The Stoned Age: Sex Differences in the Effects of Adolescent Cannabinoid Exposure on Prefrontal Cortex Structure and Function in Animal Models

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Abstract

Cannabis is the most used drug during adolescence, which is a period of enhanced cortical plasticity and synaptic remodeling that supports behavioral, cognitive, and emotional maturity. In this chapter, we review preclinical studies indicating that adolescent exposure to cannabinoids has lasting effects on the morphology and synaptic organization of the prefrontal cortex and associated circuitry, which may lead to cognitive dysfunction later in life. Additionally, we reviewed sex differences in the effects of adolescent cannabinoid exposure with a focus on brain systems that support cognitive functioning. The body of evidence indicates enduring sex-specific effects in behavior and organization of corticolimbic circuitry, which appears to be influenced by species, strain, drug, route of administration, and window/pattern of drug exposure. Caution should be exercised when extrapolating these results to humans. Adopting models that more closely resemble human cannabis use will provide more translationally relevant data concerning the long-term effects of cannabis use on the adolescent brain.

Keywords

adolescence; cannabinoids; cannabis; THC; neurobiological effects; behavior

1. Introduction

Cannabis is the most commonly used drug among adolescents in North America, with a lifetime prevalence of nearly double that of all other illicit drugs combined (NSDUH, 2020). As of 2020, 43.7% of 12th graders in the United States have reported using cannabis in their lifetime (NSDUH, 2020). Perhaps more concerning, the prevalence of *heavy* cannabis use during adolescence has seen a threefold increase in the past 25 years, with an estimated 6.9% of 12th graders reporting *daily* use in the US (NSDUH, 2020). These trends have been coupled with steady declines in the social stigma and perceived risks of cannabis use over

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the past decade (Okaneku et al., 2015; Schuermeyer et al., 2014). Similar trends are also evident in Canada, where cannabis was legalized for recreational purposes at a national level in October 2018. Data from statistics for Canada's school-based Canadian Student Tobacco, Alcohol and Drug Use Survey (CSTADS) indicate that approximately 28% of teenagers in Grade 12, and 53% for young adults in the age range of 20–24, have used cannabis in the past year (CSTADS, 2018). Moreover, roughly 75% of those surveyed in CSTADS who had ever used cannabis reported using it in the past 3 months, with 32% of that group reporting daily use (CSTADS, 2018). These alarming statistics have reignited public concerns over the effects of adolescent cannabis use and have highlighted the pressing need to better understand its long-term repercussions and the neurobiological mechanisms by which these occur.

Despite the public consensus that cannabis use during adolescence has uniformly negative consequences that increase the risk for cognitive impairment later in life, review of the scientific literature presents a more complex picture. This is in part because the vast majority of human studies on this topic employ cross-sectional designs comparing cannabis users to non-users, and as such, a direct causal relationship has yet to be established (Weiland et al., 2015; Scott et al., 2018; 2019). Rodent models provide an excellent opportunity to answer complex questions in a high throughput manner under tightly controlled conditions in the absence of confounding variables. Depending on the outcome, rodent models offer differing levels of utility, but with respect to brain development, they offer a very valuable approach. The developmental organization of the rodent brain parallels the human brain in many ways, including the ontogeny of neurocircuitry connecting subcortical and cortical structures (Spear, 2000; Casey et al., 2013). With respect to adolescence, like humans, rodents exhibit a non-linear pattern of frontal cortical development that is highlighted by an overproduction of synapses, which are then pruned back in an experience-dependent manner (Spear, 2000). As such, rodent models represent a valuable approach for examining the lasting impacts of adolescent cannabinoid exposure on brain development and behavior. With that said, the primary objective of this mini-review is not to provide an exhaustive overview of all studies conducted on the effects of cannabis and cannabinoid administration during adolescence (see Stringfield and Torregrossa, 2020 for an excellent recent review of the human and rodent literature), but rather, to summarize the preclinical literature examining effects of adolescent cannabinoid exposure, with a focus on aspects of the cortical and striatal systems that support cognitive and motivational outcomes.

There is emerging evidence that while males are traditionally more at risk for using cannabis and developing cannabis-related problems (Hasin et al., 2019), that male-female gap has narrowed in recent years, particularly among adolescent users (Johnson et al., 2015; Johnston et al., 2020). Moreover, like other drugs of abuse, women demonstrate a "telescoping effect" that results in more rapid progression from first use to cannabis use disorder (Ehlers et al., 2010; Hernandez-Avila et al. 2004; Khan et al., 2013; Scheipis et al., 2011; Westermeyer et al., 2000). Accordingly, women enter treatment for cannabis-related problems after fewer years of use and less cumulative use than men (Hernandez-Avila et al., 2004). However, observed sex differences in the effects of adolescent cannabinoid use/ exposure have not been systematically reviewed, which presents a major impediment to better understanding if and why these sex differences exist. Thus, a secondary objective of

this review is to note evidence of sex differences in the effects of adolescent cannabinoid use/exposure.

2.   The Adolescent Brain Is Exquisitely Sensitive to Exogenous Cannabinoids

Adolescence is a critical neurodevelopmental period that is characterized by enhanced cortical plasticity and remodeling that supports behavioral, cognitive, and emotional maturity. Given the plastic nature of the brain during this developmental period, it can be exquisitely sensitive to perturbation by external stimuli. For example, it has been well established that phenomena such as head trauma (Toledo et al., 2012), substance use (Spear, 2018; Squeglia et al., 2009), and significant life stress (Andersen and Teicher, 2008) can impact the normative trajectory of brain development to exert indelible changes in brain structure. Structural alterations in cortical and subcortical brain regions, following these experiences during adolescence, are thought to be a primary mechanism for disrupting cognitive and behavioral function. With respect to cannabis use, a large body of evidence has indicated that adolescents that engage in heavy cannabis use often show alterations in brain development that are associated with impaired cognitive impairments (see Fischer et al., 2020 for review). However, it remains unknown whether these alterations are caused by cannabis use or alternately reflect pre-existing conditions that increase vulnerability for problematic cannabis use (Jacobus and Tapert, 2014).

Importantly, adolescent brain development differs by sex. The female cortex reaches a point of macrostructural maturity faster than the male cortex (Lenroot et al., 2007; Mutlu et al., 2013), and males show an age-related increase in resting state functional connectivity in the prefrontal cortex (PFC), whereas females show an age-related decrease (Zuo et al., 2010). Additionally, subcortical regions involved in emotional regulation, such as the amygdala and hippocampus, also show rates of maturation that differ by sex (Goddings et al., 2014; Bezivin Frere et al., 2020). The striatum also undergoes significant remodeling during adolescence and these processes also follow a sex-specific trajectory (Hammerslag and Gulley, 2016). As with the PFC, female development of the striatum occurs more rapidly than in males, with volume changes remaining steady after 12 years of age (Raznahan et al., 2014). Furthermore, males often continue to experience striatal volume and area changes into early adulthood, whereas females have already reached a relatively consistent state (Raznahan et al., 2014). Studies in rats also indicate sex-specific fluctuations in dopamine receptor density during adolescence. Male rats experience greater alterations in both D1 and D2 receptors in the ventral striatum during adolescent development, with large increases in D1/D2 receptor density followed by synaptic pruning (Andersen et al., 1997). In contrast, females experience a slight increase in D1/D2 receptor density, which remains unchanged into adulthood (Andersen et al., 1997). This sex-dependent organization of cortical and striatal networks during adolescence could make males and females differentially susceptible to cannabis-induced perturbations, thereby producing unique cognitive and behavioral effects later in life. Along these lines, compelling clinical data indicate that sex may even moderate the relationship between adolescent cannabis use

and PFC volume (Medina et al., 2009), which supports the idea that sex is a crucial variable to consider in studies examining effects of adolescent cannabinoid exposure.

The cannabis plant contains hundreds of molecules, but it is primarily delta-9-tetrahydrocannabinol (THC) that mediates the psychoactive properties of the plant (Mechoulam et al., 2014). THC exerts its biological effects through direct interaction with the endocannabinoid (eCB) system, a chemical signaling system in the brain and body that regulates a host of physiological processes (see Katona and Freund, 2012 for review). Generally speaking, THC activates type-1 cannabinoid (CB1) receptors, which influence synaptic transmission and information processing in the brain. From a neurodevelopmental perspective, CB1 receptors are widely expressed in the brain, and their expression peaks approximately at the onset of adolescence and declines into adulthood (Heng et al., 2011). In rodents, CB1 receptor expression is highest at the onset of adolescence (around postnatal day [PND] 30), particularly in the medial prefrontal cortex (mPFC) and striatum, followed by a decline in expression into adulthood (around PND 70) (Meyer et al., 2018). The eCB system also displays sexual dimorphism, with fluctuations in eCB activity coinciding with estrous phases (Gonzalez et al., 2000), which modulates the release of gonadal hormones in both humans and rodents (Meyer et al., 2018). Interestingly, CB1 receptor density begins increasing earlier in female rodents compared to their male counterparts (Rodríguez de Fonseca et al., 1993). In addition to regulating synaptic transmission and gonadal hormone release, eCB signaling is also known to play a role in circuit formation by modulating long-range axonal pathfinding and patterning of synaptic contacts for the appropriate development of cortical circuits (Berghuis et al., 2007; Maccarrone et al., 2014). This positions the eCB system at the center of critical neurodevelopmental processes; and, as such, interfering with eCB-mediated processes via exogenous THC administration during adolescence could produce long-lasting alterations within corticostriatal and corticolimbic circuits, resulting in behavioral and cognitive impairments later in life.

3.   Effects of Adolescent Cannabinoid Exposure on Prefrontal Cortex (PFC)-Dependent Behavior

Many studies have been conducted using preclinical animal models to examine the effects of adolescent cannabinoid exposure on cognitive endpoints in adulthood. The majority of studies employing THC have used a consistent administration protocol, whereby rats receive escalating intraperitoneal doses of THC (2.5 mg/kg PND 35–37; 5 mg/kg PND 38–41; 10 mg/kg PND 42–45) or vehicle, followed by a washout period until testing around PND 70–75. This method is designed to model the pattern of use seen in heavy human adolescent cannabis users (Rubino et al., 2008). Studies examining sex-dependent effects of this escalating THC exposure regimen on PFC-dependent working memory tasks have revealed mixed results. In the radial arm maze task, deficits in spatial working memory have been documented in THC-exposed rats of both sexes (Rubino et al. 2009a, 2009b). However, in the novel object recognition task, Quinn et al. (2008) have documented impaired discrimination in THC-exposed male Wistar rats (females were not tested), whereas two groups have independently shown effects specifically in female rats receiving this escalating THC dose regimen (Zamberletti et al., 2014; Llorente et al., 2013). However, it should be

noted that others have not observed any adolescent THC- (or cannabis smoke-) induced impairments in novel object recognition memory in male or female Long Evans rats that were tested in adulthood (Bruijnzeel et al., 2019). Thus, effects of adolescent THC administration on working memory performance have been reliably observed in rodent studies (but see Bruijnzeel et al., 2019, for conflicting evidence), but the results do not paint a clear picture of whether males or females are differentially impacted.

Effects of synthetic cannabinoid exposure during adolescence produce clearer evidence of a sex-dependent effect on memory. For instance, adolescent administration of the ultra-potent CB1 receptor agonist CP55,940 (0.4 mg/kg from PND 28–43) produced sex-specific alterations, with male (more so than female) rats showing an increase in working memory as measured by a water maze task (Higuera-Matas et al., 2009). Similarly, an escalating dose regimen of CP55,940 (0.15–3 mg/kg) from PND 29–50 also produced deficits selectively in male rats (Renard et al., 2013). These deficits were recapitulated in studies using male rats that were treated with another synthetic CB1 receptor agonist, WIN 55212–2 (WIN; 1.2 mg/kg/day), from PND 35–50 (Schneider et al, 2003; Abush and Akirav, 2012; Kirschmann et al., 2017a). Moreover, Mateos et al., (2011) showed that male (but not female) rats treated with CP55,940 during adolescence exhibited impaired novel object recognition and increased hippocampal CB1 receptor activity when tested in adulthood, whereas only female rats exhibited impaired object location memory, with no changes in novel object recognition or CB1 receptor activity. Additionally, it may be that these effects are partially driven by the unintended stress produced by high doses of synthetic CB1 receptor agonists. When rats are given volitional control over intravenous WIN administration during adolescence, they achieve an average daily intake of only 0.216 mg/kg WIN and consequently, neither males nor females show deficits in novel object recognition memory in adulthood at this dose (Kirschmann et al., 2017a). In fact, WIN self-administration actually led to *enhanced* working memory performance in an operant delayed match-to-sample task (Kirschmann et al., 2017a), which is consistent with another study from the same group showing that low-dose treatment with WIN during adolescence enhances working memory performance in female rats tested in adulthood (Kirschmann et al., 2017b). Finally, a follow-up study from this group also revealed that male rats that were trained to self-administer WIN during adolescence exhibited slightly enhanced working memory performance in the delayed match-to-sample task in adulthood; and, surprisingly, better performance in this task was positively correlated with total WIN self-administered (Kirschmann et al., 2017a). These effects underscore the importance of considering dose, drug, and route of administration when attempting to draw comparisons regarding the effects of adolescent cannabinoid exposure across studies.

In addition to cognitive outcomes, cannabinoid-induced neuroadaptations could also be implicated in aberrant drug-seeking behavior as well. Chronic adolescent cannabinoid administration increases opiate self-administration in male rats (Ellgren et al., 2007) and increases cocaine self-administration in adult female rats, but the latter, surprisingly, had no effect in adult male rats (Higuera-Matas et al., 2008). Moreover, 3 mg/kg WIN exposure during early adolescence has been shown to increase alcohol preference in male mice (Frontera et al., 2018), while male rats that were exposed to THC during adolescence have been shown to self-administer more WIN in adulthood compared to THC-naïve

control rats (Scherma et al., 2016). These changes in drug-seeking behavior may be due to alterations in ventral tegmental area (VTA) dopamine transmission, as *in vivo* recording studies have revealed that THC-treated adolescent rats exhibit a dose-dependent decrease in WIN-evoked VTA dopamine firing in adulthood, with a corresponding decrease in evoked dopamine release in the nucleus accumbens (NAc) shell (Scherma et al., 2016). One mechanism by which this may occur is through cannabinoid-induced alterations in eCB-mediated long-term depression (LTD) of inhibitory transmission in VTA GABA neurons, as reported in male CB1 knockout mice (Friend et al., 2017). However, there may be sex differences in these neuroadaptations. For example, female rats exposed to THC during adolescence were found to have reduced CB1 receptor density and/or CB-1/G-protein coupling in the VTA, NAc, and amygdala (Rubino et al., 2008). These changes in the VTA and NAc were exclusively seen in females, with males experiencing reductions in hippocampal CB1/G-protein coupling and amygdala CB1 receptor density (Rubino et al., 2008). Comparatively, female rats also experience reductions in CB1 receptor binding and anandamide (AEA) (but not 2-arachidonoylglycerol [2-AG]) content in the PFC following escalating doses of THC (Rubino et al., 2015). Studies have further confirmed an upregulation of CB1 receptors on GABAergic terminals and a downregulation of CB1 receptors on glutamatergic terminals following THC exposure selectively in adolescent female mice that express a genetic polymorphism in the fatty acid amide hydrolase gene (FAAH[C/A]) that results in compromised AEA degradation (Burgdorf et al., 2020). These data highlight how cannabinoid-mediated disinhibition of VTA dopamine projections to the NAc may contribute to sex-specific brain alterations that drive aberrant and persistent drug-use and -seeking behavior in adulthood.

4. Effects of Adolescent Cannabinoid Exposure on Prefrontal Cortex (PFC) Structure and Function

A large body of literature has demonstrated a central involvement of cortical and striatal dopamine signaling in working memory and flexible decision making (Floresco 2013, see Floresco et al., 2009; and Klanker et al., 2013 for detailed reviews). The ventral striatum serves as a functional interface between limbic and motor systems (Floresco, 2015), and the mPFC provides top-down control via glutamatergic projections to striatal regions to guide decision making via indirect eCB-mediated modulation of dopamine release (Mateo et al., 2017). Thus, interference with eCB-mediated control of the corticostriatal pathway via exogenous cannabinoid administration could alter the structure and function of these synapses, thereby setting the stage for cognitive and motivational alterations. In the following section, we will briefly review evidence demonstrating morphological, synaptic, and molecular alterations following adolescent cannabinoid exposure and note sex-specific effects where possible. We conclude by describing evidence indicating effects of adolescent cannabinoid exposure on non-neuronal cell populations (i.e., glial cells and inflammatory markers), which is important to consider in future studies.

4.1. Region-Specific Structural Alterations Following Adolescent Cannabinoid Exposure

Chronic cannabinoid exposure during adolescence induces long-lasting changes to the structure of mPFC neurons and their synaptic connections in rats, including reduced

basal dendritic arborization of pyramidal neurons in layer II/III (Rubino et al., 2015), and impaired hippocampal-input driven synaptic plasticity in the male rat mPFC (Renard et al., 2017b). Additionally, dendritic length and spine formation in hippocampal granule cells is reduced in proximal and distal dendrites from male rats exposed to THC during adolescence (Rubino et al., 2009b). In contrast, females had decreased spine density at distal basal dendrites (as opposed to proximal basal or apical) specifically in layer II/III mPFC pyramidal neurons (Rubino et al., 2015) that project to other cortical areas and are thought to be a key node in the working memory network (Goldman-Rakic, 1995). However, the effects of cannabinoid administration in rats during adolescence on dendritic morphology have been somewhat inconsistent, as others have shown that cannabinoids can *increase* dendritic length, spine density and the number of dendritic branches in mPFC pyramidal neurons (Carvalho et al., 2016). Notably, these changes are in direct opposition to the ventral striatum, which experiences widespread reductions in spine density in both adolescence and adulthood following adolescent cannabinoid administration (Carvalho et al., 2016). Thus, mPFC and NAc neurons undergo major structural changes following developmental cannabinoid exposure that may be sex-dependent and could have negative ramifications for corticostriatal-dependent behavior.

RNA-sequencing of prelimbic mPFC layer III cells from adult male rats exposed to THC in adolescence has revealed robust changes in the developmental transcription trajectory (i.p. 1.5 mg/kg, every third day PND 28–49) (Miller et al. 2019). For instance, vehicle-treated rats showed expected changes for developmental age in genes related to cell morphology, morphogenesis, and signal transduction that were surprisingly absent in THC-treated rats. In fact, pyramidal cell developmental transcriptomes exhibited very little overlap between the treatment groups. In addition to changes associated with actin cytoskeleton and dendritic development, THC exposure was associated with impaired epigenetic mechanisms like chromatin modification and histone methylation across development (Miller et al. 2019). Enrichment analysis of the differentially expressed genes revealed a functional association with Kmt2a, a chromatin methyl transferase, and the histone it interacts with, H3k4me3, which is implicated in cellular processes linked to neurodevelopment that are coincidentally associated with the development of psychiatric conditions such as major depression (Cruceanu et al. 2013). THC-treated rats also exhibited different dendritic arborization, spine density, and premature spine pruning compared to vehicle-treated rats (Miller et al. 2019). Another study found that adolescent THC exposure in female rats (i.p. 2.5–10 mg/kg from PND 35–45) modified mPFC H3k9me3, a histone that regulates genes related to synaptic plasticity, and impaired performance in a novel object recognition task (Prini et al., 2018). Importantly, pharmacological blockade of H3k9me3 during development prevented the negative effects of THC on these cognitive endpoints (Prini et al. 2018). These results suggest that sex differences in adolescent THC exposure may result from differential effects on epigenetic regulation of gene expression, and that these long lasting epigenetic changes may persist across generations (Szutorisz et al. 2014; Watson et al. 2015), at least in the striatum. The male offspring of parents exposed to THC during adolescence (i.p. 1.5 mg/kg, every third day PND 28–49) exhibit 1027 differentially methylated regions (DMRs) in the NAc, both core and shell, when compared to the offspring of rats treated with vehicle as adolescents (Watson et al. 2015). Watson and colleagues (2015) also reported that functional

networks impacted by these DMRs are involved in the regulation of glutamatergic synapses such as glutamatergic receptors, G proteins, ion channels, and scaffolding proteins. These studies indicated that adolescent THC exposure induced heritable, sex-specific alterations in the epigenetic regulation of genes that affected the structure and activity of corticostriatal synapses.

4.2. Synaptic Effects of Adolescent Cannabinoid Exposure on the medial-PFC (mPFC)

As mentioned above, the eCB system fundamentally contributes to adolescent neurodevelopment, particularly in the PFC (Meyer et al., 2018). Excitatory and inhibitory transmission in the PFC is under the control of eCBs (see McLaughlin et al., 2014 for review), and both glutamatergic and GABAergic synapses undergo considerable plasticity during adolescence, which can be perturbed following adolescent cannabinoid exposure. For instance, eCB-mediated LTD of excitatory transmission is reduced in the mPFC in adult female rats and mice following adolescent cannabinoid exposure, suggesting decreased eCB-mediated control of glutamatergic transmission (Rubino et al., 2015; Lovelace et al., 2015). Moreover, escalating doses of THC during adolescence increased the expression of GluA1 subunits in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors as well as GluN2B subunits in N-Methyl-D-aspartate (NMDA) receptors in adulthood (Rubino et al. 2015), which may help explain reduced LTD at glutamatergic synapses in the mPFC previously documented (Rubino et al., 2015; Lovelace et al., 2015). Remarkably, daily administration of the FAAH inhibitor URB597 in adult rats (PND 75–105) rescued deficits in (1) mPFC CB1 receptor density, (2) eCB-mediated LTD, (3) density and dendritic arborization of newborn hippocampal neurons, and (4) synaptic plasticity within the dentate gyrus in a CB1 receptor-dependent manner (Cuccurazzu et al. 2018). Thus, re-establishing a normative baseline of AEA signaling could reverse some of the neurobiological and behavioral deficits that have been observed in female rodents following adolescent cannabinoid exposure.

At the synaptic level, the vesicle-associated protein synaptophysin and postsynaptic anchoring postsynaptic density protein 95 (PSD95) have been shown to be reduced in the mPFC of adult female rodents exposed to THC during adolescence (Rubino et al. 2009a). Notably, this reduction was unique to the mPFC, as it was not seen in the hippocampus. In contrast, male rats exposed to THC in adolescence showed altered expression of proteins involved in synaptic transmission in the hippocampus rather than mPFC (Rubino et al. 2009b). Decreased hippocampal PSD95 expression paralleled decreased NMDA receptor levels throughout the hippocampus. Since PSD95 mediates the anchoring of NMDA receptors in the synapse, these effects may be closely linked. Vesicle associated protein VAMP2, a critical mediator of neurotransmitter release and vesicle trafficking that interacts with synaptophysin (Pennuto et al. 2003), was similarly decreased.

Early adolescent exposure to WIN not only enhances excitatory transmission, but also disrupts inhibitory networks of the mPFC, at least in male rats, as evidenced by a disruption in gamma oscillations that are comprised of aggregated synchronous activity of fast-spiking interneuron populations (Cass et al., 2014; Renard et al., 2017b). Accordingly, 2 mg/kg WIN exposure from PND 35–45 (but not PND 50–55 or PND 75–80) decreased GABA

transmission onto layer V pyramidal neurons in the mPFC of male rodents (Cass et al., 2014), which suggests a specific window of vulnerability for cannabinoid-induced alterations at GABAergic synapses. Accordingly, male mice exhibited reductions in cortical oscillations at gamma, beta, alpha, and theta bandwidths (Raver et al., 2013). It has been speculated that this compromised mPFC disinhibition could be an underlying mechanism behind working memory deficits seen in male, but not female, rats following synthetic CB1 receptor agonist treatment during adolescence (Raver et al., 2013; Poulia et al., 2020). However, it should also be noted that basal GABA levels, as well as GAD67 expression in parvalbumin- and cholecystokinin-positive neurons, are also reduced in the mPFC of female rats following escalating THC injections during adolescence (Zamberletti et al., 2014). Thus, systematic interrogation of sex differences in mPFC GABA transmission following adolescent cannabinoid exposure will be required before we can fully understand whether these effects are indeed sexually dimorphic.

Overall, it appears that adolescent cannabinoid administration produces region-, and sex-dependent effects on cortical cell morphology and synaptic transmission. This causes exaggerated mPFC activation, perhaps as a result of diminished GABAergic input to mPFC pyramidal cells. This could, in turn, give rise to the cognitive deficits described above. It should be noted, however, that glutamatergic inputs from the mPFC to the dorsal striatum unexpectedly show reduced activity following escalating doses of THC in female rodents, which questions the hypothesis of uniformly overactive cortical-striatal neurotransmission (Zamberletti et al., 2014). It remains unknown whether this effect is unique to female rodents, as similar endpoints have not yet been evaluated in male rodents. Interestingly, chronic administration of increasing doses of THC during adolescence sensitizes the locomotor response to acute administration of the NMDA receptor antagonist phencyclidine in female (Zamberletti et al. 2014) but not male rats (Zamberletti et al. 2016) when tested in adulthood, which suggests that cannabinoid-induced neuroadaptations in motor pathways may be more specific to adolescent female rodents.

4.3. Sex-Dependent Molecular Alterations Following Adolescent Cannabinoid Exposure

Studies designed to characterize the molecular underpinnings of cannabinoid-induced behavioral alterations have pointed to a role for phosphorylated cyclic adenosine monophosphate response element-binding protein (pCREB), which regulates the transcription of proteins that are fundamentally involved in neurodevelopment and synaptic plasticity. THC-treated female rodents showed a reduction in pCREB levels in the mPFC and hippocampus; where, notably, many antidepressants have been shown to increase CREB activity (Blendy, 2006). THC-treated female rats also exhibited decreased neurogenesis in the hippocampus, another hallmark of depression in humans (Realini et al., 2011). Additionally, pCREB levels were increased in the NAc of THC-treated female rodents, the opposite of which has been shown to produce antidepressant-like effects (Newton et al., 2002). In contrast to female rodents, THC-treated male rodents did not differ in pCREB levels from vehicle treated males. These data suggest that females may be more susceptible to the deleterious effects of adolescent THC exposure that result in a pronounced depressive-like phenotype in adulthood.

In addition to pCREB, brain-derived neurotrophic factor (BDNF), a known mediator of cellular survival and synapse maintenance, has also been implicated in altered neurodevelopment of frontal brain regions (Reichardt, 2006; Poulia et al. 2020). Adolescent male and female rats treated with the same THC administration protocol, as above, exhibited differences in BDNF protein levels in the PFC when tested during adulthood. Western blot analysis determined that male rodents have decreased PFC BDNF expression whereas female rodents showed an increase. Since BDNF transcription is mediated by CREB family transcription factor activity (Esvald et al. 2020), altered pCREB levels may disrupt the tightly controlled autoregulation of BDNF transcripts that can account for the differential developmental responses to cannabinoids between males and females, at least in rodents.

It is tempting to posit that altered CREB and BDNF signaling may be due to neurodevelopmental disruption, which results in reduced synaptic activity. Proteomic analysis of THC-treated female rat PFC synaptosomes revealed a reduction in the mitochondrial protein cytochrome b-c1 complex subunits 1 and 2, and adenosine triphosphate synthase alpha and beta subunits, which suggests reduced synaptosomal mitochondria and less metabolically active synaptosomes (Rubino et al. 2009a). Further evidence supporting this conclusion includes reduced glycolysis enzymes pyruvate kinase isozymes M1/M2, fructose bisphosphate aldolase isoform A, and glyceraldehyde-3-phosphate dehydrogenase, as well as reduced molecular chaperone heat shock cognate 71 kDa protein, a regulator of proteostasis during periods of oxidative stress (Rubino et al. 2009a). These data support a general pattern of disruption for various metabolic markers in the mPFC of female rodents after adolescent THC exposure, whereas male rodents showed more prominent alterations in the hippocampus. However, one study indicated that adolescent THC exposure disrupts mammalian target of rapamycin (mTOR) and wingless (Wnt) signaling in the PFC of male rats, which are critical for synaptic plasticity (Renard et al., 2017a). Decreased phosphorylated Akt-thr308, glycogen synthase kinase-3, β-catenin, ribosomal protein S6 kinase, and mTOR were also reported (Renard et al., 2017a). Similar to findings in post-mortem PFC tissue from patients with schizophrenia (Beasley et al. 2001; Kozlovsky et al. 2001) and major depression (Jernigan et al. 2011), alterations in Wnt signaling may underlie some of the observed cognitive and behavioral abnormalities.

4.4. Effects of Adolescent Cannabinoid Exposure on Non-Neuronal Cells

Astrocytes are known to be key mediators of synaptic transmission and metabolic activity in neighboring neurons, particularly in the hippocampus where stimulation of astrocytic CB1 receptors results in the release of excitatory gliotransmitters at tripartite synapses (Navarrete & Araque 2008). It is possible this is another mechanism by which adolescent THC exposure disrupts circuit development and synapse maturity. There is additional evidence for a contribution of non-neuronal cells in the effects of cannabinoids on cortical neurodevelopment. For instance, escalating doses of THC exposure during adolescence induce a persistent neuroinflammatory state in the mPFC of female rats (male rats were not tested), that is characterized by increased expression of the microglia marker Iba1, upregulation of CB2 receptors on microglia, a reduction in the anti-inflammatory cytokine IL-10, and an increase in the pro-inflammatory markers TNF-α, iNOS, and COX-2 (Zamberletti et al. 2015). Notably, these effects are specific to the mPFC, with no significant

group differences observed in the hippocampus or amygdala. In contrast, male rats exhibited increased glial fibrillary acidic protein (GFAP) expression and a neuroinflammatory state similar to female rats, but in the hippocampus rather than the mPFC (Zamberletti et al. 2016). Chronic neuroinflammation that is mediated by glia are synapto- and cyto-toxic; and, as such, these changes are receiving more attention as key contributors to neurodegenerative and neuropsychiatric diseases. Strikingly, treating female rodents with ibudilast to block glial activation during adolescent THC exposure prevented mPFC neuroinflammation and rescued performance in the novel object recognition task in adulthood, but not altered passive behavior in the forced swim test (Zamberletti et al. 2015). These results suggest a novel mechanism in female rodents whereby adolescent THC exposure provokes mPFC neuroinflammation, presumably mediated by microglia and astrocytes, damaging neurons and their synaptic connections, with a concomitant memory impairment that is independent of increased passive coping behavior. Unfortunately, the ibudilast experiment has yet to be conducted in pretreated male rodents to test the contributions of hippocampal microglia, nor were the effects of ibudilast tested on sucrose preference or the morphological characteristics of mPFC neurons.

5. Conclusions

As the rates of daily, heavy cannabis use continue to rise across North America, there is an increased urgency to better understand the long-term effects of adolescent cannabinoid exposure on the brain, behavior, and cognitive functioning. The PFC is rich in CB1 receptors and undergoes substantial remodeling during adolescence, thus making it is exquisitely sensitive to cannabinoid-induced perturbations that may also differ by sex. Preclinical studies have generally supported the notion that adolescent cannabinoid administration causes enduring effects on working memory and novel object recognition memory, particularly in males that are administered high doses of synthetic cannabinoids, though these effects may be influenced by the species, strain, drug employed, route of administration, and developmental window and pattern of drug exposure. Both male and female rodents that receive cannabinoids during adolescence show alterations in drug-seeking behavior later in life, but emerging evidence indicates that females may experience more reward-related effects following adolescent THC administration than males. This appears to interact with genetic polymorphisms in components of the eCB system that synergize to augment preference for THC (and possibly other drugs) later in adulthood (Burgdorf et al., 2020). These findings are in line with the possibility that cannabinoid exposure during adolescence accelerates the "telescoping effect" of THC exposure that has been routinely demonstrated in female rodents and humans.

At the neurobiological level, cannabinoid-induced alterations in the structure and function of neurons in the mPFC results in long-term adaptations in both glutamatergic and GABAergic synapses. This culminates in increased disinhibition and hyperactivity of mPFC pyramidal output neurons. However, fully powered comparisons by sex are lacking and as such, it remains unknown whether these effects are more pronounced in either sex or whether they give rise to unique, sex-specific behavioral alterations. The effects of adolescent cannabinoid exposure at the synaptic level are supported by studies demonstrating alterations in transcription factors (pCREB) and neurotrophic factors (BDNF) in the mPFC of females,

which differs from results in males that tend to be more restricted to the hippocampus. Emerging evidence further indicates that non-neuronal cells in the mPFC are also impacted by adolescent cannabinoid exposure in females, whereas these cells are again more negatively impacted in the hippocampus of males. Remarkably, restoring eCB signaling via the inhibition of FAAH has emerged as a promising strategy for mitigating the long-term neurobiological and behavioral effects of adolescent cannabinoid exposure in rodent studies.

As with all preclinical studies, we must proceed with caution when attempting to translate findings from animal models to human populations. Except for a few notable exceptions using cannabis smoke (Bruijnzeel et al. 2019) or self-administration (Kirschmann et al. 2017a, 2017b), virtually all of the studies described in this review used forced injections of isolated cannabis constituents or synthetic CB1 receptor agonists, which exhibit a fundamentally different pharmacological profile compared to volitional inhalation of whole-plant cannabis preparations, the most common form of cannabis use in humans (McLaughlin, 2018). In the studies described herein, the doses administered are also exceptionally high, which further limits the translational value of these studies. Differences in the metabolism of THC between adolescents and adults could also be an important consideration. For instance, studies have shown 50%−70% higher brain concentrations of THC metabolites in adolescent mice relative to adult mice (Torrens et al., 2020). This is an important consideration when assessing sex differences as well, since studies now indicate that adolescent female rodents exhibit enhanced metabolism of THC compared to males, resulting in elevated production of the psychoactive and highly potent THC metabolite 11-OH-THC (Wiley and Burston, 2014; Ruiz et al., 2021). This may lead to greater unintended CB1 receptor activation in adolescent females under circumstances where metabolites are likely to accumulate, like that seen after a large bolus injection of THC. Thus, to draw meaningful conclusions regarding the effects of adolescent cannabis use and potential sex differences in these effects, it is absolutely critical that we adopt more translationally relevant models that recapitulate the drug and route of administration that is most common among human users (see Freels et al., 2020 for validation of a novel model of cannabis vapor self-administration in male rats and Glodosky et al., 2020 for a demonstration of its feasibility in females). Adopting models that more closely resemble human adolescent cannabis use, as well as the behavioral and cognitive endpoints of interest, will undoubtedly provide us with a more trustworthy indication of the long-term effects of cannabis on the developing brain. This can be hopefully leveraged to inform prospective and current cannabis users on the actual risk of using cannabis during this sensitive neurodevelopmental period.

Abbreviations

2-AG	2-arachidonoylglycerol
11-OH-THC	11-hydroxy-delta-9-tetrahydrocannabinol
AEA	anandamide
BDNF	brain-derived neurotrophic factor

CB1	type-1 cannabinoid receptor
CB2	type-2 cannabinoid receptor
COX2	cyclooxygenase-2
CREB	cyclic adenosine monophosphate response element-binding protein
CSTADS	Canadian Student Tobacco, Alcohol and Drug Use Survey
DMRs	differentially methylated regions
eCB	endocannabinoid
FAAH	fatty acid amide hydrolase
GABA	gamma-Aminobutyric acid
iNOS	inducible nitric oxide synthase
i.p.	intraperitoneal
mPFC	medial prefrontal cortex
mTOR	mammalian target of rapamycin
NAc	nucleus accumbens
NMDA	N-Methyl-D-aspartate
pCREB	phosphorylated cyclic adenosine monophosphate response element-binding protein
PFC	prefrontal cortex
PND	postnatal day
THC	delta-9-tetrahydrocannabinol
TNF-α	tumor necrosis factor alpha
VAMP2	vesicle associated membrane protein 2
VTA	ventral tegmental area
WIN	WIN 55,212
Wnt	Wingless

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