavioural deficits in male rats prenatally exposed to valproic acid. Pharmacol Res. 2016;113:228–35.
Kilb W. Development of the GABAergic system from birth to adolescence. Neuroscientist. 2012;18(6):613–30.
Kirschmann EK, et al. Effects of adolescent cannabinoid self-administration in rats on addiction-related behaviors and working memory. Neuropsychopharmacology. 2017;42(5):989–1000.
Klein C, et al. Cannabidiol potentiates Δ 9-tetrahydrocannabinol (THC) behavioural effects and alters THC pharmacokinetics during acute and chronic treatment in adolescent rats. Psychopharmacology. 2011;218:443–57.

Klug M, Van Den Buuse M. An investigation into "two hit" effects of BDNF deficiency and young-adult cannabinoid receptor stimulation on prepulse inhibition regulation and memory in mice. Front Behav Neurosci. 2013;7:149.
Kruse LC, et al. Voluntary oral consumption of Delta(9)-tetrahydrocannabinol by adolescent rats impairs reward-predictive cue behaviors in adulthood. Neuropsychopharmacology. 2019;44(8):1406–14.
Kurz R, Blaas K. Use of dronabinol (delta-9-THC) in autism: a prospective single-case-study with an early infantile autistic child. Can Underwrit. 2010;5(4):4–6.
Lee SE, Lee Y, Lee GH. The regulation of glutamic acid decarboxylases in GABA neurotransmission in the brain. Arch Pharm Res. 2019;42(12):1031–9.
Leweke FM, Schneider M. Chronic pubertal cannabinoid treatment as a behavioural model for aspects of schizophrenia: effects of the atypical antipsychotic quetiapine. Int J Neuropsychopharmacol. 2011;14(1):43–51.
Llorente-Berzal A, et al. Sex-dependent effects of maternal deprivation and adolescent cannabinoid treatment on adult rat behaviour. Addict Biol. 2011;16(4):624–37.
Long LE, et al. Transmembrane domain Nrg1 mutant mice show altered susceptibility to the neurobehavioural actions of repeated THC exposure in adolescence. Int J Neuropsychopharmacol. 2013;16(1):163–75.
Lopez-Gallardo M, et al. Maternal deprivation and adolescent cannabinoid exposure impact hippocampal astrocytes, CB1 receptors and brain-derived neurotrophic factor in a sexually dimorphic fashion. Neuroscience. 2012;204:90–103.
Lovelace JW, et al. An animal model of female adolescent cannabinoid exposure elicits a long-lasting deficit in presynaptic long-term plasticity. Neuropharmacology. 2015;99:242–55.
Lubman DI, Cheetham A, Yücel M. Cannabis and adolescent brain development. Pharmacol Ther. 2015a;148:1–16.
Lubman DI, Cheetham A, Yucel M. Cannabis and adolescent brain development. Pharmacol Ther. 2015b;148:1–16.
Maccarrone M, et al. Programming of neural cells by (endo)cannabinoids: from physiological rules to emerging therapies. Nat Rev Neurosci. 2014;15(12):786–801.
Mackie K. Distribution of cannabinoid receptors in the central and peripheral nervous system. Handb Exp Pharmacol. 2005;168:299–325.
Marsicano G, Lutz B. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. Eur J Neurosci. 1999;11(12):4213–25.
Marusich JA, et al. Evaluation of sex differences in cannabinoid dependence. Drug Alcohol Depend. 2014;137:20–8.
McCandliss B, Cohen L, Dehaene S. The visual word form area: expertise for reading in the fusiform gyrus. Trends Cogn Sci. 2003;7:293–9.
McCutcheon JE, Marinelli M. Age matters. Eur J Neurosci. 2009;29(5):997–1014.
Meyer HC, Lee FS, Gee DG. The role of the endocannabinoid system and genetic variation in adolescent brain development. Neuropsychopharmacology. 2018;43(1):21–33.
Miller ML, et al. Adolescent exposure to Δ9-tetrahydrocannabinol alters the transcriptional trajectory and dendritic architecture of prefrontal pyramidal neurons. Mol Psychiatry. 2019;24(4):588–600.
Moghaddam B, Javitt D. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. Neuropsychopharmacology. 2012;37(1):4–15.
Morris JP, Pelphrey KA, McCarthy G. Face processing without awareness in the right fusiform gyrus. Neuropsychologia. 2007;45(13):3087–91.
Moyer CE, Shelton MA, Sweet RA. Dendritic spine alterations in schizophrenia. Neurosci Lett. 2015;601:46–53.
Mulder J, et al. Endocannabinoid signaling controls pyramidal cell specification and long-range axon patterning. Proc Natl Acad Sci USA. 2008;105(25):8760–5.
O'Brien LD, et al. Effect of chronic exposure to rimonabant and phytocannabinoids on anxiety-like behavior and saccharin palatability. Pharmacol Biochem Behav. 2013;103(3):597–602.

O'Tuathaigh CM, et al. Chronic adolescent exposure to Δ-9-tetrahydrocannabinol in COMT mutant mice: impact on psychosis-related and other phenotypes. Neuropsychopharmacology. 2010;35(11):2262–73.

O'Tuathaigh CM, et al. Genetic vs. pharmacological inactivation of COMT influences cannabinoid-induced expression of schizophrenia-related phenotypes. Int J Neuropsychopharmacol. 2012;15(9):1331–42.

Poels EM, et al. Glutamatergic abnormalities in schizophrenia: a review of proton MRS findings. Schizophr Res. 2014;152(2–3):325–32.

Poleg S, et al. Cannabidiol as a suggested candidate for treatment of autism spectrum disorder. Prog Neuro-Psychopharmacol Biol Psychiatry. 2019;89:90–6.

Powell CM, Miyakawa T. Schizophrenia-relevant behavioral testing in rodent models: a uniquely human disorder? Biol Psychiatry. 2006;59(12):1198–207.

Powell SB, Weber M, Geyer MA. Genetic models of sensorimotor gating: relevance to neuropsychiatric disorders. Behav Neurogenet. 2012;12:251–318.

Pretzsch CM, et al. Effects of cannabidiol on brain excitation and inhibition systems; a randomised placebo-controlled single dose trial during magnetic resonance spectroscopy in adults with and without autism spectrum disorder. Neuropsychopharmacology. 2019a;44(8):1398–405.

Pretzsch CM, et al. The effect of cannabidiol (CBD) on low-frequency activity and functional connectivity in the brain of adults with and without autism spectrum disorder (ASD). J Psychopharmacol. 2019b;33(9):1141–8.

Prini P, et al. Chronic Δ9-THC exposure differently affects histone modifications in the adolescent and adult rat brain. Int J Mol Sci. 2017;18(10):2094.

Puighermanal E, et al. Dissociation of the pharmacological effects of THC by mTOR blockade. Neuropsychopharmacology. 2013;38(7):1334–43.

Purcell AE, et al. Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. Neurology. 2001;57(9):1618–28.

Qin M, et al. Endocannabinoid-mediated improvement on a test of aversive memory in a mouse model of fragile X syndrome. Behav Brain Res. 2015;291:164–71.

Quinn HR, et al. Adolescent rats find repeated Δ9-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. Neuropsychopharmacology. 2008;33(5):1113–26.

Rais M, et al. Cannabis use and progressive cortical thickness loss in areas rich in CB1 receptors during the first five years of schizophrenia. Eur Neuropsychopharmacol. 2010;20(12):855–65.

Rapp C, et al. Cannabis use and brain structural alterations of the cingulate cortex in early psychosis. Psychiatry Res. 2013;214(2):102–8.

Realini N, et al. Chronic URB597 treatment at adulthood reverted most depressive-like symptoms induced by adolescent exposure to THC in female rats. Neuropharmacology. 2011;60(2–3):235–43.

Renard J, et al. Long-term consequences of adolescent cannabinoid exposure in adult psychopathology. Front Neurosci. 2014;8:361.

Renard J, et al. Chronic cannabinoid exposure during adolescence leads to long-term structural and functional changes in the prefrontal cortex. Eur Neuropsychopharmacol. 2016;26(1):55–64.

Rubino T, Parolaro D. The impact of exposure to cannabinoids in adolescence: insights from animal models. Biol Psychiatry. 2016;79(7):578–85.

Rubino T, et al. Chronic Δ9-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. Neuropsychopharmacology. 2008;33(11):2760–71.

Rubino T, et al. Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. Hippocampus. 2009;19(8):763–72.

Rubino T, Zamberletti E, Parolaro D. Adolescent exposure to cannabis as a risk factor for psychiatric disorders. J Psychopharmacol. 2012;26(1):177–88.

Rubino T, et al. Adolescent exposure to THC in female rats disrupts developmental changes in the prefrontal cortex. Neurobiol Dis. 2015;73:60–9.
Schultz S, et al. Endocannabinoid system dysregulation from acetaminophen use may lead to autism spectrum disorder: could cannabinoid treatment be efficacious? Molecules. 2021;26(7):1845.
Selemon LD, Zecevic N. Schizophrenia: a tale of two critical periods for prefrontal cortical development. Transl Psychiatry. 2015;5(8):e623.
Shrader SH, et al. Cannabidiol is a behavioral modulator in BTBR mouse model of idiopathic autism. Front Neurosci. 2024;18:1359810.
Silva Junior EAD, et al. Cannabis and cannabinoid use in autism spectrum disorder: a systematic review. Trends Psychiatry Psychother. 2021;44:e20200149.
Silva L, et al. Sex-specific alterations in hippocampal cannabinoid 1 receptor expression following adolescent delta-9-tetrahydrocannabinol treatment in the rat. Neurosci Lett. 2015;602:89–94.
Silva L, et al. Sex and age specific effects of delta-9-tetrahydrocannabinol during the periadolescent period in the rat: the unique susceptibility of the prepubescent animal. Neurotoxicol Teratol. 2016;58:88–100.
Simonoff E, et al. Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. J Am Acad Child Adolesc Psychiatry. 2008;47(8):921–9.
Siniscalco D, et al. Cannabinoid receptor type 2, but not type 1, is up-regulated in peripheral blood mononuclear cells of children affected by autistic disorders. J Autism Dev Disord. 2013;43(11):2686–95.
Siniscalco D, et al. The in vitro GcMAF effects on endocannabinoid system transcriptionomics, receptor formation, and cell activity of autism-derived macrophages. J Neuroinflammation. 2014;11:78.
Smith DR, et al. Rare genetic variants in the endocannabinoid system genes CNR1 and DAGLA are associated with neurological phenotypes in humans. PLoS One. 2017;12(11):e0187926.
Sohal VS, Rubenstein JLR. Excitation-inhibition balance as a framework for investigating mechanisms in neuropsychiatric disorders. Mol Psychiatry. 2019;24(9):1248–57.
Steel RW, et al. Learning impairment by Δ9-tetrahydrocannabinol in adolescence is attributable to deficits in chunking. Behav Pharmacol. 2011;22(8):837–46.
Tai S, et al. Repeated administration of phytocannabinoid Delta(9)-THC or synthetic cannabinoids JWH-018 and JWH-073 induces tolerance to hypothermia but not locomotor suppression in mice, and reduces CB1 receptor expression and function in a brain region-specific manner. Pharmacol Res. 2015;102:22–32.
Tarazi FI, Tomasini EC, Baldessarini RJ. Postnatal development of dopamine D4-like receptors in rat forebrain regions: comparison with D2-like receptors. Brain Res Dev Brain Res. 1998;110(2):227–33.
Thibault K, et al. Activation-dependent subcellular distribution patterns of CB1 cannabinoid receptors in the rat forebrain. Cereb Cortex. 2013;23(11):2581–91.
Tournier BB, Ginovart N. Repeated but not acute treatment withΔ 9-tetrahydrocannabinol disrupts prepulse inhibition of the acoustic startle: reversal by the dopamine D2/3 receptor antagonist haloperidol. Eur Neuropsychopharmacol. 2014;24(8):1415–23.
Trezza V, et al. Altering endocannabinoid neurotransmission at critical developmental ages: impact on rodent emotionality and cognitive performance. Front Behav Neurosci. 2012;6:2.
Verdurand M, et al. Comparison of cannabinoid CB(1) receptor binding in adolescent and adult rats: a positron emission tomography study using [F]MK-9470. Int J Mol Imaging. 2011;2011:548123.
Verrico CD, et al. Repeated Δ9-tetrahydrocannabinol exposure in adolescent monkeys: persistent effects selective for spatial working memory. Am J Psychiatry. 2014;171(4):416–25.
Wang X, et al. Preferential limbic expression of the cannabinoid receptor mRNA in the human fetal brain. Neuroscience. 2003;118(3):681–94.

Weed PF, et al. Chronic Delta9-tetrahydrocannabinol during adolescence differentially modulates striatal CB1 receptor expression and the acute and chronic effects on learning in adult rats. J Pharmacol Exp Ther. 2016;356(1):20–31.

Wegener N, Koch M. Behavioural disturbances and altered Fos protein expression in adult rats after chronic pubertal cannabinoid treatment. Brain Res. 2009;1253:81–91.

Wei D, et al. Enhancement of anandamide-mediated endocannabinoid signaling corrects autism-related social impairment. Cannabis Cannabinoid Res. 2016;1(1):81–9.

Wiley JL, Burston JJ. Sex differences in Δ9-tetrahydrocannabinol metabolism and in vivo pharmacology following acute and repeated dosing in adolescent rats. Neurosci Lett. 2014;576:51–5.

Wiley JL, et al. Preclinical study: age-dependent differences in sensitivity and sensitization to cannabinoids and 'club drugs' in male adolescent and adult rats. Addict Biol. 2008;13(3–4):277–86.

Winsauer PJ, Sutton JL. Chronic administration during early adulthood does not alter the hormonally-dependent disruptive effects of delta-9-tetrahydrocannabinol (Δ9-THC) on complex behavior in female rats. Pharmacol Biochem Behav. 2014;117:118–27.

Winsauer PJ, et al. Long-term behavioral and pharmacodynamic effects of delta-9-tetrahydrocannabinol in female rats depend on ovarian hormone status. Addict Biol. 2011;16(1):64–81.

Zamberletti E, et al. Alterations of prefrontal cortex GABAergic transmission in the complex psychotic-like phenotype induced by adolescent delta-9-tetrahydrocannabinol exposure in rats. Neurobiol Dis. 2014;63:35–47.

Zamberletti E, et al. Cortical neuroinflammation contributes to long-term cognitive dysfunctions following adolescent delta-9-tetrahydrocannabinol treatment in female rats. Eur Neuropsychopharmacol. 2015;25(12):2404–15.

Zamberletti E, et al. Long-term hippocampal glutamate synapse and astrocyte dysfunctions underlying the altered phenotype induced by adolescent THC treatment in male rats. Pharmacol Res. 2016;111:459–70.

Zamberletti E, Gabaglio M, Parolaro D. The endocannabinoid system and autism Spectrum disorders: insights from animal models. Int J Mol Sci. 2017;18(9):1916.

Part IV
Psychiatric Risks and Vulnerabilities

Chapter 15
Endocannabinoid System Contributions to Psychiatric Risk



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1 Overview of the Endocannabinoid System

Discovered for its mediation of the psychoactive effects of delta-9-tetrahydrocannabinol (THC; Gaoni and Mechoulam 1964; Hill et al. 2023), the endocannabinoid (eCB) system is a neuromodulatory system critically involved in many brain functions, including metabolism, growth and development, learning and memory, and the regulation of stress and emotional processing (Lutz 2020; Skaper and Di Marzo 2012). This integral system is comprised of two major cannabinoid receptors, various endogenous ligands, and their respective synthetic and degrading enzymes. Thus, the complex physiology of the eCB system provides multiple opportunities to explore how modulation of this system may be used as a novel therapeutic target for a host of psychiatric disorders. In parallel, a deeper understanding of the functions and features of this system can provide insight into whether variation in its constituents may confer risk or resilience to the development or maintenance of such disorders.

The eCB system consists of two primary receptors with distinct functions and patterns of localization. The cannabinoid type-1 receptor (CB1R) is one of the most ubiquitously expressed G-protein coupled receptors in the brain. As a result, signaling at the CB1R is involved in a diverse range of brain functions. The CB1R binds

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both major eCBs, N-arachidonoylethanolamine (anandamide; AEA) and 2-arachidonoylglycerol (2-AG). Cannabinoid type-2 receptors (CB2R) are found to a lesser extent in the brain and are localized to immune cells. Within the central nervous system (CNS), CB2Rs are primarily localized to microglia and their expression is associated with inflammation and the regulation of immune responses (Freund et al. 2003; Mackie 2008; Palazuelos et al. 2009; Turcotte et al. 2016).

The two primary eCB ligands, AEA and 2-AG, bind CBRs but have some notable differences. AEA acts as a partial agonist with a high affinity at the CB1R while 2-AG is a full agonist with low to moderate binding affinity at both the CB1R and CB2R (Maccarrone and Finazzi-Agró 2003). Unlike other neurotransmitters, eCBs travel in a retrograde fashion. The binding of either eCB ligand to presynaptic cannabinoid receptors triggers a signaling cascade that ultimately dampens presynaptic neurotransmission, thus having a neuromodulatory effect (Freund et al. 2003; Kendall and Yudowski 2017). The biological activity of AEA is terminated by its primary catabolic enzyme, fatty acid amide hydrolase (FAAH). Therefore, FAAH enzymatic activity indirectly mediates presynaptic neurotransmission by regulating levels of available AEA (Lazary et al. 2016). The main pathway of 2-AG degradation is the hydrolysis of its ester bond to produce AA and glycerol via monoacylglycerol lipase (MAGL), though other enzymes may contribute to this process (Baggelaar et al. 2018). As a result, elucidating how these two ligands differ in mediating psychiatric risk may allow for strategies to target one, the other, or both, based on the understanding of their synthesis and degradation pathways.

Impairments in cannabinoid receptor-mediated functions have been implicated the risk, development, and progression of various psychopathologies such as anxiety disorders, major depressive disorder (MDD), eating disorders (EDs), posttraumatic stress disorder (PTSD), substance use disorders (SUDs), autism spectrum disorder (ASD), and schizophrenia. Thus, understanding the role of eCB signaling in risk of psychiatric disorder development is imperative to understanding the etiology of these conditions and elucidating novel therapeutic targets. Here, we will highlight the potential (and challenges) of eCB system components as biomarkers and how they contribute to risk for the development of psychiatric disorders.

2 Biomarker Potential of the Endocannabinoid System

The exploration of the eCB system as a potential biomarker for psychiatric risk has been a longstanding objective in psychiatric research. Elements of the eCB system have been suggested as disease or diagnostic biomarkers, used as biomarkers for drug discovery, and discussed as predictive biomarkers (Alcaraz-Silva et al. 2023; Pinna 2023). With recent clinical trials targeting the eCB system currently in the pipeline, it is highly anticipated that eCBs will be further used as biomarkers to detect drug effects. However, due to the complex interplay of various factors affecting eCB levels, not all observed differences in the eCB system in psychiatric patients are consistent enough to serve as potential biomarkers (Gowatch et al. 2024).

In the majority of studies, a central focus has been the direct determination of eCB concentrations, particularly AEA and 2-AG, in various tissues. This has occurred most notably in peripheral blood, but occasionally also in saliva and hair (Ney et al. 2023). However, meta-analytic evidence reveals significant heterogeneity in the concentrations of both AEA and 2-AG across studies examining these molecules in stress-related neuropsychiatric disorders, such as anxiety, depression, and PTSD (Gowatch et al. 2024). If the goal of clinical biomarkers is to track disease progression over time and correlate with known clinical measures, or show the effect of drug or other intervention (Jain 2017), it is essential that the biomarker has established performance characteristics such as consistency and accuracy. The high susceptibility of eCBs to in vivo and ex vivo influences (Hillard 2018; Kratz et al. 2021), coupled with the resultant variability across different study protocols, complicates their use as reliable biomarkers and might explain the heterogeneity observed in the literature (Gowatch et al. 2024).

One inherent issue with eCB measurement is that several endogenous and exogenous parameters must be considered. From a person's perspective, perhaps most obvious is the effects of cannabis consumption, which can impact eCB concentrations (Chester et al. 2024; Hillard 2018; Kratz et al. 2021). Moreover, a well-established correlation exists between eCB concentrations and body mass index (BMI; Côté et al. 2007), and other metabolic markers like triglycerides are linked to 2-AG concentrations (Baenas et al. 2023). Time of day also matters, as eCBs exhibit a diurnal profile (Jian et al. 2010) and are likely influenced by food intake (Pedersen et al. 2021). Meta-analytic evidence demonstrates that exercise impacts eCB concentrations (Desai et al. 2022), particularly at moderate to high intensity levels (Raichlen et al. 2013). It is particularly important to consider that the eCB system plays a crucial role in development and undergoes significant changes throughout life (Meyer et al. 2018). However, longitudinal data on eCB levels across different life stages—children, adolescents, adults, and older adults—are currently lacking and urgently needed. Thus, several participant-specific factors can impact the sensitivity of eCB measurements.

Beyond characteristics of the individual providing the sample, the collection, preparation, and analysis of samples is also crucial. Especially with plasma samples, the time until centrifugation is crucial, as significant concentration changes can occur ex vivo (Vogeser et al. 2006). For instance, at room temperature and on ice, AEA concentration in whole blood increases by approximately threefold and twofold, respectively (Jian et al. 2010). The isomerization of 2-AG to 1-arachidonoylglycerol (1-AG) is also highly dependent on pre-analytical processing (Vogeser and Schelling 2007). Unfortunately, many details necessary to understand these nuanced details of sample processing are often missing from publications. As a result, comparisons across, or at times even within, studies are challenging.

Due to the significant methodological challenges, and propelled by promising preclinical and translational findings, the investigation into eCBs as surrogate biomarkers for their metabolic processes has emerged as a pivotal research direction, with potential to enhance the understanding and treatment of psychiatric disorders. Investigating key aspects of eCB synthesis, transport, and degradation is now

proposed as a strategy for identifying novel biomarkers pertinent to the eCB system and psychiatric risk. The exploration of functional polymorphisms in genes encoding eCB-metabolizing enzymes has garnered substantial interest. Variations in these polymorphisms, some of which lead to differences in enzyme activity, are being recognized for their potential as reliable biomarkers (Mayo et al. 2020a). These genetic markers offer a higher degree of consistency compared to traditional metabolic biomarkers, positioning them as a significant focus in the study of the eCB system's role in psychiatric conditions.

Beyond genetic biomarkers of the metabolizing enzymes, initial studies have directly measured enzyme activity in patients with psychopathology (Kroll et al. 2024). In addition, a limited number of positron emission tomography (PET) studies have utilized ligands for these metabolizing enzymes to visualize their brain distribution (Best et al. 2023). Despite the successful application of methods for measuring FAAH enzyme activity in validating FAAH inhibitors (e.g., Ahn et al. 2011), translational studies assessing FAAH activity in the plasma of psychiatric patients have not demonstrated a clear correlation between FAAH activity and AEA concentrations (Kroll et al. 2024), complicating the interpretation of results. PET imaging studies have provided valuable insights into the distribution of FAAH in the brains of psychiatric patients. However, the widespread use of PET as a biomarker remains challenging due to its high costs and relatively invasive nature.

The challenges in establishing eCBs as predictive biomarkers for people at risk for psychiatric conditions are exacerbated by the variability in study designs and methodologies. It remains unclear whether observed changes in eCB concentrations are causative or reactive, creating ambiguity that necessitates more robust study designs. Longitudinal, high-quality design studies with eCBs, the integration of genetic biomarkers, and improved methods directly investigating enzyme activities of the eCB system are essential to better elucidate its role for psychiatric risk.

3 Genetics of the Endocannabinoid System

A growing number of studies have begun to explore how genetic variation may impact the eCB system and its contribution to psychiatric risk. For instance, the rs1049353 polymorphism of the CNR1 gene, which encodes for the CB1R, attenuated the likelihood of anhedonia following exposure to early life stress (Agrawal et al. 2012). Moreover, the rs604300 polymorphism of the monoacylglycerol lipase (MGLL) gene, which encodes for the primary degrading enzyme of 2-AG, is related to cannabis dependence symptoms in individuals with a history of childhood sexual assault (Carey et al. 2015). More recently, CNR1 and MGLL polymorphisms have been linked to risk of AUD development (Elkrief et al. 2021). These findings implicate genetic alterations of the eCB system in risk for several psychiatric conditions. However, gene-association studies have come under scrutiny for lack of replicability due to common issues, such as small sample sizes, methodological differences, and multiple testing (Gorroochurn et al. 2007). Another mechanistic concern is that

many of the SNPs do not have a direct, quantifiable outcome, resulting in challenging interpretations on specifically how the SNP impacts endogenous function of the system. To date, existing literature regarding these SNPs is limited to correlations between prevalence of the SNPs and psychiatric disorders.

One exception to this latter concern is the FAAH C385A SNP (rs324420), which occurs in ~32% (3.7% A/A, 28.3% A/C) of individuals of European descent (Sipe et al. 2002). This genetic mutation renders the FAAH enzyme of A-allele carriers more vulnerable to proteolytic degradation (Sipe et al. 2002). As the primary catabolic enzyme of AEA, reductions of FAAH activity result in elevated levels of available AEA, and putatively enhanced activation of CB1Rs. Activation of the CB1R by eCBs has an inhibitory effect on the presynaptic release of neurotransmitters such as glutamate and GABA. Therefore, FAAH activity may indirectly modulate CB1R signaling and synaptic excitation/inhibition through its regulation of AEA. In humans, AA carriers of this mutation have a ~ 50% reduction in FAAH enzymatic activity compared to CC genotype carriers (Chiang et al. 2004) and this has been confirmed to result in elevated concentrations of AEA detectable in blood (Mayo et al. 2020b). The FAAH C385A polymorphism then, provides an interesting genetic model to understand how the eCB system, particularly altered AEA levels, influences brain function, behavior, and risk of psychiatric disorder throughout development.

While there are limitations to this genetics-based approach to exploring eCB function in humans, there are notable advantages, too. Utilizing the FAAH SNP to investigate the eCB system is noninvasive and permits exploration of gene-environment interactions. Importantly, we have shown that, as predicted, the A-allele confers a gene-dose-dependent increase in AEA concentrations in humans (Mayo et al. 2020b). The polymorphism has also been reliably back translated to animal models, enabling more intensive study of behavioral phenotypes (Dincheva et al. 2015). Of particular interest, combined human and animal data has provided us with a timeline of fluctuations of eCB system components throughout development, and how the presence of the A-allele impacts this trajectory (Gee et al. 2016). Thus, studies exploring consequences FAAH C385A variation can provide initial insights into how eCB-targeting interventions, particularly those specific to AEA, may protect against risk or serve as innovative treatment opportunities for psychopathologies. Thus, here, we will explore how genetic variation at this particular locus impacts psychiatric risk.

4 Endocannabinoid Contributions to Psychiatric Risk in Children and Adolescents

Many biological systems function in unique ways during childhood (Pujol et al. 2021), and the eCB system is not an exception to this rule. ECB signaling shows complex and fluctuating patterns throughout childhood and adolescence (Meyer

et al. 2018). Even prior to birth, eCB signaling is involved in critical signal transduction cascades during neural development (Berrendero et al. 1998), and adolescence is a critical period where the synthesis and degradation enzymes of both AEA and 2-AG are in flux in the brain (Long et al. 2012; Meyer et al. 2018). Importantly, since the FAAH C385A SNP is present throughout the entire developmental process, it may exert meaningful effects on mental health in childhood and adolescence, that may be separate from those during adulthood.

There is emerging evidence that the FAAH C385A SNP is related to psychiatric symptoms and brain structure in adolescence, with some studies suggesting a relationship with psychiatric resilience in youth. For instance, in children aged 9–13 years, AA-homozygotes were found to have lower depressive symptoms compared to children with the CC and AC genotype (Desai et al. 2024). Additional evidence suggests that the A-allele may confer alterations to global functional connectivity that are related to resilience, as CC-homozygotes who had functional connectome profiles similar to A-allele carriers had decreased anxiety symptoms (Sisk et al. 2022). In youth older than 12 years, the A-allele conferred both lower anxiety and increased integrity of the uncinate fasciculus (Gee et al. 2016), a white matter tract in the brain that is involved in memory, reward, and cognitive control (Olson et al. 2015). Taken together, these findings provide preliminary evidence that the SNP is associated with lower psychiatric symptoms such as depression and anxiety in young individuals.

However, other work has painted a more complex picture between anxiety symptoms and the FAAH C385A SNP in middle childhood. In children 11 years old and younger, there was an indirect effect of the SNP on increased anxiety symptoms through the integrity of the fornix (Marusak et al. 2023), another frontolimbic white matter tract that is involved in episodic memory (Senova et al. 2020). This suggests that in this age group, anxiety symptoms may be related to the alterations in fornix white matter microstructure that is conferred by the A-allele. There may also be age-dependent effects of the FAAH C385A SNP on anxiety (Gee et al. 2016), though more work is needed to fully understand this developmental trajectory. Research to establish the definitive links between psychiatric risk, FAAH genotype, and brain structure and function is ongoing.

5 Endocannabinoid Contributions to Risk of Anxiety Disorders

5.1 The Endocannabinoid System and Anxiety

Anxiety is a future-oriented emotion that aids animals in detecting and appropriately responding to potential threats in their environment (Grupe and Nitschke 2013). However, this adaptive function can become pathological when the response is disproportionate to the perceived threat. Anxiety disorders are among the most

prevalent health-related causes of disability worldwide, with long-term impacts on well-being and high rates of comorbidity with other psychiatric conditions, such as depression (Penninx et al. 2021). Anxiety disorders are characterized by exaggerated anxiety and fear responses to perceived threats and can disrupt daily functioning of affected individuals (Penninx et al. 2021). Notably, anxiety disorders are the most common form of mental health disorder in children, whose developmental fluctuations in brain function and behavior may render them particularly vulnerable to anxiety-related impairments. In addition, these effects can be exacerbated by environmental impacts such as early life stress, which can have lasting effects into adulthood (Chaby et al. 2017; Fonzo et al. 2016; Grupe and Nitschke 2013).

The eCB system plays an integral role in regulating various aspects of anxiety, such as the stress response (Evanson et al. 2010; Hill et al. 2011; Wang et al. 2012; Yasmin et al. 2020). In particular, CB1Rs are highly expressed in brain regions that mediate anxiety, such as the medial prefrontal cortex, hippocampus, and basolateral amygdala (Mackie 2008). Pharmacological manipulation of signaling at these receptors has been shown to influence anxiety-like behaviors. Preclinical studies have shown that disruption of FAAH enzymatic activity, in particular, has anxiolytic effects. In the elevated plus maze, a common paradigm for modeling anxiety-like behaviors, mice treated with a FAAH inhibitor display increased entries and time spent in the open arms indicating an anxiolytic effect of FAAH inhibition (Moreira et al. 2008). This drug effect was blocked by the administration of rimonabant, a CB1R antagonist, indicating that enhanced CB1R signaling following FAAH inhibition is likely responsible for the observed anxiolytic effects (Moreira et al. 2008).

Endocannabinoid signaling is also an important regulator of physiological and behavioral responses to stress exposure that are dysfunctional in anxiety disorders. Following acute exposure to stress, eCB signaling shifts to mediate the hypothalamus-pituitary-adrenal (HPA) axis. Specifically, AEA signaling appears to contribute to the downstream activation of the stress response via disinhibition of the basal lateral amygdala (Bedse et al. 2017; Yasmin et al. 2020) while delayed 2-AG signaling blunts the magnitude and duration of the stress response (Evanson et al. 2010; Hill et al. 2011; Hohmann et al. 2005; Wang et al. 2012). Therefore, the activation of FAAH and its subsequent breakdown of AEA are components of a healthy acute stress response, permitting appropriate HPA responses. Exposure to chronic stress, however, prolongs FAAH activity potentially causing a chronic decrease in available AEA levels, which has been associated with heightened anxiety (Hill et al. 2013). Thus, dysregulation of eCB functioning may contribute to the development and maintenance of anxiety disorders (Petrie et al. 2021).

The efficacy of FAAH inhibition as an anxiolytic agent, however, appears to depend on environmental conditions. One study found that the administration of a FAAH inhibitor, URB597, failed to produce an anxiolytic effect when the aversiveness of testing procedures was minimized (Haller et al. 2009). Conversely, URB597 elicited robust anxiolytic effects when the aversiveness of testing was maximized through enhancing bright lighting during testing and eliminating habituation to the experimental room (Haller et al. 2009). The anxiolytic effects in this condition were blocked by administration of CB1R antagonist, AM251, indicating a

CB1R-dependency. Together, these findings demonstrate the involvement of eCB signaling in anxiety-like behaviors and illustrate FAAH inhibition as being a particularly efficacious anxiolytic mechanism under heightened stress (Haller et al. 2009).

5.2 FAAH Genetics and Anxiety

Genetic mouse models of reduced FAAH enzymatic activity have recapitulated pharmacological findings. FAAH−/− mice have been shown to enter and spend more time in the open arms of the elevated plus maze than wild type mice (FAAH+/+), indicating anxiolytic effects (Moreira et al. 2008). Translational evidence also indicates that genetically driven reductions in FAAH activity are protective against the effects of stress. In both humans and mice, humanized for the FAAH C385A SNP, A-allele carriers were protected from the typical stress-induced changes in circulating AEA concentrations (Mayo et al. 2020a). This effect was specific to AEA, as there was no effect of genotype on stress-induced neuroendocrine changes (Mayo et al. 2020a). Thus, it appears that genetically driven alterations in eCB-signaling parallel results from preclinical pharmacological investigations and translate across species. As such, investigation of the FAAH C385A SNP provides a path for preliminary exploration of the putative anxiolytic effects of FAAH inhibition and altered eCB signaling in humans, without the requirement of currently hard-to-obtain pharmacological compounds (e.g., FAAH inhibitors). It also allows us to examine the role of FAAH and AEA signaling in appropriate and dysregulated stress responding, and thereby provide insights into the role of these molecules in contributing to the risk of anxiety disorder development and maintenance in humans.

Studies exploring the behavioral consequences of the FAAH C385A SNP provided promising early data suggesting that the A-allele may confer resilience to anxiety-related phenotypes. Initial studies investigated the impact on both threat and reward processing, and found that the A-allele was associated with reduced amygdala reactivity to threat and increased ventral striatal activity during reward processing in healthy humans (Hariri et al. 2009). Interestingly, carriers of the A-allele also had brain-behavior correlations that were not present in CC homozygotes. In particular, A-allele carriers had reduced amygdala reactivity that correlated with lower trait anxiety, as measured by the State-Trait Anxiety Inventory (STAI-T). They also showed a positive correlation between ventral striatal activity and measures of impulsivity (Hariri et al. 2009). Subsequent studies also supported a role for the A-allele in reduced anxiety-related behaviors. For instance, a translational study found that reductions in FAAH expression conferred via FAAH C385A variation were evident across mice and humans, including enhanced fronto-amygdala connectivity, increased fear extinction learning ($N = 40$), and reductions in anxiety-like behaviors (STAI-T; $N = 137$) in humans (Dincheva et al. 2015). Thus, early studies

supported the hypothesis that the elevated AEA produced by this genetic variant may function to protect against heightened anxiety.

Importantly, the effects of the FAAH C385A on fronto-amygdala connectivity and anxiety-like behaviors appear to emerge during a critical period in adolescence, an effect demonstrated in both humans and animal models (Gee et al. 2016). A translational study found that connectivity within the uncinate fasciculus (UF), a white matter tract in the brain that connects limbic structures in the anterior temporal lobes with ventral and medial prefrontal cortices, demonstrated an age x genotype interaction (Gee et al. 2016). The A-allele was associated with increased fronto-amygdala connectivity, as reported in previously mentioned studies; however, this was only evident in individuals ages 12 and older. This age-dependent effect was likewise observed in a FAAH C385A knock-in mouse model. Only by day postnatal day 45, the equivalent adolescent period, did A-allele mice show increased infralimbic cortex afferent fibers to the basolateral amygdala. Another study found that, in mice, the A-allele was associated with decreased anxiety-like behaviors in the elevated plus maze exclusively during the adolescent period. In humans, only A-allele carriers over 12 years of age had reductions in self-reported anxiety (Gee et al. 2016). The emergence of anxiety-like behaviors and altered brain connectivity during adolescence in A-allele carriers suggests a critical developmental window within the eCB system that may impact psychiatric outcomes and vulnerabilities.

However, subsequent studies exploring the impacts of this FAAH SNP on anxiety in healthy humans provided a less consistent picture. The first healthy control study to link the FAAH C385A SNP to peripheral AEA levels found a gene-dose-dependent effect of more copies of the A-allele, such that more copies corresponded to greater levels of AEA (Mayo et al. 2020b). No relationship was found between the A-allele and trait anxiety or subjective stress, though there were secondary effects in relevant behavioral measures. For instance, A/A homozygotes had elevated AEA at baseline and failed to show stress-induced reductions in AEA during stress recovery. These individuals likewise displayed reduced stress-induced negative affect measured by activation of the corrugator muscle, recorded with facial electromyography (Mayo et al. 2020b). These findings were supported by a male-only study ($N = 55$) which confirmed that the A-allele was associated with levels of AEA, but not with state trait anxiety (Spohrs et al. 2021). A-allele carriers did, however, exhibit less state anxiety after a fear extinction protocol and greater connectivity between the anterior insula and anterior cingulate cortex (Spohrs et al. 2021). These inconsistent results could be a consequence of many factors, such as differences in tools used to assess anxiety, or variation in environmental circumstances, which we have discussed above as a critical mediator of the anxiolytic effects of elevated AEA on anxiety-like behaviors.

Considering the dynamic fluctuations in the eCB system throughout development, it is not surprising that severe stressors during childhood and adolescence can have lasting effects on this stress-regulating system. A significant interaction has been reported between the FAAH C385A SNP and childhood maltreatment (CM),

particularly concerning depressive and anxious phenotypes ($N = 858$; Lazary et al. 2016). A-allele carriers who experienced childhood adversity scored significantly higher in self-reported measures of anxiety than C-allele carriers. The authors proposed that permanently elevated AEA levels, such as those of A-allele carriers, may inhibit CB1R expression, which is associated with impaired eCB signaling and increased vulnerability to anxiety (Lazary et al. 2016). In contrast, a multisite international collaborative study investigating the FAAH C385A SNP in relation to cognitive behavioral therapy outcomes in youth with anxiety disorders, and observed no interaction between the FAAH SNP and improvements in anxiety scores (Lester et al. 2017). These mixed findings highlight the need for further investigations into eCB contributions to anxiety disorder risk, particularly in relation to environmental stressors.

Overall, the evidence presented here indicates a likely role for eCB signaling, and AEA in particular, in anxiety-like behaviors and phenotypes. It is possible, then, that pharmacological manipulation of the eCB system, specifically FAAH inhibition, may serve as a therapeutic target in anxiety disorders. While FAAH inhibitors are not currently approved for use in any jurisdiction, recently completed and ongoing trials will provide more definitive evidence. The efficacy of enhancing AEA through the administration of a FAAH inhibitor to treat anxiety was assessed in patients with social anxiety disorder (SAD). Unfortunately, administration of a FAAH inhibitor did not have a statistically significant effect on anxiety, measured by Liebowitz Social Anxiety Scale (LSAS), between treatment and placebo groups. However, significantly more participants given the FAAH inhibitor reported greater than 30% reductions in anxiety when compared to those who received placebo. Therefore, while the drug did not significantly improve the magnitude of anxiety scores overall, FAAH inhibition appears to marginally improve anxiety scores in a greater subset of individuals when compared to placebo (Schmidt et al. 2021). Further analysis of AEA and drug levels in the plasma revealed that the given drug dose of 25 mg may not have sufficiently inhibited FAAH enzymatic activity to produce clinical improvement across all individuals (Schmidt et al. 2021). Future investigations may explore the potential benefits of this drug with optimized dosing intervals to enhance the likelihood of sustained complete inhibition of FAAH.

Altogether, these studies point to a complicated relationship between eCB signaling, particularly reduced FAAH enzymatic activity, and anxiety disorder pathology. Initial studies suggested the presence of the A-allele, a potential proxy of elevated AEA levels, would confer resilience to anxiety phenotypes and anxiety disorders. However, subsequent studies that further explored genotype-specific therapeutic outcomes and targeted pharmacological interventions did not support these associations. Future studies should assess eCB gene x environment interactions and collect longitudinal data to better understand eCB contributions to risk of anxiety disorder development throughout the lifespan.

6 Endocannabinoid Contributions to Risk of Post-Traumatic Stress Disorder

6.1 The Endocannabinoid System and PTSD

Post-traumatic stress disorder (PTSD) is a chronic mental health condition that emerges following exposure to traumatic events. It is thought that PTSD develops from an inability to extinguish the fear memory of the traumatic event (Careaga et al. 2016), resulting in symptoms such as intrusive and distressing thoughts (flashbacks), avoidant behaviors, hyperarousal, and negative mood (American Psychiatric Association, 2013). Often, the trauma response can generalize to neutral stimuli, which results in an exacerbation of the other symptoms characterizing the disorder. PTSD remission can take decades to achieve, and relapses following a period of remission are common (Chapman et al. 2012; Kessler et al. 2017). Prolonged exposure therapy, the current gold standard in psychotherapy for PTSD, is designed to encourage fear extinction and habituation (Brown et al. 2019; Hembree et al. 2003). However, innovative treatment approaches to PTSD are needed, and may potentially be found in the eCB system (Mayo et al. 2022).

The eCB system's role in stress- and trauma-based disorders has been an especially compelling area of study. Evidence of integral eCB involvement in fear memory processes from preclinical animal models and healthy human adults has served as a main motivator for this line of inquiry (Gunduz-Cinar et al. 2013; Mayo et al. 2022). Many studies have shown that increased CB1R activity, particularly in the amygdala or prefrontal cortex, augments fear extinction processes (Lin et al. 2006, 2009; Pamplona et al. 2006; Simone et al. 2015). Specifically, the role of AEA in fear extinction has been well-established in preclinical research. Increasing endogenous AEA promotes the extinction of acquired fear in mice, rats, and nonclinical human populations (Balogh et al. 2019; Dincheva et al. 2015; Mayo et al. 2020a; Morena et al. 2014). Thus, cross-species evidence supports the notion that the eCB system, and AEA in particular, may be an innovative treatment target for PTSD.

6.2 FAAH Genetics and PTSD

Given the proposed importance of fear extinction processes to the development and maintenance of PTSD, and the subsequent association of those processes on AEA signaling, it follows that FAAH genotype may have an interaction with symptom severity, fear processing, or treatment response in those with PTSD. However, the current literature investigating the role of the FAAH C385A SNP in PTSD is not straightforward. Early work in individuals with co-occurring PTSD and alcohol dependence found that A-allele carriers had decreased hyperarousal symptoms and

less stress-induced anxiety (Spagnolo et al. 2016). This supported the notion that higher AEA conferred by the A-allele may be beneficial, particularly via reduction of the hyperarousal domain of PTSD. This was particularly promising, as it suggested that elevating AEA in PTSD would not just facilitate fear extinction, as in animal models and healthy humans, but may also attenuate anxiety. As such, elevation of AEA could potentially be combined with existing psychotherapy, such as prolonged exposure therapy, the current gold standard treatment for PTSD (Mayo et al. 2022). Prolonged exposure therapy is based on the ideas of extinction learning and is modestly effective, but can be distressing to patients, resulting in dropout. Thus, a compound that can attenuate stress while facilitating learning would be an exceptionally valuable tool in PTSD treatment. Unfortunately, subsequent studies suggested that it may not be so simple.

More recent studies exploring the impact of FAAH C385A variation in patients with PTSD have provided more nuanced findings. For instance, compared to A-allele carriers with PTSD, CC homozygotes had increased threat expectancy to a conditioned fear stimulus, which may be related to differences in functional responses to the fear conditioning task in limbic and striatal networks in the brain (Crombie et al. 2022). However, in a different sample of PTSD patients, those with the A-allele showed poorer fear extinction and greater physiological (skin conductance) responses to conditioned stimuli than CC homozygotes (Ney et al. 2021). Furthermore, a community-based observational study enrolled participants who were hospitalized for traumatic injuries and investigated the subsequent development and severity of PTSD 6-months later. In this study, individuals homozygous for the A-allele had higher PTSD symptom severity at follow-up, as compared to both CC-homozygotes and heterozygotes; this was in conjunction with increased levels of AEA in the AA-group compared to both other genotypic groups (deRoon-Cassini et al. 2022). It should be noted however, that allelic distribution varied significantly across racial groups, complicating direct interpretation of the outcomes. Specifically, the AA genotype was overrepresented in Black/African American participants than other racial groups. Given the complex interaction between systemic barriers faced by minority populations and their impact on physical and mental health (Webb et al. 2024), further investigation of how these factors interact is warranted.

Taken together, the evidence suggests that the effects of the FAAH C385A A-allele in PTSD may be different from those in preclinical animal models and healthy, nonclinical adult populations. While elevated AEA initially appeared to be advantageous in stress reactivity and fear extinction, this largely did not translate to clinical populations (Ney et al. 2022). More research into this complex topic will be essential to understanding the role that endogenous eCB signaling plays in PTSD and whether cannabinoid-based therapeutics will indeed be beneficial.

7 Endocannabinoid Contributions to Risk of Major Depressive Disorder

7.1 Depression and the Endocannabinoid System

Major depressive disorder (MDD) is a mood disorder characterized primarily by a depressed mood, loss of interest or pleasure in once enjoyable things (often referred to as anhedonia), a sense of hopelessness and persistent thoughts of death (Marx et al. 2023). The etiology of MDD is complex. Vulnerabilities to the disorder include genetic predisposition, early life stress, acute life stress, and biological deficits such as depressed levels of neurotransmitters such as serotonin, dopamine, and norepinephrine (Brown and Gershon 1993; Owens and Nemeroff 1994). Additionally, MDD often has co-occurring psychiatric disorders, meaning individuals diagnosed with MDD are also likely to be diagnosed with another disorder(s) (Kessler et al. 2003).

The eCB system has been implicated in modulating and maintaining depression. CB1R downregulation and reduced activity have been thought to contribute to depression in humans (Hillard and Liu 2014). One of the earliest indications of this is attributable to rimonabant, a CB1R antagonist originally developed for treating obesity. Though rimonabant successfully treated obesity, it was removed from the market due to the onset of severe depressive episodes it induced in patients (Christensen et al. 2007). These findings suggest a role for eCB signaling in depressive pathology. In fact, it is thought that the eCB system may play a role in modulating serotonergic functioning (Horstmann and Binder 2009), particularly within critical emotional processing areas like the prefrontal cortex, hippocampus, limbic structures and the hypothalamus (Herkenham 1991). Studies utilizing FAAH inhibitors in animals have shown that elevated AEA results in antidepressant-like effects, such as significant reductions in immobility time in the forced swim paradigm (Adamczyk et al. 2008; Gobbi et al. 2005). Such research has proposed FAAH as a potential treatment target for depression; however, research in this area is limited.

7.2 FAAH Genetics and Depression

The FAAH C385A SNP appears to have minimal impact on the risk for MDD or bipolar disorder. A study examining the impact of the FAAH C385A variation in participants with MDD or bipolar disorder and found that, when comparing A-allele carriers to CC genotypes, there was a slightly higher frequency of the A-allele in patients with MDD as compared to controls. (Monteleone et al. 2010). However, this finding was nonsignificant, and the distribution of C and A alleles did not significantly differ between participants with MDD, participants with bipolar disorder, and controls (Monteleone et al. 2010). The finding of a slightly higher AC genotype

frequency may suggest a relationship between the A-allele and risk for MDD, though more definitive evidence would be needed to confirm this.

Genetic variation in eCB function has been explored as a mediator for depression risk in those exposed to early life adversity, as well as a potential mediator of treatment in those diagnosed with MDD. Individuals with previous childhood adversity who carried at least one copy of the A-allele had a two-fold increased risk for depression (Lazary et al. 2016). These findings suggest a possible vulnerability to depressive phenotypes in A-allele carriers following exposure to aversity. However, in patients with MDD prescribed an SSRI, there was no effect of FAAH C385A on self-reported depression symptoms. Thus, while the A-allele may confer increased risk for MDD development, particularly in those who experience early life adversity, it does not appear to mediate MDD treatment via SSRIs.

Beyond MDD itself, the FAAH C385A SNP has been linked to depressive symptoms in other patient populations, apart from MDD. In particular, control participants demonstrated the expected effect of the A-allele on increased AEA concentrations, but there was no effect of genotype on AEA concentrations in patients with borderline personality disorder (BPD) (Spohrs et al. 2023). However, patients with BPD who were homozygous for the C-allele had higher self-reported depression ratings than their A-allele counterparts. Interesting, a PET study determined that levels of FAAH were higher in patients with BPD than matched healthy controls (Kolla et al. 2020), though this effect was not mediated by genotype. In addition, FAAH genotype mediated the relationship between poor sleep and depressive symptoms in emerging adults, with C/C homozygotes showing poorer sleep; an effect that was mediated by depressive symptoms (Maple et al. 2016).

Within these studies, there is no clear consensus on the FAAH polymorphism contribution to psychiatric risk of MDD development. Much of the research looking at MDD and eCB genetic correlates for psychiatric risk have focused on the CB1R SNP; CNR1 rs1049353, likely due to the established connection discovered from rimonabant which acts directly on CB1R. However, further research is needed to discern if the FAAH C385A polymorphism could confer psychiatric risk for MDD or other disorders characterized by depressive symptoms.

8 Endocannabinoid Contributions to Risk of Substance Use Disorders

8.1 The Endocannabinoid System and Substance Use Disorders

Substance use disorders (SUDs) encompass many classes of drugs of abuse, such as alcohol use disorder (AUD), psychostimulant use disorder, cannabis use disorder, and opioid use disorder. SUD is a prevalent public health concern, with 36 million people meeting clinically significant SUD criteria worldwide as of 2018 (World

Health Organization, 2018). The use and potential misuse of substances can be due to the positive reinforcement created by the pleasurable subjective effects, as well as the negative reinforcing effects that attenuate aversive states (Gilpin and Koob 2008). Individuals living with SUD may participate in polysubstance use, and whether on different occasions or at the same time, the interactions between different substances may exacerbate harmful physical and psychological effects (Crummy et al. 2020). Additionally, SUDs often co-occur with mood disorders such as anxiety and depression, further complicating treatment approaches and efficacy (Essau 2020). Thus, understanding how the eCB system is impacted by chronic substance use may point to novel therapeutic opportunities to mitigate the symptoms of SUDs.

8.2 FAAH Genetics and Substance Use Disorders

Some of the earliest research on the FAAH C385A SNP has been linked to SUD pathology, including an elevated risk for developing SUD and AUD in particular (Sipe et al. 2002; Sloan et al. 2018; Zhang et al. 2020). For instance, the A-allele was found to be associated with increased street drug use and problematic drug and alcohol use (Chiang et al. 2004; Sipe et al. 2002). A subsequent study found that the A-allele was associated with diagnosis and severity of AUD, including fewer days abstinent and more binge drinking days (Sloan et al. 2018). However, in young individuals (~21 years of age), CC homozygotes were more likely to engage in risky alcohol consumption, though they did not differ from A-allele carries in their responses to alcohol or drug cues in a laboratory setting (Bühler et al. 2014). Heavy-drinking youth carrying the A-allele reported significantly more frequent heavy drinking episodes and higher alcohol-related problems, which was mediated by coping motives (Best et al. 2021). Subsequent studies using PET imaging demonstrated that lower FAAH levels are associated with more hazardous drinking and more positive subjective effects of alcohol in heavy-drinking youth (Best et al. 2023). Thus, the response to alcohol and risk for AUD development in relation to FAAH may differ based on genetics or environmental exposure, as well as age.

Given the direct action of THC on the eCB system, it is logical that studies have explored how the FAAH C385A SNP interacts with cannabis use. The FAAH SNP appears to impact the various components of risk for cannabis use and negative consequences of use, though these effects are not always in the same direction. For example, A/A homozygotes were more likely to have tried cannabis, but less likely to develop a cannabis use disorder (Tyndale et al. 2007). In individuals who met DSM-IV criteria for cannabis dependence, there was no difference in cannabis craving based on genotype, but C/C homozygotes reported higher withdrawal severity when trying to abstain (Schacht et al. 2009), perhaps related to the effects of the SNP anxiety, as discussed above. However, others have found that C/C homozygotes do report higher craving during abstinence, though A-allele carriers report greater increases in negative mood during early abstinence (Haughey et al. 2008). Moreover, in cannabis users who abstained for 3 days, C/C homozygotes had greater

neural activity in response to cannabis cues than A-allele carriers (Filbey et al. 2010), potentially suggesting that C/C homozygotes may be more sensitive to cue-induced relapse during attempts at abstinence. This could be a consequence of differences in anxiety attributed to this variation, though this has not been directly tested.

Cannabis use is prevalent in late adolescence and early adulthood (Myhr et al. 2024), which is a critical period for maturation of white matter tracts within the brain. The eCB system also fluctuates during development (Sisk et al. 2022), potentially rendering it more sensitive to exogenous influences. Young adults aged 18–25 who were classified as chronic cannabis users, defined as having smoked a minimum of 25 joints in the past year, had reduced white matter integrity, as compared to controls who smoked 5 or fewer joints over the last 10 years (Shollenbarger et al. 2015). However, there was a cannabis group x FAAH interaction such that, within the control group, A-allele carriers had reduced white matter integrity than C/C homozygotes. In contrast, in the cannabis user group, A-allele carriers had greater white matter integrity than C/C homozygotes (Shollenbarger et al. 2015). Thus, genetics, environmental exposure, and developmental stage may all be factors impacting risks related to cannabis use.

There is also work exploring how the FAAH C385A polymorphism impacts risk for developing other substance use disorders. As mentioned above, studies assessing poly-drug or "street drug" use also suggest a higher risk of use or more severe use in carriers of the A-allele (Flanagan et al. 2006; Sipe et al. 2002). In addition, the A-allele was significantly more common in a large Chinese Han population of individuals who had a methamphetamine dependence, as compared to healthy controls (Zhang et al. 2020). In a study of individuals from the United States who had a cocaine use disorder (CUD), the A-allele was associated with greater cocaine-induced feelings of positive (high) and negative (depressed) subjective effects. In addition, the A-allele was over-represented in the CUD group, and A-allele carriers within the CUD group exhibited greater consumption of cocaine and tobacco than the C/C homozygotes. Thus, one mechanism rendering A-allele carriers more vulnerable to SUD development may be differences in sensitivities to the subjective drug effects. Whether this effect is an inherent difference or something that emerges throughout the development of the SUD remains to be determined.

While studies generally suggest the A-allele as a factor that may confer some vulnerability to risky or problematic use, C/C homozygotes appear to experience more negative effects during attempts at abstinence. Withdrawal and abstinence are times of marked stress and negative affect, so it is plausible that the effects of elevated AEA on mitigating these symptoms may be advantageous in successful attempts at reducing substance intake. As a result, some studies have been exploring the eCB system as a target in SUD treatment, particularly during this challenging period. In particular, elevated AEA during withdrawal in a population with cannabis use disorder improved cessation attempts (D'Souza et al. 2019). A large, multicenter trial expanding on this outcome has recently been conducted and completed, though results have yet to be published. However, overall, understanding how the

eCB system underpins various processes underlying the risk for SUD development or challenges to attempts at reducing use can provide novel, mechanism-based therapeutic strategies.

9 Endocannabinoid Contributions to Risk of Eating Disorders

9.1 The Endocannabinoid System and Feeding Behaviors

Eating disorders (EDs), such as anorexia nervosa, bulimia nervosa, and binge eating disorder, are psychiatric conditions with a diverse range of characteristics, including a preoccupation with weight and food, binging and/or purging behaviors, overly restrictive feeding behaviors, and body dysmorphia, which have severe consequences for overall health (Mitchell and Peterson 2020; Spohrs et al. 2021). EDs also have a high co-occurrence with other psychiatric conditions, particularly anxiety disorders, and have marked treatment resistance (Mitchell and Peterson 2020). Thus, it is imperative that research elucidate the neurological underpinnings of ED pathology to develop effective treatments.

Preclinical evidence has demonstrated a role of eCB signaling in regulating various components of metabolic functioning and feeding behaviors that are pathologically altered in ED populations (Balsevich et al. 2023; Mazier et al. 2015). For example, fasting has been shown to increase levels of AEA and 2-AG in the limbic forebrain of rats, and 2-AG in the hypothalamus, though to a lesser extent (Kirkham et al. 2002). Building on this work, 2-AG injections into the nucleus accumbens shell of rats were shown to stimulate feeding behavior in a dose-dependent manner, potentially by driving both the liking of and motivation for food consumption through regulation of dopamine release in this region (Mazier et al. 2015). This orexigenic effect of 2-AG is attenuated by the CB1R antagonist, SR141716, indicating CB1R-dependency. Similarly, pharmacological elevation of AEA levels has been demonstrated to stimulate food intake in mice (Soria-Gómez et al. 2007). Thus, the eCB system can regulate multiple aspects of feeding behavior.

Due to the regulatory role of eCB signaling in systems and behaviors relevant to ED pathology—including feeding behavior, metabolism, and anxiety—it is possible that disruption of this system contributes to risk of ED development. In humans, altered eCB signaling appears to be correlated with ED pathology. Comparisons of circulating AEA levels in women with various EDs (anorexia nervosa, $N = 15$, bulimia nervosa, $N = 12$, binge eating disorder, $N = 11$, healthy controls, $N = 15$) found AEA to be significantly elevated in both anorexia nervosa and binge eating disorder patients, but not bulimia nervosa patients or healthy controls (Monteleone et al. 2005). However, it remains to be seen if changes in eCB concentrations are a cause or consequence of disordered eating behaviors, particularly considering the established relationship between eCBs and food consumption in non-pathological states (Hillard 2018).

9.2 FAAH Genetics and Eating Disorders

Preclinical investigations using a humanized FAAH C385A knock-in mouse model have demonstrated an impact of altered eCB signaling on feeding behaviors. A recent study found that A/A mice show amplified orexigenic and diminished anorexigenic responses to feeding cues compared to C/C carriers (Balsevich et al. 2023). At baseline, homozygous A-allele mice demonstrated lower basal food intake, and a lower matching energy expenditure compared to C/C mice. On the other hand, exposure to glucocorticoid, a hormone involved in the regulation of appetite and energy homeostasis, increased food intake in the A/A mice exclusively and was associated with elevated levels of hypothalamic AEA. The authors therefore proposed that the FAAH A/A genotype may sensitize carriers to hyperphagic feeding outcomes, and by extension, predispose them to overconsumption and weight gain. Notably, there was no effect of genotype on changes to energy expenditure or substrate utilization. Thus, the FAAH C385A SNP appears to mediate glucocorticoid-induced weight gain through alterations in feeding behaviors opposed to energy expenditure (Balsevich et al. 2023). Taken together, these studies indicate a role of eCB signaling in metabolic functioning and feeding behavior that is dependent on dose, region, and neuronal type. To understand the impact of eCB signaling on these functions in relation to ED pathology in humans, researchers have explored the impact of the FAAH C385A SNP in clinical ED populations.

Early work reported that the FAACH C385A SNP was associated with obesity and overweight in White and Black, but not Asian, individuals (Sipe et al. 2005). Subsequently, the SNP has been explored in relation to both obesity-related traits and EDs (Lopez-Cortes et al. 2024). In overweight patients with or without a binge eating disorder, the frequency of the A-allele was greater than in matched healthy weight controls (Monteleone et al. 2008). Another group also found that the A-allele was marginally more frequent in women with a binge eating disorder than those without, though this effect did not reach statistical significance (Yagin et al. 2020). The A-allele has also been found to be more common in patients with anorexia or bulimia, as compared to healthy controls (Monteleone et al. 2009), though some studies report a lack of association (Arias et al. 2009).

One interesting feature of genetic studies is the ability to track how traits vary across distinct genetic populations. However, this also produces results from groups that are diverse enough that not all findings replicate uniformly. Cross-sectional evidence from a population in western Mexico suggests that the CC genotype was associated with greater body weight and body fat, but no association was found with dysfunctional eating patterns (Martínez-Rodríguez et al. 2024). However, A-allele carriers had a higher risk of stress perception scores, in contrast to other findings presented in the above sections. In a large Japanese population, the A-allele was less frequent in patients with anorexia nervosa as compared to controls (Ando et al. 2014). Further analyses revealed that this was specific to patients who had a history of the restricting subtype of anorexia, and not those with a history of binge eating or

purging. Thus, diversity in the patient population, as well as the ED subtype, can make direct inferences challenging, highlighting the need for additional research in this domain.

10 Endocannabinoid Contributions to Risk of Autism Spectrum Disorder

Autism spectrum disorder (ASD) refers to a collection of neurodevelopmental disorders that can be characterized by impairments in social processing and repetitive patterns of behavior. ASD is often accompanied by co-occurring conditions, including anxiety, seizure susceptibility, sleep disturbances, gastrointestinal disorders, and attention-deficit/hyperactivity disorder among others (Neumeyer et al. 2019). Despite an estimated 1 in 100 children diagnosed with ASD and well-defined behavioral diagnostic features, the etiology of ASD remains elusive (Autism 2020).

Dysfunction of social processing, a hallmark feature of ASD, has recently been linked to eCB system functioning. Preclinical research has shown CB1Rs to be densely expressed in regions associated with social behavior, such as the prefrontal cortex, hippocampus, dorsolateral striatum, ventral tegmental area, and to a lesser extent, the nucleus accumbens (Wei et al. 2017). It is plausible then, that eCB signaling in these regions impacts social behaviors and potentially contributes to related pathology. Indeed, manipulation of eCB signaling alters social interaction in animal models of ASD-like behaviors. Valproic acid (VPA) environmental models of ASD have reliably produced social behavioral deficits reminiscent of those observed in patients with autism, including compromised sociability and social play behaviors, diminished exploratory behaviors, reduced social communication and discrimination, and elevated anxiety (Servadio et al. 2016). Administration of an FAAH inhibitor reverses VPA-induced social deficits (Kerr et al. 2016; Servadio et al. 2016). These findings corroborate results from another group, showing that inhibition of FAAH via URB597 completely reversed social impairments in two mouse models of ASD (Wei et al. 2016). The FAAH inhibition-induced prosocial behaviors were blocked by administration of a CB1R antagonist, confirming CB1R-dependency.

To our knowledge, no studies have explored the impact of FAAH C385A on risk for ASD or in relation to social behaviors. However, as predicted from the preclinical literature, disruptions in eCB signaling have been reported in ASD populations. For instance, children with ASD have demonstrated decreased circulating levels of AEA, PEA, and OEA, implicating a potential relation between altered eCB functioning and ASD pathology (Aran et al. 2019; Karhson et al. 2018; Zou et al. n.d.). Studies have also begun exploring cannabinoid-based therapeutics in ASD treatment, though preliminary reports do not find significant effects (Aran et al. 2021). However, a recent clinical trial testing the efficacy of elevating AEA via FAAH

inhibition has been completed, but results have yet to be published (NCT03664232). Thus, it remains to be seen if targeting the eCB system will ameliorate symptoms of ASD.

11 Endocannabinoid Contributions to Risk of Schizophrenia

An area of particular interest is the role that the eCB system may play in schizophrenia and psychosis. Schizophrenia is a mental health condition characterized by positive symptoms such as hallucinations and delusions, negative symptoms like social withdrawal, and cognitive symptoms such as working memory or executive function deficits (American Psychiatric Association 2013; (McCutcheon et al. 2020). A compelling motivator for exploring eCB function in schizophrenia is the belief that individuals who have a genetic predisposition to psychotic disorders may also be prone to cannabis-induced psychosis (Malone et al. 2010). One large observational study found that 47.4% of individuals who had an episode of cannabis-induced psychosis converted to either schizophrenia or bipolar disorder within 20 years (Starzer et al. 2018), underscoring the need for more research to establish the relationship between the eCB system and conditions with psychosis-related symptomology.

While alterations of the eCB system have been associated with schizophrenia, there does not appear to be a clear relationship to the FAAH C385A polymorphism specifically. Studies have found no significant effect of the SNP on risk of schizophrenia (Morita et al. 2005), first-episode psychosis (Bioque et al. 2019), or cannabis-induced psychosis-like experiences (Hindocha et al. 2020). However, while this specific polymorphism may not be involved in the development of psychosis or schizophrenia, there is preliminary evidence suggesting that AEA is found in higher concentrations of the blood and cerebrospinal fluid of patients (Potvin et al. 2008; De Marchi et al. 2003; Leweke et al. 1999). It is possible that levels of AEA in patients with schizophrenia are increased via a mechanism independent from the action of the FAAH C385A polymorphism. Further, a number of neurodevelopmental processes that are thought to play a role in the emergence of schizophrenia can be impacted by alterations in the eCB system (Zamberletti and Rubino 2021). Further research is needed to conclusively establish the importance of the eCB system in conveying risk for psychosis-spectrum conditions, and whether it may prove to be a potential target for novel therapies.

12 Conclusions

Overall, the evidence presented throughout this chapter highlights the eCB system as an integral regulator of many brain and behavioral functions, including stress, fear, emotional regulation, metabolism, growth and development, and synaptic

physiology (Lutz 2020; Skaper and Di Marzo 2012). Dysfunction of these processes hallmarks various psychiatric conditions, such as anxiety disorders, MDD, EDs, PTSD, SUDs, ASD, and schizophrenia. Thus, disruption of eCB signaling, and the critical brain and behavioral functions it governs, may confer risk of developing such psychopathologies. Pharmacological preclinical investigations have largely supported this hypothesis, demonstrating pathological alterations in brain function and behavior following manipulation of eCB signaling. In particular, changes in AEA levels through administration of a FAAH inhibitor appear to influence anxious and depressive phenotypes, which are correlated with disorders ranging from SUDs to EDs. However, current clinical literature offers mixed conclusions. Several limitations in pharmacological tools, such as a lack of precision, and restricted translatability, may contribute to the mixed data in the field.

Utilization of the FAAH C385A SNP uniquely enables investigation of eCB contributions to psychiatric pathology in a noninvasive manner. Exploration of genetic variations in this SNP and resulting changes in AEA levels have been demonstrated to impact psychiatric outcomes. However, there is little consensus concerning the nature of these changes. The field would benefit from greater study into the complex relationship of eCB signaling and psychiatric risk, particularly investigation of gene x environment interactions that complicate psychiatric pathology. In addition, it is unclear if the impacts of the FAAH SNP are due to elevated levels of AEA at the time of testing or elevated levels of AEA throughout development. Moreover, future research can determine if the FAAH C385 SNP or AEA concentrations, which are notably dynamic, appear to be more proximally related to risk for psychiatric development. Therefore, collection of longitudinal data should be a priority of future studies assessing the influence of FAAH genetic variation on psychiatric health outcomes. The insight provided by such research may elucidate eCB-related therapeutic targets for the treatment of several psychiatric conditions discussed here.

References

Adamczyk P, Gołda A, McCreary AC, Filip M, Przegaliński E. Activation of endocannabinoid transmission induces antidepressant-like effects in rats. J Physiol Pharmacol. 2008;59(2):217–28.

Agrawal A, Nelson EC, Littlefield AK, Bucholz KK, Degenhardt L, Henders AK, Madden PAF, Martin NG, Montgomery GW, Pergadia ML, Sher KJ, Heath AC, Lynskey MT. Cannabinoid receptor genotype moderation of the effects of childhood physical abuse on anhedonia and depression. Arch Gen Psychiatry. 2012;69(7):732–40. https://doi.org/10.1001/archgenpsychiatry.2011.2273.

Ahn K, Smith SE, Liimatta MB, Beidler D, Sadagopan N, Dudley DT, Young T, Wren P, Zhang Y, Swaney S, Becelaere KV, Blankman JL, Nomura DK, Bhattachar SN, Stiff C, Nomanbhoy TK, Weerapana E, Johnson DS, Cravatt BF. Mechanistic and pharmacological characterization of PF-04457845: a highly potent and selective fatty acid amide hydrolase inhibitor that reduces inflammatory and noninflammatory pain. J Pharmacol Exp Ther. 2011;338(1):114–24. https://doi.org/10.1124/jpet.111.180257.

Alcaraz-Silva J, Feingold D, Viana-Torre G, Budde H, Imperatori C, Machado S, Murillo-Rodríguez E. The endocannabinoid system as a biomarker for diagnostic and therapeutic

applications in depression and anxiety. CNS Neurol Disord Drug Targets. 2023;22(3):417–30. https://doi.org/10.2174/1871527321666220405114402.

Ando T, Tamura N, Mera T, Morita C, Takei M, Nakamoto C, Koide M, Hotta M, Naruo T, Kawai K, Nakahara T, Yamaguchi C, Nagata T, Ookuma K, Okamoto Y, Yamanaka T, Kiriike N, Ichimaru Y, Ishikawa T, Komaki G. Association of the c.385C>a (p.Pro129Thr) polymorphism of the fatty acid amide hydrolase gene with anorexia nervosa in the Japanese population. Mol Genet Genomic Med. 2014;2(4):313–8. https://doi.org/10.1002/mgg3.69.

Aran A, Eylon M, Harel M, Polianski L, Nemirovski A, Tepper S, Schnapp A, Cassuto H, Wattad N, Tam J. Lower circulating endocannabinoid levels in children with autism spectrum disorder. Mol Autism. 2019;10:2. https://doi.org/10.1186/s13229-019-0256-6.

Aran A, Harel M, Cassuto H, Polyansky L, Schnapp A, Wattad N, Shmueli D, Golan D, Castellanos FX. Cannabinoid treatment for autism: a proof-of-concept randomized trial. Mol Autism. 2021;12(1):6. https://doi.org/10.1186/s13229-021-00420-2.

Arias F, Ampuero I, Sagredo O, Almódovar F, Sánchez S, Gorgojo JJ, Ramos JA. Lack of association between polymorphisms in cannabinoid receptor gene (CNR1) and fatty acid amide hydroxylase gene (FAAH) and eating disorders in a preliminary study. Psychiatr Genet. 2009;19(6):336. https://doi.org/10.1097/YPG.0b013e3283328de2.

Autism. 2020. https://www.who.int/news-room/fact-sheets/detail/autism-spectrum-disorders. Accessed 30 Aug 2024.

Baenas I, Miranda-Olivos R, Granero R, Solé-Morata N, Sánchez I, Pastor A, del Pino-Gutiérrez A, Codina E, Tinahones FJ, Fernández-Formoso JA, Vilarrasa N, Guerrero-Pérez F, Lopez-Urdiales R, Virgili N, Soriano-Mas C, Jiménez-Murcia S, de la Torre R, Fernández-Aranda F. Association of anandamide and 2-arachidonoylglycerol concentrations with clinical features and body mass index in eating disorders and obesity. Eur Psychiatry. 2023;66(1):e49. https://doi.org/10.1192/j.eurpsy.2023.2411.

Baggelaar MP, Maccarrone M, van der Stelt M. 2-Arachidonoylglycerol: a signaling lipid with manifold actions in the brain. Prog Lipid Res. 2018;71:1–17. https://doi.org/10.1016/j.plipres.2018.05.002.

Balogh Z, Szente L, Biro L, Varga ZK, Haller J, Aliczki M. Endocannabinoid interactions in the regulation of acquisition of contextual conditioned fear. Prog Neuro-Psychopharmacol Biol Psychiatry. 2019;90:84–91. https://doi.org/10.1016/j.pnpbp.2018.11.007.

Balsevich G, Petrie GN, Heinz DE, Singh A, Aukema RJ, Hunker AC, Vecchiarelli HA, Yau H, Sticht M, Thompson RJ, Lee FS, Zweifel LS, Chelikani PK, Gassen NC, Hill MN. A genetic variant of fatty acid amide hydrolase (FAAH) exacerbates hormone-mediated orexigenic feeding in mice. eLife. 2023;12:e81919. https://doi.org/10.7554/eLife.81919.

Bedse G, Hartley ND, Neale E, Gaulden AD, Patrick TA, Kingsley PJ, Uddin MJ, Plath N, Marnett LJ, Patel S. Functional redundancy between canonical endocannabinoid signaling Systems in the Modulation of anxiety. Biol Psychiatry. 2017;82(7):488–99. https://doi.org/10.1016/j.biopsych.2017.03.002.

Berrendero F, García-Gil L, Hernández ML, Romero J, Cebeira M, De Miguel R, Ramos JA, Fernández-Ruiz JJ. Localization of mRNA expression and activation of signal transduction mechanisms for cannabinoid receptor in rat brain during fetal development. Development. 1998;125(16):3179–88. https://doi.org/10.1242/dev.125.16.3179.

Best LM, Wardell JD, Tyndale RF, McPhee MD, Le Foll B, Kish SJ, Boileau I, Hendershot CS. Association of the fatty acid amide hydrolase C385A polymorphism with alcohol use severity and coping motives in heavy-drinking youth. Alcohol Clin Exp Res. 2021;45(3):507–17. https://doi.org/10.1111/acer.14552.

Best LM, Hendershot CS, Buckman JF, Jagasar S, McPhee MD, Muzumdar N, Tyndale RF, Houle S, Logan R, Sanches M, Kish SJ, Foll BL, Boileau I. Association between fatty acid amide hydrolase and alcohol response phenotypes: a positron emission tomography imaging study with [11C]CURB in heavy-drinking youth. Biol Psychiatry. 2023;94(5):405–15. https://doi.org/10.1016/j.biopsych.2022.11.022.

Bioque M, Mas S, Costanzo MC, Cabrera B, Lobo A, González-Pinto A, Rodriguez-Toscano E, Corripio I, Vieta E, Baeza I, Ibáñez Á, Fraile MG, Cuesta MJ, Mezquida G, Lafuente A, Bernardo M. Gene-environment interaction between an endocannabinoid system genetic polymorphism and cannabis use in first episode of psychosis. Eur Neuropsychopharmacol. 2019;29(6):786–94. https://doi.org/10.1016/j.euroneuro.2019.04.005.

Brown AS, Gershon S. Dopamine and depression. J Neural Trans. 1993;91(2):75–109. https://doi.org/10.1007/BF01245227.

Brown LA, Zandberg LJ, Foa EB. Mechanisms of change in prolonged exposure therapy for PTSD: implications for clinical practice. J Psychother Integr. 2019;29(1):6–14. https://doi.org/10.1037/int0000109.

Bühler K-M, Huertas E, Echeverry-Alzate V, Giné E, Moltó E, Montoliu L, López-Moreno JA. Risky alcohol consumption in young people is associated with the fatty acid amide hydrolase gene polymorphism C385A and affective rating of drug pictures. Mol Genet Genom. 2014;289(3):279–89. https://doi.org/10.1007/s00438-013-0809-x.

Careaga MBL, Girardi CEN, Suchecki D. Understanding posttraumatic stress disorder through fear conditioning, extinction and reconsolidation. Neurosci Biobehav Rev. 2016;71:48–57. https://doi.org/10.1016/j.neubiorev.2016.08.023.

Carey CE, Agrawal A, Zhang B, Conley ED, Degenhardt L, Heath AC, Li D, Lynskey MT, Martin NG, Montgomery GW, Wang T, Bierut LJ, Hariri AR, Nelson EC, Bogdan R. Monoacylglycerol lipase (MGLL) polymorphism rs604300 interacts with childhood adversity to predict cannabis dependence symptoms and amygdala habituation: evidence from an endocannabinoid system-level analysis. J Abnorm Psychol. 2015;124(4):860–77. https://doi.org/10.1037/abn0000079.

Chaby LE, Zhang L, Liberzon I. The effects of stress in early life and adolescence on posttraumatic stress disorder, depression, and anxiety symptomatology in adulthood. Curr Opin Behav Sci. 2017;14:86–93. https://doi.org/10.1016/j.cobeha.2017.01.001.

Chapman C, Mills K, Slade T, McFarlane AC, Bryant RA, Creamer M, Silove D, Teesson M. Remission from post-traumatic stress disorder in the general population. Psychol Med. 2012;42(8):1695–703. https://doi.org/10.1017/S0033291711002856.

Chester LA, Englund A, Chesney E, Oliver D, Wilson J, Sovi S, Dickens AM, Oresic M, Linderman T, Hodsoll J, Minichino A, Strang J, Murray RM, Freeman TP, McGuire P. Effects of Cannabidiol and Delta-9-tetrahydrocannabinol on plasma endocannabinoid levels in healthy volunteers: a randomized double-blind four-arm crossover study. Cannabis Cannabinoid Res. 2024;9(1):188–98. https://doi.org/10.1089/can.2022.0174.

Chiang KP, Gerber AL, Sipe JC, Cravatt BF. Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. Hum Mol Genet. 2004;13(18):2113–9. https://doi.org/10.1093/hmg/ddh216.

Christensen R, Kristensen PK, Bartels EM, Bliddal H, Astrup A. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet. 2007;370(9600):1706–13. https://doi.org/10.1016/S0140-6736(07)61721-8.

Côté M, Matias I, Lemieux I, Petrosino S, Alméras N, Després J-P, Di Marzo V. Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. Int J Obes. 2007;31(4):692–9. https://doi.org/10.1038/sj.ijo.0803539.

Crombie KM, Privratsky AA, Schomaker CM, Heilicher M, Ross MC, Sartin-Tarm A, Sellnow K, Binder EB, Andrew James G, Cisler JM. The influence of FAAH genetic variation on physiological, cognitive, and neural signatures of fear acquisition and extinction learning in women with PTSD. NeuroImage. 2022;33:102922. https://doi.org/10.1016/j.nicl.2021.102922.

Crummy EA, O'Neal TJ, Baskin BM, Ferguson SM. One is not enough: understanding and modeling polysubstance use. Front Neurosci. 2020;14:569. https://doi.org/10.3389/fnins.2020.00569.

D'Souza DC, Cortes-Briones J, Creatura G, Bluez G, Thurnauer H, Deaso E, Bielen K, Surti T, Radhakrishnan R, Gupta A, Gupta S, Cahill J, Sherif MA, Makriyannis A, Morgan PT, Ranganathan M, Skosnik PD. Efficacy and safety of a fatty acid amide hydrolase inhibitor (PF-04457845) in the treatment of cannabis withdrawal and dependence in men: a double-blind,

placebo-controlled, parallel group, phase 2a single-site randomised controlled trial. Lancet Psychiatry. 2019;6(1):35–45. https://doi.org/10.1016/S2215-0366(18)30427-9.

De Marchi N, De Petrocellis L, Orlando P, Daniele F, Fezza F, Di Marzo V. Endocannabinoid signalling in the blood of patients with schizophrenia. Lipids Health Dis. 2003;2(1):5. https://doi.org/10.1186/1476-511X-2-5.

deRoon-Cassini TA, Bergner CL, Chesney SA, Schumann NR, Lee TS, Brasel KJ, Hillard CJ. Circulating endocannabinoids and genetic polymorphisms as predictors of posttraumatic stress disorder symptom severity: heterogeneity in a community-based cohort. Transl Psychiatry. 2022;12(1):1–12. https://doi.org/10.1038/s41398-022-01808-1.

Desai S, Borg B, Cuttler C, Crombie KM, Rabinak CA, Hill MN, Marusak HA. A systematic review and meta-analysis on the effects of exercise on the endocannabinoid system. Cannabis Cannabinoid Res. 2022;7(4):388–408. https://doi.org/10.1089/can.2021.0113.

Desai S, Zundel CG, Evanski JM, Gowatch LC, Bhogal A, Ely S, Carpenter C, Shampine M, O'Mara E, Rabinak CA, Marusak HA. Genetic variation in endocannabinoid signaling: anxiety, depression, and threat-and reward-related brain functioning during the transition into adolescence. Behav Brain Res. 2024;463:114925. https://doi.org/10.1016/j.bbr.2024.114925.

Dincheva I, Drysdale AT, Hartley CA, Johnson DC, Jing D, King EC, Ra S, Gray JM, Yang R, DeGruccio AM, Huang C, Cravatt BF, Glatt CE, Hill MN, Casey BJ, Lee FS. FAAH genetic variation enhances fronto-amygdala function in mouse and human. Nat Commun. 2015;6(1):6395. https://doi.org/10.1038/ncomms7395.

Elkrief L, Spinney S, Vosberg DE, Banaschewski T, Bokde ALW, Quinlan EB, Desrivières S, Flor H, Garavan H, Gowland P, Heinz A, Brühl R, Martinot J-L, Paillère Martinot M-L, Nees F, Papadopoulos Orfanos D, Poustka L, Hohmann S, Millenet S, Conrod P. Endocannabinoid gene × gene interaction association to alcohol use disorder in two adolescent cohorts. Front Psych. 2021;12:645746. https://doi.org/10.3389/fpsyt.2021.645746.

Essau CA. Comorbidity of addictive problems: assessment and treatment implications. In: Essau CA, Delfabbro PH, editors. Adolescent addiction. 2nd ed. Academic Press; 2020. p. 291–317. https://doi.org/10.1016/B978-0-12-818626-8.00011-6.

Evanson NK, Tasker JG, Hill MN, Hillard CJ, Herman JP. Fast feedback inhibition of the HPA Axis by glucocorticoids is mediated by endocannabinoid signaling. Endocrinology. 2010;151(10):4811–9. https://doi.org/10.1210/en.2010-0285.

Filbey FM, Schacht JP, Myers US, Chavez RS, Hutchison KE. Individual and additive effects of the CNR1 and FAAH genes on brain response to marijuana cues. Neuropsychopharmacology. 2010;35(4):967–75. https://doi.org/10.1038/npp.2009.200.

Flanagan JM, Gerber AL, Cadet JL, Beutler E, Sipe JC. The fatty acid amide hydrolase 385 A/A (P129T) variant: haplotype analysis of an ancient missense mutation and validation of risk for drug addiction. Hum Genet. 2006;120(4):581–8. https://doi.org/10.1007/s00439-006-0250-x.

Fonzo GA, Ramsawh HJ, Flagan TM, Simmons AN, Sullivan SG, Allard CB, Paulus MP, Stein MB. Early life stress and the anxious brain: evidence for a neural mechanism linking childhood emotional maltreatment to anxiety in adulthood. Psychol Med. 2016;46(5):1037–54. https://doi.org/10.1017/S0033291715002603.

Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev. 2003;83(3):1017–66. https://doi.org/10.1152/physrev.00004.2003.

Gaoni Y, Mechoulam R. Isolation, structure, and partial synthesis of an active constituent of hashish. J Am Chem Soc. 1964;86(8):1646–7. https://doi.org/10.1021/ja01062a046.

Gee DG, Fetcho RN, Jing D, Li A, Glatt CE, Drysdale AT, Cohen AO, Dellarco DV, Yang RR, Dale AM, Jernigan TL, Lee FS, Casey BJ. Individual differences in frontolimbic circuitry and anxiety emerge with adolescent changes in endocannabinoid signaling across species. Proc Natl Acad Sci USA. 2016;113(16):4500–5. https://doi.org/10.1073/pnas.1600013113.

Gilpin NW, Koob GF. Neurobiology of alcohol dependence. Alcohol Res Health. 2008;31(3):185–95.

Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M, Cassano T, Morgese MG, Debonnel G, Duranti A, Tontini A, Tarzia G, Mor M, Trezza V, Goldberg SR, Cuomo V, Piomelli D. Antidepressant-like activity and modulation of brain monoaminergic transmission

by blockade of anandamide hydrolysis. Proc Natl Acad Sci. 2005;102(51):18620–5. https://doi.org/10.1073/pnas.0509591102.

Gorroochurn P, Hodge SE, Heiman GA, Durner M, Greenberg DA. Non-replication of association studies: "pseudo-failures" to replicate? Genet Med. 2007;9(6):325–31. https://doi.org/10.1097/GIM.0b013e3180676d79.

Gowatch LC, Evanski JM, Ely SL, Zundel CG, Bhogal A, Carpenter C, Shampine MM, O'Mara E, Mazurka R, Barcelona J, Mayo LM, Marusak HA. Endocannabinoids and stress-related Neurospsychiatric disorders: a systematic review and meta-analysis of basal concentrations and response to acute psychosocial stress. Cannabis Cannabinoid Res. 2024;9:1217. https://doi.org/10.1089/can.2023.0246.

Grupe DW, Nitschke JB. Uncertainty and anticipation in anxiety. Nat Rev Neurosci. 2013;14(7):488–501. https://doi.org/10.1038/nrn3524.

Gunduz-Cinar O, MacPherson KP, Cinar R, Gamble-George J, Sugden K, Williams B, Godlewski G, Ramikie TS, Gorka AX, Alapafuja SO, Nikas SP, Makriyannis A, Poulton R, Patel S, Hariri AR, Caspi A, Moffitt TE, Kunos G, Holmes A. Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. Mol Psychiatry. 2013;18(7):813–23. https://doi.org/10.1038/mp.2012.72.

Haller J, Barna I, Barsvari B, Gyimesi Pelczer K, Yasar S, Panlilio LV, Goldberg S. Interactions between environmental aversiveness and the anxiolytic effects of enhanced cannabinoid signaling by FAAH inhibition in rats. Psychopharmacology. 2009;204(4):607–16. https://doi.org/10.1007/s00213-009-1494-7.

Hariri AR, Gorka A, Hyde LW, Kimak M, Halder I, Ducci F, Ferrell RE, Goldman D, Manuck SB. Divergent effects of genetic variation in endocannabinoid signaling on human threat-and reward-related brain function. Biol Psychiatry. 2009;66(1):9–16. https://doi.org/10.1016/j.biopsych.2008.10.047.

Haughey HM, Marshall E, Schacht JP, Louis A, Hutchison KE. Marijuana withdrawal and craving: influence of the cannabinoid receptor 1 (CNR1) and fatty acid amide hydrolase (FAAH) genes. Addiction. 2008;103(10):1678–86. https://doi.org/10.1111/j.1360-0443.2008.02292.x.

Hembree EA, Rauch SAM, Foa EB. Beyond the manual: the insider's guide to prolonged exposure therapy for PTSD. Cogn Behav Pract. 2003;10(1):22–30. https://doi.org/10.1016/S1077-7229(03)80005-6.

Herkenham M. Characterization and localization of cannabinoid receptors in brain: an in vitro technique using slide-mounted tissue sections. NIDA Res Monogr. 1991;112:129–45.

Hill MN, McLaughlin RJ, Pan B, Fitzgerald ML, Roberts CJ, Lee TT-Y, Karatsoreos IN, Mackie K, Viau V, Pickel VM, McEwen BS, Liu Q, Gorzalka BB, Hillard CJ. Recruitment of prefrontal cortical endocannabinoid signaling by glucocorticoids contributes to termination of the stress response. J Neurosci. 2011;31(29):10506–15. https://doi.org/10.1523/JNEUROSCI.0496-11.2011.

Hill MN, Kumar SA, Filipski SB, Iverson M, Stuhr KL, Keith JM, Cravatt BF, Hillard CJ, Chattarji S, McEwen BS. Disruption of fatty acid amide hydrolase activity prevents the effects of chronic stress on anxiety and amygdalar microstructure. Mol Psychiatry. 2013;18(10):1125–35. https://doi.org/10.1038/mp.2012.90.

Hillard CJ, Liu Q. Endocannabinoid signaling in the etiology and treatment of major depressive illness. Curr Pharm Des. 2014;20(23):3795–811. https://doi.org/10.2174/13816128113196660735

Hill MN, Haney M, Hillard CJ, Karhson DS, Vecchiarelli HA. The endocannabinoid system as a putative target for the development of novel drugs for the treatment of psychiatric illnesses. Psychol Med. 2023;53(15):7006–24. https://doi.org/10.1017/S0033291723002465.

Hillard CJ. Circulating endocannabinoids: from whence do they come and where are they going? Neuropsychopharmacology. 2018;43(1):155–72. https://doi.org/10.1038/npp.2017.130.

Hindocha C, Quattrone D, Freeman TP, Murray RM, Mondelli V, Breen G, Curtis C, Morgan CJA, Valerie Curran H, Di Forti M. Do AKT1, COMT and FAAH influence reports of acute cannabis intoxication experiences in patients with first episode psychosis, controls and young adult cannabis users? Transl Psychiatry. 2020;10(1):1–13. https://doi.org/10.1038/s41398-020-0823-9.

Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R, Krey JF, Michael Walker J, Holmes PV, Crystal JD, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D. An endocannabinoid mechanism for stress-induced analgesia. Nature. 2005;435(7045):1108–12. https://doi.org/10.1038/nature03658.

Horstmann S, Binder EB. Pharmacogenomics of antidepressant drugs. Pharmacol Ther. 2009;124(1):57–73. https://doi.org/10.1016/j.pharmthera.2009.06.007.

Jian W, Edom R, Weng N, Zannikos P, Zhang Z, Wang H. Validation and application of an LC–MS/MS method for quantitation of three fatty acid ethanolamides as biomarkers for fatty acid hydrolase inhibition in human plasma. J Chromatogr B. 2010;878(20):1687–99. https://doi.org/10.1016/j.jchromb.2010.04.024.

Jain KK. Biomarkers and Regulatory Issues. In: The Handbook of Biomarkers. New York, NY: Humana Press; 2017. pp. 713–731. https://doi.org/10.1007/978-1-4939-7431-3_19.

Karhson DS, Krasinska KM, Dallaire JA, Libove RA, Phillips JM, Chien AS, Garner JP, Hardan AY, Parker KJ. Plasma anandamide concentrations are lower in children with autism spectrum disorder. Mol Autism. 2018;9:18. https://doi.org/10.1186/s13229-018-0203-y.

Kendall DA, Yudowski GA. Cannabinoid receptors in the central nervous system: their signaling and roles in disease. Front Cell Neurosci. 2017;10:294. https://doi.org/10.3389/fncel.2016.00294.

Kerr DM, Gilmartin A, Roche M. Pharmacological inhibition of fatty acid amide hydrolase attenuates social behavioural deficits in male rats prenatally exposed to valproic acid. Pharmacol Res. 2016;113:228–35. https://doi.org/10.1016/j.phrs.2016.08.033.

Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, Rush AJ, Walters EE, Wang PS. The epidemiology of major depressive DisorderResults from the National Comorbidity Survey Replication (NCS-R). JAMA. 2003;289(23):3095–105. https://doi.org/10.1001/jama.289.23.3095.

Kessler RC, Aguilar-Gaxiola S, Alonso J, Benjet C, Bromet EJ, Cardoso G, Degenhardt L, de Girolamo G, Dinolova RV, Ferry F, Florescu S, Gureje O, Haro JM, Huang Y, Karam EG, Kawakami N, Lee S, Lepine J-P, Levinson D, Koenen KC. Trauma and PTSD in the WHO world mental health surveys. Eur J Psychotraumatol. 2017;8(sup5):1353383. https://doi.org/10.1080/20008198.2017.1353383.

Kirkham TC, Williams CM, Fezza F, Di Marzo V. Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. Br J Pharmacol. 2002;136(4):550–7. https://doi.org/10.1038/sj.bjp.0704767.

Kolla NJ, Mizrahi R, Karas K, Wang C, Bagby RM, McMain S, Simpson AI, Rusjan PM, Tyndale R, Houle S, Boileau I. Elevated fatty acid amide hydrolase in the prefrontal cortex of borderline personality disorder: a [11C]CURB positron emission tomography study. Neuropsychopharmacology. 2020;45(11):1834–41. https://doi.org/10.1038/s41386-020-0731-y.

Kratz D, Thomas D, Gurke R. Endocannabinoids as potential biomarkers: it's all about pre-analytics. J Mass Spect Adv Clin Lab. 2021;22:56–63. https://doi.org/10.1016/j.jmsacl.2021.11.001.

Kroll SL, Meier P, Mayo LM, Gertsch J, Quednow BB. Endocannabinoids and related lipids linked to social exclusion in individuals with chronic non-medical prescription opioid use. Neuropsychopharmacology. 2024;49(10):1630–9. https://doi.org/10.1038/s41386-024-01881-8.

Lazary J, Eszlari N, Juhasz G, Bagdy G. Genetically reduced FAAH activity may be a risk for the development of anxiety and depression in persons with repetitive childhood trauma. Eur Neuropsychopharmacol. 2016;26(6):1020–8. https://doi.org/10.1016/j.euroneuro.2016.03.003.

Lester KJ, Coleman JRI, Roberts S, Keers R, Breen G, Bögels S, Creswell C, Hudson JL, McKinnon A, Nauta M, Rapee RM, Schneider S, Silverman WK, Thastum M, Waite P, Wergeland GJH, Eley TC. Genetic variation in the endocannabinoid system and response to cognitive behavior therapy for child anxiety disorders. Am J Med Genet. 2017;174(2):144–55. https://doi.org/10.1002/ajmg.b.32467.

Leweke FM, Giuffrida A, Wurster U, Emrich HM, Piomelli D. Elevated endogenous cannabinoids in schizophrenia. Neuroreport. 1999;10(8):1665.
Lin H-C, Mao S-C, Gean P-W. Effects of intra-amygdala infusion of CB1 receptor agonists on the reconsolidation of fear-potentiated startle. Learn Mem. 2006;13(3):316–21. https://doi.org/10.1101/lm.217006.
Lin H-C, Mao S-C, Su C-L, Gean P-W. The role of prefrontal cortex CB1 receptors in the modulation of fear memory. Cereb Cortex. 2009;19(1):165–75. https://doi.org/10.1093/cercor/bhn075.
Long LE, Lind J, Webster M, Weickert CS. Developmental trajectory of the endocannabinoid system in human dorsolateral prefrontal cortex. BMC Neurosci. 2012;13(1):87. https://doi.org/10.1186/1471-2202-13-87.
Lopez-Cortes OD, Trujillo-Sánchez F, Sierra-Ruelas E, Martinez-Lopez E, Di Marzo V, Vizmanos B. Association between the FAAH C385A variant (rs324420) and obesity-related traits: a systematic review. Int J Obes. 2024;48(2):188–201. https://doi.org/10.1038/s41366-023-01428-9.
Lutz B. Neurobiology of cannabinoid receptor signaling. Dialog Clin Neurosci. 2020;22(3):207–22. https://doi.org/10.31887/DCNS.2020.22.3/blutz.
Maccarrone M, Finazzi-Agró A. The endocannabinoid system, anandamide and the regulation of mammalian cell apoptosis. Cell Death Diff. 2003;10(9):946–55. https://doi.org/10.1038/sj.cdd.4401284.
Mackie K. Cannabinoid receptors: where they are and what they do. J Neuroendocrinol. 2008;20(s1):10–4. https://doi.org/10.1111/j.1365-2826.2008.01671.x.
Malone DT, Hill MN, Rubino T. Adolescent cannabis use and psychosis: epidemiology and neurodevelopmental models. Br J Pharmacol. 2010;160(3):511–22. https://doi.org/10.1111/j.1476-5381.2010.00721.x.
Maple KE, McDaniel KA, Shollenbarger SG, Lisdahl KM. Dose-dependent cannabis use, depressive symptoms, and FAAH genotype predict sleep quality in emerging adults: a pilot study. Am J Drug Alcohol Abuse. 2016;42(4):431–40. https://doi.org/10.3109/00952990.2016.1141913.
Marusak HA, Evanski J, Desai S, Rabinak CA. Impact of childhood trauma exposure, genetic variation in endocannabinoid signaling, and anxiety on Frontolimbic pathways in children. Cannabis Cannabinoid Res. 2023;8(6):1079–89. https://doi.org/10.1089/can.2022.0144.
Martínez-Rodríguez TY, Valdés-Miramontes EH, Muñoz-Valle JF, Reyes-Castillo Z. Genetic Evidence of Endocannabinoid System on Perceived Stress and Restricted Food Intake: The Role of Variants rs324420 in FAAH Gene and rs1049353 in CNR1 Gene. Cannabis Cannabinoid Res. 2024. https://doi.org/10.1089/can.2024.0077.
Marx W, Penninx BWJH, Solmi M, Furukawa TA, Firth J, Carvalho AF, Berk M. Major depressive disorder. Nat Rev Dis Prim. 2023;9(1):1–21. https://doi.org/10.1038/s41572-023-00454-1.
Mayo LM, Asratian A, Lindé J, Holm L, Nätt D, Augier G, Stensson N, Vecchiarelli HA, Balsevich G, Aukema RJ, Ghafouri B, Spagnolo PA, Lee FS, Hill MN, Heilig M. Protective effects of elevated anandamide on stress and fear-related behaviors: translational evidence from humans and mice. Mol Psychiatry. 2020a;25(5):993–1005. https://doi.org/10.1038/s41380-018-0215-1.
Mayo LM, Asratian A, Lindé J, Holm L, Nätt D, Augier G, Stensson N, Vecchiarelli HA, Balsevich G, Aukema RJ, Ghafouri B, Spagnolo PA, Lee FS, Hill MN, Heilig M. Protective effects of elevated anandamide on stress and fear-related behaviors: translational evidence from humans and mice. Mol Psychiatry. 2020b;25(5):993–1005. https://doi.org/10.1038/s41380-018-0215-1.
Mayo LM, Rabinak CA, Hill MN, Heilig M. Targeting the endocannabinoid system in the treatment of posttraumatic stress disorder: a promising case of preclinical-clinical translation? Biol Psychiatry. 2022;91(3):262–72. https://doi.org/10.1016/j.biopsych.2021.07.019.
Mazier W, Saucisse N, Gatta-Cherifi B, Cota D. The endocannabinoid system: pivotal orchestrator of obesity and metabolic disease. Trend Endocrinol Metab. 2015;26(10):524–37. https://doi.org/10.1016/j.tem.2015.07.007.
McCutcheon RA, Reis Marques T, Howes OD. Schizophrenia-An Overview. *JAMA*. Psychiatry. 2020;77(2):201–10. https://doi.org/10.1001/jamapsychiatry.2019.3360.

Meyer HC, Lee FS, Gee DG. The role of the endocannabinoid system and genetic variation in adolescent brain development. Neuropsychopharmacology. 2018;43(1):21–33. https://doi.org/10.1038/npp.2017.143.

Mitchell JE, Peterson CB. Anorexia Nervosa. N Engl J Med. 2020;382(14):1343–51. https://doi.org/10.1056/NEJMcp1803175.

Monteleone P, Matias I, Martiadis V, De Petrocellis L, Maj M, Di Marzo V. Blood levels of the endocannabinoid anandamide are increased in anorexia nervosa and in binge-eating disorder, but not in bulimia nervosa. Neuropsychopharmacology. 2005;30(6):1216–21. https://doi.org/10.1038/sj.npp.1300695.

Monteleone P, Tortorella A, Martiadis V, Di Filippo C, Canestrelli B, Maj M. The cDNA 385C to a missense polymorphism of the endocannabinoid degrading enzyme fatty acid amide hydrolase (FAAH) is associated with overweight/obesity but not with binge eating disorder in overweight/obese women. Psychoneuroendocrinology. 2008;33(4):546–50. https://doi.org/10.1016/j.psyneuen.2008.01.004.

Monteleone P, Bifulco M, Di Filippo C, Gazzerro P, Canestrelli B, Monteleone F, Proto MC, Di Genio M, Grimaldi C, Maj M. Association of CNR1 and FAAH endocannabinoid gene polymorphisms with anorexia nervosa and bulimia nervosa: evidence for synergistic effects. Genes Brain Behav. 2009;8(7):728–32. https://doi.org/10.1111/j.1601-183X.2009.00518.x.

Monteleone P, Bifulco M, Maina G, Tortorella A, Gazzerro P, Proto MC, Di Filippo C, Monteleone F, Canestrelli B, Buonerba G, Bogetto F, Maj M. Investigation of *CNR1* and *FAAH* endocannabinoid gene polymorphisms in bipolar disorder and major depression. Pharmacol Res. 2010;61(5):400–4. https://doi.org/10.1016/j.phrs.2010.01.002.

Moreira FA, Kaiser N, Monory K, Lutz B. Reduced anxiety-like behaviour induced by genetic and pharmacological inhibition of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) is mediated by CB1 receptors. Neuropharmacology. 2008;54(1):141–50. https://doi.org/10.1016/j.neuropharm.2007.07.005.

Morena M, Roozendaal B, Trezza V, Ratano P, Peloso A, Hauer D, Atsak P, Trabace L, Cuomo V, McGaugh JL, Schelling G, Campolongo P. Endogenous cannabinoid release within prefrontal-limbic pathways affects memory consolidation of emotional training. Proc Natl Acad Sci. 2014;111(51):18333–8. https://doi.org/10.1073/pnas.1420285111.

Morita Y, Ujike H, Tanaka Y, Uchida N, Nomura A, Ohtani K, Kishimoto M, Morio A, Imamura T, Sakai A, Inada T, Harano M, Komiyama T, Yamada M, Sekine Y, Iwata N, Iyo M, Sora I, Ozaki N, Kuroda S. A nonsynonymous polymorphism in the human fatty acid amide hydrolase gene did not associate with either methamphetamine dependence or schizophrenia. Neurosci Lett. 2005;376(3):182–7. https://doi.org/10.1016/j.neulet.2004.11.050.

Myhr A, Vesterbekkmo RK, Samarawickrema I, Sund ER. Trends in Norwegian adolescents' substance use between 2014 and 2022: socioeconomic and gender differences. BMC Public Health. 2024;24(1):2482. https://doi.org/10.1186/s12889-024-19983-9.

Neumeyer AM, Anixt J, Chan J, Perrin JM, Murray D, Coury DL, Bennett A, Farmer J, Parker RA. Identifying associations among co-occurring medical conditions in children with autism Spectrum disorders. Acad Pediatr. 2019;19(3):300–6. https://doi.org/10.1016/j.acap.2018.06.014.

Ney LJ, Matthews A, Hsu C-MK, Zuj DV, Nicholson E, Steward T, Nichols D, Graham B, Harrison B, Bruno R, Felmingham K. Cannabinoid polymorphisms interact with plasma endocannabinoid levels to predict fear extinction learning. Depress Anxiety. 2021;38(10):1087–99. https://doi.org/10.1002/da.23170.

Ney LJ, Crombie KM, Mayo LM, Felmingham KL, Bowser T, Matthews A. Translation of animal endocannabinoid models of PTSD mechanisms to humans: where to next? Neurosci Biobehav Rev. 2022;132:76–91. https://doi.org/10.1016/j.neubiorev.2021.11.040.

Ney LJ, Cooper J, Lam GN, Moffitt K, Nichols DS, Mayo LM, Lipp OV. Hair endocannabinoids predict physiological fear conditioning and salivary endocannabinoids predict subjective stress reactivity in humans. Psychoneuroendocrinology. 2023;154:106296. https://doi.org/10.1016/j.psyneuen.2023.106296.

Olson IR, Heide RJVD, Alm KH, Vyas G. Development of the uncinate fasciculus: implications for theory and developmental disorders. Dev Cogn Neurosci. 2015;14:50–61. https://doi.org/10.1016/j.dcn.2015.06.003.

Owens MJ, Nemeroff CB. Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. Clin Chem. 1994;40(2):288–95. https://doi.org/10.1093/clinchem/40.2.288.

Palazuelos J, Aguado T, Pazos MR, Julien B, Carrasco C, Resel E, Sagredo O, Benito C, Romero J, Azcoitia I, Fernández-Ruiz J, Guzmán M, Galve-Roperh I. Microglial CB2 cannabinoid receptors are neuroprotective in Huntington's disease excitotoxicity. Brain. 2009;132(11):3152–64. https://doi.org/10.1093/brain/awp239.

Pamplona FA, Prediger RDS, Pandolfo P, Takahashi RN. The cannabinoid receptor agonist WIN 55,212-2 facilitates the extinction of contextual fear memory and spatial memory in rats. Psychopharmacology. 2006;188(4):641–9. https://doi.org/10.1007/s00213-006-0514-0.

Pedersen TL, Gray IJ, Newman JW. Plasma and serum oxylipin, endocannabinoid, bile acid, steroid, fatty acid and nonsteroidal anti-inflammatory drug quantification in a 96-well plate format. Anal Chim Acta. 2021;1143:189–200. https://doi.org/10.1016/j.aca.2020.11.019.

Penninx BW, Pine DS, Holmes EA, Reif A. Anxiety disorders. Lancet (London, England). 2021;397(10277):914–27. https://doi.org/10.1016/S0140-6736(21)00359-7.

Petrie GN, Nastase AS, Aukema RJ, Hill MN. Endocannabinoids, cannabinoids and the regulation of anxiety. Neuropharmacology. 2021;195:108626. https://doi.org/10.1016/j.neuropharm.2021.108626.

Pinna G. Biomarkers and treatments for mood disorders encompassing the neurosteroid and endocannabinoid systems. J Neuroendocrinol. 2023;35(2):e13226. https://doi.org/10.1111/jne.13226.

Potvin S, Kouassi É, Lipp O, Bouchard R-H, Roy M-A, Demers M-F, Gendron A, Astarita G, Piomelli D, Stip E. Endogenous cannabinoids in patients with schizophrenia and substance use disorder during quetiapine therapy. J Psychopharmacol. 2008;22:262. Accessed 29 Aug 2024. https://doi.org/10.1177/0269881107083816.

Pujol J, Blanco-Hinojo L, Macia D, Martínez-Vilavella G, Deus J, Pérez-Sola V, Cardoner N, Soriano-Mas C, Sunyer J. Differences between the child and adult brain in the local functional structure of the cerebral cortex. NeuroImage. 2021;237:118150. https://doi.org/10.1016/j.neuroimage.2021.118150.

Raichlen DA, Foster AD, Seillier A, Giuffrida A, Gerdeman GL. Exercise-induced endocannabinoid signaling is modulated by intensity. Eur J Appl Physiol. 2013;113(4):869–75. https://doi.org/10.1007/s00421-012-2495-5.

Schacht JP, Selling RE, Hutchison KE. Intermediate cannabis dependence phenotypes and the FAAH C385A variant: An exploratory analysis. Psychopharmacology. 2009;203(3):511–7. https://doi.org/10.1007/s00213-008-1397-z.

Schmidt ME, Liebowitz MR, Stein MB, Grunfeld J, Van Hove I, Simmons WK, Van Der Ark P, Palmer JA, Saad ZS, Pemberton DJ, Van Nueten L, Drevets WC. The effects of inhibition of fatty acid amide hydrolase (FAAH) by JNJ-42165279 in social anxiety disorder: a double-blind, randomized, placebo-controlled proof-of-concept study. Neuropsychopharmacology. 2021;46(5):1004–10. https://doi.org/10.1038/s41386-020-00888-1.

Senova S, Fomenko A, Gondard E, Lozano AM. Anatomy and function of the fornix in the context of its potential as a therapeutic target. J Neurol Neurosurg Psychiatry. 2020;91(5):547–59. https://doi.org/10.1136/jnnp-2019-322375.

Servadio M, Melancia F, Manduca A, di Masi A, Schiavi S, Cartocci V, Pallottini V, Campolongo P, Ascenzi P, Trezza V. Targeting anandamide metabolism rescues core and associated autistic-like symptoms in rats prenatally exposed to valproic acid. Transl Psychiatry. 2016;6(9):e902. https://doi.org/10.1038/tp.2016.182.

Shollenbarger SG, Price J, Wieser J, Lisdahl K. Poorer frontolimbic white matter integrity is associated with chronic cannabis use, *FAAH* genotype, and increased depressive and apathy symptoms in adolescents and young adults. NeuroImage. 2015;8:117–25. https://doi.org/10.1016/j.nicl.2015.03.024.

Simone JJ, Green MR, Hodges TE, McCormick CM. Differential effects of CB1 receptor agonism in behavioural tests of unconditioned and conditioned fear in adult male rats. Behav Brain Res. 2015;279:9–16. https://doi.org/10.1016/j.bbr.2014.11.012.

Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc Natl Acad Sci USA. 2002;99(12):8394–9. https://doi.org/10.1073/pnas.082235799.

Sipe JC, Waalen J, Gerber A, Beutler E. Overweight and obesity associated with a missense polymorphism in fatty acid amide hydrolase (FAAH). Int J Obes. 2005;29(7):755–9. https://doi.org/10.1038/sj.ijo.0802954.

Sisk LM, Rapuano KM, Conley MI, Greene AS, Horien C, Rosenberg MD, Scheinost D, Constable RT, Glatt CE, Casey BJ, Gee DG. Genetic variation in endocannabinoid signaling is associated with differential network-level functional connectivity in youth. J Neurosci Res. 2022;100(3):731–43. https://doi.org/10.1002/jnr.24946.

Skaper SD, Di Marzo V. Endocannabinoids in nervous system health and disease: the big picture in a nutshell. Philos Trans Royal Society B: Biol Sci. 2012;367(1607):3193–200. https://doi.org/10.1098/rstb.2012.0313.

Sloan ME, Gowin JL, Yan J, Schwandt ML, Spagnolo PA, Sun H, Hodgkinson CA, Goldman D, Ramchandani VA. Severity of alcohol dependence is associated with the fatty acid amide hydrolase Pro129Thr missense variant. Addict Biol. 2018;23(1):474–84. https://doi.org/10.1111/adb.12491.

Soria-Gómez E, Matias I, Rueda-Orozco PE, Cisneros M, Petrosino S, Navarro L, Di Marzo V, Prospéro-García O. Pharmacological enhancement of the endocannabinoid system in the nucleus accumbens shell stimulates food intake and increases c-Fos expression in the hypothalamus. Br J Pharmacol. 2007;151(7):1109–16. https://doi.org/10.1038/sj.bjp.0707313.

Spohrs J, Ulrich M, Grön G, Prost M, Plener PL, Fegert JM, Bindila L, Abler B. Fear extinction learning and anandamide: An fMRI study in healthy humans. Transl Psychiatry. 2021;11:161. https://doi.org/10.1038/s41398-020-01177-7.

Spohrs J, Kühnle V, Mikusky D, Sanhüter N, Macchia A, Nickel S, Abler B. Plasma endocannabinoid levels in patients with borderline personality disorder and healthy controls. Int J Mol Sci. 2023;24(24):17452. https://doi.org/10.3390/ijms242417452.

Spagnolo PA, Ramchandani VA, Schwandt ML, Kwako LE, George DT, Hillard CJ, Heilig M. FAAH gene variation moderates stress response and symptom severity in patients with post-traumatic stress disorder and comorbid alcohol dependence. Alcohol Clin Exp Res. 2016;40(11):2426–34. https://doi.org/10.1111/acer.13210.

Starzer MSK, Nordentoft M, Hjorthøj C. Rates and predictors of conversion to schizophrenia or bipolar disorder following substance-induced psychosis. Am J Psychiatry. 2018;175(4):343–50. https://doi.org/10.1176/appi.ajp.2017.17020223.

Turcotte C, Blanchet M-R, Laviolette M, Flamand N. The CB2 receptor and its role as a regulator of inflammation. Cell Mol Life Sci. 2016;73(23):4449–70. https://doi.org/10.1007/s00018-016-2300-4.

Tyndale RF, Payne JI, Gerber AL, Sipe JC. The fatty acid amide hydrolase C385A (P129T) missense variant in cannabis users: studies of drug use and dependence in caucasians. Am J Med Genet B Neuropsychiatr Genet. 2007;144B(5):660–6. https://doi.org/10.1002/ajmg.b.30491.

Vogeser M, Schelling G. Pitfalls in measuring the endocannabinoid 2-arachidonoyl glycerol in biological samples. Clin Chem Lab Med. 2007;45(8):1023–5. https://doi.org/10.1515/CCLM.2007.197.

Vogeser M, Hauer D, Azad SC, Huber E, Storr M, Schelling G. Release of anandamide from blood cells. Clin Chem Lab Med (CCLM). 2006;44(4):488–91. https://doi.org/10.1515/CCLM.2006.065.

Wang M, Hill MN, Zhang L, Gorzalka BB, Hillard CJ, Alger BE. Acute restraint stress enhances hippocampal endocannabinoid function via glucocorticoid receptor activation. J Psychopharmacol. 2012;26(1):56–70. https://doi.org/10.1177/0269881111409606.

Webb EK, Stevens JS, Ely TD, Lebois LAM, van Rooij SJH, Bruce SE, House SL, Beaudoin FL, An X, Neylan TC, Clifford GD, Linnstaedt SD, Germine LT, Bollen KA, Rauch SL, Haran JP, Storrow AB, Lewandowski C, Musey PI, Harnett NG. Neighborhood resources associated with psychological trajectories and neural reactivity to reward after trauma. JAMA Psychiat. 2024;81:1090. https://doi.org/10.1001/jamapsychiatry.2024.2148.

Wei D, Dinh D, Lee D, Li D, Anguren A, Moreno-Sanz G, Gall CM, Piomelli D. Enhancement of anandamide-mediated endocannabinoid signaling corrects autism-related social impairment. Cannabis Cannabinoid Res. 2016;1(1):81–9. https://doi.org/10.1089/can.2015.0008.

Wei D, Allsop S, Tye K, Piomelli D. Endocannabinoid signaling in the control of social behavior. Trends Neurosci. 2017;40(7):385–96. https://doi.org/10.1016/j.tins.2017.04.005.

Yagin NL, Aliasgari F, Alizadeh M, Aliasgharzadeh S, Mahdavi R. Comparison of endocannabinoids levels, FAAH gene polymorphisms, and appetite regulatory substances in women with and without binge eating disorder: a cross-sectional study. Nutr Res. 2020;83:86–93. https://doi.org/10.1016/j.nutres.2020.09.001.

Yasmin F, Colangeli R, Morena M, Filipski S, van der Stelt M, Pittman QJ, Hillard CJ, Teskey GC, McEwen BS, Hill MN, Chattarji S. Stress-induced modulation of endocannabinoid signaling leads to delayed strengthening of synaptic connectivity in the amygdala. Proc Natl Acad Sci. 2020;117(1):650–5. https://doi.org/10.1073/pnas.1910322116.

Zamberletti E, Rubino T. Impact of endocannabinoid system manipulation on neurodevelopmental processes relevant to schizophrenia. Biol Psychiatr Cognit Neurosci Neuroimag. 2021;6(6):616–26. https://doi.org/10.1016/j.bpsc.2020.06.013.

Zhang W, Liu H, Deng X-D, Ma Y, Liu Y. FAAH levels and its genetic polymorphism association with susceptibility to methamphetamine dependence. Ann Hum Genet. 2020;84(3):259–70. https://doi.org/10.1111/ahg.12368.

Zou M, Liu Y, Xie S, Wang L, Li D, Li L, Wang F, Zhang Y, Xia W, Sun C, Wu L. Alterations of the endocannabinoid system and its therapeutic potential in autism spectrum disorder. Open Biol. n.d.;11(2):200306. https://doi.org/10.1098/rsob.200306.

Chapter 16
Cannabis Use and Psychiatric Symptoms in Adolescence



Meghan A. Costello, Randi M. Schuster, Jodi M. Gilman, and A. Eden Evins

1 Introduction

Adolescence is a period of profound growth and exploration, marked by dynamic changes in emotional and psychological functioning (Uhlhaas et al. 2023. Growing evidence indicates that rates of adolescent distress have continued to rise, with adolescents reporting multifold increases in mental health symptom prevalence and severity over the past decade (Ahn-Horst and Bourgeois 2024; Benton et al. 2021; Racine et al. 2021). The mean age of onset for any psychiatric condition is around 14.5 years of age, and symptoms in adolescence may be vital indicators of long-term mental illness (Solmi et al. 2022). Amid this pressing youth mental health crisis, adolescent cannabis use has emerged as a topic of concern, and its long-term impacts have become a focus of ongoing research (Gobbi et al. 2019). This chapter seeks to highlight that adolescent cannabis use is a public health concern that must also be considered as an indicator of other, less visible mental health symptoms in adolescence. This chapter begins by briefly describing the landscape of adolescent cannabis use and explaining the importance of considering mental health comorbidities associated with cannabis use. The second section then describes existing evidence regarding links from cannabis use to comorbid symptoms of five categories of major mental health concerns for adolescents: psychotic symptoms, mood symptoms, anxious symptoms, neurodevelopmental symptoms, and other substance use. The chapter concludes by suggesting future directions in intervention programming and research to promote adolescent public mental health.

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1.1 Adolescent Cannabis Use Is Prevalent

It is estimated that 45% of adolescents have used cannabis at least once by age 18 (Fischer et al. 2020), making it one of the most-used drugs by adolescence across the globe (Godin and Shehata 2022). Major health organizations such as the American Academy of Child and Adolescent Psychiatry (2017) and the American Society of Addiction Medicine (2020) have expressed concerns about youth's increased access to cannabis as a result of legalization and widespread commercialization of cannabis products. As cannabis is commercialized, new products are available that are more potent, easier to conceal, and often designed to appeal to young people (D'Souza et al. 2022; Wilson et al. 2019). Not only does legalization and commercialization facilitate access to cannabis, but it may also falsely communicate to adolescents that the effects of cannabis use are understood and deemed to be safe (Miech et al. 2024a, b). In truth, the long-term impacts of cannabis use on adolescent physical and psychological development are unknown (and particularly, the effect of rapidly growing potency is unknown), although this is an area of ongoing research. Despite preliminary conclusions from this work indicating harmful impacts of early cannabis use, the proportion of adolescents who believe cannabis use is harmful is decreasing in the US and Europe (Miech et al. 2024a, b). Given adolescents' susceptibility to influence and sensitivity to social risk and reward (Volkow et al. 2017), they may be particularly vulnerable to messages that suggest cannabis use is neutral or positive, whether via targeted marketing (Hall et al. 2019) or social exposure (Kosterman et al. 2000; Hasin et al. 2019).

Earlier initiation of cannabis use in adolescence is associated with significant lifelong challenges with addiction, and each year that cannabis initiation is delayed reduces the odds of developing a cannabis use disorder by 14% (Millar et al. 2021). Among adolescents who use cannabis, one in six qualify for a cannabis use disorder diagnosis, and cannabis use disorder is the most prevalent problem in specialized substance treatment centers that serve adolescents (Chandra et al. 2019). Adolescents who use cannabis are more than three times more likely to develop severe cannabis use disorder than adult cannabis users (Lawn et al. 2022; see Chap. 13 for a comprehensive discussion of adolescent cannabis use disorder). Use in adolescence, and particularly early use, appears to be a major risk factor for long-term cannabis dependence.

1.2 Adolescent Cannabis Use and Coping Motives

Many adolescents who use cannabis report that they do so in an attempt to cope with negative emotions (Knapp et al. 2021). Using a substance as a coping strategy is highly reinforcing for many individuals, as it provides acute, predictable changes in mood state and physiological experiences (Volkow et al. 2017). Although cannabis may provide short-term relief from a negative mood state, if adolescents use

cannabis to avoid their own negative emotions repeatedly over time, they may struggle to develop alternative coping strategies and ultimately limit their ability to tolerate negative mood states in the future (Rusby et al. 2019). Furthermore, when adolescents attempt to quit cannabis use, they may experience common emotional and uncomfortable side effects of withdrawal, including dysphoria, irritability, and anxiety (Connor et al. 2022). If adolescents who use cannabis to cope with negative emotions experience a rebound of emotional symptoms when they attempt to stop, they may be more likely to use cannabis again in the future (Stiles-Shields et al. 2021). In addition, previous work has also pointed to significant social consequences associated with heavy cannabis use, including peer disconnection and rejection that could intensify both use and mood symptoms (Cyrus et al. 2021). Therefore, although cannabis use may offer a short-term, instrumental "solution" to a negative mood state, it has been shown to exacerbate isolation, distress, and restricted coping strategies in the long-term (Knapp et al. 2021). Not only does cannabis use put adolescents at risk for developing problems with cannabis addiction, it is also associated with a host of emotional and psychological symptoms.

2 Psychological Symptoms Associated with Cannabis Use in Adolescence

The next section of this chapter will describe psychological symptoms, including psychotic symptoms, mood symptoms, anxious symptoms, neurodevelopmental symptoms, and other substance use, and explore their links to cannabis use in adolescence. Each of the discussed symptoms has been dose-dependently linked to adolescent cannabis use, such that a higher level of use is associated with more severe concurrent psychiatric symptoms, as well as more severe mental health progression in adulthood (Levine et al. 2017; Tervo-Clemmens et al. 2024).

2.1 Adolescent Cannabis Use and Psychotic Symptoms

Psychotic disorders (called "Schizophrenia spectrum and other psychotic disorders" in the DSM-5 and DSM-5-TR), describe a class of psychological conditions that involve abnormalities in one or more of the five following domains: delusions, hallucinations, disorganized thinking and speech, grossly disorganized or abnormal motor behavior (including catatonia), and negative symptoms (Heckers et al. 2013). Full-syndrome psychotic disorders often do not fully manifest until adulthood, although prodromal symptoms often begin during adolescence (Chang et al. 2017). However, subthreshold psychotic symptoms are *most* common in adolescence compared to all other age groups, with prevalence rates of up to 16% (Giocondo et al. 2021; Kelleher et al. 2012). Experiencing psychotic symptoms in adolescence has

been linked to a multifold increase in the likelihood of developing any psychiatric disorder in adulthood (Fisher et al. 2013; McGrath et al. 2016) and places adolescents at clinically high risk for psychosis (Woods et al. 2018). Psychotic disorders are relatively rare in the general population, with a lifetime prevalence of about 1% (Taylor and Huque 2021). In contrast, about 20% of adolescents at clinically high risk for psychosis eventually develop a psychotic disorder (Taylor and Huque 2021). Therefore, identifying adolescents experiencing psychotic-like symptoms could offer a significant opportunity to support a group that is highly likely to eventually progress to more significant mental health needs than their peers.

The association between psychotic symptoms and cannabis use has been an area of study for decades (Andréasson et al. 1987; Hasan et al. 2020; Lawn et al. 2022). There is compelling evidence that cannabis use often precedes the onset of psychotic symptoms and that cannabis exposure in adolescence is a significant predictor of these symptoms in adulthood (Kuepper et al. 2011; Bourque et al. 2018). Adolescents are particularly at risk for developing long-term, severe symptoms of psychosis when they initiate cannabis use earlier, use cannabis more often, and use higher-potency cannabis products (Di Forti et al. 2015; Godin and Shehata 2022; Leadbeater et al. 2019; Marconi et al. 2016; Myran et al. 2023; Schubart et al. 2011). Of note, the potency of the principal psychoactive constituent in cannabis products, delta-9-tetrahydrocannabidiol (THC), has increased dramatically over the past decade (Wilson et al. 2019). Early investigation into the downstream impacts of this potency change indicates that psychosis is more prevalent in areas with access to the highest-potency cannabis, and that 30–50% of new psychosis cases could be prevented if individuals abstained from using high-potency cannabis (Di Forti et al. 2019; Murray and Hall 2020). The rise in potency may further amplify the risk of any acute cannabis exposure for an adolescent with underlying genetic predisposition for psychosis (Hines et al. 2024).

Cannabis exposure appears to be a particularly potent environmental factor in predicting the eventual development of psychotic symptoms among those at risk for or already experiencing symptoms (Athanassiou et al. 2021). Cannabis use in adolescence is associated with a shift in the course of psychotic symptoms: among individuals with psychosis, those who report using cannabis in adolescence systematically experienced earlier prodromal symptoms, earlier first episode of psychosis, and earlier diagnosis of schizophrenia (Veen et al. 2004). Using cannabis in adolescence is associated with more intensive hospitalization usage, including both longer and more frequent psychiatric hospital stays (Manrique-Garcia et al. 2014). It has been proposed that heritable differences in neurotransmission may be related to individual differences in short- and long-term response to THC, including risk for ultimately developing psychotic symptoms and schizophrenia (Van Winkel et al. 2011). For example, when administered THC in a laboratory study, individuals diagnosed with schizophrenia are much more likely than those without to experience psychotic symptoms, even despite being treated with antipsychotic medication (D'Souza et al. 2022). Further study is needed to understand the role of theorized neurotransmitter mechanisms, such as the interaction of increased dopamine

sensitivity in psychosis and the disruption of the dopamine system when using cannabis (Kuepper et al. 2011).

Genetic risk is known to be particularly relevant in the development of psychotic disorders, with research primarily suggesting that the combination of genetic risk and exposure to cannabis use could trigger substance-induced psychosis in a subset of individuals (Colizzi et al. 2015; Di Forti et al. 2015). These theories that cannabis use triggers psychotic symptoms for individuals with genetic risk, such as the diathesis-stress model of psychosis, have prompted ongoing work to understand these associations and identify which adolescents may be most at-risk (Batalla and Maat 2022; Kiburi et al. 2021; Ksir and Hart 2016). Polygenic risk for schizophrenia also predicts a portion of the variance in cannabis use, suggesting that there are both unique and shared risk components for psychosis and cannabis use (Power et al. 2014). Although the specifics of shared vs. distinct underlying genetic etiology are still uncertain, associations between cannabis use and psychotic symptoms are strong, and remain even after controlling for both genetic risk *and* environmental factors associated with cannabis use, including socioeconomic status (SES), other drug use, urbanicity, and prior psychiatric symptoms (Giordano et al. 2015; Marconi et al. 2016). Therefore, cannabis use does seem to be a meaningful antecedent to the development of psychotic symptoms.

2.2 Adolescent Cannabis Use and Mood Symptoms

The Center for Disease Control and Prevention (CDC) reports that in 2019, over one-third of adolescents reported feeling persistently sad or helpless and approximately one-fifth had experienced a major depressive episode (Bitsko 2022). Adolescents experiencing mood-related symptoms may notice sleep changes (either too much sleep or too little sleep), appetite changes (higher or lower appetite), loss of joy (anhedonia), hopelessness, tearfulness, reductions in motivation or activation, and suicidal ideation (Malgaroli et al. 2021). Rates of reported depressive symptoms have been steadily increasing among adolescents over the past twenty years (Keyes et al. 2019; Mojtabai et al. 2016; Shorey et al. 2022). Additional evidence from hospitals and emergency rooms indicates a concurrent, commensurate increase in self-harm and suicide-related injuries (Plemmons et al. 2018; Burstein et al. 2019), hospitalizations following self-harm behaviors (Mercado et al. 2017), and rates of adolescent suicide deaths (Twenge et al. 2018; Ruch et al. 2019). This evidence suggests that the increase in adolescents' reported mood symptoms does not simply reflect this age cohort's willingness to report symptoms (Twenge et al. 2021).

Rates of adolescent cannabis use have increased in parallel with depression symptoms over the past several decades (Pacek et al. 2020). Causal understanding of the links between mood symptoms and cannabis use is limited; it is possible that cannabis use causes increased mood symptoms, that mood symptoms lead to cannabis use, or that cannabis use and mood symptoms share a common third cause (Feingold and Weinstein 2021). Individuals with diagnosed mood disorders,

including major depressive disorder and bipolar disorder, report higher rates of cannabis use and are more frequently diagnosed with cannabis use disorder in comparison to the general population (Lucatch et al. 2018). This could prompt a vicious cycle, as cannabis use has been shown to exacerbate symptoms of depression (Langlois et al. 2021). Of note, when depression and cannabis use co-occur, they are each more likely to be severe than when each of the conditions occurs in isolation (Onaemo et al. 2021). Looking longer-term, cannabis use initiated in adolescence has arisen as particularly associated with increased risk for depression and suicidality both concurrently and across time (Schmidt et al. 2020); when adolescents use cannabis, they are more likely to develop mood disorders years into the future (Lev-Ran et al. 2014).

Understanding the biological and genetic underpinnings of mood disorders is complex, in part due to the large degree of heterogeneity in symptom presentation in this class of disorders (Forbes et al. 2024). Relatedly, this heterogeneity is also a factor in attempts to distill underlying genetic predictors of mood disorders and cannabis use. In genome-wide association studies, there appears to be a shared genetic predictor underlying the development of depressive symptoms, the tendency to use cannabis, and ultimate risk for cannabis use disorder (Carey et al. 2016; Hodgson et al. 2017; Sherva et al. 2016). Other genetics work suggests that cannabis use may increase the odds of ultimately developing depressive symptoms among those with genetic risk for mood disorders (Smolkina et al. 2017). Related investigations into the neurological underpinnings of the links between cannabis use and mood symptoms have highlighted the importance of the limbic system and prefrontal cortex in regulating emotion processing and decision-making (Hammond et al. 2022). These brain regions undergo significant developmental changes through adolescence and into young adulthood and, therefore, may be particularly relevant in the links between cannabis use and mood symptoms in adolescence (Fuhrmann et al. 2015). Other work has suggested that both cannabis use and depressive symptoms are related to changes in the production of the neurotransmitter serotonin and its receptors, proposing that reduced serotonin in those predisposed to mood-related symptoms may prompt attempts to instrumentally use cannabis for symptom reduction (De Gregorio et al. 2020). Work remains to better understand the neural and genetic underpinnings of both cannabis use and depression in isolation, as well as their potential reciprocal associations. In particular, researchers have called for more work that targets the effects of neurobiological development using objective measures of cannabis exposure, to clarify these associations (Fischer et al. 2020).

2.3 Adolescent Cannabis Use and Anxious Symptoms

Anxiety disorders are another of the most common mental health problems in the general population, and anxious symptoms often have their onset in adolescence (Lijster et al. 2017). Anxiety disorders are characterized by fear, dread, or

avoidance, sometimes of a particular target or experience, but not always (Craske et al. 2011). Studies have estimated that 10–20% of adolescents qualify for a diagnosis of an anxiety disorder at any given time (Finsaas et al. 2018; Steinsbekk and Wichstrøm 2018). Generalized anxiety symptoms appear to increase in adolescence and tend to relate to challenges with anxiety and anxious symptoms into adulthood (Steinsbekk and Wichstrøm 2018).

Anxious symptoms and cannabis use in adolescence have been repeatedly associated in previous work, in relation to both brief and chronic cannabis use (Stiles-Shields et al. 2021). Frequent cannabis use has been associated with higher anxious symptoms (Kaasbøll et al. 2018; Thompson et al. 2018) and a greater likelihood of meeting criteria for an anxiety disorder diagnosis (Degenhardt et al. 2013). Earlier cannabis use initiation has been linked to greater reports of concurrent and long-term anxious symptoms (Cloak et al. 2015), and more recent cannabis use is associated with greater anxious symptom lability (Rusby et al. 2019). Longitudinal dose-dependent associations have also been identified in adolescence, with more cannabis use associated with more intense and more frequent anxious symptoms up to ten years later (Duperrouzel et al. 2018; Meier et al. 2020). A follow-up longitudinal meta-analysis identified that cannabis use in adolescence is associated with a twofold increase in the risk of developing anxiety symptoms and disorders in adulthood (Lowe et al. 2024).

Work to discern causality in the link between anxious symptoms and cannabis use has suggested a bidirectional relationship, in which associations are both symptom- and substance-driven (Davis et al. 2022). Researchers have called for more work to unpack unique contributions of associated factors—such as timing, age, diagnosis, gender, amount used, severity of symptoms, and frequency of use—each of which moderates the association between early cannabis use and anxious symptoms (Degenhardt et al. 2013; Wilson et al. 2019). Looking prospectively, one study identified that early cannabis use is associated with more persistent anxious symptoms, while early levels of anxiety did not meaningfully predict later cannabis use (Duperrouzel et al. 2018). In contrast, other work has described self-reported negative associations among cannabis use and anxious symptoms. Several studies have hypothesized that high baseline anxiety may reduce propensity to use cannabis, perhaps through negative expectations about the consequences of use (Schmits et al. 2016; Schmits and Quertemont 2018). In other cases, cannabis has been associated with reported decreases in anxious symptoms, which could ultimately reinforce and lead to increases in cannabis use over time (Krygsman and Vaillancourt 2022). Some work on biochemical mechanisms has suggested that THC may influence regulation of stress and anxiety through its effects on cannabinoid receptors in adult samples (Hillard et al. 2012; Mechoulam and Parker 2013; Parsons and Hurd 2015), although more work is needed to understand these links in the adolescent brain (Devinsky et al. 2014).

2.4 Adolescent Cannabis Use and Neurodevelopmental Symptoms

Neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) often arise during childhood and adolescence, and typically persist as lifelong conditions (Thapar et al. 2017). Youth meeting criteria for ADHD demonstrate symptoms related to attention, regulation, social competence, and cognitive processing (Soler Artigas et al. 2020). Youth with ASD demonstrate symptoms including impairments in cognitive functioning, behavioral challenges, differences in social communication, and repetitive behaviors or fixed interests (Lever and Geurts 2016). These conditions are increasingly recognized in young people across the globe, prompting a call for increased assessment and supportive intervention for individuals who demonstrate neurodevelopmental symptoms (Gyllenberg et al. 2018). Adolescents with neurodevelopmental symptoms tend to have worse mental health outcomes, including greater service use and functional impairment, than their peers who do not have neurodevelopmental symptoms (Gallant et al. 2022).

Adolescents with ADHD are nearly eight times more likely to use cannabis than their peers without ADHD (Soler Artigas et al. 2020), and are at significantly higher risk for developing cannabis use disorder (Mitchell et al. 2016). Many adolescents with ADHD report that using cannabis alleviates either the symptoms of ADHD or the side effects of stimulant medication, putting them at risk for developing problematic patterns of use (Loflin et al. 2014). Separating the symptoms associated with cannabis use, ADHD, medical treatment for ADHD, and comorbidities of ADHD is challenging because of the degree of symptom overlap, including changes in behavior, socialization, cognition, and learning (Molinero and Hinckley 2023). Some work has suggested that there may be neural underpinnings for these links. For instance, cannabis use might exacerbate ADHD symptoms through its impact on the frontal cortical regions of the brain which facilitate executive functioning and contain dopamine reward p0thways (Notzon et al. 2016), all of which are still developing during adolescence (Fuhrmann et al. 2015). Others have noted that chronic cannabis use in adolescence can lead to memory impairment and degradation of executive functioning while use persists (Ferland and Hurd 2020). Changes in executive functioning and cognitive control could make stopping regular cannabis use challenging and may contribute to the high rates of relapse among adolescents with ADHD (Mitchell et al. 2016).

Research regarding the association between ASD and cannabis use is more limited (Adhia et al. 2020; Mottron and Bzdok 2020). A recent meta-analysis indicated that most (21 of 26) studies found that individuals with ASD are at increased risk for developing any substance use disorder (Haasbroek and Morojele 2022). Cannabis is the second most-commonly used substance among individuals with ASD (following alcohol), and it has been suggested that the increasing legality of cannabis may lead to disproportionate increases in use among youth with ASD (Kronenberg et al. 2015). In this study, the researchers note that social symptoms of ASD may serve as

a protective barrier to accessing illegal drugs, and increasing legalization could particularly increase risk for increases in cannabis use among adolescents with ASD (Kronenberg et al. 2015). Furthermore, teens with ASD face a high risk for loneliness and disconnection, which may amplify the risk for ultimately developing heavy or problematic use (Kronenberg et al. 2014). Ensuring that adolescents with ASD symptoms receive the information and support that they need to understand the role of cannabis in their lives may be key to ensuring both their short- and long-term mental health.

2.5 Adolescent Cannabis Use and Other Substance Use

Adolescents who use cannabis are at elevated risk for using other substances (Tomczyk et al. 2016). Using multiple substances in adolescence, while far more common than using only one substance (Tomczyk et al. 2016), is associated with poor mental health prognosis and a higher likelihood of progression to addiction across the lifespan (Gobbi et al. 2019; Hawke et al. 2018). When cannabis use is initiated earlier in adolescence, progression to polysubstance use and polysubstance addiction becomes an increasing concern (Richmond-Rakerd et al. 2017), and mental health symptomatology becomes much more likely and much more severe across diagnostic categories (Halladay et al. 2020).

The Common Liability to Addiction Model (CLA) suggests that the use of multiple substances is primarily driven by underlying common predispositions (such as genetic risk, context sensitivity, and behavioral tendencies) that make one more likely to use substances (Vanyukov et al. 2012). From this perspective, using cannabis reflects the intersection of behavioral and contextual factors that put an adolescent at risk for using other substances, as well. Researchers have suggested that a reciprocal, iterative association exists between mental health and substance use, such that substance use may contribute to mental health decline, which could lead to increased use of more substances and subsequently worsening mental health over time (Leyton and Stewart 2014). While broad, this highlights a tenet that is true for each disorder discussed in this chapter: cannabis use and mental health appear highly associated, and problems in each put an adolescent at risk for problems with the other.

2.6 Summary: Cannabis Use and Mental Health Symptoms

In sum, cannabis use is significantly associated with greater risk for a range of psychiatric diagnoses and symptoms in adolescence (Shanahan et al. 2021). Symptoms in this chapter were discussed within diagnostic categories for ease of reading and to follow the structure of previous research, although it is important to note that a transdiagnostic approach to assessment and treatment will likely serve adolescents

best, considering the degree of symptom overlap across mental health disorder diagnoses (Forbes et al. 2024). Across diagnostic categories, subthreshold symptoms experienced in adolescence may indicate risk for clinical syndromes across the lifespan (Uhlhaas et al. 2023). Mechanisms underlying the associations between cannabis use and psychopathology are broad, ranging from genetic to neural to social to behavioral (Kuhns et al. 2022). It is challenging to disentangle these theoretical mechanisms because they often underlie psychological functioning in a very global sense; for instance, psychiatric disorders across *all* diagnostic categories share genetic risk (Giangrande et al. 2022). Behavioral and social reinforcement patterns are also linked to these genetic and neural mechanisms and could perpetuate or reduce symptoms across diagnostic domains. In combination, extant work indicates that cannabis use and mental health symptoms in adolescence inter-relate.

3 Discussion

3.1 The Smoke, Not the Fire: Cannabis Use as an Indicator of Mental Health Need

Cannabis use does not occur in a vacuum, and an integrated approach to mental health intervention may support a more comprehensive and potent treatment approach (Vidal et al. 2024). Adolescents are reticent to seek mental health care, whether due to stigma about treatment, financial and logistical barriers to accessing treatment, mistrust of mental health professionals, or downplaying of symptoms (Radez et al. 2021). As a result, adolescents' mental health symptoms may be overlooked until those symptoms become severe or until they manifest in a way that is behaviorally disruptive or noticeable by adults in their lives. Symptoms that could easily be addressed with early treatment may escalate and establish lifelong problems (Uhlhaas et al. 2023), particularly if adults interpret behavioral markers of poor functioning, such as cannabis use, as an offense rather than an indicator of broader concerns.

Stated simply, "*Where there is smoke, there is fire.*" In this case, cannabis use may be "the smoke" that draws attention to underlying, less visible mental health symptoms ("the fire"). Mental health trajectories established in adolescence set the foundation for lifelong mental health functioning, and it is vital to intervene during this window of opportunity (Uhlhaas et al. 2023). Beyond disorders that rise above the clinical threshold, adolescents endorse significant amounts of sub-clinical distress (Costello 2016). For many of these adolescents, early identification and intervention could defray the onset of severe psychological symptoms that require a higher level of treatment (Solmi et al. 2022). Suppose only punitive responses are offered to the discovery of adolescent cannabis use. In that case, adults may miss the opportunity to understand and ultimately intervene on underlying distress that could persist and intensify over time. In contrast, if an adolescent's report of cannabis use

prompts further follow-up, adolescents' mental health symptoms can be better understood and then triaged to the appropriate level of care (Levy et al. 2020; Winn et al. 2019). Replacing punishment with mental health assessment and treatment may offer more traction in treatment and facilitate longer-term wellness while also indirectly intervening in cannabis use as a strategy for coping with psychological distress (Vidal et al. 2024). Beyond just connecting to treatment—and perhaps equally or more importantly—this approach could also promote a culture of openness and trust between adolescents and the adults in their lives. If disclosing cannabis use and mental health symptoms prompts support, adolescents may feel more empowered to discuss their symptoms with adults in the future.

3.2 Implications for Public Health and Clinical Practice

Previous approaches to psychological intervention have commonly advised tackling psychological symptoms only *after* substance use is treated, although more recent work has promoted integrated intervention (Vidal et al. 2024). In treatment, it is key to acknowledge the complex interplay between mental health symptoms and cannabis use, with the goal that tackling one domain of symptoms could facilitate improvement in the other domain as well (Hammond et al. 2020; Hser et al. 2017). Viewing the adolescent as a whole, understanding their constellation of symptoms, and offering a rehabilitative response stands to provide meaningful support for an adolescent who is using cannabis, particularly if that cannabis use escalates to a risky degree.

Toward the goal of collaborative and empowering intervention, developing a person-centered approach to assessment and treatment will be a crucial step in directly and indirectly addressing cannabis use and other mental health concerns (Vidal et al. 2024). Given the severity of the adolescent mental health crisis, a multifaceted approach to comprehensive mental health care is needed; one that encompasses identification, prevention, and intervention (Costello 2016). This approach might also benefit from a degree of flexibility and one that considers problems on a spectrum as equally important (rather than waiting for a mental health crisis). For example, the Screening, Brief Intervention, and Referral to Treatment (SBIRT) framework has been introduced to identify and triage adolescents based on the severity of substance use (Ozechowski et al. 2016). This model has been recommended for use in schools to assess and treat substance use and other mental health symptoms (Cohen et al. Under Review; Rahm et al. 2015). Considering the high degree of comorbidity among cannabis use and other mental health symptoms, screening and treatment that is regular, universal, and transdiagnostic may offer the best path forward (Deas 2006; Humphrey and Wigelsworth 2016; see Chap. 13 for a comprehensive discussion of adolescent cannabis use treatment).

Public policy support for such initiatives may be necessary to truly and comprehensively identify adolescents in need and triage them to various levels of care, including partnership with physical healthcare systems. Additional policy steps

could include establishing regular mandates for adolescent screening and providing direct resource, financial, and staff support to schools, which often shoulder the burden of adolescent mental health care, as has been enacted in the Massachusetts House Bill 4056: Substance Use Treatment, Education, and Prevention "STEP" Act, in March 2016.

3.3 Future Directions for Research

As policy changes are made in response to the staggering youth mental health crisis in the United States, it is key that research and evaluation keep pace with those changes to ensure that they are in the best interest of the adolescent, make efficient use of resources available, and remain sustainable and feasible to carry out for community partners. Programmatic work should continue to evaluate the effectiveness of mental health screenings prompted by cannabis use, identify challenges to partnering with health and school systems to intervene in problematic cannabis use and develop proactive prevention programming, and iteratively improve and reallocate efforts based on research and community feedback.

Research should also continue to explore these initial links between cannabis use and both concurrent and future mental health functioning. Much remains to be understood about the risk and protective factors that drive the development of cannabis use, mental health symptoms, and the interaction among them. As longitudinal studies expand, ongoing work can disentangle within-person development, sensitive periods, and inflection points that might lend themselves to intervention. As implementation scientists work to develop targeted interventions to mitigate cannabis use and mental health symptoms, those interventions will be best served if they are attuned to the latest innovations with regard to understanding mechanisms, trajectory, and risk in community samples of adolescents.

3.4 Summary and Conclusion

Cannabis use during adolescence is relatively common, and the impacts of relaxing legal restrictions, commercialization, and increasing potency remain unclear (Hall et al. 2019; Hammond et al. 2020). In addition to the risk of developing lifelong dependence on cannabis itself, adolescent cannabis use is associated with an overall increased risk for psychological symptoms (Keyes et al. 2019). Evidence suggests more frequent and potent use of cannabis is associated with more severe psychiatric symptoms (Tervo-Clemmens et al. 2024). Some mechanisms have been proposed for long-term maintenance of the psychological comorbidities associated with cannabis use in adolescence, including the disruption of neurophysiological and biological development (Albaugh et al. 2021; Hammond et al. 2022), reinforcement learning that precludes the intrapersonal need for coping strategy development

(Moreno-Mansilla et al. 2021), and negative social consequences resulting in loneliness and isolation (Levine et al. 2017). In the case of each of these symptoms, it is challenging to establish directionality (i.e., did cannabis use "cause" mental health challenges *or* did mental health challenges "cause" adolescents to use cannabis). Regardless of direction, cannabis use should be considered an indicator of underlying mental health symptoms. Mental health trajectories established in adolescence set the stage for mental health across the lifespan, suggesting that this sensitive developmental period may be a potent time for early prevention and intervention efforts (Uhlhaas et al. 2023). Therefore, it would be prudent to treat cannabis use as not simply a punishable behavior but rather a signal that there may be other psychiatric symptoms also in need of support or intervention.

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References

Adhia A, Bair-Merritt M, Broder-Fingert S, Nunez Pepen RA, Suarez-Rocha AC, Rothman EF. The critical lack of data on alcohol and marijuana use by adolescents on the autism spectrum. Autism Adulthood. 2020;2(4):282–8.

Ahn-Horst RY, Bourgeois FT. Mental health–related outpatient visits among adolescents and young adults, 2006–2019. JAMA Netw Open. 2024;7(3):e241468. https://doi.org/10.1001/jamanetworkopen.2024.1468.

Albaugh MD, Ottino-Gonzalez J, Sidwell A, Lepage C, Juliano A, Owens MM, IMAGEN Consortium. Association of cannabis use during adolescence with neurodevelopment. JAMA Psychiatry. 2021;78(9):1031–40.

American Academy of Child and Adolescent Psychiatry. Marijuana Legalization. Policy Statements. 2017. https://www.aacap.org/AACAP/Policy_Statements/2014/AACAP_Marijuana_Legalization_Policy.aspx. Accessed 9 July 2024.

American Society of Addiction Medicine. Public Policty Statement on Cannabis. 2020. https://www.asam.org/docs/default-source/public-policy-statements/2020-public-policy-statement-on-cannabis.pdf. Accessed 9 July 2024.

Andréasson S, Engström A, Allebeck P, et al. Cannabis and schizophrenia a longitudinal study of Swedish conscripts. Lancet. 1987;330:1483–6.

Athanassiou M, Dumais A, Gnanhoue G, Abdel-Baki A, Jutras-Aswad D, Potvin S. A systematic review of longitudinal studies investigating the impact of cannabis use in patients with psychotic disorders. Expert Rev Neurother. 2021;21(7):779–91.

Batalla A, Maat A. Cannabis use and psychosis susceptibility: a call to action. Eur Neuropsychopharmacol. 2022;54:70–1.

Benton TD, Boyd RC, Njoroge WF. Addressing the global crisis of child and adolescent mental health. JAMA Pediatr. 2021;175(11):1108–10.

Bitsko RH. Mental health surveillance among children—United States, 2013–2019. MMWR Supple. 2022;71:1.

Bourque J, Afzali MH, Conrod PJ. Association of cannabis use with adolescent psychotic symptoms. JAMA Psychiatry. 2018;75:864–6.

Burstein B, Agostino H, Greenfield B. Suicidal attempts and ideation among children and adolescents in US emergency departments, 2007-2015. JAMA Pediatr. 2019;173(6):598–600.

Carey CE, Agrawal A, Bucholz KK, Hartz SM, Lynskey MT, Nelson EC, Bogdan R. Associations between polygenic risk for psychiatric disorders and substance involvement. Front Genet. 2016;7:149.

Chandra S, Radwan MM, Majumdar CG, Church JC, Freeman TP, ElSohly MA. New trends in cannabis potency in USA and Europe during the last decade (2008–2017). Eur Arch Psychiatry Clin Neurosci. 2019;269:5–15.

Chang WC, Wong CSM, Chen EYH, Lam LCW, Chan WC, Ng RMK, Bebbington P. Lifetime prevalence and correlates of schizophrenia-spectrum, affective, and other non-affective psychotic disorders in the Chinese adult population. Schizophr Bull. 2017;43(6):1280–90.

Cloak CC, Alicata D, Ernst TM, Chang L. Psychiatric symptoms, salivary cortisol and cytokine levels in young marijuana users. J Neuroimmune Pharmacol. 2015;10:380–90.

Cohen KA, Costello MA, Schleider JL, and Schuster RM. Under Review. A roadmap for leveraging schools for population mental health: the case for an integrated and person-centered screening, brief intervention, and referral to treatment (SBIRT) framework. Under Review.

Colizzi M, Iyegbe C, Powell J, Blasi G, Bertolino A, Murray RM, Di Forti M. Interaction between DRD2 and AKT1 genetic variations on risk of psychosis in cannabis users: a case–control study. NPJ Schizophr. 2015;1(1):1–4.

Connor JP, Stjepanović D, Budney AJ, Le Foll B, Hall WD. Clinical management of cannabis withdrawal. Addiction. 2022;117(7):2075–95.

Costello EJ. Early detection and prevention of mental health problems: developmental epidemiology and systems of support. J Clin Child Adolesc Psychol. 2016;45(6):710–7.

Craske MG, Rauch SL, Ursano R, Prenoveau J, Pine DS, Zinbarg RE. What is an anxiety disorder? Focus. 2011;9(3):369–88.

Cyrus E, Coudray MS, Kiplagat S, Mariano Y, Noel I, Galea JT, Wagner E. A review investigating the relationship between cannabis use and adolescent cognitive functioning. Curr Opin Psychol. 2021;38:38–48.

D'Souza DC, DiForti M, Ganesh S, George TP, Hall W, Hjorthøj C, Spinazzola E. Consensus paper of the WFSBP task force on cannabis, cannabinoids and psychosis. World J Biol Psychiatry. 2022;23(10):719–42.

Davis JP, Pedersen ER, Tucker JS, Prindle J, Dunbar MS, Rodriguez A, D'Amico EJ. Directional associations between cannabis use and anxiety symptoms from late adolescence through young adulthood. Drug Alcohol Depend. 2022;241:109704.

De Gregorio D, Dean Conway J, Canul ML, Posa L, Bambico FR, Gobbi G. Effects of chronic exposure to low-dose delta-9-tetrahydrocannabinol in adolescence and adulthood on serotonin/norepinephrine neurotransmission and emotional behavior. Int J Neuropsychopharmacol. 2020;23(11):751–61.

Deas D. Adolescent substance abuse and psychiatric comorbidities. J Clin Psychiatry. 2006;67(Suppl 7):18–23.

Degenhardt L, Coffey C, Romaniuk H, Swift W, Carlin JB, Hall WD, Patton GC. The persistence of the association between adolescent cannabis use and common mental disorders into young adulthood. Addiction. 2013;108(1):124–33.

Devinsky O, Cilio MR, Cross H, Fernandez-Ruiz J, French J, Hill C, Katz R, Di Marzo V, Jutras-Aswad D, Notcutt WG, et al. Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. Epilepsia. 2014;55:791–802. https://doi.org/10.1111/epi.12631.

Di Forti M, Marconi A, Carra E, Fraietta S, Trotta A, Bonomo M, Murray RM. Proportion of patients in South London with first-episode psychosis attributable to use of high potency cannabis: a case-control study. Lancet Psychiatry. 2015;2(3):233–8.

Di Forti M, Quattrone D, Freeman TP, Tripoli G, Gayer-Anderson C, Quigley H, van der Ven E. The contribution of cannabis use to variation in the incidence of psychotic disorder across Europe (EU-GEI): a multicentre case-control study. Lancet Psychiatry. 2019;6(5):427–36.

Duperrouzel J, Hawes SW, Lopez-Quintero C, Pacheco-Colón I, Comer J, Gonzalez R. The association between adolescent cannabis use and anxiety: a parallel process analysis. Addict Behav. 2018;78:107–13.

Feingold D, Weinstein A. Cannabis and depression. Adv Exp Med Biol. 2021;1267:67–80.

Ferland JMN, Hurd YL. Deconstructing the neurobiology of cannabis use disorder. Nat Neurosci. 2020;23(5):600–10.

Finsaas MC, Bufferd SJ, Dougherty LR, Carlson GA, Klein DN. Preschool psychiatric disorders: homotypic and heterotypic continuity through middle childhood and early adolescence. Psychol Med. 2018;48(13):2159–68.

Fischer AS, Tapert SF, Louie DL, Schatzberg AF, Singh MK. Cannabis and the developing adolescent brain. Curr Treat Options Psychiatry. 2020;7:144–61.

Fisher HL, Caspi A, Poulton R, Meier MH, Houts R, Harrington H, Moffitt TE. Specificity of childhood psychotic symptoms for predicting schizophrenia by 38 years of age: a birth cohort study. Psychol Med. 2013;43(10):2077–86.

Forbes MK, Neo B, Nezami OM, Fried EI, Faure K, Michelsen B, Dras M. Elemental psychopathology: distilling constituent symptoms and patterns of repetition in the diagnostic criteria of the DSM-5. Psychol Med. 2024;54(5):886–94.

Fuhrmann D, Knoll LJ, Blakemore SJ. Adolescence as a sensitive period of brain development. Trends Cogn Sci. 2015;19(10):558–66.

Gallant C, Luczon R, Ryan D, Good D. Investigating cannabis use and associated symptoms among university students with and without a history of concussion. Neuropsychol Rehabil. 2022;32(6):967–91.

Giangrande EJ, Weber RS, Turkheimer E. What do we know about the genetic architecture of psychopathology? Annu Rev Clin Psychol. 2022;18(1):19–42.

Giocondo JG, Salum GA, Gadelha A, Argolo FC, Simioni AR, Mari JJ, Pan PM. Psychotic-like experiences and common mental disorders in childhood and adolescence: bidirectional and transdiagnostic associations in a longitudinal community-based study. Schizophrenia Bulletin Open. 2021;2(1):sgab028.

Giordano GN, Ohlsson H, Sundquist K, Sundquist J, Kendler KS. The association between cannabis abuse and subsequent schizophrenia: a Swedish national co-relative control study. Psychol Med. 2015;45(2):407–14.

Gobbi G, Atkin T, Zytynski T, et al. Association of cannabis use in adolescence and risk of depression, anxiety, and suicidality in young adulthood: a systematic review and meta-analysis. JAMA Psychiatry. 2019;76(4):426–34.

Godin SL, Shehata S. Adolescent cannabis use and later development of schizophrenia: an updated systematic review of longitudinal studies. J Clin Psychol. 2022;78(7):1331–40.

Gyllenberg D, Marttila M, Sund R, Jokiranta-Olkoniemi E, Sourander A, Gissler M, Ristikari T. Temporal changes in the incidence of treated psychiatric and neurodevelopmental disorders during adolescence: an analysis of two national Finnish birth cohorts. Lancet Psychiatry. 2018;5(3):227–36.

Haasbroek H, Morojele N. A systematic literature review on the relationship between autism spectrum disorder and substance use among adults and adolescents. Rev J Autism and Dev Disord. 2022;9(1):1–20.

Hall W, Stjepanović D, Caulkins J, Lynskey M, Leung J, Campbell G, Degenhardt L. Public health implications of legalising the production and sale of cannabis for medicinal and recreational use. Lancet. 2019;394(10208):1580–90.

Halladay J, Woock R, El-Khechen H, Munn C, MacKillop J, Amlung M, Georgiades K. Patterns of substance use among adolescents: a systematic review. Drug Alcohol Depend. 2020;216:108222.

Hammond CJ, Chaney A, Hendrickson B, Sharma P. Cannabis use among US adolescents in the era of marijuana legalization: a review of changing use patterns, comorbidity, and health correlates. Int Rev Psychiatry. 2020;32(3):221–34.

Hammond CJ, Allick A, Park G, Rizwan B, Kim K, Lebo R, Ivanov I. A meta-analysis of fMRI studies of youth cannabis use: alterations in executive control, social cognition/emotion processing, and reward processing in cannabis using youth. Brain Sci. 2022;12(10):1281.
Hasan A, von Keller R, Friemel CM, Hall W, Schneider M, Koethe D, Hoch E. Cannabis use and psychosis: a review of reviews. Eur Arch Psychiatry Clin Neurosci. 2020;270:403–12.
Hasin DS, Shmulewitz D, Sarvet AL. Time trends in US cannabis use and cannabis use disorders overall and by sociodemographic subgroups: a narrative review and new findings. Am J Drug Alcohol Abuse. 2019;45(6):623–43.
Hawke LD, Koyama E, Henderson J. Cannabis use, other substance use, and co-occurring mental health concerns among youth presenting for substance use treatment services: sex and age differences. J Subst Abus Treat. 2018;91:12–9.
Heckers S, Barch DM, Bustillo J, Gaebel W, Gur R, Malaspina D, Carpenter W. Structure of the psychotic disorders classification in DSM-5. Schizophr Res. 2013;150(1):11–4.
Hillard CJ, Weinlander KM, Stuhr K. Contributions of endocannabinoid signaling to psychiatric disorders in humans: genetic and biochemical evidence. Neuroscience. 2012;204:207–29.
Hines LA, Heron J, Zammit S. Incident psychotic experiences following self-reported use of high-potency cannabis: results from a longitudinal cohort study. Addiction. 2024;119:1629.
Hodgson K, Almasy L, Knowles EE, Kent JW Jr, Curran JE, Dyer TD, Glahn DC. The genetic basis of the comorbidity between cannabis use and major depression. Addiction. 2017;112(1):113–23.
Hser Y-I, Mooney LJ, Huang D, Zhu Y, Tomko RL, McClure E, Gray KM. Reductions in cannabis use are associated with improvements in anxiety, depression, and sleep quality, but not quality of life. J Subst Abus Treat. 2017;81:53–8. https://doi.org/10.1016/j.jsat.2017.07.012.
Humphrey N, Wigelsworth M. Making the case for universal school-based mental health screening. Emot Behav Difficulties. 2016;21(1):22–42. https://doi.org/10.1080/13632752.2015.1120051.
Kaasbøll C, Hagen R, Gråwe RW. Population-based associations among cannabis use, anxiety, and depression in Norwegian adolescents. J Child Adolesc Subst Abuse. 2018;27(4):238–43.
Kelleher I, Connor D, Clarke MC, Devlin N, Harley M, Cannon M. Prevalence of psychotic symptoms in childhood and adolescence: a systematic review and meta-analysis of population-based studies. Psychol Med. 2012;42(9):1857–63.
Keyes KM, Gary D, O'Malley PM, Hamilton A, Schulenberg J. Recent increases in depressive symptoms among US adolescents: trends from 1991 to 2018. Soc Psychiatry Psychiatr Epidemiol. 2019;54:987–96.
Kiburi SK, Molebatsi K, Ntlantsana V, Lynskey MT. Cannabis use in adolescence and risk of psychosis: are there factors that moderate this relationship? A systematic review and meta-analysis. Subst Abus. 2021;42(4):527–42.
Knapp AA, Allan NP, Cloutier R, Blumenthal H, Moradi S, Budney AJ, Lord SE. Effects of anxiety sensitivity on cannabis, alcohol, and nicotine use among adolescents: evaluating pathways through anxiety, withdrawal symptoms, and coping motives. J Behav Med. 2021;44:187–201.
Kosterman R, Hawkins JD, Guo J, Catalano RF, Abbott RD. The dynamics of alcohol and marijuana initiation: patterns and predictors of first use in adolescence. Am J Public Health. 2000;90(3):360.
Kronenberg LM, Slager-Visscher K, Goossens PJ, van den Brink W, van Achterberg T. Everyday life consequences of substance use in adult patients with a substance use disorder (SUD) and co-occurring attention deficit/hyperactivity disorder (ADHD) or autism spectrum disorder (ASD): a patient's perspective. BMC Psychiatry. 2014;14:1–9.
Kronenberg LM, Goossens PJ, van Etten DM, van Achterberg T, van den Brink W. Need for care and life satisfaction in adult substance use disorder patients with and without attention deficit hyperactivity disorder (ADHD) or autism spectrum disorder (ASD). Perspect Psychiatr Care. 2015;51(1):4–15.
Krygsman A, Vaillancourt T. Elevated social anxiety symptoms across childhood and adolescence predict adult mental disorders and cannabis use. Compr Psychiatry. 2022;115:152302.
Ksir C, Hart CL. Cannabis and psychosis: a critical overview of the relationship. Curr Psychiatry Rep. 2016;18:1–11.

Kuepper R, Van Os J, Lieb R, et al. Continued cannabis use and risk of incidence and persistence of psychotic symptoms: 10 year follow-up cohort study. BMJ. 2011;342:d738.
Kuhns L, Kroon E, Colyer-Patel K, Cousijn J. Associations between cannabis use, cannabis use disorder, and mood disorders: longitudinal, genetic, and neurocognitive evidence. Psychopharmacology. 2022;239(5):1231–49.
Langlois C, Potvin S, Khullar A, Tourjman SV. Down and high: reflections regarding depression and cannabis. Front Psych. 2021;12:625158.
Lawn W, Mokrysz C, Lees R, Trinci K, Petrilli K, Skumlien M, Curran HV. The CannTeen study: cannabis use disorder, depression, anxiety, and psychotic-like symptoms in adolescent and adult cannabis users and age-matched controls. J Psychopharmacol. 2022;36(12):1350–61.
Leadbeater BJ, Ames ME, Linden-Carmichael AN. Age-varying effects of cannabis use frequency and disorder on symptoms of psychosis, depression and anxiety in adolescents and adults. Addiction. 2019;114:278–93.
Lever AG, Geurts HM. Psychiatric co-occurring symptoms and disorders in young, middle-aged, and older adults with autism spectrum disorder. J Autism Dev Disord. 2016;46:1916–30.
Levine A, Clemenza K, Rynn M, Lieberman J. Evidence for the risks and consequences of adolescent cannabis exposure. J Am Acad Child Adolesc Psychiatry. 2017;56(3):214–25. https://doi.org/10.1016/j.jaac.2016.12.014.
Lev-Ran S, Roerecke M, Le Foll B, George TP, McKenzie K, Rehm J. The association between cannabis use and depression: a systematic review and meta-analysis of longitudinal studies. Psychol Med. 2014;44(4):797–810.
Levy S, Wiseblatt A, Straus JH, Strother H, Fluet C, Harris SK. Adolescent SBIRT practices among pediatricians in Massachusetts. J Addict Med. 2020;14(2):145–9. https://doi.org/10.1097/ADM.0000000000000551.
Leyton M, Stewart S. Substance abuse in Canada: childhood and adolescent pathways to substance use disorders. Canadian Centre on Substance Abuse; 2014.
Lijster JMD, Dierckx B, Utens EM, Verhulst FC, Zieldorff C, Dieleman GC, Legerstee JS. The age of onset of anxiety disorders: a meta-analysis. Can J Psychiatry. 2017;62(4):237–46.
Loflin M, Earleywine M, De Leo J, Hobkirk A. Subtypes of attention deficit-hyperactivity disorder (ADHD) and cannabis use. Subst Use Misuse. 2014;49(4):427–34.
Lowe DJ, Sorkhou M, George TP. Cannabis use in adolescents and anxiety symptoms and disorders: a systematic review and meta-analysis. Am J Drug Alcohol Abuse. 2024;50(2):150–61.
Lucatch AM, Coles AS, Hill KP, George TP. Cannabis and mood disorders. Curr Addict Rep. 2018;5:336–45.
Malgaroli M, Calderon A, Bonanno GA. Networks of major depressive disorder: a systematic review. Clin Psychol Rev. 2021;85:102000.
Manrique-Garcia E, Zammit S, Dalman C, Hemmingsson T, Andreasson S, Allebeck P. Prognosis of schizophrenia in persons with and without a history of cannabis use. Psychol Med. 2014;44(12):2513–21.
Marconi A, Di Forti M, Lewis CM, Murray RM, Vassos E. Meta-analysis of the association between the level of cannabis use and risk of psychosis. Schizophr Bull. 2016;42(5):1262–9.
McGrath JJ, Saha S, Al-Hamzawi A, Andrade L, Benjet C, Bromet EJ, Kessler RC. The bidirectional associations between psychotic experiences and DSM-IV mental disorders. Am J Psychiatry. 2016;173(10):997–1006.
Mechoulam R, Parker LA. The endocannabinoid system and the brain. Annu Rev Psychol. 2013;64(1):21–47.
Mercado MC, Holland K, Leemis RW, Stone DM, Wang J. Trends in emergency department visits for nonfatal self-inflicted injuries among youth aged 10 to 24 years in the United States, 2001-2015. JAMA. 2017;318(19):1931–3.
Miech RA, Johnston LD, Patrick ME, O'Malley PM, Bachman JG. Monitoring the future National Survey Results on drug use, 1975–2023: overview and detailed results for secondary school students. Institute for Social Research; 2024a.

Miech R, Heeringa SG, Molinaro S, Benedetti E. Cannabis reduction among adolescents as spillover from successful tobacco control. Int J Drug Policy. 2024b;124:104315.
Meier MH, Beardslee J, Pardini D. Associations between recent and cumulative cannabis use and internalizing problems in boys from adolescence to young adulthood. J Abnorm Child Psychol. 2020;48(6):771–82.
Millar SR, Mongan D, Smyth BP, Perry IJ, Galvin B. Relationships between age at first substance use and persistence of cannabis use and cannabis use disorder. BMC Public Health. 2021;21(1):997.
Mitchell JT, Sweitzer MM, Tunno AM, Kollins SH, McClernon FJ. "I use weed for my ADHD": a qualitative analysis of online forum discussions on cannabis use and ADHD. PLoS One. 2016;11(5):e0156614.
Mojtabai R, Olfson M, Han B. National trends in the prevalence and treatment of depression in adolescents and young adults. Pediatrics. 2016;138(6):e20161878.
Molinero K, Hinckley JD. Adolescent cannabis use, comorbid attention-deficit/hyperactivity disorder, and other internalizing and externalizing disorders. Child Adolesc Psychiatr Clin N Am. 2023;32(1):57–68.
Moreno-Mansilla S, Ricarte JJ, Hallford DJ. Cannabis use among early adolescents and transdiagnostic mental health risk factors. Clin Child Psychol Psychiatry. 2021;26(2):531–43.
Mottron L, Bzdok D. Autism spectrum heterogeneity: fact or artifact? Mol Psychiatry. 2020;25(12):3178–85.
Murray RM, Hall W. Will legalization and commercialization of cannabis use increase the incidence and prevalence of psychosis? JAMA Psychiatry. 2020;77(8):777–8.
Myran DT, Harrison LD, Pugliese M, Solmi M, Anderson KK, Fiedorowicz JG, Tanuseputro P. Transition to schizophrenia spectrum disorder following emergency department visits due to substance use with and without psychosis. JAMA Psychiatry. 2023;80(11):1169–74.
Notzon DP, Mariani JJ, Pavlicova M, Glass A, Mahony AL, Brooks DJ, Levin FR. Mixed-amphetamine salts increase abstinence from marijuana in patients with co-occurring attention-deficit/hyperactivity disorder and cocaine dependence. Am J Addict. 2016;25(8):666–72.
Onaemo VN, Fawehinmi TO, D'Arcy C. Comorbid cannabis use disorder with major depression and generalized anxiety disorder: a systematic review with meta-analysis of nationally representative epidemiological surveys. J Affect Disord. 2021;281:467–75.
Ozechowski TJ, Becker SJ, Hogue A. SBIRT-A: adapting SBIRT to maximize developmental fit for adolescents in primary care. J Subst Abus Treat. 2016;62:28–37. https://doi.org/10.1016/j.jsat.2015.10.006.
Pacek LR, Weinberger AH, Zhu J, Goodwin RD. Rapid increase in the prevalence of cannabis use among people with depression in the United States, 2005–17: the role of differentially changing risk perceptions. Addiction. 2020;115(5):935–43.
Parsons LH, Hurd YL. Endocannabinoid signalling in reward and addiction. Nat Rev Neurosci. 2015;16(10):579–94.
Plemmons G, Hall M, Doupnik S, Gay J, Brown C, Browning W, Williams D. Hospitalization for suicide ideation or attempt: 2008–2015. Pediatrics. 2018;141(6):e20172426.
Power RA, Verweij KJ, Zuhair M, Montgomery GW, Henders AK, Heath AC, Martin NG. Genetic predisposition to schizophrenia associated with increased use of cannabis. Mol Psychiatry. 2014;19(11):1201–4.
Racine N, McArthur BA, Cooke JE, Eirich R, Zhu J, Madigan S. Global prevalence of depressive and anxiety symptoms in children and adolescents during COVID-19: a meta-analysis. JAMA Pediatr. 2021;175(11):1142–50.
Radez J, Reardon T, Creswell C, Lawrence PJ, Evdoka-Burton G, Waite P. Why do children and adolescents (not) seek and access professional help for their mental health problems? A systematic review of quantitative and qualitative studies. Eur Child Adolesc Psychiatry. 2021;30(2):183–211.
Rahm AK, Boggs JM, Martin C, Price DW, Beck A, Backer TE, Dearing JW. Facilitators and barriers to implementing screening, brief intervention, and referral to treatment (SBIRT) in primary

Care in Integrated Health Care Settings. Subst Abus. 2015;36(3):281–8. https://doi.org/10.1080/08897077.2014.951140.
Richmond-Rakerd LS, Slutske WS, Wood PK. Age of initiation and substance use progression: a multivariate latent growth analysis. Psychol Addict Behav. 2017;31(6):664.
Ruch DA, Sheftall AH, Schlagbaum P, Rausch J, Campo JV, Bridge JA. Trends in suicide among youth aged 10 to 19 years in the United States, 1975 to 2016. JAMA Netw Open. 2019;2(5):e193886.
Rusby JC, Westling E, Crowley R, Mills KL, Light JM. Associations between marijuana use and anxious mood lability during adolescence. Addict Behav. 2019;92:89–94.
Schmidt K, Tseng I, Phan A, Fong T, Tsuang J. A systematic review: adolescent cannabis use and suicide. Addict Disord Treat. 2020;19(3):146–51.
Schmits E, Quertemont E. Components of social anxiety prevent cannabis use in adolescents. J Subst Abus. 2018;23(4):441–50.
Schmits E, Mathys C, Quertemont E. Is social anxiety associated with cannabis use? The role of cannabis use effect expectancies in middle adolescence. J Child Adolesc Subst Abuse. 2016;25(4):348–59.
Schubart CD, Van Gastel WA, Breetvelt EJ, et al. Cannabis use at a young age is associated with psychotic experiences. Psychol Med. 2011;41:1301–10.
Shanahan L, Steinhoff A, Bechtiger L, Copeland WE, Ribeaud D, Eisner M, Quednow BB. Frequent teenage cannabis use: prevalence across adolescence and associations with young adult psychopathology and functional Well-being in an urban cohort. Drug Alcohol Depend. 2021;228:109063.
Sherva R, Wang Q, Kranzler H, Zhao H, Koesterer R, Herman A, Gelernter J. Genome-wide association study of cannabis dependence severity, novel risk variants, and shared genetic risks. JAMA Psychiatry. 2016;73(5):472–80.
Shorey S, Ng ED, Wong CH. Global prevalence of depression and elevated depressive symptoms among adolescents: a systematic review and meta-analysis. Br J Clin Psychol. 2022;61(2):287–305.
Smolkina M, Morley KI, Rijsdijk F, Agrawal A, Bergin JE, Nelson EC, Lynskey MT. Cannabis and depression: a twin model approach to co-morbidity. Behav Genet. 2017;47:394–404.
Soler Artigas M, Sánchez-Mora C, Rovira P, Richarte V, Garcia-Martínez I, Pagerols M, Ribasés M. Attention-deficit/hyperactivity disorder and lifetime cannabis use: genetic overlap and causality. Mol Psychiatry. 2020;25(10):2493–503.
Solmi M, Radua J, Olivola M, Croce E, Soardo L, Salazar de Pablo G, Fusar-Poli P. Age at onset of mental disorders worldwide: large-scale meta-analysis of 192 epidemiological studies. Mol Psychiatry. 2022;27(1):281–95.
Steinsbekk S, Wichstrøm L. Cohort profile: the Trondheim early secure study (TESS)—a study of mental health, psychosocial development and health behaviour from preschool to adolescence. Int J Epidemiol. 2018;47(5):1401–1401i.
Stiles-Shields C, Archer J, Zhang J, Burnside A, Draxler J, Potthoff LM, Karnik NS. A scoping review of associations between cannabis use and anxiety in adolescents and young adults, vol. 54. Child Psychiatry Hum Dev; 2021. p. 1–20.
Taylor JH, Huque ZM. Commentary: schizophrenia prevention and prodromal psychosis in children and adolescents. J Child Psychol Psychiatry. 2021;62(5):674–6.
Tervo-Clemmens B, Gilman JM, Evins AE, Bentley KH, Nock MK, Smoller JW, Schuster RM. Substance use, suicidal thoughts, and psychiatric comorbidities among high school students. JAMA Pediatr. 2024;178(3):310–3.
Thapar A, Cooper M, Rutter M. Neurodevelopmental disorders. *The lancet*. Psychiatry. 2017;4(4):339–46.
Thompson K, Merrin GJ, Ames ME, Leadbeater B. Marijuana trajectories in Canadian youth: associations with substance use and mental health. Can J Behav Sci. 2018;50(1):17.
Tomczyk S, Isensee B, Hanewinkel R. Latent classes of polysubstance use among adolescents—a systematic review. Drug Alcohol Depend. 2016;160:12–29.

Twenge JM, Joiner TE, Rogers ML, Martin GN. Increases in depressive symptoms, suicide-related outcomes, and suicide rates among US adolescents after 2010 and links to increased new media screen time. Clin Psychol Sci. 2018;6(1):3–17.

Twenge JM, Haidt J, Blake AB, McAllister C, Lemon H, Le Roy A. Worldwide increases in adolescent loneliness. J Adolesc. 2021;93:257–69.

Uhlhaas PJ, Davey CG, Mehta UM, Shah J, Torous J, Allen NB, Wood SJ. Towards a youth mental health paradigm: a perspective and roadmap. Mol Psychiatry. 2023;28(8):3171–81.

Van Winkel R, Van Beveren NJ, Simons C. AKT1 moderation of cannabis-induced cognitive alterations in psychotic disorder. Neuropsychopharmacology. 2011;36(12):2529–37.

Vanyukov MM, Tarter RE, Kirillova GP, Kirisci L, Reynolds MD, Kreek MJ, Ridenour TA. Common liability to addiction and "gateway hypothesis": theoretical, empirical and evolutionary perspective. Drug Alcohol Depend. 2012;123:S3–S17.

Veen ND, Selten JP, van der Tweel I, Feller WG, Hoek HW, Kahn RS. Cannabis use and age at onset of schizophrenia. Am J Psychiatry. 2004;161(3):501–6.

Vidal C, Simon K, Brooks C, White J, Hinckley JD. A systematic review of evidence on integrated management of psychiatric disorders in youth who use cannabis. DAD Rep. 2024;10:100216.

Volkow ND, Hampson AJ, Baler RD. Don't worry, be happy: endocannabinoids and cannabis at the intersection of stress and reward. Annu Rev Pharmacol Toxicol. 2017;57(1):285–308.

Wilson J, Freeman TP, Mackie CJ. Effects of increasing cannabis potency on adolescent health. Lancet Child Adolesc Health. 2019;3(2):121–8.

Winn LAP, Paquette KL, Donegan LRW, Wilkey CM, Ferreira KN. Enhancing adolescent SBIRT with a peer-delivered intervention: an implementation study. J Subst Abus Treat. 2019;103:14–22. https://doi.org/10.1016/j.jsat.2019.05.009.

Woods SW, Powers III AR, Taylor JH, Davidson CA, Johannesen JK, Addington J, et al. Lack of diagnostic pluripotentiality in patients at clinical high risk for psychosis: specificity of comorbidity persistence and search for pluripotential subgroups. Schizophr Bull. 2018;44(2):254–63.

Chapter 17
The Role of the Endocannabinoid System in Adolescent Suicidal Thoughts and Behaviors

Samantha L. Ely and Hilary A. Marusak

1 Introduction

Suicide is a public health crisis, particularly among adolescents, where it is the second leading cause of death for individuals ages 10–24 years in the United States (US; Heron 2021). Rates of suicidal thoughts and behaviors (STBs) among adolescents have steadily increased over the past decade. In 2011, 16% of US high school students reported seriously contemplating suicide within the past year, and this number rose to 22% in 2021, which is the highest rate recorded to date (Centers for Disease Control and Prevention 2023). Across child and adolescent populations, SI may first emerge around the age of 10, though prevalence rates remain low in the early teen years. Around age 12, SI prevalence exponentially increases until approximately age 17, before tapering off into young adulthood (see Fig. 17.1a) (Nock et al. 2013). The incidence of suicide plans and attempts, on the other hand, have a delayed emergence around the age of 12, and show slower increases through age 17, never quite reaching the same prevalence as SI (Nock et al. 2013). Although rates of STBs are alarmingly high among adolescents, they tend to decline in young adulthood. In 2022, 13.6% of US young adults (ages 18–25 years) and less than 6% of adults (aged 26 years and older) reported seriously considering suicide within the past year (Substance Abuse and Mental Health Services Administration 2022). Therefore, adolescence may be a particularly unique period for suicide risk, perhaps due to ongoing developmental changes during this period.

This chapter explores the potential implications of developmental changes in the endocannabinoid (eCB) system for adolescent suicide risk. We will begin with an

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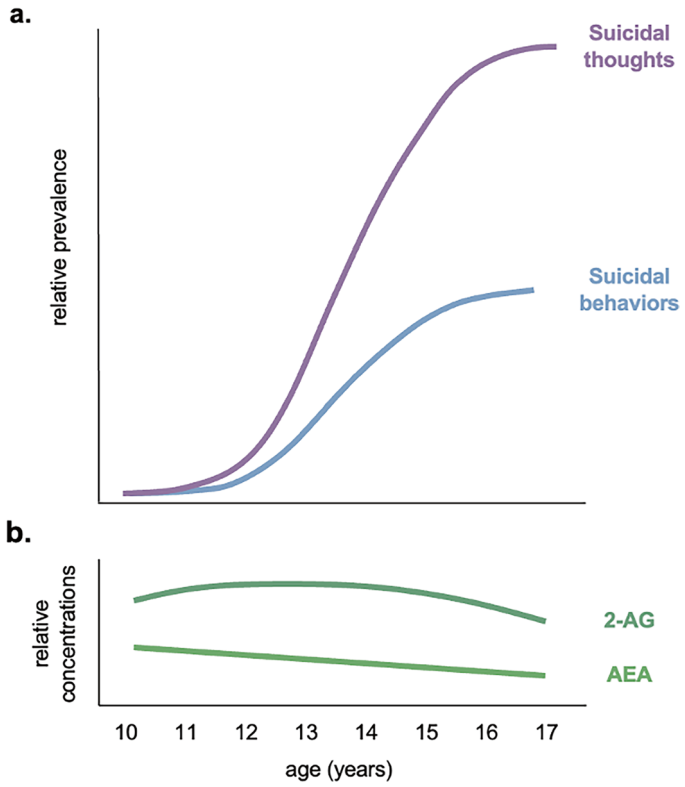


Fig. 17.1 The onset of suicidal thoughts and behaviors (**a**) overlayed with the average circulating endocannabinoid (eCB) concentrations across adolescence (**b**). Suicidal thoughts typically emerge around the age of 10 years, with prevalence dramatically increasing from ages 12–17 years. Suicidal behaviors (e.g., plan, attempt) emerge around age 12 years and become more prevalent through age 17, but at a much slower rate and lower incidence than suicidal thoughts. The eCBs, N-acylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) show negative relationships with age across adolescence, with AEA demonstrating a linear age-related decline and 2-AG concentrations peaking around age 12–13 before following a nonlinear decline. (This figure has been adapted from Nock et al. 2013 and Marusak et al. 2024)

overview of STBs, focusing on common contributing factors such as stress and emotion regulation. Next, we offer a brief overview of the eCB system, including preclinical evidence, its developmental trajectory, and summarize current evidence on eCB system alterations in adults and adolescents with STBs. We then discuss gaps in the literature and propose directions for future research. Finally, we present an integrative model that synthesizes existing knowledge, highlighting the eCB system as an important target for understanding suicide risk during adolescence.

2 Suicidal Thoughts and Behaviors

2.1 Definitions and Epidemiology

STBs encompass a spectrum of thoughts and actions related to suicide, including suicidal ideation (SI), suicide plan development, suicide attempt, and non-suicidal self-injury (NSSI). SI, as defined by the American Psychological Association (APA; 2022), refers to "thoughts about or preoccupation with killing oneself" and ranges in severity from passive to active. While definitions vary in the literature, passive and active SI are generally differentiated by intent and the presence of a plan. In particular, passive SI involves a desire for death without any specific plan or intent to act (Liu et al. 2020). On the other hand, active SI involves a desire to kill oneself, where the individual may have seriously contemplated or developed a plan for a suicide attempt (Liu et al. 2020). Although passive and active SI differ in terms of severity, both are equally likely to predict future suicide attempt (Liu et al. 2020).

A suicide attempt is defined by the APA as "a behavior that the individual has undertaken with at least some intent to die," regardless of lethality, meaning that attempts can be fatal or nonfatal (American Psychiatric Association 2022). Often discussed alongside suicide attempt are acts of nonsuicidal self-injury (NSSI), which refer to behaviors intended to inflict mild or moderate injury to oneself without any intent to die. Unlike suicide attempt, NSSI lacks suicidal intent, and these behaviors are typically aimed at emotional relief rather than causing serious injury or death (Buelens et al. 2020). Collectively, passive and active SI, suicide plan development, suicide attempts, and NSSI encompass the STBs discussed in this chapter.

Nearly a third (33.4%) of adolescents with SI will develop a plan for a suicide attempt, and an estimated 60.8% of those with a plan will subsequently make an attempt. Alarmingly, 86.1% of these cases make the transition from ideation to attempt within the first year of SI onset. Furthermore, demographic factors such as biological sex, gender and sexual identity, racial/ethnic minority status, and comorbid mental health symptoms such as anxiety and depression, are associated with increased risk for STBs (Gili et al. 2019; American Psychiatric Association 2022). Despite these known risk factors, the ability to *predict* STBs remains poor (Franklin et al. 2017), and the exact mechanisms underlying the increasing incidence of STBs across adolescence are unknown.

As a result, attention has turned toward understanding the intersection of neurobiological processes with psychosocial factors associated with STBs. Researchers posit that factors such as changing social dynamics and ongoing development may contribute to the onset of STBs through increased incidence of interpersonal conflict and a poor response to such stressors; two factors heavily discussed in theories of suicide risk.

2.2 *Stress and Emotion Regulation in Suicidal Thoughts and Behaviors*

Most modern theories of suicide risk converge on the principle that individuals at risk for suicide struggle with psychosocial stressors such as interpersonal conflict (Joiner 2005; Klonsky and May 2015; O'Connor and Kirtley 2018; Klonsky et al. 2021). Such stressors, especially when severe and/or repeated, may contribute to feelings of thwarted belongingness, perceived burdensomeness, and social isolation commonly reported in those with STBs (Klonsky et al. 2021). Individuals with STBs often show maladaptive responses to psychosocial stress (Vargas-Medrano et al. 2020), including persistent negative affect and cognitive inflexibility, which may exacerbate and intensify STBs (Nicolopoulos et al. 2018; O'Brien et al. 2021). In fact, a qualitative study of adolescents with a history of suicide attempts revealed that the inability to de-escalate negative emotions and reframe cognitive distortions was often the immediate catalyst for their decision to attempt suicide, rather than the conflict itself (O'Brien et al. 2021). Therefore, dysfunctional stress responses and emotion regulation processes may serve as key precipitants to STBs.

2.2.1 Stress Response

Research links STBs to poor stress adaptation and altered functioning in the body's stress response systems (see Miller and Prinstein 2019 for a review). Among these systems is the hypothalamic-pituitary-adrenal axis (HPA axis), a primary stress response system that becomes active in response to various stressors, including psychosocial stressors. The HPA axis produces glucocorticoids (e.g., cortisol) to facilitate biological adaptation to stress (Herman et al. 2020). This system undergoes substantial developmental changes during adolescence, typically resulting in higher cortisol reactivity to stress and a prolonged return to baseline function compared to childhood and adulthood (Westlund Schreiner et al. 2017; Miller and Eisenlohr-Moul 2019). However, adolescents with STBs often show blunted cortisol responses to acute psychosocial stress (Eisenlohr-Moul et al. 2018; Klimes-Dougan et al. 2019; Berardelli et al. 2020), despite reporting high levels of perceived stress and exhibiting stress-related behaviors (Klimes-Dougan et al. 2019). This deviation from normative development may contribute to STBs by creating a discordance between psychological experiences and physiological stress responses.

The suppressed cortisol response may arise from repeated psychosocial stress experienced by individuals with STBs (Westlund Schreiner et al. 2017). While the literature is somewhat mixed, studies predominantly show that individuals with STBs exhibit higher basal cortisol and a flattened cortisol response to stress compared to those without (Berardelli et al. 2020; Başgöze et al. 2021). It has been suggested that chronically elevated basal cortisol may lead to allostatic shifts that affect both diurnal patterns and responses to stress. In other words, when cortisol remains

high as a result of repeated stress, the body may adjust by suppressing the cortisol stress response and diurnal variations (Westlund Schreiner et al. 2017). These patterns may explain why individuals with STBs often show elevated basal cortisol but flattened response to acute stress. Collectively, these findings suggest that alterations in HPA axis function during adolescence—a sensitive period of neurodevelopment—may play a role in STBs.

In addition, STBs have been associated with altered function in other systems involved with stress response, such as the inflammatory/immune response and the autonomic nervous system. A thorough review of evidence linking alterations in these systems to STBs is beyond the scope of this chapter. Therefore, we encourage readers to find more information on these topics in reviews such as those conducted by Alacreu-Crespo et al. 2024; Johnston et al. 2022; Miller and Eisenlohr-Moul 2019, and Miller and Prinstein 2019.

2.2.2 Emotion Regulation

Emotion regulation processes are highly intertwined with stress response systems (Herman et al. 2020), and altered biological signaling in related brain regions may be implicated in STBs (Schmaal et al. 2020). Subcortical brain regions involved with emotional processing (e.g., amygdala) directly modulate the activation of stress response systems such as the HPA axis (McLaughlin et al. 2014). Abnormalities in these subcortical emotional processing areas and other interconnected regions (e.g., ventral prefrontal cortex (PFC), insula, and precuneus) may contribute to excessive negative and blunted positive mood, negative self-referential thinking, and rumination (Schmaal et al. 2020). Furthermore, cortical areas involved with emotion regulation, such as the PFC, can exert top-down control over subcortical regions and thereby regulate emotion processing and stress response systems (Herman et al. 2020). Meta-analyses of neuroimaging studies conducted in individuals free from psychiatric conditions show that top-down control from areas like the dorsolateral PFC (dlPFC) facilitates emotion regulation (Buhle et al. 2014; Denny et al. 2023). Dysfunction in the dlPFC and related regions (e.g., dorsal anterior cingulate cortex (dACC), inferior frontal gyrus (IFG)) contribute to a diminished ability to exert cognitive control over emotions and behavior, as well as a lack of cognitive flexibility and impaired decision making (Schmaal et al. 2020). Lack of top-down control may explain the diminished ability to not only quell emotions in response to stress, but also for these areas to regulate downstream stress response processes. Furthermore, regardless of STB presence, adolescents are prone to an imbalance between bottom-up and top-down emotional processes. During adolescence, the PFC matures more slowly than limbic regions, creating an imbalance that favors bottom-up emotional processes over top-down control mechanisms (Casey et al. 2019). This imbalance is believed to contribute to the difficulties typically developing adolescents face in regulating emotions compared to older or younger

individuals and may contribute to exacerbated emotion regulation difficulties in those with STBs (Miller and Prinstein 2019; Miller and Eisenlohr-Moul 2019).

Adolescents with STBs report that their difficulties stem from a lack of access to emotion regulation strategies rather than challenges in utilizing those strategies (Brausch et al. 2022). These findings align with neuroimaging evidence suggesting discrepancies in the autonomic and effortful engagement of emotion regulation processes among adolescents with STBs. When explicitly instructed to downregulate emotions toward negative stimuli, adolescents with STBs (compared to their peers without) demonstrate higher engagement in PFC emotion regulation areas such as the dlPFC, IFG, and frontal pole (Miller et al. 2018), particularly after experiencing social rejection (Miller et al. 2024). However, differences in PFC activation while passively viewing negative stimuli (i.e., without effortful regulation) have also been reported (Miller et al. 2018, 2024), suggesting an altered ability to automatically recruit emotion regulation processes (Miller et al. 2018). Notably, subjective ratings of emotional intensity toward stimuli did not differ between the groups, suggesting that neural abnormalities may be detectable even in the absence of subjective differences (Miller et al. 2018, 2024). Collectively, these findings suggest that youth with STBs can regulate negative emotions but show differences in brain activity related to emotion regulation as compared to their counterparts without STBs. Similar to the discordance between HPA axis function and feelings of stress (Klimes-Dougan et al. 2019), there appears to be a mismatch between psychological experience and physiological processes regarding emotion regulation.

In sum, STBs are associated with poor stress responses and challenges in emotion regulation, likely driven by altered biological processes. Compared to children or adults, adolescents may be particularly vulnerable to STBs due to the increased occurrence of psychosocial stress and developmental changes to stress and emotion regulation systems. This heightened vulnerability may help explain the dramatic onset and increased incidence of STBs during the adolescent period (Nock et al. 2013). It should be noted, however, that a majority of adolescents will encounter incidences of psychosocial stress and developmental changes, yet not all will develop STBs. Some investigators have posited that these factors do not sufficiently explain why some view suicide as a viable option (Miller and Prinstein 2019; Miller and Eisenlohr-Moul 2019). Instead, it has been proposed that suicide risk increases when individuals surpass their unique "threshold" for stress tolerance, which may occur during periods of high distress (e.g., excessive bullying), and may then feel unable to cope with the stress. In addition, suicide propinquity—or exposure to STBs through friends, relatives, or celebrities—may lead youth to consider suicide as an option during extreme stress. Therefore, while altered biological function in stress response and emotion regulatory systems may contribute to suicide risk, the threshold for STB development may depend on an individual's tolerance threshold for stress.

3 Role of the Endocannabinoid System in Stress and Emotion Regulation

Emerging research implicates altered eCB system function in STBs given its significant role in modulating the stress response and mood (Hillard 2018; deRoon-Cassini et al. 2020; Mannekote Thippaiah et al. 2021). ECBs and cannabinoid receptors are widely distributed throughout both the central and peripheral nervous systems (Joshi and Onaivi 2019), as reviewed extensively in Chap. 2. In the brain, the cannabinoid type 1 (CB1) receptor is predominantly located on neurons and glial cells in areas involved in emotional processing and regulation, such as limbic areas and the PFC (Donald Burns et al. 2007; Terry et al. 2009). The main function of CB1 receptor signaling is to regulate neurotransmission through inhibition of glutamate or γ-aminobutyric acid (GABA), as determined by their location on excitatory (glutamatergic) or inhibitory (GABAergic) neurons (Hillard et al. 2017).

CB1 receptors can be activated by both exogenous agents, such as delta-9-tetrahydrocannabinol (THC)—the primary intoxicating constituent of cannabis—and endogenous ligands such as the eCBs—*N*-acylethanolamine (anandamide or AEA) and 2-arachidonoylglycerol (2-AG; Lu and MacKie 2016). Notably, these eCBs act as retrograde neuromodulators, as they are synthesized and released "on-demand" from the postsynaptic neuron before binding to CB1 receptors on the presynaptic neuron or glial cell (Lu and MacKie 2016). While it is distinctive that two endogenous ligands influence the same receptor, they are thought to play unique roles in regulating the system (Martinez Ramirez et al. 2023). AEA, a partial agonist with high affinity but weak intracellular signal transduction (McLaughlin et al. 2014), exhibits a tonic effect on CB1 receptors, providing fine-tuning to maintain the system. In contrast, 2-AG, a full agonist with weaker receptor affinity but more robust intracellular response (McLaughlin et al. 2014) demonstrates phasic firing effects on CB1 receptors. This versatility of ligand-receptor signaling in the eCB system may lend the system to a widespread ability to regulate downstream processes, particularly in fine-tuned systems like those involved with stress response (deRoon-Cassini et al. 2020).

3.1 Acute Stress

Given the eCB system's critical role in stress regulation, several laboratory-based paradigms have been employed to study the eCB response to acute stress and its involvement in various neuropsychiatric disorders. These paradigms encompass both physiological and psychosocial stressors, such as acute exercise and public speaking tests like the Trier Social Stress Task (TSST) in humans. Analogous research in animal models uses techniques such as footshock and social defeat stress/maternal separation as translational physical and psychosocial stressors.

Research in animal models indicates that AEA concentrations are rapidly and significantly reduced in the brain in response to an acute stressor (deRoon-Cassini et al. 2020). Acute stress triggers the quick release of corticotropin releasing hormone, which binds to CRH type 1 receptors. Once CRH type 1 receptors are activated, fatty-acid amide hydrolase (FAAH), a catabolizing enzyme of AEA, increases and ultimately leads to the breakdown of AEA (Morena et al. 2016; deRoon-Cassini et al. 2020). Contrary to stress-related reductions in AEA, acute stress *increases* 2-AG signaling (deRoon-Cassini et al. 2020), although these changes occur with a delay compared to changes in AEA (Morena et al. 2016). Acute stress produces an increase in glucocorticoids (e.g., cortisol in humans and corticosterone in rodents) which, once bound to their receptor, triggers the synthesis of 2-AG (deRoon-Cassini et al. 2020). Further, administration of corticosterone in rodents (analogous to cortisol in humans) has delayed effects on the brain, which may explain the lag in 2-AG elevations following acute stress (Morena et al. 2016).

Importantly, the patterns of eCB response vary by the nature of the stressor, whether eCB concentrations are measured in a particular brain region vs. in circulation, and between rodent models and humans. For example, analogous psychosocial stressors such as social defeat and maternal separation have consistently produced increases in FAAH and decreases in AEA in the rodent amygdala and hippocampus, but not in the medial PFC (mPFC). In contrast, painful stressors, such as footshock, produced rapid and immediate *increases* in AEA concentrations in the mPFC, amygdala, and hippocampus (Hohmann et al. 2005; Morena et al. 2014), but may lead to overall AEA reductions 24 hours later (Bluett et al. 2014).

Furthermore, humans without psychiatric disorders display circulating eCB stress response patterns that somewhat oppose psychosocial stress response patterns seen in the brain tissue of rodents, but instead mimic those seen with footshock stressor. Recent systematic reviews from our group found several studies reporting pre-to-post stress *increases* in AEA following acute physical (i.e., exercise; Desai et al. 2022) and psychosocial stress (e.g., the TSST; Gowatch et al. 2024); however, only elevations following exercise were statistically supported by meta-analyses (Desai et al. 2022). With regard to circulating 2-AG, meta-analyses also support concentration increases following physical (Desai et al. 2022) and psychosocial stress (Gowatch et al. 2024), although again the latter did not reach significance through a meta-analysis. Collectively, this suggests that, among both humans and animal models, the nature of the stressor (i.e., physical vs. psychosocial) may differentially impact the eCB system's response to stress and there is some variability across studies. While there is some discrepancy between evidence from human and rodent studies, the exact reasoning is unknown. Likely, this may be due to the inherent limitations of current methods. For example, in humans, eCB response measured in circulation to common laboratory-based psychosocial stressors may not compare to rodent models of eCBs measured in brain tissue.

3.2 Chronic Stress

The eCB system has also been studied in the context of chronic stressors, which can include repeated stressors that are homotypic (one type of repeated stressor, which can follow a temporally predictable pattern), heterotypic (repeated stressors that vary in nature and temporal predictability), and/or unpredictable (temporally variable). Most animal research employs stressors similar to those used to study acute stress (e.g., social defeat), but the chronicity of the stressor paradigms is sometimes used to induce anhedonia-like symptoms as an animal model for depression (Hill et al. 2008). However, in studies of humans, chronic stress can be more challenging to model given the feasibility and ethical considerations of inducing significant, repeated, and/or long-term stress. Some studies in humans have employed approaches such as exercise interventions (e.g., 10-week programs) to capture chronic physical stress, while others have investigated individuals with stress-related psychiatric disorders, such as depression or posttraumatic stress disorder (PTSD), in an attempt to model chronic stress.

Similar to the effects of acute stress, evidence from animal models shows that chronic homotypic stressors can reduce AEA concentrations in brain regions such as the amygdala, hippocampus, and mPFC by way of CRH increasing FAAH activity (Morena et al. 2016; deRoon-Cassini et al. 2020). Conversely, research employing chronic *heterotypic* stress paradigms produced mixed findings, with some showing reduced AEA across brain regions (Hill et al. 2008), while others report no significant changes in AEA concentrations (Hill et al. 2005; Bortolato et al. 2007; Wang et al. 2010). In contrast to AEA, 2-AG concentrations have been shown to increase in various brain regions as a result of chronic homotypic stress (deRoon-Cassini et al. 2020), although this effect tends to be short lived (Morena et al. 2016). Like AEA, however, the evidence for chronic heterotypic stress exposure impacting 2-AG remains inconsistent (Morena et al. 2016). This inconsistency of eCB response to heterotypic chronic stress may stem from differences in the nature of the stressor, similar to the inconsistency with stressor type noted above with acute stress. Alternatively, the timing of eCB stress response may differ depending on the type of stressor. Studies employing chronic heterotypic stress paradigms often measure the eCB response at the same time interval after each stressor, which might not accurately reflect the optimal timing for capturing more rapid or delayed dynamics.

While CB1 receptor density typically remains unchanged in response to acute stressors, it may decrease following chronic homotypic and heterotypic stress. Chronic stress generally leads to a downregulation of CB1 receptor expression across the brain, potentially as a compensatory response to altered eCB concentrations (deRoon-Cassini et al. 2020). For example, chronic corticosterone administration, which elevates 2-AG concentrations, is associated with downregulated CB1 receptor density. Conversely, blocking glucocorticoid receptors—whose signaling typically reduces AEA concentrations—prevents stress-induced changes in CB1 receptor density (Rossi et al. 2010; Hong et al. 2011). Notably, this pattern of CB1 receptor downregulation in response to stress, whether homotypic or heterotypic, is

not observed in the mPFC. Instead, CB1 receptor expression in the mPFC is upregulated (Morena et al. 2016). While the exact reasoning for this region-specific difference remains unclear, it may be due to the localization of CB1 receptors on GABAergic neurons in the PFC, as opposed to glutamatergic neurons in other brain regions. This difference could provide a unique mechanism for fine-tuning neurotransmitter signaling in the system (Hillard et al. 2017).

To summarize, acute stress exposure reduces AEA levels in the brain by increasing FAAH activity through CRH signaling, while it increases 2-AG levels through elevated glucocorticoid signaling. Chronic stress, especially when repeatedly exposed to the same stressor, may amplify these effects; though evidence is limited regarding how variable stressors influence this dynamic.

3.3 HPA Axis Regulation

Given the clear link between the eCB system and stress, it is not surprising that the eCB system plays a role in regulating the HPA axis. The tonic function of AEA is thought to help regulate the baseline activity of the HPA axis, primarily by constraining its activity in the absence of stressors (McLaughlin et al. 2014; Morena et al. 2016). However, if AEA concentrations fluctuate in response to stress, tonic regulation may become disrupted and contribute to increased HPA axis signaling (Morena et al. 2016). In contrast, the phasic nature of 2-AG may be well-suited to regulate the magnitude of HPA axis activity and to terminate the system's response after a stressor (Morena et al. 2016).

3.4 Alterations of the eCB System in Stress-Related Disorders

Our recent meta-analysis indicates that circulating eCB concentrations are altered in individuals with stress-related neuropsychiatric disorders, including depression, anxiety, and PTSD (Gowatch et al. 2024). Compared to adults without psychiatric conditions, those with stress-related disorders exhibited, on average, higher circulating concentrations of both AEA and 2-AG while at rest. These elevated eCB concentrations may reflect eCB system alterations due to repeated psychosocial stress, given that these disorders are commonly associated with chronic stress. However, individuals with stress-related disorders exhibited a *blunted* 2-AG response to psychosocial stress compared to those without, suggesting an impaired ability to appropriately mount a 2-AG response to acute stress. The meta-analysis did not support statistically significant differences in AEA response to stress in individuals with stress-related disorders compared to those without. However, there was substantial variability across studies, and few studies examined eCB response to acute stress in individuals with vs. without psychiatric disorders. Nevertheless, these data suggest that circulating eCB concentrations are out of balance at rest and

may not have an adequate response to stress in individuals with stress-related disorders. This may contribute to the HPA axis dysregulation and dysfunctional biological responses commonly associated with these disorders (Naughton et al. 2014). Furthermore, as STBs may be a common characteristic across these stress-related disorders (American Psychiatric Association 2022), it is possible that these eCB patterns may be observed in individuals with STBs as well.

3.5 *Endocannabinoids, Mood, and Affect Regulation*

Endocannabinoids play a key role in regulating mood and affect, though research in this area is much more limited as compared to stress-related research. A large portion of research on this topic comes from evidence of the mood-enhancing effects of exercise and its links to changes in circulating eCBs (Hillard 2018; Mannekote Thippaiah et al. 2021). Our previous meta-analysis demonstrated that acute exercise elevates circulating AEA and 2-AG concentrations in animal models and in adults with and without psychiatric and physical health conditions (Desai et al. 2022). In studies of healthy adults, elevations in both 2-AG and AEA have been correlated with positive mood outcomes including reduced feelings of depression, mood disturbance, and increased vigor, and generally positive affect (Raichlen et al. 2012; Brellenthin et al. 2017). However, there is some evidence linking elevated circulating AEA concentrations *at rest* to greater affect intensity and lability (i.e., instability) in adults regardless of psychiatric disorder presence (Coccaro et al. 2018). Collectively, this evidence suggests that elevated circulating eCBs at rest may contribute to difficulties regulating affect and that minimal elevations in eCBs (i.e., blunted response) as a response to stress may foster a negative mood response in the face of stress.

3.6 *Age-Related Changes in the Endocannabinoid System Across Childhood and Adolescence*

Studies examining the eCB system across the lifespan in animal models suggest heightened activity of the eCB system during adolescence compared to earlier or later stages of life, primarily due to peak CB1 receptor expression and higher concentrations of AEA and 2-AG (Meyer et al. 2018). At the onset of adolescence, CB1 receptor expression peaks in the PFC and then declines into adulthood. In contrast, eCBs show more dynamic changes: 2-AG concentrations rise at both the beginning and end of adolescence, while AEA concentrations show an overall increase with consistent fluctuations throughout the adolescent period (Meyer et al. 2018).

While these studies highlight dynamic fluctuations in the eCB system during adolescence, it is important to note that this evidence is derived from rodent models.

As previously noted, it is possible that the eCB system functions differently in humans than in animal models, including during development. In fact, data from our recent, cross-sectional, in vivo study in human adolescents demonstrate slightly different patterns: across adolescence (ages 10–17 years), plasma AEA concentrations follow a linear decrease, while 2-AG follows a quadratic decrease, peaking at approximately 13 years (see Fig. 17.1b; Marusak et al. 2024). These discrepancies may result from developmental differences between animals and humans, including temporal differences in corresponding ages and patterns of expression, and from the comparison of circulating eCB samples in humans to brain tissue samples in rodents. Future studies employing a longitudinal design with larger samples are needed to confirm and extend these findings.

It is interesting to note that dynamic fluctuations in the eCB system correspond with the increase in STB incidence during adolescence (Fig. 17.1a). Given the eCB system's role in regulating stress response and mood—two domains of dysfunction frequently associated with STBs—it is possible that developmental changes in the eCB system may make adolescence a heightened period of risk for the emergence of STBs. The next section explores emerging evidence on alterations in the eCB system to support this notion.

4 The Endocannabinoid System in Suicidal Thoughts and Behaviors

As of this writing, we identified only one published abstract by Ferger and colleagues that examined alterations in the eCB system in adolescents with STBs. This abstract, presented at the 2023 Society of Biological Psychiatry meeting, reported initial associations between plasma eCB concentrations at rest and trauma exposure in female adolescents with ($n = 110$) and without ($n = 38$) NSSI (Ferger et al. 2023). The study found that adolescents with NSSI had significantly lower circulating AEA concentrations compared to controls without a psychiatric condition, with the lowest concentrations observed in those with trauma exposure. These findings provide preliminary evidence for eCB system dysregulation in youth with STBs, underscoring the need for further research.

Although studies in adults are also limited, we identified seven publications reporting differences in the eCB system between adults with and without STBs. These studies highlight alterations in receptor density, signaling, and circulating eCB concentrations. Below, we summarize findings from these studies, which include four postmortem investigations using brain tissue and three in vivo studies examining circulating eCBs at rest. Notably, most postmortem research focused on the dlPFC, likely due to early evidence of region-specific alterations in CB1 receptor density in individuals with schizophrenia (Dean et al. 2001)—a disorder often associated with suicide risk (Siris 2001))—and the established links between dlPFC

dysfunction and STBs (Miller and Prinstein 2019; Miller and Eisenlohr-Moul 2019; Schmaal et al. 2020).

Further supporting this focus on the dlPFC, a recent proteomics study (Kim et al. 2022) found that individuals who died by suicide exhibited a greater number of proteins associated with eCB neuron development and eCB synapse pathways. These findings emphasize the need for continued investigation in the role of the eCB system in STBs, particularly in the dlPFC.

4.1 Cannabinoid Receptor Density in Postmortem Research of Suicide

The first evidence of eCB system alterations in humans with STBs emerged from a postmortem investigation of brain tissue from the right dlPFC (Brodmann Area 9), a region involved in top-down emotion regulation (Hungund et al. 2004). This study matched cases based on age, sex, ethnic background, and postmortem interval, and compared CB1 receptor density and signaling between 10 individuals with major depressive disorder (MDD) who died by suicide and 10 controls free of psychopathology who died by other means (e.g., homicide, accidental injury, or natural causes). Compared to controls, individuals who died by suicide exhibited higher CB1 receptor density, as measured by both [^{3}H]CP-55,940 binding assay (24% higher, $p < 0.0001$) and Western blot analysis (38%, $p < 0.001$). In addition, when exposed to the synthetic agonist CP-55,940, CB1 receptor-stimulated [^{35}S]GTPγS binding showed a 45% higher coupling efficiency in the suicide group. These findings suggest enhanced signaling of the eCB system in the dlPFC of individuals who died by suicide, although the higher signaling may also be attributed to the greater CB1 receptor density. Interestingly, receptor affinity and basal [^{35}S]GTPgS binding did not differ significantly between groups, suggesting that the observed differences in receptor density and binding are not due to differences in ligand binding ability or intrinsic activity at rest in the absence of a ligand. Therefore, individuals with MDD who died by suicide exhibited higher CB1 receptor density and stimulated signaling in the dlPFC. However, it remains unclear whether these findings are specifically linked to suicide or MDD, given that the comparator group was free from psychopathology. Nonetheless, studies examining these differences in other populations may provide further insight into the role of eCB system dysfunction in suicide.

A second study (Vinod et al. 2005) compared 11 individuals with alcohol use disorder (AUD) who died by suicide to 11 AUD control cases (who died by other means) matched on alcohol use, age, sex, race, postmortem interval, and psychotropic medication use. Aside from one individual with remitted MDD and schizoaffective disorder, all subjects were free of other psychiatric and substance use conditions. Similar to findings in individuals with MDD (Hungund et al. 2004), those with AUD who died by suicide showed significant CB1 receptor upregulation in the dlPFC (Brodmann Area 9) compared to controls. This upregulation was observed through

both CP-55,940 binding (39% greater, $p < 0.0021$) and Western blot analysis (67% greater, $p = 0.014$). In addition, individuals with AUD who died by suicide demonstrated 34% greater CB1 receptor signaling in the dlPFC compared to controls. In contrast, receptor stimulation in the visual association cortex showed no significant difference, suggesting regional specificity in the eCB system's involvement in suicide.

A third study (Erdozain et al. 2015) examined eCB system differences in 11 adults with AUD who died by suicide, comparing them to three other groups: (1) individuals who died by suicide without AUD, (2) individuals who died naturally with AUD, and (3) individuals with neither AUD nor suicide death. All groups were matched by age, sex, and postmortem interval, and no other psychiatric conditions were present in any group. The results showed that individuals with AUD who died by suicide had higher CB1 receptor density in the dlPFC (Brodmann Area 9) compared to the non-suicide groups. However, mRNA expression did not differ, likely due to the localization of CB1 receptors on axon terminals from other brain regions. Unlike prior studies, no significant differences in CB1 receptor signaling or G-protein coupling efficiency were found, which may be attributable to the use of a different cannabinoid agonist (WIN 55,212–2 compared to CP-55,940). The authors suggest that post-translational modifications of the CB1 receptor may account for the increased CB1 receptor density without corresponding changes in functionality.

A fourth study (Mato et al. 2018) examined postmortem dlPFC brain tissue from 12 individuals with MDD who died by suicide, compared to 12 controls matched for age, sex, and postmortem interval. A key objective of the study was to assess the influence antidepressant treatment on CB1 receptor density and signaling. However, unlike previous studies, no differences in CB1 receptor density were found between groups. The study did reveal that individuals who died by suicide exhibited 23% higher [^{35}S]GTPgS binding in response to WIN 55,212–2 stimulation, indicating increased CB1 receptor signaling compared to controls. Further subgroup analyses suggested that this effect was largely driven by individuals who were not treated with antidepressants. Specifically, medication-free individuals showed a 38% increase in binding compared to controls, while no significant differences were observed in individuals treated with antidepressants. In addition, the study found that individuals who died by suicide demonstrated greater efficiency in CB1 receptor-G_{α} coupling overall, particularly due to higher $G_{\alpha o}$ signaling in the antidepressant-free subgroup. However, when examining downstream effects on cellular signaling, such as inhibition of adenylate cyclase (AC) through WIN 55,212–2 in cAMP assays, no differences between groups were found. These results align with the conclusion that while increased CB1 receptor signaling is consistent in individuals who died by suicide, this increase does not appear to stem from higher CB1 receptor density in the dlPFC. Instead, the study suggests that higher CB1 receptor signaling is likely due to enhanced $G_{\alpha o}$ signaling, particularly in those with MDD who were not treated with antidepressants and died by suicide. This may also explain the lack of observed differences in downstream cellular signaling pathways such as AC inhibition as $G_{\alpha o}$ modulate ion channel function rather than affecting the

AC pathway. These findings highlight the complexity of CB1 receptor signaling in the context of suicide and antidepressant use.

In sum, postmortem studies generally indicate a greater density and signaling of CB1 receptors in the dlPFC among individuals who died by suicide compared to those who died from other causes. While these studies were conducted in the context of suicide and comorbid conditions (e.g., MDD, AUD), the consistent findings across different samples suggest that these patterns are frequently observed following fatal suicide attempts. In addition, since CB1 receptor affinity and basal signaling do not appear to differ in those who died by suicide, the observed differences in CB1 receptor density are likely not due to intrinsic properties of the system. Rather, these differences may arise from other mechanisms, such as increased receptor synthesis or decreased degradation. The higher signaling observed in the presence of a ligand may reflect either an increase in receptor density or altered signaling mechanisms. These findings underscore the importance of considering ligand interactions with CB1 receptors, particularly in vivo, when interpreting changes in eCB system function in the context of suicide.

4.2 Endocannabinoid Concentrations in Individuals with STBs

Given the evidence of greater CB1 receptor density in individuals who died by suicide, it is reasonable to hypothesize that this could be an adaptive feedback mechanism in response to lower in vivo concentrations of endogenous ligands (i.e., eCB). However, three studies suggest the opposite: higher postmortem and in vivo eCB concentrations in individuals who died by suicide compared to those who died by other causes.

In one such study, postmortem dlPFC tissue was compared between individuals with AUD who died by suicide and matched controls (individuals with chronic alcohol use who died by other causes; Vinod et al. 2005). The authors found that individuals who died by suicide had 79% and 47% higher concentrations of AEA and 2-AG in dlPFC tissue, respectively, compared to controls.

A later study supported these findings and was one of the first to investigate in vivo eCBs as they relate to STBs in humans (Herranz-Herrer et al. 2020). This study included 30 adults who recently made a nonfatal suicide attempt and 13 psychiatric controls, all recruited from an inpatient clinic. Participants provided serum samples for circulating eCBs and stress-related hormones, such as cortisol and adrenocorticotropic hormone (ACTH). Notably, there were no significant differences between the groups in terms of sociodemographic factors, psychiatric disorders, or the history and frequency of NSSI. Initial analyses, which included subjects who had recently used cannabis, revealed no significant differences in AEA or 2-AG concentrations between groups. However, after excluding those who used cannabis, the results showed that individuals who had attempted suicide had higher

circulating concentrations of AEA and lower concentrations of ACTH. The groups did not differ in 2-AG, cortisol, or beta-endorphin concentrations.

A third study conducted a comparison of plasma eCB concentrations between 17 combat-exposed military veterans with a history of nonfatal suicide attempt in the past 5 years and 17 combat-exposed veterans without a lifetime history of suicide attempt (Sher et al. 2020). The two groups were matched on sociodemographic factors and psychiatric conditions (e.g., PTSD, mood disorders, substance use), although they did differ in SI (measured by the Beck Scale for Suicidal Ideation). When biological sex was considered, those with a history of suicide attempt had higher plasma 2-AG concentrations compared to those without. However, no significant differences were found between groups for AEA or cortisol concentrations. Interestingly, a moderate negative correlation ($r = -0.524$, $p = 0.031$) was observed between AEA concentrations and the severity of SI, indicating lower AEA concentrations were associated with more severe SI, but this relationship was present only in individuals with a history of suicide attempt.

In a fourth study, the effects of electroconvulsive therapy (ECT)—an FDA-approved treatment for severe depression—on plasma eCB concentrations were investigated in 17 individuals with MDD (Bloemhof-Bris et al. 2024). Both 2-AG and AEA concentrations generally increased across 12 sessions of ECT, with some correlation to STBs. Prior to the first ECT session, STB severity (measured by the Columbia-Suicide Severity Rating Scale (C-SSRS)) did not correlate with plasma 2-AG, AEA, or related lipid concentrations. However, after six sessions of ECT, STB severity was negatively correlated with AEA plasma concentrations ($r = -0.86$, $p = 0.03$), indicating that higher AEA was associated with less severe STBs—consistent with findings from Sher et al. Interestingly, after six ECT sessions, depressive symptoms did not significantly correlate with plasma eCB concentrations, suggesting that these relationships may be specific to STBs, particularly in the context of ECT.

In sum, studies examining resting eCB concentrations in adults with suicide attempt histories indicate elevations in AEA and/or 2-AG in both circulation and in the dlPFC. However, evidence suggests a complex relationship between AEA concentration and SI severity, with higher AEA concentrations correlating with less severe ideation. These findings may suggest a nonlinear relationship between STB severity and AEA concentrations, variable relationships with thoughts vs. behaviors, and/or that AEA concentrations increase following a significantly stressful event, such as a suicide attempt. Larger and longitudinal studies are needed to fully discern these relationships.

4.3 Summary of Existing Literature

The collective findings from the studies in humans suggest a complex relationship between the eCB system and STBs. Postmortem studies reveal increased CB1 receptor density in the dlPFC of individuals who died by suicide, potentially

reflecting compensatory mechanisms, with some evidence that these changes may be region-specific. Although there is some inconsistency, both postmortem and in vivo studies frequently report elevated concentrations of eCBs, including AEA and 2-AG, in individuals who died by suicide or attempted suicide as compared to controls. Notably, AEA findings may be more consistent, as lower concentrations correlate with greater SI severity both in combat veterans with a history of suicide attempt and in individuals undergoing ECT. Findings from the ECT study further highlight that the relationship between eCBs and STB severity may differ from their relationship with general depressive symptoms, suggesting that eCB system alterations may be uniquely involved in STBs. Together, these studies underscore the importance of further investigating the eCB system as a potential biomarker or therapeutic target for STBs.

The CB1 receptor findings align with preclinical research on chronic stress, which is often used as an animal model for anhedonia and depressive symptoms. Chronic stress in rodents typically leads to downregulation of CB1 receptors across the brain, except in the mPFC, where CB1 receptor expression is upregulated. It has been suggested that the mPFC in rodents functions similarly to the dlPFC in humans (McLaughlin et al. 2014). Therefore, it is reasonable to expect upregulation of CB1 receptors in the dlPFC following chronic stress, a finding frequently observed in individuals who died by suicide. These findings may reflect overlap in patterns observed in response to chronic stress as well as STBs.

Although the eCB findings in humans are somewhat consistent with data from animal models on chronic stress, there are some interesting contrasts. For instance, studies in animal models suggest that AEA concentrations decrease under chronic stress conditions, while 2-AG remains relatively unaffected. However, studies in humans have reported higher circulating eCB concentrations in individuals who have attempted suicide compared to controls, which aligns with our recent meta-analysis showing higher circulating eCB concentrations in individuals with stress-related disorders, such as depression, anxiety, and PTSD—conditions commonly co-occurring with STBs (American Psychiatric Association 2022). Currently, it is challenging to disentangle effects of STBs from comorbid psychiatric symptoms. It may be that eCB system dysfunction is generally a hallmark of psychiatric risk, more general homeostatic dysregulation, pain, or an indication of tissue injury (Hillard 2018). However, as further research explores the eCB system's response to stress in individuals with STBs, it may become clearer whether specific patterns of eCB system function are indicative of STB risk, as opposed to other psychiatric or even physical health conditions.

5 Remaining Questions and Future Directions for Research

The reviewed studies highlight eCB system alterations in adults who died by suicide or previously attempted suicide compared to controls, with important differences and similarities to animal models of chronic stress—a model used to study

depression-like symptoms in animals (e.g., anhedonia)—noted. However, there are several important gaps that remain.

First, we identified only one published abstract examining associations between STBs and circulating eCB concentrations during *human adolescence*—a critical period of neurodevelopment and heightened risk for STBs. This study offered preliminary evidence supporting eCB system dysregulation in youth with NSSI, but further research is needed to validate and expand upon these findings. The eCB system, which modulates other stress response systems (e.g., autonomic nervous system, HPA axis) and regulates mood, undergoes its own developmental changes during adolescence. These fluctuations may have downstream effects on stress response and emotion regulation processes. Since these developmental changes coincide with the emergence of STBs (see Fig. 17.1) and altered eCB system function has been linked to STBs in adults, it is plausible that adolescence represents a "perfect storm" where changes to the eCB system, and/or other predisposing conditions, leave some youth ill-equipped to manage stress effectively.

In addition, the heightened incidence of stress often experienced during adolescence could further dysregulate the eCB system, mirroring patterns observed in animal models of chronic stress. These stress-induced changes, combined with ongoing developmental shifts in the eCB system may amplify the risk for STBs in adolescents. For example, early-life stress, such as childhood maltreatment or interpersonal violence exposure, has been linked to alterations in circulating eCB concentrations in adolescents and adults and is known to modulate the risk for MDD and substance use disorders (Perini et al. 2023; Marusak et al. 2024; Mazurka et al. 2024). Thus, early-life stress or other risk factors might induce eCB system fluctuations, predisposing some youth to STBs. This is particularly interesting given the well-documented associations between early-life stress and suicide risk (Ortin-Peralta et al. 2021).

Alternatively, or perhaps concurrently, alterations in the eCB system (i.e., higher receptor density and signaling, and/or elevated eCB concentrations) may predispose individuals with risk factors for STBs. In other words, in individuals at risk for STBs, the eCB system may be altered by factors other than incidences of stress. For example, the CB1 receptor antagonist, rimonabant, has been associated with worsened psychopathology, including SI and attempts, which ultimately precluded its market approval during clinical trials for obesity (Christensen et al. 2007; Topol et al. 2010; King 2010). Notably, SI and attempts were reported several months into rimonabant administration, suggesting that prolonged receptor blockade could contribute to STB risk.

Compared to adults, adolescents may be disproportionately impacted by STBs given the inherent ongoing developmental changes in the eCB system. In humans, circulating eCBs appear to decline with age across adolescence (Marusak et al. 2024), and preclinical data suggest that CB1 receptors may also follow a similar decline (Meyer et al. 2018). It may be possible that the degree of eCB system fluctuations during development play a critical role in STB risk, and that nontypical changes (e.g., more or less pronounced changes) leave youth particularly vulnerable

to STB development until the system reaches a more stable and calibrated state later in development.

Conversely, it is also plausible that STBs themselves predispose individuals to altered eCB function. The studies discussed above examined eCB system alterations primarily in adults with a history of suicide attempts, raising the possibility that these changes result from the extreme stress, pain, or tissue damage associated with such events. The proximity of STBs may induce internal stress, potentially leading to eCB system alterations. If so, the relationship between the eCB system and STBs might represent a feedback loop, akin to other neurobiological mechanisms. Alternatively, eCB system changes in STBs could be a confounding effect of another factor, such as chronic stress, or an epiphenomenon—a secondary effect or byproduct rather than a direct contributor to the pathophysiology or maintenance of STBs. This perspective aligns with recent neurobiological theories challenging the serotonin deficit model in depression, which suggest that serotonin-related changes may not be causally linked to depression but instead reflect a more complex role of the serotonin system in the pathophysiology and treatment of MDD (Page et al. 2024). These theories suggest that serotonergic antidepressants exert modulatory effects on brain circuits implicated in MDD rather than correcting a simple deficit. Similarly, the role of the eCB system in STBs may follow parallel patterns, warranting further investigation.

In summary, current evidence supports associations between the eCB system—including brain tissue and circulating concentrations, as well as alterations in CB1 receptor density and signaling—and suicide risk. However, causal inferences cannot yet be drawn. Most studies have been conducted in adults, who may be more influenced by the long-term effects of chronic stress, disease, or confounding factors such as medication use or pain. Research in adolescents, who are closer to the typical onset of STBs, is scarce, with only one published abstract identified.

Another limitation is that adult studies have primarily examined eCB system alterations in the context of recent fatal or nonfatal suicide attempts. It remains unclear whether similar—or different—eCB system patterns are present in individuals with other forms or severities of STBs, such as ideation or NSSI. While animal models of chronic stress provide relevant insights, modeling STBs in animals is inherently challenging, complicating efforts to determine whether observed findings are specific to STBs rather than reflective of depressed mood or chronic stress.

Furthermore, existing human studies have largely measured eCB concentrations in a resting state unperturbed by stress. Given the eCB system's critical role in acute and chronic stress responses, future research should investigate eCB system activity at rest, in response to acute stress, and over time. Incorporating longitudinal and co-twin study designs (see Chap. 19) could help disentangle developmental trajectories of eCB associations and account for shared genetic and environmental influences. For instance, a common genetic variant (rs324420) in the gene encoding the FAAH enzyme, which is associated with 11–20% higher AEA concentrations in adolescents and adults (Sipe et al. 2010; Mayo et al. 2018; Marusak et al. 2024), represents a promising area for exploration. Future studies should integrate

genotyping to further elucidate the genetic underpinnings of eCB system alterations in the context of STBs.

An important consideration for future research is the role of substance use—particularly cannabis—during adolescence. Cannabis use is highly prevalent, with recent data from Monitoring the Future reporting that 26% of 12th-grade US students used cannabis in the past year (Miech et al. 2024). Cannabis is known to influence the eCB system (as discussed in Chaps. 2–3), and adolescent-onset cannabis use is associated with altered psychiatric and cognitive outcomes, as well as neurodevelopmental changes (see Chaps. 11–14).

Cannabis use is also common among adolescents and adults with STBs and psychiatric comorbidities, with many individuals reporting its use as a form of self-medication for their symptoms (Osborn et al. 2015). Recent studies have found significant associations between nondisordered cannabis use and depressive symptoms or SI in adolescents (Sultan et al. 2023; Hinckley et al. 2023). Moreover, findings from studies of the eCB system in STBs have demonstrated that results can change significantly when individuals with cannabis use are excluded (Herranz-Herrer et al. 2020), underscoring the importance of accounting for this variable.

In addition, evidence suggests that adolescents who use cannabis may be at increased risk of developing MDD and SI in adulthood (Gobbi et al. 2019). Conversely, early depressive symptoms in adolescence are associated with a higher likelihood of developing cannabis use disorder later in adolescence (Rhew et al. 2017). While there are documented associations between cannabis use, depressive symptoms, and STBs, the directionality and causality of these relationships remain unclear. It is plausible that cannabis use could precipitate or exacerbate suicide risk in individuals predisposed to or already experiencing STBs. Components of cannabis, particularly THC, activate CB1 receptors and alter eCB system function. For example, chronic cannabis use has been linked to elevated circulating AEA levels during periods of abstinence (Boachie et al. 2023). Since STBs are associated with altered circulating AEA and 2-AG levels at rest, it is conceivable that chronic cannabis use could heighten vulnerability to STBs, particularly during abstinence.

Conversely, adolescents with STBs might use cannabis as a coping mechanism to manage their thoughts and behaviors (Osborn et al. 2015). Research evidence, though mixed, implicates a dysfunctional eCB system in STBs. For example, one study found evidence for elevated plasma AEA concentrations in individuals with a prior suicide attempt only after excluding individuals who used cannabis (Herranz-Herrer et al. 2020), suggesting that cannabis use might "stabilize" eCB system dysfunction. This could imply that some individuals with STBs use cannabis as a means of self-regulation through its effects on the eCB system.

Regardless of these possibilities, it is critical for future research to consider cannabis and other substance use in the design, analysis, and interpretation of studies exploring the relationship between the eCB system and STBs. This will help uncover the nuances of these interactions and their implications for understanding and addressing suicide risk.

6 Conclusion

The incidence of STBs begins to rise in late childhood and increases exponentially throughout adolescence before plateauing into young adulthood (Nock et al. 2013). While the exact cause of this dramatic onset remains unclear, evidence suggests that adolescence is a period of heightened vulnerability due to increased incidence of psychosocial stress and substantial developmental changes in biological stress and emotion regulatory systems (Miller and Prinstein 2019). The eCB system has recently garnered attention for its role in adult suicide risk, primarily due to its modulatory effects on mood, stress responding, and emotion regulation. Postmortem and in vivo studies have identified eCB system alterations in adults who died by suicide or had a history of nonfatal suicide attempts compared to controls. These findings reveal notable similarities and differences with animal models of chronic stress—a model often used to study core features of depression, such as anhedonia.

However, a critical gap exists: the lack of studies investigating the relationship between the eCB system and STBs during human adolescence. We identified only one published abstract in adolescents, showing lower circulating eCBs in adolescents with NSSI compared to controls. Addressing this gap is essential, as adolescence represents a period of substantial neurodevelopment and heightened vulnerability to suicide risk. Future research should prioritize understanding how eCB system changes during this developmental window contribute to STBs, potentially providing valuable insights for early intervention and prevention strategies.

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References

Alacreu-Crespo A, Sebti E, Moret RM, Courtet P. From social stress and isolation to autonomic nervous system dysregulation in suicidal behavior. Curr Psychiatry Rep. 2024;26:312–22.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders fifth edition text revision. 5th TR ed. American Psychiatric Association, Arlington, VA; 2022.

Başgöze Z, Wiglesworth A, Carosella KA, et al. Depression, non-suicidal self-injury, and suicidality in adolescents: common and distinct precursors, correlates, and outcomes. J Psychiatr Brain Sci. 2021;6:e210018.

Berardelli I, Serafini G, Cortese N, et al. The involvement of hypothalamus–pituitary–adrenal (HPA) axis in suicide risk. Brain Sci. 2020;10:1–12.

Bloemhof-Bris E, Meiri D, Sulimani L, et al. Alterations in plasma endocannabinoid concentrations among individuals with major depression treated with electroconvulsive therapy. Psychiatry Res. 2024;337:115967.

Bluett RJ, Gamble-George JC, Hermanson DJ, et al. Central anandamide deficiency predicts stress-induced anxiety: behavioral reversal through endocannabinoid augmentation. Transl Psychiatry. 2014;4:e408.

Boachie N, Gaudette E, Bazinet RP, et al. Circulating endocannabinoids and n-acylethanolamines in individuals with cannabis use disorder—preliminary findings. Brain Sci. 2023;13:1375.
Bortolato M, Mangieri RA, Fu J, et al. Antidepressant-like activity of the fatty acid amide hydrolase inhibitor URB597 in a rat model of chronic mild stress. Biol Psychiatry. 2007;62:1103–10.
Brausch AM, Clapham RB, Littlefield AK. Identifying specific emotion regulation deficits that associate with nonsuicidal self-injury and suicide ideation in adolescents. J Youth Adolesc. 2022;51:556–69.
Brellenthin AG, Crombie KM, Hillard CJ, Koltyn KF. Endocannabinoid and mood responses to exercise in adults with varying activity levels. Transl J Am Coll Sports Med. 2017;2:138–45.
Buelens T, Luyckx K, Kiekens G, et al. Investigating the DSM-5 criteria for non-suicidal self-injury disorder in a community sample of adolescents. J Affect Disord. 2020;260:314–22.
Buhle JT, Silvers JA, Wage TD, et al. Cognitive reappraisal of emotion: a meta-analysis of human neuroimaging studies. Cereb Cortex. 2014;24:2981–90.
Casey BJ, Heller AS, Gee DG, Cohen AO. Development of the emotional brain. Neurosci Lett. 2019;693:29.
Centers for Disease Control and Prevention. Youth risk behavior survey data summary & trends report: 2011–2021. U.S Department of Health and Human Services; 2023.
Christensen R, Kristensen PK, Bartels EM, et al. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet. 2007;370:1706–13.
Coccaro EF, Hill MN, Robinson L, Lee RJ. Circulating endocannabinoids and affect regulation in human subjects. Psychoneuroendocrinology. 2018;92:66–71.
Dean B, Sundram S, Bradbury R, et al. Studies on [^{3}H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. Neuroscience. 2001;103:9–15.
Denny BT, Jungles ML, Goodson PN, et al. Unpacking reappraisal: a systematic review of fMRI studies of distancing and reinterpretation. Soc Cogn Affect Neurosci. 2023;18:1–12.
deRoon-Cassini TA, Stollenwerk TM, Beatka M, Hillard CJ. Meet your stress management professionals: the endocannabinoids. Trends Mol Med. 2020;26:953.
Desai S, Borg B, Cuttler C, et al. A systematic review and meta-analysis on the effects of exercise on the endocannabinoid system. Cannabis Cannabinoid Res. 2022;7:388.
Donald Burns H, Van Laere K, Sanabria-Bohó Rquez S, et al. [^{18}F]MK-9470, a positron emission tomography (PET) tracer for in vivo human PET brain imaging of the cannabinoid-1 receptor. Proc Natl Acad Sci. 2007;104:9800–5.
Eisenlohr-Moul TA, Miller AB, Giletta M, et al. HPA axis response and psychosocial stress as interactive predictors of suicidal ideation and behavior in adolescent females: a multilevel diathesis-stress framework. Neuropsychopharmacology. 2018;43:2564–71.
Erdozain AM, Rubio M, Valdizan EM, et al. The endocannabinoid system is altered in the postmortem prefrontal cortex of alcoholic subjects. Addict Biol. 2015;20:773–83.
Ferger M, Höper S, van der Venne P, et al. Endocannabinoid system alterations in adolescents with non-suicidal self-injurious behavior and childhood trauma. Biol Psychiatry. 2023;93:S12.
Franklin JC, Ribeiro JD, Fox KR, et al. Risk factors for suicidal thoughts and behaviors: a meta-analysis of 50 years of research. Psychol Bull. 2017;143:187–232.
Gili M, Castellví P, Vives M, et al. Mental disorders as risk factors for suicidal behavior in young people: a meta-analysis and systematic review of longitudinal studies. J Affect Disord. 2019;245:152–62.
Gobbi G, Atkin T, Zytynski T, et al. Association of cannabis use in adolescence and risk of depression, anxiety, and suicidality in young adulthood: a systematic review and meta-analysis. JAMA Psychiatry. 2019;76:426.
Gowatch LC, Evanski JM, Ely SL, et al. Endocannabinoids and stress-related neurospsychiatric disorders: a systematic review and meta-analysis of basal concentrations and response to acute psychosocial stress. Cannabis Cannabinoid Res. 2024;9:1217–34.
Herman JP, Nawreen N, Smail MA, Cotella EM. Brain mechanisms of HPA axis regulation: neurocircuitry and feedback in context Richard Kvetnansky lecture. Stress. 2020;23:617.

Heron M. Deaths: leading causes for 2019. Natl Vital Stat Rep. 2021;70:1.
Herranz-Herrer J, Gil-Benito E, Ponte-López T, et al. Serum endocannabinoid levels in suicide attempters: a pilot study. Eur Neuropsychopharmacol. 2020;40:52–60.
Hill MN, Patel S, Carrier EJ, et al. Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. Neuropsychopharmacology. 2005;30:508–15.
Hill MN, Carrier EJ, McLaughlin RJ, et al. Regional alterations in the endocannabinoid system in an animal model of depression: effects of concurrent antidepressant treatment. J Neurochem. 2008;106:2322–36.
Hillard CJ. Circulating endocannabinoids: from whence do they come and where are they going? Neuropsychopharmacology. 2018;43:155–72.
Hillard CJ, Beatka M, Sarvaideo J. Endocannabinoid signaling and the hypothalamic-pituitary-adrenal axis. Compr Physiol. 2017;7:1–15.
Hinckley JD, Mikulich-Gilbertson SK, He J-P, et al. Cannabis use is associated with depression severity and suicidality in the national comorbidity survey−adolescent supplement. JAACAP Open. 2023;1:24–35.
Hohmann AG, Suplita RL, Bolton NM, et al. An endocannabinoid mechanism for stress-induced analgesia. Nature. 2005;435:1108–12.
Hong S, Zheng G, Wu X, et al. Corticosterone mediates reciprocal changes in CB1 and TRPV1 receptors in primary sensory neurons in the chronically stressed rat. Gastroenterology. 2011;140:627–637.e4.
Hungund BL, Vinod KY, Kassir SA, et al. Upregulation of CB1 receptors and agonist-stimulated [^{35}S]GTPγS binding in the prefrontal cortex of depressed suicide victims. Mol Psychiatry. 2004;9:184–90.
Johnston JN, Campbell D, Caruncho HJ, et al. Suicide biomarkers to predict risk, classify diagnostic subtypes, and identify novel therapeutic targets: 5 years of promising research. Int J Neuropsychopharmacol. 2022;25:197.
Joiner T. Why people die by suicide. Cambridge, MA: Harvard University Press; 2005.
Joshi N, Onaivi ES. Endocannabinoid system components: overview and tissue distribution. Adv Exp Med Biol. 2019;1162:1–12.
Kim MJ, Do M, Han D, et al. Proteomic profiling of postmortem prefrontal cortex tissue of suicide completers. Transl Psychiatry. 2022;12(1):1–10.
King A. Neuropsychiatric adverse effects signal the end of the line for rimonabant. Nature Rev Cardio. 2010;l7:602.
Klimes-Dougan B, Begnel E, Almy B, et al. Hypothalamic-pituitary-adrenal axis dysregulation in depressed adolescents with non-suicidal self-injury. Psychoneuroendocrinol. 2019;102:216–24.
Klonsky D, May AM. The three-step theory (3ST): a new theory of suicide rooted in the "ideation-to-action" framework. Int J Cogn Ther. 2015;8:114–29.
Klonsky D, Pachkowski MC, Shahnaz A, May AM. The three-step theory of suicide: description, evidence, and some useful points of clarification. Prev Med (Baltim). 2021;152:106549.
Liu RT, Bettis AH, Burke TA. Characterizing the phenomenology of passive suicidal ideation: a systematic review and meta-analysis of its prevalence, psychiatric comorbidity, correlates, and comparisons with active suicidal ideation. Psychol Med. 2020;50:367–83.
Lu HC, MacKie K. An introduction to the endogenous cannabinoid system. Biol Psychiatry. 2016;79:516–25.
Mannekote Thippaiah S, Iyengar SS, Vinod KY. Exo-and endo-cannabinoids in depressive and suicidal behaviors. Front Psych. 2021;12:636228.
Martinez Ramirez CE, Ruiz-Pérez G, Stollenwerk TM, et al. Endocannabinoid signaling in the central nervous system. Glia. 2023;71:5–35.
Marusak HA, Ely SL, Zundel CG, et al. Endocannabinoid dysregulation and PTSD in urban adolescents: associations with anandamide concentrations and FAAH genotype. Psychopharmacology (Berl). 2024:1–12.
Mato S, Pilar-Cuéllar F, Valdizán EM, et al. Selective up-regulation of cannabinoid CB1 receptor coupling to go-proteins in suicide victims with mood disorders. Biochem Pharmacol. 2018;157:258.

Mayo LM, Asratian A, Lindé J, et al. Protective effects of elevated anandamide on stress and fear-related behaviors: translational evidence from humans and mice. Mol Psychiatry. 2018;25:993–1005.
Mazurka R, Harkness KL, Hassel S, et al. Endocannabinoid concentrations in major depression: effects of childhood maltreatment and relation to hippocampal volume. Transl Psychiatry. 2024;14:431.
McLaughlin RJ, Hill MN, Gorzalka BB. A critical role for prefrontocortical endocannabinoid signaling in the regulation of stress and emotional behavior. Neurosci Biobehav Rev. 2014;42:116–31.
Meyer HC, Lee FS, Gee DG. The role of the endocannabinoid system and genetic variation in adolescent brain development. Neuropsychopharmacology. 2018;43:21–33.
Miech RA, Johnston LD, Patrick ME, O'malley PM. Monitoring the future national survey results on drug use, 1975–2024: overview and key findings for secondary school students. In: Monitoring the Future Monograph Series; 2024.
Miller AB, Eisenlohr-Moul TA. Biological responses to acute stress and suicide: a review and opportunities for methodological innovation. Curr Behav Neurosci Rep. 2019;6:141–50.
Miller AB, Prinstein MJ. Adolescent suicide as a failure of acute stress-response systems. Annu Rev Clin Psychol. 2019;15:425–50.
Miller AB, McLaughlin KA, Busso DS, et al. Neural correlates of emotion regulation and adolescent suicidal ideation emotion regulation and suicidal ideation. Biol Psychiatry Cogn Neurosci Neuroimaging. 2018;3:125–32.
Miller AB, Jenness JL, Elton AL, et al. Neural markers of emotion reactivity and regulation before and after a targeted social rejection: differences among girls with and without suicidal ideation and behavior histories. Biol Psychiatry. 2024;95:1100–9.
Morena M, Roozendaal B, Trezza V, et al. Endogenous cannabinoid release within prefrontal-limbic pathways affects memory consolidation of emotional training. Proc Natl Acad Sci. 2014;111:18333–8.
Morena M, Patel S, Bains JS, Hill MN. Neurobiological interactions between stress and the endocannabinoid system. Neuropsychopharmacology. 2016;41:80–102.
Naughton M, Dinan TG, Scott LV. Corticotropin-releasing hormone and the hypothalamic–pituitary–adrenal axis in psychiatric disease. Handb Clin Neurol. 2014;124:69–91.
Nicolopoulos A, Boydell K, Shand F, Christensen H. Why suicide?: reasons for suicide attempts as self-reported by youth: a systematic evaluation of qualitative studies. Adolesc Res Rev. 2018;3:155–72.
Nock MK, Green JG, Hwang I, et al. Prevalence, correlates and treatment of lifetime suicidal behavior among adolescents: results from the national comorbidity survey replication—adolescent supplement (NCS-A). JAMA Psychiatry. 2013;70:300–10.
O'Brien KHMM, Nicolopoulos A, Almeida J, et al. Why adolescents attempt suicide: a qualitative study of the transition from ideation to action. Arch Suicide Res. 2021;25:269–86.
O'Connor RC, Kirtley OJ. The integrated motivational-volitional model of suicidal behaviour. Philos Trans R Soc Lond B Biol Sci. 2018;373:20170268.
Ortin-Peralta A, Myruski S, Rosario-Williams B, Xu M. Early childhood adversity, stress, and developmental pathways of suicide risk. In: Miranda R, Jeglic EL, editors. Handbook of youth suicide prevention: integrating research into practice. Switzerland: Springer Nature; 2021.
Osborn LA, Lauritsen KJ, Cross N, et al. Self-medication of somatic and psychiatric conditions using botanical marijuana. J Psychoactive Drugs. 2015;47:345–50.
Page CE, Epperson CN, Novick AM, et al. Beyond the serotonin deficit hypothesis: communicating a neuroplasticity framework of major depressive disorder. Mol Psychiatry. 2024;29:3802.
Perini I, Mayo LM, Capusan AJ, et al. Resilience to substance use disorder following childhood maltreatment: association with peripheral biomarkers of endocannabinoid function and neural indices of emotion regulation. Mol Psychiatry. 2023;28:2563–71.
Raichlen DA, Foster AD, Gerdeman GL, et al. Wired to run: exercise-induced endocannabinoid signaling in humans and cursorial mammals with implications for the 'runner's high. J Exp Biol. 2012;215:1331–6.

Rhew IC, Fleming CB, Vander Stoep A, et al. Examination of cumulative effects of early adolescent depression on cannabis and alcohol use disorder in late adolescence in a community-based cohort. Addict. 2017;112:1952–60.

Rossi S, De Chiara V, Musella A, et al. Preservation of striatal cannabinoid CB1 receptor function correlates with the antianxiety effects of fatty acid amide hydrolase inhibition. Mol Pharmacol. 2010;78:260–8.

Schmaal L, van Harmelen AL, Chatzi V, et al. Imaging suicidal thoughts and behaviors: a comprehensive review of 2 decades of neuroimaging studies. Mol Psychiatry. 2020;25:408–27.

Sher L, Bierer LM, Flory J, et al. Endogenous cannabinoid levels and suicidality in combat veterans. Psychiatry Res. 2020;287:112495.

Sipe JC, Scott TM, Murray S, et al. Biomarkers of endocannabinoid system activation in severe obesity. PLoS One. 2010;5:e8792.

Siris SG. Suicide and schizophrenia. J Psychopharmacol. 2001;15:127–35.

Substance Abuse and Mental Health Services Administration. Key substance use and mental health indicators in the United States: results from the 2021 National Survey on Drug Use and Health (HHS Publication No. PEP22–07–01-005, NSDUH Series H-57). Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration; 2022.

Sultan RS, Zhang AW, Olfson M, et al. Nondisordered cannabis use among US adolescents. JAMA Netw Open. 2023;6:E2311294.

Terry GE, Liow JS, Zoghbi SS, et al. Quantitation of cannabinoid CB1 receptors in healthy human brain using positron emission tomography and an inverse agonist radioligand. NeuroImage. 2009;48:362–70.

Topol EJ, Bousser MG, Fox KA, et al. Rimonabant for prevention of cardiovascular events (CRESCENDO): a randomised, multicentre, placebo-controlled trial. Lancet. 2010;376:517–23.

Vargas-Medrano J, Diaz-Pacheco V, Castaneda C, et al. Psychological and neurobiological aspects of suicide in adolescents: current outlooks. Brain Behav Immun. Health. 2020;7:100124.

Vinod KY, Arango V, Xie S, et al. Elevated levels of endocannabinoids and CB1 receptor-mediated G-protein signaling in the prefrontal cortex of alcoholic suicide victims. Biol Psychiatry. 2005;57:480–6.

Wang W, Sun D, Pan B, et al. Deficiency in endocannabinoid signaling in the nucleus accumbens induced by chronic unpredictable stress. Neuropsychopharmacology. 2010;35:2249–61.

Westlund Schreiner M, Cullen KR, Klimes-Dougan B. Multi-level analysis of the functioning of the neurobiological threat system in adolescents: implications for suicide and nonsuicidal self-injury. Curr Behav Neurosci Rep. 2017;4:79–86.

Chapter 18
Does Cannabis Use Increase Risk of Psychotic Disorders? A Critical Examination of Causality and Differential Vulnerability

Ali Sloan and Jonathan D. Schaefer

1 Introduction

The recent liberalization of cannabis policies across the United States has increased concern about the potentially detrimental effects of cannabis on mental health—and, in particular, psychosis. Nearly 50% of people who use cannabis report psychotic symptoms or psychotic-like experiences following the use of cannabis, most commonly paranoia and hallucinations, which typically resolve within a few hours of use (Murray et al. 2017; Schoeler et al. 2022). Human laboratory studies have further demonstrated that acute cannabis administration reliably produces a wide range of transient positive (e.g., delusions, hallucinations, disorganization), negative (e.g., avolition, diminished emotional expression), and cognitive symptoms that resemble psychotic disorders in a time locked and dose-dependent fashion in non-psychiatric controls (D'Souza et al. 2004; Morrison et al. 2011; Sherif et al. 2016). The primary mechanism responsible for this effect is thought to be the activation of cannabinoid—specifically CB1—receptors in the brain by tetrahydrocannabinol (THC), leading to altered dopaminergic signaling in the mesolimbocortical system and prefrontal cortex, which, in turn, contributes to the emergence of psychotic symptoms (Fiorentini et al. 2021; Garson et al. 2023; Luzi et al. 2008).

While THC's ability to induce short-term psychotic symptoms is well-established, the nature of the relationship between cannabis use and chronic psychotic disorders has sparked much controversy. While cannabis use has been associated with mood disorders that can involve psychotic symptoms (e.g., bipolar disorder), the relationship with nonaffective psychotic disorders has received far more empirical attention and subsequent support (Sideli et al. 2020b). Nonaffective psychotic disorders

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include diagnoses of schizophreniform disorder, schizoaffective disorder, schizophrenia, brief psychotic disorder, delusional disorder, and psychosis not otherwise specified. These disorders are chronic illnesses characterized by positive, negative, mood, and cognitive symptoms with a typical onset in late adolescence or early adulthood (Charlson et al. 2018; Solmi et al. 2023). Those who are diagnosed with nonaffective psychotic disorders may experience complete remission from psychotic symptoms, but these symptoms can also reoccur, leading to additional psychotic episodes throughout one's lifetime. While the prevalence of nonaffective psychotic disorders is estimated to be less than one percent (Radua et al. 2018), it is associated with a considerable burden of disease (Charlson et al. 2018) and affects approximately 5% of individuals with cannabis use disorder (Libuy et al. 2018).

Some have claimed that reducing cannabis use reduces risk for developing psychotic disorders (Fischer et al. 2023), implying that cannabis use plays a causal role in their etiology. However, research on the relationship between cannabis use and nonaffective psychotic disorders conducted to date has generated mixed results, leading to three, nonexclusive possibilities: (1) cannabis is a causal risk factor for psychosis (simple causation), (2) predisposition to psychosis leads to cannabis use (reverse causation), or (3) correlations between cannabis and psychosis arise due to shared vulnerability factors (e.g., genetics, childhood adversity) (Hamilton and Monaghan 2019).

The simple causation hypothesis suggests that cannabis use may trigger the onset of a nonaffective psychotic disorder in vulnerable individuals who may not have otherwise developed it if they had never used the substance (or, perhaps, if they had used less). Support for this hypothesis comes from a large body of cross-sectional and longitudinal epidemiological studies, with a recent review of systematic reviews and meta-analyses concluding both that psychotic illnesses are more prevalent in cannabis users compared to nonusers and that cannabis use is associated with increased risk of developing psychotic illness (Hasan et al. 2020). Although various mechanistic hypotheses have been proposed regarding the biological pathways linking cannabis use to chronic psychotic disorders, no single hypothesis has received consistent support. Just as the activation of cannabinoid receptors by THC leading to altered dopaminergic signaling is hypothesized to explain the ability of cannabis to generate short-term psychotic symptoms, chronic THC exposure is hypothesized to impact the reactivity of the endocannabinoid system, which disrupts the regulation of glutamatergic signaling and contributes to altered dopaminergic activity favoring psychotic phenomena (Leiderman 2021; Rentero et al. 2021; Sánchez-Blázquez et al. 2013; Sideli et al. 2020b; Xenaki et al. 2023).

It is also possible that individuals with a predisposition to psychosis are more likely to use cannabis. One version of "reverse causation," termed the "self-medication hypothesis," suggests that individuals who begin experiencing prodromal symptoms of psychosis may turn to cannabis and other drugs in an attempt to ameliorate them (van Winkel and Kuepper 2014). The initial onset of a psychotic

disorder—termed a "first episode" of psychosis—is often preceded by a period ranging from several days to years in which the individual begins experiencing attenuated symptoms. At this time, the person is then considered to be at clinical high risk for psychosis (abbreviated "CHR") and, should they develop a psychotic disorder, this initial period of attenuated symptoms is then considered their prodromal phase of the illness. CHR individuals who use cannabis have reported doing so for mood enhancement, suggesting that emerging negative—not positive—symptoms (e.g., affective flattening, anhedonia, or avolition) may drive cannabis use in this population (Gill et al. 2015). However, evidence consistently suggests cannabis use precedes both prodromal symptoms and first episodes of psychosis for most individuals diagnosed with a psychotic illness (Andréasson et al. 1987; Arseneault 2002; van Winkel and Kuepper 2014; Volkow et al. 2016). One meta-analysis found the onset of cannabis use to precede that of psychosis by 6 years, and that cannabis use significantly declined after the onset of psychosis (Myles et al. 2016). Further, in a sample of 246 individuals within the first three years of psychotic illness onset, 94% of those who reported lifetime cannabis use reported use prior to their first episode of psychosis and 78% reported use prior to prodromal symptom onset (Kline et al. 2022). Finally, another study found that predisposition to psychosis—defined as scoring highly in paranoid ideation and psychoticism—did not predict future cannabis use (Henquet et al. 2005). Taken together, the evidence suggests that the self-medication hypothesis may only be true for a small fraction of individuals who develop a nonaffective psychotic disorder.

Lastly, the association between cannabis and psychosis may be attributed to shared vulnerability factors. For example, genetic predisposition has been shown to influence the risk of both cannabis involvement and psychotic disorders—including schizophrenia—and numerous studies suggest certain genetic variants simultaneously increase risk of both phenotypes (Bogdan et al. 2016; Hamilton and Sumnall 2021; Kendler et al. 2021; Murray et al. 2016; Power et al. 2014; Robinson and Bergen 2021). Similarly, environmental risk factors may also be shared between cannabis use and psychotic disorders (Shakoor et al. 2015). For instance, childhood adversity has been associated with increased risk for both cannabis use (Bogdan et al. 2016) and psychosis (Robinson and Bergen 2021). The concurrent use of other substances, such as tobacco and alcohol, has also been considered a potential confounder, yielding mixed results. Some studies have found that concurrent substance use confounds the relationship between cannabis use and psychosis (Ryan et al. 2020), while others report that the association persists after controlling for other substance use (Kuepper et al. 2011). Notably, cannabis use has been linked to increased use of other substances, and almost every type of substance use has been independently associated with risk for psychosis (Kuepper et al. 2011). Considering these shared vulnerability factors, it is imperative to rigorously examine the extent to which the relationship between cannabis use and the risk of developing psychotic disorders is explained by confounding variables, to determine whether cannabis plays a causal role.

2 Causally Informative Research Designs

Because the effects of long-term cannabis use on psychosis risk cannot be studied experimentally, some researchers have attempted to distinguish between these different etiological hypotheses using quasi-experimental, causally informative observational research designs. To date, these studies have generally fallen into two categories: (1) co-relative comparisons or (2) cannabis legalization studies. This section examines the evidence drawn from these two categories of causally informative research designs to evaluate the potential causal role of cannabis in the development of psychotic disorders.

2.1 Co-relative Comparisons

Co-relative designs most commonly compare siblings who differ in their exposure to cannabis, exploiting genetic and environmental similarities to rule out possible confounders. Full siblings, for example, share 50% of their segregating genes, as well as aspects of their shared family, school, and neighborhood environments. Thus, if the member of the sibling pair who uses more cannabis is found to also be more likely to develop a psychotic disorder, this supports the hypothesis that there is a causal relationship. If, on the other hand, siblings who differ in their cannabis use are equally likely to develop a psychotic disorder, this would suggest the relationship between cannabis and psychosis is likely noncausal, driven instead by shared environmental or genetic factors (sometimes also referred to as "familial liability"). Perhaps the most significant limitation of all co-relative comparison studies, however, is that they cannot rule out the possibility of confounding due to nonshared factors, which includes anything that makes members of a sibling pair different (e.g., personality, IQ, victimization history, etc.).

Sibling comparison studies of cannabis and psychosis have generated mixed results, suggesting that a substantial portion of the association reflects familial liability, but also finding some evidence of potential causal effects. One study of 228 sibling pairs from an Australian birth cohort reported that siblings who initiated cannabis use earlier scored higher on a measure of delusional-like experiences at the age 21 follow-up ($F_{1,213} = 18.5$; $p < 0.001$) but were not significantly more likely to receive a diagnosis of nonaffective psychosis ($F_{1,5} = 0.60$; $p = 0.49$) (McGrath et al. 2010). A Swedish registry study, on the other hand, reported that associations between cannabis abuse and schizophrenia observed in the general population (OR: 10.44, 95% CI: 8.99–12.11) shrank to roughly half when analyses were repeated in discordant full-sibling pairs (OR: 5.07, 95% CI: 4.17–6.16) (Giordano et al. 2015). However, even in these models the sibling with a diagnosis of cannabis abuse was significantly more likely to also receive a diagnosis of schizophrenia, consistent with a possible causal effect.

Twin comparison studies afford researchers all the advantages of full-sibling comparison designs while additionally providing more robust controls for shared environmental factors (given that twins, unlike non-twin siblings, experience these factors at the same age), as well as genetic propensity (in the case of monozygotic twins, who are genetically identical). Results from these studies have also been mixed. One study of a population-based Norwegian twin cohort reported that controlling for shared genetic and environmental factors in co-twin control analyses reduced the individual-level association between cannabis use disorder (CUD) and psychotic-like experiences by roughly 50% (Nesvåg et al. 2017). However, the within-pair effect was still significant (IRR: 3.5, 95% CI: 1.5, 8.2), indicating that twins with CUD were still more likely to report psychotic-like experiences relative to their co-twin without CUD. Similarly, a second study combining data from the Human Connectome Project and Australian Twin Registry reported that, in their sample of over 300 twin pairs, twins reporting frequent cannabis use (≥100 lifetime uses), diagnosed with CUD, or testing positive for current cannabis use were significantly more likely to report psychotic-like experiences than their co-twins who reported less use (βs = 0.23–0.41, Ps < 0.05) (Karcher et al. 2019). A third study, however, using longitudinal data from over 600 twin pairs participating in the Minnesota Twin and Family Study, found that twins diagnosed with CUD or scoring higher on a measure of cumulative adolescent cannabis use were statistically indistinguishable from their less-cannabis-using co-twins on a measure of adult psychoticism (estimates ranging from −0.04 to 0.06 for CUD and from −0.01 to 0.01 for cannabis use), suggesting no causal effect of cannabis use (Schaefer et al. 2021b). Given the many methodological differences that characterize these studies, it is difficult to determine whether the mixed results reflect differences in geography, age, sample size, cannabis use and psychoticism ascertainment strategies, or all the above. It is worth noting, however, that only one co-relative control study conducted to date has used a prospectively assessed, continuous measure of cannabis use, and this study found no evidence of a causal effect (Schaefer et al. 2021b).

2.2 Cannabis Legalization Studies

Cannabis legalization studies are a type of quasi-experimental study design that leverage natural policy changes to infer causal relationships. By examining changes in psychosis rates before and after legalization within states, and comparing these changes to states without legalization, researchers can better isolate the impact of cannabis policy from other confounding factors. This approach offers several advantages over traditional observational studies that examine the cannabis-psychosis relationship at the individual level. Legalization studies clearly establish temporal precedence, addressing the issue of reverse causality. They implicitly control for time-invariant confounders by comparing changes within states over time. In addition, these studies reduce selection bias by affecting entire populations rather than self-selected cannabis users, and the quasi-random timing of legalization across

states approximates conditions of a randomized controlled trial at the population level. Research on cannabis legalization in the United States conducted examining other outcomes has linked shifts to less restrictive cannabis policies to both positive and negative outcomes, including decreased arrest rates (Plunk et al. 2019), increased clearance rates for violent crimes (Wu et al. 2022), increased rates of cannabis use disorder (Cerdá et al. 2020; Hasin et al. 2017), and increased rates of self-harm among men younger than 40 years (Matthay et al. 2021).

One Colorado-based study used hospital administrative data on county-level emergency department visits between January 1, 2013 and December 31, 2018 to examine how new exposure to recreational cannabis dispensaries following statewide legalization in 2014 differentially influenced rates of emergency department visits for psychosis and schizophrenia. This study found that while psychosis visits increased by 24% following legalization, there was no significant increase in rates of schizophrenia diagnoses (Wang et al. 2022). Two similar studies examined how emergency departments presentations for psychosis and psychotic disorders changed before and after cannabis legalization in Canada, finding no evidence of significant legalization effects on post-legalization counts of cannabis-induced psychosis, schizophrenia, or other psychotic disorders (Callaghan et al. 2022; Vignault et al. 2021). Finally, a broad-scale, US-based study conducted a year later using commercial and Medicare Advantage claims data for beneficiaries between 2003 and 2017 also concluded that states with recreational legalization policies and cannabis retail outlets experienced no statistically significant increase in rates of psychosis-related diagnoses or prescribed antipsychotics following the institution of these policy changes, and that effects were even smaller for states passing more restrictive policies (e.g., medical legalization only, no retail outlets) (Elser et al. 2023).

3 Differential Vulnerability

While the causally informative literature thus does not converge neatly on a single answer regarding the causal status of cannabis-psychosis associations, the mixed results observed across different cohorts and study designs suggest that if cannabis use is a causal risk factor for psychosis at the population-level, its effects are likely to be small, and their detection may also depend on a number of yet-unknown study characteristics. Some proponents of the simple causation hypothesis have advanced the notion that cannabis use may appreciably increase risk of adverse outcomes only in certain, more vulnerable individuals—a variation on simple causation we'll refer to as "the differential vulnerability hypothesis" (e.g., Curran et al. 2016). A corollary of the differential vulnerability hypothesis is that the proportion of "vulnerable" individuals is likely to vary across study samples. While this variability could, in

theory, explain some of the mixed results reviewed above, a significant drawback of the differential vulnerability hypothesis is that it is difficult to falsify, given the many ways in which "vulnerability" can be defined. For example, it could be that only individuals with severe use pattern characteristics are at elevated risk for psychosis. Alternatively, it could also be the case that only individuals with elevated psychosis-proneness are vulnerable to the effects of cannabis use. The following section thus summarizes the research testing the differential vulnerability hypothesis using operationalizations of vulnerability that span these two overarching categories.

3.1 Use Pattern Characteristics

Cannabis use characteristics—including frequency, product potency, and age of onset—are the most widely reported moderators for the relationship between cannabis and risk for psychotic illnesses. Below, we summarize the findings of the studies that have tested the role of different patterns of use in the relationship between cannabis and psychosis.

3.1.1 Use Severity

Epidemiological studies consistently demonstrate that increased cannabis use frequency is associated with increased risk for psychosis, but not linearly. Instead, there appears to be a thresholding effect such that less frequent use is not associated with significant elevations in risk for psychotic disorders compared to no use, but that risk significantly increases for weekly use and even more so for daily (Kiburi et al. 2021; Marconi et al. 2016; Robinson et al. 2023). Similarly, psychotic disorder risk has also been shown to increase with cannabis product potency. Lifetime use of high-potency cannabis products (THC $\geq$ 10%) is associated with a threefold increase in the likelihood of developing a psychotic illness compared to non-use, whereas lifetime use of low-potency cannabis products (THC $\leq$ 10%) is associated with a twofold increase in likelihood compared to nonuse (Di Forti et al. 2019a; van der Steur et al. 2020). Moreover, daily use of high-potency cannabis is associated with an almost sixfold increase in the likelihood of developing a psychotic illness compared to nonuse, while daily use of low-potency cannabis is associated with a threefold increase in likelihood (Di Forti et al. 2019a). Together, these two lines of research suggest the mechanistic hypothesis that perhaps exogenous THC exposure needs to reach a particular level to induce lasting neurobiological changes in the endocannabinoid system, ultimately triggering the cascading effects that give rise to psychotic illness.

3.1.2 Age of Use Onset

Earlier age of cannabis use onset has also been investigated as a potential moderator, in line with the hypothesis that the developing brain may be particularly sensitive to the neurodevelopmental and/or neurotoxic effects of THC (Kiburi et al. 2021). This hypothesis draws support primarily from animal research, which indicates that cannabis administered to adolescent rodents causes disrupted hippocampal signaling and associated behavioral deficits akin to those seen in rodent models of schizophrenia, but these same deficits are not seen when cannabis is administered to adult animals (Gleason et al. 2012). However, human studies examining cannabis use age of onset have returned mixed results, with some reporting that use at or before age 15 is associated with greater risk of psychosis (Arseneault 2002), while others reporting no significant difference in risk associated with use that begins before versus after age 18 (Gage et al. 2016). As previously mentioned, one study of sibling pairs from an Australian birth cohort found no significant difference in the age of cannabis use onset between siblings with and without psychotic disorder diagnoses; however, the sibling reporting early use did tend to score higher on a measure of delusion-like experiences (McGrath et al. 2010).

3.2 Indicators of Psychosis-Proneness

3.2.1 Genetic Risk

Several studies conducted to date have examined whether cannabis use might be more likely to lead to psychosis for individuals with certain types of measured genetic risk—a classic example of gene-by-environment interaction. Early research in this area primarily focused on specific candidate genes, particularly those involved in modulating dopaminergic activity (Halldorsdottir and Binder 2017). The AKT1 and COMT genes have been the most extensively studied, with preliminary investigations also examining DRD2 and FAAH. However, the results of these studies have been mixed. While some research has shown that these genotypes may moderate the relationship between cannabis use and the risk of developing a psychotic disorder, these findings have yet to be consistently replicated (van der Steur et al. 2020), in line with evidence showing that candidate gene-by-environment studies are plagued by power issues and low replication rates (Duncan and Keller 2011).

Another approach to measuring genetic risk is the polygenic risk score (PRS), which provides an estimate of an individual's genetic liability based on the cumulative effect of multiple genetic variants. PRSs for schizophrenia can be estimated by calculating a weighted sum of associated risk alleles of each individual, and statistical analysis using PRSs to predict their respective phenotypes have demonstrated good replicability across independent samples, unlike candidate gene analyses (Adorjan and Papiol 2019; Schaefer et al. 2023). Studies testing whether the

association between cannabis use and psychosis differs as a function of schizophrenia polygenic risk have returned mixed results. One large, cross-sectional study reported that cannabis use was associated with significantly greater odds of schizophrenia in individuals falling above the 75th percentile in the schizophrenia PRS score (OR: 11.54, 95% CI: 7.60–17.51) versus those scoring below this cut-off (OR: 4.10, 95% CI: 3.13–5.36) (Guloksuz et al. 2019). However, a second case-control study of the first-episodes within that same sample revealed no such interaction between schizophrenia PRS and a measure of heavy cannabis use (daily or high-potency users), and only a weak interaction between schizophrenia PRS and a frequency-type composite cannabis use measure (Di Forti et al. 2019b). Other studies have found little-to-no moderation effect of cannabis use by continuous schizophrenia PRSs in predicting adult psychoticism (Schaefer et al. 2021b) or psychotic-like experiences (Elkrief et al. 2021), suggesting that the cannabis-psychosis link is not limited to those with genetic predisposition to schizophrenia.

A third approach to operationalizing genetic risk is through family history. A family history of schizophrenia is consistently identified as one of the most significant predictors of developing the disorder and has been linked to cannabis use and abuse (Mowla and Bahrami 2020). The diagnosis of a psychotic disorder has been associated with high rates of cannabis use independent of family history (Guloksuz et al. 2019). However, only a few studies have examined family history in the context of the relationship between cannabis use and risk for developing a psychotic disorder, and the two studies that have were conducted within high-risk for psychosis (e.g., CHR) populations. One of those studies found no difference in the family history of psychosis between cannabis users and nonusers (Dragt et al. 2012), and the only study that has tested the moderating effect of family history reported that cannabis use did not increase the risk of developing a psychotic disorder more in individuals with a family history of psychosis versus those without such a family history (Valmaggia et al. 2014). Although preliminary evidence suggests family history does not moderate the relationship between cannabis and psychosis, more work is needed to replicate these findings and examine these relationships outside of high-risk populations.

3.2.2 Subclinical Psychotic Symptoms

As previously described, individuals who begin experiencing attenuated or subclinical symptoms of psychosis are considered to be at clinical high risk (CHR) for developing a psychotic disorder. Because CHR status is often defined by symptom presentation alone, these individuals may or may not also carry genetic risk for psychosis. It is estimated that 20–30% of CHR individuals convert to a psychotic disorder (Caballero et al. 2023; George et al. 2017). The prevalence of lifetime cannabis use in CHR samples is also often high, ranging between 50 and 75% (Farris et al. 2020; Kline et al. 2022). Although more frequent cannabis use, lifetime cannabis abuse, and lifetime cannabis dependence have been associated with increased likelihood of transition in isolated reports, recent systematic reviews and

meta-analyses found no significant association between any measures of cannabis use (frequency, type, age of onset) and transition to psychosis in CHR populations (Andreou et al. 2023; Chester et al. 2023; Farris et al. 2020; Kraan et al. 2016; van der Steur et al. 2020). Taken together, this suggests a possible threshold effect, such that more severe use patterns may increase risk of developing a psychotic disorder in vulnerable individuals. Importantly, however, CHR status has yet to be tested as a true moderator; the research to date has instead compared rate of transition to psychotic disorders between cannabis users and nonusers within CHR populations. A more robust test of moderation by CHR status (and thus the hypothesis that cannabis affects psychosis risk only in those already vulnerable to psychosis) would entail recruiting a more clinically heterogeneous sample to examine whether the strength of the association differs between CHR individuals and those without subthreshold symptoms.

3.2.3 Cannabis-Induced Psychosis

For an estimated 0.5% of cannabis users, their use can trigger delusions and/or hallucinations severe and persistent enough to warrant clinical attention (Pearson and Berry 2019). These individuals receive a diagnosis of cannabis-induced psychosis, a disorder characterized by psychotic symptoms clinically indistinguishable from chronic psychotic disorders (e.g., schizophrenia) that persist beyond the typical period of cannabis intoxication and withdrawal but subside within one month of abstinence, potentially with the aid of antipsychotic medications (Garson et al. 2023; Rentero et al. 2021). This cessation of symptoms following abstinence is what differentiates cannabis-induced psychosis from chronic psychotic illnesses. Of note, the etiology of cannabis-induced psychosis is not yet well understood, and the factors that might increase one's vulnerability remain largely unknown.

Those who experience cannabis-induced psychosis may be at increased risk for developing a nonaffective psychotic disorder. Although the mechanisms responsible for this effect are not well understood, the leading hypothesis is that those who ultimately convert were already at increased likelihood of developing a psychotic disorder (e.g., due to genetic risk), or that the individuals who develop cannabis-induced psychosis are more vulnerable to the psychogenic effects of THC. The few empirical studies that have investigated transition rates from cannabis-induced psychosis to persistent psychotic disorders report rates ranging from 18 to 47%, although the cumulative hazard for transition to nonaffective psychotic disorders is consistently highest for cannabis-induced psychosis compared to substance-induced psychosis precipitated by all other substances (e.g., amphetamines, alcohol, polysubstance use) (Rognli et al. 2023). Further, a recent population-based cohort study found that over 40% of adolescent and young adult males aged 14–24 who discharged from the emergency department with cannabis-induced psychosis developed schizophrenia spectrum disorder within 3 years (Myran et al. 2023). Despite this strikingly high transition rate reported in recent work, these studies rely on the validity of diagnostic codes retroactively extracted from medical records and cannabis-induced

psychosis has yet to be tested as a true moderator of the cannabis-psychosis relationship. A more robust test of differential vulnerability would require utilizing standardized diagnostic practices and prospective, longitudinal study designs to examine whether the strength of the association between cannabis and risk for psychotic disorders differs between individuals who have experienced cannabis-induced psychosis and those who haven't.

3.2.4 Other Environmental Risk Factors

Several environmental factors have been associated with increased risk for developing a psychotic disorder, in addition to cannabis use. These include obstetric complications, migration, urbanicity, low socioeconomic status, and adverse childhood experiences (Amar 2020; Robinson and Bergen 2021; Xenaki et al. 2023). Each of these factors—including cannabis use—rarely act in isolation, yet the majority of the literature has investigated each factor independently rather than considering their interactions. For instance, adolescent-onset cannabis use has been associated with low-socioeconomic status, which has also independently been linked to increased risk for psychosis, yet potential interactions between these risk factors remained largely uninvestigated (Brasso et al. 2021; Forman-Hoffman et al. 2017; Xenaki et al. 2023).

Several studies have examined childhood adversity as a potential moderator of the relationship between cannabis use and psychosis risk. Childhood adversity has been associated with both increased risk for psychotic disorders as well as cannabis use and abuse, suggesting it may play a role in the link between cannabis and psychosis (Amar 2020; Hines et al. 2023; Varese et al. 2012). Exposure to childhood adversity and cannabis use has been repeatedly associated with increased risk for experiencing psychotic symptoms (Harley et al. 2010; Konings et al. 2012) and disorders (Kiburi et al. 2021; van der Steur et al. 2020) beyond the risk associated with each exposure independently, suggesting a potential synergistic interaction. Mechanistically, this is thought to be possible due to the combination of cannabis exposure and early life adversity suppressing the hypothalamic-pituitary-adrenal (HPA) axis response to stress, leading to abnormal stress regulation and the emergence of psychosis (Xenaki et al. 2023). However, a recent systematic review reported that interactions were largely found in epidemiological surveys but not replicated in case-control studies while also highlighting that most studies defined cannabis exposure in terms of lifetime use rather than taking into account continuous differences in use characteristics (Sideli et al. 2020a). Some studies further suggest that the moderating effect of childhood adversity depends on the type of adversity endured. For example, one study reported that the combination of cannabis use under the age of 16 and sexual abuse was associated with a 12-fold increase in risk for a psychotic disorder diagnosis, yet the relationship between sexual abuse and psychosis was not significant for those who did not use cannabis by age 16 (Houston et al. 2008). Likewise, individuals who endorsed lifetime cannabis use and sexual assault prior to age 16 were at a sevenfold increase in risk for a psychotic

disorder diagnosis, but the relationship between sexual assault and psychosis was not significant in nonusers (Houston et al. 2011). Taken together, early cannabis use may increase strength of the relationship between childhood trauma and psychosis, however, these studies do not test whether childhood adversity moderates the relationship between cannabis use and psychosis. A true test of differential vulnerability by childhood adversity would involve examining whether the strength of the association between cannabis use and risk for psychotic disorders differs between individuals with different degrees of exposure to childhood adversity (e.g., none versus severe) or, potentially, with exposure to different types of childhood adversity (e.g., none versus sexual abuse versus physical abuse).

4 Conclusion

Although experimental studies have repeatedly shown that cannabis can induce short-term psychotic symptoms during acute intoxication, the relationship between long-term cannabis use and risk of chronic psychotic illnesses is significantly more complex and controversial. Much of this controversy stems from the fact that the reliable associations seen between cannabis use and nonaffective psychotic disorders in observational studies could be consistent with any number of multiple, interwoven processes. In this chapter, we reviewed evidence both for and against the perspective that cannabis use is a causal risk factor for later psychotic disorders, ultimately showing that studies capable of ruling out alternative possibilities generally converge to suggest that if these causal effects exist, they are likely to be either (a) modest or (b) substantial only for a particularly vulnerable subset of the general population.

Causally informative, quasi-experimental studies on cannabis and psychosis extend the general observational literature on this topic in several important ways. Co-relative control studies facilitate causal inference by controlling for shared genetic and environmental factors, with longitudinal designs additionally helping to rule out the possibility of reverse causation. When both reverse causation and confounding factors are eliminated, only simple causation or confounding by nonshared environmental factors remain as plausible explanations. Findings from this literature are mixed, with associations found between cannabis use and some measures of psychosis but not others. Cannabis legalization studies provide another perspective, establishing temporal precedence and controlling for time-invariant confounders, but also aggregating effects at the hospital or regional level. These studies generally find no evidence connecting legalization of recreational cannabis use to increased rates of psychotic disorders—a finding that is also at odds with observational studies suggesting higher rates of these conditions among cannabis users.

Combined, these literatures provide only weak support for the notion that cannabis use is a causal risk factor for the development of long-term psychotic illnesses, and strong support for the notion that associations between these constructs observed in observational studies are inflated by numerous, potential confounds.

The mixed, modest effects observed in the causally informative literature on cannabis and psychosis also mean that proponents of the simple causation hypothesis must contend with the strong possibility that either the magnitude of cannabis's effects on psychosis risk are quite small at the individual level, or else unevenly distributed in the population, perhaps increasing risk of psychosis only in particularly vulnerable individuals.

This latter possibility, which we termed "the differential vulnerability hypothesis" has also received mixed support in the literature to date. We focused our review on two main categories: cannabis use characteristics encompassing use frequency, product potency, and use age-of-onset, and measures of psychosis-proneness, capturing indices of genetic and environmental vulnerability. Research in the first category provides mixed support for the possibility of moderation. Several studies of cannabis use frequency and potency suggest a thresholding effect, where risk of psychotic disorder increases significantly only beyond a certain level of exposure. Findings on cannabis use age-of-onset, however, are more equivocal, with some studies indicating increased risk for adolescent users and others finding no age-related differences.

Research examining the interaction of cannabis use and indicators of psychosis-proneness also returned mixed results. Most studies of genetic risk factors (i.e., specific genetic variants, polygenic risk of schizophrenia, and family history of psychosis) report no significant interaction with indicators of cannabis use, suggesting that each factor exerts independent, additive effects on the risk of psychotic disorder. Studies in populations judged to be at high risk of psychosis based primarily on clinical presentation (i.e., participants judged to be at clinical high-risk or diagnosed with at least one instance of cannabis-induced psychosis) have also produced inconsistent findings. While isolated reports suggest a potential threshold effect, whereby more severe patterns of use may increase risk of transition in this population, evidence from recent systemic reviews and meta-analyses largely support no difference in the rate of transition to psychotic disorders between cannabis users and nonusers within clinical high-risk populations. Further, recent work has demonstrated that individuals who experience cannabis-induced psychosis may be at elevated risk for developing a persistent psychotic disorder, although no tests of differential vulnerability have been conducted. Collectively, these findings are equivocal, with slightly stronger evidence supporting no moderation by these indicators of psychosis-proneness; more robust tests of moderation are needed to better understand whether—and, if so, how—these psychosis vulnerability markers interact with cannabis use to influence risk for developing a psychotic disorder.

The moderating effects of other environmental risk factors for psychotic disorders remain largely untested. A handful of studies have found a potential synergistic interaction between exposure to childhood adversity—particularly sexual abuse—and cannabis use, suggesting that the risk for developing psychotic symptoms and disorders associated with this combination of exposures is greater than the risk associated with each exposure independently. However, these studies did not conduct true tests of moderation of the cannabis-psychosis relationship by childhood adversity, thus it remains uncertain whether the effect of cannabis on psychosis risk is

elevated for individuals exposed to childhood adversity. Far more work is needed to better understand the nuanced interactions between environmental risk factors for psychosis and cannabis use in risk for developing psychotic disorders.

The scientific impact of empirical tests of the differential vulnerability hypothesis conducted to date is also constrained by several limitations. First, most studies are observational and not causally informative, making it difficult to determine whether putative moderators are truly augmenting a causal effect of cannabis use versus simply identifying groups of individuals at greater risk of psychosis for other reasons. For example, positive associations between earlier age of cannabis use onset and risk of psychosis in the literature could indicate that the deleterious effects of cannabis use on neurodevelopment are greater when use occurs during a developmental "sensitive period," or simply that individuals who use cannabis at an early age tend to do so in a context characterized by multiple other risk factors (e.g., easy access to addictive substances, minimal parental supervision, limited opportunities for other extracurricular activities, etc.). Second, many studies examining cannabis use and clinical indicators of psychosis-proneness (i.e., CHR status, cannabis-induced psychosis diagnosis) neglect to conduct formal tests of moderation, choosing instead simply to examine whether cannabis use in these populations is associated with increased risk of transition into a chronic psychotic illness. Further, few environmental risk factors have been tested as potential moderators of the cannabis–psychosis relationship, which is a significant limitation of this body of work considering the growing evidence that psychotic disorders and cannabis use share many of these risk factors, which often coexist and have the potential to interact (Radua et al. 2018; Stone et al. 2012). These limitations thus underscore the need for more robust and comprehensive research designs in larger samples to ensure the statistical power needed to test interactions. Causally informative research designs—particularly twin studies—may be especially informative in this context, given that randomized controlled trials examining the outcomes of cannabis use are not feasible.

In addition to the limitations outlined above, the wider cannabis–psychosis literature is also characterized by several limitations that constrain our ability to draw firmer conclusions than those previously articulated. A primary concern is the variability in operationalizing psychosis, with some studies measuring symptoms rather than formal diagnoses. This distinction is crucial, as different forms of psychosis manifest on varying timescales and arise from distinct mechanisms. Moreover, many symptom-based measures (e.g., of psychotic-like experiences or psychoticism) fail to differentiate between experiences occurring under the influence of substances—like cannabis—versus those occurring in a drug-free state. Additional longitudinal studies examining associations between cannabis use, symptoms that persist across periods of abstinence, and psychotic disorder diagnoses assessed via standardized, gold-standard measures are therefore needed to elucidate the role of cannabis in the emergence of psychotic symptoms and disorders.

Another key limitation concerns variability in the operationalization of cannabis exposure. Many studies of psychosis define exposure (particularly heavy use) in terms of cannabis abuse or dependence diagnoses, which is not optimal as these conditions involve significantly more than simply high levels of cannabis exposure (i.e., failure to fulfill major role obligations, repeated use in physically hazardous situations, or continued use despite persistent interpersonal problems) and therefore invite the possibility that associations with psychotic disorder exist due to other confounded exposures or individual characteristics. Other studies rely on relatively coarse, binary measures of cannabis exposure including lifetime use (yes/no), frequent use (>100×/not), or early use (before age 18 years/after 18) characterized by reduced variability relative to more continuous measures of cannabis use, and thus a reduced power to detect effects. Additional studies that use repeated, dimensional measures of cannabis use over time are thus needed, particularly those that embed these strengths in the context of a causally informative, quasi-experimental study design (e.g., a sibling or twin comparison study).

The complex relationship between cannabis use and psychosis presents significant challenges for clinical practice and public policy. For clinicians, formulating concrete recommendations is challenging given that the causal effect of cannabis on psychosis risk does not appear to be large or robust. Moreover, while continued cannabis use following psychotic illness onset—compared with discontinued use upon onset—has been associated with increased symptom severity, higher rates of relapse, longer hospitalization time, and decreased measures of quality-of-life among diagnosed individuals, some report benefits from cannabis use, which is supported by research demonstrating antipsychotic effects of cannabidiol (CBD), another one of the chemicals found in cannabis (Amar 2020; Patel et al. 2020). Future investigations should focus on teasing apart the effects of cannabis products containing different ratios of THC and CBD to better inform clinical recommendations. For public policy, we argue that the benefits of cannabis legalization, such as reduced incarceration rates, must be weighed against the potential negatives, which include possible small effects on psychosis risk and other outcomes like reduced educational attainment (Schaefer et al. 2021a). By embracing the complexity of this relationship and continuing to refine our understanding of the relationship between cannabis and psychotic illness through rigorous research, we can work towards a future where both individual and public health concerns are effectively addressed by both evidence-based clinical practices and public policies.

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References

Adorjan K, Papiol S. Genetic risk of psychosis in relation to cannabis use: findings from polygenic risk score approaches. J Psychiatry Brain Sci. 2019;4(6):e190018. https://doi.org/10.20900/jpbs.20190018.

Amar MB. Cannabis and psychosis: a review of the risk factors involved. Am J Plant Sci. 2020;11(12):12. https://doi.org/10.4236/ajps.2020.1112139.

Andréasson S, Allebeck P, Engström A, Rydberg U. Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. Lancet. 1987;2(8574):1483–6. https://doi.org/10.1016/s0140-6736(87)92620-1.

Andreou C, Eickhoff S, Heide M, de Bock R, Obleser J, Borgwardt S. Predictors of transition in patients with clinical high risk for psychosis: an umbrella review. Transl Psychiatry. 2023;13(1):1–11. https://doi.org/10.1038/s41398-023-02586-0.

Arseneault L. Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. BMJ. 2002;325(7374):1212–3. https://doi.org/10.1136/bmj.325.7374.1212.

Bogdan R, Winstone JMA, Agrawal A. Genetic and environmental factors associated with cannabis involvement. Curr Addict Rep. 2016;3(2):199–213. https://doi.org/10.1007/s40429-016-0103-1.

Brasso C, Giordano B, Badino C, Bellino S, Bozzatello P, Montemagni C, Rocca P. Primary psychosis: risk and protective factors and early detection of the onset. Diagnostics. 2021;11(11):11. https://doi.org/10.3390/diagnostics11112146.

Caballero N, Machiraju S, Diomino A, Kennedy L, Kadivar A, Cadenhead KS. Recent updates on predicting conversion in youth at clinical high risk for psychosis. Curr Psychiatry Rep. 2023;25(11):683–98. https://doi.org/10.1007/s11920-023-01456-2.

Callaghan RC, Sanches M, Murray RM, Konefal S, Maloney-Hall B, Kish SJ. Associations between Canada's cannabis legalization and emergency department presentations for transient cannabis-induced psychosis and schizophrenia conditions: Ontario and Alberta, 2015-2019. Can J Psychiatr. 2022;67(8):616–25. https://doi.org/10.1177/07067437211070650.

Cerdá M, Mauro C, Hamilton A, Levy NS, Santaella-Tenorio J, Hasin D, Wall MM, Keyes KM, Martins SS. Association between recreational marijuana legalization in the United States and changes in marijuana use and cannabis use disorder from 2008 to 2016. JAMA Psychiatry. 2020;77(2):165–71. https://doi.org/10.1001/jamapsychiatry.2019.3254.

Charlson FJ, Ferrari AJ, Santomauro DF, Diminic S, Stockings E, Scott JG, McGrath JJ, Whiteford HA. Global epidemiology and burden of schizophrenia: findings from the global burden of disease study 2016. Schizophr Bull. 2018;44(6):1195–203. https://doi.org/10.1093/schbul/sby058.

Chester LA, Valmaggia LR, Kempton MJ, Chesney E, Oliver D, Hedges EP, Klatsa E, Stahl D, van der Gaag M, de Haan L, Nelson B, McGorry P, Amminger GP, Riecher-Rössler A, Studerus E, Bressan R, Barrantes-Vidal N, Krebs M-O, Glenthøj B, Nordentoft M, Ruhrmann S, Sachs G, McGuire P, EU-GEI High Risk Study Group. Influence of cannabis use on incidence of psychosis in people at clinical high risk. Psychiatry Clin Neurosci. 2023;77(9):469–77. https://doi.org/10.1111/pcn.13555.

Curran HV, Freeman TP, Mokrysz C, Lewis DA, Morgan CJA, Parsons LH. Keep off the grass? Cannabis, cognition and addiction. Nat Rev Neurosci. 2016;17(5):293–306. https://doi.org/10.1038/nrn.2016.28.

D'Souza DC, Perry E, MacDougall L, Ammerman Y, Cooper T, Wu Y, Braley G, Gueorguieva R, Krystal JH. The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacology. 2004;29(8):8. https://doi.org/10.1038/sj.npp.1300496.

Di Forti M, Quattrone D, Freeman TP, Tripoli G, Gayer-Anderson C, Quigley H, Rodriguez V, Jongsma HE, Ferraro L, La Cascia C, La Barbera D, Tarricone I, Berardi D, Szöke A, Arango C, Tortelli A, Velthorst E, Bernardo M, Del-Ben CM, Menezes PR, Selten JP, Jones PB, Kirkbride JB, Rutten BP, de Haan L, Sham PC, van Os J, Lewis CM, Lynskey M, Morgan C, Murray RM,

EU-GEI WP2 Group. The contribution of cannabis use to variation in the incidence of psychotic disorder across Europe (EU-GEI): a multicentre case-control study. Lancet Psychiatry. 2019a;6(5):427–36. https://doi.org/10.1016/S2215-0366(19)30048-3.

Di Forti M, Wu-Choi B, Quattrone D, Richards AL, Freeman TP, Tripoli G, Gayer-Anderson C, Rodriguez V, Jongsma H, Ferraro L, Cascia CL, Tosato S, Tarricone I, Berardi D, Szoke A, Arango C, Bobes J, Sanjuan J, Santos JL. The independent and combined influence of schizophrenia polygenic risk score and heavy cannabis use on risk for psychotic disorder: a case-control analysis from the EUGEI study. 2019b. bioRxiv. https://doi.org/10.1101/844803.

Dragt S, Nieman DH, Schultze-Lutter F, van der Meer F, Becker H, de Haan L, Dingemans PM, Birchwood M, Patterson P, Salokangas RKR, Heinimaa M, Heinz A, Juckel G, Graf von Reventlow H, French P, Stevens H, Ruhrmann S, Klosterkötter J, Linszen DH. Cannabis use and age at onset of symptoms in subjects at clinical high risk for psychosis. Acta Psychiatr Scand. 2012;125(1):45–53. https://doi.org/10.1111/j.1600-0447.2011.01763.x.

Duncan LE, Keller MC. A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. Am J Psychiatry. 2011;168(10):1041–9. https://doi.org/10.1176/appi.ajp.2011.11020191.

Elkrief L, Lin B, Marchi M, Afzali MH, Banaschewski T, Bokde ALW, Quinlan EB, Desrivières S, Flor H, Garavan H, Gowland P, Heinz A, Ittermann B, Martinot J-L, Martinot M-LP, Nees F, Orfanos DP, Paus T, Poustka L, The IMAGEN Consortium. Independent contribution of polygenic risk for schizophrenia and cannabis use in predicting psychotic-like experiences in young adulthood: testing gene × environment moderation and mediation. Psychol Med. 2021;53(5):1759–69. https://doi.org/10.1017/S0033291721003378.

Elser H, Humphreys K, Kiang MV, Mehta S, Yoon JH, Faustman WO, Matthay EC. State cannabis legalization and psychosis-related health care utilization. JAMA Netw Open. 2023;6(1):e2252689. https://doi.org/10.1001/jamanetworkopen.2022.52689.

Farris MS, Shakeel MK, Addington J. Cannabis use in individuals at clinical high-risk for psychosis: a comprehensive review. Soc Psychiatry Psychiatr Epidemiol. 2020;55(5):527–37. https://doi.org/10.1007/s00127-019-01810-x.

Fiorentini A, Cantù F, Crisanti C, Cereda G, Oldani L, Brambilla P. Substance-induced psychoses: an updated literature review. Front Psych. 2021;12:694863. https://doi.org/10.3389/fpsyt.2021.694863.

Fischer B, Hall W, Fidalgo TM, Hoch E, Foll BL, Medina-Mora M-E, Reimer J, Tibbo PG, Jutras-Aswad D. Recommendations for reducing the risk of cannabis use-related adverse psychosis outcomes: a public mental health-oriented evidence review. J Dual Diagn. 2023;19(2-3):71–96. https://doi.org/10.1080/15504263.2023.2226588.

Forman-Hoffman VL, Glasheen C, Batts KR. Marijuana use, recent marijuana initiation, and progression to marijuana use disorder among young male and female adolescents aged 12–14 living in US households. Substance Abuse Res Treat. 2017;11:1178221817711159. https://doi.org/10.1177/1178221817711159.

Gage SH, Hickman M, Zammit S. Association between cannabis and psychosis: epidemiologic evidence. Biol Psychiatry. 2016;79(7):549–56. https://doi.org/10.1016/j.biopsych.2015.08.001.

Garson E, Castle DJ, George TP. Substance-induced psychosis: a narrative review. Curr Addict Rep. 2023;10(2):335–40. https://doi.org/10.1007/s40429-023-00475-6.

George M, Maheshwari S, Chandran S, Manohar JS, Sathyanarayana Rao TS. Understanding the schizophrenia prodrome. Indian J Psychiatry. 2017;59(4):505–9. https://doi.org/10.4103/psychiatry.IndianJPsychiatry_464_17.

Gill KE, Poe L, Azimov N, Ben-David S, Vadhan NP, Girgis R, Moore H, Cressman V, Corcoran CM. Reasons for cannabis use among youths at ultra high risk for psychosis. Early Interv Psychiatry. 2015;9(3):207–10. https://doi.org/10.1111/eip.12112.

Giordano GN, Ohlsson H, Sundquist K, Sundquist J, Kendler KS. The association between cannabis abuse and subsequent schizophrenia: a Swedish national co-relative control study. Psychol Med. 2015;45(2):407–14. https://doi.org/10.1017/S0033291714001524.

Gleason KA, Birnbaum SG, Shukla A, Ghose S. Susceptibility of the adolescent brain to cannabinoids: long-term hippocampal effects and relevance to schizophrenia. Transl Psychiatry. 2012;2(11):e199. https://doi.org/10.1038/tp.2012.122.

Guloksuz S, Pries L-K, Delespaul P, Kenis G, Luykx JJ, Lin BD, Richards AL, Akdede B, Binbay T, Altınyazar V, Yalınçetin B, Gümüş-Akay G, Cihan B, Soygür H, Ulaş H, Cankurtaran E, Kaymak SU, Mihaljevic MM, Petrovic SA, van Os J. Examining the independent and joint effects of molecular genetic liability and environmental exposures in schizophrenia: results from the EUGEI study. World Psychiatry. 2019;18(2):173–82. https://doi.org/10.1002/wps.20629.

Halldorsdottir T, Binder EB. Gene × environment interactions: from molecular mechanisms to behavior. Annu Rev Psychol. 2017;68:215–41. https://doi.org/10.1146/annurev-psych-010416-044053.

Hamilton I, Monaghan M. Cannabis and psychosis: are we any closer to understanding the relationship? Curr Psychiatry Rep. 2019;21(7):48. https://doi.org/10.1007/s11920-019-1044-x.

Hamilton I, Sumnall H. Are we any closer to identifying a causal relationship between cannabis and psychosis? Curr Opin Psychol. 2021;38:56–60. https://doi.org/10.1016/j.copsyc.2020.07.027.

Harley M, Kelleher I, Clarke M, Lynch F, Arseneault L, Connor D, Fitzpatrick C, Cannon M. Cannabis use and childhood trauma interact additively to increase the risk of psychotic symptoms in adolescence. Psychol Med. 2010;40(10):1627–34. https://doi.org/10.1017/S0033291709991966.

Hasan A, von Keller R, Friemel CM, Hall W, Schneider M, Koethe D, Leweke FM, Strube W, Hoch E. Cannabis use and psychosis: a review of reviews. Eur Arch Psychiatry Clin Neurosci. 2020;270(4):403–12. https://doi.org/10.1007/s00406-019-01068-z.

Hasin DS, Sarvet AL, Cerdá M, Keyes KM, Stohl M, Galea S, Wall MM. US adult illicit cannabis use, cannabis use disorder, and medical marijuana laws: 1991-1992 to 2012-2013. JAMA Psychiatry. 2017;74(6):579–88. https://doi.org/10.1001/jamapsychiatry.2017.0724.

Henquet C, Krabbendam L, Spauwen J, Kaplan C, Lieb R, Wittchen H-U, van Os J. Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. BMJ. 2005;330(7481):11. https://doi.org/10.1136/bmj.38267.664086.63.

Hines LA, Jones HJ, Hickman M, Lynskey M, Howe LD, Zammit S, Heron J. Adverse childhood experiences and adolescent cannabis use trajectories: findings from a longitudinal UK birth cohort. Lancet Public Health. 2023;8(6):e442–52. https://doi.org/10.1016/S2468-2667(23)00095-6.

Houston JE, Murphy J, Adamson G, Stringer M, Shevlin M. Childhood sexual abuse, early cannabis use, and psychosis: testing an interaction model based on the National Comorbidity Survey. Schizophr Bull. 2008;34(3):580–5.

Houston JE, Murphy J, Shevlin M, Adamson G. Cannabis use and psychosis: re-visiting the role of childhood trauma. Psychol Med. 2011;41(11):2339–48. https://doi.org/10.1017/S0033291711000559.

Karcher NR, Barch DM, Demers CH, Baranger DAA, Heath AC, Lynskey MT, Agrawal A. Genetic predisposition vs individual-specific processes in the association between psychotic-like experiences and cannabis use. JAMA Psychiatry. 2019;76(1):87–94. https://doi.org/10.1001/jamapsychiatry.2018.2546.

Kendler KS, Ohlsson H, Sundquist J, Sundquist K. Family genetic risk scores and the genetic architecture of major affective and psychotic disorders in a Swedish National Sample. JAMA Psychiatry. 2021;78(7):735–43. https://doi.org/10.1001/jamapsychiatry.2021.0336.

Kiburi SK, Molebatsi K, Ntlantsana V, Lynskey MT. Cannabis use in adolescence and risk of psychosis: are there factors that moderate this relationship? A systematic review and meta-analysis. Subst Abus. 2021;42(4):527–42. https://doi.org/10.1080/08897077.2021.1876200.

Kline ER, Ferrara M, Li F, D'Souza DC, Keshavan M, Srihari VH. Timing of cannabis exposure relative to prodrome and psychosis onset in a community-based first episode psychosis sample. J Psychiatr Res. 2022;147:248–53. https://doi.org/10.1016/j.jpsychires.2022.01.039.

Konings M, Stefanis N, Kuepper R, de Graaf R, ten Have M, van Os J, Bakoula C, Henquet C. Replication in two independent population-based samples that childhood maltreatment and cannabis use synergistically impact on psychosis risk. Psychol Med. 2012;42(1):149–59. https://doi.org/10.1017/S0033291711000973.

Kraan T, Velthorst E, Koenders L, Zwaart K, Ising HK, van den Berg D, de Haan L, van der Gaag M. Cannabis use and transition to psychosis in individuals at ultra-high risk: review and meta-analysis. Psychol Med. 2016;46(4):673–81. https://doi.org/10.1017/S0033291715002329.

Kuepper R, van Os J, Lieb R, Wittchen H-U, Höfler M, Henquet C. Continued cannabis use and risk of incidence and persistence of psychotic symptoms: 10 year follow-up cohort study. BMJ. 2011;342:d738. https://doi.org/10.1136/bmj.d738.

Leiderman EA. Cannabis and psychosis: a causal association. In: Gargiulo PÁ, Mesones Arroyo HL, editors. Psychiatry and neuroscience update: from epistemology to clinical psychiatry, vol. IV. Cham: Springer International Publishing; 2021. p. 599–611. https://doi.org/10.1007/978-3-030-61721-9_42.

Libuy N, de Angel V, Ibáñez C, Murray RM, Mundt AP. The relative prevalence of schizophrenia among cannabis and cocaine users attending addiction services. Schizophr Res. 2018;194:13–7. https://doi.org/10.1016/j.schres.2017.04.010.

Luzi S, Morrison PD, Powell J, di Forti M, Murray RM. What is the mechanism whereby cannabis use increases risk of psychosis? Neurotox Res. 2008;14(2-3):105–12. https://doi.org/10.1007/BF03033802.

Marconi A, Di Forti M, Lewis CM, Murray RM, Vassos E. Meta-analysis of the association between the level of cannabis use and risk of psychosis. Schizophr Bull. 2016;42(5):1262–9. https://doi.org/10.1093/schbul/sbw003.

Matthay EC, Kiang MV, Elser H, Schmidt L, Humphreys K. Evaluation of state cannabis laws and rates of self-harm and assault. JAMA Netw Open. 2021;4(3):e211955. https://doi.org/10.1001/jamanetworkopen.2021.1955.

McGrath J, Welham J, Scott J, Varghese D, Degenhardt L, Hayatbakhsh MR, Alati R, Williams GM, Bor W, Najman JM. Association between cannabis use and psychosis-related outcomes using sibling pair analysis in a cohort of young adults. Arch Gen Psychiatry. 2010;67(5):440–7. https://doi.org/10.1001/archgenpsychiatry.2010.6.

Morrison PD, Nottage J, Stone JM, Bhattacharyya S, Tunstall N, Brenneisen R, Holt D, Wilson D, Sumich A, McGuire P, Murray RM, Kapur S, ffytche, D. H. Disruption of frontal theta coherence by Δ9-tetrahydrocannabinol is associated with positive psychotic symptoms. Neuropsychopharmacology. 2011;36(4):827–36. https://doi.org/10.1038/npp.2010.222.

Mowla A, Bahrami S. Study family history of psychiatry disorders in schizophrenia patients. J Neurol Res. 2020;10(6):6. https://doi.org/10.14740/jnr.v10i6.631.

Murray RM, Quigley H, Quattrone D, Englund A, Di Forti M. Traditional marijuana, high-potency cannabis and synthetic cannabinoids: increasing risk for psychosis. World Psychiatry. 2016;15(3):195–204. https://doi.org/10.1002/wps.20341.

Murray RM, Englund A, Abi-Dargham A, Lewis DA, Di Forti M, Davies C, Sherif M, McGuire P, D'Souza DC. Cannabis-associated psychosis: neural substrate and clinical impact. Neuropharmacology. 2017;124:89–104. https://doi.org/10.1016/j.neuropharm.2017.06.018.

Myles H, Myles N, Large M. Cannabis use in first episode psychosis: meta-analysis of prevalence, and the time course of initiation and continued use. Austr N Z J Psychiatry. 2016;50(3):208–19. https://doi.org/10.1177/0004867415599846.

Myran DT, Harrison LD, Pugliese M, Solmi M, Anderson KK, Fiedorowicz JG, Perlman CM, Webber C, Finkelstein Y, Tanuseputro P. Transition to schizophrenia spectrum disorder following emergency department visits due to substance use with and without psychosis. JAMA Psychiatry. 2023;80(11):1169–74. https://doi.org/10.1001/jamapsychiatry.2023.3582.

Nesvåg R, Reichborn-Kjennerud T, Gillespie NA, Knudsen GP, Bramness JG, Kendler KS, Ystrom E. Genetic and environmental contributions to the association between cannabis use and psychotic-like experiences in young adult twins. Schizophr Bull. 2017;43(3):644–53. https://doi.org/10.1093/schbul/sbw101.

Patel S, Khan S, Hamid P. The association between cannabis use and schizophrenia: causative or curative? A systematic review. Cureus. 2020;12(7):e9309. https://doi.org/10.7759/cureus.9309.

Pearson NT, Berry JH. Cannabis and psychosis through the lens of DSM-5. Int J Environ Res Public Health. 2019;16(21):4149. https://doi.org/10.3390/ijerph16214149.

Plunk AD, Peglow SL, Harrell PT, Grucza RA. Youth and adult arrests for cannabis possession after decriminalization and legalization of cannabis. JAMA Pediatr. 2019;173(8):763–9. https://doi.org/10.1001/jamapediatrics.2019.1539.

Power RA, Verweij KJH, Zuhair M, Montgomery GW, Henders AK, Heath AC, Madden PAF, Medland SE, Wray NR, Martin NG. Genetic predisposition to schizophrenia associated with increased use of cannabis. Mol Psychiatry. 2014;19(11):1201–4. https://doi.org/10.1038/mp.2014.51.

Radua J, Ramella-Cravaro V, Ioannidis JPA, Reichenberg A, Phiphopthatsanee N, Amir T, Yenn Thoo H, Oliver D, Davies C, Morgan C, McGuire P, Murray RM, Fusar-Poli P. What causes psychosis? An umbrella review of risk and protective factors. World Psychiatry. 2018;17(1):49–66. https://doi.org/10.1002/wps.20490.

Rentero D, Arias F, Sanchez-Romero S, Rubio G, Rodriguez-Jimenez R. Cannabis-induced psychosis: clinical characteristics and its differentiation from schizophrenia with and without cannabis use. Adicciones. 2021;33(2):95–108.

Robinson N, Bergen SE. Environmental risk factors for schizophrenia and bipolar disorder and their relationship to genetic risk: current knowledge and future directions. Front Genet. 2021;12:686666. https://doi.org/10.3389/fgene.2021.686666.

Robinson T, Ali MU, Easterbrook B, Hall W, Jutras-Aswad D, Fischer B. Risk-thresholds for the association between frequency of cannabis use and the development of psychosis: a systematic review and meta-analysis. Psychol Med. 2023;53(9):3858–68. https://doi.org/10.1017/S0033291722000502.

Rognli EB, Heiberg IH, Jacobsen BK, Høye A, Bramness JG. Transition from substance-induced psychosis to schizophrenia spectrum disorder or bipolar disorder. Am J Psychiatry. 2023;180(6):437–44. https://doi.org/10.1176/appi.ajp.22010076.

Ryan JE, Veliz P, McCabe SE, Stoddard SA, Boyd CJ. Association of early onset of cannabis, cigarette, other drug use and schizophrenia or psychosis. Schizophr Res. 2020;215:482–4. https://doi.org/10.1016/j.schres.2019.10.002.

Sánchez-Blázquez P, Rodríguez-Muñoz M, Garzón J. The cannabinoid receptor 1 associates with NMDA receptors to produce glutamatergic hypofunction: Implications in psychosis and schizophrenia. Front Pharmacol. 2013;4:169. https://doi.org/10.3389/fphar.2013.00169.

Schaefer JD, Hamdi NR, Malone SM, Vrieze S, Wilson S, McGue M, Iacono WG. Associations between adolescent cannabis use and young-adult functioning in three longitudinal twin studies. Proc Natl Acad Sci. 2021a;118(14):e2013180118. https://doi.org/10.1073/pnas.2013180118.

Schaefer JD, Jang S-K, Vrieze S, Iacono WG, McGue M, Wilson S. Adolescent cannabis use and adult psychoticism: a longitudinal co-twin control analysis using data from two cohorts. J Abnorm Psychol. 2021b;130(7):691–701. https://doi.org/10.1037/abn0000701.

Schaefer JD, Jang S-K, Clark DA, Deak JD, Hicks BM, Iacono WG, Liu M, McGue M, Vrieze SI, Wilson S. Associations between polygenic risk of substance use and use disorder and alcohol, cannabis, and nicotine use in adolescence and young adulthood in a longitudinal twin study. Psychol Med. 2023;53(6):2296–306. https://doi.org/10.1017/S0033291721004116.

Schoeler T, Ferris J, Winstock AR. Rates and correlates of cannabis-associated psychotic symptoms in over 230,000 people who use cannabis. Transl Psychiatry. 2022;12(1):1–8. https://doi.org/10.1038/s41398-022-02112-8.

Shakoor S, Zavos HMS, McGuire P, Cardno AG, Freeman D, Ronald A. Psychotic experiences are linked to cannabis use in adolescents in the community because of common underlying environmental risk factors. Psychiatry Res. 2015;227(2):144–51. https://doi.org/10.1016/j.psychres.2015.03.041.

Sherif M, Radhakrishnan R, D'Souza DC, Ranganathan M. Human laboratory studies on cannabinoids and psychosis. Biol Psychiatry. 2016;79(7):526–38. https://doi.org/10.1016/j.biopsych.2016.01.011.

Sideli L, Murray RM, Schimmenti A, Corso M, Barbera DL, Trotta A, Fisher HL. Childhood adversity and psychosis: a systematic review of bio-psycho-social mediators and moderators. Psychol Med. 2020a;50(11):1761–82. https://doi.org/10.1017/S0033291720002172.

Sideli L, Quigley H, La Cascia C, Murray RM. Cannabis use and the risk for psychosis and affective disorders. J Dual Diagn. 2020b;16(1):22–42. https://doi.org/10.1080/15504263.2019.1674991.

Solmi M, Seitidis G, Mavridis D, Correll CU, Dragioti E, Guimond S, Tuominen L, Dargél A, Carvalho AF, Fornaro M, Maes M, Monaco F, Song M, Il Shin J, Cortese S. Incidence, prevalence, and global burden of schizophrenia—data, with critical appraisal, from the Global Burden of Disease (GBD) 2019. Mol Psychiatry. 2023;28(12):5319–27. https://doi.org/10.1038/s41380-023-02138-4.

Stone AL, Becker LG, Huber AM, Catalano RF. Review of risk and protective factors of substance use and problem use in emerging adulthood. Addict Behav. 2012;37(7):747–75. https://doi.org/10.1016/j.addbeh.2012.02.014.

Valmaggia LR, Day FL, Jones C, Bissoli S, Pugh C, Hall D, Bhattacharyya S, Howes O, Stone J, Fusar-Poli P, Byrne M, McGuire PK. Cannabis use and transition to psychosis in people at ultra-high risk. Psychol Med. 2014;44(12):2503–12. https://doi.org/10.1017/S0033291714000117.

van der Steur SJ, Batalla A, Bossong MG. Factors moderating the association between cannabis use and psychosis risk: a systematic review. Brain Sci. 2020;10(2):2. https://doi.org/10.3390/brainsci10020097.

van Winkel R, Kuepper R. Epidemiological, neurobiological, and genetic clues to the mechanisms linking cannabis use to risk for nonaffective psychosis. Annu Rev Clin Psychol. 2014;10(1):767–91. https://doi.org/10.1146/annurev-clinpsy-032813-153631.

Varese F, Smeets F, Drukker M, Lieverse R, Lataster T, Viechtbauer W, Read J, van Os J, Bentall RP. Childhood adversities increase the risk of psychosis: a meta-analysis of patient-control, prospective- and cross-sectional cohort studies. Schizophr Bull. 2012;38(4):661–71. https://doi.org/10.1093/schbul/sbs050.

Vignault C, Massé A, Gouron D, Quintin J, Asli KD, Semaan W. The potential impact of recreational cannabis legalization on the prevalence of cannabis use disorder and psychotic disorders: a retrospective observational study. Can J Psychiatry. 2021;66(12):1069–76. https://doi.org/10.1177/0706743720984684.

Volkow ND, Swanson JM, Evins AE, DeLisi LE, Meier MH, Gonzalez R, Bloomfield MAP, Curran HV, Baler R. Effects of cannabis use on human behavior, including cognition, motivation, and psychosis: a review. JAMA Psychiatry. 2016;73(3):292. https://doi.org/10.1001/jamapsychiatry.2015.3278.

Wang GS, Buttorff C, Wilks A, Schwam D, Tung G, Pacula RL. Impact of cannabis legalization on healthcare utilization for psychosis and schizophrenia in Colorado. Int J Drug Policy. 2022;104:103685. https://doi.org/10.1016/j.drugpo.2022.103685.

Wu G, Li Y, Eric Lang X. Effects of recreational marijuana legalization on clearance rates for violent crimes: evidence from Oregon. Int J Drug Policy. 2022;100:103528. https://doi.org/10.1016/j.drugpo.2021.103528.

Xenaki L-A, Dimitrakopoulos S, Selakovic M, Stefanis N. Stress, environment and early psychosis. Curr Neuropharmacol. 2023;22(3):437–60. https://doi.org/10.2174/1570159X21666230817153631.

Chapter 19
Cannabis Effects on Brain and Behavior: The Problem of Residual Confounding and Utility of Twin Studies



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1 Introduction

The primary psychoactive compound in cannabis, Δ9-tetrahydrocannabinol (THC), induces acute changes in mood and cognition, with more pronounced effects at higher doses and in occasional users (Karoly et al. 2020). As cannabis use grows globally, improving scientific understanding of potential long-term effects on the brain and mental health becomes increasingly critical. This knowledge is crucial not only for informing consumer behavior but also for guiding the formulation and implementation of evidence-based cannabis policies. These aims are particularly relevant given that cannabis legalization has been associated with several positive outcomes, including declines in cannabis-related arrests (Grucza et al. 2018; Willits et al. 2022), reduced opioid-related mortality (Bachhuber et al. 2014; Chan et al. 2020), and decreased alcohol and non-cannabis drug use (Alley et al. 2020; Fedorova et al. 2022). Policymakers must therefore carefully weigh these benefits against potential adverse effects on brain development and mental health, especially in vulnerable populations such as adolescents and those predisposed to psychiatric disorders.

Accurately assessing the long-term effects of cannabis use presents significant challenges. Practical and ethical constraints preclude experimental studies of chronic use in humans, and, while animal models offer a viable alternative, they, too, have limitations. Neuroanatomical and neurochemical differences between human and animal brains, especially in regions involved in complex cognitive and emotional processes, hinder the direct translation of findings. Moreover, the animal analogues of human psychiatric conditions such as depression or substance use

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disorder are inherently approximate, often failing to capture the full complexity of these conditions in humans (Salgado and Sandner 2013).

Consequently, most human cannabis research relies on observational studies, which present their own interpretive challenges. Cannabis use is not randomly distributed in the population; instead, it often correlates with other environmental exposures that may independently influence mental health outcomes (Hasin 2018). This constellation of correlated risk factors complicates the process of isolating the specific causal effects of cannabis use. For instance, increasing levels of cannabis use in adolescence is associated with lower educational attainment (Fergusson and Boden 2008), which itself is a risk factor for various mental health issues (Montez and Friedman 2015). Similarly, childhood trauma is associated with both increased likelihood of cannabis use and heightened risk for mental health disorders (Schaefer et al. 2018; Scheidell et al. 2018).

Genetic factors that predispose individuals to cannabis use may also increase vulnerability to other adverse outcomes, a phenomenon known as genetic confounding. Cannabis use and use disorder—a clinical diagnosis characterized by problematic, uncontrolled cannabis use causing significant impairment—are both moderately heritable ($h^2 \sim 0.4$–0.5 and $h^2 \sim 0.5$–0.6, respectively), meaning that about half of the variation in each phenotype in a population can be attributed to genetic differences between individuals (Gerring et al. 2024). A recent genome-wide association study, which analyzed genetic variations across the entire human genome in a large, combined sample to identify specific variants associated with cannabis use behaviors, identified significant, positive genetic correlations between a polygenic measure of liability to cannabis use and analogous measures for smoking initiation, schizophrenia, major depressive disorder, and living in an area with high-material poverty. Parallel analyses on cannabis use disorder revealed significant, positive genetic correlations with genetic liability for the same constructs as well as positive associations with genetic liability to attention-deficit hyperactivity disorder (ADHD), alcohol use, nicotine dependence, and post-traumatic stress disorder (PTSD), among other adverse phenotypes (Johnson et al. 2020). These genetic correlations suggest that some of the same genes affect both cannabis use and mental health issues. As a result, it's hard to tell if cannabis use causes mental health problems, or if people who use cannabis are simply more likely to have mental health issues because of their genes.

This intricate web of interrelated factors—socioeconomic status, educational attainment, childhood adversity, genetic predispositions, and other unmeasured variables—creates a complex landscape for cannabis researchers. These confounding elements may artificially inflate the apparent association between cannabis use and various outcomes in observational studies. Therefore, scientists must approach findings from such studies with caution, considering that observed associations may be partially or wholly attributable to these confounding influences rather than cannabis use itself.

Although some observational studies attempt to control for potential confounds by measuring them and including them in their statistical models as covariates, this approach has several fundamental limitations:

1. Incomplete identification of confounds: It is extremely difficult, if not impossible, to identify and measure *all* possible confounding factors. Therefore, it is also possible that some unmeasured variables influence both cannabis use and the outcome of interest.
2. Measurement error: Even when potential confounds are identified, they are often measured imperfectly. For example, self-reported measures of complex constructs like childhood adversity or socioeconomic status may not fully capture these factors. This measurement error can lead to residual confounding, where the impact of these factors is not fully accounted for in the analysis.
3. Statistical power and model overfit: Including a large number of covariates in a statistical model can lead to reduced ability to detect true effects (decreased power) and excessive fitting to noise in the data rather than underlying relationships (overfit), potentially compromising the model's generalizability and reliability. Paradoxically, including too many covariates can sometimes reduce even a true causal effect to statistical nonsignificance, particularly in smaller studies.
4. Collider bias: In some cases, controlling for certain variables can introduce new biases into the analysis, particularly if the controlled variable is a consequence of both cannabis use and the outcome of interest.

Given these challenges in controlling for confounding factors in observational studies, some researchers have turned to more sophisticated approaches. One powerful approach that can be used to obtain a more accurate estimation of cannabis's true effects is known as "co-twin control" or "twin difference" analyses (McGue et al. 2010). This method relies on samples of both dizygotic (fraternal) and monozygotic (identical) twins who differ in their cannabis use. While assembling a large, representative sample of cannabis-using twins poses logistical challenges, the strength of this approach lies in its ability to control for genetic and shared environmental factors that might otherwise confound the relationship between cannabis use and various outcomes by study design, rather than statistical adjustment.

In these studies, researchers compare outcomes between same-sex twin pairs raised in the same home where one twin uses cannabis and the other does not (discordant twin), or where twins differ in their level of cannabis use (twin difference). These studies frequently use the term *individual-level associations* to refer to the covariation between cannabis use and outcomes observed when comparing across all individuals in the sample (i.e., treating participants as if they were all singletons, while controlling for clustering by family). This effect is then often compared to the *within-pair association*, which refers to the covariation between differences in cannabis use and differences in outcomes observed within twin pairs. This method is particularly effective because estimating a "within-pair" association implicitly controls for many potential confounding factors, including:

(a) Genetic factors: monozygotic twins share 100% of their genes, while dizygotic twins share approximately 50% of their segregating genes (i.e., those that vary between individuals in a population).
(b) Prenatal environment: Twins share the same intrauterine environment during gestation.

(c) Rearing environment: Twins are typically raised in the same household, exposed to similar parenting styles, socioeconomic conditions, and neighborhood influences.

By comparing outcomes within twin pairs, researchers can effectively control for these shared genetic and environmental factors, thus isolating the specific effects of cannabis use to a greater degree than studies of unrelated individuals.

The following sections of this chapter will provide an overview of recent research examining the relationships between cannabis use and two critical domains: mental health and brain structure and function. We will explore findings from both twin and non-twin studies, highlighting how twin research has refined or challenged our understanding of the associations observed in traditional observational studies. This approach will allow us to disentangle the complex web of genetic, environmental, and cannabis-specific factors influencing mental health and neurological outcomes. By doing so, we hope to offer a clearer picture of cannabis's true effects, helping to inform both public health policies and individual decision-making in an era of increasing cannabis use and legalization. As we proceed, we will maintain a critical lens, acknowledging the strengths and limitations of each research approach and emphasizing the importance of continued, rigorous investigation in this rapidly evolving field.

2 The Non-twin Literature on Cannabis and Mental Health

2.1 Major Depressive Disorder

The relationship between cannabis use and major depressive disorder (MDD) is complex and has been the subject of numerous studies. One meta-analysis of studies examining patient-reported reasons for medical cannabis use identified improvements in mood as the third-most common reason (34%) for medical cannabis use, behind only management of pain (64%) and anxiety (50%) (Kosiba et al. 2019). Animal studies, however, suggest a biphasic effect of cannabinoids on mood regulation, such that lower doses of cannabinoid receptor agonists in rodents produce short-term antidepressant effects, while higher doses led to pro-depressive effects (Crippa et al. 2009; Hasbi et al. 2023). Chronic administration in adolescent animals has also been shown to contribute to anhedonic and depressive behaviors in adulthood (Bambico et al. 2010; Realini et al. 2011; Rubino et al. 2008; Schneider and Koch 2003).

Epidemiological surveys have consistently found associations between cannabis use and depression. The United States National Comorbidity Survey reported that nondependent cannabis users were 1.6 [95% CI: 1.1, 2.2] times more likely to experience a major depressive episode relative to nonusers (Chuan-Yu et al. 2002). A more recent, nationally-representative, epidemiological survey, sampling 10,123 adolescents from the United States, found that those who had used cannabis were

twice as likely as nonusers to experience mild depression and three times as likely to experience severe depression (Hinckley et al. 2023). Canadian data showed daily cannabis users were about twice as likely to have anxiety and mood disorders compared to nonusers (Cheung et al. 2010). Similarly, Australian studies found cannabis users were over two times more likely to meet criteria for mood disorders than nonusers (Degenhardt et al. 2001), with adolescent users being three times more likely to meet criteria for depression (Rey et al. 2002). These findings consistently demonstrate a higher prevalence of depressive disorders among cannabis users, particularly those with heavy use or dependence.

Longitudinal studies show that cannabis use is also a reliable predictor of future depression. An initial meta-analysis of fourteen longitudinal studies reported that cannabis users had 1.17 [95% CI: 1.05–1.30] times greater odds of developing depression compared to nonusers, and that heavy users (defined as those diagnosed with cannabis use disorder or reported at least weekly use) had 1.62 [95% CI: 1.21–2.16] times greater odds (Lev-Ran et al. 2014). A subsequent meta-analysis of eight longitudinal studies that specifically assessed cannabis use in adolescence and depression in young adulthood confirmed this finding, reporting that cannabis users had 1.37 [95% CI: 1.16–1.62] times greater odds of developing depression, and attributing 7.2% of young adult depression cases to adolescent cannabis use (Gobbi et al. 2019). Importantly, however, some longitudinal studies have indicated that associations between cannabis and depression become nonsignificant after adjusting for covariates such as comorbid drug use, demographic characteristics, and childhood conduct disorder (De Graaf et al. 2010; Fergusson and Horwood 1997; Harder et al. 2008), consistent with the notion that many of these associations may reflect residual confounding.

2.2 Anxiety Disorders

Many of the findings concerning the link between cannabis use and anxiety mirror those seen for depression. A systematic review and meta-analysis of human experimental studies concluded that while low doses of THC (≤7.5 mg) did not significantly affect anxiety levels, moderate doses (7.5–12.5 mg) increased anxiety. Interestingly, cannabidiol (CBD) administration was found to reduce THC-induced anxiety, suggesting a potential protective role of CBD in cannabis preparations (Twomey 2017). Similarly, animal studies have consistently shown that cannabinoid receptor agonists produce anxiolytic effects at lower doses but anxiogenic effects at higher doses (Crippa et al. 2009; Hasbi et al. 2023). Multiple animal studies have also concluded that repeated cannabinoid exposure, at both low and high doses, during adolescence was sufficient to induce persistent anxiety behaviors in adulthood (Bambico et al. 2010; Realini et al. 2011; Rubino et al. 2008; Schneider and Koch 2003).

Epidemiological studies of cannabis use and anxiety have returned mixed results. For example, a review of human cross-sectional studies examining associations

between cannabis use and social anxiety in young adulthood reported no significant association between social anxiety and cannabis use frequency ($r = 0.002$, $k = 16$, $p = 0.929$), but a significant positive association between social anxiety and cannabis-related problems ($r = 0.197$, $k = 16$, $p < 0.001$), including symptoms of cannabis use disorder (Single et al. 2022). Another recent review of cross-sectional and longitudinal studies concluded that evidence most strongly supports the "self-medication hypothesis" (wherein individuals with anxiety are more likely to initiate cannabis use), but also noted that the literature contains contradictory findings including non-significant associations, as well as several studies reporting effects that become insignificant with covariate adjustment (Beletsky et al. 2024). This conclusion has received mixed support from two meta-analyses focusing specifically on longitudinal studies assessing cannabis use in adolescence and subsequent anxiety, which have concluded that adolescents who used cannabis at baseline both were and were not significantly more likely to develop anxiety relative to nonusers (pooled ORs 1.18 [95% CI: 0.84–1.67] and 2.14 [95% CI: 1.37–3.36], respectively) (Gobbi et al. 2019; Lowe et al. 2024).

2.3 Other Substance Use and Use Disorders

While human experimental studies examining effects of cannabis use on consumption of other drugs are few and far between, animal research on this topic has provided substantial support for the "gateway drug" hypothesis, which posits that the use of one substance (i.e., cannabis) may increase the likelihood of using other drugs. For example, animal studies using intravenous drug self-administration paradigms have shown that adolescent cannabinoid exposure can increase vulnerability and reward sensitivity to certain psychoactive drugs in adulthood including opiates and cocaine (albeit in a sex-specific manner). These effects were also shown to co-occur alongside various neurobiological changes, including alterations in μ-opioid receptor function, levels of proenkephalin (a precursor molecule for several opioid peptides), and dopaminergic signaling (Biscaia et al. 2008; Ellgren et al. 2008; Higuera-Matas et al. 2010; Tomasiewicz et al. 2012).

Human observational data is largely consistent with these animal studies. For example, data from the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC) conducted in 2012–2013 suggest a lifetime diagnosis of cannabis use disorder is associated with 14.5 [95% CI: 12.0–17.6] times greater odds of any other substance use disorder, including 7.8 [95% CI: 7.0–8.7] times greater odds of alcohol use disorder, 6.6 [95% CI: 5.8–7.6] times greater odds of nicotine use disorder, and 10.0 [95% CI: 8.6–11.8] times greater odds of any other drug use disorder (Hasin et al. 2016). Reviews of epidemiological studies also suggest robust associations between cannabis use and use of tobacco (Agrawal et al. 2012; Fingesi et al. 2024; Ramo et al. 2012), alcohol (Karoly et al. 2020), and opiates (Wilson et al. 2022), among other substances.

2.4 Antisocial Personality Disorder

Antisocial Personality Disorder (ASPD) is a complex condition characterized by a pervasive pattern of disregard for, and violation of, the rights of others. Individuals with ASPD often display a lack of empathy, impulsivity, manipulative behavior, and a history of rule-breaking or criminal activity. Although ASPD is therefore a uniquely human phenomenon, early animal studies provide some mechanistic insights by suggesting that acute THC administration has been shown to reduce aggression, likely due to its short-term antidepressant effects (Alves and Carlini 1973; McDonough et al. 1972), whereas chronic THC use appears to increase aggressive behavior, possibly due to the persistent state of irritability induced by long-term exposure (Ueki et al. 1972).

Cross-sectional studies have provided mixed support for an association between cannabis use and ASPD or related behaviors. One nationally representative study used data from the first wave of the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC), which sampled 43,093 U.S. adults, and reported strong and significant associations between marijuana abuse/dependence and ASPD (Compton et al. 2005). In contrast, a second epidemiological study which used data from the second wave of the NESARC, which sampled 34,653 U.S. adults, reported that ASPD was not significantly more prevalent in individuals with cannabis use disorder compared to those without, although those with comorbid nicotine and cannabis use disorders exhibited more antisocial behaviors (Peters et al. 2014).

Studies have also explored the relationship between cannabis use and behaviors linked to antisocial personality disorder, such as criminality and violence. A meta-analysis of 30 studies using adolescent and young adult samples found significant associations between heavy use (OR: 2.81 [95% CI: 1.68–4.74]), past year use (OR: 2.05 [95% CI: 1.5–2.8]), and lifetime use (OR: 1.94 [95% CI: 1.29–2.93]) of cannabis and perpetration of physical violence. This suggests a dose-response relationship between cannabis use and violent behavior, although the direction of causality remains unclear (Dellazizzo et al. 2020). However, one longitudinal study, sampling 702 Black Americans from a community-based cohort, reported that heavy adolescent cannabis use was associated with increased risk of drug- and property-related crime rather than other types of criminal behavior (e.g., violent crime) (Green et al. 2010). Another longitudinal study, sampling 1353 Norwegian adolescents and following them into young adulthood, even reported that associations between cannabis use and crime disappeared entirely when drug-specific charges were excluded (Pedersen and Skardhamar 2010).

Longitudinal studies have provided more nuanced insights into the temporal relationship between cannabis use and antisocial behaviors. One early study, sampling 100 White individuals at baseline with an average age of 22, observed that regular cannabis users had a significantly higher rate of ASPD diagnoses at the follow-up (average age of 28) compared to nonregular users, suggesting a possible effect of prolonged cannabis use on ASPD emergence (Weller and Halikas 1985). However, two subsequent longitudinal studies, with an average of 745 Black and

Puerto Rican participants, found that the use of cannabis in adolescence alone was not significantly associated with increased risk of ASPD or earlier symptom emergence in young adulthood. Instead, these studies highlighted the importance of polysubstance use, finding that comorbid use of alcohol, tobacco, and cannabis in adolescence increased the risk of ASPD in young adulthood (Brook et al. 2014; Lee et al. 2022).

2.5 Suicidality

Another uniquely human phenomenon, suicidality has attracted the attention of cannabis researchers due to the steadily increasing number of publications suggesting links between suicidal ideation, attempts, and long-term cannabis use. The emphasis on "long-term use" is crucial, as acute cannabis intoxication shows a different pattern. A review of six studies found no association between acute cannabis intoxication and imminent risk of suicidal ideation or attempts. These studies assessed imminent suicide risk through toxicology reports and patient self-reports of recent cannabis use among individuals receiving treatment for recent suicide attempts in clinical settings. The review concluded that cannabis intoxication did not increase imminent suicide risk, with only one case study suggesting a potential link between cannabis intoxication and suicidal ideation (Borges et al. 2016).

Interestingly, a case-crossover study of 363 recent suicide attempters found that cannabis intoxication might even have a protective effect on suicide attempt risk. This epidemiological study design, which compares exposure status during a hazard period just before an event to control periods when the event did not occur, revealed that individuals had decreased odds of a suicide attempt shortly after cannabis use compared to periods of no use (OR: 0.39 [95% CI: 0.16–0.93]) (Bagge and Borges 2017).

Research on the relationship between long-term cannabis use and suicidality, however, paints a different picture. For example, a review of 14 longitudinal studies identified a moderate association between any cannabis use and later suicidal ideation (OR 2.53 [95% CI: 1.00, 6.39]) and attempts (OR 2.23 [95% CI: 1.24, 4.00]), with an even stronger link between attempts and heavy use (OR 3.20 [95% CI: 1.72, 5.94]) (Borges et al. 2016). Supporting these findings, a meta-analysis of 13 cross-sectional and seven longitudinal studies, reported that cannabis use was associated with significant elevations in risk of suicidal ideation (OR: 2.04 [95% CI: 1.64, 2.53]), suicide plans (OR: 1.67 [95% CI: 1.55, 1.80]), and suicide attempts (OR: 2.33 [95% CI: 1.78, 3.05]) among adolescents and young adults (Fresán et al. 2022). Echoing these results, a recent, nationally representative, epidemiological survey of 10,123 U.S. adolescents found that those who had used cannabis were nearly seven times as likely to report a suicide attempt compared to nonusers (Hinckley et al. 2023).

2.6 Summary

Research concerning the relationship between cannabis use and mental health outcomes is extensive and multifaceted, with links to some outcomes supported by stronger evidence than others. For major depressive disorder, there is consistent epidemiological evidence linking cannabis use, particularly heavy use, to increased risk of depression. Longitudinal studies further support this association, though some findings become nonsignificant after adjusting for covariates, suggesting possible residual confounding. The relationship between cannabis and anxiety is less clear, with mixed results from epidemiological studies and conflicting findings from longitudinal research, with strongest support for the "self-medication hypothesis" (i.e., the notion that individuals who develop anxiety may be more likely to subsequently use cannabis).

Evidence for the "gateway drug" hypothesis appears relatively strong, with both animal studies and human observational data supporting associations between cannabis use and increased use of other substances. The literature on antisocial personality disorder (ASPD) and related behaviors is more mixed, with some studies showing significant associations between cannabis use and ASPD or violent behavior, while others find no relationship or suggest that observed links may be due to polysubstance use rather than cannabis alone. Regarding suicidality, the evidence distinguishes between acute and long-term effects. While acute cannabis intoxication does not appear to increase imminent suicide risk, longitudinal studies consistently show moderate to strong associations between long-term cannabis use and increased risk of suicidal ideation and attempts, particularly among heavy users.

Overall, the non-twin literature most strongly links cannabis to depression, other substance use, and long-term suicide risk, with less clarity for anxiety and antisocial behaviors. These associations are consistently strongest for frequent use and adolescent-onset use, suggesting potential dose-response effects and heightened vulnerability during brain development. However, the problem of residual confounding remains—with several studies showing that significant associations between cannabis use and mental health outcomes become nonsignificant after statistical adjustment for key confounds.

3 Twin Studies Examining the Relationship Between Cannabis and Mental Health

To date, a total of 13 twin studies have examined the relationship between cannabis use and common mental health problems (i.e., depression, anxiety, ASPD, other illicit drug use, and suicidality) (see Table 19.1). Across studies and outcomes, the individual-level associations from these studies largely resemble associations from the non-twin literature, with increased cannabis use correlating with increased risk of each outcome. Some of these twin studies also mirror the non-twin literature in

Table 19.1 Summary of 13 twin studies that examined the relationship between cannabis use and common mental health problems

First author (publication year)	*N* twin pairs (MZ/DZ)	% male	Sample	Assessment of cannabis	Assessment of outcome	Ages assessed	Twin-specific covariates	Co-twin control/twin difference results
Depression and anxiety								
Lynskey et al. (2004)	277–311 (136–137 MZ/140–175 DZ)	52%	Australian twin registry	Early cannabis use, cannabis dependence	Major depressive disorder	Young and middle adulthood (mean age 30, range 24–36)	Conduct disorder, alcohol dependence, nicotine dependence early tobacco use, early regular alcohol use, and childhood sexual abuse	DZ twins with cannabis dependence were significantly more likely to receive diagnoses of major depressive disorder (MDD) than their co-twins (OR 3.40, 95% CI: 1.91–6.05). This association was not significant in MZ twins (OR 1.25, 95% CI: 0.69–2.25). DZ twins reporting early cannabis use were significantly more likely to receive diagnoses of MDD than their co-twins (OR 1.69, 95% CI: 1.07–2.67). This association was not significant in MZ twins (OR 0.86, 95% CI: 0.50–1.48)
Smolkina et al. (2017)	63 MZ	33%[a]	Australian twin registry	Cannabis use disorder	Major depressive disorder	Young and middle adulthood (mean age 32)	None reported	MZ twins with cannabis use disorder were significantly more likely to have MDD compared to their co-twins without cannabis use disorder (OR 2.83, 95% CI: 1.12–7.19)
Agrawal et al. (2017)	431–571 (190–278 MZ/ 241–293 DZ)	40%[a]	Australian twin registry	Lifetime cannabis use, early cannabis use, frequent cannabis use (>100 times, lifetime)	Major depressive disorder	Young and middle adulthood (mean age 33.1)	Early alcohol use, early tobacco smoking, conduct disorder, childhood sexual abuse, early suicidal thoughts, early suicidal behavior, and early anhedonia and/or dysphoria	Lifetime cannabis use was not examined in the discordant twin model due to null individual-level findings. DZ twins reporting early cannabis use were significantly more likely to receive diagnoses of MDD than their co-twins (OR 1.82, 95% CI: 1.22–2.70). This association was not significant in MZ twins (OR 1.34, 95% CI: 0.85–2.12). Both MZ and DZ twins reporting frequent cannabis use were significantly more likely to receive diagnoses of MDD than their co-twins. For MZ twins, the odds ratio was 2.12 (95% CI: 1.33–3.37), which remained significant after adjustment (aOR 1.68, 95% CI: 1.01–2.80). For DZ twins, the odds ratio was 1.72 (95% CI: 1.05–2.82)

Schaefer et al. (2021)	1205 MZ	48%[a]	Minnesota twin and family study (MTFS)	Cannabis use index (capturing frequency of cannabis use/number of uses)	Major depressive disorder, anxiety disorders (including generalized anxiety disorder, social phobia, specific phobia, panic disorder, and agoraphobia)	Cannabis: adolescence (three assessments targeting ages 11, 14, 17); Outcomes: Young adulthood (two assessments targeting ages 24 and 29)	Cumulative adolescent alcohol use, cumulative adolescent tobacco use, adolescent externalizing problems	MZ twins with higher cumulative adolescent cannabis use frequency were not significantly more likely to receive a diagnosis of MDD (OR 1.11, 95% CI: 0.90–1.36) or an anxiety disorder (OR 1.58, 95% CI: 0.98–2.54) compared to their co-twins with lower cumulative adolescent cannabis use frequency. These associations remained non-significant after covariate adjustment
Other drug use and use disorders								
Eisen et al. (2002)	56 MZ	100%	Vietnam era twin registry (VET-R)	The minimum number of days on which marijuana was used during the participants' lifetime	Lifetime alcohol and nicotine abuse/ dependence, current drinking and smoking status, duration of drinking (in years), average number of drinks/day in the past year, and number of cigarette packs/ day	Adulthood (mean age 46.2, range 38–51)	None reported	MZ twins who were former heavy cannabis users did not show significant differences in any of the assessed alcohol or nicotine outcomes compared to their non-cannabis using co-twins (χ^2 ranging from −0.24 to 2.13)

(continued)

Table 19.1 (continued)

First author (publication year)	*N* twin pairs (MZ/DZ)	% male	Sample	Assessment of cannabis	Assessment of outcome	Ages assessed	Twin-specific covariates	Co-twin control/twin difference results
Lynskey et al. (2003)	311 (136 MZ/175 DZ)	49%	Australian twin register	Early cannabis use (before age 17)	Lifetime use of other illicit drugs (i.e., sedatives, hallucinogens, cocaine or other stimulants, opioids), drug abuse/ dependence (i.e., of cannabis, sedatives, cocaine or other stimulants, opioids, alcohol dependence	Adulthood (median age 30, range 24–36)	Lifetime depression, conduct disorder, a nondiagnostic measure of social anxiety, regular tobacco use before age 17, regular alcohol use before age 17	Twins who reported early cannabis use were significantly more likely than their co-twins to report lifetime use of other drugs, with odds ratios ranging from 2.57 to 5.15. These same twins were also significantly more likely to meet criteria for lifetime illicit drug abuse/dependence diagnoses for most substances, with odds ratios ranging from 2.13 to 4.13, except for sedatives which did not show a significant association. Early cannabis use in twins was also associated with a higher likelihood of alcohol dependence (OR 2.17, 95% CI: 1.46–3.24). These associations remained significant in adjusted models
Agrawal et al. (2004)	2130 (1196 MZ/934 DZ)	53%	Virginia twin registry	Early cannabis use (before age 18)	Use of other illicit drugs (cocaine, sedatives, stimulants, opiates, hallucinogens), drug abuse/ dependence after age 18	Adulthood (mean age 35.7, range 20–62)	Years of education, regular alcohol use before or at 18, regular nicotine use before or at 18, major depression, generalized anxiety disorder, conduct disorder	DZ twins with early cannabis use showed higher odds of using any other illicit drugs compared to their co-twins (ORs 1.5–1.8). This association remained significant in MZ twins (ORs 2.6–3.6). DZ twins with early cannabis use had higher odds of meeting criteria for cannabis, opiate, or any illicit drug abuse/dependence diagnosis relative to their co-twins (ORs 1.2–2.7). This association remained significant in MZ twins for cannabis, cocaine, sedative, stimulant, hallucinogen, alcohol, or any illicit drug abuse/dependence diagnosis (ORs 1.5–5.1)

Lynskey et al. (2006)	219 (110 MZ/109 DZ)	49%	Netherlands twin register	Early cannabis use (before age 18)	Lifetime illicit drug use (divided into party drugs and hard drugs), regular cannabis use	Adulthood (mean age 26.8, SD 11.8)[b]	Regular tobacco use before age 18, regular alcohol use before age 18, aggressive behavior, rule-breaking behavior	Twins with early cannabis use were significantly more likely to report party drug use compared to their co-twins who did not report early use (OR 6.8, 95% CI: 2.7–17.4). This association remained significant for hard drug use (OR 14.0, 95% CI: 3.3–58.8), party and/or hard drug use (OR 6.3, 95% CI: 2.7–15.0), and regular cannabis use (OR 4.7, 95% CI: 2.1–10.6). After adjustment for covariates, these associations remained significant for party drug use, hard drug use, and party and/or hard drug use. The association with regular cannabis use became nonsignificant in adjusted models (aOR 1.3, 95% CI: 0.3–5.1)
Grant et al. (2010)	130–293[c]	100%	Vietnam era twin registry (VET-R)	Early cannabis use (before age 18)	Lifetime illicit drug use (i.e., cannabis, cocaine, stimulants, sedatives, opiates, or hallucinogens/ PCP), drug or alcohol abuse/ dependence	Adulthood (mean age 42.0 years, SD 2.8)	Lifetime depression, conduct disorder (before age 16), lifetime PTSD, early regular alcohol use, early regular nicotine use, stationed in Southeast Asia during their Vietnam-era military service	Twins who used cannabis early were at significantly greater risk of trying other illicit drugs compared to their non-using or later-using cotwins (ORs 1.89–3.63). This association remained significant in adjusted models (aORs 2.71–4.09). These twins were also at greater risk of other illicit drug abuse/dependence (OR 1.66, 95% CI: 1.07–2.58). This association remained significant in adjusted models (aOR 2.02, 95% CI: 1.18–3.45). Twins with early cannabis use had a higher risk of alcohol dependence (OR 2.41, 95% CI: 1.66–3.51). This association remained significant in adjusted models (aOR 2.36, 95% CI: 1.53–3.63)
Vink et al. (2020)	1302 MZ	37%	Netherlands twin register	Lifetime use	Lifetime use of ecstasy, stimulants, and any illicit drug	Adulthood (detailed age information not reported)	None reported	MZ twins who ever used cannabis were not significantly more likely to use ecstasy compared to their co-twins who never used cannabis (8.3% vs. 2.5%, $p = 0.065$). MZ twins who ever used cannabis were significantly more likely to use stimulants compared to their non-using co-twins (9.2% vs. 2.2%, $p = 0.002$). MZ twins who ever used cannabis were also significantly more likely to use any drugs compared to their non-using co-twins (15.8% vs. 4.2%, $p < 0.001$)

(continued)

Table 19.1 (continued)

First author (publication year)	*N* twin pairs (MZ/DZ)	% male	Sample	Assessment of cannabis	Assessment of outcome	Ages assessed	Twin-specific covariates	Co-twin control/twin difference results
Zellers et al. (2024)	2039 (1081 MZ/958 DZ)	43%	Twin cohorts from the Minnesota center for twin and family research and Colorado center for antisocial drug dependence	Average cannabis use frequency	Past-year substance use and use disorder, past-180-days use of alcohol and tobacco, number of non-cannabis illicit substances consumed across 11 categories of substances (e.g., stimulants, hallucinogens, steroids, etc.)	Cannabis: Adolescence to adulthood (ages 11–35); Middle adulthood (range 25–49)	Current residence, prospective alcohol frequency, prospective tobacco frequency, adolescent externalizing	Twins with higher cannabis use frequency did not show significant differences in alcohol use disorder, nicotine dependence, or alcohol use frequency relative to their co-twins. These twins showed higher rates of cannabis use disorder, other illicit drug use, and tobacco use frequency compared to their co-twins, although these were all modest effects (Bs 0.06–0.15). This association remained significant only for cannabis use disorder in MZ-only models (B 0.16, SE 0.21). This association with cannabis use disorder remained significant across all adjusted models in specification curve analysis
Antisocial personality disorder								
Scherrer et al. (1996)	99 MZ	100%	Vietnam era twin registry (VET-R)	Lifetime cannabis abuse and/or dependence	Lifetime symptoms of antisocial personality disorder	Middle adulthood (mean age 44.6, range 36–55)	Conduct disorder, alcohol abuse/ dependence, and combat exposure	MZ twins diagnosed with cannabis abuse/dependence were significantly more likely to endorse the symptom of "failure to conform to social norms of lawful behavior" compared to their non-disordered co-twins (OR 2.8, 95% CI: 1.5–5.5). This association remained significant in adjusted models (OR 2.42, 95% CI: 1.12–5.21). MZ twins with cannabis abuse/dependence were also significantly more likely to endorse the symptom of "reckless regard for one's own or other's personal safety" relative to their non-disordered co-twins (OR 2.4, 95% CI: 1.0–5.4). This association was not significant in adjusted models

Schaefer et al. (2021)	1205 MZ	48%[a]	Minnesota Twin and Family Study (MTFS)	Cannabis use index (capturing frequency of cannabis use/number of uses)	Antisocial personality disorder	Cannabis: adolescence (three assessments targeting ages 11, 14, 17); outcomes: young adulthood (two assessments targeting ages 24 and 29)	Cumulative adolescent alcohol use, cumulative adolescent tobacco use, adolescent externalizing problems	MZ twins with higher cumulative adolescent cannabis use frequency were not significantly more likely to have a diagnosis of antisocial personality disorder (ASPD) compared to their co-twins with lower cumulative adolescent cannabis use frequency (OR 1.18, 95% CI: 0.93–1.50). This association remained non-significant in adjusted models
Suicidality								
Lynskey et al. (2004)	277–311 (136–137 MZ/140–175 DZ)	52%	Australian twin registry	Early cannabis use (before age 17), cannabis dependence	Suicidal ideation, attempts	Young and middle adulthood (mean age 30, range 24–36)	Conduct disorder, alcohol dependence, nicotine dependence early tobacco use, early regular alcohol use, and childhood sexual abuse	Twins with cannabis dependence were significantly more likely to report suicidal ideation compared to their co-twins (OR 3.43, 95% CI: 2.16–5.46). This association remained significant in adjusted models (aOR 2.89, 95% CI: 1.76–4.76). Twins with cannabis dependence were also significantly more likely to report suicide attempts (OR 3.44, 95% CI: 1.64–7.23). This association remained significant in adjusted models (aOR 2.53, 95% CI: 1.12–5.67). Twins with early cannabis use were significantly more likely to report suicidal ideation compared to their co-twins without early cannabis use (OR 1.73, 95% CI: 1.18–2.54). This association was not significant in adjusted models (aOR 1.10, 95% CI: 0.70–1.73). Twins with early cannabis use were also significantly more likely to report suicide attempts (OR 3.38, 95% CI: 1.53–7.43). This association remained significant in adjusted models (aOR 3.49, 95% CI: 1.42–8.58)

(continued)

Table 19.1 (continued)

First author (publication year)	*N* twin pairs (MZ/DZ)	% male	Sample	Assessment of cannabis	Assessment of outcome	Ages assessed	Twin-specific covariates	Co-twin control/twin difference results
Few et al. (2016)	180-414 MZ (sample 1), 137–441[c] (sample 2)	40%[a] (sample 1), 0% (sample 2)	Australian twin registry (sample 1), Missouri adolescent female twin study (sample 2)	Lifetime cannabis use, early cannabis use (before age 16/17)	Non-suicidal self-injury	Young and middle adulthood (range 24-40) (sample 1), young adulthood (range 18–29) (sample 2)	Depressed mood, childhood sexual abuse, regular smoking, regular alcohol use, illicit drug use, conduct disorder, and social anxiety (sample 1); none (sample 2)	MZ twins reporting lifetime cannabis use were not significantly more likely to report engaging in non-suicidal self-injury (NSSI) compared to their non-using co-twins (OR 1.08, 95% CI 0.49, 2.37). However, MZ twins reporting early cannabis use were significantly more likely to report engaging in NSSI than their co-twins (OR 3.20, 1.17–8.73), and this association remained significant after adjusting for covariates (OR 3.51, 95% CI 1.19, 10.33)
Agrawal et al. (2017)	431–571 (190–278 MZ/241–293 DZ)	40%[a]	Australian twin registry	Lifetime cannabis use, early cannabis use (before age 17/18), frequent cannabis use (>100 lifetime uses)	Major depressive disorder, suicidal ideation, persistent suicidal ideation (lasting >1 day), suicide plans, suicide attempts	Young and middle adulthood (mean age 33.1)	Early alcohol use, early tobacco smoking, conduct disorder, childhood sexual abuse, early suicidal thoughts, early suicidal behavior, and early anhedonia and/or dysphoria	DZ twins with early cannabis use were significantly more likely to report suicidal ideation (OR 2.23, 95% CI: 1.45–3.44), persistent ideation (OR 4.00, 95% CI: 1.93–8.30), suicide plans (OR 2.80, 95% CI: 1.36–5.76), and suicide attempts (OR 6.50, 95% CI: 2.27–18.62) compared to their co-twins without early cannabis use. These associations were not significant in MZ twins for suicidal ideation (OR 1.50, 95% CI: 0.91–2.46), persistent ideation (OR 1.67, 95% CI: 0.82–3.41), suicide plans (OR 1.17, 95% CI: 0.54–2.52), or suicide attempts (OR 1.90, 95% CI: 0.88–4.09). DZ twins with frequent cannabis use were significantly more likely to report suicidal ideation (OR 2.63, 95% CI: 1.69–4.10), persistent suicidal ideation (OR 3.50, 95% CI: 1.60–7.68), and suicide attempts (OR 4.40, 95% CI: 1.67–11.62), but not suicide plans (OR 1.64, 95% CI: 0.77–3.47) compared to co-twins without frequent cannabis use. These associations remained significant in MZ twins for suicidal ideation (OR 2.71, 95% CI: 1.47–5.01) and persistent suicidal ideation (OR 3.14, 95% CI: 1.34–7.36), but were not significant for suicide plans (OR 2.14, 95% CI: 0.87–5.26) or suicide attempts (OR 2.29, 95% CI: 0.94–5.56). The within-MZ-pair association with suicidal ideation remained significant in adjusted models (OR 2.35, 95% CI: 1.24–4.48). This association was not significant for persistent suicidal ideation in adjusted models (OR 2.23, 95% CI: 0.89–5.66)

Brain outcomes								
Harper et al. (2021)	186 (120 MZ/66 DZ)	42%[a]	Minnesota twin family study enrichment sample	Cannabis use disorder	Medial orbitofrontal cortex thickness	Young adulthood (mean age 24.3 years, SD 0.8)[b]	Subthreshold cases, twin differences in comorbid substance use disorders, and recent use of other substances	Twins with cannabis use disorder had significantly thinner medial orbitofrontal cortices compared to their co-twins without cannabis use disorder ($\beta = -0.108$, 95% CI: −0.199 to −0.015). This association was statistically equivalent for both MZ and DZ twins. This association remained significant in adjusted models accounting for potential confounds
Sepe-Forrest et al. (2022)	229 (147 MZ/82 DZ)	40%	Human connectome project (HCP)—S1200 release	Lifetime number of uses of cannabis, age of cannabis use onset	Cerebellar-cortical functional connectivity	Young and middle adulthood (mean age 29.11 years, range 22–37 years)	None reported	MZ twins reporting higher numbers of lifetime cannabis uses did not show significant differences in resting-state functional connectivity across any of the seven cerebellar-cortical networks compared to their less-cannabis-using co-twins (bs ranging from -3.23×10^{-6} to 1.00×10^{-5}). This association was also not significant for MZ twins reporting earlier age of cannabis use onset (bs ranging from −0.031 to 0.017). Tests of interaction with zygosity were significant for several lifetime cannabis use models, specifically those predicting connectivity in the dorsal attention, limbic, and somatomotor networks. These interactions showed significant associations in DZ twins but smaller, nonsignificant associations in MZ twins
Harper et al. (2023)	112 (69 MZ/43 DZ)	0%	Minnesota twin family study enrichment sample	Cannabis use index scores (average of use frequency and number of uses measures)	Left and right hippocampal volume	Young adulthood (mean age 24.3 years, SD 0.8)[b]	None reported	Twins with higher cannabis use index scores were not significantly different in hippocampal volume compared to their co-twins with lower cannabis use index scores ($b = -54.51$, 95% CI: −114.71 to 10.17)

[a] % male reflects characteristics of the full sample because data for the twin subsample was not provided
[b] Age data reflect characteristics of the full sample because data for the twin subsample was not provided
[c] Breakdown of zygosity not provided

reporting that these individual-level associations were rendered nonsignificant after adjustment for one or more covariates (e.g., Agrawal et al. 2017).

3.1 Depression and Anxiety

A total of four studies have examined the relationship between cannabis use and depression or anxiety using a co-twin control or twin differences design. The first study on this topic investigated the relationships between early cannabis use and research diagnoses of cannabis dependence and major depressive disorder using data from twin pairs participating in the Australian Twin Registry. Despite the cross-sectional design of the study, the results accounted for temporal ordering by excluding twins who reported experiencing depression before they had ever used cannabis. Co-twin control models indicated that the twin reporting early cannabis use was significantly more likely to be diagnosed with depression compared to their later or nonusing co-twin. Similarly, twins diagnosed with cannabis dependence were also more likely to be diagnosed with depression compared to their nondependent co-twin. Tests of zygosity revealed that both within-pair associations were significant only in dizygotic twins, suggesting cannabis-depression associations likely reflected genetic confounding. In addition, the within-pair association between cannabis dependence and depression became nonsignificant after adjusting for the covariates of conduct disorder, early alcohol and tobacco use, alcohol dependence, and childhood sexual abuse (Lynskey et al. 2004).

A second study using data from an even larger sample of twin pairs participating in the Australian Twin Registry examined the relationships between lifetime cannabis use, early cannabis use (before age 17 or 18, cohort dependent), high-frequency cannabis use (>100 lifetime uses), and research diagnoses of major depressive disorder. Again, the authors controlled for temporal ordering by excluding twins reporting onset of depression prior to ever using cannabis. Associations between lifetime cannabis use and depression were nonsignificant in individual-level models, so only early and high-frequency cannabis use were examined using twin comparisons. Co-twin control models indicated that the twin reporting early cannabis use was significantly more likely to be diagnosed with depression than their later-using co-twin. Again, tests of zygosity revealed that these within-pair associations were significant only in dizygotic twins, suggesting the association likely reflected genetic confounding. Interestingly, however, co-twin control models also showed that twins reporting high-frequency cannabis use were significantly more likely to report depression than their less-cannabis-using co-twin. This within-pair association persisted in models restricted to monozygotic twins only to fully control for genetic confounding, consistent with a potential causal effect (Agrawal et al. 2017).

A third study investigated the relationship between research diagnoses of cannabis use disorder (CUD) and major depressive disorder through both co-twin control and comorbidity model-fitting analyses using additional data from the Australian

Twin Registry. Co-twin control models restricted to a subset of monozygotic twin pairs found that the twin diagnosed with CUD was significantly more likely to also receive a diagnosis of major depressive disorder than the co-twin without CUD. Comorbidity model fitting analysis found that the models suggesting causal effects from CUD to MDD fit the data best, providing further support for the idea that CUD may be a causal risk factor in the etiology of MDD (Smolkina et al. 2017).

A final study used longitudinal data from twin pairs participating in the Minnesota Twin and Family Study to examine the relationship between cumulative cannabis use assessed every few years in adolescence and research diagnoses of major depressive disorder and anxiety disorders made in young adulthood. Co-twin control models restricted to monozygotic twins indicated that twins reporting greater cumulative cannabis use were not significantly more likely than their less-cannabis-using co-twins to receive either diagnosis. It is worth noting, however, that the within-pair effect for receiving an anxiety disorder diagnosis was, in this case, larger than the individual-level effect, making it unclear if the nonsignificant within-pair effect should be interpreted as evidence of confounding by familial factors or reduced statistical power due to exclusion of dizygotic twin pairs (Schaefer et al. 2021).

3.2 Other Drug Use and Use Disorders

A total of seven twin studies have tested associations between cannabis use and use of other drugs as well as drug use disorders. Two used cross-sectional data from a national registry of male–male twin pairs who served in the military during the Vietnam War era. Co-twin control models indicated that twins who were former heavy cannabis users did not significantly differ from their less-cannabis-using co-twins in terms of their rates of alcohol and nicotine use disorders (Eisen et al. 2002). Twins who used cannabis early, however, were at significantly greater risk of trying other illicit drugs, being diagnosed with a non-cannabis illicit drug use disorder, and being diagnosed with alcohol dependence (Grant et al. 2010).

A third study used cross-sectional data from the Australian Twin Registry. Unadjusted co-twin control models indicated that twins with early cannabis use were significantly more likely to endorse lifetime use of other drugs as well as meet criteria for a lifetime illicit drug abuse/dependence diagnosis corresponding to each substance other than sedatives or alcohol dependence in unadjusted models. These associations all remained significant in adjusted models, suggesting they could not be explained by twin-specific covariates capturing other psychopathology or types of substance use (Lynskey et al. 2003).

A fourth study used cross-sectional data from the Virginia Twin Registry to also probe the effects of early cannabis use. Co-twin control models indicated that twins reporting early use were more likely to report using other illicit drugs. They also were more likely to meet criteria for a cannabis, opiate, or any illicit drug abuse/dependence diagnosis relative to their co-twin in dizygotic-only models, and more likely to meet criteria for a cannabis, cocaine, sedative, stimulant, hallucinogen,

alcohol, or any illicit drug abuse/dependence diagnosis in monozygotic-only models (Agrawal et al. 2004).

Two additional studies used longitudinal data from the Netherlands Twin Register. Co-twin control models of early cannabis use found that twins reporting early use were significantly more likely to report using party drugs, hard drugs, and regular cannabis use than their co-twins. However, after adjustment for covariates, the within-pair association with regular cannabis use became nonsignificant (Lynskey et al. 2006). Similarly, co-twin control models of lifetime cannabis use from this cohort found that twins reporting lifetime use were also more likely to report higher use of any drug and stimulants specifically, but not ecstasy use (Vink et al. 2020).

A final study used longitudinal data from a combined sample consisting of cohorts from the Minnesota Center for Twin Family Research and the Colorado Center for Antisocial Drug Dependence. Co-twin control models indicated that twins reporting more frequent cannabis use also were more likely to receive a diagnosis of cannabis use disorder, report other illicit drug use, and report higher tobacco use frequency than their less-cannabis-using co-twins. However, they were not significantly more likely to receive a diagnosis of alcohol use disorder or nicotine dependence and did not report significantly higher alcohol use frequency. Furthermore, only within-pair associations with cannabis use disorder remained significant in monozygotic-only twin models, suggesting that associations between cannabis and other outcomes may have reflected shared genetic propensity (Zellers et al. 2024).

3.3 Antisocial Personality Disorder

Two studies have used co-twin control models to examine associations between cannabis use and risk of antisocial personality disorder (ASPD). The first study used cross-sectional data from male, middle-adult monozygotic twin pairs in the Vietnam Era Twin Registry to investigate the relationship between cannabis abuse/dependence diagnoses and ten antisocial personality disorder symptoms. Co-twin control models restricted to monozygotic twins indicated that twins with a diagnosis of cannabis abuse or dependence were more likely to endorse only the ASPD symptoms of "failure to conform to social norms of lawful behavior" and "reckless regard for one's own or other's personal safety" than their non-diagnosed co-twins. In addition, only the symptom of "failure to conform to social norms of lawful behavior" remained significantly associated with these diagnoses in co-twin control models adjusted for alcohol abuse/dependence, conduct disorder, and combat experience, suggesting the relationship between this symptom and cannabis may be attributable to these factors rather than cannabis use per se (Scherrer et al. 1996).

The second study used longitudinal data from twin pairs participating in the Minnesota Center for Twin and Family Research to examine the relationship between cumulative cannabis use frequency in adolescence and research diagnoses

of ASPD made in young adulthood. Co-twin control models restricted to monozygotic twins indicated adolescent cumulative cannabis use frequency was not significantly associated with antisocial personality disorder in young adulthood, suggesting associations seen in individual-level models were primarily due to confounding by shared genetic and environmental risk factors (Schaefer et al. 2021).

3.4 Suicidality

Three studies have used a co-twin control design to examine the relationship between cannabis use and suicidality, operationalized as suicidal ideation, suicide plans, suicide attempts, and non-suicidal self-injury. All three used cross-sectional data on young and middle–adult twin pairs drawn from the Australian Twin Registry.

One cross-sectional study examined the relationships between research diagnoses of cannabis dependence, early cannabis use (before age 17), suicidal ideation, and suicide attempts. The study controlled for temporal ordering by excluding individuals who reported suicidality before starting cannabis use from the analysis. Co-twin control models indicated that twins with a cannabis dependence diagnosis were significantly more likely to report suicidal ideation and suicide attempts compared to their nondependent co-twins. Similarly, twins with early cannabis use were significantly more likely to report suicide attempts compared to co-twins who denied early use. Tests of interaction with zygosity indicated that the magnitudes of these within-pair effects were roughly equivalent for both monozygotic and dizygotic twin pairs, suggesting that within-pair effects remained significant even when controlling fully for shared genetic propensity. However, the within-pair effect of early cannabis use on subsequent suicidal ideation became nonsignificant in co-twin control models adjusting for conduct disorder, early alcohol and tobacco use, alcohol dependence, and childhood sexual abuse, raising the possibility that the observed relationship might be due to confounding by these twin-specific factors (Lynskey et al. 2004).

Another study investigated the relationship between both lifetime cannabis use and early cannabis use and non-suicidal self-injury, a known risk factor for suicidal ideation and suicide attempts (Wang et al. 2022). The co-twin control model restricted to monozygotic twins participating in the Australian Twin Registry found twins with early cannabis use (but not lifetime cannabis use) were significantly more likely to report engaging in non-suicidal self-injury relative to their co-twins. Results from the second co-twin control model utilizing data from pairs participating in the Missouri Adolescent Female Twin Study, which accounted for temporal ordering to ensure non-suicidal self-injury occurred after or around the onset of cannabis use, reported identical results to those of the Australian sample (Few et al. 2016).

A third study using data from twin pairs participating in the Australian Twin Registry assessed the relationships between lifetime cannabis use, early cannabis use (before age 17 or 18, cohort dependent), high-frequency cannabis use (using

cannabis >100 times), and suicidality measures (momentary and persistent suicidal ideation, suicide plan, and suicide attempt). Results from the study controlled for temporal ordering such that those reporting suicidality prior to cannabis onset were excluded from the analysis. Lifetime cannabis use was dropped from the co-twin control analysis due to null associations in the individual-level model. Co-twin control models indicated that compared to their non-early cannabis use co-twin, the early cannabis use co-twin was significantly more likely to report experiencing all measures of suicidality. Zygosity tests indicated that these relationships were only significant in dizygotic twins. This suggests that the individual-level relationships were largely due to shared familial factors. Further co-twin control models indicated the high-frequency cannabis use co-twin was significantly more likely to report both momentary and persistent suicidal ideation than their non-high-frequency cannabis use co-twin with null results for the associations with both suicide attempts and suicide plans. Co-twin control models restricted to monozygotic twins indicated that momentary suicidal ideation was still significantly associated with high-frequency cannabis use, consistent with a potential causal relationship (Agrawal et al. 2017).

3.5 Summary

Like its non-twin counterpart, the twin literature on cannabis use and mental health outcomes also provides varying levels of support for causal relationships across different conditions. For depression, the evidence generally does not support a causal effect of cannabis use. Within-pair associations between cannabis use and depression were significant in dizygotic but not monozygotic twin comparisons, suggesting that associations between cannabis use and major depressive disorder likely reflect genetic confounding rather than causation. The sole exception was for cannabis use disorder (CUD), where one study found significant within-pair associations with depression in models using monozygotic twins (Smolkina et al. 2017). The same conclusion can also be reached for associations between cannabis use and anxiety, although this finding was only examined in one study (Schaefer et al. 2021).

Regarding other drug use, the twin literature generally supports a causal relationship between cannabis use and increased risk of using other illicit drugs. Early cannabis use and lifetime cannabis use are consistently associated with greater likelihood of using other substances and developing substance use disorders, even after controlling for genetic and shared environmental factors. For antisocial personality disorder (ASPD), the evidence for a causal effect of cannabis use is weak, with most associations becoming nonsignificant in monozygotic twin comparisons or after adjusting for covariates. Finally, the literature on cannabis use and suicidality provides some evidence for causal effects, particularly for frequent cannabis use and early onset use. These associations persist in some monozygotic twin comparisons, suggesting that they cannot be fully explained by genetic or shared environmental factors. Overall, the twin literature provides the strongest support for causal

effects of cannabis use on other drug use and suicidality, with limited evidence supporting causal links to depression and little to no support for causal effects on anxiety or ASPD.

4 The Non-twin Literature on Cannabis and Brain Outcomes

Understanding the impact of cannabis use on brain structure and function can help improve our understanding of potential effects on mental health. The brain, as the primary organ responsible for cognition, emotion regulation, and behavior, serves as a critical mediator between cannabis exposure and psychological outcomes. By examining how cannabis use relates to various aspects of brain architecture and activity, researchers can therefore gain insights into the neurobiological mechanisms that may underlie observed associations between cannabis use and mental disorders.

This section will review current evidence on the relationship between cannabis use and three key aspects of brain structure and function: gray matter, white matter, and functional connectivity. Each of these components plays a unique and essential role in neural processing and communication. Advances in neuroimaging techniques, particularly various forms of magnetic resonance imaging (MRI), have enabled researchers to non-invasively assess multiple aspects of brain structure and function in vivo. Structural MRI is used to examine gray and white matter characteristics, while diffusion tensor imaging (DTI) provides insights into white matter integrity and connectivity. Functional MRI (fMRI) allows for the investigation of brain activity and functional connectivity. By synthesizing findings from both animal models and human studies across these different neuroimaging modalities, we aim to provide an overview of how cannabis might influence brain organization and activity, thereby informing our understanding of its potential impact on mental health outcomes. We will then compare findings from observational human studies to those generated by co-twin control studies of cannabis use, which extend this literature by providing key insights into the nature of observed individual-level associations.

4.1 Gray Matter

Gray matter is a crucial component of the central nervous system, comprising neuronal cell bodies, dendrites, unmyelinated axons, and glial cells. It plays a fundamental role in neural processing, synaptic plasticity, and the execution of cognitive functions. Gray matter is distributed throughout various regions of the brain, including the cerebral cortex, subcortical nuclei, and limbic structures such as the

hippocampus and amygdala. The volume, density, and integrity of gray matter in specific brain regions are often utilized as quantitative markers of neural health and function. Alterations in gray matter morphometry have been associated with a range of neurological and psychiatric conditions, as well as variation in cognitive performance. Consequently, understanding the potential impact of cannabis use on gray matter could provide several clues regarding its effects on mental health and cognitive functioning.

The effects of cannabis and its primary psychoactive constituents (THC, CBD, and their combination) on the brain have been studied in a variety of model organisms, including mice and rats. Studies comparing the brains of mice who received vaporized cannabis or cannabis constituents to nonexposed controls have reported that exposure leads to reduced gray matter volume in areas in the forebrain (Sadaka et al. 2023), midbrain (Taylor et al. 2023), and basal ganglia (Orihuel et al. 2023). One of these studies also identified changes in gray matter microstructure in the striatum, globus pallidus, lateral ventricles, thalamus, and septal nuclei, however, these changes were also affected by sex such that changes in regional microstructure were more pronounced in female (vs. male) rats (Orihuel et al. 2023). Although the research does not indicate whether changes are associated with adverse effects on brain function, the paper does suggest that the changes may be associated with different behavioral outcomes, such as apathy, depressive-like symptoms, and altered reward processing. Given that this study was conducted during adolescence, the observed sex-specific effects might be attributed to differences in hormonal influences and neurodevelopmental trajectories between males and females, with hormonal fluctuations potentially interacting with cannabinoid exposure to produce differential effects on gray matter microstructure.

Human studies investigating the effects of cannabis on brain structure and function have been limited to observational research designs, due to obvious logistical and ethical concerns. One meta-analysis of MRI studies from 30 different cross-sectional studies comparing cannabis users and nonusers reported that individuals who used cannabis consistently showed evidence of reduced gray matter volume in the hippocampus and orbitofrontal cortex relative to nonusers (Lorenzetti et al. 2019).

Longitudinal studies of cannabis use have helped to tease apart the effects of cannabis use on the brain from neurological factors that may predispose people to cannabis use. For example, one study initially identified reduced volume in the left hippocampus, amygdala, and superior temporal gyrus in cannabis users compared to nonusers. However, their three-year follow-up revealed that continued cannabis use did not further affect gray matter volume, suggesting potential limitations to cannabis-induced structural changes beyond adolescence (Koenders et al. 2016). This finding contrasts with cross-sectional studies but aligns with other longitudinal research. For instance, a longitudinal study of 181 participants from the Pittsburgh Youth Study found no associations between cannabis use patterns between ages 13 and 19 and adult brain structure (Meier et al. 2019). Conversely, a longitudinal analysis of European participants from the IMAGEN study reported greater gray matter volume in bilateral medial temporal lobes as well as the bilateral posterior cingulate, lingual gyri, and cerebellum in individuals reporting 1–2 uses of cannabis

by age 14 years (vs. those who did not use cannabis), but no significant differences in brain structure between individuals who were cannabis-naive at 14 years and went on to use cannabis versus those who were cannabis-naive and never used cannabis (Orr et al. 2019). Finally, a more recent longitudinal study of 1037 participants from the Dunedin Longitudinal Study reported that long-term cannabis users showed significantly smaller volumes in the left and right total hippocampus and 12 subfields compared to both nonusers and midlife recreational cannabis users (Meier et al. 2022).

4.2 White Matter

White matter, comprising myelinated axons and glial cells, plays a crucial role in facilitating communication between different brain regions. It forms the structural foundation for neural networks, enabling efficient information transfer and cognitive processing. The integrity and organization of white matter are essential for optimal brain function and have been implicated in various cognitive abilities and mental health outcomes. Two key measures used to assess white matter microstructure are fractional anisotropy (FA) and mean diffusivity (MD). FA quantifies the directional dependence of water diffusion in tissue, with higher values generally indicating greater white matter integrity. MD, on the other hand, measures the average molecular motion of water in tissue, independent of direction. Higher MD values suggest less restricted water movement, which can indicate tissue damage or inflammation. Together, these measures provide complementary information about white matter microstructure, with lower FA and higher MD often suggesting compromised structural connectivity.

Animal models of cannabis exposure suggest widespread effects on white matter. For example, mice exposed to THC were found to have reduced white matter FA compared to their healthy counterparts (Orihuel et al. 2023), suggesting that cannabis may disrupt these communication pathways, potentially affecting how different parts of the brain work together. Two additional animal studies using mouse models, however, found that exposure to THC resulted in increased white matter myelination and oligodendrocyte development, suggesting that THC may also have some positive effects on the brain (Aguado et al. 2021; Huerga-Gómez et al. 2021).

Cross-sectional, case-control studies comparing cannabis users and nonusers have also identified differences in white matter microstructure, though results have been somewhat inconsistent. A meta-analysis of 30 MRI studies comparing the white matter of cannabis users and nonusers found that nearly all studies found differences in white matter between the two groups, with many reporting reduced FA in the white matter of the superior longitudinal fasciculus and corpus callosum of cannabis users. Of the nine studies investigating correlations between FA of white matter microstructure and cannabis use age of onset, four reported significant positive correlations. Of the 16 studies comparing mean diffusivity (MD) between cannabis users and controls, 10 found significant differences. However, there were

notable inconsistencies across studies regarding FA and MD. For example, associations with FA in other brain regions, including the anterior cingulate gyrus and other white matter tracts, were reported in some studies but generally did not replicate consistently (Robinson et al. 2023).

Results from longitudinal studies have generally corroborated those from the case-control, cross-sectional literature. A two-year follow-up study of 23 regular cannabis users and 23 age, sex, and IQ-matched non-cannabis-using controls recruited from university advertisements reported reductions in FA in the superior longitudinal fasciculus in cannabis users (vs. nonusers) over the study period (Becker et al. 2015). Similarly, another 18-month follow-up study of 19 adolescents with CUD and 29 "healthy controls" reported that adolescents with CUD showed greater changes in FA in the left inferior longitudinal fasciculus (vs. controls), with greater consumption of cannabis in the inter-scan interval predicting greater FA decreases (Epstein and Kumra 2015).

4.3 Functional Connectivity

Functional connectivity refers to the temporal correlation of neural activity between spatially distinct brain regions, providing insights into the dynamic interactions within and between neural networks. This measure is crucial for understanding how different brain areas communicate and coordinate to support cognitive processes and behavior. Functional magnetic resonance imaging (fMRI) allows researchers to assess functional connectivity by measuring synchronous fluctuations in blood oxygen level-dependent (BOLD) signals across brain regions. Changes in functional connectivity may precede or accompany structural alterations in the brain, offering a potentially more sensitive measure of cannabis-related neural effects.

Studies investigating the effects of cannabis on functional connectivity have produced varied results, suggesting complex and region-specific changes in brain network dynamics. One study reported that male mice exposed to vaporized cannabis containing THC and CBD experienced altered connectivity in the hippocampus, increased connectivity in the hypothalamus and thalamus, and decreased connectivity in the prefrontal cortex and olfactory system (Coleman et al. 2022). These findings highlight the diverse effects of both cannabinoids on different brain regions, likely mediated through CB1 receptors in areas such as the dorsal and ventral striatum, hippocampal complex, prefrontal/cortical areas, and cerebellum. Another animal study examining the effects of CBD on global brain connectivity reported that male and female mice given CBD showed enhanced connectivity relative to controls (Sadaka et al. 2023).

Human studies of cannabis use and functional connectivity are relatively few in number, but provide an important complement to animal research. A review of 13 studies examining differences in functional connectivity across cannabis users and nonusers reported that use was associated with decreased functional connectivity in the nucleus accumbens (Lorenzetti et al. 2023). In addition, one 18-month

follow-up study of 22 treatment-seeking adolescents with cannabis use disorder (CUD) reported that the amount of cannabis used was correlated with reductions in functional connectivity between the caudal anterior cingulate cortex and the orbitofrontal cortex, regions implicated in executive function and decision-making processes (Camchong et al. 2017).

4.4 Summary

Like the literature on cannabis and mental health, the literature on cannabis and brain outcomes is also wide-ranging and marked by notable inconsistencies. For gray matter, animal studies consistently show reduced volume in various brain regions following cannabis exposure. Human studies, particularly cross-sectional research, have identified reduced gray matter volume in areas like the hippocampus and orbitofrontal cortex among cannabis users. However, longitudinal studies have produced mixed results, with some finding no significant changes in brain structure associated with continued cannabis use, while others report volume reductions in specific regions like the hippocampus in long-term users.

Regarding white matter, animal studies suggest that cannabis exposure may affect white matter integrity, though some studies indicate potential positive effects on myelination. Human studies have generally found differences in white matter microstructure between cannabis users and nonusers, with reduced FA in areas like the superior longitudinal fasciculus and corpus callosum being the most consistent findings. Longitudinal studies have largely supported these observations.

For functional connectivity, both animal and human studies report altered connectivity patterns associated with cannabis use, particularly in regions involved in reward processing and executive function. However, the specific nature and extent of these alterations vary across studies, with some reporting decreased connectivity and others finding no significant differences in global network organization. Overall, while there is evidence for cannabis-related effects on brain structure and function, the strength and consistency of this evidence vary across outcomes, with white matter changes appearing to be the most robustly supported findings.

5 Twin Studies Examining the Relationship Between Cannabis and Brain Outcomes

The twin literature on cannabis and brain outcomes is less extensive than the twin literature on cannabis and mental health, but nevertheless affords several interpretive advantages. To date, only three twin studies have examined the relationship between cannabis use and various region-specific brain outcomes (i.e., hippocampal volume, orbitofrontal cortex thickness, and resting-state cerebellar-cortical

functional connectivity) (see Table 19.1). Across studies and outcomes, the individual-level associations from these studies largely resemble associations from the non-twin literature.

Two of the three twin studies conducted their research using same-sex twins from the population-based Minnesota Twin Family Study Enrichment Sample. This twin sample was assessed at the age of 24 years and comprises 120 complete monozygotic (MZ) pairs and 66 complete dizygotic (DZ) pairs. One twin study using these twins investigated associations between cannabis use in the past 7 years and hippocampal volume. At the individual level, participants reporting greater cannabis use tended to have smaller hippocampi. This effect was moderated by sex, with women showing a significant relationship compared to men. However, co-twin control analyses suggested no significant differences in hippocampus size between twins reporting greater cannabis use and their lesser-using co-twins, even when analyses were conducted only using female twin pairs. This pattern of results suggests that associations between cannabis use and hippocampus volume likely reflect familial confounding rather than a causal effect of cannabis use (Harper et al. 2023).

Another study conducted using the same sample examined associations between orbitofrontal cortex (OFC) thickness and cannabis use disorder during emerging adulthood. Co-twin control models indicated that twins with cannabis use disorders had significantly thinner medial orbitofrontal cortices than their non-disordered co-twins. Interactions with zygosity were tested and revealed that the within-pair effects were statistically comparable between monozygotic and dizygotic twin pairs, suggesting that associations survived controls for both shared environmental and genetic factors. These within-pair effects were also robust to adjustment for twin-specific covariates, including subthreshold cases, unshared confounding from twin differences in comorbid substance use disorders, and recent substance use, strengthening the possibility they represent causal effects (Harper et al. 2021).

Finally, a third study included 1206 participants in the Human Connectome Project and examined the relationship between cannabis use and resting-state cerebellar-cortical functional connectivity. The primary ways the study operationalized cannabis exposure were age of cannabis use onset and the number of lifetime cannabis uses. Co-twin control models indicated that the associations between cannabis use measures and cerebellar-cortical connectivity were no longer significant after accounting for familial confounding, suggesting that connectivity differences associated with cannabis use were likely due to residual confounding (Sepe-Forrest et al. 2022).

5.1 Summary

Twin studies evaluating impacts of cannabis use on brain outcomes extend non-twin findings in several ways. While the non-twin studies generally suggested associations between cannabis use and various brain structural and functional changes, the

twin studies offer a more mixed picture, highlighting the potential role of genetic and shared environmental factors in these relationships.

The twin studies on hippocampal volume and cerebellar-cortical functional connectivity suggest that the associations observed in non-twin studies may be largely attributable to familial confounding rather than causal effects of cannabis use. This contrasts with some of the non-twin longitudinal studies that reported volume reductions in the hippocampus among long-term cannabis users. However, the twin study on orbitofrontal cortex thickness provides evidence more consistent with a potential causal effect of cannabis use disorder on brain structure, aligning with some findings from the non-twin literature.

These results underscore the importance of controlling for genetic and shared environmental factors when investigating cannabis-related brain outcomes. They suggest that some previously reported associations between cannabis use and brain structure or function may be overestimated due to unmeasured confounding. However, the limited number of twin studies in this area and their focus on specific brain regions means that caution should be exercised in generalizing these findings too widely. The mixed results also highlight the need for further research using genetically informed designs to better understand the complex relationship between cannabis use and brain outcomes across different regions and measures of brain structure and function.

6 Conclusion

Deciphering the lasting effects of cannabis use on the brain and behavior presents a formidable challenge to researchers, policymakers, mental health professionals, and consumers alike. Animal studies offer experimental controls but uncertain generalizability, whereas observational studies offer significantly greater ecological validity but struggle to identify the causal processes that give rise to the associations they generate. In other words, associations from observational studies can represent various causal processes, including direct causal effects, reverse causation, or shared risk factors (genetic or environmental) that influence both cannabis use and the outcome of interest. This complexity underscores the utility of genetically informed designs like twin studies, which offer a nonexperimental approach to disentangling these etiological possibilities (McGue et al. 2010).

The non-twin literature on cannabis use and mental health outcomes reveals a complex landscape with varying degrees of evidence strength across different conditions. Epidemiological and longitudinal studies consistently show associations between cannabis use and increased risk of depression, other substance use disorders, and suicidality, particularly among heavy users and those who initiate use in adolescence. The evidence for anxiety and antisocial behaviors is less clear, with mixed results across studies.

The twin literature extends these findings by providing crucial insights into the potential causal nature of these relationships (Table 19.1). For depression, the twin studies generally do not support a causal effect of cannabis use, as most within-pair associations in monozygotic twin models were nonsignificant (even if corresponding dizygotic estimates were not), suggesting genetic confounding. The exception is for high-frequency cannabis use and cannabis use disorder, where some evidence of a potential causal relationship emerges. The twin literature provides stronger support for causal effects of cannabis use on other drug use and suicidality, particularly for frequent and early onset use. However, there is little support for causal effects on anxiety or antisocial personality disorder.

Similarly, the non-twin studies consistently find that cannabis use is associated with alterations in brain structure and function. Cross-sectional and longitudinal studies have identified reduced gray matter volume in areas such as the hippocampus and orbitofrontal cortex among cannabis users. White matter studies have consistently found differences in microstructure between users and nonusers, particularly reduced fractional anisotropy in regions like the superior longitudinal fasciculus and corpus callosum. Functional connectivity studies report altered patterns associated with cannabis use, though results vary across brain regions.

The limited twin literature on brain outcomes provides important nuance to these findings (Table 19.1). Twin studies on hippocampal volume and cerebellar-cortical functional connectivity suggest that observed associations may be largely attributable to familial confounding rather than causal effects of cannabis use. However, a twin study on orbitofrontal cortex thickness provides evidence more consistent with a potential causal effect of cannabis use disorder on brain structure. These mixed results underscore the importance of controlling for genetic and shared environmental factors when investigating cannabis-related brain outcomes and suggest that some previously reported associations are likely overestimated due to unmeasured confounding.

Across twin studies, findings were least likely to indicate potential causal effects when cannabis exposure was operationalized as "lifetime use of any cannabis" and most likely when the exposure was "cannabis use disorder diagnosis." This pattern could be consistent with a potential thresholding effect, wherein cannabis use must reach a sufficient severity to have detectable effects on the brain and behavior. However, it could also reflect increased residual confounding in cannabis use disorder-outcome associations. To receive a cannabis use disorder diagnosis, an individual must demonstrate not only heavy cannabis use but also a pattern of problematic use impacting various aspects of their life. These diagnostic criteria inherently capture a broader range of individual characteristics beyond mere cannabis consumption, such as poor self-regulation, risk-taking tendencies, or difficulties in stress management. Consequently, associations between cannabis use disorder and various outcomes may be more susceptible to confounding by these unmeasured, nonshared individual traits, which could independently influence both the likelihood of developing a use disorder and the outcomes of interest. This complexity

underscores the need for caution in interpreting cannabis use disorder-outcome associations as purely causal, even in genetically informed designs.

The twin literature on cannabis use and its effects on mental health and brain outcomes, while valuable, is subject to several important limitations. One significant issue is the heterogeneity in how cannabis use and outcomes are assessed across studies, making direct comparisons challenging. In addition, the operationalization of cannabis use often relies on binary measures or cannabis use disorder diagnoses, which may not capture the full spectrum of use patterns and can reduce statistical power to detect effects. The reliance on retrospective measures and cross-sectional designs in many twin studies further limits efforts to establish temporal ordering of effects and introduces several measurement-related issues. Retrospective measures are prone to elevated risks of recall failure, particularly for less salient or more frequent behaviors, potentially leading to underreporting of cannabis use or mental health symptoms (Collins et al. 1985; Moffitt et al. 2010). Other limitations of retrospective measures include mood-congruent recall bias, where current mood states can influence the recollection of past experiences (Gaddy and Ingram 2014). These measurement issues can introduce significant noise into the data, potentially obscuring true associations or creating spurious ones.

Because twin studies are rare compared to studies of singletons, the generalizability of their findings is also less certain. Many studies draw data from the same twin registries, leading to an overrepresentation of twins who are Australian, Vietnam-era veterans, or Minnesotans, particularly for certain outcomes. This geographical and demographic concentration clearly does not represent the broader population of cannabis users. Furthermore, the community-based samples used in most studies may not capture the effects of particularly severe cannabis use, potentially underestimating the impact on certain outcomes. The literature is also characterized by a paucity of studies on specific outcomes, particularly brain-related measures, and a low number of studies examining longitudinal associations between cannabis use in adolescence and later mental health outcomes compared to non-twin studies.

Finally, it's crucial to recognize that while twin designs effectively control for genetic and shared environmental factors, they cannot distinguish between causal effects and confounding by nonshared environmental factors. Even significant within-pair effects observed in monozygotic twin comparisons could represent confounding by twin-specific factors like differences in personality, other substance use, or unique experiences, rather than true causal effects. Furthermore, exposure measurement error tends to bias within-twin-pair estimates more dramatically than corresponding unpaired associations (Fresán et al. 2022), and discordant twin designs generally provide less statistical power than twin difference designs. These limitations underscore the need for cautious inference regarding causality, even in a twin context.

The literature on cannabis and mental health presents interpretive challenges for clinicians working with patients who use cannabis, given the multiple causal possibilities associations might represent. Clinicians should be mindful that the twin literature suggests reducing cannabis use may not necessarily improve certain mental health outcomes, such as depression, anxiety, or ASPD. Indeed, these results suggest that for some outcomes, identifying and treating other underlying factors (e.g., environmental stressors, other substance use) might be more crucial than focusing solely on cannabis use reduction. However, clinicians should remain vigilant about the potential risks associated with heavy use and early onset, particularly regarding outcomes like other substance use and suicidality, where the evidence for causal effects is stronger.

For policymakers, these findings underscore the need for a nuanced understanding of cannabis effects when crafting legislation and public health strategies. Policymakers should be aware that many associations between cannabis use and brain/mental health outcomes reported in observational studies are likely inflated due to genetic and environmental confounding and keep this information in mind when weighing potential adverse effects of cannabis use against potential benefits of more liberal regulatory policies.

Future research should prioritize several key areas to address current limitations and enhance our understanding of cannabis effects. More twin difference studies with repeated, prospective assessments of cannabis use from adolescence into adulthood are needed to better establish temporal relationships and capture developmental effects. Greater homogeneity in outcome assessment across studies, or studies that operationalize outcomes in multiple ways, would facilitate more robust comparisons and meta-analyses. Replication efforts in both representative samples and those enriched for heavy cannabis use would enhance generalizability and capture effects at the extreme end of use. The creation of additional twin registries to examine associations in diverse demographics would address current limitations in sample representation. Finally, implementing high-frequency assessment strategies of cannabis exposure and outcomes, possibly smartphone-assisted, could minimize measurement error and maximize rates of twin discordance, enhancing the power of twin designs (Monk et al. 2015). As cannabis use policies continue to evolve globally, rigorous research remains crucial. By embracing methodological innovations and addressing current limitations, we can build a more comprehensive picture that balances potential risks and benefits, ultimately serving both individual and public health interests.

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References

Agrawal A, Neale MC, Prescott CA, Kendler KS. A twin study of early cannabis use and subsequent use and abuse/dependence of other illicit drugs. Psychol Med. 2004;34(7):1227–37. https://doi.org/10.1017/S0033291704002545.

Agrawal A, Budney AJ, Lynskey MT. The co-occurring use and misuse of cannabis and tobacco: a review. Addiction. 2012;107(7):1221–33. https://doi.org/10.1111/j.1360-0443.2012.03837.x.

Agrawal A, Nelson EC, Bucholz KK, Tillman R, Grucza RA, Statham DJ, Madden PA, Martin NG, Heath AC, Lynskey MT. Major depressive disorder, suicidal thoughts and behaviours, and cannabis involvement in discordant twins: a retrospective cohort study. Lancet Psychiatry. 2017;4(9):706–14. https://doi.org/10.1016/S2215-0366(17)30280-8.

Aguado T, Huerga-Gómez A, Sánchez-de la Torre A, Resel E, Chara JC, Matute C, Mato S, Galve-Roperh I, Guzman M, Palazuelos J. Δ9-Tetrahydrocannabinol promotes functional remyelination in the mouse brain. Br J Pharmacol. 2021;178(20):4176–92. https://doi.org/10.1111/bph.15608.

Alley ZM, Kerr DCR, Bae H. Trends in college students' alcohol, nicotine, prescription opioid and other drug use after recreational marijuana legalization: 2008–2018. Addict Behav. 2020;102:106212. https://doi.org/10.1016/j.addbeh.2019.106212.

Alves CN, Carlini EA. Effects of acute and chronic administration of Cannabis sative extract on the mouse-killing behavior of rats. Life Sci. 1973;13(1):75–85. https://doi.org/10.1016/0024-3205(73)90279-8.

Bachhuber MA, Saloner B, Cunningham CO, Barry CL. Medical cannabis laws and opioid analgesic overdose mortality in the United States, 1999-2010. JAMA Intern Med. 2014;174(10):1668–73. https://doi.org/10.1001/jamainternmed.2014.4005.

Bagge CL, Borges G. Acute substance use as a warning sign for suicide attempts: a case-crossover examination of the 48 hours prior to a recent suicide attempt. J Clin Psychiatry. 2017;78(6):20169. https://doi.org/10.4088/JCP.15m10541.

Bambico FR, Nguyen N-T, Katz N, Gobbi G. Chronic exposure to cannabinoids during adolescence but not during adulthood impairs emotional behaviour and monoaminergic neurotransmission. Neurobiol Dis. 2010;37(3):641–55. https://doi.org/10.1016/j.nbd.2009.11.020.

Becker MP, Collins PF, Lim KO, Muetzel RL, Luciana M. Longitudinal changes in white matter microstructure after heavy cannabis use. Dev Cogn Neurosci. 2015;16:23–35. https://doi.org/10.1016/j.dcn.2015.10.004.

Beletsky A, Liu C, Lochte B, Samuel N, Grant I. Cannabis and anxiety: a critical review. Med Cannabis Cannabinoids. 2024;7(1):19–30. https://doi.org/10.1159/000534855.

Biscaia M, Fernández B, Higuera-Matas A, Miguéns M, Viveros M-P, García-Lecumberri C, Ambrosio E. Sex-dependent effects of periadolescent exposure to the cannabinoid agonist CP-55,940 on morphine self-administration behaviour and the endogenous opioid system. Neuropharmacology. 2008;54(5):863–73. https://doi.org/10.1016/j.neuropharm.2008.01.006.

Borges G, Bagge CL, Orozco R. A literature review and meta-analyses of cannabis use and suicidality. J Affect Disord. 2016;195:63–74. https://doi.org/10.1016/j.jad.2016.02.007.

Brook JS, Lee JY, Rubenstone E, Brook DW, Finch SJ. Triple comorbid trajectories of tobacco, alcohol, and marijuana use as predictors of antisocial personality disorder and generalized anxiety disorder among urban adults. Am J Public Health. 2014;104(8):1413–20. https://doi.org/10.2105/AJPH.2014.301880.

Camchong J, Lim KO, Kumra S. Adverse effects of cannabis on adolescent brain development: a longitudinal study. Cereb Cortex. 2017;27(3):1922–30. https://doi.org/10.1093/cercor/bhw015.

Chan NW, Burkhardt J, Flyr M. The effects of recreational marijuana legalization and dispensing on opioid mortality. Econ Inq. 2020;58(2):589–606. https://doi.org/10.1111/ecin.12819.

Cheung JTW, Mann RE, Ialomiteanu A, Stoduto G, Chan V, Ala-Leppilampi K, Rehm J. Anxiety and mood disorders and cannabis use. Am J Drug Alcohol Abuse. 2010;36(2):118–22. https://doi.org/10.3109/00952991003713784.

Chuan-Yu C, Wagner FA, Anthony JC. Marijuana use and the risk of major depressive episode epidemiological evidence from the United States National Comorbidity Survey: epidemiological evidence from the United States National Comorbidity Survey. Soc Psychiatry Psychiatr Epidemiol. 2002;37(5):199–206. https://doi.org/10.1007/s00127-002-0541-z.

Coleman JR, Madularu D, Ortiz RJ, Athanassiou M, Knudsen A, Alkislar I, Cai X, Kulkarni PP, Cushing BS, Ferris CF. Changes in brain structure and function following chronic exposure to inhaled vaporised cannabis during periadolescence in female and male mice: a multimodal MRI study. Addict Biol. 2022;27(3):e13169. https://doi.org/10.1111/adb.13169.

Collins LM, Graham JW, Hansen WB, Johnson CA. Agreement between retrospective accounts of substance use and earlier reported substance use. Appl Psychol Meas. 1985;9(3):301–9. https://doi.org/10.1177/014662168500900308.

Compton WM, Conway KP, Stinson FS, Colliver JD, Grant BF. Prevalence, correlates, and comorbidity of DSM-IV antisocial personality syndromes and alcohol and specific drug use disorders in the United States: results from the national epidemiologic survey on alcohol and related conditions. J Clin Psychiatry. 2005;66(6):677–85.

Crippa JA, Zuardi AW, Martín-Santos R, Bhattacharyya S, Atakan Z, McGuire P, Fusar-Poli P. Cannabis and anxiety: a critical review of the evidence. Hum Psychopharmacol Clin Exp. 2009;24(7):515–23. https://doi.org/10.1002/hup.1048.

De Graaf R, Radovanovic M, Van Laar M, Fairman B, Degenhardt L, Aguilar-Gaxiola S, Bruffaerts R, De Girolamo G, Fayyad J, Gureje O, Haro JM, Huang Y, Kostychenko S, Lepine J-P, Matschinger H, Mora MEM, Neumark Y, Ormel J, Posada-Villa J, Anthony JC. Early cannabis use and estimated risk of later onset of depression spells: epidemiologic evidence from the population-based World Health Organization World Mental Health Survey Initiative. Am J Epidemiol. 2010;172(2):149–59. https://doi.org/10.1093/aje/kwq096.

Degenhardt L, Hall W, Lynskey M. The relationship between cannabis use, depression and anxiety among Australian adults: findings from the National Survey of Mental Health and Well-Being. Soc Psychiatry Psychiatr Epidemiol. 2001;36(5):219–27. https://doi.org/10.1007/s001270170052.

Dellazizzo L, Potvin S, Dou BY, Beaudoin M, Luigi M, Giguère C-É, Dumais A. Association between the use of cannabis and physical violence in youths: a meta-analytical investigation. Am J Psychiatry. 2020;177(7):619–26. https://doi.org/10.1176/appi.ajp.2020.19101008.

Eisen SA, Chantarujikapong S, Xian H, Lyons MJ, Toomey R, True WR, Scherrer JF, Goldberg J, Tsuang MT. Does marijuana use have residual adverse effects on self-reported health measures, socio-demographics and quality of life? A monozygotic co-twin control study in men. Addiction. 2002;97(9):1137–44. https://doi.org/10.1046/j.1360-0443.2002.00120.x.

Ellgren M, Artmann A, Tkalych O, Gupta A, Hansen HS, Hansen SH, Devi LA, Hurd YL. Dynamic changes of the endogenous cannabinoid and opioid mesocorticolimbic systems during adolescence: THC effects. Eur Neuropsychopharmacol. 2008;18(11):826–34. https://doi.org/10.1016/j.euroneuro.2008.06.009.

Epstein KA, Kumra S. White matter fractional anisotropy over two time points in early onset schizophrenia and adolescent cannabis use disorder: a naturalistic diffusion tensor imaging study. Psychiatry Res Neuroimaging. 2015;232(1):34–41. https://doi.org/10.1016/j.pscychresns.2014.10.010.

Fedorova EV, Ataiants J, Wong CF, Iverson E, Lankenau SE. Changes in medical cannabis patient status before and after cannabis legalization in california: associations with cannabis and other drug use. J Psychoactive Drugs. 2022;54(2):129–39. https://doi.org/10.1080/02791072.2021.1926604.

Fergusson DM, Boden JM. Cannabis use and later life outcomes. Addiction. 2008;103(6):969–76. https://doi.org/10.1111/j.1360-0443.2008.02221.x.

Fergusson DM, Horwood LJ. Early onset cannabis use and psychosocial adjustment in young adults. Addiction. 1997;92(3):279–96. https://doi.org/10.1111/j.1360-0443.1997.tb03198.x.

Few LR, Grant JD, Nelson EC, Trull TJ, Grucza RA, Bucholz KK, Verweij KJH, Martin NG, Statham DJ, Madden PAF, Heath AC, Lynskey MT, Agrawal A. Cannabis involvement and

nonsuicidal self-injury: a discordant twin approach. J Stud Alcohol Drugs. 2016;77(6):873–80. https://doi.org/10.15288/jsad.2016.77.873.

Fingesi TS, Kimaru LJ, Okusanya BO, Ehiri JE, Rosales C. Nicotine and alcohol use as predictors of recreational cannabis use in adolescence: a systematic review and narrative synthesis. Subst Use Misuse. 2024;59(9):1367–82. https://doi.org/10.1080/10826084.2024.2342008.

Fresán A, Dionisio-García DM, González-Castro TB, Ramos-Méndez MÁ, Castillo-Avila RG, Tovilla-Zárate CA, Juárez-Rojop IE, López-Narváez ML, Genis-Mendoza AD, Nicolini H. Cannabis smoking increases the risk of suicide ideation and suicide attempt in young individuals of 11–21 years: a systematic review and meta-analysis. J Psychiatr Res. 2022;153:90–8. https://doi.org/10.1016/j.jpsychires.2022.06.053.

Gaddy MA, Ingram RE. A meta-analytic review of mood-congruent implicit memory in depressed mood. Clin Psychol Rev. 2014;34(5):402–16. https://doi.org/10.1016/j.cpr.2014.06.001.

Gerring ZF, Thorp JG, Treur JL, Verweij KJH, Derks EM. The genetic landscape of substance use disorders. Mol Psychiatry. 2024;29(11):3694–705. https://doi.org/10.1038/s41380-024-02547-z.

Gobbi G, Atkin T, Zytynski T, Wang S, Askari S, Boruff J, Ware M, Marmorstein N, Cipriani A, Dendukuri N, Mayo N. Association of cannabis use in adolescence and risk of depression, anxiety, and suicidality in young adulthood: a systematic review and meta-analysis. JAMA Psychiatry. 2019;76(4):426–34. https://doi.org/10.1001/jamapsychiatry.2018.4500.

Grant JD, Lynskey MT, Scherrer JF, Agrawal A, Heath AC, Bucholz KK. A cotwin-control analysis of drug use and abuse/dependence risk associated with early-onset cannabis use. Addict Behav. 2010;35(1):35–41. https://doi.org/10.1016/j.addbeh.2009.08.006.

Green KM, Doherty EE, Stuart EA, Ensminger ME. Does heavy adolescent marijuana lead to criminal involvement in adulthood? Evidence from a multiwave Longitudinal Study of Urban African Americans. Drug Alcohol Depend. 2010;112(1–2):117–25. https://doi.org/10.1016/j.drugalcdep.2010.05.018.

Grucza RA, Vuolo M, Krauss MJ, Plunk AD, Agrawal A, Chaloupka FJ, Bierut LJ. Cannabis decriminalization: a study of recent policy change in five U.S. states. Int J Drug Policy. 2018;59:67–75. https://doi.org/10.1016/j.drugpo.2018.06.016.

Harder VS, Stuart EA, Anthony JC. Adolescent cannabis problems and young adult depression: male-female stratified propensity score analyses. Am J Epidemiol. 2008;168(6):592–601. https://doi.org/10.1093/aje/kwn184.

Harper J, Wilson S, Malone SM, Hunt RH, Thomas KM, Iacono WG. Orbitofrontal cortex thickness and substance use disorders in emerging adulthood: causal inferences from a co-twin control/discordant twin study. Addiction. 2021;116(9):2548–58. https://doi.org/10.1111/add.15447.

Harper J, Wilson S, Bair JL, Hunt RH, Thomas KM, Malone SM, Iacono WG. Testing the consequences of alcohol, cannabis, and nicotine use on hippocampal volume: a quasi-experimental cotwin control analysis of young adult twins. Psychol Med. 2023;53(6):2671–81. https://doi.org/10.1017/S0033291721004682.

Hasbi A, Madras BK, George SR. Endocannabinoid system and exogenous cannabinoids in depression and anxiety: a review. Brain Sci. 2023;13(2):325. https://doi.org/10.3390/brainsci13020325.

Hasin DS. US epidemiology of cannabis use and associated problems. Neuropsychopharmacology. 2018;43(1):195–212. https://doi.org/10.1038/npp.2017.198.

Hasin DS, Kerridge BT, Saha TD, Huang B, Pickering R, Smith SM, Jung J, Zhang H, Grant BF. Prevalence and correlates of DSM-5 cannabis use disorder, 2012-2013: findings from the national epidemiologic survey on alcohol and related conditions–III. Am J Psychiatry. 2016;173(6):588–99. https://doi.org/10.1176/appi.ajp.2015.15070907.

Higuera-Matas A, Botreau F, Del Olmo N, Miguéns M, Olías Ó, Montoya GL, García-Lecumberri C, Ambrosio E. Periadolescent exposure to cannabinoids alters the striatal and hippocampal dopaminergic system in the adult rat brain. Eur Neuropsychopharmacol. 2010;20(12):895–906. https://doi.org/10.1016/j.euroneuro.2010.06.017.

Hinckley JD, Mikulich-Gilbertson SK, He J-P, Bhatia D, Ellingson JM, Vu BN, Merikangas KR, Sakai JT. Cannabis use is associated with depression severity and suicidality in the National

Comorbidity Survey−Adolescent Supplement. JAACAP Open. 2023;1(1):24–35. https://doi.org/10.1016/j.jaacop.2023.02.002.

Huerga-Gómez A, Aguado T, Sánchez-de la Torre A, Bernal-Chico A, Matute C, Mato S, Guzmán M, Galve-Roperh I, Palazuelos J. Δ9-tetrahydrocannabinol promotes oligodendrocyte development and CNS myelination in vivo. Glia. 2021;69(3):532–45. https://doi.org/10.1002/glia.23911.

Johnson EC, Demontis D, Thorgeirsson TE, Walters RK, Polimanti R, Hatoum AS, Sanchez-Roige S, Paul SE, Wendt FR, Clarke T-K, Lai D, Reginsson GW, Zhou H, He J, Baranger DAA, Gudbjartsson DF, Wedow R, Adkins DE, Adkins AE, Agrawal A. A large-scale genome-wide association study meta-analysis of cannabis use disorder. Lancet Psychiatry. 2020;7(12):1032–45. https://doi.org/10.1016/S2215-0366(20)30339-4.

Karoly HC, Ross JM, Ellingson JM, Feldstein Ewing SW. Exploring cannabis and alcohol co-use in adolescents: a narrative review of the evidence. J Dual Diagn. 2020;16(1):58–74. https://doi.org/10.1080/15504263.2019.1660020.

Koenders L, Cousijn J, Vingerhoets WAM, van den Brink W, Wiers RW, Meijer CJ, Machielsen MWJ, Veltman DJ, Goudriaan AE, de Haan L. Grey matter changes associated with heavy cannabis use: a longitudinal sMRI study. PLoS ONE. 2016;11(5):e0152482. https://doi.org/10.1371/journal.pone.0152482.

Kosiba JD, Maisto SA, Ditre JW. Patient-reported use of medical cannabis for pain, anxiety, and depression symptoms: systematic review and meta-analysis. Soc Sci Med. 2019;233:181–92. https://doi.org/10.1016/j.socscimed.2019.06.005.

Lee JY, Pahl K, Kim W. Latent triple trajectories of substance use as predictors for the onset of antisocial personality disorder among urban African American and Puerto Rican adults: A 22-year longitudinal study. Subst Abus. 2022;43(1):442–50. https://doi.org/10.1080/08897077.2021.1946890.

Lev-Ran S, Roerecke M, Le Foll B, George TP, McKenzie K, Rehm J. The association between cannabis use and depression: a systematic review and meta-analysis of longitudinal studies. Psychol Med. 2014;44(4):797–810. https://doi.org/10.1017/S0033291713001438.

Lorenzetti V, Chye Y, Silva P, Solowij N, Roberts CA. Does regular cannabis use affect neuroanatomy? An updated systematic review and meta-analysis of structural neuroimaging studies. Eur Arch Psychiatry Clin Neurosci. 2019;269(1):59–71. https://doi.org/10.1007/s00406-019-00979-1.

Lorenzetti V, Gaillard A, Thomson D, Englund A, Freeman TP. Effects of cannabinoids on resting state functional brain connectivity: a systematic review. Neurosci Biobehav Rev. 2023;145:105014. https://doi.org/10.1016/j.neubiorev.2022.105014.

Lowe DJE, Sorkhou M, George TP. Cannabis use in adolescents and anxiety symptoms and disorders: a systematic review and meta-analysis. Am J Drug Alcohol Abuse. 2024;50(2):150–61. https://doi.org/10.1080/00952990.2023.2299922.

Lynskey MT, Heath AC, Bucholz KK, Slutske WS, Madden PAF, Nelson EC, Statham DJ, Martin NG. Escalation of drug use in early-onset cannabis users vs co-twin controls. JAMA. 2003;289(4):427–33. https://doi.org/10.1001/jama.289.4.427.

Lynskey MT, Glowinski AL, Todorov AA, Bucholz KK, Madden PAF, Nelson EC, Statham DJ, Martin NG, Heath AC. Major depressive disorder, suicidal ideation, and suicide attempt in twins discordant for cannabis dependence and early-onset cannabis use. Arch Gen Psychiatry. 2004;61(10):1026–32. https://doi.org/10.1001/archpsyc.61.10.1026.

Lynskey MT, Vink JM, Boomsma DI. Early onset cannabis use and progression to other drug use in a sample of Dutch twins. Behav Genet. 2006;36(2):195–200. https://doi.org/10.1007/s10519-005-9023-x.

McDonough JH, Manning FJ, Elsmore TF. Reduction of predatory aggression of rats following administration of delta-9-tetrahydrocannabinol. Life Sci. 1972;11(3):103–11. https://doi.org/10.1016/0024-3205(72)90223-8.

McGue M, Osler M, Christensen K. Causal inference and observational research: the utility of twins. Perspect Psychol Sci. 2010;5(5):546–56. https://doi.org/10.1177/1745691610383511.

Meier MH, Schriber RA, Beardslee J, Hanson J, Pardini D. Associations between adolescent cannabis use frequency and adult brain structure: a prospective study of boys followed to adulthood. Drug Alcohol Depend. 2019;202:191–9. https://doi.org/10.1016/j.drugalcdep.2019.05.012.

Meier MH, Caspi AR, Knodt A, Hall W, Ambler A, Harrington H, Hogan SM, Houts R, Poulton R, Ramrakha S, Hariri AR, Moffitt TE. Long-term cannabis use and cognitive reserves and hippocampal volume in midlife. Am J Psychiatry. 2022;179(5):362–74. https://doi.org/10.1176/appi.ajp.2021.21060664.

Moffitt TE, Caspi A, Taylor A, Kokaua J, Milne BJ, Polanczyk G, Poulton R. How common are common mental disorders? Evidence that lifetime prevalence rates are doubled by prospective versus retrospective ascertainment. Psychol Med. 2010;40(06):899–909. https://doi.org/10.1017/S0033291709991036.

Monk RL, Heim D, Qureshi A, Price A. "I have no clue what i drunk last night" using smartphone technology to compare in-vivo and retrospective self-reports of alcohol consumption. PLoS ONE. 2015;10(5):e0126209. https://doi.org/10.1371/journal.pone.0126209.

Montez JK, Friedman EM. Educational attainment and adult health: under what conditions is the association causal? Soc Sci Med. 2015;127:1–7. https://doi.org/10.1016/j.socscimed.2014.12.029.

Orihuel J, Capellán R, Casquero-Veiga M, Soto-Montenegro ML, Desco M, Oteo-Vives M, Ibáñez-Moragues M, Magro-Calvo N, Luján VM, Morcillo MÁ, Ambrosio E, Higuera-Matas A. The long-term effects of adolescent Δ9-tetrahydrocannabinol on brain structure and function assessed through neuroimaging techniques in male and female rats. Eur Neuropsychopharmacol. 2023;74:47–63. https://doi.org/10.1016/j.euroneuro.2023.05.005.

Orr C, Spechler P, Cao Z, Albaugh M, Chaarani B, Mackey S, D'Souza D, Allgaier N, Banaschewski T, Bokde ALW, Bromberg U, Büchel C, Quinlan EB, Conrod P, Desrivières S, Flor H, Frouin V, Gowland P, Heinz A, Garavan H. Grey matter volume differences associated with extremely low levels of cannabis use in adolescence. J Neurosci. 2019;39(10):1817–27. https://doi.org/10.1523/JNEUROSCI.3375-17.2018.

Pedersen W, Skardhamar T. Cannabis and crime: findings from a longitudinal study. Addiction. 2010;105(1):109–18. https://doi.org/10.1111/j.1360-0443.2009.02719.x.

Peters EN, Schwartz RP, Wang S, O'Grady KE, Blanco C. Psychiatric, psychosocial, and physical health correlates of co-occurring cannabis use disorders and nicotine dependence. Drug Alcohol Depend. 2014;134:228–34. https://doi.org/10.1016/j.drugalcdep.2013.10.003.

Ramo DE, Liu H, Prochaska JJ. Tobacco and marijuana use among adolescents and young adults: a systematic review of their co-use. Clin Psychol Rev. 2012;32(2):105–21. https://doi.org/10.1016/j.cpr.2011.12.002.

Realini N, Vigano D, Guidali C, Zamberletti E, Rubino T, Parolaro D. Chronic URB597 treatment at adulthood reverted most depressive-like symptoms induced by adolescent exposure to THC in female rats. Neuropharmacology. 2011;60(2-3):235–43. https://doi.org/10.1016/j.neuropharm.2010.09.003.

Rey JM, Sawyer MG, Raphael B, Patton GC, Lynskey M. Mental health of teenagers who use cannabis: results of an Australian survey. Br J Psychiatry. 2002;180(3):216–21. https://doi.org/10.1192/bjp.180.3.216.

Robinson EA, Gleeson J, Arun AH, Clemente A, Gaillard A, Rossetti MG, Brambilla P, Bellani M, Crisanti C, Curran HV, Lorenzetti V. Measuring white matter microstructure in 1,457 cannabis users and 1,441 controls: a systematic review of diffusion-weighted MRI studies. Front Neuroimaging. 2023;2:1129587. https://doi.org/10.3389/fnimg.2023.1129587.

Rubino T, Vigano D, Realini N. Chronic Δ-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. Neuropsychopharmacology. 2008;33(11):2760–71. https://doi.org/10.1038/sj.npp.1301664.

Sadaka AH, Canuel J, Febo M, Johnson CT, Bradshaw HB, Ortiz R, Ciumo F, Kulkarni P, Gitcho MA, Ferris CF. Effects of inhaled cannabis high in Δ9-THC or CBD on the aging brain: a translational MRI and behavioral study. Front Aging Neurosci. 2023;15:1055433. https://doi.org/10.3389/fnagi.2023.1055433.

Salgado JV, Sandner G. A critical overview of animal models of psychiatric disorders: challenges and perspectives. Braz J Psychiatry. 2013;35:S77–81. https://doi.org/10.1590/1516-4446-2013-1156.

Schaefer JD, Moffitt TE, Arseneault L, Danese A, Fisher HL, Houts R, Sheridan MA, Wertz J, Caspi A. Adolescent victimization and early-adult psychopathology: Approaching causal inference using a longitudinal twin study to rule out noncausal explanations. Clin Psychol Sci. 2018;6(3):352–71. https://doi.org/10.1177/2167702617741381.

Schaefer JD, Hamdi NR, Malone SM, Vrieze S, Wilson S, McGue M, Iacono WG. Associations between adolescent cannabis use and young-adult functioning in three longitudinal twin studies. Proc Natl Acad Sci USA. 2021;118(14):e2013180118. https://doi.org/10.1073/pnas.2013180118.

Scheidell JD, Quinn K, McGorray SP, Frueh BC, Beharie NN, Cottler LB, Khan MR. Childhood traumatic experiences and the association with marijuana and cocaine use in adolescence through adulthood. Addiction. 2018;113(1):44–56. https://doi.org/10.1111/add.13921.

Scherrer JF, Lin N, Eisen SA, Goldberg J, True WR, Lyons MJ, Tsuang MT. the association of antisocial personality symptoms with marijuana abuse/dependence: a monozygotic co-twin control study. J Nerv Ment Dis. 1996;184(10):611–5. https://doi.org/10.1097/00005053-199610000-00005.

Schneider M, Koch M. Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. Neuropsychopharmacology. 2003;28(10):1760–9. https://doi.org/10.1038/sj.npp.1300225.

Sepe-Forrest L, Kim D-J, Quinn PD, Bolbecker AR, Wisner KM, Hetrick WP, O'Donnell BF. Evidence of familial confounding of the association between cannabis use and cerebellar-cortical functional connectivity using a twin study. NeuroImage Clin. 2022;36:103237. https://doi.org/10.1016/j.nicl.2022.103237.

Single A, Bilevicius E, Ho V, Theule J, Buckner JD, Mota N, Keough MT. Cannabis use and social anxiety in young adulthood: a meta-analysis. Addict Behav. 2022;129:107275. https://doi.org/10.1016/j.addbeh.2022.107275.

Smolkina M, Morley KI, Rijsdijk F, Agrawal A, Bergin JE, Nelson EC, Statham D, Martin NG, Lynskey MT. Cannabis and depression: a twin model approach to co-morbidity. Behav Genet. 2017;47(4):394–404. https://doi.org/10.1007/s10519-017-9848-0.

Taylor A, Nweke A, Vincent V, Oke M, Kulkarni P, Ferris CF. Chronic exposure to inhaled vaporized cannabis high in Δ9-THC alters brain structure in adult female mice. Front Neurosci. 2023;17:1139309. https://doi.org/10.3389/fnins.2023.1139309.

Tomasiewicz HC, Jacobs MM, Wilkinson MB, Wilson SP, Nestler EJ, Hurd YL. Proenkephalin mediates the enduring effects of adolescent cannabis exposure associated with adult opiate vulnerability. Biol Psychiatry. 2012;72(10):803–10. https://doi.org/10.1016/j.biopsych.2012.04.026.

Twomey CD. Association of cannabis use with the development of elevated anxiety symptoms in the general population: a meta-analysis. J Epidemiol Community Health. 2017;71(8):811–6.

Ueki S, Fujiwara M, Ogawa N. Mouse-killing behavior (muricide) induced by Δ9-tetrahydrocannabinol in the rat. Physiol Behav. 1972;9(4):585–7. https://doi.org/10.1016/0031-9384(72)90016-9.

Vink JM, Veul L, Abdellaoui A, Hottenga J-J, Boomsma DI, Verweij KJH. Illicit drug use and the genetic overlap with Cannabis use. Drug Alcohol Depend. 2020;213:108102. https://doi.org/10.1016/j.drugalcdep.2020.108102.

Wang Y-J, Li X, Ng CH, Xu D-W, Hu S, Yuan T-F. Risk factors for non-suicidal self-injury (NSSI) in adolescents: a meta-analysis. eClinicalMedicine. 2022;46:101350. https://doi.org/10.1016/j.eclinm.2022.101350.

Weller RA, Halikas JA. Marijuana use and psychiatric illness: a follow-up study. Am J Psychiatry. 1985;142(7):848–50. https://doi.org/10.1176/ajp.142.7.848.

Willits DW, Solensten B, Meize M, Stohr MK, Makin DA, Hemmens C, Stanton DL, Lovrich NP. Racial disparities in the wake of cannabis legalization: documenting persistence and change. Race Justice. 2022;15(1):4–21. https://doi.org/10.1177/21533687221087355.

Wilson J, Mills K, Freeman TP, Sunderland M, Visontay R, Marel C. Weeding out the truth: a systematic review and meta-analysis on the transition from cannabis use to opioid use and opioid use disorders, abuse or dependence. Addiction. 2022;117(2):284–98. https://doi.org/10.1111/add.15581.

Zellers S, Alexander J, Ellingson JM, Schaefer JD, Corley RP, Iacono W, Hewitt JK, Hopfer CJ, McGue MK, Vrieze S. Limited psychological and social effects of lifetime cannabis use frequency: evidence from a 30-year community study of 4,078 twins. J Psychopathol Clin Sci. 2024;133(1):115–28. https://doi.org/10.1037/abn0000867.

Part V
Social and Environmental Contexts

Chapter 20
Childhood Maltreatment, Pain, and the Endocannabinoid Signaling System



Tehmina Shakir and Hilary A. Marusak

1 Childhood Maltreatment: Definitions, Prevalence and Correlates

Childhood maltreatment constitutes all forms of physical and emotional maltreatment, including physical, emotional and sexual abuse, physical and emotional neglect of a child and commercial or other exploitation experienced before the age of 18 years (Administration on Children, Youth and Families, Administration for Children and Families 2022). According to the WHO, *physical abuse* of a child results in actual or potential physical harm from an interaction, reasonably within the control of a parent or person in a position of responsibility, power and trust (WHO_HSC_PVI_99.1). *Emotional abuse* involves the failure to provide a developmentally appropriate, safe and supportive environment, including the availability of a primary attachment figure, enabling the child to develop a stable and full range of emotional and social competencies commensurate with their potential and the context of their society. *Neglect* is the failure to provide for a child's development across all areas and has a high probability of causing harm to the child's health or physical, mental, spiritual, moral, or social development. Child *sexual abuse* involves engaging a child in sexual activities that they do not fully understand, cannot give informed consent to, are not developmentally prepared for, or that violate societal laws or social norms. *Commercial or other exploitation* refers to using a child in work or other activities for the benefit of others, such as child labor and child prostitution (WHO_HSC_PVI_99.1).

Maltreatment often co-occurs with other stressors, such as *trauma* and *adversity* (Brown et al. 2019). Trauma refers to a broader range of distressing events,

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including both interpersonal (e.g., assault, abuse) and non-interpersonal events (e.g., car accidents, natural disasters) (Brown et al. 2019; SAMHSA 2024). Adversity encompasses a wide array of negative early life experiences that can affect development including having a household member with substance use disorder or mental illness, separation from a parent or loved one due to death, poverty, parental divorce or incarceration, and systemic racism and discrimination (www.nctsn.org). Here, we focus on childhood maltreatment given its strong correlation with later development of pain and related pain syndromes (Afari et al. 2014).

Unfortunately, childhood maltreatment is common in the United States. Each day, more than 1000 youth are treated in emergency departments for physical assault-related injuries (SAMHSA 2024). According to the National Child Abuse and Neglect Data System, during FY 2022, a total of 558,899 victims of child abuse and neglect were reported in the United States, with a national rate of 7.7 victims per 1000 children in the population. Among these victims, 74.3% experienced neglect, while 17%, 10.6%, and 6.8% were physically, sexually, and psychologically maltreated, respectively. The same year, 45,756 infants with prenatal substance exposure were referred to child protective services. A nationally estimated 1990 children died from childhood maltreatment at a rate of 2.73 per 100,000 children (Administration on Children, Youth and Families, Administration for Children and Families 2022). Studies have shown mixed results of childhood maltreatment during the COVID-19 pandemic, depending on the study design and data source (Carsley et al. 2024). There is also possible under detection, underreporting and delays in the presentation of abusive injuries at treatment centers due to the COVID-19 pandemic (Carsley et al. 2024; Russell et al. 2022; Rapp et al. 2021; Kovler et al. 2021). More studies are needed to evaluate the direct effects of childhood maltreatment during the pandemic, its correlates and long-term effects.

Global statistics also report high prevalence rates of maltreatment against children. According to the World Health Organization (WHO)'s Global Status Report on Preventing Violence Against children, 2020, globally, an estimated one out of two children aged 2–17 years suffer some form of violence each year. Worldwide, close to 300 million children aged 2–4 years regularly experience violent discipline by their caregivers. A third of students aged 11–15 years worldwide have been bullied by their peers in the past month, and 120 million girls are estimated to have suffered some form of forced sexual contact before the age of 20 years. Emotional violence affects one in three children, and worldwide one in four children lives with a mother who is the victim of intimate partner violence. An estimated 40, 150 children worldwide were victims of homicide in the year 2017. The global homicide rate for 0–17-year-olds was 1.7 per 100,000 population, and the rate for boys of 2.4 per 100,000 was over twice that in girls (1.1 per 100,000 population) (WHO 2020). A study by Stoltenborgh et al., estimated the global prevalence of child sexual abuse to be 11.8%, based on a sample of 331 studies including a total of 991,148 participants (Stoltenborgh et al. 2011).

Different forms of childhood maltreatment often co-occur (Brown et al. 2019; Yeung et al. 2016). According to a report from the National Survey of Children's Exposure to Violence found that a large proportion of children surveyed (38.7%)

reported more than one type of direct victimization in the past year, such as sexual abuse and physical violence (Slowikowski 2009). Youth exposed to multiple domains of victimization, or "poly-victimization," may be at particularly high risk for lasting physical, mental and emotional problems, when compared with children who experience repeated exposures to a single form of violence (Haahr-Pedersen et al. 2020) especially when sexual abuse is present along with other maltreatment types (Vaughn et al. 2015). This has prompted interest into identifying different profiles or sub-groups with differing childhood maltreatment types, their chronicity, and relationships to outcomes, such as depression, anxiety, post-traumatic stress disorder (PTSD), self-harm and suicide attempts, and various pain conditions (Cay et al. 2022; Chandan et al. 2020; Mishra and Marceau 2020).

There is evidence that the rates of childhood maltreatment have been rising, especially among females and minority groups. According to Center for Disease Control and Prevention's (CDC) Youth Risk Behavior Survey from 2013 to 2023 (CDC 2024), there were worsening trends among youth in protective sexual behaviors, experiences of violence, persistent sadness or hopelessness, and suicidal thoughts and behaviors. Negative school experiences, including bullying, increased from 2021 to 2023. Female students fared worse than male students across almost all measures of substance use, experiences of violence (including sexual violence), mental health, and suicidal thoughts and behaviors. Disparities based on gender identity persisted, with LGBTQ+ students more likely than their peers to experience substance use, violence, and poor mental health. Almost a third of students reported experiencing racism in school, 1 in 5 students reported being unfairly disciplined, and only about half of students felt a sense of connection to others at school (CDC 2024).

Childhood maltreatment is associated with a range of negative psychological and physical outcomes including chronic pain, depression, anxiety, eating disorders, substance use disorders, suicidality, bipolar disorder, PTSD, psychosis, personality disorders, and poorer treatment responses (Cay et al. 2022; Green et al. 2010). Experiencing maltreatment during early development can disrupt biological stress systems, adversely impacting brain development and functioning in multiple developmental domains (Teicher and Samson 2016; Teicher et al. 2012, 2003; De Bellis and Zisk 2014). The CDC-Kaiser Permanente Adverse Childhood Experiences (ACEs) Study (Felitti et al. 1998) examined the relationship between childhood adversities such as emotional, physical, or sexual abuse, and household dysfunction—and health risk behaviors and chronic diseases during adulthood. The study revealed a graded relationship between the number of childhood adversities and adult health risk behaviors, as well as chronic physical and mental conditions, including ischemic heart disease, diabetes, cancer, chronic lung disease, skeletal fractures, liver disease, depression and suicide attempts, alcohol and drug abuse, smoking, decreased physical activity and obesity (Felitti et al. 1998). Moreover, female childhood maltreatment is correlated with various chronic conditions, including PTSD, obsessive-compulsive disorder, anxiety and phobias, paranoid ideation, early menarche, sleep disorders, metabolism disorders, cardiovascular diseases, asthma, chronic pain and early mortality (Liveri et al. 2023).

Childhood maltreatment has significant long-term deleterious effects on child development across multiple domains, often resulting in negative mental representations of self and others (Hildyard and Wolfe 2002). Childhood maltreatment is a significant risk factor for post-traumatic stress disorder (PTSD), which is similarly associated with changes in mental representations. Cognitive factors that increase risk of development and severity of PTSD include cognitive processing style during the traumatic experience, appraisal of trauma outcomes, negative beliefs about oneself and the world, and maladaptive control strategies (Dunmore et al. 2001). Parental childhood maltreatment is also associated with a range of parenting behaviors that contribute to the intergenerational transmission of childhood victimization. Parents with a history of childhood maltreatment, especially poly-victimization, are at an increased risk of abusing and neglecting their own children, leading to problematic behaviors, such as role reversal, rejection, and withdrawal in their relationships with their children (Greene et al. 2020). In addition to the individual burden, childhood maltreatment and other forms of early life adversity have substantial societal costs. The total annual costs attributable to ACEs are estimated to be $581 billion USD in Europe and $748 billion USD in North America (Bellis et al. 2019), with more than 75% of these costs arising from individuals with two or more ACEs (Bellis et al. 2019).

2 Associations Between Childhood Maltreatment and Pain

Pain is defined by the International Association for the Study of Pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (Raja et al. 2020). While pain serves an adaptive function, it can also negatively impact overall functioning, social interactions, and psychological well-being (Gureje et al. 2008). Childhood maltreatment significantly increases the risk of chronic and multisite pain, defined as recurrent, high-intensity pain lasting more than 3 months (Abrahamyan et al. 2022, 2024), which, in turn, increases the prevalence of mental health comorbidities (Gureje et al. 2008; Macedo et al. 2019). The combination of chronic pain and psychiatric disorders leads to worse outcomes compared to chronic pain alone (Craner et al. 2022; Johnston and Huckins 2023). In epidemiological studies, various factors influence the relationship between childhood maltreatment and pain, such as the definitions and types of childhood maltreatment, study population characteristics (e.g., gender, geographical area, cultural beliefs and values), methodology, and statistical analysis, confounding factors, sample size, whether prevalence or incidence is measured, and the period studied (Stoltenborgh et al. 2011; Marin et al. 2021).

Pain and emotions interact in several ways and have a bidirectional relationship (Yao et al. 2023). Stress can either reduce (stress-induced hypoalgesia) or exacerbate (stress-induced hyperalgesia) pain and emotional distress, stress, fear, and anxiety can amplify the perception of pain, and vice versa (Corcoran et al. 2015). Early indications of pain and somatosensory abnormalities following childhood

maltreatment may predict the development of future pain syndromes (Cay et al. 2022). Individuals with a history of childhood maltreatment are 2–3 times more likely to experience chronic pain in adulthood compared to those without such a history (Afari et al. 2014). Those with functional abdominal pain are 8.2 times more likely to report childhood experience of maltreatment compared to those without the condition (Hashemi et al. 2020).

Among youth in foster care, a higher frequency of self-reported maltreatment (i.e., a greater number of different maltreatment events across types) is significantly associated with increased likelihood of chronic pain in a dose-response relationship (Huffhines and Jackson 2019). Individuals with a history of childhood sexual trauma often present with pain symptoms resulting from the trauma or related psychological distress, including genito-pelvic pain, irritable bowel syndrome, interstitial cystitis/painful bladder, headaches, pregnancy and labor-related complications, and severe nausea (Tsur et al. 2022; Jonsdottir et al. 2022). In one study, 58% of participants with migraine headaches reported experiencing childhood maltreatment (Tietjen et al. 2010), with specific types of maltreatment linked to specific pain conditions: emotional abuse with irritable bowel syndrome, chronic fatigue syndrome, and arthritis; physical neglect with arthritis and uterine fibroids; and physical abuse with endometriosis. Overall, all forms of childhood maltreatment were associated with a greater total number of comorbid pain conditions. To summarize, there is a graded relationship between childhood maltreatment and chronic pain with certain pain disorders correlated with specific types of childhood maltreatment.

One possible mechanism through which childhood maltreatment may lead to pain conditions is central sensitization. Central sensitization results from an increased inflammatory response and abnormal hypothalamic-pituitary-adrenal (HPA) axis activity, which may be caused by or exacerbated by childhood maltreatment, leading to elevated circulating levels of inflammatory markers and blunted cortisol responses (Bourke et al. 2015). Central sensitization is associated with the enhancement of nociceptive neuronal pathways (Bourke et al. 2015) and activation and recruitment of glial cells within the nervous system, which modulate neuronal responses through initiation of multiple signaling cascades (Gosselin et al. 2010). Central sensitization syndromes include both somatic conditions (e.g., fibromyalgia, chronic fatigue syndrome, temporomandibular joint disorder, chronic lower back pain, chronic headache, myofascial pain syndrome, restless leg syndrome) and visceral conditions (e.g., interstitial cystitis, vulvodynia, chronic prostatitis and irritable bowel syndrome) (Chandan et al. 2019). Juvenile fibromyalgia and central sensitization have been positively correlated with high pain intensity, anxiety, depression, and experiences of childhood maltreatment (Mansiz-Kaplan et al. 2020).

Chronic pelvic pain is prevalent among individuals with a history of childhood maltreatment (Netzl et al. 2022). Chronic pelvic pain is the leading reason patients with conditions such as irritable bowel syndrome, interstitial cystitis/painful bladder syndrome, vulvodynia, and chronic prostatitis/chronic pelvic pain syndrome seek medical attention (Pierce and Christianson 2015). These syndromes are often comorbid with each other and with non-pelvic functional pain disorders, such as

migraine, fibromyalgia, and temporomandibular joint disorder, as well as mood and anxiety-related disorders (Pierce and Christianson 2015). In patients with overactive bladder, a history of childhood sexual abuse is associated with more severe bladder pain, greater non-urologic pain, poorer mood, heightened anxiety, and a higher physical and psychological burden (Lai et al. 2016). Similarly, patients with interstitial cystitis/bladder pain syndrome frequently report a history of childhood maltreatment, with symptoms of dissociation and anxiety being particularly common among those who experienced harm from close others (De Chiu et al. 2017).

Chronic pain is similarly associated with mood and anxiety disorders across different cultures (Gureje et al. 2008). Individuals with a history of child maltreatment and PTSD are at significantly increased risk of developing chronic pain in adulthood (Marin et al. 2021). The overlap between chronic pain and PTSD, as well as chronic pain and borderline personality disorder, often centers around a history of trauma (Johnston and Huckins 2023). Both PTSD and chronic pain share vulnerabilities, predispositions, and common symptoms, including hypervigilance, avoidance behavior, and elevated somatic focus (Macdonald et al. 2018).

Several mechanisms contribute to the comorbidity between chronic pain and psychiatric conditions, including genetic overlap, psychological factors, immune system involvement, dysregulation of the HPA axis, and alterations in the opioid and endocannabinoid signaling systems (Johnston and Huckins 2023). Anxiety sensitivity, defined as a fear of anxiety-related sensations and symptoms, along with the belief that these sensations are dangerous or harmful, may predispose individuals to both PTSD and chronic pain (Asmundson et al. 2002). Individuals who experience persistent interpersonal trauma, such as childhood maltreatment, may develop posttraumatic orientation of bodily signals, interpreting them as catastrophic and frightful. The negative orientation toward the body, manifested through pain catastrophizing, fear of pain, and body vigilance, reflects assumptions about one's own body shaped by traumatic experiences (Tsur and Talmon 2023; Tsur et al. 2018). In addition, childhood maltreatment and certain cases of chronic, unusual, or unexplained pain can be understood through the concept of pain flashbacks (Macdonald et al. 2018). These flashbacks involve intense but fragmented re-experiencing of traumatic events (Van Der Kolk 2015). Pain flashbacks are associated with more severe instances of child abuse and are linked to an increased risk of developing chronic pain (Tsur 2023).

Pain is a complex phenomenon with inputs from biological nociceptive and hypothalamic-pituitary-adrenal axis activity, as well as psychosocial and socioeconomic factors. In fact, the biological processes contributing to pain have roots in psychosocial processes including both emotions and cognition (Gatchel et al. 2014). As such, the biopsychosocial model is a widely accepted heuristic approach to the understanding and management of chronic pain (Gatchel et al. 2007). The biopsychosocial model of pain provides an integrative perspective, incorporating biological, psychological, and social factors. Attention is placed on sensory, cognitive, affective, behavioral and homeostatic factors that influence the experience of pain. Therefore, to fully understand an individual's experience of pain, it is essential to consider the interplay of these factors (Gatchel et al. 2007). The biopsychosocial

model not only provides a comprehensive approach for evaluating chronic pain but has also opened avenues for integrated and interdisciplinary management of pain in individuals with chronic pain (Gatchel et al. 2014).

To conclude, everyone's experience of pain, including its cognitive appraisal, is unique and influenced by multiple biopsychosocial variables (Gatchel et al. 2007), with childhood maltreatment as an important risk factor (Craner et al. 2022; Sonagra et al. 2022; Nelson et al. 2021). Traditional biomedical interventions of chronic pain like opioid medications and surgery may lack long-term benefits and in fact may increase psychological distress and healthcare utilization (Gatchel et al. 2014). In addition to the biopsychosocial model utilizing interdisciplinary care, there is need to explore therapeutic potential of novel agents, such as those targeting the endocannabinoid signaling system, as potential biomarker for evaluation of pain and trauma related symptoms and treatment response, treatment of chronic pain and related disorders and mitigate the effects of childhood maltreatment. We will now move on to our next section and discuss the role of the endocannabinoid signaling system in pain regulation and homeostasis.

3 The Endocannabinoid System as a Potential Link Between Childhood Maltreatment and Pain

The endocannabinoid (ECB) signaling system begins to develop in utero and continues to mature during childhood and adolescence until adult levels are reached, playing an important role in neuronal development and neural circuit tuning (Lu and Mackie 2021). ECBs, the endogenous lipophilic ligands for cannabinoid receptors, are arachidonic acid derivatives, specifically *N*-arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG) (Lu and Mackie 2021; Hillard 2018). Both AEA and 2-AG act as agonists for the cannabinoid receptors—CB1R and CB2R—which are G-protein coupled receptors found in the central and peripheral nervous systems (Castillo et al. 2012). ECBs can be measured in circulation, originating from various organs and tissues, including the brain, muscle, adipose tissue, and circulating cells, and are involved in multiple physiological functions, including regulation of metabolism at systems and cellular level, reward processes, fear learning and extinction, modulation of pain, and dampening the effects of the stress signaling systems (Hillard 2015, 2018; deRoon-Cassini et al. 2020; Hillard et al. 2017; Di Marzo et al. 2004).

Cannabinoid receptors were named because delta-9-tetrahydrocannabinol (THC), a compound found in organic extracts of *Cannabis sativa L.*, is a partial agonist at CB1R (Howlett 2002). While both AEA and 2-AG bind to CB1R and CB2R, they do so with different affinities due to the existence of distinct binding sites for these ligands on the cannabinoid receptors (Castillo et al. 2012; Howlett 2002). CB1R density is moderate to high in regions involved in pain transmission and modulation, such as the dorsal root ganglion, spinal cord, thalamus,

periaqueductal grey, amygdala, and rostroventromedial medulla (Sagar et al. 2012). CB1Rs are also found in immune cells, the reproductive system, gastrointestinal tract, adipose tissue, and the lungs, as well as on glutamate and GABA releasing neurons and the terminals of dopaminergic, serotonergic, and noradrenergic neurons (deRoon-Cassini et al. 2020). In contrast, CB2Rs are most abundant in the immune system, particularly in the tonsils, the spleen, macrophages, and lymphocytes (Lu and Mackie 2021).

The enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) are responsible for the inactivation of the endogenous cannabinoids AEA and 2-AG, respectively (Hillard 2018; Castillo et al. 2012). ECB-like compounds include the *N*-acylethanolamines (NAEs) such as N-oleoyl ethanolamine (OEA), N-stearoyl ethanolamine (SEA), and N-palmitoyl ethanolamine (PEA). Although these compounds do not have affinity for CB1R or CB2R, they can modulate ECB activity indirectly via substrate competition at FAAH (Bourke et al. 2022).

The ECB system plays an important role in modulating both pain and the stress responses (Bourke et al. 2022). Within the central nervous system, ECBs mediate activity-dependent retrograde signaling in various brain regions, including the cerebellum, hippocampus, amygdala, and prefrontal cortex (Hillard 2015). These brain regions are crucial for the perception and experience of pain (Yang and Chang 2019) and childhood maltreatment negatively affects the development of these regions (Teicher and Samson 2013, 2016; Teicher et al. 2003). The amygdala, hippocampus and ventromedial prefrontal cortex also play an important role in fear extinction and its retention which are impaired in anxiety and stress-related disorders (Rabinak and Luan 2014). Research indicates that childhood maltreatment can lead to ECB dysfunction (Mazurka et al. 2024), which may help explain the elevated risk of pain-related conditions and psychiatric disorders, such as depression and anxiety (Patel and Hillard 2009; Hillard and Liu 2014) following childhood maltreatment.

Stress exposure provokes a shift in many neurobehavioral processes, such as anxiety/vigilance, memory, reward salience, pain sensitivity, and coping behaviors (McEwen 2012). There are data highlighting the interactions between the ECB signaling system and the hypothalamic-pituitary-adrenal (HPA) axis (Morena et al. 2016; Hill et al. 2010a) and pain perception (Morena et al. 2016). Exposure to stress, whether physical or psychological, activates the acute stress response via the sympathetic nervous system and HPA axis, along with coordinated behavioral, endocrine, and neuronal changes to facilitate survival (Hillard 2018). During the initial phase of acute stress, the concentration of AEA decreases due to FAAH hydrolysis in the amygdala and prefrontal cortex, facilitating HPA axis activation and optimal rapid cortisol response. Subsequently, 2-AG helps to restore the HPA axis to baseline levels by acting as an effector in the negative feedback mechanism initiated by glucocorticoid receptor activation in the hippocampus and prefrontal cortex (Hillard et al. 2017; Hill et al. 2009) and contributes to pain perception, memory and synaptic plasitcity (Morena et al. 2016).

The ECB signaling system possesses unique properties that underlie its role in analgesia (Hillard 2018). Unlike classic neurotransmitters, ECBs are not stored in vesicles (Di Marzo et al. 2004; Kaczocha and Haj-Dahmane 2021), are produced on

demand following neuronal activation, and are synthesized in the postsynaptic cell. Following synthesis, ECBs travel across the synapse to interact with receptors in the presynaptic cell in a retrograde fashion (Hillard 2018; Vaughn et al. 2010; Martinez Ramirez et al. 2023). There is also evidence to suggest that ECBs signal in a non-retrograde or autocrine manner, where they can modulate neural function and synaptic transmission by engaging transient receptor potential (TRPV1) and CB1Rs located on or within the postsynaptic cell (Castillo et al. 2012). TRPVI receptors are largely expressed in afferent peripheral sensory neurons where its activation regulates synaptic transmission associated with pain sensation (Castillo et al. 2012; Frias and Merighi 2016). The peripheral ECB system, with both neuronal and non-neuronal ECB receptors, also plays an important part in the cannabinoid-receptor-mediated antinociception (Burston and Woodhams 2014). ECB action on CB1R results in the release of various neurotransmitters such as GABA, glutamate and dopamine within fear, anxiety, stress and reward circuits and its action on CB2R produces anti-inflammatory effects in immune cells (Freund et al. 2003; Katona and Freund 2012; Dantzer et al. 2008).

In animal models of pathological pain, the ECB system undergoes adaptive changes, including altered cannabinoid receptor expression and functionality. ECB concentrations vary depending on the model and anatomical site used (Sagar et al. 2012; Guindon et al. 2013). In rodent models of inflammatory and neuropathic pain, CB1R and CB2R agonists, as well as PEA, consistently demonstrate antinociceptive efficacy, reducing pain-related behaviors (Finn et al. 2021). Additionally, FAAH and MAGL inhibitors have been employed to prolong ECB effects. Systemic inactivation of FAAH through compounds like URB597, OL135 and PF-3845 has shown analgesic effects in inflammatory pain models (Jayamanne et al. 2006). Similarly, peripheral MAGL blockade via JZ184 has been found to attenuate capsaicin-induced pain behavior and thermal hypersensitivity (Spradley et al. 2010).

In a study using a rat model of osteoarthritis, the FAAH inhibitor URB597 targeting CB1R reduced pain and mechanical allodynia, which is defined as pain from a typically non-painful stimulus (Kędziora et al. 2023). Another study identified direct interactions between CB1Rs and delta opioid receptors in the brain in a rodent model of neuropathic pain (Bushlin et al. 2012), suggesting a potential mechanism for ECB-opioid crosstalk in pain modulation.

Animal studies have also revealed sex differences in ECB-mediated analgesia. These differences include variations in ECB system expression and activity in the brain, responses to specific types of pain (thermal, inflammatory or, neuropathic), hormonal impacts on cannabinoid signaling and antinociception, ECB metabolism and receptor expression, and cross reactivity of cannabinoids and hormones (Blanton et al. 2021).

Chronic pain states are associated with dynamic changes in the ECB systems that influence pain modulation. The plasticity of the systems affecting nociceptive processing can significantly impact ECB concentrations and receptor expression (Sagar et al. 2012). Evidence suggests alterations in the ECB system in patients with fibromyalgia, including single nucleotide polymorphisms and increased concentrations of circulating ECBs, particularly 2-AG and related NAEs (Bourke et al. 2022;

Stensson et al. 2018). In addition, AEA concentrations in these patients have been positively correlated with depressive symptoms (Stensson et al. 2018).

In diabetic neuropathy, AEA concentrations were found to be elevated (more so than 2-AG and NAEs), suggesting a potential maladaptive mechanism contributing to disease or a compensatory mechanism to restore homeostasis (Bäckryd et al. 2024). In a case-control study examining endometriosis-associated pain, ECBs and related lipids were measured in serum and peritoneal fluid in 23 cases and 19 controls. Results indicated a positive link between ECB system and inflammatory pain in endometriosis, with elevated 2-AG concentrations correlating positively with abdominal pain and dysmenorrhea (Andrieu et al. 2022).

Childhood maltreatment exerts long-lasting psychological and biological alterations and emerging data indicate it also impacts the affects the ECB system, which modulates inflammation and the endocrine stress response (Hitzler et al. 2023). Using a rat model, Hill, et al. looked at effects of early life stress, due to repeated maternal separation, on the developmental trajectory of the corticolimbic and ECB system and found that early life stress not only alters the development of the corticolimbic system, it also disrupts the normal ontogeny of the ECB signaling system, resulting in bidirectional changes in AEA and 2-AG tissue concentrations within the amygdala and hippocampus, leading to sustained reduction in ECB function and CB1R densities across all brain regions (Hill et al. 2019). In addition, the extent to which stressful stimuli reduce amygdala AEA/CB1R signaling influences the magnitude of the HPA response (Hill et al. 2009). Animal studies, using FAHH inhibitor AM3506, show that elevated tonic AEA signaling is associated with synaptic plasticity, with reduced anxiety, blunted activation and accelerated habituation of the amygdala in response to threat cues, and greater coupling of prefrontal cortical and amygdala circuits (Gunduz-Cinar et al. 2013), all of which may promote stress resilience.

Although studies in humans are less common than in animal models, emerging research suggests that the ECB system is affected by childhood maltreatment and is implicated in psychiatric risk. A recent case-control study investigated the impact of childhood maltreatment on circulating ECB concentrations and emotion regulation in relation to susceptibility or resilience to developing substance use disorders (Perini et al. 2023). The authors found that adults with a history of childhood maltreatment who did not have a substance use disorder had increased AEA concentrations at baseline and during stress compared to those with both childhood maltreatment and substance use disorders. These findings may indicate a potential mechanism that renders them resilient to developing substance use disorders following childhood maltreatment (Perini et al. 2023). In another study, individuals with major depressive disorder showed higher AEA concentrations compared to healthy controls, regardless of childhood maltreatment history. Major depressive disorder was also associated with higher 2-AG concentrations but only among participants with childhood maltreatment (Mazurka et al. 2024). Furthermore, our recent systematic review and meta-analysis revealed higher concentrations of both AEA and 2-AG at rest in individuals with stress-related psychiatric disorders, including PTSD, anxiety, and depression, compared to controls (Gowatch et al.

2024). Therefore, the ECB system appears to be dysregulated in those exposed to childhood maltreatment or other forms of early adversity and may be related to risk or resilience to psychiatric and substance use disorders.

Enzymatic processes involved in the metabolism of ECBs can be modulated by both acute and chronic stress, leading to individual variability in stress responses. Chronic stress can downregulate the ECB system, resulting in poor adaptation and an excessive stress response (deRoon-Cassini et al. 2020), while sensitization of the ECB signaling system may contribute to stress habituation and facilitate inadequate adaptation to repeated stress exposure (Patel and Hillard 2008). Stress exposure also increases inflammation in the body and the brain (Hillard 2018), and the ECB system has been shown to activate anti-inflammatory signaling pathways that modulate immune cell functions (Tanaka et al. 2020). Specifically, activation of ECB signaling can suppress microglial activation, thereby reducing the release of inflammatory mediators that could otherwise harm neuronal cells and contribute to neurodegeneration (Tanaka et al. 2020). A reduction of ECB signaling, which may be triggered by early life stress like childhood maltreatment, is proposed as a predisposing factor for the development of migraine headaches and related painful comorbid conditions, including fibromyalgia, irritable bowel syndrome, and interstitial cystitis (Russo 2016).

To summarize, the ECB system plays an important role in maintaining homeostasis (Lowe et al. 2021), serving both neuro-modulatory and immune-regulatory functions (Botsford et al. 2023). It plays a crucial role in modulating various processes, including cognition, motor control, stress responses, feeding behavior, and pain (Hillard 2018; Fine and Rosenfeld 2013). Enhancing ECB signaling can dampen the effects of acute and chronic stress by limiting the magnitude of the stress response and facilitating recovery to baseline function post-stress (Morena et al. 2016). In addition, due to its unique signaling properties, the ECB system plays a vital role in pain modulation and may therefore be an important link between childhood maltreatment and greater risk of pain-related conditions.

4 Gaps and Future Research Directions

Within the central nervous system, ECBs mediate activity-dependent retrograde signaling in various brain regions, including those implicated in development of pain conditions (Teicher and Samson 2013, 2016; Teicher et al. 2003, 2012; Hillard 2015; Yang and Chang 2019). Emerging research indicates that childhood maltreatment can lead to ECB dysfunction (Mazurka et al. 2024), which may help explain the elevated risk of pain-related conditions and psychiatric disorders, such as depression and anxiety (Patel and Hillard 2009; Hillard and Liu 2014), following childhood maltreatment. Although prior studies have separately highlighted dysregulations in the ECB system among individuals with chronic pain conditions and those with a history of childhood maltreatment, no studies have systematically linked these concepts. We propose a conceptual model that links the ECB system

with pain in the context of childhood maltreatment (see Fig. 20.1). Specifically, we hypothesize that childhood maltreatment alters the ECB system in ways that heighten susceptibility to pain conditions and comorbid disorders (Yang and Chang 2019).

Further studies are needed to understand the biopsychosocial correlates of the ECB signaling system, including its functioning, adaptability, and overlap with other signaling systems and pain trajectories. Research should examine the influence of childhood maltreatment across the lifespan, beginning in the prenatal period, with a focus on intrinsic and extrinsic childhood factors with an ecological framework. In addition, more research is needed to understand the pathways leading to specific chronic pain conditions and how these relate to various types of childhood maltreatment. Investigating the role of ECBs in the manifestation, perception, intensity, severity, and location of pain will also be crucial.

Prior studies have reported alterations in circulating ECB concentrations in individuals with a history of childhood maltreatment (deRoon-Cassini et al. 2020; Hitzler et al. 2023), as well as in relation to risk or resilience for psychiatric disorders and pain conditions (Perini et al. 2023). However, much of this research is cross-sectional and affected by various confounding factors (e.g., medication use) and diverse populations, which limits our understanding of the associations between childhood maltreatment, ECB signaling, and the risk of pain conditions. Future longitudinal studies are necessary to elucidate the temporal dynamics and bidirectional associations involved. In addition, identifying ECB biomarkers of neurobiological dysfunction and therapeutic response to both pharmacologic and nonpharmacologic treatments could enhance the monitoring and management of patients with complex presentations, such as comorbid psychiatric and physical conditions.

Targeting the ECB signaling system offers an important avenue for treatment of chronic pain and psychiatric symptoms related to early life stressors including childhood maltreatment. Indeed, changes to the ECB signaling are associated with analgesia, sedation, anxiolysis and decreased depression in endurance exercisers (Heijnen et al. 2016). Exercise is a promising approach for modulating the ECB system. Aerobic exercise generates ECBs in circulation, which may lead to anxiolytic and antinociceptive responses (Fuss et al. 2015; Da Silva Santos and Galdino 2018; Matei et al. 2023; Desai et al. 2022). In mice, acute long distance running decreases anxiety (by activation of forebrain GABAergic neurons) and pain (by activation of peripheral CB1R and CB2R) and blocking ECB receptors, diminishes the anxiolytic and analgesic effects of running (Fuss et al. 2015). In another study, exercise and ECB activity in rodents have been linked to hippocampal plasticity and

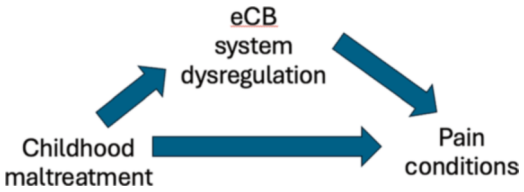


Fig. 20.1 Conceptual model linking childhood maltreatment to elevated risk of pain-related conditions in adulthood, via disruptions to the ECB signaling system

cell proliferation (Hill et al. 2010b). In a study using 63 healthy human participants, Siebert et al. found that participants had increased euphoria and decreased anxiety, and higher plasma concentrations of AEA and 2-AG after 45 min of running. The euphoria and anxiolytic effects remained the same after opioid blockade, suggesting the key role of ECBs—rather than endorphins—in exercise-induced euphoria and decreases in anxiety (Siebers et al. 2021). Our prior systematic review and meta-analysis supported consistent increases in AEA and 2-AG concentrations following acute exercise, in humans and animal models (Desai et al. 2022). This review included studies of individuals with psychiatric and chronic pain conditions, such as depression and chronic neck and shoulder pain, suggesting that exercise may be a promising approach for reducing risk of chronic pain and psychiatric symptoms for individuals exposed to childhood maltreatment.

The ECB signaling system has also been explored in fear extinction and its retention, which is important in the management of anxiety disorders and PTSD (Rabinak and Luan 2014), which can result from childhood maltreatment. For example, in humans, low-dose THC, which targets the CB1R, can produce anxiolytic effects, modulate threat-related amygdala activation, and enhance functional coupling between amygdala and medial prefrontal cortex and rostral cingulate cortex—regions important for emotion regulation, in trauma-exposed individuals (Rabinak et al. 2018, 2020). Animal research has shown bimodal effect of THC on fear responding with low dose being anxiolytic and higher doses being anxiogenic (Rabinak and Luan 2014; Genn et al. 2004). Other studies have investigated the use of FAAH inhibitors, which enhances AEA concentrations to target pain and related conditions like fibromyalgia, arthritis. and migraines (Stacey et al. 2022). Together, these data suggest that the ECB system is a promising target for interventions for pain and related conditions. Therefore, future studies should examine pharmacological targets for the ECB signaling system to stem the etiology of psychiatric and pain-related conditions following childhood maltreatment.

Due to the involvement of the ECB system in multiple pathophysiological processes, including but not limited to chronic pain, genetic and pharmacologic manipulation of the ECB system has garnered significant interest in medicine, research, and drug discovery, and development (Lowe et al. 2021). The distribution of ECB system components throughout the body presents unique opportunities for developing novel cannabinergic, cannabimimetic, and cannabinoid-based therapeutic drugs. These drugs may genetically or pharmacologically modulate the ECB system through the inhibition of metabolic pathways and/or the agonism or antagonism of ECB receptors (Lowe et al. 2021). Activation of extracerebral ECB receptors has been shown to alleviate pain, while inhibition of ECB degradation has been implicated in modulation of pain and anxiety (Jayamanne et al. 2006; Piomelli and Tagne 2024). Behavioral strategies such as aerobic exercise generate ECBs leading to anxiolytic and antinociceptive responses (Da Silva Santos and Galdino 2018; Matei et al. 2023; Desai et al. 2022). Low-dose THC has shown to have anxiolytic effects and facilitates in fear extinction and its retention (Rabinak and Luan 2014; Rabinak et al. 2018). Further research is needed to identify prophylactic pharmacologic and nonpharmacologic strategies, targeting the ECB system, for mitigating the effects of

childhood maltreatment like chronic pain. Research is also warranted to investigate modifying existing cannabinergic agents for medical use, identify potential targets in the ECB signaling system and develop novel agents for management of pain and other chronic conditions resulting from childhood maltreatment.

Another aspect of the ECB signaling system worth exploring is the characterization of ECB receptors. These receptors are G protein-coupled receptors (GCPRs), and their diversity allows them to recognize and respond to a wide range of ligands through biased agonism (Wootten et al. 2018) and heterodimerization with other GPCRs, including D2 dopamine, hypocretin, and opioid receptors (Lu and Mackie 2021). These unique properties of ECB receptors can be investigated to identify specific unit or subunits for targeted treatment of chronic pain in individuals with a history of childhood maltreatment. In addition, this research could enhance our understanding of the interactions between ECBs and other neurochemicals.

5 Clinical Implications

Childhood maltreatment is one of the most significant preventable risk factors for the future development of physical and psychological conditions, often leading to an earlier age of onset, increased severity, and poorer treatment response to. This situation results in a challenging prognosis for the patient and poses significant difficulties for health care providers.

Early identification and the use of a trauma-informed biopsychosocial model for managing childhood maltreatment and its associated conditions, such as chronic pain, are essential to prevent further comorbidities and improve quality of life. However, it is important to note that not everyone who experiences childhood maltreatment develops physical or psychological conditions as a result. Recognizing mitigating and protective factors within an ecological framework, as well as exploring signaling systems like the ECB signaling system, may provide insights into how some individuals avoid long-term adverse effects.

Similarly, the principles of a biopsychosocial approach can be utilized to develop trauma-informed, integrated multidisciplinary care models for treating individuals with a history of childhood maltreatment and chronic pain, incorporating novel therapeutic modalities such as ECBs.

6 Conclusion

Childhood maltreatment is a pervasive problem worldwide. We propose that childhood maltreatment alters the ECB system, which plays a critical role in pain modulation, stress responding, and a variety of other crucial homeostatic process, in ways that increase risk for the development of pain-related health conditions and other

comorbid physical and mental disorders. Understanding the complex interactions of these systems can open doors to developing novel therapeutic modalities to help manage patients with a history of childhood maltreatment and mitigate its effects.

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References

Abrahamyan A, Lucas R, Soares S, Talih M, Fraga S. Adverse childhood experiences and bodily pain at 10 years of age: findings from the generation XXI cohort. Child Abuse Negl. 2022;128:105620.

Abrahamyan A, Lucas R, Severo M, Talih M, Fraga S. Association between adverse childhood experiences and bodily pain in early adolescence. Stress Health. 2024;40(4):e3383.

Administration on Children, Youth and Families, Administration for Children and Families. Child maltreatment 2022 h Administration for Children and Families Administration on Children, Youth and Families Children's Bureau; 2022.

Afari N, Ahumada SM, Wright LJ, et al. Psychological trauma and functional somatic syndromes: a systematic review and meta-analysis. Psychosom Med. 2014;76(1):2–11.

Andrieu T, Chicca A, Pellegata D, et al. Association of endocannabinoids with pain in endometriosis. Pain. 2022;163(1):193–203.

Asmundson GJ, Coons MJ, Taylor S, Katz J. PTSD and the experience of pain: research and clinical implications of shared vulnerability and mutual maintenance models. Can J Psychiatr. 2002;47:930–7.

Bäckryd E, Themistocleous A, Stensson N, et al. Serum levels of endocannabinoids and related lipids in painful vs painless diabetic neuropathy: results from the pain in neuropathy study. Pain. 2024;165(1):225–32.

Bellis MA, Hughes K, Ford K, Ramos Rodriguez G, Sethi D, Passmore J. Life course health consequences and associated annual costs of adverse childhood experiences across Europe and North America: a systematic review and meta-analysis. Lancet Public Health. 2019;4(10):e517–28.

Blanton HL, Barnes RC, McHann MC, Bilbrey JA, Wilkerson JL, Guindon J. Sex differences and the endocannabinoid system in pain. Pharmacol Biochem Behav. 2021;202:173107.

Botsford C, Brellenthin AG, Cisler JM, Hillard CJ, Koltyn KF, Crombie KM. Circulating endocannabinoids and psychological outcomes in women with PTSD. J Anxiety Disord. 2023;93:102656.

Bourke JH, Langford RM, White PD. The common link between functional somatic syndromes may be central sensitisation. J Psychosom Res. 2015;78(3):228–36.

Bourke SL, Schlag AK, O'Sullivan SE, Nutt DJ, Finn DP. Cannabinoids and the endocannabinoid system in fibromyalgia: a review of preclinical and clinical research. Pharmacol Ther. 2022;240:108216.

Brown SM, Rienks S, McCrae JS, Watamura SE. The co-occurrence of adverse childhood experiences among children investigated for child maltreatment: a latent class analysis. Child Abuse Negl. 2019;87:18–27.

Burston JJ, Woodhams SG. Endocannabinoid system and pain: an introduction. Proc Nutr Soc. 2014;73(1):106–17.

Bushlin I, Gupta A, Stockton SD, Miller LK, Devi LA. Dimerization with cannabinoid receptors allosterically modulates delta opioid receptor activity during neuropathic pain. PLoS One. 2012;7(12):e49789.

Carsley S, Thomas S, Oei T, et al. Child abuse and neglect during the COVID-19 pandemic: an umbrella review. Child Abuse Negl. 2024;149:106645.
Castillo PE, Younts TJ, Chávez AE, Hashimotodani Y. Endocannabinoid signaling and synaptic function. Neuron. 2012;76(1):70–81.
Cay M, Gonzalez-Heydrich J, Teicher MH, et al. Childhood maltreatment and its role in the development of pain and psychopathology. Lancet Child Adolesc Health. 2022;6(3):195–206.
CDC. Youth risk behavior survey data summary & trends report: 2013-2023. 2024.
Chandan JS, Thomas T, Raza K, Bandyopadhyay S, Nirantharakumar K, Taylor J. Association between child maltreatment and central sensitivity syndromes: a systematic review protocol. BMJ Open. 2019;9(2):e025436.
Chandan JS, Keerthy D, Zemedikun DT, et al. The association between exposure to childhood maltreatment and the subsequent development of functional somatic and visceral pain syndromes. EClinicalMedicine. 2020;23:100392.
Corcoran L, Roche M, Finn DP. The role of the brain's endocannabinoid system in pain and its modulation by stress. Int Rev Neurobiol. 2015;125:203–55.
Craner JR, Lake ES, Barr AC, Kirby KE, O'Neill M. Childhood adversity among adults with chronic pain: prevalence and association with pain-related outcomes. Clin J Pain. 2022;38(9):551–61.
Da Silva Santos R, Galdino G. Endogenous systems involved in exercise-induced analgesia. J Physiol Pharmacol. 2018;69(1):3–13.
Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008;9(1):46–56.
De Bellis MD, Zisk A. The biological effects of childhood trauma. Child Adolesc Psychiatr Clin N Am. 2014;23(2):185–222.
De Chiu C, Lee MH, Chen WC, Ho HL, Wu HC. Childhood trauma perpetrated by close others, psychiatric dysfunction, and urological symptoms in patients with interstitial cystitis/bladder pain syndrome. J Psychosom Res. 2017;93:90–5.
deRoon-Cassini TA, Stollenwerk TM, Beatka M, Hillard CJ. Meet your stress management professionals: the endocannabinoids. Trends Mol Med. 2020;26(10):953–68.
Desai S, Borg B, Cuttler C, et al. A systematic review and meta-analysis on the effects of exercise on the endocannabinoid system. Cannabis Cannabinoid Res. 2022;7(4):388–408.
Di Marzo V, Bifulco M, De Petrocellis L. The endocannabinoid system and its therapeutic exploitation. Nat Rev Drug Discov. 2004;3(9):771–84.
Dunmore E, Clark DM, Ehlers A. A prospective investigation of the role of cognitive factors in persistent posttraumatic stress disorder (PTSD) after physical or sexual assault. Behav Res Ther. 2001;39:1063–84.
Felitti VJ, Anda RF, Nordenberg D, et al. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: the adverse childhood experiences (ACE) study. Am J Prev Med. 1998;14(4):245–58.
Fine PG, Rosenfeld MJ. The endocannabinoid system, cannabinoids, and pain. Rambam Maimonides Med J. 2013;4(4):e0022.
Finn DP, Haroutounian S, Hohmann AG, Krane E, Soliman N, Rice ASC. Cannabinoids, the endocannabinoid system, and pain: a review of preclinical studies. Pain. 2021;162:S5–S25.
Freund AF, Katona A, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev. 2003;83(3):1017–66.
Frias B, Merighi A. Capsaicin, nociception and pain. Molecules. 2016;21(6):797.
Fuss J, Steinle J, Bindila L, et al. A runner's high depends on cannabinoid receptors in mice. Proc Natl Acad Sci USA. 2015;112(42):13105–8.
Gatchel RJ, Peng YB, Peters ML, Fuchs PN, Turk DC. The biopsychosocial approach to chronic pain: scientific advances and future directions. Psychol Bull. 2007;133(4):581–624.
Gatchel RJ, McGeary DD, McGeary CA, Lippe B. Interdisciplinary chronic pain management. Am Psychol. 2014;69(2):119–30.

Genn RF, Tucci S, Marco EM, Viveros MP, File SE. Unconditioned and conditioned anxiogenic effects of the cannabinoid receptor agonist CP 55,940 in the social interaction test. Pharmacol Biochem Behav. 2004;77(3):567–73.

Gosselin RD, Suter MR, Ji RR, Decosterd I. Glial cells and chronic pain. Neuroscientist. 2010;16(5):519–31.

Gowatch L, Evanski JM, Ely SL, et al. Endocannabinoids and stress-related neurospsychiatric disorders: a systematic review and meta-analysis of basal concentrations and response to acute psychosocial stress. Cannabis Cannabinoid Res. 2024;9(5):1217–34.

Green JG, McLaughlin KA, Berglund PA, et al. Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: Associations with first onset of DSM-IV disorders. Arch Gen Psychiatry. 2010;67(2):113–23.

Greene CA, Haisley L, Wallace C, Ford JD. Intergenerational effects of childhood maltreatment: a systematic review of the parenting practices of adult survivors of childhood abuse, neglect, and violence. Clin Psychol Rev. 2020;80:101891.

Guindon J, Lai Y, Takacs SM, Bradshaw HB, Hohmann AG. Alterations in endocannabinoid tone following chemotherapy-induced peripheral neuropathy: effects of endocannabinoid deactivation inhibitors targeting fatty-acid amide hydrolase and monoacylglycerol lipase in comparison to reference analgesics following cisplatin treatment. Pharmacol Res. 2013;67(1):94–109.

Gunduz-Cinar O, MacPherson KP, Cinar R, et al. Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. Mol Psychiatry. 2013;18(7):813–23.

Gureje O, Von Korff M, Kola L, et al. The relation between multiple pains and mental disorders: results from the World Mental Health Surveys. Pain. 2008;135(1-2):82–91.

Haahr-Pedersen I, Ershadi A, Hyland P, et al. Polyvictimization and psychopathology among children and adolescents: a systematic review of studies using the Juvenile Victimization Questionnaire. Child Abuse Negl. 2020;107:104589.

Hashemi SM, Yousefichaijan P, Salehi B, et al. Comparison of child abuse history in patients with and without functional abdominal pain: a case-control study. BMC Psychiatry. 2020;20(1):258.

Heijnen S, Hommel B, Kibele A, Colzato LS. Neuromodulation of aerobic exercise-A review. Front Psychol. 2016;6:1890.

Hildyard KL, Wolfe DA. Child neglect: developmental issues and outcomes. Child Abuse Negl. 2002;26:679–95.

Hill MN, McLaughlin RJ, Morrish AC, et al. Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic-pituitary-adrenal axis. Neuropsychopharmacology. 2009;34(13):2733–45.

Hill MN, Patel S, Campolongo P, Tasker JG, Wotjak CT, Bains JS. Functional interactions between stress and the endocannabinoid system: from synaptic signaling to behavioral output. J Neurosci. 2010a;30:14980–6.

Hill MN, Titterness AK, Morrish AC, et al. Endogenous cannabinoid signaling is required for voluntary exercise-induced enhancement of progenitor cell proliferation in the hippocampus. Hippocampus. 2010b;20(4):513–23.

Hill MN, Eiland L, Lee TTY, Hillard CJ, McEwen BS. Early life stress alters the developmental trajectory of corticolimbic endocannabinoid signaling in male rats. Neuropharmacology. 2019;146:154–62.

Hillard CJ. Endocannabinoids and the endocrine system in health and disease. In: Endocannabinoids. Cham: Springer International Publishing; 2015. p. 317–39.

Hillard CJ. Circulating endocannabinoids: from whence do they come and where are they going? Neuropsychopharmacology. 2018;43(1):155–72.

Hillard CJ, Liu QS. Endocannabinoid signaling in the etiology and treatment of major depressive illness. Curr Pharm Des. 2014;20(23):3795–811.

Hillard CJ, Beatka M, Sarvaideo J. Endocannabinoid signaling and the hypothalamic-pituitary-adrenal axis. Compr Physiol. 2017;7(1):1–15.

Hitzler M, Matits L, Gumpp AM, et al. Longitudinal course of endocannabinoids and N-acylethanolamines in hair of mothers and their children in the first year postpartum: investigating the relevance of maternal childhood maltreatment experiences. Psychol Med. 2023;53(15):7446–57.

Howlett AC. The cannabinoid receptors; 2002.

Huffhines L, Jackson Y. Child maltreatment, chronic pain, and other chronic health conditions in youth in foster care. J Child Adolesc Trauma. 2019;12(4):437–45.

Jayamanne A, Greenwood R, Mitchell VA, Aslan S, Piomelli D, Vaughan CW. Actions of the FAAH inhibitor URB597 in neuropathic and inflammatory chronic pain models. Br J Pharmacol. 2006;147(3):281–8.

Johnston KJA, Huckins LM. Chronic pain and psychiatric conditions. Complex Psychiatry. 2023;9(1-4):24–43.

Jonsdottir IV, Sigurdardottir S, Halldorsdottir S, Jonsdottir SS. 'We experienced lack of understanding in the healthcare system'. Experiences of childhood sexual abuse survivors of the childbearing process, health and motherhood. Scand J Caring Sci. 2022;36(3):673–85.

Kaczocha M, Haj-Dahmane S. Mechanisms of endocannabinoid transport in the brain. Br J Pharmacol. 2021;179(17):4300–10.

Katona I, Freund TF. Multiple functions of endocannabinoid signaling in the brain. Annu Rev Neurosci. 2012;35:529–58.

Kędziora M, Boccella S, Marabese I, et al. Inhibition of anandamide breakdown reduces pain and restores LTP and monoamine levels in the rat hippocampus via the CB1 receptor following osteoarthritis. Neuropharmacology. 2023;222:109304.

Kovler ML, Ziegfeld S, Ryan LM, et al. Increased proportion of physical child abuse injuries at a level I pediatric trauma center during the Covid-19 pandemic. Child Abuse Negl. 2021;116:104756.

Lai HH, Morgan CD, Vetter J, Andriole GL. Impact of childhood and recent traumatic events on the clinical presentation of overactive bladder. Neurourol Urodyn. 2016;35(8):1017–23.

Liveri K, Dagla M, Sarantaki A, Orovou E, Antoniou E. Abuse of girls during childhood and its impacts on the health of their adult lives: a systematic review. Cureus. 2023;15(2):e34981.

Lowe H, Toyang N, Steele B, Bryant J, Ngwa W. The endocannabinoid system: a potential target for the treatment of various diseases. Int J Mol Sci. 2021;22(17):9472.

Lu HC, Mackie K. Review of the endocannabinoid system. Biol Psychiatry Cogn Neurosci Neuroimaging. 2021;6(6):607–15.

Macdonald B, Salomons TV, Meteyard L, Whalley MG. Prevalence of pain flashbacks in posttraumatic stress disorder arising from exposure to multiple traumas or childhood traumatization. Can J Pain. 2018;2(1):48–56.

Macedo BBD, Von Werne Baes C, Menezes IC, Juruena MF. Child abuse and neglect as risk factors for comorbidity between depression and chronic pain in adulthood. J Nerv Ment Dis. 2019;207(7):538–45.

Mansiz-Kaplan B, Ayhan FF, Cagli M, Atik F, Ece İ. A preliminary study of the child abuse and central sensitization in adolescent patients with chronic non-organic chest pain and an overlooked condition: Juvenile fibromyalgia syndrome. Pediatr Rheumatol. 2020;18(1):28.

Marin TJ, Lewinson RE, Hayden JA, et al. A systematic review of the prospective relationship between child maltreatment and chronic pain. Children. 2021;8(9):806.

Martinez Ramirez CE, Ruiz-Pérez G, Stollenwerk TM, Behlke C, Doherty A, Hillard CJ. Endocannabinoid signaling in the central nervous system. Glia. 2023;71(1):5–35.

Matei D, Trofin D, Iordan DA, et al. The endocannabinoid system and physical exercise. Int J Mol Sci. 2023;24(3):1989.

Mazurka R, Harkness KL, Hassel S, et al. Endocannabinoid concentrations in major depression: effects of childhood maltreatment and relation to hippocampal volume. Transl Psychiatry. 2024;14(1):431.

McEwen BS. Brain on stress: how the social environment gets under the skin. Proc Natl Acad Sci USA. 2012;109(suppl.2):17180–5.

Mishra AA, Marceau K. Co-occurring childhood maltreatment exposure and depressive symptoms in adulthood: testing differential effects of stress dysregulation and perceived stress. Aging Ment Health. 2020;24(11):1837–46.

Morena M, Patel S, Bains JS, Hill MN. Neurobiological interactions between stress and the endocannabinoid system. Neuropsychopharmacology. 2016;41(1):80–102.

Nelson S, Beveridge JK, Mychasiuk R, Noel M. Adverse childhood experiences (ACEs) and internalizing mental health, pain, and quality of life in youth with chronic pain: a longitudinal examination. J Pain. 2021;22(10):1210–20.

Netzl J, Gusy B, Voigt B, Sehouli J, Mechsner S. Chronic pelvic pain in endometriosis: cross-sectional associations with mental disorders, sexual dysfunctions and childhood maltreatment. J Clin Med. 2022;11(13):3714.

Patel S, Hillard CJ. Adaptations in endocannabinoid signaling in response to repeated homotypic stress: a novel mechanism for stress habituation. Eur J Neurosci. 2008;27(11):2821–9.

Patel S, Hillard CJ. Role of endocannabinoid signaling in anxiety and depression. Curr Top Behav Neurosci. 2009;1:347–71.

Perini I, Mayo LM, Capusan AJ, et al. Resilience to substance use disorder following childhood maltreatment: association with peripheral biomarkers of endocannabinoid function and neural indices of emotion regulation. Mol Psychiatry. 2023;28(6):2563–71.

Pierce AN, Christianson JA. Stress and chronic pelvic pain. In: Progress in molecular biology and translational science, vol. 131. Amsterdam: Elsevier; 2015. p. 509–35.

Piomelli D, Tagne AM. Endocannabinoid-based therapies. Annu Rev Pharmacol Toxicol. 2024;35:30.

Rabinak CA, Luan PK. Cannabinoid modulation of fear extinction brain circuits: a novel target to advance anxiety treatment. Curr Pharm Des. 2014;20(13):2212–7.

Rabinak CA, Peters C, Marusak HA, Ghosh S, Phan KL. Effects of acute Δ9-tetrahydrocannabinol on next-day extinction recall is mediated by post-extinction resting-state brain dynamics. Neuropharmacology. 2018;143:289–98.

Rabinak CA, Blanchette A, Zabik NL, et al. Cannabinoid modulation of corticolimbic activation to threat in trauma-exposed adults: a preliminary study. Psychopharmacology. 2020;237(6):1813–26.

Raja SN, Carr DB, Cohen M, et al. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. Pain. 2020;161(9):1976–82.

Rapp A, Fall G, Radomsky AC, Santarossa S. Child maltreatment during the COVID-19 pandemic: a systematic rapid review. Pediatr Clin N Am. 2021;68(5):991–1009.

Russell KW, Acker SN, Ignacio RC, et al. Child physical abuse and COVID-19: trends from nine pediatric trauma centers. J Pediatr Surg. 2022;57(2):297–301.

Russo EB. Clinical endocannabinoid deficiency reconsidered: current research supports the theory in migraine, fibromyalgia, irritable bowel, and other treatment-resistant syndromes. Cannabis Cannabinoid Res. 2016;1(1):154–65.

Sagar DR, Burston JJ, Woodhams SG, Chapman V. Dynamic changes to the endocannabinoid system in models of chronic pain. Philos Trans R Soc B Biol Sci. 2012;367(1607):3300–11.

SAMHSA. Understanding child trauma. Rockville: SAMHSA; 2024.

Siebers M, Biedermann SV, Bindila L, Lutz B, Fuss J. Exercise-induced euphoria and anxiolysis do not depend on endogenous opioids in humans. Psychoneuroendocrinology. 2021;126:105173.

Slowikowski J. Children's exposure to violence children's exposure to violence. Rockville: OJJDP Publications; 2009.

Sonagra M, Jones J, McGill M, Gmuca S. Exploring the intersection of adverse childhood experiences, pediatric chronic pain, and rheumatic disease. Pediatr Rheumatol. 2022;20(1):14.

Spradley JM, Guindon J, Hohmann AG. Inhibitors of monoacylglycerol lipase, fatty-acid amide hydrolase and endocannabinoid transport differentially suppress capsaicin-induced behavioral sensitization through peripheral endocannabinoid mechanisms. Pharmacol Res. 2010;62(3):249–58.

Stacey S, Brzozowski K, Daily M, et al. Shut the F(AAH) up: inhibiting fatty acid amide hydrolase as a novel approach to pain. FASEB J. 2022;36:L7533. https://doi.org/10.1096/fasebj.2022.36.S1.L7533.

Stensson N, Ghafouri N, Ernberg M, et al. The relationship of endocannabinoidome lipid mediators with pain and psychological stress in women with fibromyalgia: a case-control study. J Pain. 2018;19(11):1318–28.

Stoltenborgh M, van Izendoorn MH, Euser EM, Bakermans-Kranenburg MJ. A global perspective on child sexual abuse: meta-analysis of prevalence around the world. Child Maltreat. 2011;16(2):79–101.

Tanaka M, Sackett S, Zhang Y. Endocannabinoid modulation of microglial phenotypes in neuropathology. Front Neurol. 2020;11:87.

Teicher MH, Samson JA. Childhood maltreatment and psychopathology: a case for ecophenotypic variants as clinically and neurobiologically distinct subtypes. Am J Psychiatry. 2013;170(10):1114–33.

Teicher MH, Samson JA. Annual research review: Enduring neurobiological effects of childhood abuse and neglect. J Child Psychol Psychiatry. 2016;57(3):241–66.

Teicher MH, Andersen SL, Polcari A, Anderson CM, Navalta CP, Kim DM. The neurobiological consequences of early stress and childhood maltreatment. Neurosci Biobehav Rev. 2003;27:33–44.

Teicher MH, Anderson CM, Polcari A. Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus, and subiculum. Proc Natl Acad Sci USA. 2012;109(9):563–72.

Tietjen GE, Brandes JL, Peterlin BL, et al. Childhood maltreatment and migraine (part III). Association with comorbid pain conditions. Headache. 2010;50(1):42–51.

Tsur N. Pain flashbacks: exploration of intrusive pain symptoms following child abuse. Child Youth Serv Rev. 2023;145:106765.

Tsur N, Talmon A. Post-traumatic orientation to bodily signals: a systematic literature review. Trauma Violence Abuse. 2023;24(1):174–88.

Tsur N, Defrin R, Lahav Y, Solomon Z. The traumatized body: long-term PTSD and its implications for the orientation towards bodily signals. Psychiatry Res. 2018;261:281–9.

Tsur N, Katz C, Klebanov B. Peritraumatic pain in child sexual abuse: children's descriptions of pain as conveyed in their testimonies following child sexual abuse. J Interpers Violence. 2022;37(7-8):4393–414.

Vaughn LK, Denning G, Stuhr KL, De Wit H, Hill MN, Hillard CJ. Endocannabinoid signalling: has it got rhythm? Br J Pharmacol. 2010;160(3):530–43.

Vaughn MG, Salas-Wright CP, Underwood S, Gochez-Kerr T. Subtypes of non-suicidal self-injury based on childhood adversity. Psychiatry Q. 2015;86(1):137–51.

WHO. Global status report on preventing violence against children 2020. Geneva: WHO; 2020.

Wootten D, Christopoulos A, Marti-Solano M, Babu MM, Sexton PM. Mechanisms of signalling and biased agonism in G protein-coupled receptors. Nat Rev Mol Cell Biol. 2018;19(10):638–53.

Yang S, Chang MC. Chronic pain: structural and functional changes in brain structures and associated negative affective states. Int J Mol Sci. 2019;20(13):3130.

Yao C, Zhang Y, Lu P, et al. Exploring the bidirectional relationship between pain and mental disorders: a comprehensive Mendelian randomization study. J Headache Pain. 2023;24(1):82.

Yeung EW, Davis MC, Ciaramitaro MC. Cortisol profile mediates the relation between childhood neglect and pain and emotional symptoms among patients with fibromyalgia. Ann Behav Med. 2016;50(1):87–97.

Chapter 21
Secondhand Cannabis Smoke Exposure, Particulate Matter, and Effects on Physical and Neurobehavioral Health in Youth

Clara G. Zundel, Katya Parker, and Hilary A. Marusak

1 Introduction

While the neurodevelopmental effects associated with secondhand tobacco smoke exposure are well documented (Herrmann et al. 2008), less is known about the impact of secondhand cannabis smoke (SHCS), making it a significant public health concern. Recent studies show that SHCS contains higher concentrations of air pollutants, such as $PM_{2.5}$ (particulate matter with an aerodynamic diameter of 2.5 microns or less), compared to tobacco smoke (Janssen et al. 2024; Nguyen and Hammond 2022). This is alarming given the increasing prevalence of cannabis use. According to the World Health Organization (WHO), about 147 million people (2.5% of the world's population) consume cannabis, with higher rates observed in developed countries, particularly in North America (Cannabis 2024). In 2022, 27% of Canadians reported using cannabis in the past year, where recreational use is legal nationwide (Government of Canada 2022). In contrast, 22% of Americans—over 61 million people—reported past-year use despite recreational cannabis being legal in only 21 states as of 2022, and still remains federally illegal in the US (Substance Abuse and Mental Health Services Administration 2023). In addition, more than 35% of US adults believe that cannabis smoke is safer than tobacco smoke, a perception that has been growing from 2017 to 2021 (Chambers et al. 2023). This belief is more prevalent among younger individuals (ages 18–29) compared to older adults (ages 60 and above) (Chambers et al. 2023). A 2017 national survey found that 18% of US adults report that SHCS is somewhat or completely

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safe for adults, while 7.6% felt it was somewhat or completely safe for children (Keyhani et al. 2018). These statistics are concerning because exposure to toxicants during sensitive developmental windows, such as during ongoing brain development into young adulthood, may have lifelong consequences.

Research on SHCS has documented elevated concentrations of cannabinoid metabolites, such as 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH), in the blood or urine of exposed adults (Cone et al. 2015; Herrmann et al. 2015). However, cannabinoids are not the only compounds emitted in cannabis smoke. Recent research has shown that various methods of cannabis inhalation, including smoking, vaping, and dabbing, produce very high concentrations of aerosolized pollutants, particularly $PM_{2.5}$ (Janssen et al. 2024; Nguyen and Hammond 2022; Ott et al. 2021). $PM_{2.5}$ is a significant concern due to its small size, which allows it to deeply penetrate the lungs and pass through the blood–brain barrier (Costa et al. 2020). $PM_{2.5}$ can trigger widespread immune and inflammatory reactions both peripherally and within the central nervous system, potentially impacting brain structure and function (Costa et al. 2020; Genc et al. 2012).

Most research examining the effects of $PM_{2.5}$ on human health has focused on outdoor air pollution as the major source. These studies have identified that youth may be especially vulnerable to the effects of $PM_{2.5}$ compared to adults. Children have greater inhalation of air per body weight and a faster respiratory rate than adults (Kim 2004). Moreover, natural barriers in the body, such as the blood–brain barrier and nasal epithelium, are still developing during childhood and adolescence, making them less effective at filtering or trapping pollutants (Brockmeyer and D'Angiulli 2016). The brain undergoes significant development during the first two decades of life (Gogtay et al. 2004), and therefore, exposure to air pollutants during this period may have lifelong consequences. In fact, $PM_{2.5}$ exposure in youth has been associated with widespread structural and functional changes in the brain (Herting et al. 2019). $PM_{2.5}$ exposure has also been associated with several neurobehavioral outcomes in youth, including a higher incidence of psychiatric disorders (e.g., autism, attention-deficit/hyperactivity disorder (ADHD), anxiety, depression, bipolar disorder, and increased risk of suicide) (Suades-Gonzalez et al. 2015; Braithwaite et al. 2019; Donzelli et al. 2019; Zundel et al. 2022).

Given the rising prevalence of cannabis use and the misconception that cannabis smoke is safer than tobacco smoke, there is an urgent need for a comprehensive understanding of the neurodevelopmental effects of SHCS. This chapter aims to address this need by reviewing the current literature on the prevalence of SHCS exposure among youth, the concentrations of $PM_{2.5}$ in SHCS, and the associated physical and neurobehavioral health outcomes. In reviewing the literature, we will synthesize the results and major themes, highlight gaps, and end by discussing directions for future research and implications of both these neurobehavioral and physical alterations for the prevention and intervention of youth exposed to SHCS.

1.1 Objective of Scoping Review

The primary objective of this chapter is to review the literature utilizing peer-reviewed published articles found on PubMed prior to August 2024, on (1) the exposure prevalence of SHCS in youth, (2) concentrations of $PM_{2.5}$ in SHCS, as well as (3) associated physical health (e.g., respiratory, cardiovascular), and (4) neurobehavioral health outcomes (e.g., anxiety, depression, ADHD, autism) in children, adolescents, and young adults (under 25 years old). We also include a review of the preclinical/animal literature to date. For preclinical studies, we followed Semple et al. (2013) benchmarks of maturation and vulnerability to injury across species which considers postnatal day (PND) 1–21 as "early-life," PND 25-26 as "childhood," PND 35–49 as "adolescence," and PND 60+ as "adulthood." The overarching hypothesis is that SHCS, which contains increased concentrations of $PM_{2.5,}$ will be associated with an increased risk for or prevalence of both physical and neurobehavioral outcomes in youth. Prenatal cannabis exposure is out of the scope of this review. For a review on prenatal exposure see De Genna et al. (2022).

In considering SHCS exposure, we reviewed various routes of cannabis administration that contribute to secondhand smoke, including smoking methods such as joints, blunts, pipes, bongs, and more recently, cannabis vaping. Each of these methods can generate substantial particle concentrations, with varying degrees of impact depending on the method of combustion or vaporization (Ott et al. 2021). With respect to cannabis vaping, where possible, we specified the type of vaping product (i.e., oil pen/E-cigarette vs. dry flower vaporizing), used in the included studies. For a more detailed discussion on cannabis vaporization, see MacCallum et al. (2024).

2 Literature Review

2.1 Prevalence of SHCS Exposure in Youth

Youth can be exposed to SHCS in various ways, such as being in the same room as a smoker, being in an adjacent room, or through poor ventilation in multiunit housing (MUH) where smoke can travel from neighboring units. The following sections review studies that examine the prevalence of SHCS exposure in youth and families either through parental self-report, or through urine or plasma biomarkers.

2.1.1 Parental Self-Report of Cannabis Smoking in the Home

Sangmo et al. (2021a) examined the prevalence of SHCS in a convenience sample of 450 parents utilizing a pediatric clinic within the Mt. Sinai Health System in New York, NY between the years 2018 and 2019, prior to state legalization of recreational use. SHCS was assessed using a parent-reported questionnaire, where

parents were asked the following: Have you ever "smelled marijuana smoke in your room/apartment/condominium coming from another apartment, when you are with your child?" Approximately 31% of parents reported smelling cannabis smoke within their home while the child was present. This reported exposure varied by race, housing type, and education level; non-White families, families living in public housing (New York City Housing Authority), and families with lower educational achievement were more likely to report SHCS exposure than White families, families living in private housing, and families wherein a parent had completed college. In addition, parents were asked if "anyone who lives in your home or who cares for your child smokes marijuana." Only 5.5% of families reported having an individual in the family who smokes cannabis, and nearly 70% of families reported that smelling cannabis smoke could be harmful to their child.

Similarly, Wade et al. (2023) assessed SHCS using parent self-report ($N = 5580$) in the nationwide Adolescent Brain and Cognitive Development (ABCD®) study. Parents were asked, "Did anyone smoke marijuana (medical OR non-medical) inside the house when your child was home or in a vehicle your child was in?" In this sample, only 1.6% of parents reported SHCS exposure. Exposure varied by race and parental history of substance use problems, with White families and parents with a history of substance use problems more likely to report exposure than non-White families and parents without substance problems.

Findings from the 2020 Marijuana Use and Environmental Survey revealed that among adult cannabis users who report no in-home cannabis smoking restrictions—including smoking, vaping, and dabbing—26.2% live with at least one child under age 18 (Tripathi et al. 2023). In a study assessing air quality in tobacco smoker homes with one child under 14 years old ($N = 221$), 15.1% of parents or guardians reported indoor cannabis smoking (Posis et al. 2019). Children in cannabis-smoking homes were significantly older than those in non-cannabis-smoking homes. Parents/guardians who reported indoor cannabis smoking had higher educational attainment (high school and above) and were more likely to be non-Hispanic than those reporting no indoor cannabis smoking.

The National Survey on Drug Use and Health (NSDUH) showed an increase in past-month cannabis use among parents with children in the home, from 4.9% in 2002 to 6.8% in 2015 (Goodwin et al. 2018). Further, an increase in daily cannabis use was also observed, from 0.7% of parents in 2002 to 1.6% in 2015. Interestingly, cannabis use increased in both cigarette-smoking parents (from 11.0% to 17.4%) and in non-cigarette smoking parents (2.4–4%, see Fig. 21.1) (Goodwin et al. 2018). A follow-up study in 2017 assessed whether these increases were associated with cannabis legalization (Goodwin et al. 2021). The study found that past-month cannabis use rates among adults living with children were higher in states with recreational cannabis laws (11.9%), followed by states with medical cannabis laws (9.4%), and states without legal cannabis use (6.1%). Interestingly, past-month cannabis use among adults with children in the home was associated with the

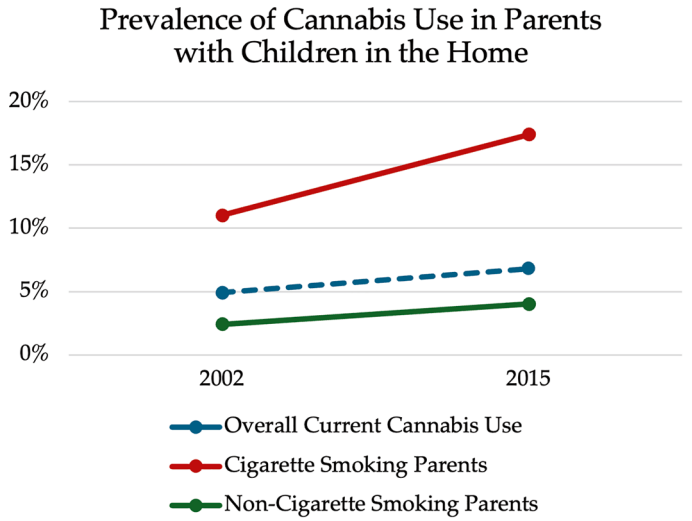


Fig. 21.1 Increasing rates of cannabis use among cigarette smoking and non-cigarette smoking parents with children in the home from 2002 to 2015. Adapted from Goodwin et al. (2018)

legalization of recreational cannabis across nearly all sociodemographic groups. In contrast, associations between past-month cannabis use and the legalization of medical cannabis were observed only among older adults, those with higher incomes, and those with higher educational attainment (Goodwin et al. 2021).

Several studies have been conducted in the state of Colorado, which was the first US state to legalize recreational cannabis for adults ages 21 and above in 2014. In a cross-sectional survey of 1500 parents/caregivers presenting for tertiary care at a children's hospital in Colorado between 2015 and 2017, 10.5% reported regular smoking/vaping of cannabis. Of the regular cannabis users, 95% reported daily or weekly use. Caregivers who smoked only cannabis were more likely to be younger, non-Hispanic, have more advanced educational achievement (i.e., college, graduate school), and a higher income than nonsmoking or tobacco-only smoking caregivers (Johnson et al. 2022; Johnson and Watson 2021). No differences in race were observed by smoking status.

Another study from Colorado examined the efficacy of a tobacco cessation program for parents ($N = 83$). In that study, most parents (85.4%) reported lifetime use of cannabis, with 11% reporting daily smoking (Wilson et al. 2018). While most parents reported that no individuals smoke cannabis inside their homes, 7.4% reported daily cannabis smoking in the home. Parents were also asked about their policies regarding cannabis smoking in the presence of children. Over half (51.8%) reported that smoking is not allowed in the home when children are present, 21.7% smoke outside, and 9.6% smoke in another room or floor of the house.

2.1.2 Biological Markers of SHCS Exposure in Drug-Naïve Youth

In a study conducted within the Mt. Sinai healthcare system in New York, NY from 2017 to 2018, prior to state legalization of recreational use, 20.8% of infants and children up to 3 years old ($N = 53$) had detectable THC metabolites (THC-COOH) in their urine, despite only 15.1% of parents reporting cannabis use by a caregiver in the home (Sangmo et al. 2021b). Black children were more likely to have detectable THC-COOH than non-Black children, and those living in MUH where smoking is permitted were nearly three times more likely to have detectable THC-COOH concentrations compared to those in MUH where smoking is prohibited.

A similar study in Colorado, focusing on 43 children aged 1 month to 2 years who were hospitalized for bronchiolitis, found that 16% of children had detectable levels of THC metabolites in their urine, mirroring the 16% of caregivers who self-reported cannabis use in the home (Wilson et al. 2017). Among children whose parents or caregivers reported cannabis use, 75% had detectable THC metabolites, compared to 10% of those with no reported use. Although no differences in gender, age, or MUH status were observed between children with and without detectable THC metabolite concentrations, non-White children were more likely to have detectable THC metabolite concentrations compared to White children.

Another Colorado study of 83 hospitalized children (ages 0–17) whose parents participated in a tobacco smoking cessation study found that 46% of children had detectable THC metabolites in their urine, and 11% had detectable THC concentrations (Wilson et al. 2018). Among those with detectable THC, three were adolescents (14–16 years old), and could have possibly been using cannabis themselves, and six were under 8 years old. Of the younger children with detectable THC, 83% had parents who reported household cannabis use some days or every day. Daily parental cannabis smoking was significantly correlated with detectable levels of THC metabolites, whereas the use of edibles was not. Furthermore, parental report of smoking cannabis in another room was significantly associated with detectable levels of THC metabolites; in particular, 100% of children whose parents smoked inside in another room had detectable levels compared to 44.2% of children whose parents reported never smoking when the children were home, and 28.4% who parents reported smoking outside. There was no significant difference in age, sex, race/ethnicity, or socioeconomic status between children with and without detectable levels of THC metabolites. However, children with detectable THC levels, suggestive of active exposure, were more likely to have parents who use cannabis daily in the home, preferred smoking as the method of use, and smoked cannabis in another room when children were present compared to those who smoked outside.

Moore et al. (2023) examined child urine samples ($N = 81$) at age 5 collected from a pre-birth cohort of 81 children at the University of Colorado Hospital for cannabinoid and cannabinoid metabolite levels. Cannabinoids were detected in 12% of the child urine samples, with the most commonly detected metabolite being cannabidiol glucuronide (CBD-gluc; 11%) followed by 7-carboxy cannabidiol (CBD-COOH, 1%)—both metabolites of cannabidiol (CBD). None of the children had detectable levels of THC or THC metabolites. Children with detectable CBD

metabolites were more likely to have mothers with higher educational attainment, come from higher household incomes, and to identify as Hispanic compared to children with undetectable levels.

A study of 10 young adults (mean age 23.3 years old, range 22–25) exposed to secondhand cannabis smoke in coffee shops in the Netherlands examined oral fluid concentrations of THC and metabolites (Moore et al. 2011). All volunteers tested negative for cannabinoids prior to entering the shop. However, THC was detected in all samples taken between 20 min to 3 h of exposure. Detectable CBD concentrations were found in three volunteers at the 3-h mark, and the THC metabolite, THC-COOH, was detected in 98.2% of the samples.

In a similar study involving 19 young adults (mean age 28.7 $\pm$ 8.8 years), nonsmokers exposed to SHCS in an unventilated lab session had detectable levels of THC in their blood samples postexposure. THC levels peaked immediately after exposure and remained detectable for 1–3 h (Herrmann et al. 2015). In a ventilated lab session, four of six nonsmokers had detectable blood cannabinoids levels immediately after exposure, but these levels were not detectable in later assessments.

While these findings provide valuable insights into SHCS exposure prevalence and its detection in biological samples, several limitations should be noted. Reporting bias may influence parental-reported cannabis use, particularly in states where cannabis is illegal and therefore, child protective services involvement would be a concern, leading to underreporting. In addition, while these studies assess cannabis "smoking," they often do not differentiate between specific routes of administration such as smoking, dabbing, or vaping, which could have different implications for exposure and health outcomes. Furthermore, the detection of THC metabolites in children's biological samples may reflect more proximal exposures and may not necessarily provide information about chronic exposure or broader patterns of exposure in youth.

2.2 $PM_{2.5}$ Concentrations in SHCS in Various Environments

Recent research indicates that SHCS contains higher concentrations of air pollutants, specifically $PM_{2.5}$, known neurotoxicants, compared to tobacco smoke. Several studies have monitored $PM_{2.5}$ concentrations generated by various cannabis routes of administration and environments (e.g., indoor homes, vehicles).

2.2.1 Indoor Home Environments

Posis et al. (2019) conducted an intervention-based study in San Diego County, California, a state where cannabis was legalized for recreational adult use (ages 21 and above) in 2016. The intervention aimed to reduce indoor SHS (tobacco and cannabis) and fine particle concentrations in low-income family homes. Particle monitors were placed in the rooms where most smoking occurred, collecting data

continuously for approximately 7 days. The findings showed that homes with cannabis-only, cigarette-only, and dual-smoking had significantly higher particle concentrations than nonsmoking homes, with no significant difference between cannabis-only and cigarette-only smoking homes. However, households that reported smoking both cannabis and tobacco had twice the concentrations compared to nonsmoking homes.

Ott et al. (2021) examined particle concentrations and emissions produced by various cannabis routes of administration (i.e., joints, bongs, glass pipes, electronic vape pens) using a 3-puff protocol in a 43 m^3 bedroom. The results showed that cannabis joints, up to 2.5 h after smoking, produced the highest average $PM_{2.5}$ concentrations (540 $\mu g/m^3$), concentrations that are 3.5 times higher than the average for tobacco cigarettes (mean = 154 $\mu g/m^3$), see Fig. 21.2. Bongs, glass pipes, and vape pens followed, with cannabis vape pens exhibiting the largest decay rate. Similarly, Nguyen and Hammond (2022) studied social bong smoking in a 20 m^2 living room and found that $PM_{2.5}$ concentrations increased significantly compared to baseline during all sessions, with elevated concentrations (50 $\mu g/m^3$) persisting for 12 h post-smoking. These concentrations were nearly 1.5 times the Environmental Protection Agency (EPA) 24-h standard limit (35 $\mu g/m^3$).

A study by Ott et al. (2022) measured $PM_{2.5}$ concentrations in a multi-room home and found elevated concentrations in all rooms after smoking cannabis joints (with varying concentrations of THC and CBD), with the highest concentrations in rooms where smoking occurred. Opening the windows reduced $PM_{2.5}$ concentrations by 67%, but concentrations remained higher than those produced by tobacco cigarettes in a room with closed windows. Further, Wallace et al. (2020) investigated $PM_{2.5}$ concentrations from vaping cannabis oil, with varying concentrations of

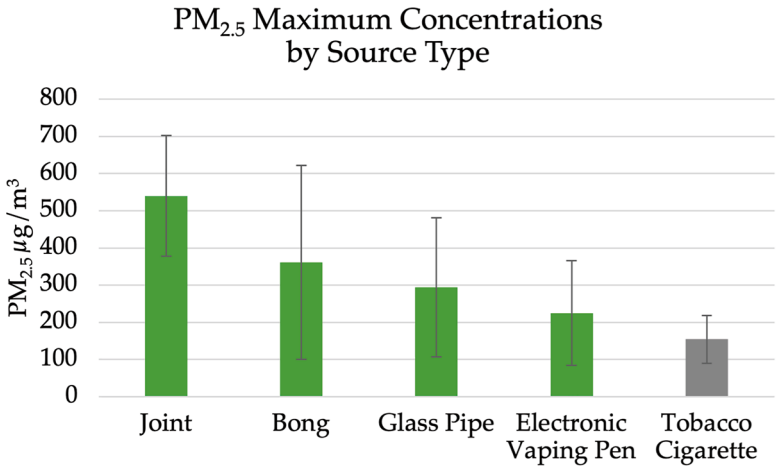


Fig. 21.2 Different $PM_{2.5}$ concentrations by cannabis source type, compared to tobacco cigarettes. Experiments were conducted using a 3-puff protocol in a 43 m^3 bedroom, with monitors placed at the midpoint of the room. This figure is adapted from Ott et al. (2021)

THC and CBD, in a sealed unventilated 30 m^3 room. They found that $PM_{2.5}$ concentrations averaged 62.9 μg/m^3 during vaping and remained elevated for approximately 9 h after the session.

Cheng et al. (2022) examined $PM_{2.5}$ concentrations from both joint (0% CBD, 9.6% THC) and electronic vape pen (46.1% CBD, 21.9% THC) use indoors and outdoors. They found that indoor vaping resulted in higher SHCS concentrations at a 1m distance compared to indoor smoking. However, outdoor smoking or vaping reduced exposure concentrations to less than 5% of those observed indoors.

Overall, these studies highlight the significant variation in $PM_{2.5}$ concentrations associated with different routes of cannabis administration in indoor home environments, demonstrating that both smoking and vaping can lead to elevated particle concentrations that persist over time, with indoor environments posing higher exposure risks compared to outdoor settings.

2.2.2 Commercial and Public Spaces

Murphy et al. (2021) studied $PM_{2.5}$ concentrations in a cannabis dispensary where the use of vaporizers and dab rings was allowed, but smoking (combustion) of cannabis and tobacco was prohibited. They found that the average $PM_{2.5}$ concentration during business hours was 84 μg/m^3, more than twice the EPA's 24-h standard limit, compared to 3–7 μg/m^3 when the dispensary was closed. In a similar study, Huang et al. (2022) assessed $PM_{2.5}$ concentrations in a cannabis store where smoking was permitted and compared them to a nearby coffee shop, during peak business hours. They found that the dispensary lounge had average $PM_{2.5}$ concentrations of 840 μg/m^3, while the coffee shop had average $PM_{2.5}$ concentrations of 3 μg/m^3. These findings underscore the significant elevation of $PM_{2.5}$ concentrations across commercial and public spaces associated with cannabis use, highlighting potential health risks associated with high levels of air pollutants in environments where cannabis consumption is permitted.

2.2.3 Vehicles

Zhao et al. (2020) examined $PM_{2.5}$ concentrations from four common cannabis smoking modalities (i.e., glass pipe, joint, bong, and vape pen) inside a car. Particle monitors were placed on the backseat behind the driver's seat to simulate secondhand exposure. Joints produced the highest $PM_{2.5}$ emission rate (6.6 mg/puff), followed by bongs (3.1 mg/puff), vape pens (3.3 mg/puff), and glass pipes (2.5 mg/puff). Emissions rates were approximately 2–6 times higher than those of a tobacco cigarette (1.1 mg/puff). This study reveals that various routes of cannabis administration in vehicles produce significantly higher $PM_{2.5}$ emission rates compared to tobacco cigarettes, emphasizing the elevated exposure risk for passengers and drivers in enclosed vehicle environments.

2.2.4 Tobacco Smoke Comparative Studies

Janssen et al. (2024) showed that pure cannabis cigarettes had $PM_{2.5}$ concentrations 123% higher (average 2193 μg/m^3) than those of tobacco cigarettes (range 948–11,173 μg/m^3), with similarly elevated concentrations observed for cannabis mixed cigarettes (average 1858 μg/m^3).

These studies collectively highlight the substantial $PM_{2.5}$ concentrations associated with various cannabis use modalities, underscoring the potential health risks of SHCS exposure in different environments. However, these findings largely focus on short-term, immediate exposure levels, which may not fully capture the cumulative health impacts or chronic exposure patterns associated with long-term SHCS in various environments.

2.3 Preclinical Studies of SHCS

Understanding the biological and health impacts of SHCS exposure necessitates a review of the preclinical and animal literature. These studies provide insights into the mechanisms and potential effects of SHCS on health.

Wang et al. (2016) examined the impact of SHCS on endothelial function, a marker of cardiovascular harm, in 9–10-week-old rats. SHCS was delivered using a modified cigarette smoking system, whereby sidestream smoke is collected from the burning tip of a cigarette in an exposure chamber as a ventilator pump simulates human puffing. Flower cannabis cigarettes were used in this study. Their findings revealed that SHCS significantly impaired endothelial function, with no recovery observed 40 min postexposure. Interestingly, exposure to THC-free cannabis smoke also impaired endothelial function to the same extent as SHCS, indicating that THC is not necessary for this effect. Instead, the authors suggest that the impairment may be related to other combustion byproducts or persistent chemicals from the cannabis plant. In addition, the study showed that even a brief SHCS exposure of just 1 min at high $PM_{2.5}$ concentrations can significantly and substantially decrease endothelial function.

In a similar study, Liu et al. (2023) examined the impact of SHCS from vaporizers, specifically aerosols from ground cannabis, on endothelial function in 9–11-week-old rats. To generate the aerosols, hot air was passed through the ground cannabis to fill a balloon. The balloon was connected to a vaping machine, and to mimic human use of vaporizing, the vaping machine pulled aerosol from the balloon into the vaping device. Their results showed that exposure to SHCS from vaping, whether the ground cannabis contained cannabinoids or not, impaired endothelial function by 50%.

Bruijnzeel et al. (2019) examined the effects of adolescent exposure to cannabis smoke on emotional behavior and cognitive functioning later in life in both male and female rats. The rats were exposed to clean air or cannabis smoke from PND29 to 49, which corresponds to human childhood and adolescence. Cannabis smoke

was from cannabis cigarettes (0%CBD, 5.6%THC), using a cigarette smoking-machine with a standardized smoking procedure of 1 puff/min. Mainstream and sidestream smoke was transported to the exposure chamber, and rats were whole-body exposed. Behavioral testing began after PND70, which is equivalent to human adulthood. The authors found no significant effects of cannabis smoke exposure on anxiety-like or depression-like behaviors, as measured by the large open field and elevated plus maze tests and sucrose preference and forced swim tests, respectively, nor on working memory, measured by the novel object recognition test. A similar study conducted only in male rats also found no significant effects of PND29-49 exposure on anxiety-like behaviors in adulthood (PND70) measured on the large open field and elevated plus maze tests (Hernandez et al. 2021). The authors also found null effects on various tests of learning, memory, motivation, reward, cognitive flexibility, and impulsivity in adulthood (Hernandez et al. 2021).

These preclinical studies collectively underscore that SHCS exposure can adversely affect endothelial function, with impacts observed across various exposure modalities and time points; however, they also highlight the need for further research to elucidate the long-term behavioral and cognitive consequences of SHCS exposure, particularly given the mixed findings related to emotional and cognitive outcomes.

2.4 Physical Health Outcomes Associated with SHCS Exposure in Human Children, Adolescents, and Young Adults

The most common physical health outcomes associated with SHCS exposure in youth are respiratory issues. Posis et al. (2019) examined parent-reported respiratory and allergenic outcomes in 221 children (mean age of 4 years old), including past-year emergency department visits for coughing or difficulty breathing, and physician diagnoses of infections, bronchitis, asthma, or allergenic skin conditions. They found that children from families who self-reported indoor cannabis smoking had higher odds (odds ratio [OR] = 1.75) of reporting a greater number of these adverse health outcomes compared to families who reported no indoor cannabis smoking. However, no significant associations were observed when examining the health outcomes individually.

In another study, Johnson et al. (2022) investigated the relationship between SHCS, measured by parental report of smoking cannabis use, and respiratory infections in 1500 children up to 11 years old. While no significant differences were found in emergency department visits between caregivers who used cannabis, tobacco, or did not smoke, caregivers who used cannabis reported a significantly higher rate of past-year viral respiratory infections in their children (1.31 infections) compared to children of nonsmoking caregivers (1.00 infections), see Fig. 21.3.

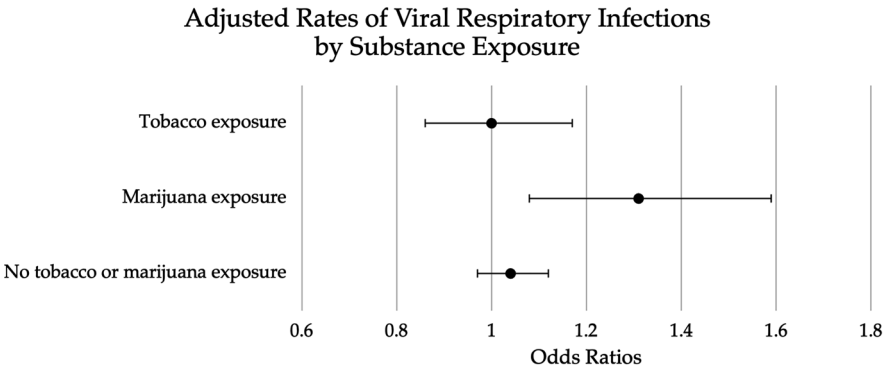


Fig. 21.3 Rates of viral respiratory infections among 1500 children (up to 11 years old) by substance exposure, as measured by parental report of use. Rates were adjusted for household income, caregiver age, child age, Hispanic ethnicity, and caregiver education. This figure is adapted from Johnson et al. (2022)

Herrmann et al. (2015) examined physiological responses to SHCS in 19 young adults (mean age 28.7 $\pm$ 8.8 years old) during unventilated and ventilated lab sessions. They observed no significant effects on heart rate or blood pressure in either session. However, there was a small but significant increase in heart rate (65.7 beats per minute (BPM) pre and 71.7 BPM post) and a nonsignificant trend toward increased systolic blood pressure (127 mmHg pre and 132 mmHg post) immediately postexposure in the unventilated session. These changes are comparable to those observed in individuals who use cannabis directly, suggesting that secondhand exposure may elicit similar acute cardiovascular responses. No effects were observed in the ventilated session.

Overall, these studies suggest that SHCS exposure is associated with increased respiratory issues in children, while findings on cardiovascular effects in young adults remain less clear, indicating a need for further research to fully understand the impact of SHCS on physical health across development.

2.5 Neurobehavioral Outcomes Associated with SHCS Exposure in Human Children, Adolescents, and Young Adults

Due to ongoing brain development, youth may be particularly vulnerable to the effect of SHCS on brain and behavioral outcomes. The following studies have investigated the associations between SHCS and neurocognitive outcomes in youth and young adults.

A study from the University of Colorado Hospital examined the impact of SHCS exposure, measured by urinary cannabinoid concentrations, on cognition and behavior in 5-year-old children (Moore et al. 2023). The findings revealed that

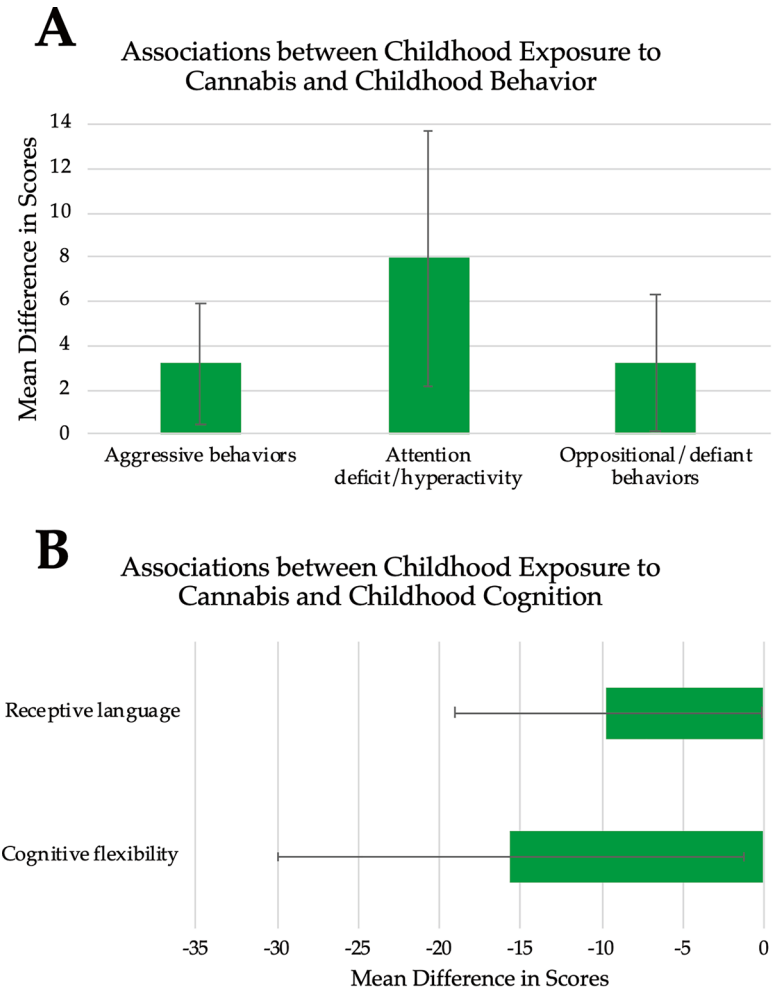


Fig. 21.4 Mean difference in behavior (**a**) and cognitive scores (**b**) between children (age 5) exposed to secondhand cannabis smoke (SHCS) vs. unexposed children, as measured by urinary cannabinoid concentrations. Higher values in panel (**a**) indicate higher problem behaviors in cannabis exposed vs. unexposed youth. Lower values in panel (**b**) indicate lower cognition scores in exposed vs. unexposed youth. This figure is adapted from Moore et al. 2023

children exposed to SHCS exhibited significantly more aggressive behavior, ADHD symptoms, and oppositional/defiant behaviors compared to those not exposed. In addition, children exposed to SHCS demonstrated less cognitive flexibility and receptive language skills than their unexposed peers (see Fig. 21.4).

The nationwide ABCD® study explored the impact of SHCS exposure, measured by parental report of smoking cannabis inside the house or vehicle when a child was present, on cognition during early adolescence (mean age 11.81 $\pm$ 0.63 years) in a

sample of 5580 youth (Wade et al. 2023). While SHCS was not significantly associated with a global cognitive performance score, a marginal association ($p = 0.08$) was observed with poorer performance on a visuospatial/attention task. No significant associations were found between SHCS exposure and language, attention, processing speed, visual memory, verbal learning and memory, reading ability, or academic achievement.

Herrmann et al. (2015) examined the effects of SHCS exposure in 19 young adults (mean age 28.7 ± 8.8 years old) during both unventilated and ventilated lab sessions. Six nonsmoker participants were seated around a table in a sealed exposure chamber (10 × 13 × 7 ft) for 60 min while six participants smoked cannabis cigarettes, each containing approximately 1 g of cannabis (11.3% THC). In the unventilated sessions, nonsmoker performance on tasks measuring global cognitive abilities—including attention, processing speed, and executive function—significantly decreased from pre- to postexposure. Conversely, in the ventilated sessions, nonsmoker performance on tests of global cognitive ability and attention significantly increased from pre- to postexposure, which the authors interpret as practice effects on speed and accuracy measures.

These studies highlight potential neurobehavioral effects of SHCS exposure, with evidence suggesting adverse impacts on cognition and emotional behavior in children and young adults; however, variability in findings indicates the need for further research to clarify these associations and their underlying neurobiological mechanisms.

3 Discussion

While cannabis use continues to climb in the United States, with about 20% of the population reporting past-year use in 2022 (Substance Abuse and Mental Health Services Administration 2023), there remains a significant gap in knowledge surrounding the potential negative effects of SHCS exposure, particularly in vulnerable populations such as children, adolescents, and young adults. Further, about 40% of US adults believe that cannabis smoke is somewhat-to-much safer than tobacco smoke, and this perception has increased over time (from 2017 to 2021) (Chambers et al. 2023). This belief is exceptionally concerning given the growing body of research documenting that cannabis smoke contains much higher concentrations of particulate matter (i.e., $PM_{2.5}$), a known neurotoxicant, compared to tobacco smoke (Janssen et al. 2024). This chapter is the first, to our knowledge, to review the existing literature on SHCS and its effects, including: (1) the prevalence of exposure in youth, (2) $PM_{2.5}$ concentrations, (3) preclinical studies, (4) physical health impacts, and (5) neurobehavioral effects. We summarize the findings of this review, discuss gaps in the literature, and identify future directions for research.

3.1 Summary of Findings

3.1.1 Prevalence of SHCS

Prevalence rates of SHCS exposure in youth vary across studies (1.6–31%), likely due to differences in demographics, legal landscape, and assessment methods, such as parental self-report versus biological assessments.

Numerous studies have explored SHCS exposure using parental self-report, though the specific language of the questionnaires varies considerably. For instance, some studies, such as Sangmo et al. (2021a), asked parents if "anyone who lives in your home or who cares for your child smokes marijuana?" More specific questionnaires, such as the one used in Wade et al. (2023), ask if anyone smokes *in the presence of the child*, thereby addressing exposure more directly. However, it is also important to consider that children can be exposed to SHCS even if they are not directly present, such as if they are in a different room or in MUH where smoking is allowed. These variations in questionnaire specificity—such as not asking about the child's presence during smoking or whether smoking occurs inside the home (in the same or different room), as well as ventilation and MUH—may contribute to discrepancies in reported prevalence rates. Many studies did not inquire about vaping or other routes of administration, or chronicity, frequency, or cannabinoid content of material consumed, which should be explored in future studies. Preliminary associations have suggested potential links between SHCS exposure and participant characteristics such as race, income, parental educational attainment, and MUH. However, these findings are not yet conclusive, and further research is needed to more definitively establish risk factors and their implications.

Several studies examined prevalence of SHCS exposure in youth using biological assessments through urine and blood samples to test for cannabinoids and cannabinoid metabolites. The prevalence rates in these studies were much higher than parent-reported rates and ranged from 12 to 46% of children. However, cannabinoids and cannabinoid metabolites inhaled from passive cannabis smoke exposure are excreted from the body relatively quickly, with urine metabolites lasting up to 24–48 h and plasma levels becoming undetectable within hours postexposure (Röhrich et al. 2010; Cone et al. 1987). Therefore, these markers are indicators of more immediate or acute exposure and do not necessarily capture more long-term exposure. While hair samples have been successfully used to detect secondhand tobacco exposure in children (Groner et al. 2012), similar methodologies are now being applied to assess longer-term cannabis exposure (past 3 months) in cannabis users (Taylor et al. 2017). Future research should explore whether these techniques can also be effectively utilized to evaluate SHCS exposure. Interestingly, in studies that assessed both parental self-report of exposure and urinalysis, there was moderate concordance, suggesting that parental self-reports may underestimate the true extent of children's exposure to SHCS (Wilson et al. 2018; Sangmo et al. 2021b; Wilson et al. 2017). This highlights the importance of using more objective

biological and/or repeated assessments to more accurately gauge SHCS exposure over sensitive developmental windows (Sangmo et al. 2021b; Wilson et al. 2017).

Most clinical studies have been conducted in urban environments, where outdoor air pollution and $PM_{2.5}$ exposure are already elevated relative to non-urban environments (Hu et al. 2014). Notably, while many studies assessing $PM_{2.5}$ concentrations across various routes of cannabis administration controlled for background or outdoor $PM_{2.5}$ concentrations, none of the included clinical studies that evaluated the links between exposure and health outcomes accounted for outdoor air pollution exposure. Further research is needed to investigate the combined impact of elevated outdoor $PM_{2.5}$ concentrations and SHCS exposure, potentially through a "double hit" model, to better understand the cumulative effects on health.

Only one study to date has combined the use of personal air monitors, a more objective measure of $PM_{2.5}$ exposure, and parental-self report of SHCS in youth (Posis et al. 2019). This study revealed that homes with cannabis smokers had higher concentrations of $PM_{2.5}$ than homes without smokers; however, the particle concentration was not used as the measure of exposure in health analyses. Instead, parental self-report was used as a proxy for exposure. Future research needs to take an integrated approach, examining associations not only with parental self-report but also with objective measures of exposure. This will inform future intervention-based studies on what constitutes an appropriate or "safe" concentration of $PM_{2.5}$.

3.1.2 $PM_{2.5}$ Concentrations in SHCS

Several studies have documented the high concentration of $PM_{2.5}$, a known neurotoxicant, in SHCS. One study found that $PM_{2.5}$ concentration in cannabis smoke is 123% higher than in tobacco smoke (Janssen et al. 2024), a well-documented health hazard. Further studies have shown that various cannabis administration methods (e.g., glass pipe, joint, bong, dab ring) all produce elevated $PM_{2.5}$ concentrations, compared to background or baseline concentrations and tobacco smoke. This includes vaping, which is often touted as safer than smoking since vaporizers heat substances without burning them. However, emerging evidence suggests that vaping cannabis concentrates or oils produce aerosols that contain extremely high concentrations of $PM_{2.5}$ (Ott et al. 2021; Wallace et al. 2020; Cheng et al. 2022; Murphy et al. 2021; Zhao et al. 2020). This is concerning given the increasing use of cannabis vaping and perceptions of its safety, especially among adolescents and young adults (Boakye et al. 2021; Popova et al. 2017).

Several studies were conducted in laboratory sessions without individuals present, making it challenging to directly link increased $PM_{2.5}$ exposure from SHCS to adverse health outcomes. However, most studies examining toxicant concentration in SHCS have taken place in homes or other environments (i.e., vehicles) where children and youth are most likely to be exposed, suggesting a plausible link to real-life exposure. Another likely exposure setting, especially for teenagers and young adults, may be concerts and rave events. For example, a recent study found elevated

levels of THC metabolites in urine samples of law enforcement officers with passive SHCS exposure during an outdoor concert, with 1 in 5 officers reporting headaches and respiratory symptoms following exposure (Wiegand et al. 2020).

3.1.3 Preclinical Studies on the Health Effects of SHCS

Preclinical studies have demonstrated an immediate impact on the cardiovascular system following exposure to SHCS (Wang et al. 2016; Liu et al. 2023). However, these studies did not find enduring effects on anxiety- or depression-like behaviors or cognition (i.e., learning, memory, motivation) later in life (Bruijnzeel et al. 2019; Hernandez et al. 2021). These findings suggest that SHCS exposure may produce immediate health impacts on the cardiovascular system. However, future research should aim to explore both short-term and long-term consequences of SHCS exposure on various health outcomes and investigate potential cumulative effects or latent impacts that may not be immediately apparent. In addition, studies incorporating a wider range of behavioral, physiological, and neurobiological assessments over extended time periods could provide more comprehensive insights into the lasting effects of SHCS exposure.

3.1.4 Clinical Studies on the Health Effects of SHCS

The most prevalent physical health outcomes associated with SHCS are respiratory issues, including asthma, bronchitis, and viral infections. Neurobehavioral outcomes in children exposed to SHCS include less cognitive flexibility and receptive language skills and increased aggressive behavior and ADHD symptoms. Young adults with SHCS exposure exhibit a decrease in cognitive abilities immediately after exposure.

Most clinical studies investigating the health outcomes associated with SHCS exposure have focused on infants and children (up to 12 years old). This focus may be primarily driven by the need to evaluate SHCS exposure in drug-naïve youth, given that the median age for the initiation of cannabis use is 15–16 years old (Richmond-Rakerd et al. 2017). However, it is important to note that brain regions important for learning, memory, and emotion regulation undergoes significant development and refinement during childhood, adolescence, and into early adulthood (Gogtay et al. 2004). Consequently, childhood, adolescence, and young adulthood may represent sensitive windows of vulnerability to SHCS exposure, with potential lifelong implications. Future research must develop methodologies to distinctly differentiate between SHCS exposure and direct cannabis use to parse out the effects on neurodevelopment. Longitudinal studies, such as the ABCD study®, that commence prior to the onset of cannabis use may be particularly well-suited to address this gap in the literature.

3.1.5 Limitations and Recommendations for Future Research

Notably, no studies to date have examined associations between SHCS exposure and brain outcomes in either animal models or humans. This gap in the literature is not unexpected, as early research on outdoor air pollution, specifically $PM_{2.5}$, initially identified associations with respiratory outcomes, such as asthma. It is only recently that studies have begun to uncover changes in brain structure and function following outdoor $PM_{2.5}$ exposure (Zundel et al. 2022). Similarly, the literature on secondhand tobacco smoke exposure began with a focus on respiratory issues, such as asthma (He et al. 2020), before expanding to include cognitive and behavioral outcomes, such as ADHD (Elbeeh 2023). Given that SHCS is a growing public health concern, it is anticipated that the health effects associated with outdoor $PM_{2.5}$ exposure, albeit at much lower concentrations, may similarly apply to SHCS. However, more research is needed.

In addition, future research should investigate the health impacts of particles smaller than $PM_{2.5}$, particularly ultrafine particles (UFPs), which may pose even greater risks due to their ability to penetrate deeper into the lungs, cause more inflammation, and persist longer in organs (Schraufnagel 2020). Notably, only one study in this review, Janssen et al. (2024) examined smaller particles (PM_1), highlighting the need for more comprehensive research in this area. Furthermore, other potential contributors to indoor $PM_{2.5}$ concentrations, such as combustion and heating sources, cooking, and household dust, must be measured and controlled for in future studies to accurately isolate the specific impact of SHCS on indoor air quality (National Academies of Sciences, Engineering, and Medicine 2016).

While the findings from this literature review underscore the paucity in research assessing neurodevelopmental outcomes related to exposure, an equally critical avenue is intervention-based strategies. Ott et al. (2022) found that opening three windows in the home during a cannabis joint smoking session decreased the $PM_{2.5}$ concentrations by 67%. However, the concentrations did not fall below the EPA's 24-h $PM_{2.5}$ standard (35 $\mu g/m^3$), demonstrating that ventilation alone is not sufficient to reduce $PM_{2.5}$ concentrations indoors to safe limits. Cheng et al. (2022) found that smoking and vaping outdoors reduced the average $PM_{2.5}$ concentrations to less than 5% of what was observed during indoor smoking sessions, suggesting a viable intervention strategy to protect youth from exposure.

From a housing policy perspective, implementing and enforcing smoke-free policies within MUH can be an effective intervention to reduce SHCS exposure. Sangmo et al. (2021a) found that parents living in MUH with policies that prohibited smoking inside apartments/units were less likely to report SHCS exposure than parents living in MUH where smoking was allowed. However, while these policies are effective, they highlight a critical gap in broader regulatory oversight. Although the EPA regulates outdoor air pollutants like $PM_{2.5}$, there is no federal regulation for indoor air quality, where SHCS can result in significantly higher $PM_{2.5}$ concentrations. Addressing this gap, policymakers could consider extending regulations to cover indoor environments, particularly in settings where vulnerable populations, such as children, are at risk. In addition, providing families with low-cost portable

personal air monitors, such as the PurpleAir indoor monitors (PurpleAir, Inc. 2024), to track indoor air quality may be a valuable and testable intervention for modifying exposure. Wallace et al. (2020) demonstrated that these low-cost air monitors can effectively assess and monitor exposure concentrations, offering a practical tool for families to manage and reduce SHCS in their homes.

Another intervention-based strategy would be to increase education and awareness for parents and providers. Research has shown that secondhand tobacco smoke education programs significantly decrease tobacco use within homes, thereby decreasing exposure in youth (Rosen et al. 2014). To our knowledge, no such programs have been developed for SHCS education. Further education is also needed for educators and healthcare providers on this emerging exposure. Specifically, physicians who treat children and families need to incorporate questions on cannabis use in the home in addition to tobacco use when assessing potential SHS exposure. Johnson and Watson (2021) found that 90% of cannabis-using caregivers had not been asked about their cannabis use by their child's pediatrician. Similarly, Brooks et al. (2017) found that only 23% of physicians and healthcare providers working with children assessed cannabis use at most visits. These studies underscore the necessity for healthcare providers to assess cannabis use in the homes of children as a first-line intervention in clinical settings. Without these regulations and interventions, indoor environments may continue to harbor $PM_{2.5}$ concentrations that far exceed safe levels, contributing to the adverse health effects identified in this review.

4 Conclusion

SHCS is an emerging and prevalent exposure among youth, containing extremely high concentrations of $PM_{2.5}$ that are up to 1.5 times the EPA 24-hour limit, exceed tobacco smoke, and are observed across various inhalation-based routes of administration (smoking, dabbing, vaping). SHCS exposure is also associated with adverse physical and neurobehavioral health outcomes. The neurobehavioral impacts are particularly concerning, as youth undergo rapid and dynamic changes in brain structure and function during development, making them more susceptible to SHCS effects. Further research is needed to address several key gaps in the literature. First, more preclinical and human mechanistic studies are needed to better characterize the neurobiological impact of SHCS exposure. Next, more longitudinal studies are needed to assess the impact of SHCS specifically during adolescence and young adulthood, as well as before and after the initiation of cannabis use, to examine the lifelong effects of exposure. Lastly, increased efforts are needed to raise public awareness and educate both communities and healthcare providers about the adverse health effects associated with SHCS, to reduce exposure. Addressing these gaps will be crucial for developing effective intervention and prevention strategies to mitigate the adverse health effects of SHCS on the brain and behavior of youth.

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References

Boakye E, et al. Cannabis vaping among adults in the United States: prevalence, trends, and association with high-risk behaviors and adverse respiratory conditions. Prev Med. 2021;153:106800.

Braithwaite I, et al. Air pollution (particulate matter) exposure and associations with depression, anxiety, bipolar, psychosis and suicide risk: a systematic review and meta-analysis. Environ Health Perspect. 2019;127(12):126002.

Brockmeyer S, D'Angiulli A. How air pollution alters brain development: the role of neuroinflammation. Transl Neurosci. 2016;7(1):24–30.

Brooks E, et al. The clinical implications of legalizing marijuana: are physician and non-physician providers prepared? Addict Behav. 2017;72:1–7.

Bruijnzeel AW, et al. Effects in rats of adolescent exposure to cannabis smoke or THC on emotional behavior and cognitive function in adulthood. Psychopharmacology. 2019;236(9):2773–84.

Cannabis. Alcohol, drugs and addictive behaviours unit. 2024. https://www.who.int/teams/mental-health-and-substance-use/alcohol-drugs-and-addictive-behaviours/drugs-psychoactive/cannabis.

Chambers J, et al. Perceptions of safety of daily cannabis vs tobacco smoking and secondhand smoke exposure, 2017-2021. JAMA Netw Open. 2023;6(8):e2328691.

Cheng KC, et al. PM(2.5) exposure close to marijuana smoking and vaping: a case study in residential indoor and outdoor settings. Sci Total Environ. 2022;802:149897.

Cone EJ, et al. Passive inhalation of marijuana smoke: urinalysis and room air levels of delta-9-tetrahydrocannabinol. J Anal Toxicol. 1987;11:89–96.

Cone EJ, et al. Non-smoker exposure to secondhand cannabis smoke. I. Urine screening and confirmation results. J Anal Toxicol. 2015;39(1):1–12.

Costa LG, et al. Effects of air pollution on the nervous system and its possible role in neurodevelopmental and neurodegenerative disorders. Pharmacol Ther. 2020;210:107523.

De Genna NM, Willford JA, Richardson GA. Long-term effects of prenatal cannabis exposure: pathways to adolescent and adult outcomes. Pharmacol Biochem Behav. 2022;214:173358.

Donzelli G, et al. Particulate matter exposure and attention-deficit/hyperactivity disorder in children: a systematic review of epidemiological studies. Int J Environ Res Public Health. 2019;17(1):67.

Elbeeh ME. Secondhand smoke's effects on brain development: ADHD and associated behaviors in children. J Umm Al-Qura Univ Appl Sci. 2023;9(4):591–608.

Genc S, et al. The adverse effects of air pollution on the nervous system. J Toxicol. 2012;2012:782462.

Gogtay N, et al. Dynamic mapping of human cortical development during childhood through early adulthood. PNAS. 2004;101(21):8174–9.

Goodwin RD, et al. Trends in cannabis and cigarette use among parents with children at home: 2002 to 2015. Pedatrics. 2018;141(6):e20173506.

Goodwin RD, et al. Trends in cannabis use among adults with children in the home in the United States, 2004-2017: impact of state-level legalization for recreational and medical use. Addiction. 2021;116(10):2770–8.

Government of Canada. Canadian cannabis survey 2022: summary. 2022.

Groner JA, et al. Secondhand smoke exposure and hair nicotine in children: age-dependent differences. Nicotine Tob Res. 2012;14(9):1104–9.

He Z, et al. The association between secondhand smoke and childhood asthma: a systematic review and meta-analysis. Pediatr Pulmonol. 2020;55(10):2518–31.
Hernandez CM, et al. Effects of repeated adolescent exposure to cannabis smoke on cognitive outcomes in adulthood. J Psychopharmacol. 2021;35(7):848–63.
Herrmann M, King K, Weitzman M. Prenatal tobacco smoke and postnatal secondhand smoke exposure and child neurodevelopment. Curr Opin Pediatr. 2008;2:184–90.
Herrmann ES, et al. Non-smoker exposure to secondhand cannabis smoke II: effect of room ventilation on the physiological, subjective, and behavioral/cognitive effects. Drug Alcohol Depend. 2015;151:194–202.
Herting MM, et al. Outdoor air pollution and brain structure and function from across childhood to young adulthood: a methodological review of brain MRI studies. Front Public Health. 2019;7:332.
Hu X, et al. 10-year spatial and temporal trends of PM(2.5) concentrations in the southeastern US estimated using high-resolution satellite data. Atmos Chem Phys. 2014;14(12):6301–14.
Huang AS, et al. PM2.5 concentrations in the smoking lounge of a cannabis store. Environ Sci Technol Lett. 2022;9(6):551–6.
Janssen F, et al. Comparison between smoked tobacco and medical cannabis cigarettes concerning particulate matter. Cannabis Cannabinoid Res. 2024;9(6):1492–9.
Johnson AB, Watson DB. Are marijuana-using caregivers being asked about their marijuana use by their child's pediatrician? Prev Med Rep. 2021;24:101548.
Johnson AB, et al. Association between secondhand marijuana smoke and respiratory infections in children. Pediatr Res. 2022;91(7):1769–74.
Keyhani S, et al. Risks and benefits of marijuana use: a national survey of U.S. adults. Ann Intern Med. 2018;169(5):282–90.
Kim JJ. Ambient air pollution: health hazards to children. Pedatrics. 2004;114(6):1699–707.
Liu J, et al. Impairment of endothelial function by aerosol from marijuana leaf vaporizers. J Am Heart Assoc. 2023;12(23):e032969.
MacCallum CA, et al. Cannabis vaporisation: understanding products, devices and risks. Drug Alcohol Rev. 2024;43(3):732–45.
Moore C, et al. Cannabinoids in oral fluid following passive exposure to marijuana smoke. Forensic Sci Int. 2011;212(1-3):227–30.
Moore BF, et al. Associations between prenatal and postnatal exposure to cannabis with cognition and behavior at age 5 years: the healthy start study. Int J Environ Res Public Health. 2023;20(6):4880.
Murphy MB, Huang AS, Schick SF. PM2.5 concentrations in a cannabis store with on-site consumption. Environ Health Perspect. 2021;129(6):67701.
National Academies of Sciences, Engineering, and Medicine. Sources of indoor particulate matter in health risks of indoor exposure to particulate matter: workshop summary. Washington: National Academies of Sciences, Engineering, and Medicine; 2016.
Nguyen PK, Hammond SK. Fine particulate matter exposure from secondhand cannabis bong smoking. JAMA Netw Open. 2022;5(3):e224744.
Ott WR, et al. Measuring indoor fine particle concentrations, emission rates, and decay rates from cannabis use in a residence. Atmos Environ X. 2021;10:100106.
Ott WR, et al. Measuring PM(2.5) concentrations from secondhand tobacco vs. marijuana smoke in 9 rooms of a detached 2-story house. Sci Total Environ. 2022;852:158244.
Popova L, et al. Perceived harms and benefits of tobacco, marijuana, and electronic vaporizers among young adults in Colorado: implications for health education and research. Addiction. 2017;112(10):1821–9.
Posis A, et al. Indoor cannabis smoke and children's health. Prev Med Rep. 2019;14:100853.
Richmond-Rakerd LS, Slutske WS, Wood PK. Age of initiation and substance use progression: a multivariate latent growth analysis. Psychol Addict Behav. 2017;31(6):664–75.
Röhrich J, et al. Concentrations of Δ9-tetrahydrocannabinol and 11-Nor-9 carboxytetrahydrocannabinol in blood and urine after passive exposure to cannabis smoke in a coffee shop. J Anal Toxicol. 2010;34:196–203.

Rosen LJ, et al. Meta-analysis of parental protection of children from tobacco smoke exposure. Pediatrics. 2014;133(4):698–714.
Sangmo L, et al. Reported marijuana and tobacco smoke incursions among families living in multiunit housing in New York city. Acad Pediatr. 2021a;21(4):670–6.
Sangmo L, et al. Secondhand marijuana exposure in a convenience sample of young children in New York City. Pediatr Res. 2021b;4:905–10.
Schraufnagel DE. The health effects of ultrafine particles. Exp Mol Med. 2020;52(3):311–7.
Semple BD, et al. Brain development in rodents and humans: identifying benchmarks of maturation and vulnerability to injury across species. Prog Neurobiol. 2013;106-107:1–16.
Suades-Gonzalez E, et al. Air pollution and neuropsychological development: a review of the latest evidence. Endocrinology. 2015;156(10):3473–82.
Substance Abuse and Mental Health Services Administration. Key substance use and mental health indicators in the United States: results from the 2022 National Survey on Drug Use and Health. Rockville: Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration; 2023.
Taylor M, et al. Comparison of cannabinoids in hair with self-reported cannabis consumption in heavy, light and non-cannabis users. Drug Alcohol Rev. 2017;36(2):220–6.
Tripathi O, et al. Location and home rules of cannabis use - findings from marijuana use and environmental survey 2020, a nationally representative survey in the United States. Prev Med Rep. 2023;35:102289.
Wade NE, et al. Clouding up cognition? Secondhand cannabis and tobacco exposure related to cognitive functioning in youth. Biol Psychiatry Glob Open Sci. 2023;3(2):233–42.
Wallace L, et al. Secondhand exposure from vaping marijuana: concentrations, emissions, and exposures determined using both research-grade and low-cost monitors. Atmos Environ X. 2020;8:100093.
Wang X, et al. One minute of marijuana secondhand smoke exposure substantially impairs vascular endothelial function. J Am Heart Assoc. 2016;5(8):e003858.
Wiegand DM, et al. Occupational exposure to secondhand cannabis smoke among law enforcement officers providing security at outdoor concert events. Ann Work Expo Health. 2020;64(7):705–14.
Wilson KM, et al. Detecting biomarkers of secondhand marijuana smoke in young children. Pediatr Res. 2017;81(4):589–92.
Wilson KM, et al. Marijuana and tobacco coexposure in hospitalized children. Pediatrics. 2018;142(6):e20180820.
Zhao T, et al. Characteristics of secondhand cannabis smoke from common smoking methods: calibration factor, emission rate, and particle removal rate. Atmos Environ. 2020;242:117731.
Zundel CG, et al. Air pollution, depressive and anxiety disorders, and brain effects: a systematic review. Neurotoxicology. 2022;93:272–300.

Chapter 22
Cannabis Use and Misuse Among Sexual and Gender Minority Youth: Implications for Practice



Suzanne Brown

1 Background

Sexual and gender minority (SGM) populations are classified as health disparities populations by the National Institutes of Health (NIH). The recognition of health disparities among SGM populations inspired the development of NIH's Sexual and Gender Minority Research Center that coordinates research on these populations. SGM have rates of substance misuse far above those of the general population, are at greater risk for physical and mental health conditions such as obesity, cancer, cardiovascular disease, mood disorders, and substance misuse, and experience significant barriers to accessing physical, mental health, and substance misuse treatment due to societal stigma and discrimination. SGM individuals are more likely than non-SGM individuals to report both illicit drug and cannabis use. Previous research found that 29% of SGM individuals reported illicit drug use and 25% reported cannabis use, while 10% of non-SGM individuals reported illicit drug use and 8% cannabis use (James et al. 2016). Youth are especially at risk for stigma and discrimination-related conditions such as social isolation, mental health difficulties, and suicide, and more at risk for substance misuse specifically. The purposes of this chapter are to discuss what is known about current cannabis use patterns, specifically among SGM youth, identify barriers to help-seeking for SGM youth, and consider current approaches to assessment and intervention for SGM youth.

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1.1 Who are "Sexual and Gender Minorities"?

SGM youth include those who identify their sexual orientation as gay, lesbian (same sex emotional, romantic, and/or sexual attraction) or bisexual (both sex emotional, romantic, and/or sexual attraction), as well as those who are transgender or gender diverse (TGD). The term "TGD" refers to those whose current gender identity is inconsistent with their assigned sex at birth, those whose experiences and/or expressions of gender do not align with a male/female binary (Rajkovic et al. 2022; Riddle and Safer 2022), and those with differences of sexual development (previously termed "intersex") (Acién and Acién 2020). According to the Pew Research Center (2024), approximately 7% of American adults across all age groups identify as gay, lesbian, or bisexual, while approximately 1.6% identify as TGD. Young adults ages 18–29 identify as SGM at significantly higher rates than older adults. Pew reports that 17% of young adults identify as lesbian, gay or bisexual, while 5.1% of young adults identify as TGD.

1.2 SGM Health Disparities

Significant health disparities exist for SGM individuals in terms of risk factors, health outcomes, and access to healthcare. SGM have increased rates of mental health (specifically anxiety and depression) disorders, substance abuse, suicide attempts and deaths by suicide, increased rates of cigarette smoking and binge drinking or heavy drinking, and less access to and use of preventive health care services such as cancer screenings (Burgess et al. 2021). SGM also experience increased rates of some infectious diseases, such as human immunodeficiency virus (HIV) and other sexually transmitted infections (STIs), increased rates of cancer, cardiovascular disease, and obesity. Lower access to physical and mental health care and treatment is an additional health disparity for SGM individuals as they have significantly lower access to routine, stable and preventive healthcare than non-SGM individuals. Although increased visibility, awareness, and civil rights legislation has reduced this disparity for sexual minorities over the past decade, gender minorities continue to experience significant barriers in access to physical and mental health treatment and services.

Specific to substance misuse, disparities exist for SGM individuals not just in rates of substance misuse but also in access to substance abuse treatment services. In general, approximately 89% of individuals who need treatment for substance use do not receive treatment. Compared to heterosexuals, the disparity between needing and receiving treatment is even higher for SGM who are twice as likely to have unmet substance abuse treatment needs due to lack of insurance or inadequate insurance coverage (Haney 2021). However, few studies have examined the relationships between sexual orientation and unmet treatment needs or relationships between sexual orientation and specific barriers to treatment. One study (Ji and Cochran

2022) found that few SGM specific substance abuse treatment programs exist across the country and that of the programs reporting to have SGM specific services on the National Survey of Substance Abuse Treatment Services only 1 in 5 actually had such services. In one qualitative study, Spalding (2023) identified both proximal and distal barriers to accessing substance abuse treatment for SGM. Proximal barriers included a sense of needing to conceal identity and anticipation of negative responses, while distal barriers included negative experiences with group and family treatment programming, policies that don't consider specific circumstances of SGM (such as sleeping arrangements), negative experiences with peers, and lack of psychological safety. Family and social support were one facilitator to treatment access. This is especially salient for SGM youth who may require more assistance from parents and families than SGM adults.

1.3 Youth Development

While there is some variation in the categorization of youth age groups, the US Agency for International Development defines youth as those aged 10–14 (early adolescence), 15–19 (adolescence), 20–24 (emerging adulthood), and 25–29 (transition to adulthood) (Sawyer et al. 2018). For the purposes of this chapter, I will focus on the periods of middle adolescence (ages 15–19), and emerging adulthood (ages 20–24), age groups most at risk for substance misuse and other high-risk behaviors. These are also the ages at which developmental tasks related to identity development become most salient. Given the importance of identity development and consolidation throughout this period, SGM individuals are often grappling with the implications of having a stigmatized identity and the societal messages in reaction to that stigma as part of general identity development that occurs in adolescence and emerging adulthood.

Middle adolescence (ages 15–19) marks a period of tremendous neuroplasticity (Michero 2025). During this period, significant increases in Dopamine receptors in the brain make adolescents more sensitive to rewards during this period than at any other time in development. Also, during this developmental period, the adolescent brain has lower baseline Dopamine, increasing the likelihood that youth will experience boredom and seek to relieve that boredom through exciting or novel experiences such as psychoactive substance use (Siegel 2015). During adolescence individuals become capable of abstract thought and have the cognitive decision-making capacities of adults. However, in what Icenogle and Cauffman (2021) termed the "maturity gap," while adolescents have a developed capacity for competent decision-making, this capacity is more situationally dependent than it is for adults. Youth are much more likely than adults to compromise competent decision making in emotionally charged circumstances, in the presence of psychosocial stressors, and when in the company of peers.

Arnett (2000), a developmental psychologist, advanced our theoretical discussions of emerging adulthood and conceptualized this as a distinct developmental

period characterized by the ongoing, intensified exploration of identity that began in adolescence. This stage is specific to cultures such as the United States, where youth are allowed a prolonged period of role exploration during their early twenties (Brandell and Brown 2015). Neurobiological findings have identified key components of brain structure and functional connectivity specific to this developmental period. Neurobiological research on this age group has established that the prefrontal cortex continues to develop throughout adolescence and emerging adulthood, even until the age of 30. Structural changes in the prefrontal cortex (specifically myelination and synaptic pruning) that continue to occur during this period of emerging adulthood (Blakemore and Choudhury 2006; Lebel and Beaulieu 2011), contribute to impulse control and planning, decision-making, and the ability to evaluate risks and rewards. They also increase the emerging adult's capacity for executive functioning, social cognition, and perspective taking. Functional connectivity between the subcortical and cortical regions is not complete until adulthood (Lebel and Beaulieu 2011; Brandell and Brown 2015).

During late adolescence and emerging adulthood, the subcortical areas, including the limbic system, mature more rapidly than the cortical regions responsible for regulation. This imbalance between subcortical and cortical brain areas accounts for the increased risky behavior that may occur during late adolescence and emerging adulthood (Casey et al. 2011). While emerging adults are quite capable of rational thought and decision-making, as well as behavioral control, in situations where intense affect is aroused, the more developed subcortical limbic system may supersede the control centers in the less-developed cortical regions. This increases the likelihood of impulsivity, emotional dysregulation, and maladaptive behaviors during adolescence and emerging adulthood. For SGM youth these neurobiological vulnerabilities to emotional and behavioral dysregulation and their impact on decision making may be exacerbated by stigma, isolation, lack of family support, and minority stress.

1.4 Cannabis Use Among SGM Youth

Several recent studies have identified sexual orientation disparities in substance use and misuse. Compared to their heterosexual and counterparts, SGM individuals report higher overall rates of past-30-day-use for disposable, THC, and CBD e-cigarettes (McCauley et al. 2024). Among youth, research within the United States and cross nationally has found that sexual minority youth are more likely than heterosexual youth to use alcohol, tobacco and nicotine products, marijuana, synthetic marijuana, and illicit drugs both recently and within their lifetime (Goldbach et al. 2017; Johns et al. 2018; Demant et al. 2016). While substance misuse in general and cannabis use specifically have been decreasing among youth over the past decade, this has not been true for SGM youth, and disparities in use between SGM and non-SGM youth may be increasing (Mereish 2019).

Related to gender minorities, TGD individuals are also more vulnerable to substance misuse disorders than cisgender people, and TGD adults are more likely than cisgender adults to repost cannabis use (25% versus 8%) (James et al. 2016). While young adults in general report among the highest alcohol and cannabis use levels in the United States, research specific to TGD young adults is somewhat limited as TGD identity is assessed in only a limited number of epidemiological surveillance systems (Zhang et al. 2020). Existing evidence suggests that substance use is common among TGD youth with rates equal to or greater than cisgender youth. For example, in an examination of undergraduate and graduate student data from the 2013 National College Health Assessment, Messman and Leslie (2019) found that transgender students were significantly more likely to use cannabis than cisgender students. Similarly, one cohort study of TGD youth and young adults identified elevated rates of substance use among transgender participants, with 34.5% of transgender women at moderate to high risk for developing a cannabis use disorder (Newcomb et al. 2020).

Research is increasingly identifying negative cognitive and neurobiological effects of persistent and prolonged cannabis use during middle adolescence and emerging adulthood. Results of one longitudinal study found that consistent cannabis use in adolescence was associated with neuropsychological impairment in adulthood (Meier et al. 2012). A large body of research supports the negative impact of persistent cannabis use on cognitive functioning among youth (Fischer et al. 2020). Cognitive impairments due to cannabis use include decreased attention, impairments in verbal and learning memory, slower cognitive processing speed, and impaired executive functioning (Broyd et al. 2016). In addition, earlier age of first use and heavier use during adolescence are more likely to produce impairments in cognitive functioning into adulthood (Meier et al. 2012; Ogeil et al. 2019). Neurobiologically, cannabis use impairs sensitivity to rewards and stress, resulting in an increase in depressive symptoms (Ceccarini et al. 2015). While SGM youth may experience a reduction in minority stress in response to cannabis use, the physiological stress reduction effects may be accompanied by decreases in reward sensitivity, cognitive impairments, and sleep disturbances. Depending on the age of onset and the intensity of cannabis use, these impairments may impact youth into middle and late adulthood.

1.5 Minority Stress Theory

Minority stress is one significant predictor of substance use and misuse for SGM youth (Meyer 1995). The increased risk for substance misuse among SGM youth is likely related to the discrimination and stigma experienced by this population (Connolly and Gilchrist 2020). The minority stress model proposes that the empirically validated disparities in health outcomes for SGM youth result from the stress

load related to social exclusion and stigma (Weeks et al. 2023). These include both distal stressors and proximal stressors. Distal stressors include external factors such as housing discrimination, trans-/homo-phobic cultural and political discourse, family rejection, and violence, that force and SGM youth to conceal their identity. Proximal stressors are the internal appraisals of these distal stressors, or the internal, psychological responses an SGM individual may have to transphobic, homophobic, and heterosexist policies and practices, including negative expectancies and self-stigma. Distal stressors have been found to increase more proximal stressors and research strongly supports a mediated model in relation to health disparities whereby distal stressors increase proximal stressors, leading to negative health outcomes within this population.

Through often daily transphobic, homophobic, and heterosexist social processes, SGM individuals may also experience intersecting forms of social marginalization intensified by the addition of intersecting marginalized identities based on factors such as gender, race, or social class (Wesp et al. 2019). For example, intersectional stresses created by gender discrimination, racism, poverty, and unemployment status may contribute to substance use as a way that SGM youth cope with gay and trans exclusionary discrimination and victimization (Hatchel and Marx 2018). Thus, economic stressors such as low income (Gonzalez et al. 2017), financial hardship (Cerezo et al. 2020), and unemployment (Connolly and Gilchrist 2020) have been correlated with greater substance use among trans and gender diverse individuals. Stress related to stigma and discrimination create a need for elevated social, cognitive, and biological coping regulation strategies that pose increased risk for negative health outcomes such as substance misuse (Durso and Johnson 2013; Hatzenbuehler 2009). For example, researchers have documented that these heterosexism-based stressors may increase craving for cannabis among SGM youth specifically (Mereish and Miranda 2024).

Minority stress is also related to short-term increases in substance use (Flentje et al. 2024). One study that examined patterns of cannabis use over 4 years among SGM people asked whether or not cannabis use trajectories related to reported minority stressors in a sample of almost 12,000 SGM people. Researchers identified five different cannabis use trajectories: "low or no risk," "low moderate risk," "high moderate risk," "steep risk increase," and "highest risk." Participants who reported past-year discrimination and/or victimization at baseline had greater odds of membership in any cannabis risk category compared to the "low risk" category. In addition, internalized stigma was related to "high moderate" and "highest risk" cannabis use over time. After accounting for minority stress, gender expansive people and transgender men had higher odds than cisgender men to have "low moderate risk" or "high moderate risk." Transgender men had higher odds of having "highest risk" cannabis use compared to the "low or no risk" category. Researchers concluded that minority stress was related to cannabis use risk trajectories, and gender diverse people and transgender men had greater cannabis use risk even after accounting for minority stress.

1.6 Neurobiology of Minority Stress and Youth

Empirical research has begun to examine the neurobiological consequences of minority stress, which has implications for substance misuse as well. During adolescence and emerging adulthood, the chronic minority stress due to proximal and distal stressors has been associated with brain functional connectivity patterns similar to those found in individuals with chronic childhood stress (Loso et al. 2023). This includes heightened activation in areas of the brain responsible for emotion and memory such as the fusiform and orbitofrontal areas of the brain (Loso et al. 2023). In one systematic review of neural correlates associated with SGM stress, Nicholson et al. (2022) summarized evidence supporting associations between minority stress and decreased activation and functional connectivity within the brain's default—mode network—a set of brain regions involved in social cognition and one's sense of self. Such brain changes would be especially salient for adolescents and emerging adults whose primary developmental task is identity development and the development of a sense of self.

Neural adaptations related to minority stress have also been identified in areas of the brain involved in executive functioning and emotion regulation (the central executive network) (Hu and Jiang 2014), and areas involved in threat detection and fear processing (the salience network) (Eckstrand et al. 2019). These neurobiological adaptations match those previously identified among individuals with posttraumatic stress disorder (PTSD) (Nicholson et al. 2022). Both the central executive network and the salience network have been associated with cannabis misuse in individuals with PTSD (Schifano et al. 2018). Cannabinoid receptors are abundant in areas of the brain involved in reward, cognition, emotional regulation and stress responsivity, and all of these regions underly vulnerability to cannabis misuse (Ferland and Hurd 2020). In addition, cannabis misuse usually begins in adolescence and emerging adulthood, and among those vulnerable due to environmental factors such as the distal conditions of minority stress. Neurobiological effects of chronic minority stress may also result in stress-related increases in heart rate, cortisol, blood pressure, and other physiological processes that underpin health-related disorders such as cardiovascular disease and behavioral health disorders such as substance misuse among SGM individuals (Parent et al. 2019).

2 Clinical Implications

2.1 Assessment

Cannabis is more widely used than any other psychoactive substance in the United States (Carliner et al. 2017), in spite of myriad short-term and long-term problems associated with youth cannabis use (Silins et al. 2014). Recent changes in laws regulating cannabis use in the United States have contributed to the lowered

perception of risk associated with regular cannabis use (Johnston et al. 2017). The availability and legality of cannabis along with its relation to neurophysiological impairment stresses the importance of identifying valid assessment tools to evaluate youths' problems related to cannabis use.

Given the complex needs and specific developmental tasks of adolescence and emerging adulthood, youth-specific assessment tools and processes have been developed. The evidence-based, Screening Brief Intervention and Referral to Treatment (SBIRT) for adolescents is one such assessment process useful in primary care and school settings (Mitchell et al. 2013). SBIRT is a process that begins by screening the individual for substance misuse and risky substance behavior. Screening tools may include the CRAFT, a brief questionnaire used to identify adolescents at risk for drug use or alcohol problems (Knight et al. 1999), or the AUDIT (Alcohol Use Disorders Identification Test), another standardized screening instrument used in randomized controlled trials of adolescent SBIRT (Bernstein et al. 2010). Both tools identify risky substance use patterns among adults and youth. Once risky behavior is identified, brief motivational interviewing techniques are utilized to encourage the individual to access intervention services and a referral to treatment is made. SBIRT can be implemented in school settings, community centers and in youth-serving primary care settings. SBIRT has been found to be effective in identifying risky substance use behaviors among youth (Fischer et al. 2020), and in enhancing treatment engagement among youth when used in primary care settings (D'Amico et al. 2008), school settings (Grenard et al. 2007), and community-based settings (Peterson et al. 2006).

Two scales have been commonly used to identify the severity of problematic cannabis use among youth. The Marijuana Adolescent Problems Inventory (MAPI) (Johnson et al. 1992) was designed to measure problems related to marijuana use for youth. The MAPI is a 23-item, problem index where youth are instructed to rate each item on a 0–4 scale and eventually yields one total scale score. Another measure used to assess youth marijuana-related problems is the 27-item Adolescent Cannabis Problems Questionnaire (CPQ-A) (Martin et al. 2006). In this measure youth endorse yes or no to each problem item, yielding a total score and scores on three problem subscales (i.e., financial/psychosocial, physical, and acute negative consequences).

2.2 Evidence-Based Practices for Cannabis Misuse among Youth

Motivational Enhancement Therapy Recent evidence supports the use of motivational interviewing (MI) and motivational enhancement therapy (MET) as effective approaches with adolescents and young adults with both general substance misuse and cannabis misuse specifically (Naar-King 2011). Both MI and MET prioritize value identification and goal development as central to the intervention. This

focus on value identification is easily integrated with the general identity development tasks central to adolescence and emerging adulthood. MI is based on four central premises that support what is termed *MI Spirit*, including *Acceptance*, *Compassion*, *Evocation*, and *Clarification. Acceptance* promotes practitioner behaviors that communicate empathy, affirmation, and a sense of the youth's worth. *Compassion* is reflected in practitioner behaviors that prioritize the youth's best interests and needs. Through *Evocation* practitioners elicit the youth's own perspectives, interpretations, and motivations for engaging in substance use behaviors and change. Through *Collaboration* practitioners act as coaches and companions by collaborating with the youth's own goals, knowledge about themselves, and values (Miller and Rollnick 2012).

The Transtheoretical Model of behavior change (Prochaska et al. 1992) supports MET as it provides a framework for identifying an individual's stage of behavior change, which informs their level of motivation for change and the development of treatment goals. The Transtheoretical Model offers an opportunity for MET practitioners to match their interventions to the youth's stage of change to enhance their motivation to change. Providers of MET interventions recognize that different individuals are at different places in the process of behavior change. MET sessions help individuals understand and characterize negative and positive aspects of their relationship with cannabis use, including main reasons for using cannabis, alternative behaviors, values and goals, and pros and cons of behavior change (Kells et al. 2019). MET was found to reduce substance misuse for both youth and adults through influencing the individual's intentions to change substance use behaviors (Li et al. 2016), and has been effectively used to help youth with cannabis misuse problems (Lenz et al. 2016).

Cognitive Behavioral Therapy Cognitive-behavioral therapies (CBT) focus on thoughts, behaviors, and emotions, and have been highly successful in helping youth maintain abstinence from cannabis (Walther et al. 2016). This approach encourages youth to change substance use behaviors by identifying nonfunctional beliefs, modifying behaviors that lead to substance misuse, and learning and utilizing skills to manage emotions that make one vulnerable to substance misuse. CBT is commonly used to treat youth with behavioral health disorders, such as substance misuse and mood disorders. CBT focuses on the development and utilization of skills, such as identifying and coping with substance use triggers, drug refusal, problem solving, and emotion regulation, including modulation of emotions and reducing vulnerability to negative emotions (Budney et al. 2007).

During CBT interventions, practitioners help youth complete behavioral analyses which assist youth to analyze past cravings, identify external and internal triggers to use, and identify both precursors and consequences of cannabis use (Budney et al. 2007). Once the analysis is complete, practitioners can assist youth to plan out strategies and skills to help them manage situations that trigger cravings to use cannabis and urges toward that behavior (Budney et al. 2007; Wells et al. 2013). Practitioners may also engage youth in completing homework assignments and

role-playing tasks—where youth have the opportunity to practice skills learned in treatment to decrease cannabis use. Barriers to the effective use of new skills in the youth's own environment might also be reviewed and discussed in individual and or group treatment settings. Research on CBT interventions for youth with cannabis misuse found significant reductions in cannabis and other drug/alcohol use and significant improvements in readiness scores on the youths' Readiness to Change Scale (Hides et al. 2010). CBT is also effective in helping youth recover from behavioral health problems such as depressive disorder that frequently co-occur with cannabis misuse (March et al. 2004), and may be offered in combination with other interventions, such as motivational interviewing (Babor 2004).

Combined MET and Cognitive Behavioral Therapy (CBT) More recently, practitioners have begun to integrate CBT and MET together in response to cannabis use among youth. While motivational enhancement therapy motivates youth to change behaviors that trigger and reinforce cannabis use and to avoid cannabis use, cognitive behavior therapy teaches youth skills which are necessary for decreasing vulnerability to cannabis use and ultimately stopping their cannabis use (Budney et al. 2007). Compared to motivational enhancement therapy, cognitive behavior therapy is much more direct in the need for individuals to change their cannabis use behaviors and in offering specific interventions to facilitate this change (Wells et al. 2013). CBT teaches youth how to change their behaviors by guiding them in adapting new techniques to change their behavior and enhance general well-being (Wells et al. 2013). Cognitive behavior therapy emphasizes skill development and use, while MET sets the foundation for the use of CBT skills by enhancing motivation to change through value identification and goal development.

Contingency Management In some instances, cognitive behavior therapy has been combined with other strategies such as contingency management, to improve its effects on the treatment of substance misuse. Contingency management increases the treatment success for adults with substance misuse in learning and utilizing CBT skills through reinforcement of homework completion and treatment session attendance (Carroll et al. 2012). Contingency management provides incentives like money to enhance an individual's motivation to change substance use behaviors and increase treatment success (Carroll et al. 2012). Contingency management (CM) is a behavioral approach that utilizes incentives such as cash or vouchers that are redeemable for goods and services. These incentives are contingent on evidence that clients have engaged in behavior change behaviors such as reductions in substance use and increases in treatment engagement (Davis et al. 2016). Research on the effectiveness of CM has demonstrated its effectiveness in promoting abstinence from cannabis (Kadden et al. 2007) among adults seeking treatment for or interested in changing their substance use behavior. However, CM has had mixed results either alone or combined with CBT in treating cannabis misuse among youth, with some researchers finding no effect (Kaminer et al. 2014), and others offering support for the use of contingency management with youth primarily with cannabis use disorder (Stanger et al. 2009).

Multidimensional Family Therapy Multidimensional Family Therapy (MDFT) (Liddle 2010) is a family-based outpatient treatment intervention for youth problem behavior, including substance misuse. The term "multidimensional" reflects the ecological framework foundational to this intervention. The model's assumption is that each major domain in the life of an adolescent contributes to the likelihood of substance misuse behavior, through risk factors, and may help in resolving such problems, through protective factors. The life domains include the individual youth, their parents, family, friends and peers, school and work, and leisure time. The practitioner's goal in this intervention model is to improve life domain conditions for the adolescent and the family through direct outreach, support for new behaviors and relationships, and skill building to enhance relationships between and among other domains in the youth's life. In MDFT family functioning is central to creating new, developmentally adaptive behavioral and relational alternatives for youth. Skills training in this model may include substance use relapse prevention, family communication, and parenting. While this model has been successful in treating youth with substance misuse, it has not been evaluated with SGM youth specifically. SGM youth may be less likely to engage in this form of treatment depending on their relationships with their own parents and families, as these youth are less likely to have functional relationships with parents and more likely to experience youth homelessness than non-SGM youth. However, its focus on the youth's larger environment may offer opportunities for practitioners to assist SGM youth in identifying and engaging in affirmative social networks and settings, thereby increasing protective factors for SGM youth.

3 SGM Affirmative Practices

Ensuring that SGM youth have access to treatment for cannabis misuse, and healthcare services more generally, requires that providers understand affirming principles of care for SGM individuals as well as evidence-based assessment and treatment for youth. Broadly, affirmative practices for working with SGM individuals actively challenge the traditional view that lesbian, gay, bisexual, or TGD individuals are pathological. Affirmative treatment perspectives regard transphobia, homophobia, and heterosexism, as opposed to homosexuality or TGD, as central to the development of health disparity conditions among SGM, such as depression, suicidality, and substance misuse (Kort 2018). Within the psychotherapy field, SGM affirming care proposes that there is nothing inherently wrong with being SGM, and that experiencing homophobia, heterosexism and transphobia create behavioral and psychological difficulties, not SGM orientation itself. Affirming psychological care seeks to repair the harm done to these SGM clients, helping them reduce the impact of stigma on their sense of self (Friedman and Downey 2008).

In medicine, SGM "affirming care is high-quality, culturally responsive treatment delivered in a safe and respectful environment" and "it recognizes the substantial impact of sexuality or gender identity on one's health" (Turner 2024). According

to the American Medical Association, SGM affirmative healthcare refers to medical services and practices that recognize, respect, and support the unique health needs and experiences of SGM individuals. This type of healthcare is designed to create an inclusive environment where patients feel safe and understood, addressing both physical and mental health concerns that may arise from discrimination or social stigma. SGM individuals deserve inclusive and affirming care at every stage of life, from adolescence to advanced age. The vulnerability of youth and the pivotal nature of this developmental period make affirmative healthcare even more vital for SGM youth. There are a range of affirmative healthcare practices that may be generated at the system or agency level, and those that may be generated by the individual practitioner. Individual providers, agencies, and health care systems can initiate actions that explicitly reflect an affirming health care environment, and in so doing can address and reduce health disparities in this population, especially disparities related to access to care and services.

3.1 System-Level Affirmative Practices

The World Health Organization has outlined system-level principles for working with SGM youth that include *availability*, *accessibility*, *acceptability*, and *equity*. *Availability* refers to the importance of having enough health care providers in one's geographic area with knowledge, competence and experience working with youth with current or in-process SGM identities, feelings and behaviors (Hadland et al. 2016, p. 3). *Accessibility* refers to how easy or difficult it is for SGM youth to access care and treatment from an available provider. *Acceptability* is the degree to which available services and treatments are BOTH culturally competent and developmentally appropriate for SGM youth. This also includes the degree to which parents are involved when appropriate, confidentiality is assured, collaboration is developed, and boundaries are maintained with parents, school personnel and others in the interest of the youth's safety. Finally, *equity* is the extent to which services and treatment are friendly, open, and accessible to ALL SGM youth regardless of sexual orientation, gender identity, gender expression, race, ethnicity, language, ability to pay, housing status, and insurance status.

Lund and Burgess et al. (2021), in their review of barriers to care for SGM, identified specific guidelines for SGM affirming healthcare that encompass both system level and practitioner level behaviors. At the system level, their suggestions include the following (pp. 184–185):

- Use inclusive and positive SGM-related posters, brochures, or signage in the clinic's physical environment to signal an affirmative environment where individuals can safely disclose.
- Include SGM-inclusive language or signage on clinic, provider, or health care system web sites or social media profiles to identify the clinic and provider as affirming.

- Ensure intake paperwork is inclusive and affirming. For example, having an open text space in which to identify gender identity is more inclusive than a forced gender binary option (male/female), as is an intake form that allows patients to indicate their pronouns and preferred names.
- Maintain an affirmative environment by quickly noticing and correcting inappropriate or offensive comments by other staff, including both macroaggressive and microaggressive comments and invalidating or outdated language (e.g., referring to transgender patients by their previous names; referring to a transgender client as "actually" their gender-assigned at birth; referring to an asexual or bisexual patient as "confused").
- Work with the SGM community to continuously develop provider cultural competency and identify and implement best practices with SGM patients.

3.2 Practitioner-Level Affirmative Practices

At the practitioner level, Lund and Burgess (2021, pp. 184–185) suggest the following:

- Understand the minority stressors faced by SGM patients and how those may affect physical and mental health.
- Learn about health disparity profiles and social determinants of health for specific SGM subpopulations and multiply marginalized SGM populations.
- Wear clothing or jewelry that indicates SGM affirmation (e.g., a rainbow or transgender flag lapel pin or a name tag listing the provider's pronouns) that a particular provider is a community member or strong ally.
- Ask about sexual orientation and gender identity directly. Researchers have consistently found that sexual and gender minority individuals prefer to be asked about their sexual orientation and gender identity by health care providers, rather than have it assumed based on appearance, partnership status, or other factors, as long as the questioning is done in a respectful and affirmative manner.
- Show respect and sensitivity toward patients' previous experiences of discrimination, exclusion, invalidation, harassment, or violence.
- Be familiar with SGM-affirming shelters, social services agencies, and specialists in the community to refer a patient to if necessary.
- Treat each patient as an individual.
- Avoid stereotyping (e.g., assuming all gay men engage in risky sexual behaviors), underemphasizing (ignoring), or overemphasizing (i.e., assuming that all health or mental health complaints are a result of their sexual orientation or gender identity) their identities.

Hadland et al. (2016) offer a number of additional considerations when working with SGM youth. They suggest that practitioners use language that helps establish a trusting relationship between practitioner and youth. For example, use the same terms youth use to describe themselves and ask what a youth's preferred pronouns are. Be aware that SGM youth may have had prior adverse interactions with health-care providers and may be initially hesitant to disclose personal information. Being transparent and clear about confidentiality and the limits of confidentiality can build trust with youth. Understand that youth may have fluid identities in relation to sexual/romantic attraction and gender identity and expression. Fluidity in identity is not evidence of pathology but rather indicative of this developmental stage for all adolescents and emerging adults. Consider that SGM youth are more likely to be uninsured than non-SGM youth and may be estranged from family support. Be prepared to consider low cost or no cost care alternatives. Consider that health-care records usually use the individual's legal name assigned at birth. Transgender youth who have changed their name may find this problematic. Review the forms your agency uses and consider inclusive options for categorizing gender and specifying names. Handle mistakes with respect and authenticity. It is not uncommon to make mistakes with names, pronouns, and other aspects of SGM youth identity. Respond with genuine apology, non-defensiveness, and a commitment to self-correction.

4 Conclusion

A range of evidence supported interventions currently exist for treating youth with cannabis misuse, including CBT, MET, Contingency Management, and Multi-Dimensional Family Therapy. However, none of these interventions have been tested specifically with SGM youth. These youth are at higher risk for cannabis (and other substance) misuse and co-occurring mood disorders and suicidality than their non SGM youth counterparts. Their higher risk may be attributed to minority stress and stigma as well as the impact that minority stress has on neurobiological functioning. Their higher risk is also driven by disparities in their access to treatment, support, and interventions due to stigma and lack of culturally competent services for this population. In addition, due to high rates of homelessness among SGM youth, there is often little family support for preventative medical care and treatment, and SGM youth are more likely to be un-insured or under-insured. Current treatment requires a combination of evidence-supported treatment methods combined with gender and sexuality affirming care. Effective treatment with this population may also require an ecological perspective that supports youth in identifying and utilizing treatment providers, social support, and social network members affirming of sexual and gender minority identity that buttress SGM youth against stigma and isolation.

References

Acién P, Acién M. Disorders of sex development: classification, review, and impact on fertility. J Clin Med. 2020;9(11):3555. https://doi.org/10.3390/jcm9113555. PMID: 33158283; PMCID: PMC7694247.

Arnett JJ. Emerging adulthood: a theory of development from the late teens through the twenties. Am Psychol. 2000;55(5):469.

Babor TF. Brief treatments for cannabis dependence: findings from a randomized multisite trial. J Consult Clin Psychol. 2004;72(3):455.

Bernstein J, Heeren T, Edward E, Dorfman D, Bliss C, Winter M, Bernstein E. A brief motivational interview in a pediatric emergency department, plus 10-day telephone follow-up, increases attempts to quit drinking among youth and young adults who screen positive for problematic drinking. Acad Emerg Med. 2010;17(8):890–902.

Blakemore SJ, Choudhury S. Development of the adolescent brain: implications for executive function and social cognition. J Child Psychol Psychiatry. 2006;47(3-4):296–312.

Brandell JR, Brown S. The new bridge to adulthood: Searching for meaning and cohesion in the nexus of hookups, internet porn, and instance messages. Smith Coll Stud Soc Work. 2015;85(4):387–408.

Broyd SJ, Van Hell HH, Beale C, Yuecel M, Solowij N. Acute and chronic effects of cannabinoids on human cognition—a systematic review. Biol Psychiatry. 2016;79(7):557–67.

Budney AJ, Roffman R, Stephens RS, Walker D. Marijuana dependence and its treatment. Addict Sci Clin Pract. 2007;4(1):4.

Burgess CM, Batchelder AW, Sloan CA, Ieong M, Streed CG. Impact of the COVID-19 pandemic on transgender and gender diverse health care. Lancet Diabetes Endocrinol. 2021;9(11):729–31.

Carliner H, Brown QL, Sarvet AL, Hasin DS. Cannabis use, attitudes, and legal status in the U.S.: a review. Prev Med. 2017;104:13–23. https://doi.org/10.1016/j.ypmed.2017.07.008.

Carroll KM, Nich C, LaPaglia DM, Peters EN, Easton CJ, Petry NM. Combining cognitive behavioral therapy and contingency management to enhance their effects in treating cannabis dependence: less can be more, more or less. Addiction. 2012;107(9):1650–9.

Casey BJ, Somerville LH, Gotlib IH, Ayduk O, Franklin NT, Askren MK, Shoda Y. Behavioral and neural correlates of delay of gratification 40 years later. Proc Natl Acad Sci. 2011;108(36):14998–5003.

Ceccarini J, Kuepper R, Kemels D, van Os J, Henquet C, Van Laere K. [18 F] MK-9470 PET measurement of cannabinoid CB 1 receptor availability in chronic cannabis users. Addict Biol. 2015;20(2):357–67.

Cerezo A, Williams C, Cummings M, Ching D, Holmes M. Minority stress and drinking: connecting race, gender identity and sexual orientation. Couns Psychol. 2020;48(2):277–303.

Connolly D, Gilchrist G. Prevalence and correlates of substance use among transgender adults: a systematic review. Addict Behav. 2020;111:106544.

D'Amico E, Miles JN, Stern S, Meredity LS. Brief motivational interviewing for teens at risk of substance use consequences: a randomized pilot study in a primary care clinic. J Subst Abus Treat. 2008;35:53–61.

Davis DR, Kurti AN, Skelly JM, Redner R, White TJ, Higgins ST. A review of the literature on contingency management in the treatment of substance use disorders, 2009–2014. Prev Med. 2016;92:36–46.

Demant D, Hides L, Kavanagh DJ, White KM, Winstock AR, Ferris J. Differences in substance use between sexual orientations in a multi-country sample: findings from the Global Drug Survey 2015. J Public Health. 2016;39:532–41.

Durso LE, Johnson KL. Minority stress and physical health among sexual minorities. Perspect Psychol Sci. 2013;8(5):521–48. https://doi.org/10.1177/1745691613497965.

Eckstrand KL, Flores LE Jr, Cross M, Silk JS, Allen NB, Healey KL, Forbes EE. Social and non-social reward processing and depressive symptoms among sexual minority adolescents. Front Behav Neurosci. 2019;13:209.

Ferland JM, Hurd YL. Deconstructing the neurobiology of cannabis use disorder. Nat Neurosci. 2020;23:600–10. https://doi.org/10.1038/s41593-020-0611-0.
Fischer AS, Tapert SF, Louie DL, Schatzberg AF, Singh MK. Cannabis and the developing adolescent brain. Curr Treat Options Psychiatry. 2020;7:144–61.
Flentje A, Sunder G, Ceja A, Lisha NE, Neilands TB, Aouizerat BE, Lubensky ME, Capriotti MR, Dastur Z, Lunn MR, Obedin-Maliver J. Cannabis use trajectories over time in relation to minority stress and gender among sexual and gender minority people. Addict Behav. 2024;157:108079. https://doi.org/10.1016/j.addbeh.2024.108079.
Friedman RC, Downey JI. Sexual orientation and psychodynamic psychotherapy: sexual science and clinical practice. New York: Columbia University Press; 2008.
Goldbach JT, Mereish EH, Burgess C. Sexual orientation disparities in the use of emerging drugs. Subst Use Misuse. 2017;52:265–71.
Gonzalez CA, Gallego JD, Bockting WO. Demographic characteristics, components of sexuality and gender, and minority stress and their associations to excessive alcohol, cannabis, and illicit (noncannabis) drug use among a large sample of transgender people in the United States. J Prim Prev. 2017;38:419–45. https://doi.org/10.1007/s10935017-0469-4.
Grenard JL, Ames SL, Wiers RW, Thush C, Stacy AW, Sussman S. Brief intervention for substance use among at-risk adolescents: a pilot study. J Adolesc Health. 2007;40:188–91.
Hadland SE, Yehia BR, Makadon HJ. Caring for lesbian, gay, bisexual, transgender, and questioning youth in inclusive and affirmative environments. Pediatr Clin. 2016;63(6):955–69.
Haney JL. Sexual orientation, social determinants of health, and unmet substance use treatment need: findings from a national survey. Subst Use Misuse. 2021;56(2):205–13. https://doi.org/10.1080/10826084.2020.1853775.
Hatchel T, Marx R. Understanding intersectionality and resiliency among transgender adolescents: exploring pathways among peer victimization, school belonging, and drug use. Int J Environ Res Public Health. 2018;15(6):1289.
Hatzenbuehler ML. How does sexual minority stress get under the skin? Psychol Bull. 2009;135(5):707–30. https://doi.org/10.1037/a0016441.
Hides L, Samet S, Lubman DI. Cognitive behaviour therapy (CBT) for the treatment of co-occurring depression and substance use: current evidence and directions for future research. Drug Alcohol Rev. 2010;29(5):508–17.
Hu C, Jiang X. An emotion regulation role of ventromedial prefrontal cortex in moral judgment. Front Hum Neurosci. 2014;8:873.
Icenogle G, Cauffman E. Adolescent decision making: a decade in review. J Res Adolesc. 2021;31(4):1006–22.
James SE, Herman JL, Rankin S, Keisling M, Mottet L, Anafi M. The report of the 2015 U.S. transgender survey. Washington: National Center for Transgender Equality; 2016.
Ji C, Cochran B. The availability of sexual and gender minority (SGM) specific substance use services. Subst Use Misuse. 2022;57(14):2126–33. https://doi.org/10.1080/10826084.2022.2137812.
Johns MM, Beltran O, Armstrong HL, Jayne PE, Barrios LC. Protective factors among transgender and gender variant youth: a systematic review by socioecological level. J Prim Prev. 2018;39:263–301.
Johnson C, Archer RP, Sheaffer CI, Miller DL. Relationships between the MAPI and the MMPI in the assessment of adolescent psychopathology. J Pers Assess. 1992;58(2):277–86.
Johnston LD, O'Malley PM, Miech RA, Bachman JG, Schulenberg JE. Monitoring the future national survey results on drug use, 1975-2016: overview, key findings on adolescent drug use. Ann Arbor: University of Michigan; 2017.
Kadden RM, Litt MD, Kabela-Cormier E, Petry NM. Abstinence rates following behavioral treatments for marijuana dependence. Addict Behav. 2007;32(6):1220–36.
Kaminer Y, Burleson JA, Burke R, Litt MD. The efficacy of contingency management for adolescent cannabis use disorder: a controlled study. Subst Abus. 2014;35(4):391–8.

Kells M, Burke PJ, Parker S, Jonestrask C, Shrier LA. Engaging youth (adolescents and young adults) to change frequent marijuana use: motivational enhancement therapy (MET) in primary care. J Pediatr Nurs. 2019;49:24–30.
Knight JR, Shrier LA, Bravender TD, Farrell M, Vander Bilt J, Shaffer HJ. A new brief screen for adolescent substance abuse. Arch Pediatr Adolesc Med. 1999;153(6):591–6.
Kort J. LGBTQ clients in therapy: clinical issues and treatment strategies. New York: W. W. Norton and Company; 2018.
Lebel C, Beaulieu C. Longitudinal development of human brain wiring continues from childhood into adulthood. J Neurosci. 2011;31(30):10937–47.
Lenz AS, Rosenbaum L, Sheperis D. Meta-analysis of randomized controlled trials of motivational enhancement therapy for reducing substance use. J Addict Offender Couns. 2016;37(2):66–86.
Li L, Zhu S, Tse N, Tse S, Wong P. Effectiveness of motivational interviewing to reduce illicit drug use in adolescents: a systematic review and meta-analysis. Addiction. 2016;111(5):795–805.
Liddle HA. Treating adolescent substance abuse using multidimensional family therapy. Evid Based Psychotherap Child Adolesc. 2010;2:416–34.
Loso H, Chaarani B, Dube SL, Albaugh MD, Cheaito A, Garavan H, Potter A. Gender diversity associated with patterns of brain activation seen in populations that experience childhood stress. Front Integr Neurosci. 2023;17:1084748.
Lund EM, Burgess CM. Sexual and gender minority health care disparities. Prim Care. 2021;48(2):179–89. https://doi.org/10.1016/j.pop.2021.02.007.
March J, Silva S, Petrycki S, Curry J, Wells K, Fairbank J, Severe J. Fluoxetine, cognitive-behavioral therapy, and their combination for adolescents with depression: treatment for adolescents with depression study (TADS) randomized controlled trial. JAMA. 2004;292(7):807–20.
Martin G, Copeland J, Gilmour S, Gates P, Swift W. The adolescent cannabis problems questionnaire (CPQ-A): psychometric properties. Addict Behav. 2006;31(12):2238–48.
McCauley DM, Baiocchi M, Gaiha SM, Halpern-Felsher B. Sociodemographic differences in use of nicotine, cannabis, and non-nicotine E-cigarette devices. Drug Alcohol Depend. 2024;255:111061. https://doi.org/10.1016/j.drugalcdep.2023.111061.
Meier MH, Caspi A, Ambler A, Harrington H, Houts R, Keefe RS, Moffitt TE. Persistent cannabis users show neuropsychological decline from childhood to midlife. Proc Natl Acad Sci. 2012;109(40):E2657–64.
Mereish EH. Substance use and misuse among sexual and gender minority youth. Curr Opin Psychol. 2019;30:123–7. https://doi.org/10.1016/j.copsyc.2019.05.002.
Mereish EH, Miranda R. Vicarious heterosexism-based stress induces alcohol, nicotine, and cannabis craving and negative affect among sexual minority young adults: an experimental study. Neurobiol Stress. 2024;32:100668. https://doi.org/10.1016/j.ynstr.2024.100668.
Messman JB, Leslie LA. Transgender college students: academic resilience and striving to cope in the face of marginalized health. J Am Coll Heal. 2019;67(2):161–73.
Meyer IH. Minority stress and mental health in gay men. J Health Soc Behav. 1995;36:38–56.
Michero E. The 15-year old. In: Jayne KM, Purcell KE, editors. A therapist's guide to adolescent development. Boca Raton: CRC Press; 2025.
Miller WR, Rollnick S. Motivational interviewing: helping people change. New York: Guilford Press; 2012.
Mitchell AM, Puskar K, Hagle H, Gotham HJ, Talcott KS, Terhorst L, Burns HK. Screening, brief intervention, and referral to treatment: overview of and student satisfaction with an undergraduate addiction training program for nurses. J Psychosoc Nurs Ment Health Serv. 2013;51(10):29–37.
Naar-King S. Motivational interviewing in adolescent treatment. Can J Psychiatry. 2011;56(11):651–7.
Newcomb ME, Hill R, Buehler K, Ryan DT, Whitton SW, Mustanski B. High burden of mental health problems, substance use, violence, and related psychosocial factors in transgender, nonbinary, and gender diverse youth and young adults. Arch Sex Behav. 2020;49:645–59.

Nicholson AA, Siegel M, Wolf J, Narikuzhy S, Roth SL, Hatchard T, Lueger-Schuster B. A systematic review of the neural correlates of sexual minority stress: towards an intersectional minority mosaic framework with implications for a future research agenda. Eur J Psychotraumatol. 2022;13(1):2002572.

Ogeil RP, Cheetham A, Mooney A, Allen NB, Schwartz O, Byrne ML, Lubman DI. Early adolescent drinking and cannabis use predicts later sleep-quality problems. Psychol Addict Behav. 2019;33(3):266.

Parent MC, Arriaga AS, Gobble T, Wille L. Stress and substance use among sexual and gender minority individuals across the lifespan. Neurobiol Stress. 2019;10:100146.

Peterson PL, Baer JS, Wells EA, Ginzler JA, Garrett SB. Short-term effects of a brief motivational intervention to reduce alcohol and drug risk among homeless adolescents. Psychol Addict Behav. 2006;20:254–64.

Pew Research Center. Key findings about LGBTQ+ Americans for Pride month. Washington: Pew Research Center; 2024.

Prochaska JO, DiClemente CC, Norcross JC. In search of the structure of change. In: Self change: social psychological and clinical perspectives. New York: Springer; 1992. p. 87–114.

Rajkovic A, Cirino AL, Berro T, Koeller DR, Zayhowski K. Transgender and gender-diverse (TGD) individuals' perspectives on research seeking genetic variants associated with TGD identities: a qualitative study. J Community Genet. 2022;13(1):31–48.

Riddle MC, Safer JD. Medical considerations in the care of transgender and gender diverse patients with eating disorders. J Eat Disord. 2022;10(1):178.

Sawyer SM, Azzopardi PS, Wickremarathne D, Patton GC. The age of adolescence. Lancet Child Adolesc Health. 2018;2(3):223–8.

Schifano F, Papanti GD, Corkery JM, Orsolini L. Post-traumatic stress and substance misuse: neurobiological and clinical correlates. Res Adv Psychiatry. 2018;5(2):52–60.

Siegel DJ. Brainstorm: the power and purpose of the teenage brain. New York: Penguin Publishing Group; 2015.

Silins E, Horwood LJ, Patton GC, Fergusson DM, Olsson CA, Hutchinson DM, Mattick RP. Young adult sequelae of adolescent cannabis use: an integrative analysis. Lancet Psychiatry. 2014;1(4):286–93.

Spalding A. The experiences of LGBQ clients accessing substance use treatment: understanding barriers and facilitators through minority stress theory (order No. 30243752). 2023. https://www.proquest.com/dissertations-theses/experiences-lgbq-clients-accessing-substance-use/docview/2758665468/se-2.

Stanger C, Budney AJ, Kamon JL, Thostensen J. A randomized trial of contingency management for adolescent marijuana abuse and dependence. Drug Alcohol Depend. 2009;105(3):240–7.

Turner T. LGBTQ+ affirming care and why it matters. Elmer: Inspira Health; 2024.

Walther L, Gantner A, Heinz A, Majic T. Evidence-based treatment options in cannabis dependency. Deut Ärzteblatt Int. 2016;113(39):653.

Weeks SN, Renshaw TL, Vinal SA. Minority stress as a multidimensional predictor of LGB+ adolescents' mental health outcomes. J Homosex. 2023;70(5):938–62.

Wells EA, Kristman-Valente AN, Peavy KM, Jackson TR. Social workers and delivery of evidence-based psychosocial treatments for substance use disorders. Soc Work Public Health. 2013;28(3-4):279–301.

Wesp LM, Malcoe LH, Elliott A, Poteat T. Intersectionality research for transgender health justice: a theory-driven conceptual framework for structural analysis of transgender health inequities. Transgender Health. 2019;4(1):287–96.

Zhang Q, Goodman M, Adams N, Corneil T, Hashemi L, Kreukels B, Motmans J, Snyder R, Coleman E. Epidemiological considerations in transgender health: a systematic review with focus on higher quality data. Int J Transgender Health. 2020;21(2):125–37. https://doi.org/10.1080/26895269.2020.1753136.

Part VI
Education, Policy, and Practice

Chapter 23
Cannabis and Reproductive Health: Having Patient Conversations



Philip J. Molloy

1 Introduction

This chapter will not comprehensively review all that is currently known about cannabis exposure and reproductive health. The purpose of this chapter is to summarize what is known about these clinically relevant and important issues for HCPs. Its specific goals are to increase competence and knowledge for HCPs; to increase level of comfort in having conversations with patients inquiring about this topic; and to motivate HCPs to proactively call attention to these potential risks in men and women considering conception/pregnancy. It will attempt to provide basic general knowledge for HCPs as a foundation. The interested reader is encouraged to review related previous chapters in this book—for example, Chaps. 6–10 focus on prenatal and early life effects.

Selected articles and studies have been included in this chapter's bibliography, starting with animal/preclinical data, followed by a few important clinical human studies and reviews. Some of these references are appropriate to share with patients.

The ECS is ubiquitously expressed in virtually all organs and body systems. It evolved in all animals from coelenterates forward over the past ~500 million years. The ECS is critically involved—like the autonomic nervous system, the HPA axis, and other body systems—in maintaining homeostasis and responding to stress (infection, ischemia, etc.).

Much background research on cannabis and medical physiology has involved describing changes in ECS expression and activity in certain disease states, as well as in pregnancy. It is tempting to assume that—for a given medical condition—a marked overexpression of some component of the ECS is contributing to the perturbation or disease process. It is further tempting to assume that downregulating it may have postulated therapeutic benefit. For example, suppose "X" (some

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component of the ECS) is markedly upregulated in a specific disease, even in proportion to the disease severity. Perhaps one should attempt to "therapeutically" block that "X" upregulation to see if it holds promise for better outcomes. However, what if this overexpression is compensatory and potentially beneficial, having evolved to mitigate the stress or insult? Be careful. There are parallels in other corners of medicine: tumor necrosis factor (TNF) is upregulated in congestive heart failure (CHF), so perhaps it is contributing to the CHF pathogenesis. Let's try blocking it and see if that benefits the patients. Nevertheless, in this example, blocking TNF has been shown to be more likely to aggravate the CHF, presumably because the up-expression was physiologically compensatory.

The ECS is highly expressed and active in all aspects of male and female reproductive health. It is involved with male and female sex hormone levels, spermatogenesis, ovulation, fertilization, implantation, and in virtually all of the processes involved in a successful pregnancy (Brents 2026; Du Plessis et al. 2015).

The maternal and paternal use of cannabis-related substances while attempting conception, and during pregnancy is substantial and increasing. Cannabis use during pregnancy has been reported to have expanded from approximately 10% in 2009 to 14% in 2019; and a 2015 study reports that 18.1% of pregnant women reporting marijuana use in the past year met criteria for marijuana abuse, or dependence, or both (Ko et al. 2015). Furthermore, it has been widely recognized and documented that current cannabis products contain much higher doses of delta-9-tetrahydrocannabinol (THC) than in the past. Thus, in the setting of conception and pregnancy, there is progressively more use of higher THC-containing cannabis products.

As we interact with our patients, it is important to examine our own knowledge, attitudes, and biases, to provide education, and to be nonjudgmental. Individual patients present with varying levels of knowledge and assumptions (like "If it is legal and easily available, it must be safe."), and with different attitudes. Interactions and outcomes are often better when we meet patients on their grounds and starting points, and not necessarily expecting them to meet us on ours. Our challenge is to improve our own knowledge by reviewing the material in the early chapters of this book, and the selected references provided in this chapter. Then find a path to communicate messages to one specific patient at a time. This chapter intends to review and highlight relevant key information about cannabis and pregnancy to serve as a foundation in having clinical conversations with patients; and to highlight challenges to having these conversations. A baseline education should include familiarity with both the Surgeon General's advisory report and the ACOG opinion statement on cannabis use during pregnancy (U.S. Surgeon General's Advisory 2019; ACOG Committee 2017).

HCPs have expanding demands on their practice scopes and responsibilities. If you see many patients of childbearing potential, decide how you plan to approach this with them. Waiting for the patient to bring the subject up is reactive; initiating these conversations is proactive and potentially expands the number of patients reached. When patients are proactive in breaching this topic, most of the questions will relate to possible benefits of cannabis ("*Doctor, do you think cannabis could*

help my pain?"—or anxiety, nausea, sleep, etc.). There is plenty "out there" in the internet, in the lay press, or in cannabis dispensaries about the promise of cannabis helping this or that condition. Patients rarely initiate a discussion with their physicians about safety or potential adverse effects; this is our role.

2 Preclinical and Animal

The foundation of educating our patients is educating ourselves as healthcare providers. The purpose of this section is to highlight a limited number of key publications that have provided important information on the role of the ECS and exogenous cannabinoid exposure in reproductive health. It is not exhaustive, but rather intended to give the healthcare provider (HCP) illustrative examples of the type of research that has contributed to our understanding of cannabis and reproductive health.

A very interesting study by Spano et al. (2007) studied rats exposed to THC and documented resulting in increased opioid seeking and drug self-administration behavior. This was likely mediated by altered preproenkephalin mRNA expression in specific brain regions. Although not specifically focusing on pregnant animals, this study suggests early cannabis exposure may be relevant in substance abuse.

In 2021, KS Ryan and colleagues exposed rhesus macaques to various doses of THC and measured numerous hormonal outcome metrics relevant to reproduction. THC dose-dependent hormonal changes associated with ovulatory and reproductive dysfunction were well-documented (Ryan et al. 2021). This animal data suggests that cannabis use may be associated with negative outcomes in conception and pregnancy. Another study determined that paternal THC exposure for 28 days (in a rat model of pregnancy) caused adverse effects in the neurodevelopment of offspring. This was likely mediated at least in part by dose-dependent deficits in central presynaptic cholinergic activity, with effects especially in the striatum and hippocampal regions (Slotkin et al. 2020). This is important because it provides evidence that paternal THC exposure contributes to neurodevelopmental abnormalities in central pathways involved in cognition and reward. Another animal study exposed pregnant mice to 0, 5, or 10 mg/kg/day of oral THC, and extensively evaluated fetal heart development. Many structural and functional cardiac abnormalities were documented in the THC-exposed groups, compared to the nonexposed group (Robinson et al. 2024).

The above animal studies focus on THC exposure. What about animal data on CBD only exposure? In a rat model, placebo control vs. a high and a low dose of CBD were given from 6 days gestation through term. Both doses of CBD were associated with significant alterations in expression of cardiac ECS receptors, as well as with reduced cardiac function. Detailed analysis suggested this was mediated via altered cardiac mitochondrial transcription pathways in the CBD groups (Lee et al. 2024).

Some of the reproductive effects of cannabis exposure are mediated by epigenetics (Schrott et al. 2020). It is very unlikely that your patients—or the general

public—are aware that cannabis exposure is associated with heritable epigenetic changes—i.e., changes that are passed along to the offspring. A recent study of edible cannabis in pregnant rhesus monkeys examined DNA methylation changes in various tissues (including placenta, heart, cerebellum, prefrontal cortex). These documented changes included candidate genes suspected to be involved in autism spectrum disorder (ASD) and neurobehavioral development (Shorey-Kendrick et al. 2023). There is, however, some potentially encouraging news. A recent well-done study in rhesus macaques suggests that if chronic THC exposure is followed by a period of cessation of use, the observed hormonal and epigenetic changes are reversible (Hedges et al. 2023). Despite challenges associated with studying mental and behavioral health outcomes using animal models, there is consistent evidence from animal models that cannabis exposure in the perinatal period appears to be associated with long-term effects on neural systems relevant to psychiatric and substance use disorders (Motamedi et al. 2023). These animal studies tell a consistent story of potential adverse consequences of cannabis exposure in the prenatal period (Tirado-Munoz et al. 2020).

3 Clinical

The overall prevalence of cannabis use during conception/pregnancy is significant and increasing. One United States study of 367,403 pregnancies found that the self-reported use of cannabis in the year prior to pregnancy increased from 6.8% in 2009 to 12.5% in 2017 (Young-Wolff et al. 2019). In this study, daily use was 1.17% in 2009 and 3.05% in 2017; use continuing during pregnancy increased significantly from 0.28% to 0.69%, respectively. This increase raises public health concerns, and these authors point out that "71% of the pregnant women who used cannabis in the past year perceive no or slight risk in using cannabis once or twice a week." The same researchers examined whether or not cannabis use may have maternal mental health associations or sequelae. They supplemented self-reported use with prenatal urine toxicology testing, and then correlated these findings with maternal mental health associations (Young-Wolff et al. 2020). They found that maternal cannabis use was strongly associated with anxiety and depression, importantly noting a dose–response relationship with cannabis use and anxiety or depression. It is acknowledged that since cannabis is often used to manage depression and anxiety (in addition to nausea, insomnia, etc.) it was not possible to definitely conclude in which direction this association existed. Is it more likely that pregnant women with anxiety and depression use cannabis for these symptoms? Or more likely that cannabis use in this setting actually contributes to the anxiety and depression? More research is needed on this important subject (Lo et al. 2021; Leng et al. 2023).

What about "traditional" pregnancy outcome metrics? There are many studies and reviews analyzing the association between pregnancy outcomes and cannabis use. A limited number of well-done current reviews are listed in the references for this chapter (Lo et al. 2022, 2023; Luke et al. 2022; Marchand et al. 2022; Ainiti

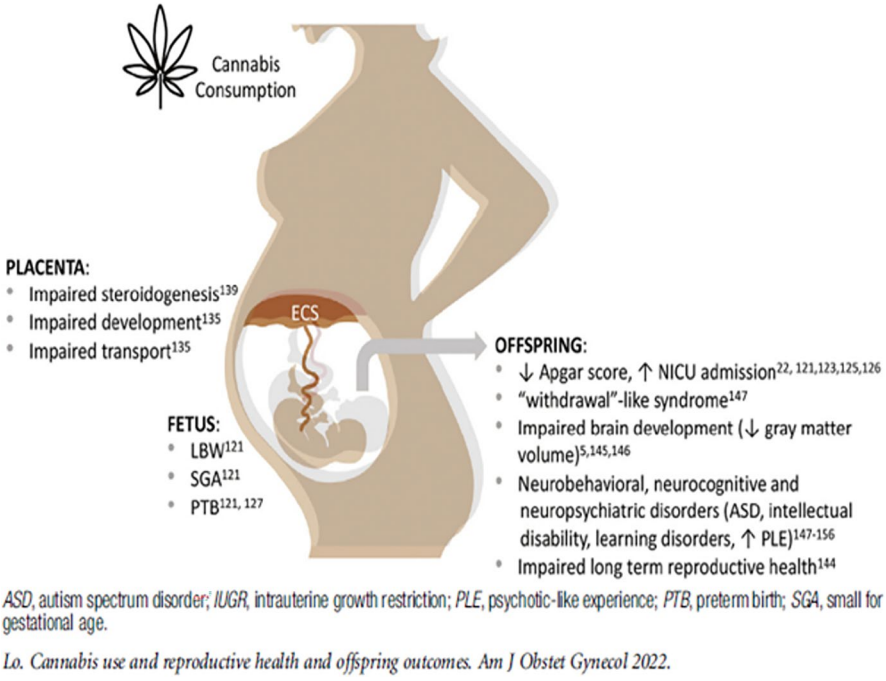


Fig. 23.1 Adverse effects of cannabis on placenta, fetus, and offspring. Used with permission Lo et al. (2023)

et al. 2023). HCPs are encouraged to become familiar with these associations and findings, which tell a clear and consistent story. For example, one Canadian epidemiologic study examined 1,280,447 singleton births, using self-reported cannabis use as the key variable. After controlling for many potentially confounding variables, the association of cannabis use with adverse pregnancy outcomes was confirmed (Marchand et al. 2022; Ainiti et al. 2023) (see Fig. 23.1 and Table 23.1). Cannabis use has been repeatedly shown to be associated with impaired sperm count and motility, lower birthweight, small for gestational age, more premature births, lower Apgar scores, and more transfers to the NICU. Specific offspring DNA methylation changes in the brain, specifically in the amygdala, have been observed, suggesting offspring emotional and behavioral impact (Lo et al. 2023).

Pregnant cannabis users are more likely to be from lower socioeconomic levels, to have less education, more likely to be underweight, and are more likely to concurrently use other substances such as alcohol (Shukla and Doshi 2024). There are even reports of prenatal cannabis use being associated with increased maternal and offspring mortality (Hayer et al. 2023), and with congenital structural birth defects (Tadesse et al. 2024a). These findings have been confirmed by others. For example, a well-done retrospective study of 2,380,446 singleton births reported that pregnant women meeting criteria for cannabis use disorder (CUD)—a more advanced severe

Table 23.1 Consensus documented adverse associations of cannabis use, compiled from numerous studies (Marchand et al. 2022; Ainiti et al. 2023)

More difficulties with fertility and conception
Low-birth weights/small for gestational age (SGA)
Increased premature births
Poorer Apgar scores
Increased transfers to neonatal intensive care units (NICU)
Possible long-term cognitive and behavioral issues in offspring

exposure than "simple" cannabis use—were clearly associated with increased mortality and morbidity in both the mothers and offspring (Prewitt et al. 2023).

Familiarity with this material can be a foundation for having informed conversations with patients. These can even be printed and shared with certain patients, as appropriate. Other patients may prefer online platforms; there are well-done, clear and concise videos covering these issues—for example, a recent 10-min YouTube video on cannabis use in males correlated use with adverse fertility and reproductive outcomes (Shahine 2019).

In addition to reviews and guidelines, several original studies are important and informative. One study methodology is to analyze archived urine samples (routinely collected during prenatal visits) for cannabis, and to correlate the findings with pregnancy and offspring outcomes. Metz et al analyzed urine specimens collected at various time points of 9257 pregnancies and utilized confirmatory quantitative assays employing liquid chromatography and mass spectrometry (LCMS). They demonstrated a dose-response effect of THC with adverse pregnancy outcomes, likely mediated by placental dysfunction (Metz et al. 2023). Other researchers use pregnant women's self-reported cannabis exposure as the input variable. In a large Canadian cohort of 661,617 women, 1.4% reported cannabis use while pregnant. They confirmed results from previous reports that cannabis use was associated with adverse pregnancy outcomes including small for gestational age, preterm birth, placental abruption, and other adverse outcomes (Corsi et al. 2019). This certainly is biologically plausible since it is known that many cannabis derived products readily do cross the placenta and have previously been demonstrated to impact maternal and placental blood flow. Another longitudinal prospective cohort study showed that daily cannabis use between age 15–17 (either maternal or paternal) was associated with a sixfold increase incidence in commonly measured adverse pregnancy outcome metrics (Corsi et al. 2020).

Challenges in doing this research included isolating the impact of cannabis distinct from other drugs and substances during pregnancy, and also comparing those women who stop when they become aware of the pregnancy to those who continue using during the pregnancy. At least one study reported that when these additional analyses were performed, the association between maternal cannabis and small for gestational age (SGA) weakened or disappeared for this and at least some other of the endpoints (Brik et al. 2024). Nonetheless, the weight of currently published evidence clearly supports the conclusion that cannabis use during pregnancy is

associated with SGA, lower birthweights, more preterm deliveries, and more NICU transfers. HCPs are advised to keep these conclusions in mind when counselling those who are pregnant of considering pregnancy.

Until recently, a critical unanswered question was, despite these consistently demonstrated negative pregnancy outcomes, is there any evidence of long-term adverse effects on offspring—cognitive, affective, neurobehavioral, or otherwise. Recent studies are shedding light on this important question. In 2024, a carefully done review and meta-analysis reported an association between maternal cannabis use and the incidence of subsequent autism spectrum disorder (ASD), intellectual disabilities, and learning disorders in offspring. Results and associations persisted after correcting for maternal mental health issues, alcohol and tobacco use (Tadesse et al. 2024b). A 2021 meta-analysis confirmed these associations of prenatal maternal cannabis exposure and ADHD/ASD in offspring (Hines et al. 2021).

The important NIH-funded ABCD study® (Adolescent Brain Cognitive Development) followed 10,719 children/parents and analyzed cannabis use before and after knowledge of pregnancy. Follow-up was when the children were ages 11–12. One analysis divided subjects into 3 groups: (1) mothers did not use cannabis at all during pregnancy; (2) mothers were using cannabis but stopped when they learned they were pregnant; (3) mothers who continued using cannabis after learning they were pregnant. Extensive endpoints were analyzed, including adverse offspring outcomes of ADHD, conduct disorders, aggressive, and rule-breaking behavior, etc.—all were statistically associated with cannabis exposure. However, the impact, however, was not noted until exposure occurred at least in the middle of the first trimester (at which time group 2 was discontinuing cannabis). This is potentially good news for the group that stopped cannabis after learning of the pregnancy (Baranger et al. 2022). A subgroup of these ABCD offspring underwent extensive neuroimaging studies, linking the above clinical outcomes with abnormal functional connectivity in numerous neurocognitive networks, especially the salience network (SN)—a core central network that processes emotional and sensory input (Faraj et al. 2022), adding to our knowledge of exactly just how cannabis use in pregnancy might "cause" some of these worrisome outcomes on a mechanistic level.

Another subgroup analysis of the ABCD cohort was further studied by fMRI. Results were controlled for parental tobacco and alcohol use, paternal psychopathology, and socioeconomic status parameters. These researchers confirmed the association between prenatal cannabis exposure and adverse behavioral outcomes in 9–10-year-old offspring but were unable to document evidence of cognitive adverse outcomes. However, despite this clinical association, patterns of fMRI activities were not significantly different in the exposed vs. the nonexposed offspring in this study (Cioffredi et al. 2022).

As important as the conclusions and messages from the ABCD study are, other researchers have not always confirmed them. The Raine study evaluated 2868 children born between 1989 and 1992 and correlated reported maternal cannabis exposure with numerous outcomes in offspring at age 10. Outcomes included language skills, behavioral metrics, neuromuscular development, autism spectrum scores, etc. This cohort did not demonstrate significant differences between the groups

(maternal cannabis-exposed vs. not exposed) (Isik et al. 2023). A 2024 meta-analysis confirmed some of the perinatal adverse outcomes noted above, focusing on long-term cognitive or behavioral adverse events; they only found significant associations for attention and externalizing problems (Sorkhou et al. 2024).

Three other 2024 reports reported on this important issue. Researchers in Spain prospectively followed offspring from thirty-two pregnant cannabis users and thirty-two nonexposed pregnant controls for 3 years. Language skill and neurodevelopmental delays (especially in male infants) were documented, and two exposed infants demonstrated severe developmental delay, adding to the literature on this concerning topic (Jarque et al. 2024). Researchers from Australia addressed a similar question by studying a database of 222,534 mother-offspring pairs. The exposure variable was CUD (indicative of a more advanced exposure than simply any cannabis use in pregnancy) and the outcome studied was ASD, which was increased threefold in offspring of mothers with CUD, compared to matched controls (Tadesse et al. 2024c). Finally a 2024 meta-analysis included 18 observational studies of 534,445 participants. These authors' analysis did not find a statistically significant association between pregnant women's cannabis use and ASD but did find a "slightly elevated" risk of offspring ADHD and a predisposition of offspring to becoming cannabis users (Bassalov et al. 2024).

Nonetheless, taking into consideration all of the published data on this important subject, the weight of evidence appears to support the possibility if not the probability of an association of cannabis exposure and long-term cognitive or behavioral adverse events. Further research is clearly needed in this area. For now, it deserves a discussion with those using cannabis and planning pregnancy. While being honest and admitting imperfect knowledge, it is reasonable to communicate to our patients that there seems to be an evolving story here about possible or even probable associations of perinatal cannabis use and adverse long-term cognitive or neurobehavioral outcomes in offspring. This is a critical point since, as we will soon learn below, pregnant women are most likely to hear, believe and respond to messages focusing on what is safe for their babies.

What about focusing on specific populations of pregnant women? One recent study of 172 Black mother–child pairs from one center in Atlanta looked at predictors for the evolution of the full spectrum of Autism Spectrum Disorder (ASD)-related behaviors in offspring. The researchers found that in this cohort, life and socioeconomic stress was more predictive of the outcome than was cannabis use (Nutor et al. 2024).

What about postnatal cannabis exposure and long-term impact on offspring health? Could women using cannabis in the perinatal period be more likely to continue using after birth, with associated early life second-hand exposure in the offspring? The Healthy Heart Study recently reported on this question, examining 81 mother–child pairs, studying both maternal exposure mid-gestation as well as child urine collected at age 5. Exposed vs. nonexposed children were compared. Comprehensive endpoints included multiple metrics of cognition and behavior. Ten children (12%) had postnatal exposure, presumably from second-hand smoke. Secondhand smoke exposure may originate from maternal, paternal, or other

sources, of course. This exposure was associated with more ADHD, aggressive behavior, oppositional/defiant behavior, and less cognitive flexibility and weaker receptive language skills (Moore et al. 2023). This important novel evidence that postnatal offspring cannabis exposure—in addition to what is being learned about perinatal maternal exposure—is indeed likely to be associated with adverse outcomes offspring; this may be appropriate to include in conversations with pregnant women/couples.

The above material is proposed to be a core foundation for having conversations with patients about this important topic. Typically, a patient will not just come out and say "*I am (or we are) considering getting pregnant. Is cannabis safe for me to continue while planning pregnancy?*" If he/she/they bring it up at all, it is often likely to be in the general context of a health or medical symptom (e.g., pain, anxiety, sleep, nausea, etc.), inquiring if a cannabis product might potentially offer relief of a symptom or complaint. It is most likely that a patient of reproductive potential will not directly inquire at all, and the HCP must be the one to proactively breach the topic. After learning that a patient (or couple) might be considering pregnancy, cannabis use is but one of many health issues that should be addressed.

There is an interesting variation on these conversations. Suppose you have a patient (either the mother or father) already with a "problem child" in an early school grade, with conduct and behavior issues. This child may or may not have already been diagnosed with an autism spectrum disorder (ASD) or ADHD issue. From the point of view of the HCP, how actively will you query regarding possible substance use during the past associated pregnancy? On the one hand, one cannot change the past, and there is a risk of imparting serious guilt issues to the parents if there is a possibility that parental perinatal cannabis use may have contributed. On the other hand, it may be an important conversation to have should the couple be planning subsequent pregnancies. This is an opportunity for non-accusatory non-judgmental education. In the best of worlds, educating such patients will continue to pay dividends by resulting in the patient being a more knowledgeable advocate in spreading caution re: cannabis use in pregnancy in his/her own family and circle of friends.

4 Clinical Implications and Conclusions

We have seen that exposure to cannabis at the time of conception and at various stages of pregnancy is associated with adverse pregnancy outcomes. There is good evidence that the ECS is ubiquitously expressed in all major organs and tissues, including male and female reproductive tissues. The ECS plays key roles in virtually all facets and phases of reproduction, including sperm mobility, fertilization, embryo implantation, successful pregnancy, and health offspring. Cannabis use in pregnancy is increasing, as are TCH concentrations. Inheritable epigenetic changes in offspring have been documented. Recent data following exposed offspring for

over a decade has shown an increased incidence of behavioral and psychological problems compared to matched nonexposed children.

Professional societies and governmental agencies have issued warnings and guidance to avoid cannabis during conception and pregnancy. It is not clear how many pregnant women or women of childbearing age are aware of this. It is not even clear how informed HCPs are on these matters. One recent study of 75 HCP's found that an amazing 34.7% or providers were not aware of the impacts of cannabis use on the health of the pregnant mother and infant, and 66.7% reported they would like further training (Kitsantas and Pursell 2023). It is reasonable for all HCPs to take a more active role in educating ourselves and communicating these issues to our patients.

Different patients need different conversations. Some have more medical knowledge, insights, curiosity, and interest and want to know more details. Hopefully this chapter has provided you with the framework to become more educated yourselves, and to have more confidence in having these patient conversations. As mentioned in the introduction to this chapter, most patients with questions do not volunteer to share these concerns with their HCPs, and when they do, there are more questions about the possible benefits than about safety and side effects. How reactive or proactive should we want to be?

Some HCPs ask a patient's permission or preferences about documenting cannabis use in the medical record; it is not unreasonable to respect the patient's preferences on this. How will you react when a patient says, "I'll only talk about this if you don't write it in my medical record?" Whose job is it anyways?—The PCP, FP, pediatrician—or should we refer and defer all of these conversations to the obstetrician? There is an expanding, seemingly endless, list of screenings, preventive and general health issues that PCPs are expected to address and document with their patients. Addressing cannabis in the context of pregnancy is urgent to add to the list.

Shown in the appendix are examples of posters for waiting rooms or exam rooms to stir interest in the topic of cannabis and reproductive health. Posting them (or including such messages on your waiting room closed circuit TV) sends the message you are yourself informed and open to these conversations. Most exam rooms and waiting rooms of adult-focused PCPs do not appear to implement this. Pediatric and OBGYN offices seem to do a better job.

Hopefully the messages in this book—that there is accumulating convincing evidence that cannabis may have negative consequences on pregnancy outcomes, including possible long-term consequences in the offspring—will motivate you to keep learning more and acting on it in your clinical settings. This is despite the public's perception and "hype" surrounding possible benefits, and relative silence surrounding possible adverse consequences. One recent study showed that in recent years, the percentage of people who believe cannabis is safe has been increasing. Between 2002 and 2017, the percentage of respondents believing cannabis was safe increased from 36.7% to 44.3%, while the percentage who believed it was of "great risk" similarly decreased (Cohen et al. 2023). This is important since it has been shown that there is an inverse relationship between perceived risk and use of cannabis (Hasin 2018). Unlike public perception, informed medical scientists seem to

Table 23.2 Generally accepted contraindications to the use of cannabis, compiled from numerous studies

Pregnancy; males and females contemplating pregnancy
Lactating females
Juveniles (various age cutoffs suggested)
Unstable mental health issues
Possibly any family history of schizophrenia

agree that pregnancy and lactation (as well as a few other medical situations) are clear contraindications to cannabis use (Table 23.2).

The perception that cannabis is relatively safe—including during conception and pregnancy—has been well documented and is associated with increased use during pregnancy—despite many national guidelines stating that cannabis should not be consumed during pregnancy. Furthermore, legalization has been associated with decreased risk perception. At the time of the writing of this chapter, there was a lot of attention being given to rescheduling cannabis from schedule 1 to schedule 3 in the United States. One might postulate that if this actually occurs, it will be accompanied by substantial news media attention and possibly yet a further movement in the minds of the public regarding the perceived relative safety of cannabis, including in the setting of pregnancy.

A recent report from a single OB practice in Baltimore, MD reported on 117 pregnant women who either self-reported cannabis use or tested positive for it. After learning of the pregnancy, only 38.1% chose to completely abstain while 61.9% chose continued cannabis use while pregnant (Cohen et al. 2023). Subjects varied in their willingness to discuss this use with their obstetrician. Some find data like this astounding, given the information presented in this chapter. There is some other evidence however that after legalization, cannabis use in pregnancy does not automatically increase (Raifman et al. 2024).

Where do pregnant women go to seek information about cannabis and pregnancy? There are reports that perinatal women tend to get substantial information from budtenders employed at cannabis dispensaries (as opposed to their doctors!—more on that below). Very little is known about details and specific recommendations. Researchers have conducted structured, detailed interviews with budtenders and also with HCPs who self-reported advising perinatal women on cannabis use, from Washington state (Barbosa-Leiker et al. 2022). Many budtenders recommended cannabis products for use in pregnancy. Most women were inquiring about cannabis use to address some medical symptom, and only 30% of budtenders recommended that these women discuss this issue with their physicians. Informed clinicians need to know where their perinatal patients are getting cannabis information, which is also frequently online or via trusted people in their social groups. Sadly, many patients do not consistently view their doctors as being knowledgeable or supportive in discussing cannabis use, especially during pregnancy (Taneja et al. 2023).

It has been postulated that some HCPs avoid having discussions re: pregnancy and cannabis use in order to preserve a more favorable therapeutic relationship with

patients (Ceasar et al. 2023). Some pregnant women are concerned that discussing cannabis use in pregnancy will end up being (unwantedly) documented in their medical record or be associated with a stigma (Daniels et al. 2023). HCPs often struggle with these conversations, and pregnant women are unclear regarding how much to trust advice they receive from their HCPs.

Pregnant patients are often distressed by medical symptoms leading them to try or ask about cannabis. It their symptoms are distressing enough and impacting quality of life, they may be tempted to view trying cannabis as an alternative that is perceived to be safer than other available medication options, even safer than tobacco (Foti et al. 2023; Chambers et al. 2023).

One recent study reported that approximately one-third of pregnant women were not queried about cannabis use, and almost two-thirds were not advised against it use during pregnancy! (Skelton 2023)

When looking for simple "soundbites" to give patients, consider starting with the 2019 Surgeon General's Report, or the 2017 (reaffirmed in 2021) ACOG Report on Cannabis and Reproductive Health (U.S. Surgeon General's Advisory 2019; ACOG Committee 2017). The message that alcohol & pregnancy is a risky combination is well-known. "I just found out I am pregnant, so I am not drinking" is a common announcement of pregnancy. Why is there not a similar widespread appreciation of the risks of cannabis use and pregnancy? The alcohol/pregnancy message has been around for decades longer and has a social media presence that the cannabis/pregnancy message does not yet seem to have.

Should cannabis dispensaries be required to participate in messaging the potential negative consequences of cannabis use on reproductive health? One report suggested that certain messaging to pregnant women about the potential adverse effects of THC on pregnancy outcomes resulted in expressed intentions to reduce cannabis use during pregnancy (Willoughby et al. 2023). However, a contrasting report showed that dispensary warning signs about adverse pregnancy outcomes do not decrease cannabis use during pregnancy (Roberts et al. 2023).

A 2024 comprehensive review of cannabis health warnings evaluated mandatory cannabis product warnings' impacts on knowledge and hypothetical effects as well as on "real world" use effects. Labelling and warnings are inconsistent across jurisdictions. One Canadian online health-warning site included rotating messages (e.g., "Do not use cannabis if pregnant or breastfeeding.") These researchers found that some messages were noticed or remembered more than others, some were easier or more difficult to understand, and that messages that included pictorial messages were more effective that text only messages (Massey et al. 2024). Overall, messaging and warnings did have various degrees of positive impact on knowledge and behavior. Messages focusing on the health outcomes of their babies tended to have more impact. This finding should motivate HCPs to press on with this and should encourage more research along these lines.

What about anonymous online resources, chat groups, or pregnancy forums? It is likely that many pregnant couples turn to such avenues either instead of, or in addition to discussions with their HCPs. A fascinating and very informative analysis of how patients use these resources has been recently published, introducing a website:

https://www.whattoexpect.com/pregnancy/marijuana/#safe. It is well done and overall offers sound advice (Micalizzi et al. 2024). For example, in the section on options for managing morning sickness, the advice given is "one remedy you should definitely NOT use for morning sickness is marijuana or any THC-containing products." In the Q&A section, in response to a question "Is smoking weed safe during pregnancy," the answer is emphatically "NO!"

Many current search engines now employ AI. If you query cannabis, safety, pregnancy, you will find summaries consistent with the data and conclusions presented in this chapter. This is reassuring, although more data is needed on how many pregnant people actually use such information and resources, or how it impacts their decisions to use or avoid cannabis. It is also reassuring that this issue of potential consequence of cannabis use in the context of conception and pregnancy is now receiving the attention of so many researchers and professional societies.

It will likely take a multifaceted approach to make a difference, and no single HCP can influence society on a large scale. What each of us can do is to stay motivated to educate ourselves. Maintain open eyes and ears to patients who could benefit from addressing this complex issue. If one sees a population of more men/women/couples of reproductive potential, a poster like the Fig. 23.1, or one of the examples in the Appendix, could be displayed in the waiting room or exam rooms; these posters just might start a patient conversation. It is unlikely that a one-time intake questionnaire with a question about cannabis use, or posters on your exam room walls, will be sufficient to adequately open the door to patient conversations (Skelton 2023). HCPs will need to be proactive, supportive, and sympathetic in order to have successful interactions.

This is an area of intense active clinical research—both the science of cannabis and pregnancy outcomes, and methodological issues of what messaging works best for communicating this topic to patients. As this chapter and textbook were being written, there continued to be numerous relevant publications appearing on this important topic. Additional basic research (animal data) has added to our understanding, and further documented the diffusion of CBD into the developing and adult brains, the CSF, and crossing the placenta (Fitzpatrick et al. 2024). Further human outcomes studies continue to be published confirming the association of cannabis use during pregnancy and adverse pregnancy outcomes (Lo et al. 2024).

A 2024 phenomenological study from Spain utilizing focus groups and in-depth interviews identified many barriers to messaging the potential risks of cannabis in pregnancy (Vila-Farinas et al. 2024). Many messaging strategy interventions were ineffective. The most impactful message was capitalizing on safeguarding the health of the offspring. Observed barriers included low perception of risk, pregnant patient concealment of cannabis use, and lack of confidence in the training and knowledge base of HCPs. Each of us can use these barriers as motivation. An additional barrier well documented in a recent 2024 UCLA report was the fear of reporting cannabis use (self-reported or discovered by urine drug screens) to Child Protective Services (CPS). There is currently significant ambiguity in professional society guidelines surrounding drug screening and what should be reported to CPS in this setting (Gould et al. 2024a).

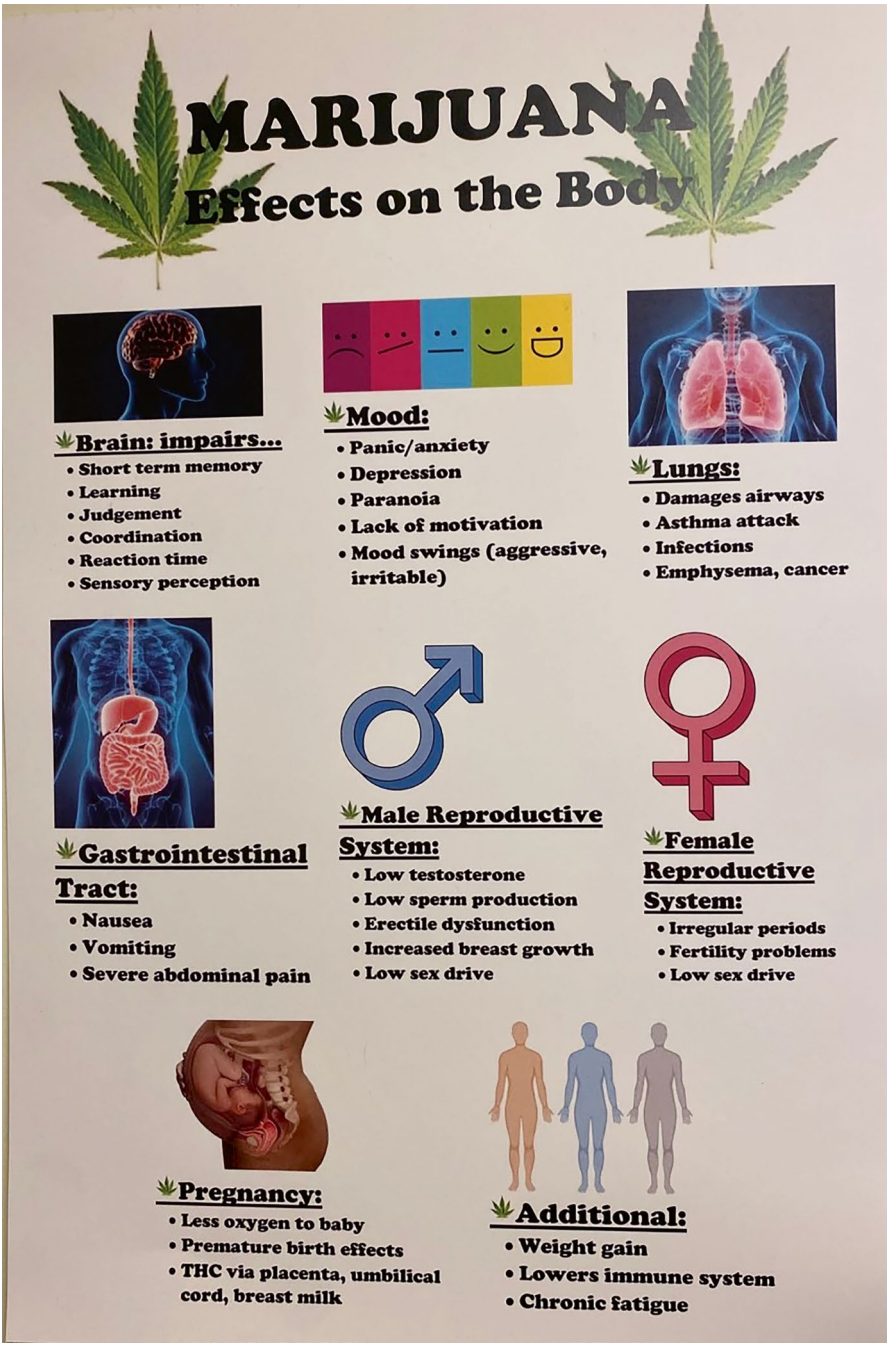
There is current research looking at pregnant cannabis users' intentions to quit showing the users' plans to quit are quite varied (Young-Wolff et al. 2024). A 2024 report assessed the efficacy of dispensary warning signs for credibility and motivation to change cannabis behavior in pregnant women. The researchers found mostly negative views on the mandatory warning signs in dispensaries. Subjects were not convinced the scientific evidence for the conclusions was sound, found the messages vague and not credible, and not motivating to change behavior regarding cannabis use in pregnancy (Gould et al. 2024b). There is clearly room for improvement and we as a profession and society overall can do a better job.

Further research might attempt to identify and predict those pregnant women who are more open-minded and amenable to stop using in order to focus on them.

Become familiar with this subject and stay up to date as medical knowledge evolves, acknowledging that medical knowledge on these issues is incomplete at this time. Remain vigilant for opportunities to bring this up to your patients as appropriate. Be honest about what we know and don't know at the present time. Be nonjudgmental and open to the patients' beliefs/fears/concerns. Maintain a therapeutic alliance with your patients who are pregnant or contemplating pregnancy. Provide balanced information and be someone your patients can trust (Vanstone et al. 2022). Remain focused on the goal of maximizing health and minimizing harm to the parents and offspring.

After synthesizing the literature, decide what you think are the most important and well-documented findings, and educate your patients one at a time. Remember that most patients are under the assumption that "*cannabis is legal so it must be safe!*" It is incumbent upon us as informed HCPs to provide perspective, education, and balance in this critical discussion.

Appendix


MARIJUANA
Effects on the Body
Brain: impairs...
• Short term memory
• Learning
• Judgement
• Coordination
• Reaction time
• Sensory perception
Mood:
• Panic/anxiety
• Depression
• Paranoia
• Lack of motivation
• Mood swings (aggressive, irritable)
Lungs:
• Damages airways
• Asthma attack
• Infections
• Emphysema, cancer
Gastrointestinal Tract:
• Nausea
• Vomiting
• Severe abdominal pain
Male Reproductive System:
• Low testosterone
• Low sperm production
• Erectile dysfunction
• Increased breast growth
• Low sex drive
Female Reproductive System:
• Irregular periods
• Fertility problems
• Low sex drive
Pregnancy:
• Less oxygen to baby
• Premature birth effects
• THC via placenta, umbilical cord, breast milk
Additional:
• Weight gain
• Lowers immune system
• Chronic fatigue



Talking to your child about cannabis

Learn how cannabis can impact a young person's development. Find out what you can do to prevent underage use.

FREE PARENT EDUCATION WORKSHOP

Join local experts to learn how marijuana use can affect your child's health, safety and educational outcomes.

May 3, 2018
6:30 to 8:30 p.m.
(6–6:30 p.m. dinner in cafeteria)
San Mateo High School
Multipurpose room
506 N. Delaware St., San Mateo

Spanish interpretation provided. For other languages, please request 5 days prior to event.
Contact Edith Cabuslay at (650) 573-2227 or ecabuslay@smcgov.org.

www.cannabiseducationsmc.com

COUNTY OF SAN MATEO


PARENT UP
HOW DOES MARIJUANA AFFECT TEENS?
GET THE FACTS
Teens whose parents tell them about the risks of marijuana are less likely to use it.
VERMONT DEPARTMENT OF HEALTH
MARIJUANA AFFECTS BRAIN DEVELOPMENT.
Brains do not fully develop until your mid-20s. Regular marijuana use during early years of life can negatively affect attention and memory in adulthood.
According to a study published in the scientific review journal PLOS One
MARIJUANA RISKS TEENS' HEALTH.
Teens who use marijuana weekly or more have 2x the risk of depression or anxiety.
According to study results featured in the Journal of the American Academy of Child and Adolescent Psychiatry and by the National Academy of Sciences
MARIJUANA IS HABIT FORMING.
Approximately 1 in 6 teens who start using marijuana before age 14 develop an addiction.
According to a study published in the journal Neuropsychopharmacology
MARIJUANA RISKS TEENS' FUTURES.
Teens who use marijuana have lower academic performance and worse job prospects—and those who continue using marijuana regularly show a decrease in IQ 20 years later.
According to a study by the National Academy of Sciences
Get simple tips, advice and tools for talking to your kids.
ParentUpVT.org

Cannabis

Before, during and after pregnancy

PublicHealth
WELLINGTON-DUFFERIN-GUELPH
Stay Well.

Fast Facts

If you use cannabis and are pregnant or are planning a pregnancy, talk to your healthcare provider.

Using cannabis (marijuana, weed, pot) during pregnancy may increase your baby's risk of developmental problems.

The chemicals in any form of cannabis (smoking, second-hand smoke, vapour, edibles, oils/creams) may be harmful for your baby.

It is not safe to use cannabis in pregnancy or when breastfeeding



Fertility

Studies have shown that daily cannabis use is linked to:

- Changes to the menstrual cycle
- Low sperm count and poor sperm quality





Pregnancy

Cannabis contains over 400 chemicals, including THC - the chemical that makes you feel "high".

- These chemicals can pass from your body to your baby when you are pregnant and may be harmful.

Cannabis smoke contains many of the same harmful chemicals as cigarette smoke.

- Smoking lowers the supply of oxygen and nutrients to your baby.

Ask your healthcare provider about safer ways to treat nausea and vomiting during pregnancy.



Baby

A baby's brain contains a lot of fat. THC is stored in fat and it could largely affect how your baby's brain develops.

Research shows that cannabis in pregnancy may increase the risk of:

- Pre-term birth
- Low birth weight
- Lower IQ scores
- Impulsivity and hyperactivity in childhood
- Learning, memory and problem-solving difficulties in teens and later in life


Marijuana
AND
Pregnancy
If you use marijuana during pregnancy, you may be putting your health and your fetus's health at risk.
Possible Effects on Your Fetus
Disruption of brain development before birth
Smaller size at birth
Higher risk of stillbirth
Higher chance of being born too early, especially when a woman uses both marijuana and cigarettes during pregnancy
Harm from secondhand marijuana smoke
Behavioral problems in childhood and trouble paying attention in school
Possible Effects on You
Permanent lung injury from smoking marijuana
Dizziness, putting you at risk of falls
Impaired judgment, putting you at risk of injury
Lower levels of oxygen in the body, which can lead to breathing problems
DID YOU KNOW?
Medical marijuana is not safer than recreational marijuana. Recreational and medical marijuana may be legal in some states, but both are illegal under federal law.
There's no evidence that marijuana helps morning sickness (ask your obstetrician–gynecologist [ob-gyn] about safer treatments).
You also should avoid marijuana before pregnancy and while breastfeeding.
Marijuana and pregnancy don't mix. If you're pregnant or thinking about getting pregnant, don't use marijuana.
If you need help quitting marijuana, talk with your ob-gyn or other health care professional.
Research is limited on the harms of marijuana use for a pregnant woman and her fetus. Because all of the possible harms are not fully known, the American College of Obstetricians and Gynecologists (ACOG) recommends that women who are pregnant, planning to get pregnant, or breastfeeding not use marijuana. ACOG believes women who have a marijuana use problem should receive medical care and counseling services to help them quit.
The American College of Obstetricians and Gynecologists
WOMEN'S HEALTH CARE PHYSICIANS
409 12th Street SW, PO Box 96920
Washington, DC 20090-6920
www.acog.org
PFS1645: This information was designed as an educational aid to patients and sets forth current information and opinions related to women's health. It is not intended as a statement of the standard of care, nor does it comprise all proper treatments or methods of care. It is not a substitute for a treating clinician's independent professional judgment. Please check for updates at www.acog.org to ensure accuracy.
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Help keep your
baby SAFE
by avoiding cannabis
while pregnant or
breastfeeding.
Natural does not
always mean safe.
Call HealthLine at 811 or
talk to your healthcare
provider if you
have questions.
saskatchewan
preventioninstitute
our goal is healthy children
CRiS
The Cannabinoid Research
Initiative of Saskatchewan

References

ACOG Committee. ACOG Committee opinion on marijuana use during pregnancy and lactation. Obstet Gynecol. 2017;130(4):e205–9.

Ainiti DF, et al. Cannabis use during pregnancy and its effect on the fetus, newborn and later childhood; a systemic review. Eur J Midwifery. 2023;7:1–9.

Baranger DAA, et al. The association of mental health burden with prenatal cannabis exposure from childhood to early adolescence: longitudinal findings from the adolescent brain cognitive development study (ABCD). JAMA Pediatr. 2022;176:1261–4.

Barbosa-Leiker B, et al. Healthcare professionals' and budtenders' perception of perinatal cannabis use. Am J Drug Alcohol Abuse. 2022;48(2):186–94.

Bassalov H, et al. Prenatal cannabis exposure and the risk for neuropsychiatric anomalies in the offspring: a systemic review and meta-analysis. Am J Gynecol. 2024;231(6):574–88. https://doi.org/10.1016/j.ajog.2024.06.014.

Brents LK. Marijuana, the endocannabinoid system and the female reproductive system. Yale J Biol Med. 2026;89:175–91.

Brik M, et al. Cannabis exposure during pregnancy and perinatal outcomes: a cohort study. Acta Obstet Gynecol Scand. 2024;103(6):1083–91. https://doi.org/10.1111/aogs.14818.

Ceasar RC, et al. Legislation has changed but issues remain: provider perceptions of caring for people who use cannabis during pregnancy in a safety net health setting. Women's Health Rep. 2023;41:400–8.

Chambers J, et al. Perceptions of safety of daily cannabis vs. tobacco smoking and secondhand smoke exposure, 2017-2021. JAMA Netw Open. 2023;6(8):e2328691.

Cioffredi LA, et al. Prenatal cannabis exposure predicts attention problems without changes on fMRI in adolescents. Neurotoxicol Teratol. 2022;91:107089. https://doi.org/10.1016/j.ntt.2022.107089.

Cohen B, et al. Perceptions of safety if daily cannabis vs. tobacco smoking and second-hand smoke exposure, 2017-2021. JAMA Netw Open. 2023;6(8):e2328691. https://doi.org/10.1001/jamanetworkopen.2023.28691.

Corsi DJ, et al. Association between self-reported prenatal cannabis use and maternal, perinatal, and neonatal outcomes. JAMA. 2019;322(2):145–52.

Corsi DJ, et al. Maternal cannabis use in pregnancy and child neurodevelopmental outcomes. Nat Med. 2020;26(10):1536–40.

Daniels S, et al. Physician communication and perceived stigma in prenatal cannabis use. J Psychoactive Drugs. 2023;55:290–8.

Du Plessis SS, Agarwal A, Syriac A. Marijuana, phytocannabinoids, the endocannabinoid system, and male fertility. J Assist Reprod Genet. 2015;32:1575–88.

Faraj MM, et al. Impact of prenatal cannabis exposure on functional connectivity of the salience network in children. J Neurosci Res. 2022;101(1):162–71.

Fitzpatrick G, et al. Entry of cannabidiol into the fetal, postnatal, and adult rat brain. Cell Tissue Res. 2024;396(2):177–95.

Foti TR, et al. Patient perceptions of prenatal cannabis use and implications for clinicians. Obstet Gynecol. 2023;142(5):1153–61.

Gould EE, et al. The unseen patient: competing priorities between patients and providers when cannabis is used in pregnancy: a qualitative study. Front Global Women's Health. 2024a;5:1355375. https://doi.org/10.3389/fgwh.2024.135575.

Gould H, et al. Mandatory warning signs for cannabis: perspectives and preferences of pregnant and recently pregnant people who use cannabis. J Stud Alcohol Drugs. 2024b;86(1):75–84. https://doi.org/10.15288/jsad.23.00214.

Hasin DS. US epidemiology of cannabis use and associated problems. Neuropharmacol Rev. 2018;43:195–212.

Hayer S, et al. Cannabis and pregnancy: a review. Obstet Gynecol Surv. 2023;78:411–28.

Hedges JC, et al. Cessation of chronic delta-9-tetrahydrocannabinol use partially reverses impacts on male fertility and the sperm epigenome in rhesus macaques. Fertil Steril. 2023;120(1):163–74.

Hines LA, et al. Cannabis and tobacco use prior to pregnancy and subsequent offspring birth outcomes: a 20-year intergenerational prospective cohort study. Sci Rep. 2021;11:16826. https://doi.org/10.1038/s41598-021-95460-2.

Isik OG, et al. Neurodevelopmental outcomes in children after prenatal marijuana exposure. Paediatr Perinat Epidemiol. 2023;37:536–46.

Jarque P, et al. Evaluation of three-year neurodevelopmental outcomes in infants prenatally exposed to substance use. Drug Alcohol Depend. 2024;259:111284. https://doi.org/10.1016/jdrugalcdep.2024.111284.

Kitsantas K, Pursell SR. Are healthcare providers caring for pregnant and postpartum women ready to confront the perinatal cannabis challenge? Am J Perinatol. 2023;41:e3249–54. https://doi.org/10.1055/s-0043-1777669.

Ko JY, Farr SL, Tong VT, Creanga AA, Callaghan WM. Prevalence and patterns of marijuana use among pregnant and nonpregnant women of reproductive age. Am J Obstet Gynecol. 2015;213:201–10.

Lee K, et al. Cannabidiol exposure during gestation leads to adverse cardiac outcomes early in postnatal life in male rat offspring. Cannabis Cannabinoid Res. 2024;9(3):781–96. https://doi.org/10.1089/can2023.0213.

Leng OL, et al. The association between preconception cannabis use and depression and anxiety during pregnancy. Gen Hosp Psychiatry. 2023;83:148–55.

Lo JO, Hanna CB, Hedges JC. Effects of marijuana use on female reproductive health and pregnancy. Contemp OBGYY. 2021;66:24–30.

Lo JO, Hedges JC, Girardi G. Impact of cannabinoids on pregnancy, reproductive health, and offspring outcomes. Am J Obstet Gynecol. 2022;227:571–81.

Lo JO, Hedges JC, Metz TD. Cannabis use and perinatal health research. JAMA. 2023;330(10):913–4. https://doi.org/10.1001/jama.2023.14697.

Lo JO, et al. Cannabis use in pregnancy and neonatal outcomes: a systemic review and meta-analysis. Cannabis Cannabinoid Res. 2024;9(2):470–85.

Luke S, et al. Cannabis use in pregnancy and maternal and infant outcomes: a Canadian cross-jurisdictional population-based cohort study. PLoS ONE. 2022;17(11):e0276824. https://doi.org/10.1371/journal.pone.0276824.

Marchand G, et al. Birth outcomes of neonates exposed to marijuana in utero. A systemic review and meta-analysis. JAMA Netw Open. 2022;5(1):e2145653. https://doi.org/10.1001/jamanetworkopen.2021.45653.

Massey ZB, Hammond D, Froeliger B. A systemic review of cannabis warning research. Prev Med Rep. 2024;37:102573. https://doi.org/10.1016/pmedr.2023.102573.

Metz TD, et al. Cannabis exposure and adverse pregnancy outcomes related to placental dysfunction. JAMA. 2023;330(22):2191–9.

Micalizzi L, et al. A descriptive analysis of a popular forum: comments on the developmental consequences of cannabis use on offspring. J Stud Alcohol Drugs. 2024;85(2):210–7.

Moore BF, et al. Associations between prenatal and postnatal exposure to cannabis with cognition and behavior at age 5 years: the healthy start study. Int J Environ Res Public Health. 2023;20:4880. https://doi.org/10.3390/ijerph20064880.

Motamedi S, et al. Cannabis during pregnancy: a way to transfer an impairment later in life. Birth Defects Res. 2023;115:1327–44.

Nutor C, et al. Prenatal cannabis use and offspring autism-related behaviors: examining maternal stress as a moderator in a Black American cohort. J Autism Dev Disord. 2024;54:2355–67.

Prewitt KC, et al. Impact of prenatal cannabis use disorder on perinatal outcomes. J Addict Med. 2023;17:e192–8. https://doi.org/10.1097/ADM0000000000001123.

Raifman S, et al. Is legal recreational cannabis associated with cannabis use during pregnancy, beliefs about safety, and perceived community stigma? Drug Alcohol Depend. 2024;255:111079.

Roberts SCM, et al. Association of mandatory warning signs for cannabis use during pregnancy with cannabis use beliefs and behaviors. JAMA Netw Open. 2023;6(6):17138. https://doi.org/10.1001/jamanetworkopen.2023.17138.

Robinson GI, et al. Maternal delta-THC exposure induces abnormalities in the developing heart in mice. Cannabis Cannabinoid Res. 2024;9:121–33.
Ryan KS, et al. The effects of delta-9-tetrahydrocannabinol exposure on female menstrual and reproductive health in rhesus macaques. F S Sci. 2021;2(3):287–94.
Schrott R, et al. Cannabis use is associated with potentially heritable widespread changes in autism candidate gene DLGAP2 DNA methylation in sperm. Epigenetics. 2020;15(1-2):161–73.
Shahine L. Marijuana and fertility: cannabis and men's reproductive health. Seattle: Pacific Northwest Fertility Clinic and University of Washington; 2019. https://www.youtube.com/watch?v=qRVUSapG3qQ
Shorey-Kendrick LE, et al. Prenatal delta-9 THC exposure is associated with changes in rhesus macaque DNA methylation enriched for autism genes. Clin Epigenetics. 2023;15(1):104.
Shukla S, Doshi H. Marijuana and maternal, perinatal, and neonatal outcomes. In: StatPearls. Treasure Island: StatPearls Publishing; 2024.
Skelton K. Maternal report of cannabis screening and recommendations during prenatal care visits in the United States. Am J OBGyn. 2023;229(4):464–6.
Slotkin TA, et al. Paternal D9-tetrahydrocannabinol exposure prior to mating elicits deficits in cholinergic synaptic function in the offspring. Toxicol Sci. 2020;174(2):210–7.
Sorkhou M, et al. Birth, cognitive and behavioral effects of intra-uterine cannabis exposure in infants and children: a systemic review and meta-analysis. Addiction. 2024;119(3):411–37.
Spano MS, et al. Prenatal cannabis exposure increases heroin seeking behavior with allostatic changes in limbic enkephalin systems in adulthood. Biol Psychiatr. 2007;61(4):554–63.
Tadesse AW, et al. The association between prenatal cannabis use and congenital birth defects in offspring: a cumulative meta-analysis. Neurotoxicol Teratol. 2024a;102:107340. https://doi.org/10.1016/j.ntt.2024.107340.
Tadesse AW, et al. Prenatal cannabis use and the risk of attention deficit hyperactivity disorder and autism spectrum disorder in offspring. J Psychiatr Rev. 2024b;171:142–51.
Tadesse AW, et al. Exposure to maternal cannabis use disorder and risk of autism spectrum disorder in offspring: a data linkage study. J Psychiatr Res. 2024c;337:115971. https://doi.org/10.1016/j.psychres.2024.115971.
Taneja S, et al. Making informed choices about cannabis use during pregnancy and lactation: a qualitative study of information use. Birth. 2023;50(3):504–12.
Tirado-Munoz J, et al. The effects of cannabis exposure in the prenatal and adolescent periods: preclinical and clinical studies in both sexes. Front Neuroendocrinol. 2020;57:100841. https://doi.org/10.1016/j.yfrne.2020.100841.
U.S. Surgeon General's Advisory. Marijuana use and the developing brain. 2019. https://www.hhs.gov/surgeongeneral/reports-and-publications/addiction-and-substance-misuse/advisory-on-marijuana-use-and-developing-brain/index.html.
Vanstone M, et al. Pregnant people's perspective on cannabis use during pregnancy: a systematic review and integrative mixed-methods research synthesis. J Midwifery Womens Health. 2022;67:354–72.
Vila-Farinas A, et al. Perception of pregnant individuals, healthcare providers, and decision makers on interventions to cease substance consumption during pregnancy: q qualitative study. BMC Public Health. 2024;24:990. https://doi.org/10.1186/s12889-024-18397-x.
Willoughby JF, et al. Examining science and media literacy communication messages to reduce intentions to use cannabis while pregnant. Psychol Addict Behav. 2023;37(6):746–57.
Young-Wolff KC, et al. Self-reported daily, weekly, and monthly cannabis use among women before and during pregnancy. JAMA Netw Open. 2019;2(7):e196471. https://doi.org/10.1001/jamanetworkopen.2019.6471.
Young-Wolff KC, et al. Association of depression, anxiety and trauma with cannabis use during pregnancy. JAMA Netw Open. 2020;3(2):e1921333. https://doi.org/10.1001/jamanetworkopen.2019.21333.
Young-Wolff KC, et al. Intentions to use cannabis postpartum: a qualitative of pregnant individuals who used cannabis during early pregnancy. J Women's Health. 2024;33(4):435–45.

Chapter 24
Empowering Teens, Parents, and Healthcare Providers in the Cannabis Dialogue



Matthew LaCasse

1 Introduction

Physical, social, and psychological maturation is a complex process with developmental lines influenced by a significant number of variables. As children and adolescents mature, they progress through different stages of change in both the internal world and their external world, each influencing the other. Youth grow up within a system comprised of the biologic traits passed down from their parents as well as the psychological traits and influences of their external communities. Adolescents' wants, desires and fantasies are often shaped by media, social media, family, friends, school, sports, etc. Substance use and exploration is a common part of adolescence (Miech et al. 2024). Adolescent cannabis exploration and misuse often occurs in a more social environment compared to the more progressed stages of problematic use and cannabis use disorder during which more isolated use occurs. Adolescents generally begin using cannabis in social contexts with strong influences from their peer groups, with transitions to cannabis dependency more often seen in those who begin using to relax or use as a coping mechanism to help deal with difficult experiences or mood states. (Lee et al. 2007;Torrejón-Guirado et al. 2023; Caouette and Feldstein Ewing 2017; van der Pol et al. 2013). With the advancement of the cannabis industry and production of inconspicuous cannabis delivery methods such as vapes, vulnerable youth can more easily transition to isolative, regular, and problematic use. In this chapter, we will discuss aspects of teen development and their relationships with substances as well as the current context of current day life for

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youth. We will explore caregivers' role, including parents and healthcare professionals, in navigating the current landscape of cannabis.

2 Cannabis Use in Teens

The number of teens using cannabis on a daily basis has increased over the last three decades, mirroring the general population's increase in cannabis use (Miech et al. 2024). There are associations highlighting the increase in cannabis use with more lenient legislation and regulation changes as well as changes in development and manufacturing cannabis products for consumption; time periods and states with legal medical or recreational cannabis show higher rates of cannabis use, adolescents included (Struble et al. 2019; Caulkins 2024; Bhatia et al. 2022). States with legal recreational cannabis have higher rates of more concealable use products such as vapes and edibles with higher potency, quicker onset and longer duration of the high (Borodovsky et al. 2016; Borodovsky et al. 2017; Struble et al. 2019; Romm et al. 2021). The new landscape of cannabis use among adolescents, including the use of vapes and edibles, is something that has not been seen before, and the consequences of these changes are still emerging.

Smoked cannabis bud, the more traditional and historic way to consume cannabis, has a combination of various cannabinoids. Research exists highlighting potential medical benefits of some of these cannabinoids as well as a synergistic or "entourage" effect of the various cannabinoids and other chemicals present in the cannabis plant, mitigating some of the more dangerous or undesirable effects of pure THC (Legare et al. 2022). Among the various cannabinoids is Delta-9-tetrahydrocannabinol, commonly known as "THC." THC is primarily responsible for the "high" associated with cannabis use. Today, THC is being isolated and is the primary ingredient in most of the cannabis products, often without or with minimal inclusion of potentially helpful cannabinoids such as cannabidiol (CBD) and others which have been shown in some research to have medical benefit for some ailments including anxiety (Legare et al. 2022; Wilson et al. 2019). Some of these products contain unscheduled, legal analogs of delta-9-THC including delta-8-THC, a compound that produces a high very similar to delta-9-THC yet escapes the regulations and laws governing cannabis (Harlow et al. 2024). The legalization and advancement in cannabis' medical utility is often misconstrued by adolescents, leading to making unvalidated conclusions such as "marijuana is legal and a medicine so it must be OK to use." What is much less often discussed in the media consumed by teens is that cannabis' medical purposes are very complex and for the most part, THC has been shown to be the compound less helpful medically, yet this is the compound most commonly included in cannabis products as it produces the most psychoactive effects or the "high."

As legalization has spread across the United States, along with the increase in medical uses of cannabis, the perceived danger of using cannabis among youth has dropped significantly (Miech et al. 2024), highlighting an often-misunderstood

notion that consuming high potency THC products is both safe and may provide some medical benefit. Unfortunately, we have seen a shift to the seemingly more dangerous latter circumstance. There is evidence to begin considering cannabis products of today as almost a "new drug" because of the vastly different chemical profiles and neurobiological and developmental implications.

The methods of delivery of cannabis have changed and have become more discrete. Adolescents can use cannabis on a near continuous basis without much paraphernalia and almost no odors or smoke; with the edible formulations being the easiest to conceal and consume. In many respects, cannabis has changed and we must consider the type of cannabis being used and think about the differences in implications this presents. For example, a 16-year-old male sharing a marijuana "joint" or cigarette of 7% THC cannabis bud among 4 friends after school has different neurodevelopmental implications compared to a 14-year-old male who uses a high potency (80%) vape THC mono product alone in his room at 6:30 AM before school.

Some teens use cannabis for "fun" and recreational purposes, enjoying the high for a short period of time. There are other teens who use cannabis to cope with underlying psychological stressors such as symptoms of depression, anxiety, PTSD, insomnia, and bullying (Patrick et al. 2024; Patrick et al. 2019). Many adolescents who use cannabis on a regular basis begin experiencing physiologic dependency to THC, something that was seen less commonly before the current era of higher potency cannabis products and the abundance of THC vapes and edibles. There are physiologic and psychologic forms of dependency. Physical dependency means that the body and brain have become accustomed to having the substance in its system to the point of making changes to accommodate for the presence of the substance, attempting to reach homeostasis, or the balanced, natural state of functioning. Psychological dependence often occurs when substances are used for self-medicating psychological distress. Individuals begin to rely on the psychoactive property of the substance to cope with difficulties and lose faith in their natural abilities to make it through hard times or to cope with distress. When someone's body and brain become dependent on a substance, they often experience cravings, tolerance, and withdrawal. Tolerance means that more of the substance is needed to produce the same or similar effects as previous. Withdrawal is a change seen when someone stops using the substance after their body and brain have made changes to accommodate for its presence. Youth who become THC dependent, either psychologically or physiologically or both, exhibit signs and symptoms of withdrawal if they stop using including anxiety, sleep difficulties, tension, sweating, cravings, nausea, anorexia, and general malaise; sometimes lasting for multiple weeks (Milin et al. 2008; Bonnet and Preuss 2017). These symptoms can be difficult to manage for an adolescent considering their academic and social demands, often reinforcing the continued use of cannabis to relieve the withdrawal symptoms, especially when the culture around them has shifted to a much more "pro-cannabis" culture with legalization and marketing efforts promoting cannabis use.

Teens' perception of cannabis is influenced by social media and the other digital platforms of communication. Although it is currently illegal in the United States to

advertise cannabis products on social media, individual companies and dispensaries are able to produce content promoting cannabis use and market themselves through their own accounts. As youth engage in social media at high rates, they are also subject to cannabis marketing and influence. There is evidence that the cannabis industry intentionally markets with proven highly effective strategies while promoting cannabis use, targeting youth, and making unsupported medical claims (Marinello et al. 2024; Asquith 2021).

Legislation also has a significant impact on rates of use as well as the understanding and perceived harms and safety of substances. There is a lot to be considered when discussing the legal status of substances. The more recent trend of individual states legalizing first medical marijuana and then recreational marijuana coincides with a co-occurring trend of adolescents believing that marijuana is safer and safer. With the legalization of marijuana, adolescents often begin to believe it is "natural" and okay to use. The term "legal" sticks in many adolescents' minds as "permission" or "safe." Often those stuck in addiction look for justification for continued use; the legalization and ubiquity of cannabis use serves this purpose. We have reached an epidemic of adolescents using THC as well as vape THC products. As we did with cigarettes, it would be important to support educational campaigns to help prevent the negative consequences of the current THC epidemic among youth.

3 Substance Use and Psychological Development

As children mature, they begin the task of mastering, or attempting to master, the world around them. They learn to build skills to control the things they can control as well as coping mechanisms to better withstand the things they cannot control. As development continues into adolescence and young adulthood, one begins to solidify a more secure and mature sense of self. Adolescents begin identifying and forming more consistent characteristics and traits of themselves and often begin associating with groups of likeminded individuals. Cannabis use during this time can be disruptive in these necessary processes for maturation. There is an adage in addiction treatment: "when addiction starts, maturation stops." Researchers and clinicians who work in the addiction field have noticed that when people are able to achieve sobriety they often display characteristics of the maturation level closely correlated to the stage during which they started heavily using substances. This proposes a difficult task for the individual affected as well as family, loved ones, and medical providers. Prevalent are adults who are unable to act their chronological age secondary to deficits in the maturation process. Those achieving sobriety are tasked with learning themselves, exploring boundaries (both personal and interpersonal), as well as deal with much of the unresolved conflict that was either present before the addiction began and/or produced along the way. As adolescents and young adults develop themselves, they begin incorporating relationships and intimacy into their daily lives with more concentrated importance and effort. Healthily maturing youth navigate their ability to achieve intimacy and closeness with the

community, including better understanding their personal psychological, spiritual, and sexual connectedness as well as boundaries. Addiction during these times often distracts from normative reflection and experiential engagement that allows for the formation of secure values. Those suffering with addiction throughout these developmental processes are often tasked with going back in time, metaphorically, and relearning themselves, their values, their boundaries in their ability to connect with each other in a healthy way.

As infants eventually develop into adult humans, it is proposed that there are developmental tasks, or stages that one must conquer for healthy development. This is a complex process, and many have proposed various formulations and hypotheses of critical processes and timing of these along the developmental lines. As an example, we can briefly explore the Eriksonian stages of development and highlight some of the interfaces and disruptions substance use may play a role in, focusing on stages most commonly occurring during the onset of adolescent substance use.

Eric Erikson proposed eight states of development (Bishop 2013). The first stage is *trust vs. mistrust* and occurs during infancy when infants are tasked with learning to trust and attach to others, beginning the process of forming self-security and wholeness. The second stage is *autonomy vs. shame and doubt* and occurs during toddlerhood. Toddlers begin to develop a more secure sense of individuality, testing their limits, capabilities, and exploring the world around them while often seeking appropriate reassurance and continued security from their caregivers. The third stage is *initiative vs. guilt* during which children continue to build their independence and practice initiating their own actions and mastery of the world around them, relying less on their caregivers' direction. It is during these stages that the foundation of healthy development is secured. Most individuals do not begin to use substances during these early years and are thus most often sheltered from the impacts of personal substance use. What is more commonly seen is a disruption in healthy development during these years through the unfortunate consequences of a parent or caregiver suffering with addiction or substance use concerns.

The fourth Eriksonian stage is *industry vs. inferiority* and is thought to occur around the school-age period. This is a time when children and young adolescents begin to develop competence and mastery of tasks and navigate opportunities and obstacles offered by the world around them. Individuality and mastery continue to form during this time. During this time, children begin to become more involved with their peers and adult role models, analyzing their own capabilities, often comparing them to others. Depending on cultural and family influences, children are often praised for their accomplishments and executions of goals, including those in the social and academic realms. With the introduction of substance use during this period, children and young adolescents often fail to internalize their capabilities and mastery of the world around them, leading to insecurities in ability and self-actualization. Individuals fail to develop self-confidence, self-reliance, and resilience needed to navigate difficulties presented to them down the road.

The fifth stage proposed by Erickson, comprising of primarily adolescent development, is that of *identity vs. role confusion.* During this stage of development adolescents begin to form their own identity. It is important to note, however, that this

stage is often interrupted without the necessary foundation of a secure sense of self, which is thought to begin to form much earlier, including early infancy and the bonding and attachment stages. By the time of adolescents, youth should have developed a sense of self and self-efficacy and further develop their individual traits and characteristics that individualize them even further. It is during this stage that adolescents begin establishing more of their likes and dislikes and currency for exchange with the external world. In adolescence, peer groups and immediate social connections play a large role in developing their own identities, for it is within these groups that adolescents begin testing their identities, hoping for acceptance.

Substance use while an adolescent is forming their identity can sometimes lead to integration of substance use into that identity. Adolescents can mistakenly assume the role or partial role of a "pot head," for example, and often find maladaptive meaning and acceptance with this integrated sense of self. This often clashes with more mature societal roles and expectations, which leaves the adolescent rebelling to protect their identity. Along the way, as substance use continues, the adolescent misses out on the opportunity to develop more naturally inherent aspects of themself and practice executing the self-efficacy and personality in the world around them. If we accept the notion that social and emotional development often become arrested at the developmental stage that heavy substance use begins, we expect many people suffering with addiction to be "stuck" in this stage with an underdeveloped sense of identity; often not quite understanding who they are, their abilities, likes and dislikes, and associated sense of internal and external acceptance for who they are. What is often seen as a consequence of failure to mature through this stage is a gravitation pull for adolescents and adults to continue to socialize with people similarly affected, often potentiating and perpetuating the dysfunction. Individuals often use substance use as a façade and maladaptive integration of their personality to bond with others in similar situations without becoming vulnerable enough to learn how to execute their own individuality through sobriety.

The sixth stage is that of *intimacy versus isolation,* which is thought to occur during late adolescence and young adulthood. This is a stage of development when individuals rely on their own security, sense of self, and self-efficacy to begin finding intimacy and connection within their community. Although some of this occurs during earlier stages of development, young adulthood is a time when individuals begin more strongly solidifying their roles and place in society, often becoming attracted to people and partners with whom they can express their sense of self and benefit from the complements of healthy connection with others in the community. Heavy substance use and addiction during this time can often lead to individuals missing out on the benefits of strong intimate connection, both romantic and platonic. Connectedness is an important part of typical maturation throughout adulthood as many of the society-given opportunities to build connectedness (school, academics, family) begin to fade away as people mature into and through young adulthood. The tragedy of underdeveloped intimacy or connectedness secondary to addiction leaves an individual feeling alone and isolated with a seemingly difficult task of building intimacy and connection in the world during which the majority of their peers already have. What is also observed is the false sense of connection

through substance use. As adolescents and young adults bond, they sometimes bond over getting high together or using together. What can easily happen is an individual begins to rely on bonding through substance use instead of sober connectedness, the latter forming much stronger and healthier bonds.

Erikson's seventh (*generativity vs. stagnation*) and eighth (*integrity vs. despair*) occur through middle and late adulthood and offer the tasks of deploying themselves and their capabilities to better the communities in which they exist along with becoming a leader while assessing their past roles and life accomplishments. Without the foundations of previous development, adults find themselves incapable of becoming leaders and executing their skills to better the communities around them. It is often during this chronological age period when individuals have to "go back" and develop more of themselves prior to being able to generate the way most of their peers around them are. Thankfully, adults who have suffered in the past can traverse these developmental milestones while in adulthood. Doing so often takes vulnerability and acceptance to experience discomfort while learning how to build self-efficacy and understand themselves and the relationships with the world around them.

It should be noted that the family and community in which a child or adolescent matures plays a large role in that individual's ability to healthily mature and progress through these developmental stages. An adolescent suffering from addiction will likely have some degree of impaired social and emotional development, even when raised in a supportive family and social network. What can be equally disruptive to development, however, is caregiver addiction. The consequences of addiction such as intoxication and withdrawal or the social consequences such as spending time seeking and recovering from drugs, missing important events and responsibilities, and impaired ability to truly care for and connect with a child disrupts the necessary bonding, attachment, modeling, scaffolding, and mirroring that is important in child development. Parents unfortunately can provide an unhealthy environment that creates difficulties for healthy maturation to occur. Although adults suffering from addiction truly suffer, it should be noted that children and adolescents within the home also suffer significantly. The contrary is also true; parents have the wonderful ability to create an environment which better allows for flourishing, healthy children.

4 Cannabis and Parents

Many parents of today's youth grew up during a time when cannabis was different compared to today. The major difference is that the cannabis today is specifically bioengineered, most often to be more potent and "stronger." Along with bioengineered cannabis flower or bud, today we have engineered THC products such as vapes and wax, which is much different compared to the previously common strains of smoked leaf cannabis. Most parents are unfamiliar with the effects of today's high-potency cannabis flower as well as cannabis vapes. Some parents who are

more permissive must be aware that although there are similarities, it is not the same cannabis as in the past; the highs come faster and stronger than ever before.

Parents and role models play a role in influencing a child's decisions and behaviors. As youth mature, they look at their parents to lead by example and teach them the world. As we grow in communities, family and community rituals become a part of the children's lives as well, often replicated when they become adults. It is important for adults to inspect their relationship with substances and the rituals they perform as it relates to their children's learning of such behaviors. For example, relaxation. Relaxation is an important part of healthy living; de-stressing and becoming more mindful and centered. The relaxation techniques of parents and role models are often learned by their children and others around them. If parents portray the use of cannabis as a way to relax after a long day at work, this will be learned by the child, especially if the friends of the parents and others in the community do the same. Soon, the children are witnessing most adults of their world turn to substances to relax. As impressionable young humans they learn that this ritual is "normal." The same can be said about other rituals and experiences. Take a celebration for example. Celebrating a birthday, graduation, or a successful life event is an appropriate thing to do. How we celebrate and how we model celebration is an important thing for parents to keep in mind. There is a difference in a child growing up in a home where celebrating a birthday included cannabis or alcohol consumption among adults versus a sober celebration that included things to increase mindfulness and connection such as song singing, storytelling, praising, nature walks, games, etc. Another example to illustrate the point would be that of recreational camping. A summer family trip camping during which the adults use cannabis at night during the time of the campfire teaches their youth that campfires and cannabis go together. Instead, imagine a campfire where true connection can be made through sober appreciation, affection, and connection. It is during these sober times that allow us to feel truly connected, let alone the impactful modeling of healthy coping and recreation.

Many people who use substances begin asking themselves "what would it be like if I did not do ___. " "What would it be like if I smoked less marijuana." "What would it be like if I did not vape THC before school". "What would it be like if I only did it on the weekends". "What would it be like if I did not do this at all." "What would it be like if I did not feel the need that I 'had to do this'." "If I had better control of my anxiety would I use less cannabis?" "What is school like sober?" "Can I have fun on vacation and not drink alcohol?". Many people who use substances wonder about their life and what it would be like if substances did not play such a significant role. The notion of pondering sobriety is being talked about more openly as "sober curious" (Lunnay et al. 2022). The "sober curious" community is one that begins wondering about the impact of substances on their lives and what sobriety would be like; often taking initial steps to limit substance use and explore sobriety. Sober curious groups often look to the community around them for role models and discuss more about what it is like to be sober. Youth are doing the same (Siconolfi et al. 2024). Sober adults and the recovery community as a whole can be very impactful in leading by example and highlighting the fact that sobriety

is attainable and when achieved, often very fulfilling and comfortable. Those in the recovery community have the opportunity to spread knowledge and be an inspiration to those who are sober curious. They can do this by sharing their story, highlight the troubles that substances caused as well as emphasis on the benefits sobriety has brought them.

Parents often have a lot of angst about raising their children the "right way." What is important for parents to keep in mind is that their role is first and foremost to love their children. Children feel their parents' love in different ways, yet the most important thing to do is to provide the *time* necessary to allow them to feel loved. Connection and healthy bonding is rooted in parents' intentional time spent attempting to connect and support their children, without distractions. These moments of connection go a very long way when it comes to raising children who feel their parents' love and presence. This groundwork makes setting expectations and rules and the subsequent consequences when the expectations are not met, or when the rules are broken a lot easier and better accepted by the children; another vitally important role of parents. It is also important for parents to think about their childhood and how it might affect their parenting of their own children. To begin this process, it is sometimes helpful to reflect on things that they did not enjoy or would have changed about their childhood and subsequently shield their own children from going through similar experiences. At the same time, identify things they cherished from their own childhood and would like to recreate for their children.

Parents are preparing their youth to navigate the world around them and eventually launch into independence. If a parent or loved one discovers a teen in trouble with cannabis or other substances, open dialogue is recommended, being sensitive to the pressures and developmental stages the youth are experiencing. Here are a few tips to keep in mind when discussing cannabis use with teens:

- First, find a time to talk that is relaxed in nature, without recent fights or escalations. Try to find a time when it seems the teen would be open for a few moments of discussion and connection.
- Allow yourself to feel the "emotional weather" of the conversation and respond accordingly—if things are seeming bright and the child is engaging, lean in and keep talking, asking questions, and reassuring them. If it seems a storm is brewing and tensions are escalating, back off, avoid escalation, and try again soon, maybe taking note of sensitive topics and being careful not to lead with those in future conversations.
- Parents often want to "fix" the problem. Please understand that if an adolescent has a strong relationship with substances they are likely going to have to continue to manage that relationship long term; there is almost never any "quick fix." With this in mind, the initial goal is simply to understand more about the relationship between the teen and the substances. Understand the relationship more intimately and allowing them to express the likes and dislikes and ways substances play a role in their lives can go a long way in building connection and moving toward sobriety or limiting the negative impacts of substances.

- Understand that part of the parents' role is to set rules and expectations as well as consequences for breaking the rules or not meeting the expectations. Be sensitive to the individual child and their capabilities and keep the "big picture" of functioning and healthy development in mind when setting these rules and expectations. Also, it is important to have consequences that are equal in degree to the offense. Parent the child in line with values of the household and avoid being overly punitive out of frustration or anger. More importantly, catch the teen while being good. Often parents focus on catching their children doing the "bad things" they want them to avoid. Catching the child while being good, while praising and rewarding those behaviors and accomplishments helps reinforce good choices and beneficial behavior. At times, it is difficult to reward the teen for something they did well after months of behaving in a way parents are frustrated with. It is important to not let frustrations with previous behavior overshadow the teens success in other areas. Continuing to support the teens as they do well reminds them that parents do care for them and this better behavior leads to more privileges and a calmer home. Please understand that the teens dislike yelling and fighting within the home just as much as the parents do. Also, it can be helpful to directly discuss the expectations, consequences, and rewards preemptively. Allowing the teen to take part in setting the rubric for expectations, punishments, and rewards can also be helpful as it empowers them to have more control at the same time still fulfilling the general expectations from parents.
- A loving and connected parent often has certain intuitions or "gut feelings" when deciding how to navigate challenging situations as well as when and how to support their children. It is ok to listen to those intuitions and do what you think is best for the overall wellbeing of the child. A loving and connected parent is doing the best they can, as long as they continue to try their best.
- Allow for access to mental health support without feeling the need to do everything yourself. The role of parents is to love and support the child while providing them resources to help with things that are out of the scope of typical parenting. Although being stewards in children's mental health is an important role of a parent, if specialty care is needed, it would be important to supply those resources.

In summary, parents play the most important role in the development of a healthy child. Parents have the ability to show their children love and connectedness while providing an environment rich for healthy maturation. Setting the groundwork by working hard to truly connect with and love the child makes navigating the more difficult aspects of parenting much easier.

5 Cannabis and the Medical Provider

Medical providers are tasked with caring for the individual in front of them. As ailments vary between individuals as do the various social circumstances and individual personality structures. Treating addiction and comorbid mental health

diseases requires patience and an understanding of the goals of treatment. First, it is important to understand the goals of the patient. The goals of patients will vary significantly; goals might include quitting substances altogether, stopping one but not the other, cutting back on cannabis use to only weekends, not using alone anymore, or continuing to use but limiting the negative consequences of use. Although sometimes patient's goals might not line up with the providers or parent's goals exactly, it is very important to recognize the patient's goals and help them work toward these goals. Along the way and down the road there will be an opportunity to continue to work with the patient's intrinsic momentum and make further change. The first task is to meet the patient where they are. It is important to consider the various unique circumstances that affect the individual in both supportive ways as well as potentially detrimental ways. Take note of risk factors as well as the non-modifiable and modifiable circumstances. After exploring the system in which the patient's cannabis use exists, one can then deploy various treatment methods to help address some of the binding conflicts or maladaptive behaviors distracting youth from living close to their natural values.

The patient's values are important to understand as most of the time, a life with problematic substance use draws them away from their values and the things they truly care about. Helping patients highlight their values and the things they care about will allow them to better understand the role cannabis plays in their life and the distraction it can cause. It is very appropriate for a primary goal of treatment to be a shift toward one's natural values, leading to a more fulfilling life. Youth often realize that they have lost connection with many or all of the things they care about when substances play a large role. Rebuilding the connections between themselves and their values is often liberating and allows for natural pleasures and fulfillment. Engaging the patients with Acceptance and Commitment Therapy (ACT) can help youth navigate the relationships between substance use, unwanted thoughts and behaviors, and their true values (Osaji et al. 2020; Thurstone et al. 2019).

Which therapeutic technique a medical provider deploys will vary largely on the treatment setting. In a setting where little time or resources are available, Screening, Brief Intervention and Referral to Treatment (SBIRT) can be very effective (Levy et al. 2016). An integral part of SBIRT is the brief intervention: when the patient is in front of a provider, the provider has the opportunity to help the patient make a change. One of the most effective ways to do this is through Motivational Interviewing (Barnett et al. 2012). Motivational interviewing (MI) has been proven to be helpful in allowing patients to reach their goals in many different medical settings including pain, chronic disease, and addiction, among others (Lundahl et al. 2013). Briefly, the spirit of MI encompasses autonomy (allowing the patient to be in charge of their own change), collaboration (working with the patient to allow the provider and the patient to better understand), and evocation (drawing out the patient's internal motivation). The spirit of MI highlights the fact that lasting change rarely comes from shaming, blaming, or being told how to change. Rather, lasting change more often comes from within and through one's own faculties, values, and desires. Motivational interviewing helps draw these out and elicits the individual's

own power for change. The principles of motivational interviewing include meeting the patient where they are and identifying their goals and rolling with resistance all while highlighting discrepancies or contradictions through an empathic connection. Empathic connection and rolling with resistance are very important to keep the patient feeling supported and engaged in care. Retention in care is highly valued in substance use treatment; after all, the patient must be seen in order to make any difference, even small. There are times when providers must intentionally work with the patient in order to keep them in care. Making baby steps and keeping someone in care is a safer way to go compared to swinging for a home run and missing. That is, being patient and collaborative can often be more effective than authoritative. Some of the skills necessary are the use of open-ended questions, affirmations, reflections, and summaries. All of this can be done through a motivated provider in only several minutes. With the appropriate time, problematic cannabis use and cannabis use disorder can be treated with other evidenced based methods including acceptance and commitment therapy, cognitive behavioral therapy, contingency management, pharmacotherapy, and various forms of family and community therapy, to name a few (Horigian et al. 2016; Stanger and Budney 2010; Stanger and Budney 2019; Wu et al. 2016; Osaji et al. 2020; Thurstone et al. 2019; Adams et al. 2023)

Medical providers and researchers are the leaders in advancing care for these individuals. Medical professionals have the power to both influence the individual as well as that individual's family and friends. Professionals can have their voice heard on the local and national political levels as well. As subject matter experts, it is important for researchers and physicians to participate in discussions regarding legislature that affects our youth.

6 Summary

Our youth are using new cannabis products at high rates with emerging evidence highlighting serious risks to their health and development, particularly the high potency vape products that are now ubiquitous in the community; especially those with legislation supporting recreational cannabis use. Adolescents are unfortunately very vulnerable to societal influences and marketing strategies surrounding the use of cannabis and the misconceived notion that it poses little to no health risks. Adolescents themselves, along with parents and caregivers, play a vital role in the cannabis dialogue and educating on cannabis use risks, as well as modeling appropriate relationships with substances. It appears that medical providers are tasked now more than ever to understand implications of cannabis use in teens and the culture around cannabis use as well as targeted approaches to identify problematic use and its interventions.

References

Adams ZW, Marriott BR, Hulvershorn LA, Hinckley JD. Treatment of adolescent Cannabis use disorders. Psychiatry Clin North Am. 2023;46(4):775–88. https://doi.org/10.1016/j.psc.2023.03.004. PMID: 37879838.

Asquith K. The visual clichés of legal cannabis promotion on social media. Crit Stud Media Commun. 2021;38(4):336–49.

Barnett E, Sussman S, Smith C, Rohrbach LA, Spruijt-Metz D. Motivational Interviewing for adolescent substance use: a review of the literature. Addict Behav. 2012;37(12):1325–34. https://doi.org/10.1016/j.addbeh.2012.07.001. Epub 2012 Jul 11. PMID: 22958865; PMCID: PMC3496394.

Bhatia D, Hinckley J, Mikulich S, Sakai J. Cannabis Legalization and Adolescent Use of Electronic Vapor Products, Cannabis, and Cigarettes. J Addict Med. 2022;16(1):e16–22. https://doi.org/10.1097/ADM.0000000000000831. PMID: 35120066; PMCID: PMC10210543.

Bishop CL. Psychosocial stages of development. In: Keith KD, editor. *The encyclopedia of cross-cultural psychology*. 1st ed. Hoboken, NJ: John Wiley & Sons, Inc.; 2013.

Bonnet U, Preuss UW. The cannabis withdrawal syndrome: current insights. Subst Abus Rehabil. 2017;27(8):9–37. https://doi.org/10.2147/SAR.S109576. PMID: 28490916; PMCID: PMC5414724.

Borodovsky JT, Crosier BS, Lee DC, Sargent JD, Budney AJ. Smoking, vaping, eating: is legalization impacting the way people use cannabis? Int J Drug Policy. 2016;36:141–7. https://doi.org/10.1016/j.drugpo.2016.02.022. Epub 2016 Mar 2. PMID: 26992484; PMCID: PMC5010515.

Borodovsky JT, Lee DC, Crosier BS, Gabrielli JL, Sargent JD, Budney AJ. U.S. cannabis legalization and use of vaping and edible products among youth. Drug Alcohol Depend. 2017;177:299–306. https://doi.org/10.1016/j.drugalcdep.2017.02.017. Epub 2017 Jun 9. PMID: 28662974; PMCID: PMC5534375.

Caouette JD, Feldstein Ewing SW. Four mechanistic models of peer influence on adolescent Cannabis use. Curr Addict Rep. 2017;4:90–9. https://doi.org/10.1007/s40429-017-0144-0.

Caulkins JP. Changes in self-reported cannabis use in the United States from 1979 to 2022. Addiction. 2024;119(9):1648–52. https://doi.org/10.1111/add.16519. Epub 2024 May 22. PMID: 38775461

Harlow AF, Miech RA, Leventhal AM. Adolescent Δ^8-THC and marijuana use in the US. *JAMA*. 2024;331(10):861–5. https://doi.org/10.1001/jama.2024.0865.

Horigian VE, Anderson AR, Szapocznik J. Family-based treatments for adolescent substance use. Child Adolesc Psychiatr Clin N Am. 2016;25(4):603–28. https://doi.org/10.1016/j.chc.2016.06.001. Epub 2016 Aug 3. PMID: 27613341; PMCID: PMC6986353.

Lee CM, Neighbors C, Woods BA. Marijuana motives: young adults' reasons for using marijuana. Addict Behav. 2007;32(7):1384–94. https://doi.org/10.1016/j.addbeh.2006.09.010. Epub 2006 Nov 13. PMID: 17097817; PMCID: PMC2723942.

Legare CA, Raup-Konsavage WM, Vrana KE. Therapeutic potential of Cannabis, Cannabidiol, and cannabinoid-based pharmaceuticals. Pharmacology. 2022;107(3–4):131–49. https://doi.org/10.1159/000521683. Epub 2022 Jan 28. PMID: 35093949.

Levy SJL, Williams JF, Ryan SA, Gonzalez PK, Patrick SW, Quigley J, Siqueira L, Smith VC, Walker LR, COMMITTEE ON SUBSTANCE USE AND PREVENTION. Substance use screening, brief intervention, and referral to treatment. Pediatrics. 2016;*138*(1):e20161210. https://doi.org/10.1542/peds.2016-1210.

Lundahl B, Moleni T, Burke BL, Butters R, Tollefson D, Butler C, Rollnick S. Motivational interviewing in medical care settings: a systematic review and meta-analysis of randomized controlled trials. Patient Educ Couns. 2013;93(2):157–68. https://doi.org/10.1016/j.pec.2013.07.012. Epub 2013 Aug 1. PMID: 24001658.

Lunnay B, Nicholls E, Pennay A, MacLean S, Wilson C, Meyer SB, Foley K, Warin M, Olver I, Ward PR. Sober curiosity: a qualitative study exploring women's preparedness to reduce alcohol by social class. Int J Environ Res Public Health. 2022;19(22):14788. https://doi.org/10.3390/ijerph192214788. PMID: 36429505; PMCID: PMC9690974.

Marinello S, Valek R, Powell LM. Analysis of social media compliance with cannabis advertising regulations: evidence from recreational dispensaries in Illinois 1-year post-legalization. J Cannabis Res. 2024;6(1):2. https://doi.org/10.1186/s42238-023-00208-6. PMID: 38173010; PMCID: PMC10762945.

Miech RA, Johnston LD, Patrick ME, O'Malley PM. Monitoring the future national survey results on drug use, 1975–2023: overview and detailed results for secondary school students. Monitoring the future monograph series. Ann Arbor, MI: Institute for Social Research, University of Michigan; 2024.

Milin R, Manion I, Dare G, Walker S. Prospective assessment of cannabis withdrawal in adolescents with cannabis dependence: a pilot study. J Am Acad Child Adolesc Psychiatry. 2008;47(2):174–9. https://doi.org/10.1097/chi.0b013e31815cdd73. PMID: 18176332.

Osaji J, Ojimba C, Ahmed S. The use of acceptance and commitment therapy in substance use disorders: a review of literature. J Clin Med Res. 2020;12(10):629–33. https://doi.org/10.14740/jocmr4311. Epub 2020 Sep 21. PMID: 33029268; PMCID: PMC7524566.

Patrick ME, Evans-Polce RJ, Kloska DD, Maggs JL. Reasons high school students use marijuana: prevalence and correlations with use across four decades. J Stud Alcohol Drugs. 2019;80(1):15–25. https://doi.org/10.15288/jsad.2019.80.15. PMID: 30807270; PMCID: PMC6396516.

Patrick ME, Peterson SJ, Terry-McElrath YM, Rogan SEB, Solberg MA. Trends in coping reasons for marijuana use among U.S. adolescents from 2016 to 2022. Addict Behav. 2024;148:107845. https://doi.org/10.1016/j.addbeh.2023.107845. Epub 2023 Sep 1. PMID: 37696065; PMCID: PMC10870514.

Romm KF, West CD, Berg CJ. Mode of marijuana use among young adults: perceptions, use profiles, and future use. Subst Use Misuse. 2021;56(12):1765–75. https://doi.org/10.1080/10826084.2021.1949724. Epub 2021 Jul 22. PMID: 34294001; PMCID: PMC8693385.

Siconolfi D, Tucker JS, Pedersen ER, Perez LG, Dunbar MS, Davis JP, Rodriguez A, Seelam R, D'Amico EJ. Sober curiosity and participation in temporary alcohol abstinence challenges in a Cohort of U.S. Emerging Adults. J Stud Alcohol Drugs. 2024;85(2):201–9. https://doi.org/10.15288/jsad.23-00137. Epub 2023 Oct 30. PMID: 37917023; PMCID: PMC10941816.

Stanger C, Budney AJ. Contingency management approaches for adolescent substance use disorders. Child Adolesc Psychiatr Clin N Am. 2010;19(3):547–62. https://doi.org/10.1016/j.chc.2010.03.007. PMID: 20682220; PMCID: PMC2916869.

Stanger C, Budney AJ. Contingency management: using incentives to improve outcomes for adolescent substance use disorders. Pediatr Clin N Am. 2019;66(6):1183–92. https://doi.org/10.1016/j.pcl.2019.08.007. PMID: 31679606; PMCID: PMC6834344.

Struble CA, Ellis JD, Lundahl LH. Beyond the bud: emerging methods of Cannabis consumption for youth. Pediatr Clin N Am. 2019;66(6):1087–97. https://doi.org/10.1016/j.pcl.2019.08.012. PMID: 31679599.

Thurstone C, Hull M, Timmerman J, Emrick C. Development of a motivational interviewing/acceptance and commitment therapy model for adolescent substance use treatment. J Contextual Behav Sci. 2019;6(4):375–9.

Torrejón-Guirado MC, Baena-Jiménez MÁ, Lima-Serrano M, de Vries H, Mercken L. The influence of peer's social networks on adolescent's cannabis use: a systematic review of longitudinal studies. Front Psych. 2023;21(14):1306439. https://doi.org/10.3389/fpsyt.2023.1306439. PMID: 38188050; PMCID: PMC10768034.

van der Pol P, Liebregts N, de Graaf R, Korf DJ, van den Brink W, van Laar M. Predicting the transition from frequent cannabis use to cannabis dependence: a three-year prospective study. Drug Alcohol Depend. 2013;133(2):352–9. https://doi.org/10.1016/j.drugalcdep.2013.06.009. Epub 2013 Jul 22. PMID: 23886472.

Wilson J, Freeman TP, Mackie CJ. Effects of increasing cannabis potency on adolescent health. Lancet Child Adolesc Health. 2019;3(2):121–8. https://doi.org/10.1016/S2352-4642(18)30342-0. Epub 2018 Dec 18. PMID: 30573419.

Wu SS, Schoenfelder E, Hsiao RC. Cognitive behavioral therapy and motivational enhancement therapy. Child Adolesc Psychiatr Clin N Am. 2016;25(4):629–43. https://doi.org/10.1016/j.chc.2016.06.002. PMID: 27613342.

Chapter 25
The Landscape of *Cannabis* Education



Jahan Marcu and Jason B. Schechter

1 Introduction

Education is simply the soul of a society as it passes from one generation to another. (G.K. Chesterton)

The increasingly complex landscape of global *Cannabis* education is undergoing transformative change, driven in large part by a rapid international legalization of both *Cannabis* and cannabinoid-based pharmaceuticals. Intensifying public understanding of cannabinoid use—for both therapeutic and so-called recreational purposes—compels an increasing public thirst for instruction, historical knowledge and pharmacological expertise. As *Cannabis* moves ever-further from the shadows of illegality into the mainstream consciousness, the need for comprehensive, evidence-based education has never been more critical. Unfortunately, the fragmented and often not empirically supported nature of cannabinoid education has led to conspicuous gaps in community knowledge and, indeed, in general understanding across various sectors of our society. This chapter explores the diverse and evolving educational landscape surrounding *Cannabis* and the cannabinoids, highlighting the many challenges, opportunities, and potential future directions for a more effective societal education on cannabinoid pharmacology and use.

With the benefit of hindsight, U.S. *Cannabis* education can be seen to have been both historically shaped and controlled largely by government reports and public health campaigns. Too-often influenced by prevailing political ideologies (and a smattering of personal vendetta), rather than empirical evidence, the "Reefer

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Madness" era of the 1930s well-exemplified how profligate use of social misinformation and propagandist fear-mongering can be skillfully utilized to both shape public perception and Federal policy. Over time, however, the narrative has shifted, driven not only by advances in scientific research, but changes in legal frameworks and a growing social recognition of both potential therapeutic benefits and risks.

Our challenge today is not just to correct the various misconceptions of the past, but to build an encompassing educational ecosystem that hopefully meets the diverse needs of a modern society. This involves not only informing the public, but also ideally provides targeted education for healthcare providers (HCPs), business professionals, policymakers, and other key stakeholders in *Cannabis* business ventures. As *Cannabis* products become more varied—and, indeed, potent—and as the synthetic cannabinoids complicate marketplaces, the complexity of the information landscape increases, requiring increasingly nuanced and well-informed approaches to education.

The sections below hope to delve into the multifaceted nature of present and emerging *Cannabis* education, examining how different sectors—from healthcare to the *Cannabis* industry and public health—are responding to evolving demands and upon our knowledge of *Cannabis* and the cannabinoids. We will explore some critical gaps in current educational efforts, the need for standardized guidelines, and the importance of integrating data-based statements into educational strategies. We will consider how historical and international reports might be better leveraged to enhance the public's understanding of their findings, and how some recent innovative technologies might make *Cannabis* education both more accessible and engaging.

It is essential to admit that *Cannabis* education is not one-size-fits-all, requiring a more-tailored approach that better considers the unique needs and perspectives of increasingly educated audiences. By fostering more well-rounded and comprehensive educational foundation, we can better ensure that *Cannabis* is more fully understood in all its related complexities—both empowering individuals to make informed decisions while contributing to responsible use, risk mitigation, and intelligent regulation of these increasingly significant substances.

2 Acute and Long-Term Effects of Government Reports

> *If politics is medicine on a larger scale, than perhaps the inverse is true. Perhaps medicine is politics on a smaller scale. (*Meili and Hewett 2016*)*

In years past, several governmental and international organizations have attempted to increase public awareness—and often to raise alarm bells—in hopes of mitigating the impact of substances such as *Cannabis* on public health. While some aspects of these reports have withstood both the test of time and scientific scrutiny, many, in hindsight, were heavily influenced by unrelated and prevailing prejudices—specifically, the demonization of particular minority groups based on race or immigration

status. In recognition of these historical biases, it does make it somewhat more difficult to take some of these reports seriously. We are still able to hear unfortunate echoes across so much modern policy and scientific literature.

Government reports generally suffer one of two inevitable fates: either (a) deemed at odds with the political desires of the time, silently relegated to the dustbin nearest the desk of whichever elected (or appointed) official, *or* (b) they take on a life of their own, becoming widely used, oft referenced hobgoblins, useful for policy-making maneuvers. In either case, *very rarely* does anyone revisit and/or coherently measure the actual studies therein, nor properly assess their actual *impact*, if any, on subsequent behavior or public health.

The following section provides a brief overview of various historical international reports on *Cannabis*, and the attempts of each to educate and/or warn the public. Each has become increasingly known (and occasionally notorious) for the leveraging of questionable statistics and anecdotes therein. Unfortunately, the true educational potential of these reports—as either cautionary tales or pillars of research—remains underutilized.

2.1 The 1893–1894 Indian Hemp Drugs Commission

The "Indian Hemp Drugs Commission" was established during Britain's colonial rule of India, in no small part to determine if restricting *Cannabis* use would be in the British Empire's best economic interests. To be sure, *other* herbally derived phytopharmaceuticals (such as opium and its derivatives) proved to be quite valuable to the Empire, not only medicinally, but for the wielding of immense political power—decisions that had obvious and lasting impacts on global society both then and now. As such, the Dutch East India Company had become the largest corporation in history, in part by trafficking over *four million kilos* of opium a year, for decades, from India to China. An estimated 20 million addicts were created in the first year of the opium trade alone, and it nearly depleted the entire supply of silver in Asia over only the course of a decade in its exchange.[1] After this motivational experience, both British industry and government decided to investigate the potential impacts of hemp.

The Commission's hemp report was one of the largest and most comprehensive reviews of *any* pharmacological subject in world history—with more than a *thousand* witnesses providing testimony for the *seven-volume* report. (Kalant 2009; Roffman et al. 2009) In short, amongst the primary findings of the Commission was that the moderate use of *Cannabis* was "*not a cause of... criminality, mental illness, or immoral behavior.*" The Commission further stated that *Cannabis* use—for religious, medical, or recreational purposes—was far more widespread than previously

[1] HSBC, a primary banking company of China, started as a bank for the opium trade, gaining notable wealth after the second opium war. Also see https://www.pbs.org/wgbh/pages/frontline/shows/heroin/etc/history.html

thought. As to true societal risk, witnesses generally (and, in hindsight, accurately) perceived a vulnerability toward the progressive use of the drug (i.e., escalation of *Cannabis* use over time).

A few limitations were acknowledged in the data as it was reported at the time, in that understanding the full societal effects of *Cannabis* was acknowledged to be difficult and, to be properly understood, one needed to more-appropriately consider not only the administration form, but frequency of usage and potency. Indeed, in the 1800s, *Cannabis* preparations (most often as ethanolic tinctures) were frequently concocted with opium, chloroform, and other drugs of potential habit, making it *extremely* difficult to determine whether resulting adverse effects of its administration were due to *Cannabis* itself. Such "polypharmacy" endures, and remains as problematic a confounding variable today as it was over 130 years ago. Poorly controlled, unregulated hemp-derived cannabinoids, chemically synthesized cannabinoids, and other nondrug component adulteration (pesticides, mold-inhibitors, etc.) continue to plague the modern marketplace. (Simon et al. 2023; Zawatsky et al. 2024)

At that time, the main conclusions of the Indian Hemp Drugs Commission stood in dramatic contrast to the diminutive view of *Cannabis* in nineteenth century Britain. Indeed, a member of Parliament had called for the inquiry because he believed, "*ganja"* was "*far more dangerous than opium.*" Much like today, *Cannabis* commerce in Britain was *somewhat* legal, but limited. And much like today as well, officials and business owners were worried that *Cannabis* use would erode the efficiency of the working class. (Al-Asmari 2024; Walters et al. 2018; Howard and Osborne 2020; Kight et al. 2019) Accordingly, the years that immediately followed the publication of the Commission's report saw a greater emphasis placed not only on drug prevention, but rather threatened outright prohibition. At a 1924 meeting of the International Opium Conference, representatives of Turkey spoke out on "chronic hashishism"—a statement which helped to push both policies and public perception against the comparatively anodyne findings of the Indian Hemp Commission. As we would see for the next hundred years, and most significantly under the Nixon administration in the United States, plans to address the opioid/opium epidemic would be repeatedly shelved, in favor of advancing an overt criminalization of another plant entirely. (Marcu and Schechter 2024)

2.2 The 1925 Panama Canal Zone Report

Concerned that *Cannabis* might undermine U.S. soldiers' and law enforcement performance in Panama, the U.S. government convened a committee to investigate "*marijuana,*" thus launching America's first significant foray into evaluating the deleterious effects of the *Cannabis* plant and its medicinal products. At that time, common local government statements were being made along the lines of "one or two puffs on *marijuana* cigarettes could cause dramatic changes if not permanent changes to the body and mind." U.S. military authorities appointed a commission and undertook a proper investigation, conducting a series of clinical studies

including observations of civilians, physicians, and policemen after smoking *Cannabis*, albeit in controlled settings. Culminating in a report published in 1925, the authors concluded "*there is no evidence that marihuana, as grown and used [in the Canal Zone] is a 'habit-forming' drug in the sense in which the term is applied to alcohol, opium, cocaine, etc., or that it has any appreciably deleterious influence on the individuals using it.*" While no action was recommended to be taken to prevent either *Cannabis* use or sale in the Panama Canal Zone report, it should be noted that historians have since theorized that the potency of the *Cannabis* plant products used in their studies appeared to have been quite low— in point of fact, some experimental tests witnessed volunteers smoking up to *12 Cannabis* cigarettes each *(*!). (Abel 1982)

Two subsequent formal inquiries produced nearly identical findings. Based on these reports and inquiries, the possession of *Cannabis* by military members was allowed in 1926. Authorities, however, refused to accept the findings of their own officially appointed committee, and the investigation was met with heavy criticism and outright hostility. (Abel 1982) Accordingly, the impact of the Panama Zone Report on informing either policy or educational interventions appears to be historically negligible. The modern-day utility of the Panama Report *could* be seen as educationally useful, though, in showing how using products with lower THC percentages might significantly steer findings and conclusions of such research—and that drug materials or products used in experimentation should always be well-defined by, and controlled for, cannabinoid profile and potency.

2.3 The 1929 Preliminary Report on Indian Hemp and Peyote Issued by U.S. Surgeon General Hugh S. Cumming

In the late 1920s, members of Congress claimed that their constituents were "greatly concerned" that "*marihuana*" was being sold to schoolchildren. This claim motivated a formal report from the fifth Surgeon General of the United States, Dr. Hugh Smith Cumming. From the outset, the report clearly sought to stigmatize people who smoked "*marijuana*," by using dehumanizing language including, but not limited to, "dope fiend" and "immoral street user" to replace more accepted, professional terms such as "addict," "dependence," or "addiction." The report, similar to past documents, also did not take into consideration *either* potency or frequency of *Cannabis* use as a factor in discerning stated *Cannabis*-associated risks. Had the author, an otherwise noted healthcare professional, utilized more appropriate professional or scientific terminology, it may have eased the report's acceptance by the medical community.

Reassuringly, *most* clinicians of the time (and surely presently) would not rely on educational materials using overtly demonizing terminology to instruct their own standard-of-care. However, Dr. Cumming's use of dehumanizing and racist language surely caused significant damage to the quality of the prevailing social

discussion (and educational efforts) surrounding *Cannabis*. The Cumming Report managed to misrepresent both the pharmacological effects and historical scientific research, in order to lend significant credence to accepted and hyped-up fears of the time, which were already largely political in nature. Cumming's report was very closely followed by the passage of the 1929 Narcotic Farms Act, authorizing the formation of two "narcotic treatment farms[2]," and resulted in the listing of "Indian hemp" as a "*habit-forming narcotic*" for which a person could/should receive treatment. **This was the first time the hemp plant (aka *Cannabis*) was referred to as a "narcotic" in Federal legislation.**

Created by the 1929 Narcotics Farm Act, the data generated by such government-funded "narcos" prisons, at very least, clearly demonstrated the value of direct treatment modalities and better scientific understanding of individual drug addictions. The prisons were eventually shut down in the 1960s, but only after the prisoners' rights movement deemed it unethical to experiment medically without consent on the patients.[3] Despite emergent evidence supporting harm-reduction practices, the political will to effectively address the issue of drug addiction did not seem to exist at the time, leaving communities to fend for themselves.[4]

2.4 1944 La Guardia Committee: "The Marijuana Problem in the City of New York"

Responding to ongoing sensationalistic, racist, and classist accounts of experiences with individuals under the influence of "*marihuana*" in New York City, Mayor Fiorello La Guardia requested the National Academies of Sciences to conduct an in-depth investigation of the drug in 1938. The La Guardia Committee's report concluded that the claims of "*marijuana... caus[ing] insanity, irrevocable damage, or addiction like opiates were unfounded and wrong.*" Further, the Committee also concluded that *Cannabis* smoking does not lead to the use of other drugs such as cocaine and opium, undermining the so-called "gateway" theory—a mischievous untruth that remains in more propagandistic materials to this day. The findings of the La Guardia Committee's report stood in stark contrast to published public warnings and educational messages issued *about* the report. At the time, paranoid fears about "*marijuana* abuse" were derived and stoked from the personal views of the particularly outspoken head of the newly formed Federal Bureau of Narcotics, Harry Anslinger. Anslinger's most popular claims were simply not based on any empirical data and many of his quotes about *Cannabis* are often cited as a modern

[2] A term for service prisons, and it remains unclear who operated these work camps in Kentucky and Texas—the Kentucky Narco transitioned seamlessly into a federal prison that is still operating today.

[3] The author William Burroughs wrote about his experiences in the Kentucky Narco.

[4] https://www.forbes.com/sites/claryestes/2019/11/18/the-narcotic-farm-and-the-little-known-history-americas-first-prison-for-drug-addicts/

source of outrageousness or hilarity. Here are a few, perhaps lesser-known Anslinger quotes:

- *"Anyone who does anything to try to legalize marijuana should be taken out and shot."*
- *"Marihuana leads to pacifism and communist brainwashing."*
- *"Marijuana is the most violence-causing drug in the history of mankind."*
- *"Those who use marijuana are simply too lazy to get off their couches and commit crimes."*
- *"The answer to the problem is simple—get rid of drugs, pushers and users. Period."*[5]

10/10. Amazing. No notes.

2.5 The 1972 Report on the National Commission on Marihuana and Drug Abuse

This 1972 report from the National Commission on *Marihuana* and Drug Abuse, which came to be known as the "Shafer Commission," was arguably the most thorough and comprehensive review of *Cannabis* knowledge to date. It was commissioned by then-President Richard Nixon after the passage of the Controlled Substances Act of 1970. The Shafer Commission's formal mandate was to recommend an appropriate "scheduling" of *Cannabis*, though Nixon's goal was quite clearly to unearth a formal justification for *Cannabis's* eventual designation as a so-called "Schedule 1" substance—i.e., a substance with no known medicinal use but a high-societal potential for abuse. Much to Nixon's chagrin, the Shafer Commission published a brilliant scientifically comprehensive report, entitled "*Marihuana*: A Signal of Misunderstanding" in March of 1972, which recommended decriminalizing *both Cannabis* use and even "casual distribution of small amounts of *marijuana.*" That is, the Commission deemed that even small-scale distribution should not be considered to be a criminal act. Among the conclusions of the 1183-page report, the Shafer Commission found that consuming the plant does not induce any significant physical dependence, though heavy-long term users can develop a *psychological* dependence—implying that *Cannabis* dependence is an appetitive process, akin to gambling or compulsive eating, as opposed to a typically "addictive" drug. The report also stated that *Cannabis*, "*does not constitute a major public health threat*" because there was no significant evidence that it leads to harder drug use such as heroin—once again contradicting the much-vaunted gateway theory. The Schafer Commission suggested there *was* indeed a heroin and opioid epidemic ongoing, and that needed to be addressed. Nixon was reportedly infuriated.

[5] https://www.ncbi.nlm.nih.gov/books/NBK234755/

The Shafer Commission's findings were largely ignored by regulators responsible for shaping subsequent Federal drug policies, as is evident from *Cannabis's* seemingly immutable (and present-day, at least at the time of writing) designation as a Schedule 1 material. We reiterate: the Shafer Commission released its recommendation that *Cannabis* be *decriminalized and remain unscheduled* immediately before *Cannabis* was listed as the highest tier of controlled substance in 1972. Only 11 states had reduced *Cannabis* penalties by 1978, and during the 1980s, *Cannabis* reform hit a wall as mandatory drug testing became more common and legislative changes sought to focus solely on minimal, medical access to *Cannabis* (permitting neither adult, nor "recreational," uses).

2.6 2017 National Academies of Science, Engineering, and Medicine

In 2017, the National Academies of Sciences, Medicine, and Engineering (NASEM) issued a cautious survey of published literature and an update to their 1999 report. (Abrams 2018) The authors sifted through all available research to find systematic reviews—over *10,000* abstracts were considered, including over 100 clinical reports. The authors reported that *Cannabis* is an effective treatment for many types of chronic pain, multiple sclerosis, and significantly ameliorates chemotherapy-induced nausea and vomiting. The Academy's report further stated, "*There is conclusive or substantial evidence that Cannabis or cannabinoids are effective… [for] the treatment for chronic pain in adults [, and]… [as] antiemetic in the treatment of chemotherapy-induced nausea* [*Cannabis*] *and* vomiting [oral cannabinoids]" and "*for improving patient-reported multiple sclerosis spasticity symptoms* [oral cannabinoids]." The report additionally states that there exists moderate evidence for *Cannabis* or individual cannabinoids being effective for improving short-term sleep outcomes in individuals with sleep disturbance associated with obstructive sleep apnea syndrome, fibromyalgia, chronic pain, and multiple sclerosis (particularly cannabinoids, primarily nabiximols).

Given the perspective of timing, the National Academies' report should likely have also addressed the final factors preventing a Federal rescheduling of *Cannabis*. Indeed, the Drug Enforcement Administration (DEA) themselves demonstrated, in their 2016 denial of a petition for a rescheduling hearing, that *Cannabis* fulfills, fully, at least *6 of the 8* factors required for rescheduling. (Pennington et al. 2023; Marcu 2016) However, DEA has long contended that there is, somehow, no recognized medical use and that its use is not accepted by health professionals—and, as such, *Cannabis* simply cannot be granted a rescheduling hearing. The DEA was sued to update their own website's "*Cannabis* health information" shortly after this announcement, but the lawsuit was met with an arguably anti-climactic end, as it coincided with its *own* internal review process and so the website was updated due to a normally scheduled review process. A nonsatisfying outcome, to be sure, but

more recently—and likely due to the National Academies' report, the FDA's begrudging approval of THC and CBD pharmaceuticals, and the recent Health and Human Services recommendation to reschedule the cannabinoids—there is now a highly-publicized societal recognition of the medicinal utility of *Cannabis*-based products afoot. (Marcu and Schechter 2024; Pennington et al. 2023)

2.7 Summary of Historical Themes

Safety findings within the handful of historical scientific reports summarized above were largely ignored during subsequent Federal drug policy reforms, from when *Cannabis* became illegal nationally in 1937 to decades later when it was deemed a Schedule 1 substance by President Nixon. Publicly available "sciencey" sources tend to often portray somewhat conflicting, or confusing, data about the societal or individual harms of cannabinoid use or abuse. In addition, there is room for improvement in how cannabinoid researchers communicate their findings to the general public, including diverse audiences. This communication gap may contribute to instances where data is overlooked or misinterpreted. Once significant findings are peer-reviewed and published, researchers often face challenges in effectively disseminating their work to nonspecialist audiences or addressing misunderstandings that may arise. These challenges can inadvertently influence decisions made by policymakers and regulators. The substantial time and effort required to compile data, combined with the complexity of interpreting results, may also limit the practical utility of this information when it is needed most.

3 The Landscape of the *Cannabis* Education Ecosystem

> *The illiterate of the 21st century will not be those who cannot read and write, but those who cannot learn, unlearn, and relearn.* (Alvin Toffler)

The modern information landscape surrounding *Cannabis* knowledge is complex and dynamic, shaped by a rapid and piecemeal evolution of legalization, research findings, delivery technology, social norms and public interest. A wide array of platforms—social media, podcasts, artificial intelligence/machine learning, online education programs, sales and industry materials, scientific publications, government agencies, private organizations, and academic institutions' websites—*all* contribute significantly to shaping both public and individual understanding of *Cannabis* and the cannabinoids. As *Cannabis* becomes an increasingly significant—and less taboo—global topic, the demand for accurate, accessible, and comprehensive education has never been more critical. Navigating this landscape is essential for educators, policymakers, and the public alike, to foster a more informed and responsible society.

One of our major challenges is to recognize the fragmentation of information sources and the widespread and destructive prevalence of misinformation. With *countless* voices and platforms offering varying perspectives on *Cannabis* and the cannabinoids, it can be challenging for any single member of the public to choose, no matter what level of education or background, wisely—to differentiate between credible information sources and potentially misleading or biased content. This diversity of source materials, however, also presents an *opportunity*—to build a more nuanced, more comprehensive educational ecosystem. By integrating peer-reviewed scientific research and appropriately sourced-and-vetted government guidelines into approved academic programs, and even industry insights, we *can* create a well-rounded understanding of cannabinoid pharmacology and use that meets the diverse needs of various audiences.

A basic education about cannabinoids is crucial to maintain both personal health and societal stability. As has been proven historically, deliberately propagandistic or malevolent, racist or classist misinformation can shred the fabric of our society. A typical education extends well beyond traditional classrooms, and surely includes informal learning through community interactions, personal experiences, and a diversity of appropriate social media consumption. The variety of ways to educate oneself are manifold—whether through YouTube videos, memes, podcasts, or scientific papers—and that many people are getting an education from YouTube *comments sections* illustrates the varied nature of our modern challenges.

Societal movement toward the safe legalization of the cannabinoids has spurred development of well over 100 separate educational programs at various institutions globally, ranging from community colleges to specialized collegiate certifications in *Cannabis* studies. (Slaven 2019; Best Cannabis Colleges 2024) Such programs aim to provide accurate, science-based knowledge that addresses gaps resulting from the rapid changes in *Cannabis* laws, public perception, and training needs for entry level employees and entrepreneurs in the cannabinoid industry. A growing legitimacy of *Cannabis* business has necessitated a corresponding evolution in educational offerings, ensuring that information about *Cannabis* and cannabinoid medicine is grounded in evidence and is made readily accessible to all those who seek it. (U.S. Cannabis Legalization and the Emergent Educational Landscape 2024)

3.1 Business and Marketing: The Role of Businesses in Providing Accurate Information About Their Products

Businesses have a particularly critical role in educating consumers about their products. We expect *non-Cannabis*, *non*-hemp industries (i.e., food, pharma, biotech, etc.) to provide detailed, accurate, accessible information—and even educational materials—about their products...from content to contraindications and potential warnings, to proper dosing/portion regimens, to safety profiles. (Simon 2024) It would seem that this should surely also apply to cannabinoid pharmaceuticals. As cannabinoid-containing products move further into the public mainstream,

businesses should unquestionably bear some responsibility to ensure that their marketing strategies are not only compliant with regulations but also provide informative, and truthful, guidance—again, as we normally require or expect for virtually all other products. (Romm et al. 2023; Nicholas et al. 2020; Cavazos-Rehg et al. 2019) This effort involves not only educating consumers about product risks, intended usage, and the distinctions between various different *Cannabis* products, but also how to translate and properly understand labeling—such as how total cannabinoid content might correlate to terms of perceived potency and effects. Businesses that prioritize consumer education as part of their marketing strategies can help mitigate risks associated with cannabinoid misuse. Conversely, broad, inappropriate claims of panacea-like qualities need to be avoided. We may well anticipate that emerging, riskier hemp or cannabinoid products require a post-marketing-type of educational commitment. Unfortunately, regulation of stated claims are often dismissed as allowable marketing tactics, as it is with so many other consumer products. It's then left to the consumer to communicate tales-of-woe to the seller—and as such, the ability to communicate experiences with the actual producer of the product may be lamentably inadequate. As the *Cannabis* product landscape continues to grow, and until a better option is made available, it is increasingly beneficial for consumers to be made aware of the FDA's MedWatch reporting system, either online (https://www.fda.gov/safety/medwatch-fda-safety-information-and-adverse-event-reporting-program) or by phone, at 1–800-FDA-1088. If one is uncomfortable with (or opposed to) the U.S. government tracking their drug use, there are nongovernmental platforms, operated by health professionals, that collect data anonymously (e.g., www.budsinfo.com).

3.2 High School and Young Adults: Importance of Age-Appropriate Education for Teenagers and Young Adults

The importance of age-appropriate education for teenagers and young adults cannot be overstated. With *Cannabis*- and hemp-derived products becoming both more prevalent in the media and more freely accessible, the need for effective, truthful, realistic early education in schools is more critical than ever. Educational institutions are *slowly* beginning to incorporate *Cannabis*-related content into both health and science curricula, country-wide, and in varying degrees, in an attempt to provide students with a balanced understanding of cannabinoid effects, risks, and legal status. (Wabwire et al. 2022) Such education is *essential* to equip young people with the knowledge they need to make informed decisions. A key positivity to this effort is that young people now have significant advantages over previous cohorts, in that the ability to fact-check and find both dissenting and informative opinions about this, or any other, drug or product has never been easier. Alas, as with all things, educators need to be both careful and science savvy, as being able to capably navigate this complex discussion risks losing credibility with the target audience.

3.3 Pregnancy and Pediatric Exposure: Specific Educational Needs for Expecting Mothers and the Risks Associated with Pediatric Exposure

Expectant parents are also particularly in need of targeted education on the risks associated with *Cannabis* and cannabinoid use, both during pregnancy and concerning pediatric exposure. (Lo et al. 2023; Berg et al. 2023) Recent articles providing meta-analysis of neonatal outcomes have similarly underscored the importance of tailored educational programs to address these specific concerns. Irrespective of systematic review of historical findings being unable to significantly link any effects of prenatal cannabinoid exposure to morbidity or mortality, it can continue to be rationally argued that the optimal exogenous cannabinoid exposure of a neonate is zero. Healthcare providers, educators, and public health campaigns must work together to disseminate accurate information, particularly as the societal norms around *Cannabis* continue to shift.

3.4 Illicit vs. Regulated Cannabis: Educating the Public on the Differences and Potential Risks

One further critical educational challenge is helping the general public to understand the differences between illicit and regulated *Cannabis*. (Botelho et al. 2024; Mallinson et al. 2021) Regulated markets are designed to ensure product safety and consistency, whereas illicit markets may still pose considerable and significant risks in comparison, due to unknown product contents, potency and the potential for adulteration and/or contamination. (Marcu and Simon 2022) While profit maximization is clearly the shared goal for both regulated and illicit producers, it is clear—as with all other products—that the value-added component of regulation provides much-needed guardrails to an otherwise potentially dangerous marketplace. Education campaigns should surely emphasize this significant distinction, to urge consumers to make safer choices, and hopefully understand the benefits of purchasing cannabinoid materials from legal sources.

The inability of authorities to be able to communicate differences between hemp (e.g., any product with less than 0.3% THC), regulated *Cannabis*, and illicit drugs continues to be detrimental to the publics' health, and is largely responsible for the wide availability of cannabinoidergic designer drugs (aka artificially derived cannabinoids). Availability of "hemp-derived," chemically altered products—generated after the President Donald J. Trump administration's update of the Farm Bill created an entirely novel industry, with poor-to-absent regulation and unbridled production and sale of synthetically derived compounds—enjoys liberties government would never afford to a proper pharmaceutical or licensed *Cannabis* company.

4 Clinical Education: The Global Deficiency

> *The greater the ignorance, the greater the dogmatism. The first duty of the physician is to educate the masses not to take medicine.* (Sir William Osler, MD)

Clinical cannabinoid education is an area of expertise that has been notably deficient, historically, despite the increasing acceptance and legalization of *Cannabis* for medical uses in so many and various regions. (Sajdeya et al. 2021) Some significant challenges that health care professionals face are specific to cannabinoid education in particular—including, but not limited to, issues of legal challenges and institutional, local or even religious barriers to educational materials and content. Local administrative pressures to curtail (or entirely omit) cannabinoid pharmacology education surely hinder any true comprehensive understanding of *Cannabis* use in medical practice.

As one example, a 2024 pilot study was conducted to assess Health Care Provider's (HCP's) confidence in their knowledge of cannabinoids, revealing that over *57%* (!) "lack confidence," while many rely primarily on news and social media to provide *Cannabis*-related information. (Marcu 2024) Nearly half (47.4%) of the surveyed HCPs reported being hesitant to discuss medical cannabinoid utilization with their own patients, due to either regulatory constraints, institutional policies, or an overall lack of adequate training. Such alarming data is in alignment with previous research on the knowledge and attitudes of HCPs regarding this issue. (Denneler et al. 2024; Worster et al. 2023; Felnhofer et al. 2021; Makki et al. 2022; Skelton et al. 2022) This clearly underscores a need for more systematic improvements, in both cannabinoid education and policy, to better provide support, education and patient-provider communication about cannabinoid pharmacology and *Cannabis*-based products, both writ large and regionally.

4.1 United States

There are nearly *50 different ways* that the United States presently goes about educating medical doctors about cannabinoid medicine—and, fully, *another* 50+ different ways that those states regulate a physician's ability to officially recommend cannabinoidergic products and/or certify a patient's access to a medical dispensary.[6] Methods and requirements range from literally nothing-at-all to *very* specific topics delivered via specific educational modules. While some states dictate specific requirements from specific providers, there are a number of educational programs available that are geared towards clinicians and nurses, including particularly outstanding examples made available through US-based medical societies. Table 25.1

[6] Note that prescriptions for medical *Cannabis* or *marijuana* products do not exist. It is common myth or falsehood often perpetrated that doctors write prescriptions for *marijuana*. The only prescriptions may relate to this are for Marinol or Epidiolex, which are FDA approved products.

provides an overview of requirements by state, and depicts the vast diversity of the educational landscape presently available to medical professionals.

4.2 Europe

Similar to healthcare providers in the United States, European doctors have access to a wide variety of educational resources to learn about *Cannabis* and the cannabinoids. From private and public social media groups and research societies (e.g., the International Cannabinoid Research Society (www.icrs.com), the International Alliance for Cannabinoid Medicines (cannabis-med.org), etc.), to universities and online platforms (e.g., www.prc-trials-plus.com), diverse educational sources can provide medical practitioners a plethora of high-quality information. As the industry continues to evolve, continuing education and training is essential to ensure the continued safe and effective use of medical cannabinoids in patient care throughout Europe. What follow are a few select summarized examples.

4.2.1 Austria

In Austria, the use of medical *Cannabis* is permitted, but it is strictly regulated. Doctors participating in cannabinoid medicine are obligated to be knowledgeable about the Austrian Narcotics Act, and are generally expected to have some manner of specialized and/or advanced training in cannabinoid medicine. The Austrian Chamber of Pharmacists has been actively involved in advocating for medical *Cannabis* use, emphasizing a need for proper education amongst healthcare providers to continue to ensure safe and effective treatment modalities made available to the populace. Specific mandated educational programs or certifications for prescribing or recommending medical *Cannabis* products, however, are not extensively detailed in currently applicable regulations.[7]

4.2.2 France

France also has a highly regulated medical *Cannabis* program. As of the most recent program updates, doctors who wish to prescribe medical cannabinoids must participate in a National experiment that involves specialized training modules and education. This specific training covers therapeutic uses of *Cannabis*, appropriate dosage, education concerning immediate and emergent side effects, and the legal framework within which they are able to prescribe *Cannabis*-based medicine. Participation in this program is mandatory, and is arguably essential to ensure that doctors are fully

[7] https://cms.law/en/int/expert-guides/cms-expert-guide-to-a-legal-roadmap-to-cannabis/austria

Table 25.1 Descriptions of educational requirements and requirements for recommending or certifying

State	CME required	Education description	Requirements to recommend or certify
Arizona	No	Does not have specific education requirements; however, a free 5-h CME is available for clinicians	No specific educational requirements. Doctors must fill out Arizona's MMJ Physician Certification. Any medical, osteopathic, or naturopathic doctor who has completed their state certification can in turn certify you to use medical cannabis
Maryland	Yes	Does not have specific education requirements. MD does have an approved CME course available: Describe the current landscape of recommending medical cannabis, evaluate the evidence for treating patient conditions with medical cannabis, discuss the pharmacology of cannabis and its effect on the endocannabinoid system, analyze use disorders and other patient precautions for recommending medical cannabis, analyze the effects of dosing and common delivery methods of medical cannabis, and examine Maryland-specific considerations for discussing medical cannabis	To legally recommend cannabis in Maryland, any licensed doctor must (a) be registered to prescribe controlled substances and (b) complete a 2-h online course on the fundamentals of cannabis medicine. After that, they're invited to register as a provider with the state. Maryland requires such providers to review every patient's medical records, complete an in-person assessment of their history and current condition, among other steps
Minnesota	No	Does not have specific education requirements	Physician assistants and advanced practice registered nurses can certify patients (in addition to doctors). The process for healthcare providers to get certified is straightforward: After they create an online account with the state's Medical Cannabis Registry, they're permitted to certify potential patients
New Mexico	No	Does not have specific education requirements	Any healthcare provider licensed to prescribe and administer controlled substances can recommend medical cannabis. This is done on a case-by-case basis using the state's Medical Cannabis Patient Application

(continued)

Table 25.1 (continued)

State	CME required	Education description	Requirements to recommend or certify
New York	Optional	Courses include the following content: The pharmacology of cannabis; contraindications; side effects; adverse reactions; overdose prevention; drug interactions; dosing; routes of administration; risks and benefits; warnings and precautions; and abuse dependence	Complete at least a 2-h course before they can begin certifying patients for medical cannabis. Seven options as of writing this
Ohio	Yes	Briefly discuss the history of marijuana in the US and Ohio and currently approved FDA drugs Understand the requirements for both prescriber and patient to appropriately recommend medical marijuana. Appropriately diagnose conditions approved in the state of Ohio. Navigate the OARRS system for patient registration. Discuss the endocannabinoid system. Understand the differences between the "species" of plants. Describe the physiologic/pharmacologic profile of marijuana and dosing. Identify clinically significant adverse effects and drug interactions that may occur with cannabinoids	Apply for a certificate to recommend from Ohio board of pharmacy. The applicant has completed at least 2 h of continuing medical education in a course or courses certified by the Ohio State Medical Association or the Ohio Osteopathic Association that assist physicians in both the following: (a) Diagnosing qualifying medical conditions as defined in section 3796.01 of the Revised Code (b) Treating qualifying medical conditions with medical marijuana, including the characteristics of medical marijuana and possible drug interactions
Washington	Yes	The proper use of marijuana (cannabis), including the pharmacology and effects of marijuana (e.g., distinction between cannabidiol (CBD) and tetrahydrocannabinol (THC); methods of administration; and potential side effects or risks)	A healthcare practitioner issuing authorizations or valid documentation for the medical use of marijuana on or after the effective date of these guidelines should complete a minimum of 3 h of continuing education related to medical marijuana
West Virginia	Yes	Course introduces the reader to the endocannabinoid system and its interaction with the components of the cannabis plant and addresses administration, therapeutic use, drug metabolism, physiologic and cognitive effects, potential risks, and drug interactions	Must complete a 4-h course established by the Dept. of Health Resources' Bureau for Public Health

Several examples are listed for comparison. Each state seems to have its own unique process and requirements. The following states have no specific educational requirements: Al, AK, CA, CO, CT, DE, DC, GA, GU, HI, ID, IL, IN, IA, KS, KY, LA, ME, MD, MI, MS, MO. MP, MT. NC, ND, NE, NH, NJ, NV, OK, PR, SC, SD, TN, TX, UT, VT, VI, VA, WI, WY. No state seems to have a course available on data collection or analysis
Figure courtesy of Marcu et al. ICSB 2024 Oxford Mississippi April 2024 Poster 41; Publication Number 1037

equipped to prescribe medical *Cannabis* responsibly and in compliance with French law. The French National Agency for the Safety of Medicines and Health Products (ANSM) oversees all cannabinoid medicine regulation.[8]

4.2.3 Germany

Germany legalized medical *Cannabis* in 2017, and all prescribing doctors must adhere to strict regulations set forth by the German Cannabis Agency. Specialized training covering medical uses of *Cannabis*, cannabinoid pharmacology, and laws governing the German regulatory environment is mandated for all medical professionals involved in *Cannabis* treatment protocols. Specific medicinal cannabinoid training serves to ensure that healthcare providers fully and appropriately understand approved therapeutic applications, dosing guidelines, and potential side effects of using cannabinoids as medicine. In addition, medical *Cannabis* must be prescribed within the framework of both the German Narcotics Act and the newly proposed Medicinal *Cannabis* Act, which aims to better streamline and comprehensively regulate the process.[9]

4.2.4 Spain

Spain's approach to medical *Cannabis* is evolving, with some regions of the country piloting governmentally controlled plant-to-patient medical *Cannabis* programs. Doctors who wish to recommend medical *Cannabis* typically need to undergo some manner of specialized training in cannabinoid medicine, which typically includes, but is not necessarily limited to, a proper understanding the endocannabinoid system, therapeutic and historical uses, and the legal aspects of prescribing cannabinoids to patients. The Spanish Agency of Medicines and Medical Devices (AEMPS) oversees these programs, in an effort to ensure that medical professionals are adequately trained. As Spain's regulatory framework around medicinal *Cannabis* continues to develop, more standardized training requirements are expected to be implemented nationwide.[10]

[8] https://cms.law/en/int/expert-guides/cms-expert-guide-to-a-legal-roadmap-to-cannabis/france

[9] https://cms.law/en/int/expert-guides/cms-expert-guide-to-a-legal-roadmap-to-cannabis/germany

[10] https://cms.law/en/int/expert-guides/cms-expert-guide-to-a-legal-roadmap-to-cannabis/spain

5 Case Studies and Examples of Educational Intervention Failure *OR* How to Lose Your Target Audience and Entirely Fail to Change Behavior

I think it's important that the science keeps up with the public debate. (Michelle Glass, Ph.D.)

Regarding social awareness and educational programs meant to protect individuals from the scourge of the health risks of drug use, there is already plenty of data available about what works—and what, often, does not. (GAO 2006; Hornik et al. 2008; Palmgreen et al. 2007; Scheier et al. 2011) Overall, the impact of implementing educational and intervention programs—specifically regarding adolescent use—is difficult to quantify. At worst, drug education programs can be somewhat overzealous—as the kids would say, *cringe*—and can even be correlated with an *increased* prevalence of recipients of said "education" using a particular drug, *Cannabis* included, particularly among specific nonreceptive populations. (Johnson et al. 2017; Dilley et al. 2019; Bailey et al. 2023; Mauro and Feng 2016) Such programs would better devote future resources to focus on formative development of more-appropriate, relatable materials—"cool" anthropomorphized and pantsless, jacked lions[11] with platitudes and/or broken eggs in pans *still* have just about as much to do with navigating actual drug-taking scenarios as they ever did. To properly ensure educational materials have their intended (and measurable) effects, sincere messaging that does not inadvertently alienate potential users (i.e., the target audience) need be effective, educationally interesting, *and* informed instead of resorting to hyperbolic language and silly aphorisms. There's *plenty* of interesting, historically notable information out there to keep our youth attentive to the conversation.

Starting in 1992, the U.S. Office of National Drug Control Policy's (ONDCP) launched a drug education effort that spent over $2 *billion* USD over only a 5-year period. (Palmgreen et al. 2007; French 2005) Television-based commercials and youth-targeted school program-based materials were generated, the bulk of which included intentionally heavy-handed "hard-hitting anti-*marijuana* messaging"-based programming. Indeed, and inadvertently, the ONDCP's efforts demonstrated an *inverted* dose–response effect on participating students—the more antidrug ONDCP materials students were exposed to, the more it seems to have normalized youth drug use. ONDCP programming of the "*Marijuana Initiative*" featuring *only* "negative outcomes of *marijuana* use," and yielded an *increased* the risk of initiating use amongst those students exposed. College students neither observed nor reported any of the effects purported by the ONDCP messaging amongst their "*weed-smoking criminal*" roommates and friends, while they did not seem to all morph into either immediately violent criminals nor turn to harder drugs with any statistical consistency. Perhaps telling of the *truth* in drug-education materials might

[11] Daren the D.A.R.E Lion; a muscular, humanoid anthropomorphized feline that walks upright wearing only a T-shirt.

perhaps be more palatable, in hindsight—indeed, there are more than enough *true* examples of the deleterious effects of chronic drug abuse.

In 2015, New South Wales, Australia's campaigns such as "stoner sloth,"[12] like Colorado's attempt at a humorous THC-DUI driving awareness campaign,[13] had similar effects—of alienating the target audience. The Colorado ads, for example, simply did not resonate with the intended audience, because they were seen, rightfully so, as both stereotyping and ridiculing all users (Davis et al. 2016). Additional use of heavy-handed and hyperbolic language tended to ultimately weaken the credibility of the messaging source. *Especially* with teens, educational materials need to be "real," need to be trustworthy, and need to be interesting...minimally...or adolescents simply will not listen. Worse still, teenagers can prove to be admirable "BS detectors"—and, as such, are likely to be an easily-lost audience. Couple to that an instant, easy access to the internet, social media, and machine learning (i.e., generative artificial intelligence programs that can provide references to counter arguments of nearly any topic), and educational materials with any hope of usefulness have to be legendarily clever. Accordingly, educators must be not only familiar with actual data, historically relevant anecdotes, and scientific process, but surely need to consider relevant communication strategies for different age-groups and audiences. One overarching principle seems be relevant to all student experiences: if you alienate your target audience, they may just do the *opposite* of your guidance...or simply will not pay attention to you at all, never to return.

Historical antidrug campaign failures (e.g., D.A.R.E., Reefer Madness, etc.) from positions of governmental leadership well illustrate messaging doomed from the outset, heavy-handedly focusing on emotions and personal opinions, as opposed to reality or, forbid, prevailing science. Indeed, the Director of NIDA appeared on stage a few years ago and legitimized a book sensationalizing scenarios positing *Cannabis* as the cause of *psychosis in the global population. (*Berenson 2019*;* Martin 2019*)* The book has since been criticized as racist, alarmist, and as confusing correlation and causation while cherry picking and misrepresenting research findings—but why let that get in the way of a good story? (Martin 2019; Agenda C on the HE of MAER and R, Practice B on PH and PH, Division H and M, Medicine NA of S Engineering 2017) Even in a statement seemingly expressing *support* for Florida's *Cannabis* policies, ex-president Donald J. Trump just warned (at time of writing): "...no one should grieve a loved one because they died from fentanyl-laced marijuana." (Trump Signals Support for Marijuana Legalization in Florida 2024)—an entirely *fictional* scourge. Again, it would seem that there are *more than enough* true tales of stark human tragedy concerning drugs-of-abuse to rely on as legitimate, and instructive, cautionary tales—without having to resort to silliness.

Since describing the quantum of negative effects from long-term use of *Cannabis* remains a challenge, things like flatly stating that *Cannabis causes* psychosis (as opposed to psychosis being a significant factor in predisposal to overall drug use)

[12] https://en.wikipedia.org/wiki/Stoner_Sloth

[13] https://www.ghsa.org/resources/colorado-drive-high-get-dui

may inadvertently discredit the speaker and organizations associated with the statement. This is not to suggest it's *all* bad news—to their great credit, the DEA has recently taken significant steps to bolster their credibility and updated their educational materials about stated negative effects of *Cannabis* not supported by scientific evidence. (Marcu 2016; DEA 2017)

Do not get it twisted—there are legitimate *and* considerable dangers inherent to *Cannabis* use. There should be no debate that *Cannabis* can acutely impair cognitive and reflexive motoric performance, yet it lingers amongst users who, for example, think they can predict their own level of impairment, or lack thereof. This is perhaps most readily apparent as it relates to driving motor vehicles while under the influence. (Marcotte et al. 2022; Burt et al. 2021) Hence, the simple "*Don't drive if you're high*" terminology confounds messaging. Research has shown that users can be impaired without being high, and users are notoriously bad at predicting their own level of impairment or "high-ness." (Marcotte et al. 2022; Burt et al. 2021) Why ask the target audience to make any self-assessment, one that they will likely fail to determine with any degree of accuracy? Considerably worse is when this ultimately influences behavior—*I do not feel high therefore I will drive*—when in fact, impairment can persist long after a "high" has worn off. It should be well-established by now that designated drivers are as important with cannabinoids as with any other perception-distorting compounds.

An educational need exists to help people understand risks regarding any lasting cognitive impacts, and the true potential impacts upon mental health. (Lorenzetti et al. 2019; Lorenzetti et al. 2016a; Lorenzetti et al. 2016b) While the issue of heavy *Cannabis* use on the developing brain and predictable negative outcomes remains contentious (Lamanna-Rama et al. 2024; Sadaka et al. 2023; Ma et al. 2022; Chye et al. 2020), communicating the risks associated with *Cannabis* and brain health further highlights the need for well-developed educational materials.

It may sound apple-pie-and-motherhood-ish to make statements everyone will agree with—such as that public health campaigns regarding *Cannabis* will need formative research funding, or that programs should be fact-based, avoiding unnecessary stereotypes and judgmental messaging. However, knowledgeable people that agree with the former, in statement, may well disagree the latter, in practice. The process by which we determine our vocabulary and *what* areas to focus our strongest educational efforts should also be scientific and pragmatic. To be sure, assessing the benefits and risks of trying to change a majority or long-held belief is where we must ask—where is there "room to move"? What fraction of the population holds the belief already? If 90% of adults *know* it's illegal to drive intoxicated, is it worth focusing on the generalities of this legal issue? Why not focus on individual responsibility...or maybe get Uber or Lyft in on the discussion? Or if a majority already *knows* that *Cannabis* helped a relative with a debilitating disease, why fight against that when there are so many other areas (e.g., end-of-life, dying with dignity, cannabinoids in hospice, etc.) that need specific attention? The ultimate factor to consider is whether-or-not a particular belief is associated with potential behavioral change. For example, the fact that driving high is *illegal* has been proven to be less strongly associated with driving high than the belief that it was *unsafe*—in other

words, no one cares that is illegal (i.e., a political issue) but they might care about safety/impairment (i.e., a scientifically measurable issue)—regardless of legal status (Walters et al. 2018; Romm et al. 2023; Nicholas et al. 2020). We can, and should, therefore, develop content to address modern *Cannabis*-related education issues with more creative messaging, especially if there are funds available for formative research.

6 Needs, Solutions and Future Directions

> *Honest and science-based drug education is essential for reducing harm and dispelling myths. By providing people with accurate information, we empower them to make safer and more informed decisions regarding drug use.* (Carl Hart, Ph.D.)

As *Cannabis* use becomes increasingly normalized in mainstream society, the need for a comprehensive and nuanced education system surrounding its use is more urgent than ever. Education should aim to liberate individuals, through encouraging personal critical thinking and an understanding of peer-reviewed scientific facts, enabling better management of their own lives and experiences. The patchwork landscape of *Cannabis* regulations, coupled to the rise of availability of cannabinoids of varying sources and potencies, demands a multifaceted approach to education.

6.1 Identifying Prevailing Needs

1. **Synthetic Cannabinoid Drugs**: There is a growing need for educational materials specifically focused on synthetic and/or artificially derived cannabinoid products. (Simon et al. 2023; Harlow et al. 2022) Non-phytologically-based substances, often more potent and unpredictable than natural *Cannabis* products, pose unique risks that both consumers and healthcare providers need to understand.
2. **Differentiating Sources and Products**: Education must also emphasize the differences between regulated and illicit products. Many consumers are unaware that regulated *Cannabis* has become both more diverse and potent over time, and may not fully understand the economic and safety trade-offs between purchasing from legal versus illegal markets. The old trope "*this ain't your daddy's weed*" surely *does* have some anchorage in fact—and escalating potencies and the growing availability of high-potency *Cannabis does* indicate that proper education on these subjects is obligatory for a better tomorrow.
3. **Consumer-Driven Research**: It is crucial to fund studies that address the questions and concerns of consumers, regulators, and the public alike. For instance, is the illicit market truly cheaper, or do hidden costs like quality and safety risks make it more expensive in the long run? Do labelling mandates of content, prov-

enance, and potency properly correlate to consumer understanding—and should labelling consistency be required guidance, not unlike the 1938 FFDCA (and its amendments) for food?
4. **Data Capture and Participation**: Engaging the community in data collection and involving them in discussions about *Cannabis* education is essential. By capturing real-world data and direct feedback from consumers, educational efforts can be more accurately tailored to meet the needs of different demographics.

6.2 Proposed Solutions

1. **Ease of Information Sharing**: One of the key roadblocks to education is the dearth of access to scientific reporting and research materials. While outlets to published, peer-reviewed information exist (e.g., PubMed, Embase, Cochrane Library, etc.), accessible and *understandable* information, made available to the general public, is insufficient. Coupled to an increased availability, effective strategies for education must include an effort to *simplify* otherwise complex concepts through analogies, pictographs, illuminated texts, comics, and social-media-friendly short-form animations.
2. **Integrating *Cannabis* Education into Healthcare**: For healthcare professionals, it is vital to integrate *Cannabis* education into existing medical and health curricula. This surely includes detailed modules on the pharmacology, therapeutic uses, health risks, and any pertinent local and Federal regulatory aspects of cannabinoid medicine, as well as relevant question-and-answer-type materials to inform providers as to contemporary and local relevancies. Providers' patients should not be consigned entirely to mass media, social media or conversations with friends to keep them informed—a movement to normalize clinical cannabinoid medicine discussion is the only way we will break our societal addiction to cannabinoid secrecy. Additionally, localized continuing education programs should be developed and modernized to keep practicing healthcare professionals up-to-date with the latest research and regulatory best practices, at least until regulations are consistent between and amongst the states.
3. **Effective Awareness Campaigns**: As previously discussed, all public education campaigns should closely focus on the legal aspects of *Cannabis* use and transport, on what factors increase general health risks and what contraindications to various conditions and polypharmacy may exist, on the differences between regulated and illicit markets, and on risks associated with unregulated products. Campaigns should be clearly written, factual, and designed to correct misconceptions without resorting to sensationalism.
4. **Utilizing International Reports**: Leveraging historical and international reports can be a powerful tool to educating the public. Reports from authoritative bodies can, and should, serve as representative case-studies to teach the public, and both regulators and scientists should be tapped to interpret results from mixed-

discipline studies to provide a deeper understanding of global *Cannabis*-related issues.

6.3 Future Directions and Conclusions

The future is bright! That is to say, the long, dark-ages of cannabinoid ignorance and secrecy seem to be genuinely in our past—we have come a very long way in a short period of time. Internet-based educational materials, social media content, healthcare provider education, and the development of rational school programs and live community events should surely be expanded and refined, but the path to a more cognizant and educated public is clear. Informed social initiatives can be powerful tools for reaching wide-ranging audiences and must be carefully designed to avoid the isolating misinformation of the past. The best future of *Cannabis* education will likely involve innovative, yet untapped technologies, such as virtual reality, augmented reality, mobile apps, and interactive online platforms. New tools should make education more engaging, more accessible, and more *fun*—particularly for younger audiences already accustomed to digital learning modalities and environments. *Cannabis* and cannabinoid education should be seen as part of a broader ecosystem—no longer as a separate or taboo subject—that includes business and marketing practices, healthcare delivery companies, legal consultancies, and public health concerns. Each of these has a role to play in ensuring that cannabinoid education is both comprehensive and accurate.

The landscape of *Cannabis* education is presently complex and somewhat labyrinthine. The truth about *Cannabis* though, in all its complexity, should be presented transparently and without overselling it—that there are true benefits and serious risks, like all things, but that cannabinoid medicine possesses far fewer risks of morbidity and mortality as compared to so many other pharmaceutical or illicit drugs. As educators, researchers, and policymakers, a priority should be placed on building cohesive educational systems that attempt to address the diverse needs of society while avoiding the damaging name-calling, pigeonholing or propagandistic platitudes of the past.

Moving forward, it is likely that the integration of informed and more standardized guidelines, based on peer-reviewed science findings and consumer-driven research, will be essential to creating a sophisticated and responsible populace. By embracing emergent educational tools and focusing on the unique challenges posed by both the synthetic cannabinoids and even novel cannabinoid sources, we can ensure that the resulting guidance is not only comprehensive but also responsive to the evolving landscape of cannabinoid pharmacology.

References

Abel EL. Marijuana on trial: the Panama Canal zone report. Int J Addict. 1982;17:667–78.

Abrams DI. The therapeutic effects of Cannabis and cannabinoids: an update from the National Academies of Sciences, Engineering and Medicine report. Eur J Intern Med. 2018;49:7–11.

Agenda C on the HE of MAER and R, Practice B on PH and PH, Division H and M, Medicine NA of S Engineering, and (2017) The health effects of Cannabis and cannabinoids. https://doi.org/10.17226/24625

Al-Asmari AI. A critical review of workplace drug testing methods for old and new psychoactive substances: gaps, advances, and perspectives. Saudi Pharm J: SPJ. 2024;32:102065.

Bailey JA, Tiberio SS, Kerr DCR, Epstein M, Henry KL, Capaldi DM. Effects of Cannabis legalization on adolescent Cannabis use across 3 studies. Am J Prev Med. 2023;64:361–7.

Berenson A. Marijuana is more dangerous than you think. Mo Med. 2019;116:88–9.

Berg CJ, Romm KF, Pannell A, Sridharan P, Sapra T, Rajamahanty A, Cui Y, Wang Y, Yang YT, Cavazos-Rehg PA. Cannabis retailer marketing strategies and regulatory compliance: a surveillance study of retailers in 5 US cities. Addict Behav. 2023;143:107696.

Best Cannabis Colleges | Cannabis Degrees, Courses, & Certifications | Leafly. https://www.leafly.com/news/industry/best-cannabis-college-degrees-and-certifications. Accessed 27 Aug 2024.

Botelho D, Boudreau A, Rackov A, et al. Analysis of illicit and legal Cannabis products for a suite of chemical and microbial contaminants. https://rpc.ca/english/press/Comparison%20of%20Illicit%20and%20Legal%20Cannabis%20Samples.pdf. Accessed 19 Aug 2024.

Burt TS, Brown TL, Schmitt R, McGehee D, Milavetz G, Gaffney GR, Berka C. Perceived effects of Cannabis and changes in driving performance under the influence of Cannabis. Traffic Inj Prev. 2021;22:S8–S13.

Cavazos-Rehg PA, Krauss MJ, Cahn E, Lee KE, Ferguson E, Rajbhandari B, Sowles SJ, Floyd GM, Berg C, Bierut LJ. Marijuana promotion online: an investigation of dispensary practices. Prev Sci. 2019;20:280–90.

Chye Y, Kirkham R, Lorenzetti V, McTavish E, Solowij N, Yücel M. Cannabis, cannabinoids, and brain morphology – a review of the evidence. Biol Psychiatry Cogn Neurosci Neuroimaging. 2020;6(6):627–35. https://doi.org/10.1016/j.bpsc.2020.07.009.

Davis KC, Allen J, Duke J, Nonnemaker J, Bradfield B, Farrelly MC, Shafer P, Novak S. Correlates of marijuana drugged driving and openness to driving while high: evidence from Colorado and Washington. PLoS One. 2016;11:e0146853.

DEA Removes Marijuana Misinformation from Website after Months of Public, Legal Pressure. 2017; https://www.safeaccessnow.org/iqa_victory

Denneler T, Mahling M, Hermann S, Stengel A, Zipfel S, Herrmann-Werner A, Festl-Wietek T. Medical students' attitudes and perceived competence regarding medical Cannabis and its suggestibility. BMC Méd Educ. 2024;24:149.

Dilley JA, Richardson SM, Kilmer B, Pacula RL, Segawa MB. Prevalence of Cannabis use in youths after legalization in Washington State. JAMA Pediatr. 2019;173(192):193.

Felnhofer A, Kothgassner OD, Stoll A, Klier C. Knowledge about and attitudes towards medical Cannabis among Austrian university students. Complement Ther Med. 2021;58:102700.

French A. Up in smoke: Office of the National Drug Control Policy's wasted efforts in the War on Drugs. 14; 2005

GAO-06-818 Youth Anti-Drug Media Campaign; 2006.

Harlow AF, Leventhal AM, Barrington-Trimis JL. Closing the loophole on hemp-derived Cannabis products. JAMA. 2022;328:2007–8.

Hornik R, Jacobsohn L, Orwin R, Piesse A, Kalton G. Effects of the National Youth Anti-Drug Media Campaign on youths. Am J Public Health. 2008;98:2229–36.

Howard J, Osborne J. Cannabis and work: need for more research. Am J Ind Med. 2020;63(11):963–72. https://doi.org/10.1002/ajim.23170.

Johnson J, Hodgkin D, Harris SK. The design of medical marijuana laws and adolescent use and heavy use of marijuana: analysis of 45 states from 1991 to 2011. Drug Alcohol Depen. 2017;170:1–8.

Kalant OJ. Report of the Indian hemp drugs commission, 1893–94: a critical review. Int J Addict. 2009;7:77–96.

Kight R, Marcu J, Phifer R. Medical marijuana and DWIC: medical and legal considerations. Am J Endocann Med. 2019;2(1).

Lamanna-Rama N, Romero-Miguel D, Casquero-Veiga M, MacDowell KS, Santa-Marta C, Torres-Sánchez S, Berrocoso E, Leza JC, Desco M, Soto-Montenegro ML. THC improves behavioural schizophrenia-like deficits that CBD fails to overcome: a comprehensive multilevel approach using the Poly I:C maternal immune activation. Psychiatry Res. 2024;331:115643.

Lo JO, Shaw B, Robalino S, Ayers CK, Durbin S, Rushkin MC, Olyaei A, Kansagara D, Harrod CS. Cannabis use in pregnancy and neonatal outcomes: a systematic review and meta-analysis. Cannabis Cannabinoid Res. 2023;9(2):470. https://doi.org/10.1089/can.2022.0262.

Lorenzetti V, Batalla A, Cousijn J. Cannabis use disorders and altered brain morphology: where is the evidence? Curr Addict Reports. 2016a;3:189–98.

Lorenzetti V, Cousijn J, Solowij N, Garavan H, Suo C, Yücel M, Verdejo-García A. The neurobiology of Cannabis use disorders: a call for evidence. Front Behav Neurosci. 2016b;10:86.

Lorenzetti V, Chye Y, Silva P, Solowij N, Roberts CA. Does regular Cannabis use affect neuroanatomy? An updated systematic review and meta-analysis of structural neuroimaging studies. Eur Arch Psy Clin N. 2019;269:59–71.

Ma J, Lyoo IK, Renshaw PF, Yurgelun-Todd DA. Effect of cannabinoids on brain metabolites: a review of animal and human studies. Exp Clin Psychopharmacol. 2022;31:248–62.

Makki I, Zheng-Lin B, Kohli M. Medical marijuana knowledge and attitudes amongst internal medicine residents. BMC Prim Care. 2022;23:38.

Mallinson DJ, Hannah AL, Cunningham G. The consequences of fickle federal policy: administrative hurdles for state Cannabis policies. State Local Gov Rev. 2021:0160323X2098454.

Marcotte TD, Umlauf A, Grelotti DJ, et al. Driving performance and Cannabis users' perception of safety. JAMA Psychiatry. 2022;79:1–9.

Marcu JP. The DEA's Denial of Existing Medical Cannabis Research; 2016. www.safeaccessnow.org/deareport_report. Accessed 27 Aug 2024.

Marcu J. Empowering the Healthcare Ecosystem with BudsinfoTM & CeriumTM for Comprehensive Data Collection. International Conference on the Science of Botanicals (ICSB) 2024 Oxford Mississippi April 2024 Poster 41; Publication Number 1037.

Marcu JP, Schechter JB. Regulated Cannabis and Hemp market navigation, business, public health, and research challenges. De Gruyter; 2024. p. 15–40.

Marcu JP, Simon TA. In Flagrante Delicto Derivatives of Hemp-CBD (5047). Presented at session: Modern Public Health Issues Associated with Cannabis and Hemp-Products. American Public Health Association Annual Meeting & Expo; Boston, MA; 2022.

Martin N. Marijuana in Mass. should come with warnings about psychosis, group says; 2019. https://www.bostonglobe.com/metro/2019/06/05/marijuana-mass-should-come-with-warnings-about-psychosis-group-says/LlJrfKslcpIQgcXMO5JPAO/story.html.

Mauro C, Feng T. Prevalence of marijuana use does not differentially increase among youth after states pass medical marijuana laws: commentary on and reanalysis of US National Survey on Drug Use in Households data 2002–2011. Int J Drug Policy. 2016;29(9):13.

Meili R, Hewett N. Turning Virchow upside down: medicine is politics on a smaller scale. J R Soc Med. 2016;109:256–8.

Nicholas W, Washburn F, Lee G, Loprieno D, Greenwell L, Berg C. Assessing the retail environments of licensed and unlicensed Cannabis dispensaries: adapting the marijuana retail surveillance tool to inform Cannabis Regulation in Los Angeles County. J Public Health Man. 2020; https://doi.org/10.1097/phh.0000000000001224.

Palmgreen P, Lorch EP, Stephenson MT, Hoyle RH, Donohew L. Effects of the Office of National Drug Control Policy's Marijuana Initiative Campaign on high-sensation-seeking adolescents. Am J Public Health. 2007;97:1644–9.

Pennington S, Kline A, Mullin HB, Marcu J, Simon T. Coalition for Cannabis scheduling reform report; 2023. https://schedulingreform.org.

Roffman R, Stephens RS, Marlatt GA. Themes in the history of Cannabis dependence. In: Roffman R, Stephens S, editors. Cannabis dependence. Cambridge University Press; 2009. p. 3–20.

Romm KF, Cavazos-Rehg PA, Williams R, et al. Cannabis retailer communication about Cannabis products, health benefits, and risks: a mystery shopper study of licensed retailers in five U.S. Cities. J Stud Alcohol Drugs. 2023;85:100–8.

Sadaka AH, Canuel J, Febo M, Johnson CT, Bradshaw HB, Ortiz R, Ciumo F, Kulkarni P, Gitcho MA, Ferris CF. Effects of inhaled Cannabis high in Δ9-THC or CBD on the aging brain: a translational MRI and behavioral study. Front Aging Neurosci. 2023;15:1055433.

Sajdeya R, Goodin AJ, Tighe PJ. Cannabis use assessment and documentation in healthcare: priorities for closing the gap. Prev Med. 2021;153:106798.

Scheier LM, Grenard JL, Holtz KD. An empirical assessment of the above the influence advertising campaign. J Drug Educ. 2011;41:431–61.

Simon TA. Regulated Cannabis and Hemp market navigation, business, public health, and research challenges – how to keep a product on the shelf. de Gruyte; 2024. p. 155–68.

Simon TA, Simon JH, Heaning EG, Gomez-Caminero A, Marcu JP. Delta-8, a Cannabis-Derived Tetrahydrocannabinol Isomer: Evaluating Case Report Data in the Food and Drug Administration Adverse Event Reporting System (FAERS) Database. Drug Healthc Patient Saf. 2023;15:25–38.

Skelton KR, Donahue E, Benjamin-Neelon SE. Measuring Cannabis-related knowledge, attitudes, perceptions, motivations, and influences among women of reproductive age: a scoping review. BMC Women's Heal. 2022;22:95.

Slaven S. The Canna(business) of Higher Education. SSRN Electron J. 2019; https://doi.org/10.2139/ssrn.3370265.

Trump Signals Support for Marijuana Legalization in Florida – The New York Times. https://www.nytimes.com/2024/08/31/us/elections/trump-marijuana-legalization-florida.html. Accessed 3 Sep 2024.

U.S. Cannabis Legalization and the Emergent Educational Landscape – The EvoLLLution. https://evolllution.com/revenue-streams/market_opportunities/u-s-cannabis-legalization-and-the-emergent-educational-landscape. Accessed 26 Aug 2024.

Wabwire M, Smale T, Ward SJ, Simon TA, Simon TA. 5047—Get your thinking cap on; Implementation of a Cannabis awareness program (CAP) for high school students. 2022 American Public Health Association Annual Meeting & Expo; 2022.

Walters KM, Fisher GG, Tenney L. An overview of health and safety in the Colorado Cannabis industry. Am J Ind Med. 2018;57:3.

Worster B, Ashare RL, Hajjar E, Garber G, Smith K, Kelly EL. Clinician attitudes, training, and beliefs about Cannabis: an interprofessional assessment. Cannabis Cannabinoid Res. 2023;8:547–56.

Zawatsky CN, Mills-Huffnagle S, Augusto CM, Vrana KE, Nyland JE. Cannabidiol-derived cannabinoids: the unregulated designer drug market following the 2018 farm bill. Méd Cannabis Cannabinoids. 2024;7:10–8.

Chapter 26
A Patchwork of Progress: Perspectives on Cannabis Regulation and Legalization: An Author's Review of "Cannabis Policy in the Age of Legalization"



Brad Thomas Rowe

1 A Patchwork of Progress: Perspectives on Cannabis Regulation and Legalization

Cannabis legalization is a dynamic journey shaped by varying perspectives, challenges, and policy innovations. The transition from prohibition to regulation has created a global laboratory of ideas, with policymakers, researchers, and advocates exploring the interplay between public health, social equity, and economic opportunity.

UCLA drug policy researcher and lecturer Brad Rowe illustrates this journey in this section. The following interviews, conducted by Rowe over the last year, shed light on this transition, fraught with complexities in the United States and beyond. These include managing the competition between illicit and legal markets and crafting regulatory frameworks that foster equity while maintaining market viability. The challenges and opportunities are as diverse as the stakeholders involved—from government officials and researchers to activists and industry pioneers.

This expert interview series compiles thought leaders' insights on the evolving cannabis landscape. Andrew Freedman, Colorado's first Cannabis Czar, offers a blueprint for transitioning from illicit to regulated markets, emphasizing the importance of implementation and equity. Similarly, Jay Shukle from Alberta Gaming highlights Canada's early missteps and lessons learned.

Beau Kilmer of RAND proposes innovative regulatory solutions to address economic dynamics, such as price pressures and the rise of high-potency products. Contributions from FDA advisors like Norman Birenbaum and equity advocates like Shaleen Title add depth to the discussion, balancing federal aspirations with

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state realities. Perspectives from international voices, including Alejandro Hope of Mexico and Simon Howell of South Africa, further contextualize the challenges of legalization within unique sociopolitical landscapes.

Ultimately, this document seeks to illuminate the patchwork of progress in cannabis regulation, highlighting the successes and pitfalls of this burgeoning industry. It underscores the importance of thoughtful implementation, equity, and adaptability in shaping cannabis policy. Through the voices captured here, a nuanced picture reveals cannabis's potential to revolutionize industries, redefine public health priorities, and serve as a vehicle for social justice.

Pioneers, advocates, researchers, and policymakers have shaped cannabis legalization. From Amy O'Gorman Jenkins' fight for equity in California to Alejandro Hope's reflections on Mexico's tumultuous marijuana landscape, the narratives unfold with triumph, frustration, and hope. This collection of voices delves into the multifaceted world of cannabis policy, regulation, and innovation, painting a vivid picture of an industry in flux.

Pioneering Implementation in Cannabis Regulation: **Andrew Freedman**, a trailblazer in cannabis policy, reflects on his unique role as Colorado's inaugural "Cannabis Czar," a position that defined the blueprint for adult-use cannabis markets. Since 2014, he has advised governments worldwide on establishing regulatory frameworks with an eye on the intricate dynamics of implementation. Freedman emphasizes that creating a regulated market isn't merely about legislation; it demands addressing endless logistical challenges—shutting down illicit operations without heavy-handed law enforcement, ensuring rigorous product testing, and fostering equitable opportunities for small businesses and marginalized groups.

He cites regulators proposing, "We're going to require this amount of testing for every product on the shelves by July 1st—but we're not going to get there. So now we're going to push that out three years." Well, people paid a lot of money—a lot of capital in place to have the only tested product on the market. And suddenly, that didn't end up being a worthwhile business expenditure. So suddenly, they're behind on debt. They're not reaching projected revenue. They're still competing with an illicit market that is also untested. It's just one example of a cascading effect of getting one logistic wrong."

His insights spotlight a critical hurdle: the enduring competition between the illicit and regulated markets. Consumers are swayed by price, and the legal market struggles to dominate if illicit products remain significantly cheaper. This issue is exacerbated by the lack of interstate commerce in the regulated sector, a problem that could undermine the very foundation of legalization. Freedman's optimism lies in the potential of regulated interstate commerce to outcompete illicit operations, provided the framework avoids overregulation and overtaxation. He warns that the regulated market might falter in critical regions without swift action, eroding businesses and governments' substantial investments.

Freedman's vision extends to fostering a craft marketplace akin to the craft beer industry. He advocates for policies that empower small businesses, emphasizing that the last mile of cannabis delivery offers a unique opportunity to ensure local, minority-owned enterprises thrive. Yet, he tempers this vision with realism,

acknowledging the inevitable consolidation of cultivation operations due to economies of scale. His overarching message is that implementation is paramount. Without thoughtful execution, even the most well-intentioned policies risk failure.

Canada's Experience—Lessons from Alberta: **Jay Shukle**, Senior Director of Cannabis at Alberta Gaming, Liquor, and Cannabis, offers a Canadian perspective on legalization's growing pains. Alberta's experience illustrates the pitfalls of mismatched supply and demand. Initial shortages left retailers scrambling, while subsequent oversupply sparked a price war that decimated profitability. Shuckle shared, "In Alberta, we did a great job of opening up retailers, but we had to put a moratorium on those stores because there was just not enough product, and it would be like inviting people to a famine."

Shukle underscores the delicate balance required to stabilize the market while navigating federal regulations that restrict THC levels in products like beverages and edibles. He envisions a future where cannabis is normalized alongside alcohol, with regulatory evolution fostering broader acceptance and accessibility.

Economic and Public Health Dynamics: Beau Kilmer, Director of the RAND Drug Policy Research Center, delves into the economic forces shaping cannabis markets. He outlines the downward price pressures in legalized states like Oregon and Michigan, attributing them to oversupply and increasing competition. Federal legalization, Kilmer predicts, would amplify these trends, potentially consolidating production into a handful of industrial-scale operations. Kilmer added, "We did some work estimating that you can probably produce all the cannabis consumed in the United States on a few dozen industrial-sized farms. That's it! And so right now, federal prohibition creates these inefficiencies. Still, if you could have this all concentrated in one or most of it in a couple of states, increasing returns to scale, you could see the prices plummet even lower."

This shift would challenge illicit growers but also reshape the market landscape. Kilmer also highlights the emerging concern over high-potency products and their implications for public health, particularly among youth. He aspires to focus research and support on the heaviest consumers, saying, "Ideally, I would like to have some measure of the total amount of THC consumed per day. Then, you can begin to get it because even if you focus on daily users, some people may just have a few hits off a vape pen late at night. And other people are daily users who are under the influence most of the day." He proposes innovative solutions, such as taxing cannabis based on THC content, to encourage the consumption of lower-potency products.

Federal Policy Challenges: **Norman Birenbaum**, Senior Public Health Advisor at the FDA, reflects on the challenges of regulating an industry evolving at breakneck speed. He notes the transition from basic plant counts to sophisticated product forms like transdermal patches and Delta-8 THC. The rise of hemp-derived intoxicants poses an additional regulatory dilemma, as these products often escape the stringent controls applied to traditional cannabis.

Birenbaum identifies seismic regulatory shifts, sharing, "No one had heard of isomerizing Delta-8 THC or Delta-9 THC from CBD three, four years ago. And now, not only do we know about it, but it is also one of the most significant

movements we've seen in the industry over the last 18 to 24 months. Looking at what's happened with hemp products and intoxicating hemp products as a rival and largely unregulated market has also been impactful on cannabis policy and regulation. We're seeing a whole host of states now move to ban these products, to regulate these products, and great parity between the two systems, so that's just another example of how this is constantly changing." Birenbaum calls for a balanced approach integrating public health priorities with market realities. He predicts that comprehensive federal reform will take years and be hindered by political and legal complexities. However, he stresses the importance of addressing regulatory gaps to safeguard consumers and support equitable market development.

Advocating for Fairness in Federal Legalization: **Shaleen Title**, a former Massachusetts Cannabis Control Commissioner, sharply critiques the pitfalls of federal legalization if implemented hastily or unfairly. She warns against repeating the mistakes of other industries, where monopolistic practices and unethical behavior flourish unchecked, saying, "We've seen that with monopolistic industries, and we've seen that with companies that have grown so large, especially when they are in the drug business that they can't be regulated effectively; and we end up with products that are addictive and dangerous and harmful companies. And I don't think we have seen that yet at the same scale when it comes to cannabis."

Title advocates for a deliberate, data-driven approach prioritizing consumer protection and equitable opportunities for small businesses. Her perspective underscores the importance of transparency and public participation in shaping cannabis policy, emphasizing that success hinges on collaboration and a willingness to adapt.

The Advocate for Change: **Amy O'Gorman Jenkins**, a seasoned lobbyist and founder of Precision Advocacy, has spent two decades at the crossroads of legislative action and public policy. Her passion for cannabis reform focuses on breaking down barriers that inhibit the industry's potential. In California, she faces the challenge of widespread municipal bans, with 62% of jurisdictions still prohibiting commercial cannabis activities.

Jenkins is committed to advancing social equity. She recognizes that many applicants for cannabis licenses remain trapped in local bureaucratic limbo, sharing, "We have thousands and thousands of applicants stuck in local processes that cannot move forward and qualify for a license. We are exploring some policy changes in that area." Her advocacy extends to environmental concerns. She critiques the state's track-and-trace program, which burdens cultivators with costly and environmentally detrimental requirements. Jenkins argues that removing these inefficiencies is essential for a more sustainable industry.

As the cannabis market expands to include hemp-derived products, Jenkins emphasizes the need for rigorous enforcement to ensure these products meet California's high standards. She envisions a future where hemp and cannabis coexist within a regulated framework, but her primary focus remains on increasing retail access and curbing the influence of the unlicensed market.

Overcoming Stigma in Research: **John Hudak**, Director of the Office of Cannabis Policy in Maine, brings a unique perspective shaped by his tenure at the Brookings Institution. Hudak highlights the persistent stigma surrounding cannabis

research, a barrier that permeates think tanks, universities, and legal systems. Researchers often justify their work in ways that would be unnecessary in other fields, especially given the lingering biases stratified along generational lines.

Hudak also points to the structural challenges of cannabis policy implementation. Ballot initiatives and rushed legislative processes leave state administrations scrambling to establish functional regulatory systems. He does acknowledge the silver lining of using the states as laboratories of democracy, saying, "And because cannabis is a relatively new area of public policy, there is some learning that can happen from state to state, county to county, and municipality to municipality."

These hurried efforts often fail, fueling public backlash and skepticism. Hudak emphasizes the need for a comprehensive guide to best practices as states grapple with the complexities of transitioning from prohibition to legalization.

Looking to the future, Hudak foresees a seismic shift when federal legalization opens the door to interstate commerce. With ideal growing conditions, states like California and Oregon will likely become net exporters, while regions like North Dakota may shift to importing cannabis. This economic specialization, Hudak warns, will demand careful planning to avoid harm to small businesses and ensure equitable market structures.

Ensuring Integrity in Testing: **Ron Brooks**, a veteran of the cannabis testing industry, has witnessed firsthand the pitfalls of insufficient oversight. Laboratory shopping and potency inflation remain pressing issues, eroding consumer trust and undermining the industry's credibility. Brooks advocates for standardized regulations and thorough vetting processes to ensure testing laboratories uphold the highest scientific standards. Brooks wants to "help government and entities that work with government bodies to craft appropriate legislation so that you don't have manufacturers and cultivators questioning why lab regulations are so stringent versus other markets so that everyone is a shareholder."

Federal legalization, he argues, could bring much-needed stability to the tumultuous market, but thoughtful policy frameworks must accompany it. As a consultant, Brooks seeks to guide policymakers and industry stakeholders toward solutions that balance stringent oversight with fair practices, ensuring everyone involved has a stake in the system's success.

A Cautionary Tale of Industry Capture: For **Jon Caulkins**, a public policy professor at Carnegie Mellon University, the story of cannabis legalization is one of unintended consequences. He notes a dramatic shift in consumption patterns, with daily or near-daily use now accounting for over a third of cannabis consumption—up from just 10% a few decades ago. The rise in potency and increased frequency of use raises questions about long-term health impacts that remain unanswered. Caulkins elaborates, "About same proportion as for alcohol. It was a weekend recreational drug. Now, the proportion of cannabis users who use daily or near-daily is upwards of 35%. The patterns of consumption are beginning to look more like tobacco. And also, on every day of use, the amount of THC consumed has gone up."

Caulkins is deeply concerned about regulatory capture, where the industry's profit motives overshadow public health priorities. Instead of adopting a protective stance akin to tobacco regulation, many state regulators have allowed the cannabis

industry to shape policy to its advantage. This approach, he warns, risks creating a market that prioritizes profits over the well-being of consumers.

Internationally, Caulkins highlights the ripple effects of legalization in countries like Uruguay and Canada. The United States' departure from international drug control treaties poses a diplomatic challenge as nations with differing views on cannabis struggle to reconcile their policies. The fragmentation of the global drug control regime, Caulkins suggests, could have far-reaching and unpredictable consequences.

The Frustrated Activist: **Andrew DeAngelo**, co-founder of Harborside and a pioneer in the California cannabis industry, has spent decades advocating for meaningful reform. Despite the progress made, he laments the industry leaders' lack of commitment to advancing better public policy. Big cannabis companies, he notes, often prioritize protecting their market share over expanding access or lowering barriers to entry, saying, "You have big cannabis companies now, publicly traded cannabis companies that have hundreds of millions if not billions of dollars on their cap table. They're lobbying not to open up the market, create level playing fields, or even reduce the barriers to entry. Still, they're lobbying to build moats around their current operations and companies."

DeAngelo reflects on the compromises made to achieve legalization, many of which he believes were short-sighted. He questions whether California should have stuck with its medical cannabis framework rather than rushing into adult-use legalization. For DeAngelo, the path forward requires more significant investment in lobbying and activism and stronger collaborations between academia, industry, and policymakers.

Unlocking Cannabis' Medical Potential: **Dr. Daniele Piomelli**, Director of the Center for the Study of Cannabis at UC Irvine, is at the forefront of research into cannabis' therapeutic applications. Chronic pain, he believes, represents a critical area where cannabis could make a significant impact. While existing studies suggest substantial evidence of cannabis' efficacy, regulatory restrictions and funding limitations have hindered the large-scale clinical trials needed to confirm these findings. He shares, "This is so important because we are very close to having an answer. Many studies have been done. Unfortunately, all are small because of regulatory restrictions and funding limitations—but they are compelling."

Piomelli is also intrigued by cannabis as a "chemical laboratory," producing a range of compounds with diverse pharmacological effects. Cannabidiols, in particular, hold promise for conditions beyond chronic pain. However, he laments the cannabis industry's focus on marketing over rigorous scientific exploration, a gap that he hopes to bridge through academic research. Despite the challenges, Piomelli remains hopeful that cannabis can revolutionize medicine, providing safer alternatives to opioids and unlocking new treatments for conditions like migraines, PTSD, and epilepsy.

Championing Medicinal Cannabis: **Dr. Bonni Goldstein** has dedicated her career to improving the lives of patients with severe and chronic medical conditions through cannabis medicine. Her practice, Canna-Centers Wellness and Education, has treated over 18,000 patients, including 2000 children. Goldstein's work is

particularly impactful for conditions like epilepsy, autism spectrum disorder, and chronic pain, where traditional treatments often fall short.

Goldstein's insights into the endocannabinoid system shed light on cannabis' potential to restore balance in the brain and body, expanding, "The job of the endocannabinoid system to put the brakes on your brain so you are not always constantly having, what we call, a neuro-excitatory brain. Pain nausea and vomiting are 'over' messages. We make compounds that look just like THC in our brain, in our immune system, in our skin, and everywhere in our body. We make those so that they go backward. They are sending feedback to the cell that's sending too much message, calling it out to turn down the message."

She sees cannabis as a first-line treatment for conditions like autism, and argues that it offers a safer alternative to FDA-approved antipsychotics. Through her research and advocacy, Goldstein continues to challenge stigmas and advance the science of medical cannabis.

Bridging Legalization and Equity: **Sam Kamin**, a law professor at the University of Denver, played a crucial role in implementing Colorado's Amendment 64, the nation's first measure to legalize adult-use cannabis. He recalls that early efforts were focused on minimizing federal intervention, resulting in conservative regulations that have since evolved to address issues like social equity.

Kamin's primary concern lies in preventing the pitfalls of industries like tobacco and alcohol, where problematic use is often exploited for profit. He advocates for regulations that balance consumer choice with protections against predatory practices, sharing, "How do we evolve, avoid what a lot of people call the 80/20 rule where 20% of your customers are 80% of your business, and companies have an incentive to take advantage of people who consume a lot, maybe too much, marijuana. How do we avoid marketing to children?"

As federal legalization looms, Kamin emphasizes the need for a cohesive framework that promotes equity and avoids the mistakes of the past.

Mexico's Cannabis Crossroads: **Alejandro Hope**, a Mexican security analyst, provides a sobering perspective on cannabis policy in Mexico. The country's marijuana trade, once a dominant force in the U.S. illicit market, has seen a dramatic decline since two dozen states have legalized recreational cannabis, saying, "By the eighties, Mexico was probably providing 70 to 80% of all cannabis consumed in the US. Most of that cannabis was not necessarily funneled through what we now call cartels. It was a very decentralized trade." Legalization in the United States. has reduced the risks associated with domestic production, driving down demand for Mexican marijuana. "That situation has radically changed over the past decade as was predicted when Washington and Oregon first legalized the recreational use of marijuana in 2012—that led to something of an input substitution effect."

Despite this shift, Mexico remains in legal limbo. Court rulings have declared absolute prohibition unconstitutional, but political gridlock has stalled efforts to create a regulated market. Hope highlights the paradox of high-grade cannabis flowing from the United States into Mexico, a reversal of historical trade patterns driven by the allure of California's premium products. Hope's analysis underscores the

interconnectedness of drug policies across borders and the need for collaborative solutions that address both economic and social dimensions.

Navigating Financial Hurdles in Cannabis: **Kris Krane**, Chair of the National Cannabis Industry Association and Managing Partner at 4Front Ventures knows the struggle of accessing capital in an industry mired in federal restrictions. Traditional funding avenues remain closed to cannabis businesses, forcing Krane and his peers to rely on friends, family, angel investors, and cannabis-specific investment funds. These options often come at steep costs, with loans carrying 14–18% interest rates, far above those available in other industries, adding, "Frankly, there isn't a hack for this. You've just got to deal with the available capital sources to the industry."

For Krane's company, going public on the Canadian Securities Exchange (CSE) became the most viable solution. This granted access to institutional capital otherwise unavailable in the United States.

Despite these obstacles, Krane remains optimistic about the future of retail cannabis. He predicts that federal legalization or even a softening of federal laws will unleash a flood of tools and technologies to help retailers better profile their customers, manage inventory, and refine their offerings, expanding, "You may have other point of sale companies, or online menu companies. Right now, those are all done through companies that are kind of specific." As more established industries enter the space, the resources available to cannabis businesses will only expand, potentially easing some of the current burdens.

California's Scaling Challenge: **Nicole Elliott**, Director of the California Department of Cannabis Control, reflects on the monumental task of transitioning the world's largest and oldest cannabis market into a legal framework. Elliott emphasizes that this process is not a one-time event but an ongoing evolution requiring sustained attention. California faces six interlocking challenges: scale, mistrust, lack of federal participation, data deficiencies, perfectionism, and holistic enforcement.

Elliott underscores the importance of rebuilding trust with communities disproportionately harmed by past drug policies, saying, "Unwinding decades of harmful drug policies on generations of people is something that cannot be done in one generation. When we look at things like equity programs, it's important to prioritize those. It's also important to reinvest in communities and do all we can to acknowledge our government's role in perpetuating those harms."

Efforts like equity programs, technical assistance, and fee waivers aim to create pathways for historically marginalized groups to thrive in the legal market. However, a lack of federal participation complicates this work, driving up prices, limiting access to financing, and perpetuating the illegal market. California has embraced creative administrative solutions to combat these issues, such as crafting agreements for potential interstate commerce and improving data-sharing infrastructure to stabilize the market.

Oakland's Pioneering Equity Program: **Greg Minor**, Assistant to the City Administrator in Oakland, recounts the city's journey to creating the nation's first cannabis equity program. Initially inclined to follow the legalization models of Colorado and Washington, Oakland shifted direction after then-Councilmember

Desley Brooks raised a crucial question: Who benefits from legalization? This inquiry led to the development of equity analysis, ensuring that policy solutions were guided by data and focused on uplifting communities most impacted by the War on Drugs. Minor shares, "That allowed us to identify a goal clearly and do an analysis that led us to that goal as opposed to jumping to policy solutions and then trying to work backward or people being inconvenienced by a particular policy solution and then people fighting over different policy outcomes but with no basis in actual data or clear guidance."

Looking ahead, Minor envisions a future where cannabis mirrors other industries, with opportunities expanding beyond the novelty of legalization. He stresses the need for adaptability, acknowledging that federal legalization will bring new challenges and possibilities, like the evolution of alcohol from prohibition to a diverse market of bars, restaurants, and entertainment venues.

Systemic Inequities in South Africa: In South Africa, **Dr. Simon Howell** of the University of Cape Town paints a sobering picture of the systemic inequities surrounding cannabis enforcement. Police officers, incentivized by arrest quotas, often target users rather than dealers, ensuring a steady stream of arrests to meet performance metrics, sharing, "And drug users were a very easy cohort to arrest ultimately to the extent that they used to leave the dealers in place. So they would have a handy pool of people to arrest when needed. Because if you arrest a dealer, your users tend to move out of your precinct."

This practice perpetuates cycles of poverty and marginalization, particularly among communities of color. Howell's research reveals that colored men are six to seven times more likely to be arrested for drug-related offenses than their white counterparts, underscoring the racial disparities embedded in the system.

Howell advocates for a shift away from punitive approaches, arguing that the War on Drugs has failed to achieve its goals. Legalization, he suggests, could address both economic and social injustices by providing marginalized communities with new opportunities and reducing the harms associated with criminalization.

Northern California's Unique Approach: **Dominic Corva** of Cal Poly Humboldt highlights the distinct challenges faced by Northern California's cannabis cultivators. Unlike urban retailers, rural growers often operate within tight-knit communities where their contributions to local economies are recognized and valued. Humboldt County, for example, has used state funding to invest in environmental infrastructure, such as solar and water systems, benefiting small businesses and promoting sustainability.

Corva emphasizes the importance of addressing consumer preferences for illicit markets, which often offer lower prices and have perceived better quality. He argues that meeting these preferences through regulated markets is critical to reducing unlicensed activity, expanding, "The central question is how we do something that's not just prohibition again—to deal with these issues [of suppressing the illicit market]. And there are genuine solutions to open up that supply chain and fix the retail bottleneck—by any means necessary."

Corva's insights also reveal the diverse faces of cannabis entrepreneurs, from young men to grandmothers and single mothers, underscoring the broad societal impact of the industry.

Social Equity and Survival: **Amber Senter**, Chair of the Board for Supernova Women, passionately discusses the barriers facing social equity operators. Federal legalization poses significant risks for small, Black-owned businesses like hers. While large companies stand to benefit from improved access to capital and lower borrowing costs, equity operators remain excluded from traditional banking and financial systems.

Senter provides details, "When something like the Safe Banking Act passes, they'll be able to borrow that money closer to about 2%. A small Black-owned, women-owned, queer-owned, veteran-owned Cannabis Company like mine won't be able to access those funds at all." She adds, "I'm not very hopeful for small businesses to survive federal legalization and what's to come."

Regulatory frameworks often hinder their ability to merge or scale, leaving them vulnerable in an increasingly competitive market. Senter laments the shift away from cannabis' craft roots, noting that small, independent operators built the industry. Without systemic changes, many social equity businesses may be forced out of the legal market, returning to the illicit economy out of necessity.

Zimbabwe's Green Revolution: **Dr. Clemence Rusenga** of the University of Bristol explores the nascent cannabis industry in Zimbabwe, where hopes of a "Green Revolution" have been tempered by the realities of production. Although the government has issued numerous licenses, only a handful of producers have begun operations, hindered by high costs, stringent security requirements, and a lack of resources. Rusenga's research reveals a widespread belief among Zimbabweans that legalization could bring economic benefits, even among those critical of cannabis' social impacts. He shares some of the findings, "There was a consensus among people who don't even take cannabis, who are critical of its negative effects, but they were of the view that the War on Drugs or the way the government legislates around it, the issue about policing, it has failed. So, they believe it should be legalized."

Zimbabwe's reliance on agriculture, particularly tobacco, positions cannabis as a potential alternative crop for export. However, realizing this potential will require significant investment in infrastructure and a strategic approach to integrating cannabis into the country's broader economic framework.

Building a Brand in Cannabis: **Shawn Gold**, Founder and CEO of Pilgrim Soul, delves into brand-building in a restricted advertising landscape. With platforms like Google and Facebook off-limits, cannabis companies must rely on creative strategies such as content marketing and in-store promotions. Gold emphasizes the role of budtenders, who influence 70–80% of purchases, and advocates for educating them as brand ambassadors.

Gold's brand focuses on cannabis for creativity, and its products are designed to enhance the cannabis experience, "What I create is journals and games, and art supplies, and other things that are meant to be used *while you're high*, to enhance the efficacy of cannabis and creativity. And I can market those on a national basis." He

views federal legalization as a double-edged sword, bringing both opportunities—such as safer banking and tax relief—and challenges, including increased regulation and potential market consolidation.

A Veteran Advocate for Legalization: U.S. Representative **Earl Blumenauer** of Oregon (D) reflects on his four-decade fight for cannabis reform. Despite broad public support and significant progress at the state level, federal legalization remains elusive, mainly due to a lack of political prioritization. The representative provides insights into the challenge, "The facts are known. Support has built a majority of people in the house—and the Senate actually supports it, even though it's been hard to get the Republican leadership to place a priority on it. This is almost no one's top priority. I'm sort of an outlier."

Blumenauer highlights the manifold harms of prohibition, from racial disparities in enforcement to the economic inefficiencies of an illicit market. He envisions a future where cannabis legalization generates tax revenue, supports public health initiatives, and provides Veterans with safer, more effective treatments. While frustrated by the slow pace of change, he remains hopeful that federal reform is within reach, driven by public awareness and the compelling case for legalization.

The Federalism Challenge: **Robert Mikos**, a law professor at Vanderbilt University, identifies the conflict between state and federal cannabis laws as a central challenge. For decades, states have navigated this tension by creating insular markets, with cultivation, processing, and sales confined within state borders. However, federal legalization would upend this structure, enabling interstate commerce and reshaping the industry. Mikos forewarns of a massive shift in the US industry, "The big reason is that once the federal government legalizes cannabis, and we don't know if that's a year from now or 10 years from now, but *once* the federal government legalizes cannabis, that's going to fundamentally change the cannabis markets that we have today. Those markets are very peculiar. They don't look like anything else we have out there right now."

Mikos predicts that a national cannabis market could lead to industry consolidation, potentially sidelining small businesses and social equity initiatives. He calls for careful planning to ensure that federal reform fosters a balanced and inclusive market while addressing new regulatory challenges.

2 Conclusion

Cannabis legalization represents a profound shift in societal norms, economic opportunities, and public health strategies. The journey from prohibition to regulation is marked by a patchwork of successes and setbacks, shaped by the diverse voices and priorities of policymakers, industry stakeholders, and community advocates. Key figures illuminate the intricate balancing act required to ensure that legalization delivers on its promises of equity and economic opportunity while mitigating commercialization risks and consumer harm.

One of the most pressing challenges remains integrating equity initiatives within a rapidly consolidating industry. Contributors highlight persistent disparities, even in markets designed to prioritize inclusion. Their work underscores the need for targeted interventions, from social equity programs to comprehensive demographic studies, to ensure that cannabis becomes a tool for reparative justice rather than perpetuating systemic inequalities. Internationally, these experts reveal the interconnectedness of drug policies across borders. Mexico's legal limbo and South Africa's struggles with systemic inequities underscore the global implications of cannabis reform, where decisions in one jurisdiction ripple across economies and communities worldwide.

From a market perspective, contributors emphasize the critical role of pricing and innovation. Meanwhile, public health advocates call for a science-driven approach to cannabis medicine, urging the industry to prioritize safety and efficacy. As the industry evolves, federal legalization is both an opportunity and a challenge. Experts caution against hastily implemented policies that could entrench monopolies and undermine equity goals.

Ultimately, this patchwork of interviews paints a nuanced picture of an industry at a crossroads. It calls for continued collaboration, innovation, and vigilance to ensure that cannabis legalization fulfills its potential as a vehicle for economic growth, public health improvement, and social justice. These voices remind us that the journey is far from over and that success lies not in the act of legalization itself but in the ongoing effort to create systems that serve all stakeholders equitably.

Chapter 27
Science Translated to Public Policy: Selected Review and Analysis of Cannabis Risks and Therapeutic Potential

Brad Thomas Rowe

1 Introduction

Brad Rowe, M.P.P., a cannabis policy researcher and lecturer at the UCLA Luskin School of Public Affairs, presents a focused review of cannabis-related research to bridge the gap between scientific evidence and public policy. His work highlights key studies with direct implications for public health and emphasizes actionable, politically feasible recommendations for lawmakers, health officials, and community leaders. The review addresses critical areas like adolescent cannabis use, prenatal exposure, secondhand smoke, and the therapeutic potential of cannabidiol (CBD).

A primary concern in the analysis is adolescent cannabis use, which is linked to increased risks of schizophrenia, anxiety, and depression, especially with early use or exposure to high-potency products. Cannabis affects brain development, impairing cognitive functioning and emotional regulation. The second part of each section makes policy recommendations, such as stricter age restrictions, marketing regulations, and the inclusion of THC content labeling. He also calls for SBIRT (Screening, Brief Intervention, and Referral to Treatment) in schools and public education campaigns for youth and parents.

Another key issue is prenatal cannabis exposure, which has been shown to disrupt fetal brain development, leading to cognitive delays, emotional issues, and behavioral disorders in children. This translational analysis calls for enhanced public health education targeting expectant mothers, warning labels on cannabis products, and healthcare provider training to support early intervention.

Secondhand cannabis smoke (SHCS) is also a critical public health issue. Research reveals that SHCS contains high levels of particulate matter (PM2.5),

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which poses risks to children's respiratory and cognitive health. Recommendations include smoke-free multi-unit housing (MUH) policies, public awareness campaigns, and pediatric health screenings to address these risks.

This section also highlights CBD's therapeutic potential for treating pediatric epilepsy. FDA-approved treatments like Epidiolex® reduce seizures for conditions like Dravet Syndrome. To ensure safety and accessibility, the author recommends broader insurance coverage, financial support for families, and regulatory standards for non-FDA CBD products.

Finally, this chapter, "Translating Science to Public Policy," discusses twin studies that explore cannabis's cognitive effects while controlling for genetic and environmental factors. To improve policy, Rowe calls for funding longitudinal twin and neuroimaging studies using cannabis tax revenue to better understand cannabis's causal effects. Through data-driven, health-focused policy recommendations, he aims to protect vulnerable populations while promoting cannabis's therapeutic potential.

2 Youth Cannabis Use: Challenges and Solutions

This section examines three papers addressing subgroups and their health challenges attributed to cannabis use. The "Areas of Overlap and Commonality" section examines some of the public health challenges and solutions these three studies share. As evidenced by the findings, this review will incorporate some translations into public policy.

2.1 "Cannabis Use and Psychiatric Symptoms in Adolescence"

By Meghan A. Costello, Ph.D., Randi M. Schuster, Ph.D., Jodi M. Gilman, Ph.D., and A. Eden Evins, M.D., M.P.H. (referred to here as "Costello")

Summary of the Science

This chapter investigates the relationship between adolescent cannabis use and psychiatric issues such as psychosis, mood and anxiety disorders, neurodevelopmental problems, and polysubstance use. Key findings include: (1) Early cannabis use, particularly with high-potency products, is associated with increased risk of psychosis and earlier onset of symptoms. (2) Cannabis use is linked to worsened depression and suicidality, with adolescents with mood disorders more likely to use cannabis. (3) Evidence suggests a two-way relationship, where cannabis use is both a response to and a trigger for anxiety symptoms. (4) Adolescents with ADHD or ASD often use cannabis to self-medicate, increasing their risk of dependence. (5) Cannabis use often precedes the use of other substances, further complicating mental health outcomes.

Cannabis affects adolescent brain development, especially in areas tied to emotion and decision-making (e.g., prefrontal cortex, limbic system). The chapter highlights the need for early intervention rather than punitive responses, given that adolescent cannabis use often signals broader mental health issues.

Translated to Public Policy
Localities and the cannabis industry should evaluate access restrictions and marketing regulations to minimize youth appeal, enforce strict age limits, limit the promotion of high-potency products, and initiate educational programs to correct misconceptions among youth regarding cannabis safety.

School districts and providers should implement SBIRT (Screening, Brief Intervention, Referral to Treatment) in schools, allocate funding for mental health services, train parents, and promote person-centered care for individuals facing co-occurring mental health challenges and cannabis use issues. They should also support family-focused and peer education programs while working to reduce stigma. Universities and public health institutions ought to investigate the effects of high-potency cannabis on adolescent brain development and mental health.

2.2 "Cannabis Use and Misuse Among Sexual and Gender Minority (SGM) Youth"

By Suzanne Brown, PhD, LMSW (referred to here as "Brown")

This chapter explores cannabis use disparities among SGM youth, emphasizing factors like minority stress, stigma, and barriers to care. (1) SGM youth use cannabis at significantly higher rates than their heterosexual and cisgender peers due to discrimination, family rejection, and internalized stigma. (2) Cannabis use during adolescence disrupts brain development, leading to cognitive impairments and stress-related vulnerabilities. (3) SGM youth face obstacles in accessing culturally competent care, including stigma in healthcare, lack of SGM-specific services, and underinsurance.

The chapter emphasizes the need for affirmative, stigma-reducing practices for SGM youth, advocating for systemic change and culturally competent substance misuse treatment.

Translated to Public Policy Federal lawmakers and agency leadership should work to make treatment accessible and equitable. This could be done by expanding Medicaid/insurance coverage for SGM-specific substance misuse care and ensuring healthcare forms include inclusive language and gender options.

Governments at all levels should require SGM-specific training for healthcare providers and promote affirmative care practices to address minority stress. Use SBIRT in schools and community centers to identify early risks of cannabis misuse and establish directories of affirming mental health providers.

Grassroots efforts could focus on creating youth-focused centers offering integrated mental health and substance misuse services. This could be done in tandem with the establishment of peer and family support systems that promote peer mentorship, family education programs, and community support networks to reduce isolation and promote acceptance of SGM youth.

State and county agencies should launch public campaigns to reduce the stigma surrounding cannabis misuse while affirming SGM identities. These programs should be evaluated using evidence-based metrics to assess the success of SGM-focused programs, ensuring interventions like Cognitive Behavioral Therapy (CBT), Motivational Enhancement Therapy (MET), and Multidimensional Family Therapy (MDFT) are adapted for SGM youth.

2.3 "Cognitive Effects of Regular Cannabis Use During Adolescence and Young Adult Regular Consumers"

By Krista Lisdahl (referred to here as "Lisdahl")

This chapter focuses on cannabis use and its cognitive effects during adolescence, a period when the brain is susceptible to endocannabinoid (eCB) system disruptions. Key findings include: (1) High-eCB receptor expression in the prefrontal cortex and hippocampus during adolescence makes these areas vulnerable to cannabis exposure. (2) Regular cannabis users experience impairments in memory, IQ, decision-making, processing speed, executive function, and attention. (3) Males show more significant deficits in IQ and decision-making, while females have more difficulty with verbal memory and attention. (4) Some cognitive impairments may recover with sustained abstinence despite limited evidence. The severity of effects is influenced by cannabis potency, modes of use (e.g., edibles, vaping), and concurrent use of other substances like alcohol.

The chapter emphasizes the need for further research to clarify causal relationships and distinguish cannabis-induced effects from shared genetic and familial risk factors.

Translated to Public Policy

States and counties should launch public health campaigns targeting adolescents and parents about cannabis-related cognitive risks and educate students on these risks as part of school curricula. They should impose stricter regulations on high-potency cannabis products, enforce clear THC/CBD labeling, and prohibit marketing that appeals to youth.

State and Federal agencies should allocate cannabis tax dollars for longitudinal studies on cannabis's long-term cognitive effects, sex-specific vulnerabilities, and neurodevelopmental impacts. Providers and institutions should use CBD-based treatments and abstinence support programs to address cognitive deficits in cannabis-affected youth.

Legislators should work with regulators and scientists to improve policy coordination and research standardization. These improvements could include ensuring consistent cannabis regulation across localities, aligning health, education, and regulatory policies, and collaborating with international research bodies to standardize methodologies.

3 Areas of Overlap and Commonality Across Costello, Lisdahl, and Brown Studies

All three chapters emphasize the following:

- The critical impact of cannabis use on adolescent neurodevelopment, with a focus on regions like the prefrontal cortex, hippocampus, and limbic system.
- Cannabis use is both a coping mechanism and a risk factor for mental health issues. Costello focuses on psychiatric conditions like psychosis, mood, and anxiety disorders.
- There is a need for systemic support, whether through mental health services or culturally competent treatment options for vulnerable populations.
- Knowledge gaps. Costello calls for a better understanding of the mental health link.

These overlaps point to the intertwined relationship between cannabis use, adolescent brain development, cognitive impairment, and mental health, especially for vulnerable groups like SGM youth and those with neurodevelopmental or mood disorders.

All three chapters point toward an emphasis on the need for:

- Education campaigns and prevention efforts targeting adolescents.
- Stricter regulation of high-potency cannabis.
- Public health education and awareness campaigns.
- Long-term research and evidence-based interventions.
- State and local government alignment in policy responses to cannabis use.
- The protection of vulnerable youth.

Table 27.1 identifies some of the areas where the three translational analyses produce different approaches to policy remediation.

The following chapter (Watt and Karl) specifically focuses on schizophrenia, ASD, and their co-occurring link with genetic predispositions. Some policy recommendations were novel from the previous three chapters reviewed above. Thus, Watt and Karl served as a standalone for analysis.

Table 27.1 Key differences in policy translation

Category	Costello (adolescents and mental health)	Brown (SGM youth and health equity)	Lisdahl (cognitive deficits and regulation)
Primary focus	Adolescent mental health and neurodevelopment	Equity and care for SGM youth	Cognitive effects of cannabis use on IQ, memory
Core strategy	Prevention, early intervention, mental health support	Health equity, culturally competent care	Public education, potency regulations, research
Main populations	Adolescents at risk for psychiatric disorders	Sexual and gender minority (SGM) youth	Adolescent cannabis users (all youth)
Prevention strategy	SBIRT, school-based mental health services	SBIRT, family support, community hubs	Cannabis awareness campaigns in schools
Regulatory focus	Age restrictions, high-potency limits	Equity in care, cultural competence in healthcare	THC potency caps, childproof edibles, tax revenue
Research focus	Longitudinal studies on mental health impacts	Evidence-based interventions (CBT, MET, MDFT)	Tax-funded longitudinal studies on cognition
Unique approach	Parent involvement and school-based services	Health equity and support for SGM youth	Cognitive impairment and global collaboration

3.1 "Early Cannabis Use in the Context of Developmental Brain Disorders"

by Georgia Watt and Tim Karl

Recap and Takeaways The chapter explores the complex effects of cannabis use during adolescence on mental health, focusing on schizophrenia and autism spectrum disorder (ASD). Some of the key findings include associations with cannabis use and the development of mental health conditions: (1) Adolescent cannabis use is associated with an increased risk of developing schizophrenia, particularly in individuals with genetic predispositions. (2) Cannabis use during adolescence disrupts neuro-maturation, including alterations to the endocannabinoid system (eCBS), neurotransmitter systems, and (3) Long-term effects include cognitive impairments, behavioral changes like social withdrawal and emotional blunting, and increased psychotic relapse risk.

Watt and Karl also examine the effects of cannabis use on Autism Spectrum Disorder (ASD), including: (1) Changes in the eCBS are commonly observed in individuals with ASD, including impaired endocannabinoid signaling. (2) Cannabidiol (CBD)-rich cannabis formulations show promise in alleviating ASD-related symptoms, such as anxiety, aggression, and sleep disorders, without significant side effects; and (3) The therapeutic use of cannabis and cannabinoids for ASD remains understudied, particularly regarding long-term effects.

Translated to Public Policy

Governments and their proxies can launch public health campaigns highlighting the risks of cannabis use during adolescence, especially its links to schizophrenia and other mental health issues. They can improve regulation and access by restricting access to cannabis for minors through robust age-verification systems and enforcement. This includes addressing easy access to untested, unregulated hemp-derived cannabinoid (HDC) products available in smoke shops, cafes, and gas stations across the country. At the level of the supply chain, we can monitor the potency of cannabis products, particularly by disincentivizing the production and consumption of high-THC strains and products that pose more significant risks to adolescent brain development.

Research funding through states that have legalized cannabis and through federal grants looking to uncover the mechanisms for aid and obstruction of the brain's abilities could be prioritized. These could take the form of investments in longitudinal studies to understand the long-term effects of adolescent cannabis use on brain health and mental disorders and the support of research on genetic and environmental factors influencing susceptibility to cannabis-induced mental health issues.

To support positive outcomes from cannabis research, state and private sources could promote clinical trials of CBD-rich cannabis formulations to establish standardized protocols for treating ASD symptoms. This and open research policies at universities and private labs would encourage collaboration between medical professionals, researchers, and policymakers to integrate findings into practice.

A framework for therapeutic use guidelines could develop evidence-based guidelines for using medicinal cannabis in pediatric populations, with a focus on minimizing risks. Provide training for healthcare providers on the potential benefits and risks of cannabis treatments for neurodevelopmental disorders.

Cannabis is used by many as an alternative to standard medicine, especially those with limited access to insurance and pharmaceuticals. Access and affordability can take the form of financial subsidization of CBD-rich medicinal cannabis products for families managing ASD-related challenges to ensure equitable access.

The following chapter (LaCasse) examined the state and tools for empowering impacted youth; as such, policy recommendations and public health messaging were built into the research.

3.2 "Empowering Teens, Parents, and Healthcare Providers in the Cannabis Dialogue"

by Matthew LaCasse, D.O.

Recap and Takeaways This chapter, which is a combination of solid research and practical application, explores the rising rates of cannabis use among adolescents, shaped by social influences, legalization, and the development of high-potency and inconspicuous cannabis products like vapes and edibles.

LaCasse examines adolescent cannabis use trends and finds that rates of daily cannabis use among teens have increased alongside the legalization of cannabis in many states and that the reduced perceived harm of cannabis and the availability of high-potency products contribute to problematic use.

These phenomena create neurodevelopmental impacts from cannabis use, such as the disruption of critical neurodevelopmental processes, leading to potential long-term cognitive and emotional impairments in adolescents. Other findings include that cannabis dependence is increasingly observed in teens using high-potency products and that peer groups and marketing heavily influence adolescent perceptions of cannabis safety.

The chapter does a good job of converting the findings into real-world environments, including where adolescents often use cannabis as a coping mechanism for anxiety and depression and the role of parents and medical providers who can play a crucial role in shaping attitudes toward substance use.

Translated to Public Policy

As mentioned, this chapter includes recommendations in its research. Some additional steps might include youth education campaigns that implement comprehensive public health initiatives to educate teens on the risks of high-potency cannabis use, focusing on neurodevelopmental impacts and dependency risks.

This could shape the development of digital media campaigns targeting the misleading portrayal of cannabis safety on social platforms and the enforcement of stricter advertising regulations to prevent cannabis marketing from targeting youth.

The author recommends that healthcare providers address cannabis use in a non-judgmental manner by utilizing evidence-based interventions such as Motivational Interviewing (MI) and Acceptance and Commitment Therapy (ACT).

Empowerment of younger consumers can start by mandating clear labeling of THC content and potential risks on cannabis products, particularly high-potency vapes and edibles. Parental support programs can be effective if community-based workshops help them understand the risks of adolescent cannabis use, equip them with strategies to address it and emphasize healthy coping mechanisms and substance-free celebrations.

Provider training and resources can be helpful as well, like training healthcare providers in screening, brief intervention, and referral to treatment (SBIRT) for adolescent cannabis use and allocating funding for specialized adolescent substance use treatment programs or integrating family-based therapy approaches.

Some evidence-based interventions could include expanding access to ACT and MI for teens struggling with cannabis use, helping them reconnect with supportive peers, and supporting research into adolescent-specific treatments for cannabis use disorder, including alternatives to high-potency products.

Examples include community and school initiatives, such as establishing peer-led sober curiosity groups in schools to normalize substance-free lifestyles and foster resilience among teens and developing extracurricular programs that promote mindfulness, stress management, and healthy social bonding.

For optimal impact, cities and states could coordinate efforts across public health, education, and legislative sectors to create a consistent message on cannabis risks and to provide grants for local governments to implement tailored interventions in communities with high rates of adolescent cannabis use.

4 Cannabis and the Family at Home

The following chapters focus on the care of young children and the prevention of risks associated with secondhand cannabis smoke.

4.1 "Purified Cannabidiol in Pediatric Epilepsy"

by Aimee F. Luat, MD, and Kayla A. Hetrick, Pharm.D.

Summary of the Science

This chapter discusses the clinical use of purified cannabidiol (CBD), specifically Epidiolex®, for treating severe pediatric-onset epilepsy syndromes, including Dravet Syndrome (DS), Lennox-Gastaut Syndrome (LGS), and Tuberous Sclerosis Complex (TSC).

Purified CBD significantly reduces seizure frequency in drug-resistant epilepsies (DREs), and its efficacy has been demonstrated in randomized controlled trials (RCTs) and open-label extension studies. Depending on the syndrome and seizure type, median seizure reductions across studies range from 40% to 90%.

Common adverse effects (AEs) include diarrhea, drowsiness, and decreased appetite. Long-term data suggest sustained seizure control with manageable side effects for many patients. Unlike THC, CBD lacks euphoric effects and has minimal abuse potential.

High costs and limited insurance coverage for pharmaceutical-grade CBD create barriers to access. Misinformation and variability in nonregulated CBD products pose risks to patients relying on unverified alternatives.

Translated to Public Policy

Given these findings, it would be advisable to encourage insurance providers to cover FDA-approved CBD treatments for eligible patients with DREs. Implement subsidies or financial assistance programs to reduce the cost burden for low-income families.

Further, governments could mandate rigorous testing and labeling requirements for non-FDA-approved CBD products to ensure safety and accuracy and penalize misleading claims about unregulated CBD products, emphasizing the risks of contaminants and mislabeling.

State and federal agencies should increase funding for studies exploring CBD's broader applications and long-term effects, particularly for pediatric populations.

These jurisdictions should train healthcare providers to counsel patients on the differences between pharmaceutical-grade and unregulated CBD products.

CBD should be integrated into standard care by encouraging the inclusion of CBD as a standard adjunctive therapy in epilepsy treatment guidelines and by supporting physician training on the therapeutic uses of CBD and its pharmacokinetics to optimize patient outcomes.

Governments and public health organizations should educate families and caregivers on the potential benefits and risks of CBD therapies for epilepsy and highlight the importance of medical supervision when using CBD to manage seizures.

Community organizations and cities should ensure equitable access to CBD therapies for underserved and minority communities disproportionately affected by health disparities.

By implementing these policies, stakeholders can improve the accessibility, safety, and integration of CBD therapies for pediatric epilepsy, leading to better outcomes for affected children and their families.

4.2 "Secondhand Cannabis Smoke Exposure, Particulate Matter, and Effects on Physical and Neurobehavioral Health in Youth"

by Clara G. Zundel, Ph.D., Katya Parker, M.S., and Hilary A. Marusak, Ph.D.

Summary of the Science

This chapter reviews the current literature on secondhand cannabis smoke (SHCS) exposure, highlighting its physical and neurobehavioral effects on youth. The findings reveal significant health risks due to high concentrations of particulate matter in SHCS, surpassing levels found in tobacco smoke.

Based on methodologies and demographics, SHCS exposure rates among youth vary widely (1.6%–31%). Cannabis use in homes and vehicles is a major contributor to exposure, with detectable THC metabolites in the biological samples of exposed children.

SHCS exposure is linked to increased respiratory issues, including asthma, bronchitis, and viral infections. High particulate matter levels in SHCS can penetrate the lungs and blood-brain barrier and pose cardiovascular and inflammatory risks.

SHCS exposure correlates with cognitive deficits, reduced language skills, ADHD symptoms, and behavioral problems in children. Adolescents exposed to SHCS exhibit immediate declines in cognitive performance, including attention and executive functioning.

Cannabis joints and bongs generate higher particulate matter concentrations than tobacco cigarettes, with indoor levels exceeding the EPA's air quality standard. Ventilation reduces but does not eliminate SHCS risks, and vaping is not a risk-free alternative.

Few studies examine the long-term neurodevelopmental impacts of SHCS exposure, particularly during sensitive developmental periods. Current research lacks integration of biological measures and parental self-reports for accurate exposure assessment.

Translated to Public Policy

Federal guidelines for indoor air quality should be implemented, explicitly targeting SHCS in homes, vehicles, and public spaces and enforcing smoke-free policies in multi-unit housing (MUH) and vehicles, mainly when children are present.

States and counties should launch public education campaigns to debunk myths about SHCS's safety, emphasizing its risks to children and adolescents. They should also educate healthcare providers to routinely screen for SHCS exposure during pediatric visits and counsel families on harm reduction strategies.

We should fund longitudinal studies to assess the chronic effects of SHCS on neurodevelopment and respiratory health and develop and distribute affordable personal air monitors to families to track indoor particulate matter levels in real-time.

Public health advocacy groups should encourage the use of outdoor spaces for cannabis consumption to reduce SHCS exposure in enclosed environments and provide subsidies for ventilation upgrades in MUH to lower indoor pollutant levels.

These objectives can be realized by integrating SHCS into Existing Frameworks, like expanding tobacco smoke education programs to include cannabis, focusing on the health risks of SHCS, and leveraging existing public health infrastructure to integrate SHCS education and mitigation into broader air quality and environmental health policies.

Communities can also provide toolkits for parents to minimize SHCS exposure at home, including portable air filters and educational materials, and introduce school-based programs to teach children and adolescents about the dangers of SHCS.

5 Cannabis, the Mother, and the Baby

This section focuses on the impacts of cannabis on the developing fetus. The chapters aim to dispel misinformation related to the potential harms of cannabis use by pregnant women. The policy implications take complicated science and distill it into actionable pieces for legislators, regulators, providers, and researchers.

5.1 "The Endocannabinoid System and the Early Development of the Brain"

by Shiwani Thapa, B.S., and Anna Bukiya, Ph.D.

Summary of the Science

This chapter investigates the critical role of the endocannabinoid system (ECS) in prenatal brain development, focusing on neurogenesis and cerebrovascular formation. While ECS components like cannabinoid receptors and enzymes are essential for brain development, the misuse of cannabis during pregnancy poses significant risks.

ECS regulates neuronal development. Endocannabinoids are active during critical developmental periods. CB1 receptors are widely expressed in the fetal brain and play a key role in synapse formation and neuronal migration. Disruption of ECS signaling can impair these processes, resulting in long-term neurodevelopmental consequences.

ECS influences neural homeostasis and modulates cerebral blood flow, essential for supplying oxygen and nutrients during rapid brain development.

Cannabis use during pregnancy disrupts ECS signaling, leading to developmental delays, cognitive impairments, and altered synaptic activity. Studies report increased vulnerability in infants exposed to cannabinoids, emphasizing the need for preventive measures.

Translated to Public Policy

State and local health agencies should implement public health campaigns targeting expectant mothers, emphasizing the risks of prenatal cannabis exposure and mandating warning labels on cannabis products regarding potential neurodevelopmental harm to fetuses.

States, federal agencies, and research organizations should fund studies on the long-term effects of prenatal cannabis exposure and therapeutic interventions for affected children, train healthcare providers to screen for cannabis use during pregnancy, and provide resources for cessation support. These institutions should also develop initiatives to monitor and support children exposed to cannabis in utero, focusing on cognitive and emotional development.

Investigating non-cannabinoid ECS modulators to address conditions in preterm infants and exploring safe ECS-targeting therapies for developmental disorders would help support positive outcomes.

Public Health media departments should disseminate knowledge about the ECS's role in brain development to reduce the stigma around seeking help for substance use during pregnancy and collaborate with schools and communities to educate youth on the developmental risks of cannabis use.

Legislators and agencies should regulate THC potency in cannabis products to minimize unintended exposure risks, particularly among vulnerable populations, and standardize testing for cannabinoid residues in maternal and neonatal health screenings.

5.2 *"Cannabis and Reproductive Health: Having Patient Conversations"*

by Philip J. Molloy, MD

Summary of the Science

This chapter explores the effects of cannabis use on reproductive health and pregnancy outcomes. It emphasizes the critical role of the endocannabinoid system (ECS) in male and female reproductive processes and the adverse impacts of cannabis use during conception and pregnancy.

Cannabis use affects sperm quality, testicular volume, and hormonal balance in men. It disrupts ovulation, implantation, and other reproductive processes in women, and increased THC potency in modern cannabis products exacerbates these effects.

Prenatal cannabis use is associated with adverse outcomes, including low birth weight, preterm birth, small infants, and increased NICU admissions. Offspring of cannabis users show a higher incidence of cognitive, behavioral, and neurodevelopmental disorders, including ADHD, autism spectrum disorder (ASD), and emotional regulation difficulties.

Cannabis use during pregnancy leads to inheritable changes, potentially affecting multiple generations. Neuroimaging studies reveal altered brain connectivity in offspring exposed to cannabis in utero.

Many patients, influenced by legalization and societal perceptions, view cannabis as safe, including during pregnancy. Pregnant individuals often turn to sources like dispensary staff or online forums rather than healthcare providers (HCPs) for information about cannabis use.

A significant number of HCPs lack adequate knowledge about cannabis's impact on reproductive health and pregnancy, leading to missed opportunities for patient education and counseling.

Translated to Public Policy

Public agencies should launch evidence-based campaigns targeting misconceptions about cannabis safety during pregnancy and reproductive health, focusing on the risks to offspring, as messages emphasizing child health resonate most with pregnant individuals.

These agencies should mandate training for HCPs on cannabis's reproductive and prenatal effects to enhance their confidence in initiating patient conversations and provide tools like decision aids and posters to support these discussions in clinical settings.

Localities and states should prohibit advertising that downplays the risks of cannabis use during pregnancy and reproduction and require dispensaries to display warnings about the potential reproductive and developmental harms of cannabis use.

Providers can incorporate cannabis use screening into routine prenatal care and preconception counseling and develop nonjudgmental, patient-centered approaches to encourage disclosure of cannabis use.

Legislators and regulators should enforce consistent labeling requirements for cannabis products, including pictorial warnings on pregnancy-related risks, and use clear, accessible language to reach a broader audience.

Institutions should fund studies on the long-term effects of prenatal cannabis exposure on offspring development and epigenetics and support research on effective public health messaging to change perceptions about cannabis use during pregnancy.

Harm reduction and prevention efforts could expand access to addiction support and cessation programs tailored to pregnant individuals and provide resources for alternative symptom management (e.g., nausea, anxiety) during pregnancy.

The following chapter (Lanigan) examines novel twin studies to clear up some confusion around the effects of cannabis.

5.3 "Cannabis Effects on Brain and Behavior: The Problem of Residual Confounding and Utility of Twin Studies"

by Joseph Lanigan, B.A.; Stefania Yee; and Jonathan Schaefer, Ph.D.

Summary of the Science

This chapter investigates the effects of cannabis use on brain and behavioral outcomes, focusing on residual confounding—unmeasured variables that complicate causal interpretations. The authors emphasize the utility of twin studies in controlling for genetic and environmental factors to provide more precise insights into cannabis's potential effects.

Cannabis use correlates with increased risks for depression, substance use disorders, and suicidality, especially among heavy users and those who begin use in adolescence. Evidence for causal links to anxiety and antisocial personality disorder is weaker, with mixed results from studies.

Observational studies show associations between cannabis use and reduced gray matter volume in the hippocampus and orbitofrontal cortex, altered white matter microstructure, and changes in functional connectivity. Twin studies suggest that many of these associations may be due to genetic or shared environmental factors rather than direct causal effects.

Observational studies often fail to fully account for confounding variables, such as socioeconomic status, genetic predispositions, and childhood adversity. Twin studies control for these shared factors and suggest that some associations (e.g., with brain structure and depression) may not be causal. In contrast, others (e.g., substance use disorders and suicidality) may involve genuine cannabis-related effects.

Cannabis use disorder (CUD) is associated with more pronounced effects on mental health and brain outcomes, but these findings are also susceptible to confounding by unmeasured individual traits.

Translated to Public Policy

State and local lawmakers and regulatory agencies should prioritize enforcing strict age restrictions on cannabis sales to minimize adolescent use, given heightened vulnerability during brain development, and introduce penalties for noncompliance with age restrictions in legal markets.

State governments could allocate cannabis tax dollars to support longitudinal twin studies to disentangle the causal effects of cannabis on mental health and brain outcomes and fund research into cannabis's effects at varying doses and durations on vulnerable populations like adolescents.

Counties and state public health agencies should educate the public on the risks of early and heavy cannabis use, emphasizing associations with mental health and substance use disorders and developing targeted messaging to dispel myths about cannabis safety, particularly for young people and parents.

The messaging could encourage moderation in cannabis use and highlight the risks associated with high-potency products, as well as provide support for the regulation of product labeling to ensure clear communication of THC content and potential side effects.

Agencies and private providers could expand access to mental health services for individuals with cannabis use disorders and train clinicians to address cannabis-related mental health issues using evidence-based approaches, such as cognitive-behavioral therapy.

Policymakers and voter advocacy groups should incorporate findings from twin studies into policy discussions to account for confounding factors when evaluating the risks and benefits of cannabis legalization. They should also use evidence to inform balanced policies that weigh cannabis's therapeutic potential against its risks.

6 Conclusion

The "Science Translated to Public Policy: Selected Review and Analysis of Cannabis Risks and Therapeutic Potential" highlights research-driven policy recommendations to address cannabis's effects on brain development, public health, and social equity. This exercise prioritizes actionable, politically feasible measures that protect vulnerable populations while maximizing therapeutic benefits.

A key focus of this examination is adolescent cannabis use, which is linked to heightened risks of psychosis, anxiety, depression, and cognitive impairments like poor memory, executive dysfunction, and slower processing speed. These effects are exacerbated by early use and high-potency products. The author recommends stricter age limits, marketing restrictions, and clear THC labeling. He advocates for school-based interventions like SBIRT (Screening, Brief Intervention, and Referral to Treatment), parent education programs, and public health campaigns to correct youth misperceptions about cannabis safety.

This section also emphasizes the unique challenges faced by Sexual and Gender Minority (SGM) youth, who have higher rates of cannabis misuse due to stigma,

discrimination, and family rejection. To address these inequities, the author proposes expanding Medicaid to cover SGM-specific substance misuse care, provider training, and affirmative care models. Community support systems like peer mentorship and family education aim to create inclusive care pathways and reduce isolation for SGM youth.

This review of a cross-section of the chapters in this book also highlights the impact of prenatal cannabis exposure, which disrupts fetal brain development via the endocannabinoid system (eCB). This exposure is linked to cognitive delays, emotional dysregulation, and behavioral disorders in children. The translational analysis points to public education campaigns warning pregnant individuals about cannabis risks, warning labels on cannabis products, and healthcare provider training to screen for cannabis use during pregnancy and offer cessation support.

Another key area worthy of guidance is secondhand cannabis smoke (SHCS), which exposes children to high levels of particulate matter linked to asthma, respiratory infections, and cognitive impairments. The author calls for indoor air quality regulations, smoke-free multi-unit housing (MUH) policies, and public education campaigns on the dangers of SHCS exposure. He also proposes screening for SHCS exposure during pediatric visits.

This chapter's investigation explored cannabis's therapeutic potential, focusing on CBD-based epilepsy treatments like Epidiolex®, which reduces seizures in pediatric epilepsy syndromes like Dravet Syndrome and Lennox-Gastaut Syndrome. To increase access to these treatments, he recommends insurance coverage for FDA-approved CBD therapies, financial assistance for families, and stricter product testing and labeling standards for non-FDA CBD products to ensure safety and accuracy.

Finally, this analytical bridge between science and policy application highlights the importance of twin studies in clarifying cannabis's impact on mental health and brain development. While observational studies often link cannabis to reduced gray matter and increased depression, twin studies show some of these effects may be driven by genetic or environmental factors. It calls for funding twin and neuroimaging studies using cannabis tax revenue to reduce confounding variables and inform evidence-based cannabis policy.

The comparisons urge policy alignment and coordination at the federal, state, and local levels in all areas. By leveraging cannabis tax revenue for research, education, and support for vulnerable groups, the analysis and recommendations offer a balanced approach to cannabis policy reform that protects public health, promotes social equity, and maximizes therapeutic potential.

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