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SPECIAL ISSUE

Neurobiological consequences of maternal cannabis on human fetal development and its neuropsychiatric outcome

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Abstract Despite the high prevalence of marijuana use among pregnant women and adolescents, the impact of cannabis on the developing brain is still not well understood. However, growing evidence supports that the endocannabinoid system plays a major role in CNS patterning in structures relevant for mood, cognition, and reward, such as the mesocorticolimbic system. It is thus clear that exposure to cannabis during early ontogeny is not benign and potential compensatory mechanisms that might be expected to occur during neurodevelopment appear insufficient to eliminate vulnerability to neuropsychiatric disorders in certain individuals. Both human longitudinal cohort studies and animal models strongly emphasize the long-term influence of prenatal cannabinoid exposure on behavior and mental health. This review provides an overview of the endocannabinoid system and examines the neurobiological consequences of cannabis exposure in pregnancy and early life by addressing its impact on the development of neurotransmitters systems relevant to neuropsychiatric disorders and its association with these disorders later in life. It posits that studying in utero cannabis exposure in association with genetic mutations of neural systems that have strong relationships to endocannabinoid function, such as the dopamine, opioid, glutamate, and GABA, might help to identify individuals at risk. Such data could add to existing knowledge to guide public health platform in regard to the use of cannabis and its derivatives during pregnancy.

Keywords Endocannabinoid · Cannabinoid receptor · Drug addiction · Schizophrenia · THC

Abbreviations

2-AG	2-Arachidonoyl glycerol
5HT3	5-hydroxytryptamine 3 receptor
AAT	*Adenosine*-adenosine-thymine
ABHD4	Alpha/beta hydroxylase-4
AEA	Anandamide
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
CB_1R	Cannabinoid receptor type 1
CB_2R	Cannabinoid receptor type 2
CNR1	Cannabinoid receptor type 1 gene
CNS	Central nervous system
COMT	Catechol-*O*-methyltransferase
D_1	Dopamine receptor type 1
D_2	Dopamine receptor type 2
DAGL	Diacylglycerol lipase
eCB	Endocannabinoid
FAAH	Fatty acid amide hydrolase
GABA	Gamma-aminobutyric acid
GABA-B	Gamma-aminobutyric acid type B receptor

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GDE1	Glycerophosphodiester phosphodiesterase 1
GluR1	Glutamate receptor type 1
GluR2/3	Glutamate receptor type 2 and 3
GPCR	G protein-coupled receptor
GPR55	G-protein coupled receptor 55
GTPγS	Guanosine gamma thio-phosphate
IQ	Intelligence quotient
MAPK	Mitogen-activated protein kinase
Met	Methionine
MGL	Monoglyceride lipase
MHPCD	Maternal Health Practices and Child Development Project
mRNA	Messenger ribonucleic acid
NAPE-PLD	N-acyl-phosphatidylethanolamine-specific phospholipase D
NRG1	Neuregulin 1
OPPS	Ottawa Prenatal Prospective Study
THC	Δ^9-tetrahydrocannabinol
TRPV1	Transient receptor potential vanilloid 1
Val	Valine
WIN55,212-2	R(+)-[2,3-Dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo [1,2,3-de]1,4-benzoxazinyl]-(1-naphthalenyl) methanone mesylate

Introduction

Approximately 4% of women in the United States report using illegal drugs with marijuana being by far the illicit drug most commonly abused during pregnancy (75%) [164; Fig. 1]. The prevalence of prenatal cannabis exposure is also between 2 and 5% in European countries [38, 43, 112], reaching even up to 13% in high-risk populations [196]. One-third of Δ^9-tetrahydrocannabinol (THC), the major psychoactive component of cannabis [143], undergoes cross-placental transfer upon cannabis smoking [100], raising important concerns about the potential impact of maternal cannabis use on the developing fetus. A number of studies have reported increased rates of fetal distress, growth retardation, and adverse neurodevelopmental outcomes with prenatal cannabis exposure [28, 50, 100]. The pathogenic impact of phytocannabinoids on the CNS is underscored by several epidemiological and clinical studies documenting impulsive behavior, social deficit, cognitive impairment, consumption of addictive substances, and psychiatric disorders (e.g., schizophrenia, depression, and anxiety) in adult individuals with in utero and early adolescent marijuana exposure [6, 57, 98, 101, 139, 150, 153].

Despite documented adverse outcomes, there is limited information regarding the neurobiological consequences of cannabis exposure during human fetal brain development. As outlined below, recent evidence suggests that perinatal THC exposure alters fundamental developmental processes, and particularly impairs the establishment of connectivity between brain regions that play a role in mood, motivation, and cognition. These mesocorticolimbic neuronal circuits remain vulnerable to dysfunction later in life and thus could be sensitive to developmental events and environmental stressors that themselves can influence the onset and course of neuropsychiatric disorders. This review provides an overview of the endocannabinoid (eCB) system, the primary molecular target of THC and an important modulator of neurotransmission, and examines the neurobiological consequences of cannabis exposure in pregnancy and early life. This is achieved by addressing THC's impact on the development of neurotransmitters systems relevant to neuropsychiatric disorders and its association with these disorders later in life. Although this review focuses on human studies, findings from animal models are also discussed in order to fill present gaps of knowledge given the limited neurobiological studies in human. Moreover, many experimental rodent studies have recapitulated findings in human suggesting evolutionary conservation of the eCB system at least in mammalian species [121]. The consequences of developmental cannabinoid exposure in animal models are summarized by Schneider et al. in this special issue.

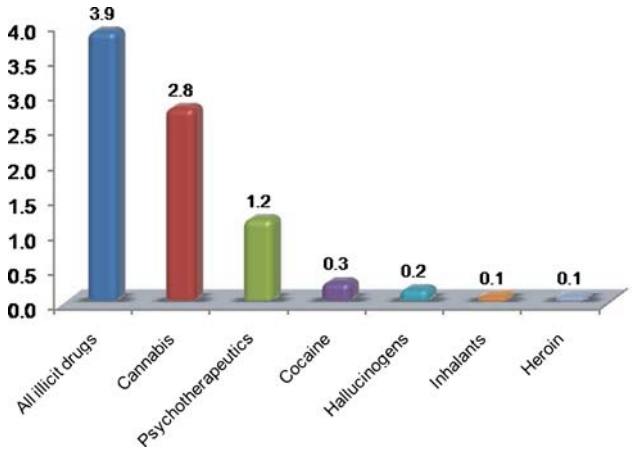

Fig. 1 Percentage of current illicit drug use in pregnant women showing cannabis as the most commonly used drug, followed by non-medical use of medication and cocaine [163]

Neurobiology of the endocannabinoid system and relevance to the developing brain

A fundamental role of eCB signaling is at the synapse with a clear continuum of action from synapse establishment during early neurodevelopment to synaptic function in the

adult brain [84]. Endocannabinoids [e.g., anandamide (AEA), 2-arachidonoyl glycerol (2-AG)], endogenous cannabinoid receptor agonists, and THC, a phytocannabinoid that mimics eCB action, exert most of their effects via the cannabinoid CB_1 [69, 120] and CB_2 [77, 132] receptors as well as the novel orphan G-protein coupled receptor GPR55 [105, 161]. The CB_1 receptor (CB_1R) belongs to the $G_{i/o}$ family of seven transmembrane G protein-coupled receptors (GPCRs) that can signal through several transduction pathways, including adenylate cyclase, MAPK and ion channels [97]. Recruitment of the signaling pathways is context-dependent based on the requirements of distinct developmental stages determining a cell's identity: stem cell proliferation, lineage commitment, neuronal morphogenesis, and axon patterning [85].

CB_1R is one of the most abundant GPCRs in the adult brain and is localized in regions important for movement (e.g., basal ganglia: striatum and substantia nigra), cognition and attention (e.g., cerebral cortex), as well as emotion and memory (e.g., amygdala, hippocampus) [14, 72, 90, 92, 117, 144]. The CB_1R emerges during very early stages of brain development [8, 22, 158]. Its receptors are localized in white matter areas and cell proliferative regions [13, 158, 191], and are intricately involved in neurodevelopmental events such as proliferation, migration, and synaptogenesis of neuronal cells [9, 10, 45, 64, 83, 194]. The CB_1R is detected from gestation day 11 in the murine CNS (comparable to around 5–6 weeks in the human embryo) with gradually increasing levels for both mRNA and receptor density throughout the prenatal period [10, 13, 45, 65, 191]. We characterized the expression pattern of the CB_1R mRNA in the midgestation (17–22 weeks) human fetus and demonstrated that in contrast to the adult brain, in which the CB_1R is widely distributed, high CB_1R expression is restricted during early to midgestational development to limbic structures such as the amygdaloid complex (primarily the basal nuclear group) and hippocampus (in particular the CA2,3 regions). In contrast, only moderate to low CB_1R expression was seen in other regions such as the medial/ventral striatum, thalamus, cerebral cortex, and subventricular zone [131, 191; Figs. 2 and 3]. CB_1R sites expressed in the fetal brain [72, 116] are functional as evidenced by WIN55,212-2, a cannabinoid receptor agonist, significantly stimulating [^{35}S]GTPγS binding in both the rodent [gestational day 16; 12] and human fetal brain [34, 119, 191].

In addition to the CB_1R, THC and eCBs also function as ligands for other receptors. One such receptor target, CB_2R, is primarily restricted to the peripheral immune system. However, circumstantial evidence also suggests that the CB_2R may be expressed in microglia cells of the CNS [5, 189] as well as in the rodent brainstem, cortex, and cerebellum in low levels [137, 183]. This, however, has remained controversial since some studies failed to detect the CB_2R in the rodent [32] or the human fetal [191] brain. THC and several eCBs also bind to GPR55 [105, 161], which has been identified in the striatum of the human adult brain [165]. Many questions remain regarding the GPR55 receptor, including its expression during development, pharmacology, and signaling capabilities, but the data is unequivocal that THC directly activates GPR55 [105]. Other lipid receptors that sense eCBs and exogenous cannabinoids are currently being explored. For example, THC and AEA, but not 2-AG, have been shown to be agonists at the transient receptor potential vanilloid 1 (TRPV1), a non-selective cation channel [160, 174]. Interestingly, cannabidiol, an apparent neuroprotective

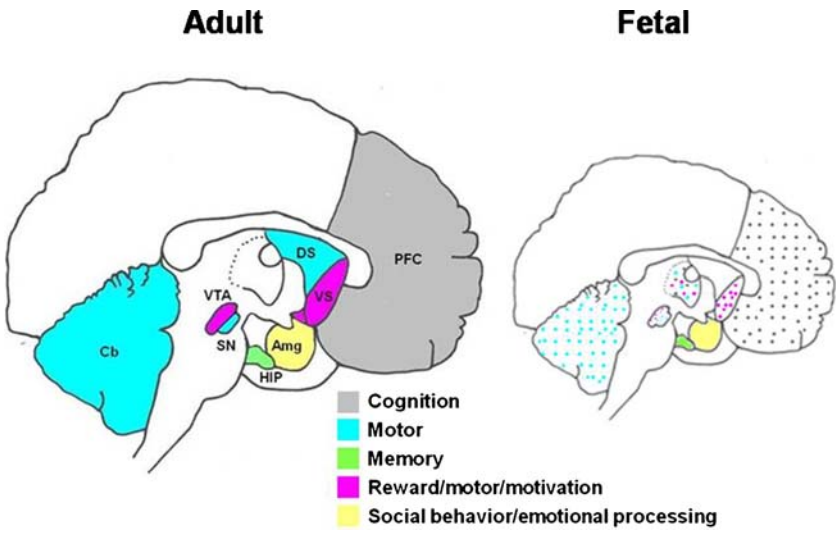

Fig. 2 Schematic overview of CB_1R mRNA expression in selective regions of the adult (*left*) and fetal (*right*) human brain in relation to behavioral and cognitive functions. *Cb* cerebellum, *VTA* ventral tegmental area, *SN* substantia nigra, *Hipp* hippocampus, *Amg* amygdala, *DS* dorsal striatum, *VS* ventral striatum, *Ctx* cortex

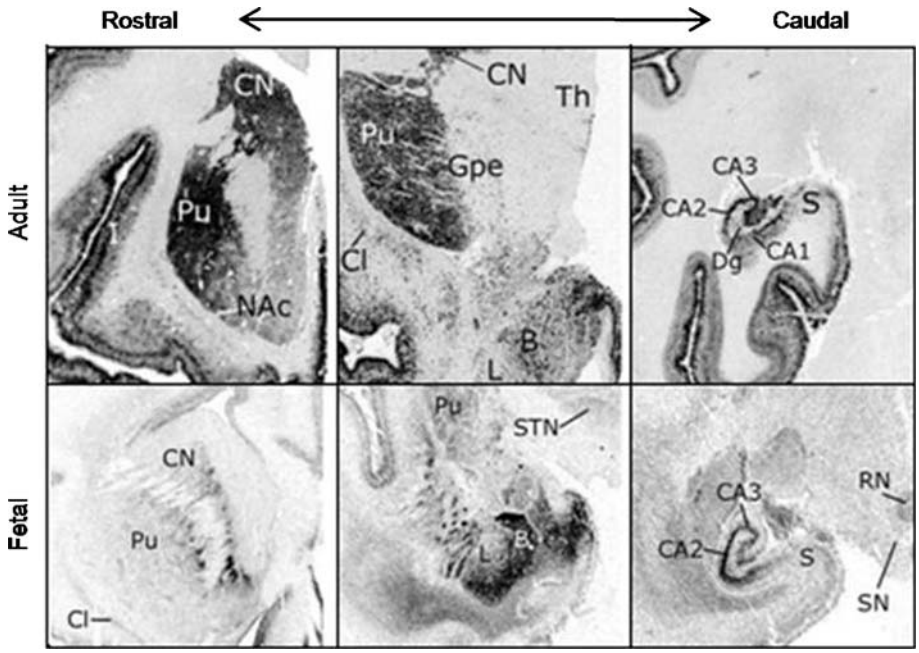

Fig. 3 CB_1R mRNA expression in rostral to caudal levels of the human adult (*top*) and midgestational (week 20) fetal brain (*bottom*). *B* basal amygdaloid nucleus, *Cl* claustrum, *CN* caudate nucleus, *Dg* dentate gyrus, *Gpe* external globus pallidus, *I* insula cortex, *L* lateral amygdaloid nucleus, *NAc* nucleus accumbens, *Pu* putamen, *RN* red nucleus, *S* subiculum, *SN* substantia nigra, *STN* subthalamic nucleus, *Th* thalamus. Scale bar = 1 cm. Adapted from Wang et al. [191]

component of cannabis, also binds TRPV1 [16]. This might have important implications for the developing brain: the expression pattern of TRPV1 during human fetal development has yet, however, to be determined, but TRPV1 has been identified in the cerebral cortex of adult human brain and in mesocorticolimbic- and basal ganglia-related areas of the adult rat brain [27, 124].

The ontogeny of the eCB system has been described in recent reviews [83, 84] and by Galve-Roperh et al. in this special issue. The evidence is clear that eCBs (in particular 2-AG), their associated enzymes [2-AG: synthesized by sn-1-selective diacylglycerol lipase (DAGL) alpha and beta and degraded by monoglyceride lipase (MGL); AEA: likely synthesized by alpha/beta hydroxylase-4 (ABHD4) and glycerophosphodiester phosphodiesterase 1 (GDE1), and inactivated by fatty acid amide hydrolase (FAAH)] and receptors (CB_1R and CB_2R, GPR55, TRPV1) are expressed from very early life and are tightly related to the regulation of neuronal generation, maturation, and cell specification within neuronal networks. Interestingly, the CB_1R is localized from early gestation to developing axonal projections of glutamatergic neurons in the cerebral cortex and hippocampus, with expression during late gestation observed in GABAergic interneurons on axons and axonal growth cones [9, 10, 84, 85, 131]. In fact, CB_1R expression levels peak as synaptic connectivity is established by cortical pyramidal cells [131] and GABAergic interneurons [10]. Hence, cannabis exposure, which induces a supraphysiological modulation of the eCB system and disrupts the temporal precision of eCB signaling, has the potential to alter synaptogenesis and the development of several neuronal circuitries.

Impact of cannabinoid exposure on the maturation of neurotransmitter systems

Given that cannabis is the most commonly abused illicit drug among pregnant women and that prenatal cannabis use is associated with cognitive, behavioral, and neuropsychiatric deficits, it is important to understand the impact of prenatal cannabis exposure on the maturation of neurotransmitter systems, which play key roles in mood, motivation, and reward. Few investigations have directly examined the human fetal brain given the obvious challenges of conducting such studies. We developed a postmortem human fetal brain collection of midgestational subjects with maternal cannabis use [100] that has begun to provide the first insights into the molecular and biochemical alterations associated with in utero cannabis exposure on human neurodevelopment. This section summarizes some of our observations from this human fetal population, which had a sufficient sample size to enable the ability to tease out cannabis-related effects from other substances such as alcohol and cigarettes used by the mothers. The human information is integrated with findings from experimental animal studies in which neurobiological alterations can be more definitively linked to cannabinoid exposure and temporal fluctuations can be monitored. Unfortunately, the existing literature does not currently allow the ability to decipher the complexity of this topic in regard to time- and brain region-dependent alterations in neurotransmitter systems or sexual dimorphic differences as a consequence of developmental cannabinoid exposure. However, some consistent themes are beginning to emerge and will be briefly reviewed below.

Endocannabinoid system

As mentioned above, the eCB system is critical for the hardwiring of the developing brain. Recently eCBs have been shown to aid in the establishment of long-range axonal connections [131] and to act as local axon guidance cues for GABAergic interneurons in the developing cerebrum [9, 10]. Given that developmental events such as postsynaptic target selectivity and functional differentiation of developing axons take place early in the prenatal period and need to be carefully orchestrated to ensure proper patterning of the brain, the introduction of cannabis during this critical period has the potential to alter neuronal connectivity.

Surprisingly, few studies have investigated the effects of developmental cannabis exposure on the eCB system. Most of the available data are related to THC effects on the CB_1R. Our study in the midgestation human brain failed to detect significant alterations in CB_1R mRNA expression in the striatum, hippocampus, and amygdala or other cortical regions examined (parietal, temporal, insula, and parahippocampal) as a consequence of in utero cannabis exposure [192]. Perinatal THC exposure in rodent models also failed to alter CB_1R mRNA levels or receptor binding in the basal ganglia and limbic-related structures examined in adulthood [67]. However, other studies have shown that perinatal THC exposure induces subtle sex-dependent alterations in CB_1R activity such as decreased receptor binding in the cerebral cortex of adult male, but not female rats [67]. The lack of marked disturbance of the CB_1R mRNA and receptor binding in association with cannabinoid exposure is perhaps not surprising considering that THC administration in adult rats only results in a transient alteration of CB_1R mRNA levels in, e.g., the hippocampus and caudate-putamen, which normalizes in approximately 3 weeks after ceasing THC treatment [159, 198]. Thus, the observations in the human midgestation fetuses could reflect normalized CB_1R mRNA expression since the mothers repeatedly used marijuana during pregnancy [100]. However, it is impossible to discount potential impairment of the functional coupling of the CB_1R because though CB_1R mRNA expression normalizes following repeated administration to THC, the drug produces time-dependent and region-specific down-regulation and desensitization of brain CB_1Rs, consistent with tolerance [21, 198]. Overall, there is still limited knowledge regarding the developmental ontogeny of the intracellular signaling pathways relevant to eCB functioning which confound predictions as to the impact of THC exposure during the different developmental stages.

No studies to date have evaluated the effects of prenatal THC exposure on AEA and 2-AG, the two predominant eCBs. However, it has been demonstrated that adolescent THC exposure increases AEA concentrations in the nucleus accumbens during early adolescence and alters the normal correlation that exists between AEA and 2-AG concentrations in the striatum and prefrontal cortex [40]. Additional work is clearly needed to determine the effects of developmental THC exposure on the enzymes involved in the synthesis and degradation of the eCBs as well as on other cannabinoid-sensing receptors such as GPR55 and TRPV1.

Dopamine

Dopamine plays a critical role in regulating behaviors that are disrupted as a consequence of developmental THC exposure. Two major components of the dopamine system are the mesocorticolimbic and nigrostriatal dopamine pathways. Each of these pathways is distinct in its anatomical connectivity and modulates different behavioral functions. The mesocorticolimbic pathway, which originates in the ventral tegmental area and projects to the cerebral cortex, as well as to mesocorticolimbic structures such as the amygdala and nucleus accumbens, participates in the control of emotion and reward, whereas the nigrostriatal pathway, which originates in the substantia nigra pars compacta and projects to the dorsal striatum, is associated with motor control [80, 99]. Dopaminergic neurons in the ventral tegmental area and substantia nigra pars compacta are present in the human fetal brain at an early developmental stage [around the 5th through 6th embryonic week; 186] and have neurotrophic actions on the maturation of their target neurons. CB_1Rs are not expressed on midbrain dopamine neurons in adult [91] but instead on inhibitory GABAergic interneurons and glutamatergic terminals that indirectly regulate dopaminergic cells [114, 156]. In contrast, TRPV1 are directly localized to dopamine cells [124]. In the adult brain, THC and cannabinoid agonists enhance striatal and mesocorticolimbic dopamine levels, which directly regulate motor function, cognition, motivation, and emotional processes [18, 41, 118].

Developmental cannabinoid exposure affects the maturation of the dopamine system. Many studies have examined cannabinoid-induced changes in the activity of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis. Tyrosine hydroxylase is expressed in growing axons before they contact their neuronal targets and is thought to aid in establishing the functional connections between neurons. Most studies have found that perinatal cannabinoid exposure disrupts tyrosine hydroxylase activity, but the direction of change varies. For example, prenatal administration with a moderate dose of THC decreased striatal tyrosine hydroxylase activity in adolescent offspring [157, 190], but increased activity has also been reported following perinatal cannabinoid exposure [17, 94]. Despite the various discrepancies as to

cannabinoid-mediated effects on tyrosine hydroxylase in rodent models, potentially due, e.g., to differences in the time period and duration of drug exposure and time of testing, the studies all emphasize a significant impact on the activity of this enzyme, which could influence the maturation of dopaminergic target structures.

THC exposure during early development also alters dopamine receptors. Of the five known dopamine receptor subtypes, D_1 and D_2 have been most studied. In our human fetal population, maternal cannabis use was selectively associated with decreased D_2 mRNA expression levels in the amygdala, an effect that was most prominent in male subjects [192; Fig. 4]. The reduction of amygdala D_2 mRNA levels was directly correlated with the degree of maternal marijuana intake reported during pregnancy such that moderate and high maternal marijuana use was associated with the lowest expression of mRNA levels (Fig. 4). Similar cannabis-associated reduction of D_2 mRNA expression was also evident in the fetal striatum. Maternal cannabis use was not, however, associated with alterations of the D_1 mRNA levels in either the amygdala or striatum in the human fetal brain though it was influenced by maternal use of other substances such as alcohol [192]. These observations are intriguing given the apparent relationship that exists between the eCB and D_2 at various levels of regulation. For example, stimulation of the D_2, but not D_1, receptor activates in vivo AEA release [71]. Moreover, CB_1R heterodimerizes with the D_2 receptor which changes the normal CB_1R $G_{i/o}$ coupling to a G_s intracellular signaling cascade [102]. Altogether, the various lines of evidence suggest a strong interaction between the eCB system and D_2 receptor, which is highly implicated in the pathophysiology of drug addiction [133, 188], schizophrenia [167], and depression [104], neuropsychiatric disorders that are significantly vulnerable to neurodevelopmental insults.

Opioid system

There is overwhelming evidence in support of a strong interaction between the eCB and opioid systems, especially in relation to reward and addictive behaviors [26, 70, 181]. Of the major endogenous opioid neuropeptides, dynorphin and enkephalin have been most studied. These neuropeptides are enzymatically produced from separate precursors, proenkephalin and prodynorphin, which are encoded by preproenkephalin and preprodynorphin genes, respectively [for review see 1]. Enkephalins have high affinity for mu and delta opioid receptors and are associated with reward [7, 168], whereas dynorphins preferentially bind to kappa opioid receptors and are linked to dysphoria and negative mood states [7, 145]. Opioid ligands and receptors are expressed early in development. In the human brain, prodynorphin- and proenkephalin-positive neurons are present in the striatum from at least 12 weeks of development [20], and opioid

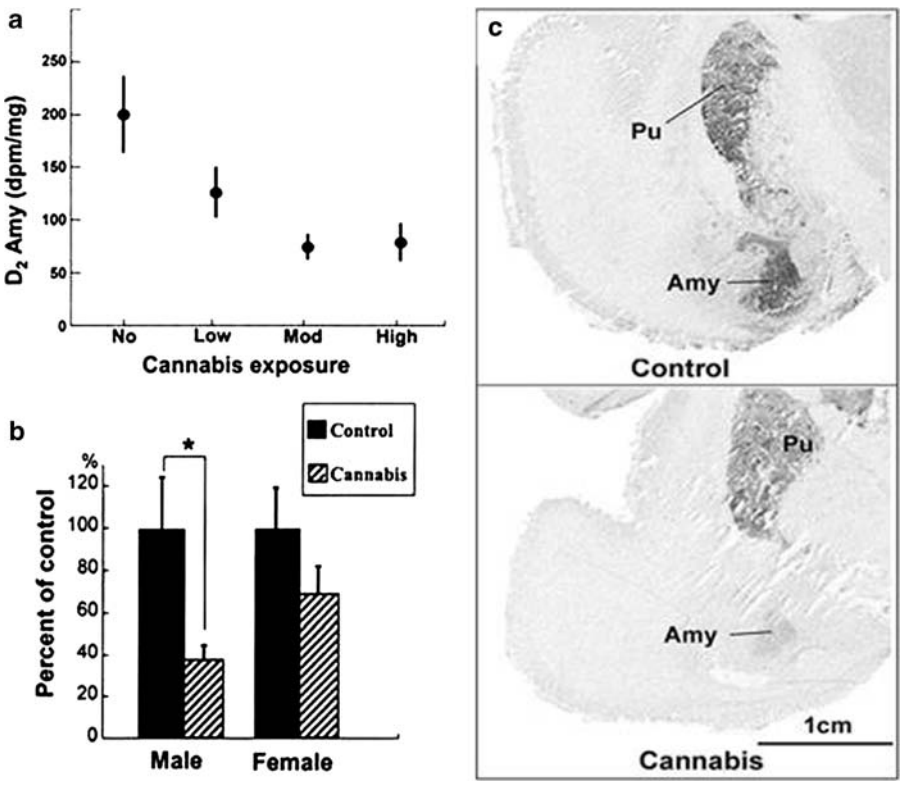

Fig. 4 **a** Dopamine D_2 mRNA expression levels in the amygdala (Amy) in relation to the amount of maternal marijuana intake reported during pregnancy. *No* no report of cannabis use, *Low* light use (average daily joints = 0.4), *Mod* moderate cannabis use (0.4–0.89 joints/day), *High* heavy maternal marijuana use (0.89 joints/day), *Pu* putamen. **b** Dopamine D_2 mRNA expression levels (mean ± SEM) in the basal amygdala nucleus of control and cannabis-exposed male and female fetuses (approximately 20 gestational weeks); $^*P < 0.05$. **c** Distribution pattern of the D_2 mRNA expression in the amygdala of a control and cannabis-exposed 20-week male subject. Modified from Wang et al. [192]

receptors are apparent at 20–21 gestational weeks [115, 179, 193].

Proenkephalin containing neurons appear to be more sensitive to prenatal THC exposure than cells containing prodynorphin. In our human fetal population, proenkephalin mRNA levels were decreased in the striatum, whereas prodynorphin levels were not significantly related to maternal cannabis use [193]. In rats, prenatal THC exposure also decreased proenkephalin mRNA levels in the nucleus accumbens at a similar ontogenic period of neurodevelopment as that studied in the human fetal brain at midgestation [173]. A number of THC rodent studies also confirm alterations in proenkephalin expression in the dorsal striatum and cerebral cortex [141], with no significant effect on prodynorphin mRNA levels in males [142]. A particularly intriguing observation is the fact that THC-induced disturbances of enkephalin persist into adulthood [39, 173]. These data suggest selective sensitivity of proenkephalin containing neurons in relation to prenatal THC exposure. This observation is interesting given that proenkephalin is colocalized with D_2 receptor in the striatum, which was also selectively decreased in our fetal population in association with prenatal THC exposure. Together the data suggest that the striatal enkephalin/D_2 receptor, but not the dynorphin/D_1 receptor-containing neuronal population is vulnerable to prenatal cannabis exposure.

Developmental cannabinoid exposure also influences the expression and activity of opioid receptors. In the midgestation human fetus, prenatal cannabis exposure was associated with increased mu receptor expression in the amygdala [193]. There are no reports from animal studies regarding the effects of prenatal cannabinoid exposure on mu opioid receptor expression during fetal development. However, prenatal THC exposure has been shown to affect mu opioid receptors changes in adulthood [185], an effect that depends on brain region and sex, with disturbances evident in the striatum and amygdala as well as other mesocorticolimbic structures such as the prefrontal cortex. In addition to the mu opioid receptor in human fetal subjects, cannabis exposure was also associated with discrete effects on the kappa receptor which contrasted the pattern of alterations related to maternal alcohol use that was associated with widespread disturbance of the kappa receptor in various brain areas [193; Fig. 5]. Instead, in utero cannabis was specifically associated with reduced kappa receptor mRNA levels in the mediodorsal thalamus [193], the limbic-related thalamic nucleus that connects subcortical structures such as the nucleus accumbens and amygdala with the prefrontal cortex. Taken together, these data suggest that cannabis exposure during early ontogeny influences specific components of the endogenous opioid system, especially within limbic structures, and that these disturbances endure into adulthood.

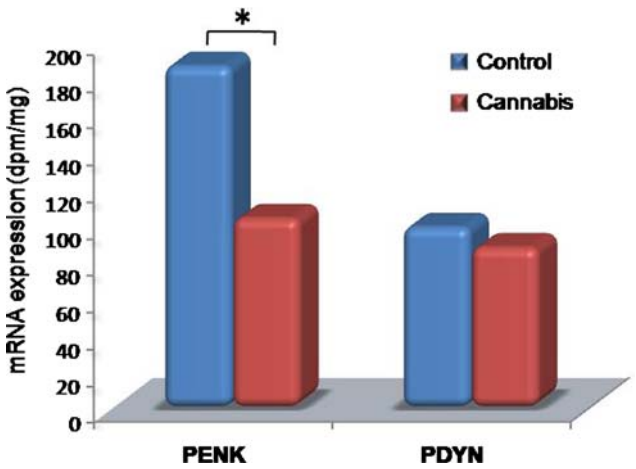

Fig. 5 Prenatal cannabis exposure decreases proenkephalin (PENK) but not prodynorphin (PDYN) mRNA expression levels in the human fetal striatum. *$P < 0.05$. Modified from Wang et al. [193]

Serotonin

Although dysfunction of the serotonergic system is not thought to fully explain mood disorders, it is recognized as a major entity in the genesis of depression [134]. Notably, the raphe nuclei, the primary source of serotonin in the forebrain, is critically implicated in depression [125]. Experimental animal studies have shown that manipulation of serotonergic transmission during the perinatal period induces profound alteration of anxiety-like and drug-seeking behaviors in adults [47, 135, 184]. In the mature brain, dorsal raphe neurons express CB_1R [127] and FAAH [35, 36], but this anatomical organization has not yet been characterized during early ontogeny. If CB_1R is expressed in serotonergic cells of the raphe or in forebrain regions that co-express CB_1R and serotonin receptors during perinatal development, as evident in adulthood, then early cannabis exposure would have the potential to impact behaviors linked to serotonergic dysfunction. It has recently been documented that interneurons in the hippocampal CA region identified by 5HT3 receptor expression do indeed express the CB_1R in the developing mouse brain [187]. No study to date has examined the influence of developmental cannabis exposure on maturation of the serotonin system in the human brain, but animal studies indicate significant effects of perinatal THC exposure [128]. Interestingly, maternal exposure to THC decreases serotonin levels in the raphe nuclei and ventral hippocampus of both genders and in the rostral striatum in male offspring [129]. Long-lasting impairment of serotonergic transmission induced by prenatal cannabis exposure could thus potentially contribute to mood dysregulation and risk of depression disorders in later life.

Glutamate and GABA

Glutamate and GABA are the major excitatory and inhibitory transmitters, respectively, driving neuronal activity in the mature brain, and are also critical for proliferation, migration, differentiation, and survival processes during neural development. In addition to instructing GABAergic interneuron development [9, 10], eCB signaling influences the acquisition of the glutamatergic neuronal phenotype during corticogenesis [131, 187]. The impact of in utero cannabis exposure on GABA and glutamate neurotransmission has yet to be examined in humans. In rats, prenatal THC exposure induces long-term reduction of glutamate neurotransmission by down-regulating GluR1 and GluR2/3 subtypes of AMPA glutamate receptors and by reducing expression of glutamine synthetase (a major glutamate precursor) in the cerebellum [175–177]. In addition, THC appears to disrupt expression of glutamatergic genes involved in synaptic transmission in the prefrontal cortex of adults [23]. Moreover, postnatal glutamate output is also lower in the frontal cortex and hippocampus of rats exposed to the CB_1R/CB_2R but not GPR55 receptor agonist WIN55,212-2 during the prenatal period, and some of the glutamatergic alterations correlate with long-term cognitive impairment and emotional reactivity [3, 4, 23, 123]. The frontal cortex and the hippocampus mediate cognition [79, 170] and the cerebellum, which is well-known to coordinate balance and motor control, has more recently been implicated in processing specific domains of cognitive function and emotional regulation through corticocerebellar circuits [75]. Given that a glutamatergic tone is instrumental for cognition and learning and memory [155], the aforementioned long-lasting alterations of glutamatergic neurotransmission may be relevant to cognitive deficits reported in association with developmental cannabis exposure.

GABA is the main inhibitory neurotransmitter in the mature brain, but it may serve as an excitatory neurotransmitter in early life [108]. Several lines of evidence suggest a significant impact of eCB disturbance during development on GABAergic function. For example, low dose prenatal THC exposure selectively increases the density of CB_1R-positive, but not CB_1R-negative, GABAergic interneurons in neonatal hippocampus, which would alter the inhibitory drive on pyramidal cells thereby inducing aberrant excitatory output from hippocampal circuitries [9]. Electrophysiological studies have also demonstrated that CB_1R controls GABA-driven neuronal activity in the hippocampus such that overactivation of CB_1R by exogenous cannabinoid agonists during early development disrupts network activity [11]. Intriguingly, the effects of elevated eCB tone and CB_1R activation on GABAergic postsynaptic activity are specific to the early stage of neurodevelopment because they are not seen in hippocampal tissues of older animals (4). Moreover, the finding that perinatal THC exposure increases the motor inhibition induced by activation of GABA-B receptors, which control GABA release in adult rats, [66] further emphasizes the potential for early developmental disturbance of the eCB system to have enduring impact into adulthood on GABAergic function that is relevant to various neurobiological mechanisms.

Consequences of developmental cannabis exposure on behavior and cognition

Several epidemiological and longitudinal studies have documented specific long-term behavioral and cognitive abnormalities in offspring of women who used marijuana during pregnancy even when accounting for maternal use of other psychoactive substances, socioeconomic status, and other environmental variables. Two longitudinal cohort studies in particular have been insightful regarding the impact of in utero cannabis exposure on CNS development and behavioral consequences. The Maternal Health Practices and Child Development Project (MHPCD) has been underway since 1982 and has examined the effects of prenatal marijuana and alcohol exposure in a low-income, African-American population in Pittsburgh, Pennsylvania [74]. The Ottawa Prenatal Prospective Study (OPPS), which was initiated in 1978, has assessed the neurobehavioral and developmental effects in association with prenatal exposure to cigarettes and cannabis in a low-risk, Caucasian, predominantly middle-class cohort in Canada [62].

Though not unequivocal, the majority of studies have observed that newborns and infants born to cannabis users have increased tremors, exaggerated startle response, and poor habituation to novel stimuli [49, 53, 58]. As children age, there are increasing reports of neurobehavioral disturbances so by age ten, prenatal marijuana-exposed children have increased hyperactivity, inattention, and impulsive symptoms [60, 73]. It has also been reported that these children show increased delinquency and externalizing behavioral problems as compared to age-appropriate non-exposed children [73].

Given the important role of cannabinoid receptors and eCB system in the development of the cerebral cortex, hippocampus, and basal ganglia, a natural question is to what extent maternal cannabis use affects cognitive function in their offspring. Studying cognitive outcomes in relation to prenatal cannabis exposure is complex since such measures are influenced by multiple factors including, e.g., the time of examination, the intensity of maternal use, the trimester during which exposure occurs, and the specific cognitive domain that is assessed [29, 52, 74, 78]. Using oversimplified outcome such as overall intelligence

have led to conflicting results [52, 74], suggesting that this measure may not be accurate to describe the multifaceted impact of prenatal cannabis exposure on cognitive performance. A detailed overview of the developmental neurobehavioral and cognitive outcomes that are influenced by numerous variables is beyond the scope of this article, but has been previously reviewed elsewhere [51, 52, 98]. Despite a lack of consistency in some studies as to the impact of in utero cannabis during the first few years of life [55, 56, 153], at approximately age three the data are more congruent. They show that children of regular cannabis users are impaired on short-term memory, abstract/visual reasoning and verbal outcomes measures [29, 56, 59, 60, 62, 74]. Based on their detailed examination of the OPPS cohort as well as other investigations, Peter Fried and colleagues proposed that the impact of prenatal cannabis exposure was associated with behaviors and cognitive abilities aligned to "executive function", a multistage process with different cognitive functions maturing at different ages [68, 110, 195] which requires the integrity of the prefrontal cortex and connectivity with other structures such as the hippocampus and cerebellum [33, 126]. Altogether, the existing literature points to significant deficits associated with prenatal cannabis exposure on domains such as attentional behavior, cognitive flexibility, and planning [42, 54, 73, 106, 154].

In utero cannabis exposure appears to be associated with distinct disturbances of top-down processes that requires integration, analysis, and synthesis of events in comparison to prenatal exposure to other substances such as cigarettes that affects IQ and more fundamental domains of cognition (e.g. basic visuoperceptual skills) [54]. Impairment of top-down integrative processes can have significant relevance for normal adaptive behaviors important for decision-making and inhibitory control. This is particularly intriguing considering that animals exposed prenatally to THC have selective disturbance of the frontostriatopallidal proenkephalin/D_2 dopamine receptor circuit, which maps onto inhibitory control behavior [48]. Moreover, it has been repeatedly observed that maternal marijuana use is predictive of impulsive behavior in their offspring, evident from childhood, throughout adolescence and into young adulthood [29, 78, 106]. Using functional magnetic resonance imaging, increased neural activity has been observed in the prefrontal cortex of subjects with in utero cannabis exposure during a Go/NoGo task, which is routinely used to assess inhibitory control [171]. Increased activation was also noted in the parahippocampal gyrus and cerebellum during the task [172]. Interestingly, adolescent cannabis exposure is also associated with a similar pattern of brain activation during inhibitory processing and spatial working memory tasks even after a few weeks of abstinence [138, 166, 178], but no residual cognitive impairment is apparent at that time period [61, 63]. The contrasting findings in regard to long-lasting cognitive outcome following prenatal and adolescent cannabis exposure could suggest that any compensatory remodeling of neural systems involved in cognition may not be sufficient when cannabis exposure occurs during the prenatal period.

Cognitive performance related to cannabis use has been primarily studied in adulthood and the long-term impact of such exposure remains controversial [113, 147, 148]. The extant literature supports a similar negative impact of cannabis on cognitive performance in adolescents, notably on psychomotor speed, complex attention, planning and sequencing ability, executive functions, and working memory [86, 122, 146]. These acute effects of cannabis on cognitive function appear more deleterious during adolescence than in adulthood [25, 136, 151]. Studies conducted in adults who still actively use cannabis also showed that users with an early onset of cannabis exposure have worse cognitive outcomes [37, 103]. Some of these alterations are still detectable in early-onset heavy users after 4 weeks of abstinence [149], but not after protracted abstinence of 3 months or more [61, 63]. These results suggest that cannabis exposure has a delirious impact on cognition, particularly when exposure begins early in adolescence, but this effect may be reversible with long-term abstinence. However, prenatal marijuana exposure predicts poorer performance on memory tasks in preteens and adolescents [154], again emphasizing the important enduring impact of exposure during the prenatal period.

Prenatal cannabis exposure in relation to neuropsychiatric disorders

It is now acknowledged that most psychiatric disorders are developmental in nature, thus the significant role of the eCB system in CNS development suggests that cannabis exposure during the prenatal period could potentially contribute to neuropsychiatric illnesses later in life. Neuropsychiatric disorders that have been primarily examined in regard to developmental cannabis exposure include drug addiction, mood and anxiety disorders, and schizophrenia. There remain, however, critical questions as to a direct causal relationship between cannabis exposure and these disorders. Similar to the observations regarding cognitive and behavioral abnormalities, the findings suggest that psychiatric vulnerabilities may depend on early versus late cannabis developmental exposure.

Drug addiction

Considering the importance of the eCB system to synaptogenesis and synaptic plasticity, an obvious concern is

the extent to which developmental cannabis exposure contribute to addiction, a disorder epitomized by neuroplastic dysfunction. Both the OPPS and MHPCD longitudinal studies have reported a significant association between prenatal cannabis exposure and cannabis use in adolescents and young adults [30, 150]. Maternal cannabis use predicted early onset and increased frequency of use among adolescent offspring. This developmental association remained evident even when controlling for multiple variables including the use of other substances and the home environment. Animal studies have confirmed a causal relationship between prenatal cannabinoid exposure and increased long-term vulnerability for drug use behavior in later life. Using a self-administration paradigm where animals directly control their own drug intake behavior, prenatal THC exposure increased impulsivity for heroin intake with shorter latency to press the first drug lever, induced a greater response for low heroin doses, and enhanced heroin seeking during drug extinction and conditions that involved mild stress in adult rats [173]. Gender-dependent effects have also been noted by other investigators with decreased opioid intake [15] and increased cocaine self-administration [95] in females as compared to male rats following perinatal administration of CB_1R agonists. These behaviors were associated with alteration in brain metabolic activity in the frontal and amygdala/entorhinal cortical regions [95]. The endogenous opioid and dopamine systems mediate reward, motivation and goal-directed behavior and preclinical animal studies have repeatedly documented perinatal cannabinoid interference on rodent ontogenic processes, particularly on enkephalin- [39, 141, 173, 185] and dopamine-related [39, 93, 173] neuronal systems. Our studies in the human fetal brain also substantiate a preferential disturbance of proenkephalin, mu opioid receptor and dopamine D_2 receptors in subjects exposed in utero to cannabis [192, 193].

A provocative genetic study has also suggested an important interaction between individual developmental disturbances of eCB signaling and drug abuse. A single nucleotide polymorphism in the human FAAH gene, 385A, was strongly associated with drug use in a large Caucasian population [169] and a predominant African-American cohort [46]. Subjects homozygous for the FAAH 385A/385A genotype have significantly higher frequency of street drug use and problem drug/alcohol use [169]. This observation raises a number of intriguing questions since the FAAH 385A variant significantly increases sensitivity to proteolytic degradation of the enzyme [169], which would result in lower FAAH levels and increased AEA tone. As such supraphysiological modulation of eCB signaling, as would be mimicked by cannabis exposure during development, could be speculated to contribute in part to individual risk to drug-related disorders.

Mood and anxiety disorders

The strong expression of the CB_1R in the human amygdala during early development [191], the amygdala disturbances evident in the midgestational human fetuses with maternal cannabis use [192], and the serotonergic impairments associated with prenatal THC exposure would suggest a potential long-term impact of in utero cannabis exposure on mood and emotional regulation. Very limited data is, however, available regarding the effect of prenatal cannabis exposure on depression and anxiety symptoms. Of the existing data, Gray et al. [76] reported that cannabis exposure during the first and third trimesters was associated with a significant increase in the levels of depressive symptoms among 10-year-old children. Marijuana exposure during gestation was also marginally observed to predict depression and anxiety at age ten in the MHPCD cohort [107].

In contrast to the prenatal developmental exposure period, there is a growing body of evidence to suggest that cannabis exposure during adolescence is linked to the subsequent development of symptoms that characterize mood and anxiety disorders [44, 87, 139]. An important longitudinal study examined the frequency of cannabis use and psychosocial outcomes in adolescent/young adults in New Zealand over a 21-year period [44]. Cannabis use was associated with an increase in depressive-like symptoms including suicidal ideation. Furthermore, there was a relationship between age and the strength of the association between cannabis use and psychosocial outcome, with younger (14–15 years old) users being more affected by regular cannabis use than older (20–21 years old) users. Not all investigations though have reported a similar association between cannabis and major depression [6, 82, 140], but there is still evidence even in some of those studies that adolescent cannabis used is associated with, e.g., later suicidal thoughts and attempts [6, 82, 140]. Overall, most investigations suggest that cannabis use in adolescence is related to subthreshold depressive and anxiety symptoms in adulthood. This association is strongest in girls, and the relationship is inconsistent when it includes major depressive disorder diagnosis as the outcome [44, 87, 139].

Schizophrenia

The pathogenesis of schizophrenia has yet to be determined, but a growing body of evidence posits that neurodevelopmental factors, both genetic and environmental, contribute substantially to the liability for developing the disease. Longitudinal studies have repeatedly identified adolescent cannabis use as one of the predictive factors associated with schizophrenia. This relationship is

particularly strong when the exposure occurs early in development and over a prolonged period [2, 6, 81]. The association between prenatal cannabis exposure and schizophrenia has not, however, been truly examined. The fact that subjects in the MHPCD and OPPS prenatal longitudinal studies are now in young adulthood when onset of schizophrenia would normally occur should potentially begin to provide some insights about such interactions. However, the relatively small population size of these cohorts might be a limitation to directly address this question given that the risk of schizophrenia is approximately 0.7% of the population [162]. Moreover, genetics is an important contribution to schizophrenia and the cohort populations in the longitudinal studies might not reflect gene mutations relevant to the liability of the disorder. Genetic vulnerability is particularly important given that the vast majority of individuals who use cannabis in adolescence and young adulthood do not develop schizophrenia. Moreover, young people with a genetic susceptibility for schizophrenia appear vulnerable to psychological symptoms such as disorganization and hyperactivity/inattention induced by cannabis use [96].

A number of genes have already been identified as potential candidates in association with schizophrenia. Some of these candidates initially focused on dopamine since dysfunction in dopaminergic signaling had been implicated in the pathophysiology of schizophrenia. Hyperdopaminergic transmission in the mesolimbic circuitry is associated with positive psychotic symptoms of schizophrenia, whereas hypodopaminergic transmission in the prefrontal cortex is linked to negative psychotic symptoms [152]. Since activation of the eCB system is known to alter dopamine transmission in these key brain regions, the interaction between cannabis exposure and genes linked to the dopamine transmission may be relevant to the pathogenesis of schizophrenia [182]. Functional polymorphism in the catechol-*O*-methyltransferase (COMT) gene, which is involved in the metabolism of catecholamines such as dopamine, has been repeatedly observed to predict the emergence of adult psychosis with adolescent-onset cannabis use. Specifically, individuals carrying the *COMT* Val allele, which is associated with high COMT activity and thus low dopamine tone in the prefrontal cortex, were more likely to exhibit psychotic symptoms and to develop schizophrenia if they used cannabis than individuals carrying the Met/Met genotype [24, 88, 89]. Additionally, a single nucleotide polymorphism in the *CNR1* gene, which codes for the CB_1R, has been associated with schizophrenia in a French Caucasian population [109], and the AAT triplet repeat in the 3' region of this gene has been associated with schizophrenia in a Japanese population [180]. The association between early cannabis exposure and schizophrenia outcomes may also be related to cannabinoid-induced changes in CB_1R activity since schizophrenics have increased CB_1R expression in the prefrontal cortex [31]. However, one study failed to find a similar interaction effect of the *CNR1* and *COMT* genes with cannabis use in regard to psychosis risk [197]. The age at onset of cannabis use was not systematically evaluated in all subjects in that investigation, which might underline the importance of the specific timing of cannabis exposure regarding psychosis vulnerability. Relatively few studies have investigated the relevance of other eCB-related genes and no significant association was found thus far between, e.g., polymorphism of the FAAH and schizophrenia [130]. It has been demonstrated that variation in neuregulin 1 (NRG1), a gene related to schizophrenia, modulates sensitivity to the behavioral effects of cannabinoids [19]. NRG1 is involved in axonal guidance and connectivity patterning, e.g., in the thalamocortical track [111], so disrupting NRG1 signaling may have direct consequences on postnatal cognitive functions that may be relevant to predisposition to schizophrenia. Altogether, the existing data does suggest that interactions between gene and early environmental cannabis exposure are relevant to schizophrenia liability, but data is lacking regarding specific contributions of prenatal cannabis exposure.

Conclusions

Despite the high prevalence of marijuana use among women of childbearing age, the potential impact of cannabis on the developing brain and the long-term influence on behavior and mental health are still not understood due to the paucity of scientific studies that have been directed toward this critical question. However, knowledge garnered to date unequivocally documents that the eCB system plays a pivotal role in CNS patterning by modulating cell fate decisions in neural progenitor cells and by influencing migration, survival, and differentiation of committed neurons. Moreover, there is a strong mesocorticolimbic relationship between the eCB developmental organization and neuronal systems relevant for mood, cognition, reward, and goal-directed behavior—cannabinoid receptors are predominantly expressed in mesocorticolimbic cell populations in the midgestation human fetal brain (Fig. 2) and the eCB system is integral to the formation of projection pathways and local interneuronal circuits within mesocorticolimbic structures. There remain numerous unanswered questions as to the exact consequence of prenatal cannabis exposure on human neurodevelopment and future mental health. However, it is clear that exposure to cannabis during early ontogeny is not benign and potential compensatory mechanisms that might be expected to occur during neurodevelopment appear insufficient to eliminate vulnerability to neuropsychiatric disorders in certain individuals. Both

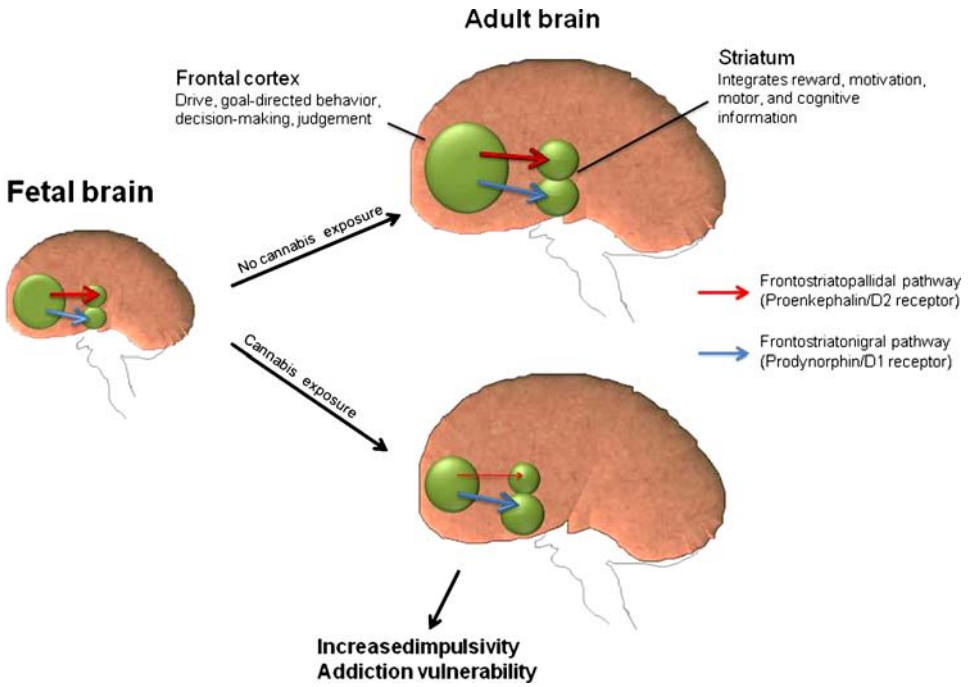

Fig. 6 Conceptual diagram depicting the hypothesized effects of prenatal cannabis exposure on the functional organization of the frontostriatal pathways, with associated behavioral consequences. It is speculated that fetal cannabis exposure alters prefrontal cortical development and the frontostriatopallidal circuit, which underlies inhibitory control

human longitudinal cohort studies and animal models strongly emphasize the enduring impact of prenatal cannabinoid exposure on behavior in later life. Discrete neuronal disturbances on, e.g., striatopallidal circuits (proenkephalin and dopamine D_2 receptor) evident in the human fetal brain are mimicked in animal models and may underlie impulsivity and enhanced drug use disturbances seen in adults with maternal cannabis use (Fig. 6). Given the significant relevance of gene × environment associations with early adolescent cannabis use for psychiatric liability, the role of such interactions needs to be explored in relation to prenatal cannabis exposure. Based on the current knowledge regarding the organization of the eCB system, future studies focused on in utero cannabis exposure in association with genetic mutations of neural systems that have strong relationships to endocannabinoid function, such as the dopamine, opioid, glutamate, and GABA, might help to identify individuals at risk.

While ongoing and future studies will no doubt add more definitive insights, the existing data already begins to establish a foundation on which a public health platform can be built in guiding women about the potential impact of cannabis use during pregnancy on the mental health outcome of their offspring. This platform should not only focus on the population of young women who might still consider cannabis to be a safe drug, but even directed towards medical practioners who consider prescribing cannabinoid drugs as anti-emetic medication to pregnant women.

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