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Mechanisms underlying cognitive impairment induced by prenatal cannabinoid exposure: a literature review

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Abstract. – Human and animal studies have revealed that prenatal cannabinoid exposure alters fetal brain development and leads to persistent impairment in the cognitive function of offspring. However, the mechanism underlying the effect of prenatal cannabinoid exposure on cognitive function in offspring is still not fully understood. Therefore, the goal of this literature review is to discuss the published studies on the mechanisms underlying the effects of prenatal cannabinoid exposure on cognitive impairment.

The articles used in this prenatal cannabinoid exposure review were retrieved by electronic search of the Medline database for literature describing human and animal models of prenatal cannabinoid exposure from 2006 to 2022. The findings from the studies reviewed revealed that the cognitive impairment associated with prenatal cannabinoid exposure is caused by alterations in the expression and function of endocannabinoid receptor 1 (CB1R), decreased glutamate transmission, reduced neurogenesis, alterations in protein kinase B (PKB/Akt) and extracellular signal-regulated kinase 1 and 2 (ERK1/2) activity, and increased mitochondrial function in the hippocampus, cortex, and cerebellum. This review briefly touches upon the currently available measurement and prevention methods and their limitations.

Key Words:
Cannabinoid, Prenatal cannabinoid exposure, Neurogenesis, Cognitive impairment.

Introduction

Marijuana is used to refer to derivatives of the *Cannabis sativa* and *Cannabis indica* plants[1,2]. The medicinal properties of marijuana are well known, and this plant has been in use throughout history. Despite this, the use of cannabis products is punishable by law in most parts of the world. However, over the last couple of decades, based on data from a vast amount of research[3], many countries have begun legalizing and decriminalizing the use of cannabis products. While the medicinal benefits of cannabis products are acknowledged, rodent studies[4] have demonstrated a strong correlation between maternal use of cannabinoids during pregnancy and lactation and cognitive outcomes in offspring, and this has been confirmed in studies[4] on other animals and human cohorts too. Specifically, the children of mothers who use cannabinoids during pregnancy appear to be at a higher risk of severe adverse health conditions[6], including chronic neurobehavioral changes, such as deficits in learning, memory, and social development[7]. Further, several lines of evidence[8] have demonstrated neurobehavioral alterations in the children of women who used cannabinoids during pregnancy.

There have been three large-scale prospective longitudinal cohorts on the consequences of prenatal cannabinoid exposure in terms of the neurodevelopmental and cognitive effects: The Ottawa Prenatal Prospective Study[9], The Maternal Health Practices and Child Development Study[9], and The Generation R Study[10]. The most recent data from studies[9-11] on these cohorts (reported between 1998 and 2012) indicate that prenatal cannabinoid exposure affects several cognitive and behavioral domains, including executive function, visuospatial working memory processing, verbal reasoning, concentration, and attention, in both infants and adolescents, and these effects even continue into late adulthood. Unfortunately, the findings of studies on these cohorts have been largely inconsistent, and any alterations observed in cognitive performance were not significant, as indicated by a recent systematic review[12]. Further, these past studies do not account for recent trends in cannabis

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legalization, the increase in both recreational and medicinal use of cannabis products, and the rise in Δ9-tetrahydrocannabinol (THC) concentrations in these products that have occurred over the last two decades[13]. Despite the shortcomings of data on these cohorts, studies[11] on cohorts from 2020 (the most recent to date) do indicate that prenatal cannabinoid exposure causes some level of cognitive impairment that is worthy of investigation. For example, a positive maternal Δ9-THC urine test at the first prenatal visit was associated with abnormal 12-month developmental scores in infants, as measured by the Ages and Stages: Social-Emotional Questionnaire[14], and a moderate increase in the incidence of intellectual disability and learning disorders was observed in children born between 2007 and 2012 who were exposed to cannabis prenatally in a cohort[15] in Ontario, Canada. Both these studies are limited by their retrospective nature, but their findings are supported by recent results from the cross-sectional, large-scale ongoing Adolescent Brain Cognitive Development (ABCD) study[16] on children aged 9-11 years, which reported that prenatal exposure to cannabis showed a correlation with deficits in attention, thought, and social problems that persisted even after potential confounders were controlled for. Another study[17] that used data from the ABCD study showed that while prenatal cannabis exposure was associated with lower attention, externalizing, and total problem scores on the Child Behavior Checklist, it did not affect cognitive performance on Functional magnetic resonance imaging (fMRI) tasks. It should be noted that these recent studies[12] (understandably) do not shed light on the long-term effects of prenatal cannabis exposure in adolescents and adults. Given the paucity of recent data from large-scale human studies on the cognitive effects of prenatal cannabis exposure, our current understanding of these effects and their mechanisms are largely based on animal models of prenatal cannabis exposure.

Among the recent studies based on rodent models, there are those reported by Silva et al[18] and de Salas-Quiroga et al[19]. In the former study[18], the offspring of rats exposed to 0.15 mg/kg/day of THC intraperitoneally throughout the gestation period performed poorly on a set of learning and long-term memory tests, and these deficits were more pronounced in male offspring. In the latter study[18], the offspring of mice that were intraperitoneally administered THC at 3.0 mg/kg/day from gestational day 12.5 to 16.5 showed deficits in motor skills and increased vulnerability to seizures. In addition, a recent meta-analysis[20] of rodent studies reported that prenatal THC administration had a moderate effect on the cognitive abilities of offspring, after accounting for differences between studies in terms of strain, type of THC administered, amount of dose administered, route of administration, and type of task.

The mechanistic features of the cognitive effects of prenatal THC are believed[8] to be mainly associated with the effects of cannabinoids on endogenous cannabinoid receptors (CB1Rs), which play a critical role in regulating the release of several neurotransmitters involved in learning and memory, including glutamate, gamma-aminobutyric acid (GABA), and acetylcholine. Exogenous cannabinoids, such as THC from marijuana, modify the function of CB1Rs in the brain. Chronic exposure of cannabinoid receptors to these exogenous cannabinoids leads to suppression of neuronal plasticity in the hippocampus as well as neighboring brain regions and reduced cognitive function[21,22]. Several studies[23,24] have shown that exposure to the cannabinoid WIN 55,212-2, which is a full agonist of CB1Rs and is more potent than THC (a partial agonist), is associated with memory deficits and brain development in adult rats. The other mechanisms *via* which cognitive function is affected in rats prenatally exposed to cannabinoids have been explored in several studies, but we do not have a comprehensive understanding of all these mechanisms. Therefore, in this review, we seek to fill in this gap in the literature by discussing the recent studies that have provided insights into the mechanisms by which prenatal cannabinoid exposure can cause cognitive impairment in offspring. We believe that the findings of this review will improve our understanding of the mechanisms underlying the cognitive effects of prenatal cannabinoid exposure and contribute to the development of preventive strategies to improve the adverse effects of prenatal cannabinoid exposure in children.

Search Strategy

The articles used in this prenatal cannabinoid exposure review were retrieved by electronic search of the Medline database for literature describing human and animal models of prenatal cannabinoid exposure from 2006 to 2022 with the following search terms: “human,” “models,” “animal” OR “behavior,” “animal/physiology”

OR "prenatal cannabinoid exposure induced cognitive impairment/dysfunction." The results were further screened by title and abstract only to include studies on rats, mice, and human primates.

Prenatal Cannabinoid Exposure and Neurogenesis

Neurogenesis, which is the process by which new neurons are generated, starts in the embryonic period and continues into adulthood in some brain regions such as the hippocampus and dentate gyrus[25,26]. This process is essential for the proliferation of neural stem cells, which differentiate into mature neurons that are ultimately integrated into the hippocampal circuitry[27]. Neurons are integrated together and communicate with other neurons through synapses[28]. The formation of new synaptic connections between neurons in the hippocampus, cerebral cortex, and dentate gyrus is essential for learning and memory formation and also plays a vital role in the establishment of new aspects of memory[29,30]. Accordingly, reduction in hippocampal neurogenesis has been shown[31,32] to lead to memory impairment. Hippocampal neurogenesis has been found to be impaired in male adolescent rats after chronic exposure to the synthetic cannabinoid HU-210[33] and WIN 55,212-2[34]. In addition, adult rats treated with cannabinoid drugs during adolescence were found[35,36] to exhibit a depression-like phenotype that was associated with decreased neurogenesis in the dentate gyrus of the hippocampus. These findings might mean that prenatal exposure could have similar effects on neuronal development. In fact, in rodent models[15] of prenatal cannabis exposure, it has been reported that prenatal exposure to WIN 55,212-2 (0.5 mg/kg daily from gestational day 5 to 20) caused a reduction in the cortical neuronal population, which may reflect reduced neurogenesis in this region. However, there is very little robust evidence from brain imaging and neuronal cell-based studies to demonstrate such an effect, and further studies in rodent models and human brain samples would help establish this effect.

Prenatal Cannabinoid Exposure and Synaptic Plasticity

The hippocampus, along with the amygdala and other parts of the temporal lobe, is the part of the brain that is in charge of learning acquisition and memory formation. Several events occur during these processes as a result of alterations in the synaptic structure – a phenomenon that is also known as synaptic plasticity[37,38]. Synaptic plasticity defines the cellular levels of synaptic neuron communication during memory encoding based on the capacity for shape and structural modifications[39,40]. Synaptic plasticity can be measured by long-term potentiation (LTP), but it can also be measured based on long-term depression (LTD) as well as other biomarkers[41,42]. The response of the neuronal synapse is based on the presynaptic release of neurotransmitters that are bound by and activate postsynaptic receptors[43]. Changes in the levels of neurotransmitters released from presynaptic neurons or the response of receptors expressed on the postsynaptic neurons can cause an increase or decrease in LTP[44]. The link between synapse changes and memory function has been demonstrated[45] under both *in vivo* and *in vitro* settings, and electrophysiological studies[46,47] have demonstrated changes in hippocampal synaptic activity following behavioral tasks. Since other regions in the brain, such as the cortex and cerebellum, can also affect hippocampal function, alterations in synaptic activity or LTP in these regions can also modulate cognitive function. Accordingly, a reduction in LTP in the hippocampus has been demonstrated[48-50] in the offspring of pregnant rats exposed to WIN 55,212-2 (0.5 mg/kg daily from gestational day 5 to 20), and the findings of the study by Antonelli et al[51], which used the same protocol, also indicated changes in LTP based on a reduction in hippocampal and cortical glutamatergic neurotransmission. This is supported by data[41] that show that subcutaneous maternal administration of WIN 55,212-2 (0.5 mg/kg daily) during lactation (from postnatal day 1 to 10) causes alteration in LTP in the prefrontal cortex and nucleus accumbens. However, the findings of Pinky et al[47] contradict these findings. In their study, they administered WIN 55,212-2 to pregnant mice at a dose of 2 mg/kg daily (the equivalent of low-to-moderate doses in humans) from gestational day 2 until delivery, but their findings indicated that this cannabinoid did not affect pup behavior or synaptic plasticity and, in fact, had an overall neuroprotective effect[14]. This inconsistency could be explained by differences in the doses administered in the Pinky et al[47] study and the other studies. Although there is some evidence[9] to indicate that the cognitive effects of cannabinoid exposure during pregnancy on the offspring are caused by a reduction in syn-

aptic plasticity, there is still a lack of robust data from both animal and human studies. However, newly emerging data from experiments on human cerebral organoids could fill in this research gap. For example, Ao et al[51] found that THC exposure to human cerebral organoids assembled from human embryonic stem cells caused a reduction in neuronal maturation, neuronal firing, and neurite outgrowth, all of which may affect neuronal plasticity. More in-depth studies in the future on such organoid models could shed light on this.

Prenatal Cannabinoid Exposure and Mitochondrial Function

The mitochondria are cellular organelles that play a crucial role in regulating processes such as ATP production, cellular respiration, and apoptosis[52,53]. Cellular respiration requires the coordinated interaction of five complexes – complex I to complex V[54]. Complex I is the major and most complicated part of the respiratory chain, and alterations in this complex are associated[55,56] with various conditions, including neurodegenerative diseases and cognitive dysfunction. Studies[57] on the relationship between prenatal cannabinoid exposure and mitochondrial dysfunction have shown that cannabinoid exposure during pregnancy can cause mitochondrial dysfunction and an increase in oxidative stress. For example, Oke et al[58] administered THC to pregnant Wistar rats at a dose of 3 mg/kg body weight from embryonic day 6.5 to 22 and found that at postnatal 6 months, the male offspring exhibited hepatic changes that were indicative of mitochondrial dysfunction and increased oxidative stress. In contrast, the earlier mentioned Pinky et al[49] study showed that prenatal administration of WIN 55,212-2 caused an increase in mitochondrial respiration and a decrease in oxidative stress that was indicative of an overall neuroprotective effect, but it is difficult to compare these two studies[49,58] because they used different cannabinoids. A recent study[59] on the BeWo human trophoblast cell line as an *in vitro* model of the human placenta showed that exposure to 3-30 μM of THC for 24 h resulted in a dose-dependent decrease in mitochondrial respiration and ATP coupling that was associated with lower abundance of mitochondrial chain complex proteins. These findings are corroborated by another study[60] in which the placental trophoblast cell lines HTR8/SVneo and BeWo were treated with 20 μM THC for 48 h and exhibited reduced mitochondrial respiration, ATP production, and loss of mitochondrial membrane polarity, which were associated with reduced trophoblast invasion and syncytialization and reduced levels of human chorionic gonadotropin, human placental lactogen and insulin-like growth factor 2. These findings have important implications for offspring health, and the potential of such changes in the embryonic period to cause mitochondrial dysfunction in offspring needs to be explored.

Prenatal Cannabinoid Exposure and Epigenetic Modifications

Chromatin is a complex of DNA and proteins that is located in the nucleus of eukaryotic cells[61]. Chromatin consists of four subunits called histones[62], which can be modified *via* acetylation, methylation, or phosphorylation, and thereby regulate gene transcription[62,63]. In particular, histone acetyltransferases and histone deacetylases (HDACs) play essential roles in the chromatin modifications involved in various cellular functions, including memory formation and synaptic plasticity[64,65]. For instance, inhibition of class I HDACs (HDAC1, HDAC2, HDAC3, and HDAC8) can increase the transcription of crucial genes involved in learning and memory processes[66,67], while the inhibition of some class II HDACs (e.g., HDAC4 and HDAC6) can impair cognitive function and synaptic plasticity[68,69]. The contribution of prenatal cannabis exposure to epigenetic reprogramming has been demonstrated in several studies[70]. For example, in a study by Innocenzi et al[71], male mice were interperitoneally administered JWH-133, a selective agonist of cannabinoid receptor type 2, at a dose of 1.5 mg/kg at regular intervals. This chronic exposure was associated with decreased sperm count, impaired placental development, and reduced offspring growth, and the defects were found to be caused by altered DNA methylation/hydroxymethylation of imprinted genes in sperm that were conserved in the placenta. These findings are confirmed in a study by Schrott et al[72] in which male mice were chronically exposed to cannabis through intraperitoneal administration of 4 mg/kg body weight of THC in cannabis extract. In this study, methylation changes in the *Mtss1l* gene in paternal sperms were transmitted to the *Mtss1l* gene in the nucleus accumbens and hippocampus of

the offspring. Further, DiNieri et al[73] reported disturbances in the histone modification profile of the nucleus accumbens in adult rats with prenatal THC exposure (pregnant dams were administered 0.15 mg/kg THC intravenously from gestational day 5 to postnatal day 2). Specifically, they[73] observed decreased levels of trimethylation of lysine 4 on histone H3 (H3K4me3) and increased levels of dimethylation of lysine 9 on histone H3 (H3K9me2), as well as a decrease in the association of RNA polymerase II with the promoter and coding regions of the *Drd2* gene in the nucleus accumbens. In addition, a study by Wanner et al [74] showed that prenatal exposure to CBD resulted in increased anxiety and improved memory behavior in offspring that were associated with thousands of differentially methylated loci. In their study, nulliparous female wild-type Agouti viable yellow mice were exposed to 20 mg/kg CBD or vehicle daily from two weeks prior to mating through gestation and lactation. Their findings are corroborated by another study[75] in which the offspring of female mice administered 0.15 THC mg/kg body weight exhibited psychiatric vulnerabilities that were associated with transcriptional and epigenetic deviations in the nucleus accumbens of the offspring *via Kmt2a* dysregulation. Thus, cannabis exposure throughout the development period seems to have effects on epigenetic modifications in the offspring that are associated with cognitive function.

MicroRNAs (miRNAs) are small molecules that are typically 22 nucleotides in length and are involved in the regulation of gene expression[76], regulation of cellular function *via* binding to mRNAs, and inhibition or interference with translation[76,77]. The deregulation of miRNA expression is associated with immunosuppressive response[78]. With regard to prenatal cannabinoid exposure, it has been found[79] to enhance immunosuppression in offspring by increasing the expression of some miRNAs such as miR-690. Further, hepatic epigenetic modifications characterized by decreased expression of the hepatic miRNAs miR-203a-3p and miR-29a/b/c have been associated[58] with an increase in hepatic triglycerides and mitochondrial dysfunction in rats with prenatal Δ9-THC exposure. The immunosuppressive effects of prenatal cannabinoid exposure have been found[80] to be mediated through epigenetic mechanisms such as altered miRNA, DNA methylation, and histone modification profiles in animal models, but there is no evidence for the effects of prenatal cannabinoid exposure on miRNA alterations that affect cognitive function. Nonetheless, the available findings lay a rather sound basis for exploring whether cannabinoid-induced modulation of epigenetic changes in the prenatal period could also affect miRNA expression and function.

Prenatal Cannabinoid Exposure and Molecular Signaling

Endogenous cannabinoids or endocannabinoids (eCBs) are abundantly expressed in the hippocampus[81], and expression of eCB ligands at the mRNA level and functional receptor signaling are initiated as early as the embryonic period [82]. Cannabinoids in the hippocampus are believed[83] to regulate synaptic plasticity and memory formation. However, the regulatory role of the eCB system in the development of neuronal circuitry and synapse formation may be impaired by chronic cannabinoid exposure during fetal development. Specifically, chronic exposure to cannabinoids has been found[84] to significantly decrease the levels of synaptic mGluR5, which is known to stimulate eCB synthesis. This reduction is also associated[85] with the upregulation of monoacylglycerol lipase and enzymes associated with eCB degradation. The level of eCBs is, therefore, reduced due to the combined effects of their decreased production and increased degradation, and this results in reduced activation of CB1R. Activation of CB1R inhibits GABA release[86]; therefore, the decreased activation of CB1R caused by reduced levels of eCB results in an increase in GABA release[87,88]. This effect has been observed[89] in rats that were prenatally exposed to WIN 55,212-2 (pregnant dams were subcutaneously administered 0.75 mg/kg WIN 55,212-2 from gestational days 5 to 20), as these animals exhibited altered migration of early-born GABAergic interneurons in the cerebral cortex.

Ionotropic glutamate receptors also play an important role in the functionality of CB1R; for example, glutamate α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and N-methyl-d-aspartate (NMDA) receptors, which are tetramers that are assembled in different subunits combinations[90,91]. AMPA receptors are assembled in different combinations of four subunits, namely, AMPAR subunit glutamate receptor 1 (GluR1), GluR2, GluR3, and GluR4, which define the functionality of the receptor[92]. It is widely reported[93,94] that alterations in the struc-

ture of AMPA and NMDA receptors lead to alterations in the brain that affect cognitive function. Accordingly, attempts to identify the molecular mechanisms involved in the effects of prenatal cannabis exposure on cognitive function have focused specifically on the physiological features of the glutamatergic synapses[48]. Preliminary studies[95] have demonstrated reduced AMPA receptor-mediated synaptic currents and decreased expression of GluR1 and GluR2 subunits at 30 and 70 days postnatally in the offspring of rats that were orally administered 5 mg/kg THC daily from gestational day 5 to postnatal day 20. In addition, Sarikahya et al[96] reported that THC induces an increase in the expression of AMPA-GluR2 but does not affect AMPA-GluR1 in the offspring of female Wistar rats treated with 3 mg/kg THC daily from gestational day 7 to 22, and this was associated with impaired Ca^{++} influx and cognitive function. Further, an overall disruption of glutamatergic/GABAergic function was observed in the nucleus accumbens of these animals.

NMDA receptors, which are also tetramers of four subunits, play an important role in regulating synaptic plasticity and memory formation[97,98]. At the synaptic level, NMDA receptors are involved in synaptic maintenance after their induction. Overactivation of NMDA receptors can cause developmental changes that result in hyperactivation of the GluN2A subunit, which in turn, may lead to cytotoxicity through the reduction of pro-survival signaling[99]. On the other hand, the knockdown of GluN2A may have neuroprotective effects[100,101], and this effect was observed[48] in prenatal cannabinoid-exposed animals that exhibited a significant reduction in the GluN2A subunit. However, in another rodent study[15], prenatal exposure through administration of 0.5 mg/kg WIN 55,212-2 from gestational days 5 to 20 was associated with a reduction in cortical glutamatergic neurotransmission and NMDA receptor activity. The observed reduction in glutamate receptors and transporters might be the result of a decrease in glutamate release in the brain of offspring, as prenatal cannabinoid exposure has been reported to reduce the levels of glutamate and glutamate transporters in the brain in several studies using animal models[102].

Molecular data from prenatal cannabinoid exposure studies[103] have confirmed a dose-dependent decrease in dopamine receptor (DA) subtype D_2 in the amygdala, basal nucleus, and hippocampus. Further, the extracellular levels of dopamine were not altered in the prenatal cannabinoid exposure group compared to the control group[104]; however, the sensitivity of the dopaminergic receptors in the brain was increased in prenatal cannabinoid-exposed humans and animals (after subcutaneous administration of 2 mg/kg THC daily to pregnant dams from gestational days 5 to 20)[105]. At the gene expression level, prenatal THC exposure was found to diminish the mRNA expression of the dopaminergic receptor D_2 in the amygdala and nucleus accumbens[73]. Together, these data suggest that impairment in the mesocorticolimbic neural system, which is essential in regulating emotional behavior, could be a result of the imbalance in the response of dopaminergic receptors[106]. In addition to dopaminergic and glutamatergic receptors, opioid receptors have also been found to be affected by prenatal cannabis exposure. In midgestational human fetal brain tissue with *in-utero* cannabis exposure, an increase in μ opioid receptors was observed[107] in the amygdala and decreased levels of the κ opioid receptors were observed in the mediodorsal thalamus. These findings have been corroborated in an animal experiment[108] in which pregnant Wistar rats were orally administered 5 mg/kg THC daily from gestational day 5 to postnatal day 24: the female offspring exhibited an increase in the density of μ opioid receptors in the hippocampus, amygdala, prefrontal cortex, the ventral tegmental area, and periaqueductal grey matter.

CB1R is expressed at high density in the hippocampus, cortex, and cerebellum, and changes in its activation can alter neurotransmission and gene expression, as well as protein synthesis and activation[109]. The proteins present in these regions of the brain play an important role in memory formation through the activation of different protein kinases *via* various mechanisms, such as alterations in channel properties, changes in synaptic ion channel density, and gene expression regulation and protein synthesis. For instance, the levels of phosphorylated extracellular signal-regulated kinase 1 and 2 (ERK1/2) and c-jun N-terminal kinase (JNK) are increased in the hippocampus following hippocampal-dependent behavioral tasks. However, in rats exposed to cannabinoids prenatally, the levels of phosphorylated ERK1/2 and JNK were reduced in the brain[49]. In addition, phosphorylation of Superior cervical ganglion 10 (SCG10)/stathmin-2 by JNK1 has been implicated in THC-induced CB1-mediated rapid axonal degradation of SCG10, which has been recently discovered[110] to be a key molecular effector mediating adverse effects of cannabis

in the hippocampal neurons. Specifically, maternal cannabis smoking was associated with reduced SCG10/stathmin-2 mRNA and protein expression in midgestational fetal brain samples, and these findings were replicated in embryonic tissue models[109] of prenatal cannabinoid exposure in which pregnant dams were intraperitoneally administered THC (3 mg/kg), WIN 55,212-2 (5 mg/kg), or cannabinoid 1 receptor (CB1) inverse agonist (AM251) (5 mg/kg) from embryonic day 5.5 to 17.5. Recently, increased expression of the neuroactive metabolite kynurenic acid was implicated in prenatal THC-associated abnormalities in short-term memory in the offspring of pregnant Wistar rats that were orally administered 5 mg/kg THC daily from gestational days 5 to 20[111]. The proteins implicated so far may be part of a complex network of proteins and kinases involved in cognitive function that are affected by prenatal cannabis exposure, and therefore, future molecular studies are required to identify other molecular markers of this effect (Table I).

Methods for Detection of Prenatal Cannabinoid Exposure

Gas chromatography and liquid chromatography-tandem mass spectrometric (LC-MS/MS) analysis are typically used to determine the concentration of cannabis compounds, but LC-MS/MS methods are more common than gas chromatography. Until recently, the analyses were mainly conducted on plasma, blood, and urine samples. Further, the major compounds analyzed were THC and 11-nor-carboxy-THC (THCCOOH, an inactive metabolite). Over the last two decades, technological developments in analytical methods have allowed for the detection of a wide array of cannabinoid compounds that it was difficult to analyze otherwise (e.g., cannabidiols and cannabichromenes), and it is also now possible to use a wide range of both maternal and fetal samples (e.g., maternal hair, umbilical cord, dried blood spots, and meconium)[112]. Even though there are more options available, the interpretation of the data may differ according to the detection ability of the various methods for the different types of samples and cannabinoid compounds being analyzed. For example, one study[114] compared the levels of various metabolites in maternal hair, meconium, placenta, and umbilical cord samples and found that maternal hair had a low level of agreement with the meconium (34.3%), umbilical cord (39.1%), and placental samples (34.6%), while there was a high level of agreement between the meconium samples and the umbilical cord (91.3%) and placental samples (92.6%). Further, THCCOOH and 8,11-dihydroxy-THC (8,11-THC-OH) were the major cannabis metabolites in the meconium, while THCCOOH-glucuronide was the predominant metabolite in the placenta and umbilical cord[113]. Among the known samples, the umbilical cord has been demonstrated[115,116] to be an objective tool, and LC-MS/MS methods with high sensitivity and specificity have been reported for the detection of THC, THCCOOH, 8-β-11-dihydroxyTHC (THC-diOH), cannabinol (CBN), 11-hydroxy-THC (11-OH-THC), and THC and THCCOOH glucuronides in umbilical cord samples. Recently, a rapid ultra-high-performance liquid chromatography method that can be used to detect even single use of THC compounds in keratin matrices, such as nails and hair, was reported[117]. This method might have immense potential for the detection of even small amounts of *in utero* exposure to cannabis.

The accurate determination of cannabinoid exposure during pregnancy has become important in the current environment of widespread cannabis decriminalization and legalization, as the regulations with regard to cutoffs and labeling differ across states and countries. In fact, two studies[118,119] have documented mislabeling of the CBD and THC contents of cannabis products sold online and in the United States. Moreover, the presence of dangerous compounds in CBD products has also been detected. For example, dextromethorphan and a dangerous cannabimimetic were found in several commercially available cannabidiol e-liquids[120], and a synthetic cannabinoid was detected in a cannabidiol oil given to a pediatric patient[121]. With regard to the cutoff levels, positive drug screening results after oral consumption of CBD products seem to be unlikely as a result of *in vivo* conversion to THC and THC products[113]. Thus, in the future, detection methods need to cover cannabinoid mimetics and potential contaminants, too, and the cutoff levels need to be re-evaluated.

Monitoring and Preventing Cannabis Use in Pregnant Women

The increase in the decriminalization and legalization of cannabis use has led to easy access to and availability of a wide variety of medicinal

Table I. Mechanisms underlying the cognitive effects of prenatal cannabinoid exposure.

Model	Cannabinoid exposure				Results		
	Drug	Dose/ Route	Duration	Offspring sex	Time point	Observation	Ref.
Impaired neurogenesis and synaptic plasticity							
Female Wistar rats	WIN 55,212-2	0.5 mg/kg, subcutaneous	GD 5 to 20, daily	M	PND 1	↓ Cortical neurons ↓ Glu (cortex and hippocampus)	14
Female Wistar rats	WIN 55,212-2	0.5 mg/kg, subcutaneous	GD 5 to 20, daily	M	PND 1 PND 40	↓Glu (hippocampus) ↓LTP (hippocampus)	48
Mitochondrial dysfunction							
BeWo human trophoblast cell line	Δ9-THC	3 to 30 µM, cell culture	24 h	--	--	↓mitochondrial respiration ↓ATP coupling efficiency	58
BeWo human trophoblast cell line	Δ9-THC	20 µM, cell culture	48 h	--	--	↓mitochondrial respiration ↓mitochondrial membrane potential	59
HTR8/SVNEO human extravillous trophoblast cell line	Δ9-THC	20 µM, cell culture	48 h	--	--	↓mitochondrial respiration ↓mitochondrial membrane potential	59
Epigenetic reprogramming							
Male CD-1 mice	JWH-133	1.5 mg/kg, intraperitoneal	Daily, 5 days a week (2-day interval) for 5 weeks	--	E 13.5	Altered DNA methylation of *Peg10* and *Plagl1*	70
Male Sprague-Dawley rats	THC	4 mg/kg, intraperitoneal	Daily, 28 consecutive days	M & F	Postnatally	Altered methylation of the *Mtss1l* gene (hippocampus and nucleus accumbens)	71
Female Long Evans rats	THC	0.15 mg/kg, intravenous	Daily, gestational day (GD) 5 to 20	M & F	PND 62	Altered histone modification of the *Drd2* gene (nucleus accumbens)	72
Female wild-type Agouti viable yellow mice	CBD	20 mg/kg, oral	Two weeks before mating till lactation	M & F	Postnatal 12 weeks	Altered genome-wide methylation (cortex)	73
Female Long Evans rats	THC	0.15 mg/kg, intravenous	Daily, GD 5 to postnatal day (PND) 2	M	PND 21	Altered histone modification of *Kmt2a* (nucleus accumbens)	74
Impaired GABAergic, glutamatergic, dopaminergic, and opioid neurotransmission*							
Female Wistar rats	WIN 55,212-2	0.75 mg/kg, subcutaneous	Daily, GD 5 to 20	M & F	E 12.5 to 20.5	↑GABAergic cortical neurons Alteration in glutamatergic cortical neurons	89
Female Wistar rats	THC	3 mg/kg, intraperitoneal	Daily, GD 7 to 22	M & F	PND 100 and 120	↑AMPA-GluR2	96

Continued

Table I *(Continued)*. Mechanisms underlying the cognitive effects of prenatal cannabinoid exposure.

Model	Cannabinoid exposure				Results		
	Drug	Dose/ Route	Duration	Offspring sex	Time point	Observation	Ref.
Female rats	THC	5 mg/kg, oral	Daily, GD 5 to PND 20	M & F	PND 30 and 70	↓AMPA-GluR1 and AMPA-GluR2	95
Female Wistar rats	WIN 55,212-2	0.5 mg/kg, subcutaneous	Daily, GD 5 to PND 20	M	PND 1	↓cortical NMDA receptor activity	15
Female Sprague-Dawley rats	THC	2 mg/kg, subcutaneous	Daily, GD 5 to 20	M	PND 15 to 28	↑dopaminergic neuron firing	105
Female Wistar rats	Δ9-THC	5 mg/kg, oral	Daily, GD 5 to PND 24	F	--	↑ density of μ opioid receptors in the prefrontal cortex, hippocampus, amygdala, ventral tegmental area, periaqueductal grey matter	107
Impaired expression of proteins							
Wild-type and CB1R−/− pregnant mice on C57Bl/6 background	THC, WIN 55,212-2, AM251	3 mg/kg, 5 mg/kg, 5 mg/kg, intraperitoneal	Daily, E 5.5 to 17.5	M & F Hippocampal sections from E 18.5	--	↓mRNA and protein expression of SCG10/ stathmin-2	109
Female Wistar rats	THC	5 mg/kg, oral	Daily, GD 5 to 20	M	PND 65 to 90	↑extracellular kynurenic acid in the prefrontal cortex	110

*See Higuera-Matas et al1[12] for more information about the effect of prenatal cannabis exposure on these pathways.

and recreational cannabis products that can be consumed in oral, inhaled, and topical forms. While it is too early for the documentation of any solid evidence of an increase in cannabis use[122], monitoring the use of cannabis products in pregnant women is important, given its cognitive effects in newborns and even later on in life. In this regard, the preconception period is an important time window for monitoring[123]. It has been reported[124] that one-third of women who use in this period continue to use cannabis products during their pregnancy; further, the number of women who start using during pregnancy is low. Accordingly, the majority of women who use cannabis during pregnancy report[125] preconception cannabis use. Thus, designing education programs and interventions that target women in the reproductive age group who report cannabis use would help identify women at risk and potentially prevent prenatal exposure. In particular, women who report the use of alcohol and tobacco and who are diagnosed with depression need to be screened, as these factors have been linked[126] to preconception cannabis use. In addition, these programs should focus on areas where recreational cannabis use has been legalized, as one study[127] showed that legalization of recreational use was linked with cannabis use in the pre-gestational, gestational, and post-gestational periods. With regard to potential interventions that could be implemented in this period, one study[128] showed that a two-session brief motivational intervention among 18- to 24-year-old women who reported cannabis use had long-term effects, especially among those who expressed the desire to quit. Further, the screening, brief intervention, and referral to treatment or screening, brief intervention and referral to treatment (SBRIT) technique proposed by the Institute of Medicine[123] has been applied successfully in individuals at risk of developing substance abuse disorders, and it could be beneficial for reducing the risk of cannabis use during pregnancy too. In addition to these tools, creating settings where patients feel at ease and are able to communicate with healthcare providers without feeling stigmatized is also a potentially beneficial approach[122]. That is, both pregnant and pregnancy-planning women require non-punitive healthcare that is

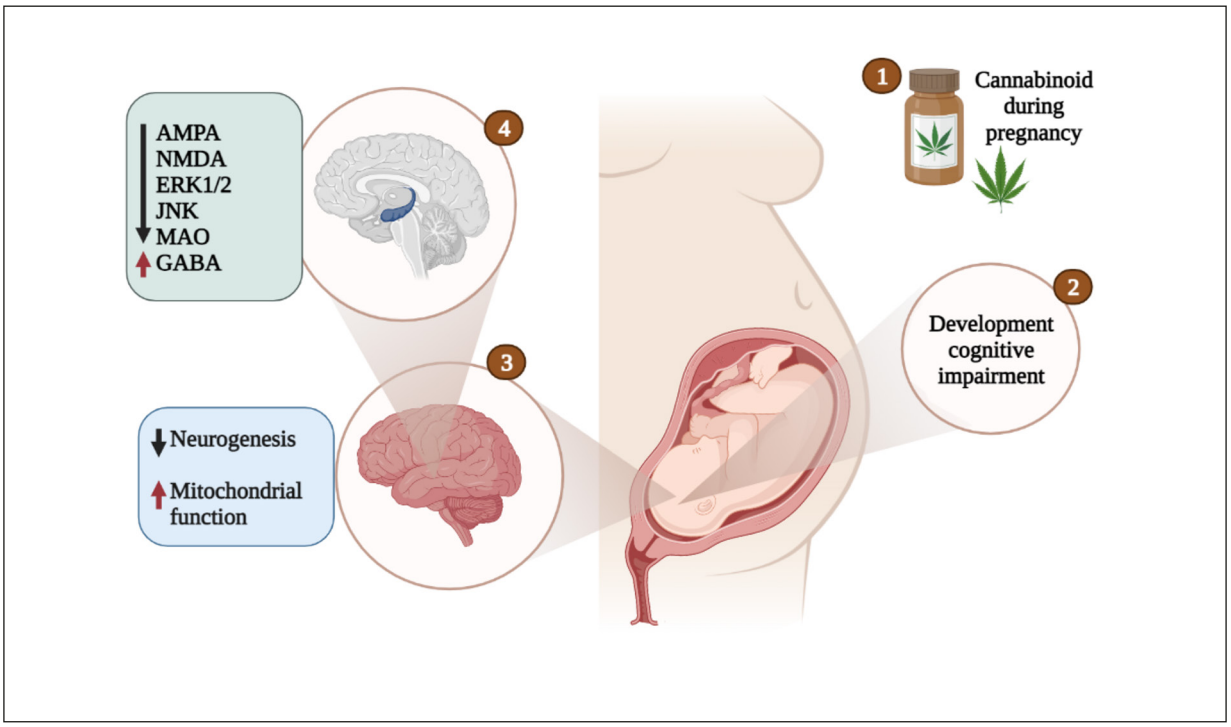

Figure 1. Conceptual diagram of the onset of cognitive impairment caused by embryonic cannabinoid exposure.

respectful of the patient's autonomy[123,129]. The dissemination of accurate scientific data related to cannabis use among the public[128] and evidence-based medicine approaches[123] is also important for preventing cannabis use in pregnant women. Finally, addressing and treating the health issues or socioeconomic contexts that necessitate cannabis use in the first place would have long-term implications for patients[123].

Although the general consensus is that cannabis use must be avoided during pregnancy based on the evidence available for its detrimental effects on the newborn, there exist certain ethical dilemmas around the implementation of prevention programs. That is, whether the rights of the mother should be prioritized over the rights of the fetus is an ongoing discussion in this field[130]. In this light, using a harm minimization strategy wherein pregnant women are provided with the information they need to make an informed decision and are also supported, irrespective of their decision, is recommended[131]. In addition, given the potential of gestational cannabis use for "harm-to-others," it is important to evaluate its public health impact and frame public health policies around it[132].

Conclusions

This review provides considerable evidence from published studies that prenatal cannabinoid exposure has a long-term effect on the function of the brain. Overall, the findings of this review imply that cannabinoid use during pregnancy is not safe. In particular, early exposure in the first trimester may have long-lasting effects on cognitive impairment in humans and experimental animals. With regard to the mechanisms underlying these cognitive effects, cannabinoids can alter the expression and function of CB1R, decrease glutamate transmission activity, reduce neurogenesis, increase mitochondrial function, and downregulate GluR1 and GluR2 expression to inhibit synaptic plasticity in the hippocampus, cortex, and cerebellum (Figure 1). However, some limitations of these findings need to be mentioned. For one, there are no standard methods for measuring cannabinoid exposure, and the measured levels differ according to the sample and metabolite being analyzed. Another limitation of this review was that not many studies have investigated the molecular mechanisms in the children of mothers exposed to canna-

binoids during pregnancy, and most molecular studies use animal models. The strengths of this review were that the studies on the effect of prenatal cannabinoid exposure showed significant changes in behavioral tendencies, electrophysiological recordings, and biomarkers related to cognitive function that lay a strong basis for future research on this topic. In addition, this review highlights the importance of preventive programs that can help women quit cannabinoid consumption when planning for pregnancy.

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The Authors declare that they have no conflict of interests.

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