

Is the Use of Cannabis During Pregnancy a Risk Factor for Autism?

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ABSTRACT

The use of marijuana by healthy adults is commonly viewed as having limited adverse health effects; however, its potential risks for fetal developmental abnormalities when used during pregnancy have not been thoroughly evaluated. It is, therefore, important that the effects of prenatal marijuana on the developing fetus be fully assessed in order to create a proper set of guidelines for use before, during and after pregnancy as is standard with other drugs such as alcohol and nicotine.

Keywords: Pregnant; Autism; Schizophrenia; Epilepsy

INTRODUCTION

The use of marijuana by healthy adults is commonly viewed as having limited adverse health effects; however, its potential risks for fetal developmental abnormalities when used during pregnancy have not been thoroughly evaluated. This area of research is of increased concern as the incidence of individuals using marijuana is continuing to rise with changes in legislation moving toward legalization in many parts of the United States. Further, the use of marijuana among pregnant women is rising and is predicted to continue to increase [1]. This may be in part to elevate symptoms of pregnancy such as nausea. It is, therefore, important that the effects of prenatal marijuana on the developing fetus be fully assessed in order to create a proper set of guidelines for use before, during and after pregnancy as is standard with other drugs such as alcohol and nicotine.

The endocannabinoid system is heavily involved in both neural development, especially cell migration, neuronal growth and synaptic plasticity, as well as lifelong processes such as motivation, motor control, emotional responses, cognition, and homeostasis [2-5]. This system is altered in many neuropsychiatric conditions, including autism, schizophrenia and epilepsy, but it may also be altered upon fetal exposure to drugs and alcohol [3,5-7].

Autism Spectrum Disorder is known for its severe cognitive, social and behavioral impairments [8]. Since the endocannabinoid system is involved in cognition, behavioral, emotional and social regulations, it seems possible that alterations in the endocannabinoid system, particularly during critical developmental stages, could cause or put one at risk for an autism spectrum disorder. Marijuana is known to bind to endogenous cannabinoid receptors and induce the same effects in the body as endogenous

cannabinoids [9]. It is, therefore, possible that the over-excitation of the endocannabinoid system during development causes changes in the endocannabinoid system which makes one at risk for development of autism spectrum disorder.

The present study aims to briefly outline the endocannabinoid system and its interaction with marijuana, prenatal marijuana exposure effects on the endocannabinoid system, and how this interaction may increase risk of developing autism spectrum disorder.

OVERVIEW OF THE ENDOCANNABINOID SYSTEM

The endogenous cannabinoid or endocannabinoid system is a signaling system present in all vertebrates and consists of endogenous ligands and cannabinoid receptors. This system received its name as it responds to cannabinoid drugs [3]. Endogenous ligands are cannabis-like substances which are derived from arachidonic acid [6]. Several endogenous ligands have been identified, but the most notable are anandamide (arachidonylethanolamide; AEA), and 2-arachidonoyl glycerol (2-AG) [3,5,7]. AEA is generated by N-acyl-phosphatidylethanolamine-selective phospholipase D and degraded by fatty acid amide hydrolase (FAAH) into arachidonic acid and ethanolamine. 2-AG is generated by alpha and beta isoforms of sn-1-diacylglycerol lipases and degraded by monoacyl glycerol lipase (MAGL) into arachidonic acid and glycerol [10-14]. Unlike other neurotransmitters, these compounds work in a retrograde fashion, on the presynaptic cell, and are synthesized when and where they are needed rather than produced then stored [5].

There are two major endocannabinoid receptors (cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2)). These two

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receptors differ based on amino acid sequence, tissue distribution in the body and signaling mechanisms [3,7]. CB1 was first discovered in the brain and CB2 was first discovered in the spleen [5,7,15,16]. Both CB1 and CB2 are G protein-coupled receptors (GPCR). The CB1 receptor was subsequently found in peripheral organs in addition to the CNS. CB1 is the most abundant GPCR in the brain with the greatest concentrations on presynaptic terminals of glutamatergic and γ -aminobutyric acid-ergic (GABAergic) neurons in the basal ganglia, substantia nigra, globus pallidus, cerebellum, olfactory bulb, amygdala, cerebral cortex, septum, hypothalamus, brainstem, spinal cord and hippocampus making the receptor important for motivation, motor control, emotional responses, cognition, and homeostasis [3,5-7]. Additionally, CB1 presence begins in the early ontogenetic developmental stage, suggesting its role in embryonic neural development [17]. The presynaptic location of these receptors allows for the inhibition of neurotransmitter release [5]. CB1 activation leads to a decrease in cyclic adenosine monophosphate (cAMP), inhibition of cAMP-dependent protein kinase (PKA), and stimulation of mitogen-activated protein kinase (MAPK), which influences cell migration, neuronal growth and synaptic plasticity [2-5]. Additionally, other protein phosphorylation cascades are triggered by CB1 receptor activation as well as inhibition of voltage-activated calcium ion channels and stimulation of potassium channels causing inhibition of neurotransmitter [4,18-21].

Like CB1 receptors, CB2 were later discovered to be present in both the CNS and peripheral organs [22,23]. Unlike CB1 receptors, however, the effects of this receptor are much more widespread. CB2 receptors have been identified in pathological conditions of the heart, liver, gut, kidney, lung, bone, reproduction, cancer, pain, neurodegenerative disorders and psychiatric disorders [24]. While the effects are widespread, they are most highly involved with immune responses and are thus hypothesized to belong to a general protective system [3,24]. Although CB2 receptors have been found in the CNS, their role in endocannabinoid-mediated synaptic transmission is still ambiguous [7].

MARIJUANA AND THE ENDOCANNABINOID SYSTEM

Marijuana or *Cannabis sativa* is a plant which contains cannabinoid compounds. These compounds produce physiological responses when ingested and inhaled such as analgesia, reduction of nausea and vomiting, appetite suppression, relief from muscle spasms and spasticity, and decreased intestinal motility, among others [3]. It is for these reasons that the plant has been used for medical purposes for the last 5000 years [7].

In an effort to better understand its physiological effects, researchers attempted to isolate the active component of marijuana. It was then discovered that there are actually more than 60 cannabis constituents, thus making a singular isolation difficult [5]. Eventually, two major principles were isolated and synthesized, Δ^9 -Tetrahydrocannabinol, commonly referred to as THC and cannabidiol, commonly referred to as CBD. CBD is much more complex than THC and has over 100 metabolites [13,25-27].

When the cannabinoid components enter the body, they make their way into the blood stream and eventually bind to CB receptors in the body. THC acts as a partial agonist on CB1 and CB2 receptors. The effects caused by this interaction are influenced by endogenous cannabinoid release, expression level, and signaling efficiency of receptors. When THC binds to CB1 receptors, there is a decrease

in glutamate transmission followed by a decrease in post synaptic excitation. CBD on the other hand acts as an antagonist of CB1 and CB2 receptor agonists in CB1 and CB2 expressing cells and tissues [9,28,29].

EFFECTS OF AUTISM SPECTRUM DISORDER ON THE ENDOCANNABINOID SYSTEM

Autism spectrum disorder is a neurodevelopmental disorder with an early life onset that is characterized by social and communication deficits, and unusual restricted repetitive behaviors resulting in significant social and occupational impairments [8]. A subset of these patients also experiences seizures, anxiety, intellectual disabilities, motor dysfunctions, altered sleep, disrupted response to sensory stimuli and metabolic disturbances [30,31]. These symptoms are caused by widespread neural impairments/alterations. One of the neural systems affected by the disease is the endocannabinoid system [2,31-34]. The endocannabinoid system also plays a role in social behavior and emotionality, which are two aspects of human behavior that are altered in individuals with autism.

One animal model of autism which is commonly used to assess novel drug and behavioral therapies is the valproic acid exposure model (VPA) in which the animals are prenatally exposed to valproic acid. This exposure results in an animal with a condition that mimics autism, both structurally and behaviourally [31]. A recent study used this model to test whether the endocannabinoid alterations seen in autism relate to the autism symptoms [35]. The results of this study suggest that while CB1, CB2, AEA, 2-AG and endocannabinoid levels remain unaltered in the autism model, the expression of the mRNA for diacylglycerol lipase α (an enzyme necessary for 2-AG synthesis) was reduced in the cerebellum, and the activity of this enzyme was increased in the hippocampus. The VPA rats also exhibited reduced PPAR α and GPR55 expression in the frontal cortex and reduced PPAR γ and GPR55 expression in the hippocampus. PPAR α , PPAR γ , and GPR55 are additional endocannabinoid receptor targets [35]. Another recent VPA study found that there are, in fact, alterations in the expression of phosphorylated CB1 receptors [36]. This effect was not seen uniformly, however. The altered expression was present in the amygdala, hippocampus and dorsal striatum, but not in the prefrontal cortex, cerebellum and nucleus accumbens. This expression was further assessed and is hypothesized to be the result of changes in anandamide metabolism. An increase in fatty acid amide hydrolase (FAAH) was also identified [36]. The results of these papers support the idea that endocannabinoid dysfunction may be a contributing factor to the behavioral abnormalities in autism spectrum disorder.

Fragile X mental retardation (Fmr1) knockout mice are commonly used to assess autism spectrum disorder since Fragile X Syndrome is one of the leading genetic causes of autism spectrum disorder [31]. Zhang and Alger, 2010 used the Fmr1 model to assess GABAergic synapses in the hippocampus [37]. The result of this study suggests enhanced response in the cells hypothesized to contribute to the cognitive deficits seen in individuals with autism [37]. Maccarrone 2010 found similar effects on the GABAergic neurons in the striatum [38]. The results further suggested that the metabotropic Glutamate 5 receptor (mGlu5R)-driven endocannabinoid signalling in the striatum is controlled by fragile x mental retardation protein and BC1 RNA. Jung et al., 2012 further built upon these findings by identifying a lack of mGlu5R depression at excitatory neurons in the ventral striatum and prefrontal cortex [39]. This effect is due

to a decrease in diacylglycerol lipase- α and mGlu5R dependent 2-A and results in deficits in endocannabinoid-dependent long-term depression [39]. These studies combined support the idea that the endocannabinoid system may be altered in autistic individuals.

Neurologin-3 (NLGN3) mouse models are also used in autism spectrum disorder animal studies. NLGNs are important for postsynaptic cell adhesion, maturation and function of excitatory and inhibitory neurons; their mutation has been shown to induce autism and autism associated behaviors in humans [31]. These animals have alterations in the NLGN3 (either NLGN3R451C knockin or NLGN3 knockout) and display autism-like behaviors [31]. Foldy et al., 2013 evaluated the synaptic transmission of hippocampal GABAergic synapses in both NLGN3R451C knockin and NLGN3 knockout models and found that tonic but not plastic endocannabinoid signalling was altered in both models [40]. Other research has also indicated an increase in inhibitory transmission in mutant mice as a result of a loss of endocannabinoid signalling through CB1 receptors [41].

Another animal model used is generated by postnatal lipopolysaccharide (LPS) administration in rats [31]. This model is used to mimic the bacterial and viral infections which are believed to contribute to the development of autism spectrum disorder. While this model is not a widely-accepted animal model for autism, work done on these animals has found decreases in CB1 binding, elevated anandamide levels and increased FAAH in the amygdala [42].

Research using animal models gives insight into the effects of experimental manipulations that cannot be performed in humans, but not all animal work translates to humans. A recent study looked at the levels of plasma AEA in children with autism spectrum disorder. The results indicated that plasma AEA concentrations are lower in children with autism [43]. This work was supported by Aran et al., 2019 who also found lower levels of AEA in individuals with autism spectrum disorder. This work further looked at other related endogenous compounds and found

that there is also a decrease in N-palmitoylethanolamine (PEA), and N-oleoylethanolamine (OEA) but not 2-AG or arachidonic acid in these patients [44].

Additional work suggests that other areas of the endocannabinoid system are also affected. For example, Siniscalco et al., 2013 found a significant increase in the mRNA level for CB2 receptors in individuals with autism spectrum disorder, but CB1 and FAAH levels remained the same [45]. Follow up work from the same group further showed that CB2 levels in peripheral blood are increased in patients with ASD. This study also indicated FAAH expression is decreased in autistic children [46]. Functional imaging studies have been performed on individuals with ASD to understand the alterations in connectivity seen in this disease. McFadden and Minshew, 2013 found that the abnormalities in connectivity seen in these patients may be due to a lack of CB1 axon guidance (Table 1) [47].

Research on individuals with autism has also suggested a down regulation in both glutamatergic and GABAergic signaling. Specifically, a reduction in GABA_A receptors has been identified. Alterations in GABA functioning have been directly linked to negative symptoms in individuals with autism [48]. Similarly, a reduction in glutamatergic protein and mRNA expression has been identified (Table 2) [49,50].

Additional reviews in both human and animal models of endocannabinoid signalling in individuals with autism support the idea the endocannabinoid system is altered in autism spectrum disorder [2,31-34].

EFFECTS OF PRENATAL MARIJUANA EXPOSURE ON THE ENDOCANNABINOID SYSTEM

The endocannabinoid system has been called the gatekeeper of neural development as it plays widespread critical roles in uterine implantation, neurodevelopment, neural stem cell proliferation

Table 1. Alterations in the Endocannabinoid System in Animal Models of Autism Spectrum Disorders.

Model	Study	Effects
Valproic acid (Rat)	Kerr (2013)	· Expression of mRNA for diacylglycerol lipase α reduced
		· Reduced PPAR α and GPR55 expression in frontal cortex
	Servadio (2016)	· Reduced PPAR γ and GPR55 expression in the hippocampus
		· Alterations in the CB1 receptors in amygdala, hippocampus and dorsal striatum but not in prefrontal cortex, cerebellum and nucleus accumbens
Fragile X Mental Retardation Knockout (Mouse)	Zhang & Alger (2010)	· Changes in anandamide metabolism
		· Increase in FAAH
	Maccarrone (2010)	· Enhanced responses in GABAergic synapses in the hippocampus
		· Enhanced responses in GABAergic synapses in the striatum
Jung (2012)	· mGlu5R-driven endocannabinoid signaling in the striatum is controlled by fragile x mental retardation protein and BC1 RNA	
	· Lack of mGlu5R depression at excitatory neurons in the ventral striatum and prefrontal cortex.	
Postnatal Lipopolysaccharide administration (Rat)	Doenni (2016)	· Decrease in diacylglycerol lipase- α
		· Decrease in mGlu5R dependent 2-A
		· Deficits in endocannabinoid-dependent long-term depression
Neurologin-3 (Mouse)	Foldy (2013)	· Decreases in CB1 binding
		· Elevated anandamide levels
	Speed (2015)	· Increased FAAH in the amygdala
Neurologin-3 (Mouse)	Speed (2015)	· Tonic but not plastic endocannabinoid signaling was altered
		· Increase in inhibitory transmission in mutant mice as a result of a loss of endocannabinoid signaling through CB1 receptors

Table 2. Alterations in the Endocannabinoid System in Individuals with Autism Spectrum Disorders.

Study	Effects
Karhson (2018)	plasma AEA concentrations are lower in children with autism
Aran (2019)	lower levels of AEA, PEA and OEA in individuals with ASD
Siniscalco (2013)	significant increase in the mRNA level for CB2 receptors in individuals with ASD, but CB1 and FAAH levels remained the same
Siniscalco (2014)	CB2 levels in peripheral blood are increased in patients with ASD and FAAH expression is decreased in ASD
McFadden and Minshew (2013)	abnormalities in connectivity seen in these patients may be due to a lack of CB1 axon guidance
Purcell (2001)	Decrease in glutaminergic signaling in ASD postmortem
Fatemi (2009)	Down regulation in GABAergic neurons in ASD

and differentiation, synapse formation, axon migration, synaptogenesis, and excitatory and inhibitory synapse modulation in brain and spinal cord [1,17,51-59]. Since the endocannabinoid system is present and functions beginning in very early embryonic development, the exposure to THC in the womb could have effects on the endocannabinoid system from very early on [17,60,61]. This may lead to lifelong impairments in cognition, visual-motor coordination, social behavior, attention deficits and anxiety and depression [62-69].

Despite marijuana being the most commonly used illicit drug among pregnant women, there is not a great deal of research into the effects of fetal exposure to marijuana. Two large longitudinal studies provide most of the human data to date. These studies focused on demographics, anatomical changes, behavior, cognitive and neuropsychological characteristics over time [70,71]. More specifically, both of these studies looked at individuals exposed to marijuana in utero as a fetus, a neonate, infant, child, adolescent and young adult [1]. As a fetus, the researchers evaluated gestational age and birth weight. As a neonate, responses to light, reflexes and physiological information (such as height) were assessed. As infants and children characteristics such as, motor skills, cognition, neuropsychology (i.e. memory, attention, impulsivity, hyperactivity, IQ), and mental development were assessed. As adolescents and young adults, again neuropsychology, and cognition were assessed in addition to delinquency functional activity through fMRI. While these studies provide great evidence for the long term behavioral and neuropsychological effects of individuals prenatally exposed to marijuana, there is still a need to evaluate the biological mechanisms behind these symptoms.

The active component in marijuana, THC, readily enters the bloodstream upon intake. In a pregnant woman, this chemical will then readily cross into the placenta [72]. The well-known effects of this fetal interaction include distress and growth retardation [73-76]. Richardson et al., 2016 proposed the concept of “first hit” and “second hit” effects [1]. Upon a first hit or first exposure to prenatal marijuana, the developmental deficits are minimal, which the authors refer to as a “buckling in the developing nervous system”. The second hit or second exposure is where much of the detrimental effects lie. THC binds CB1 receptors mimicking the effects of AEA; however, this unregulated stimulation activates dopamine production due to its depressive effects on GABAergic neurons producing a paradoxical effect that ultimately depresses CB1 receptors. The authors describe the exposure as a “power punch” which causes massive negative effects on the nervous system resulting in life long neurodevelopmental and behavioral abnormalities [1]. These lifelong negative effects may be the result of malformation of the endocannabinoid system during

development or disruptions in the developmental processes with which endocannabinoids are involved [77,78].

Jutras-Asward et al., 2009 looked at post-mortem human neural tissue of fetuses exposed to marijuana in utero [79]. This study found no significant changes to CB1 receptor mRNA in the striatum, hippocampus, amygdala, insula, temporal cortex, parietal cortex, or parahippocampus [79]. This group further assessed dopamine 1 and 2 receptors (D1 and D2 respectively) and observed a reduction in D2 receptor mRNA in the amygdala of individuals exposed to marijuana in utero [51,80]. Effects may not be seen at the mRNA expression of the CB1 receptor because these data could be representing normalized levels of expression as a result of repeated marijuana exposure [79]. Interestingly, de Salas-Quiroga et al., 2015 found significant down regulation in CB1 receptor protein levels in rats embryonically exposed to THC but levels returned to those similar to control at the perinatal stage [81]. Additionally, other work suggests a decrease in CB1 receptor mRNA expression following prenatal THC exposure associated with an increase in MAG lipase and a decrease in diacylglycerol lipase enzymes [78]. This work was further supported by Bara et al., 2018 in which they observed decreases in diacylglycerol lipase alpha mRNA levels in the frontal cortex. However, this finding was only observed in females [82].

Keimpema et al., 2011 describes two cellular foci of action of prenatal marijuana exposure using animal models. The first focus is THC displacing 2-AG from CB1 receptors, specifically in the growth cones [51]. This results in alternations in directional axonal growth. The second focus of cellular action is that THC can hijack CB1 receptors by circumventing MAGL barriers while the axons are being trafficked. The authors further suggest that these cellular processes cause the indiscriminate activation of CB1 receptors which would have not been activated otherwise during neurite outgrowth [51].

Additionally, effects of prenatal marijuana exposure on GABAergic and glutamatergic neurons have been seen. This relationship is important because the endocannabinoid system is vital to GABAergic interneuron development and glutamatergic development in corticogenesis [56,60]. Suarez and colleagues exposed rats to THC prenatally; this exposure caused several glutamatergic related effects. First, the rats exhibited decreased glutamine synthetase in the cerebellum. Glutamine is an important precursor to glutamate [83]. The researchers then observed a down-regulation of Glu1 and Glu2/3 receptors [84]. Finally, a down-regulation in glutamate transporter expressions was observed. These findings suggest that prenatal marijuana exposure during cerebellar development can have long last effects. A similar glutamatergic gene down regulation was also observed in the cortex [85].

Effects of prenatal marijuana exposure on GABA-ergic neurons have been seen in hippocampal neurons. Specifically, CB1 receptor-positive GABA-ergic interneurons in marijuana exposed rats are increased in density while CB1 receptor-negative GABA-ergic interneuron concentrations do not change [86]. This work has recently been corroborated and further suggests these specific GABA-ergic hippocampal alternations lead to cognitive deficits [87]. Additionally, over-excitation of these neurons in development has been seen to disrupt neural development. More specifically, the maternal use of cannabis while pregnant can result in the shutdown of cortical activity as a result of GABA-ergic neurotransmission alterations [88].

Genetic alterations have also been seen in rats exposed to prenatal marijuana. Kittler et al., 2000 looked at rats exposed to THC and found that 49 different genes were altered following this exposure [89]. Of the 49, many are involved in the biochemical cascades of endogenous cannabinoid synthesis or effector systems. Similar work has evaluated the effects of prenatal THC on levels of expression of neurotrophins important for CB1 receptor mediated growth responses and further found alterations in the neurotrophins [85,90].

Finally, work has also been done using CB1 and CB2 agonist WIN55,212-2 to gain insights into the effects of prenatal over-stimulation of these receptors. Results indicate no change in CB1 receptor density in any neural region but increased AEA and N-acyl-phosphatidylethanolamine-specific phospholipase D,

and decreased FAAH in the striatum. The study also indicated decreased levels of AEA in the limbic system. Therefore, this work suggests that cannabinoid receptor over-excitation in utero can have long lasting effects [91].

In summary, the exposure to marijuana in utero may have lifelong effects on the offspring. Excessive amounts of exposure can be lethal while lower doses can have serious implications in the endocannabinoid system leading to many cognitive, behavioral and social implications (Table 3) [1].

DISCUSSION

Autism spectrum disorder is associated with alterations in the endocannabinoid system, including but not limited to reduction in mRNA expression of diacylglycerol lipase α , PPAR α , GPR55, PPAR β , mGlu5R dependent 2-AG, CB1 binding, AEA (in humans), PEA, and OEA; alterations in CB1 receptors, and anandamide metabolism; and increases in FAAH, GABAergic synapse response, AEA levels (in animals), mRNA expression of CB2, and CB2 in peripheral blood [35-39,41-44,46]. These alterations in the endocannabinoid system occur at a young age and persist throughout the life of the individual with autism. Since the endocannabinoid system is highly involved in social, behavioral, emotional and cognitive function, it is likely that some of the symptoms caused by autism spectrum disorder are direct results of the disruptions in the endocannabinoid system [3,5-7].

Table 3. Effects of fetal marijuana exposure on the endocannabinoid system.

Effect	Study
Malformation of the endocannabinoid system during development	Wu, Jew & Lu (2011)
DISRUPTIONS in axonal growth	Tortoriello (2014)
No significant changes to CB1 receptor mRNA expression in the striatum, hippocampus, amygdala, insula, temporal cortex, parietal cortex, and parahippocampus	Jutras-Aswad, DiNieri, Harkany, & Hurd (2009)
Reduction in D ₂ gene expression in the amygdala	Wang, Dow-Edwards, Anderson, Minkoff & Hurd (2004)
Down regulation in CB1 receptor protein levels in rats embryonically exposed to THC but levels returned to those of control at the perinatal stage	Salas-Quiroga (2015)
Decrease in CB1 receptor mRNA expression following prenatal THC exposure although with an increase in MAG lipase and decrease in diacylglycerol lipase enzymes	Tortoriello (2014)
Decreases in diacylglycerol lipase alpha mRNA levels in the frontal cortex in female offspring	Bara et al., 2018
THC displaces 2-AG from CB1 receptors in the growth cones	Keimpema et al., 2011
THC can hijack CB1 receptors by circumventing MAGL barriers while the axons are being trafficked and cause the silencing of CB1 receptors	Keimpema (2011)
Decreased glutamine synthetase in the cerebellum	I. Suárez (2002)
Down-regulation of Glu1 and Glu2/3 receptors	Isabel Suárez (2004)
Down-regulation in glutamate transporter expressions	I. Suárez (2004)
Glutamatergic gene down regulation observed in the cortex	Campolongo (2007)
CB1 receptor-positive GABA-ergic interneurons increased in density while CB1 receptor-negative GABA-ergic interneuron concentrations do not change	Berghuis (2005)
Long lasting alternations in hippocampal GABA-ergic neurons	Beggiato (2017)
Down of cortical activity as a result of GABA-ergic neurotransmission alterations	Bernard (2005)
49 different genes were altered. many are involved in the biochemical cascades of endogenous cannabinoid synthesis or effector systems	Kittler (2000)
Alterations in expression of neurotrophins important for CB1 receptor mediated growth responses	Campolongo (2007); Maison, Walker, Walsh, Williams, & Doherty (2009)
Prenatal exposure to CB1 and CB2 agonist WIN55,212-2 results in no change in CB1 receptor density in any neural region but increased AEA and N-acyl-phosphatidylethanolamine-specific phospholipase D, and decreased FAAH in the striatum. Also, decreased levels of AEA in the limbic system	Castelli (2007)

Prenatal exposure to marijuana is also known to alter the endocannabinoid system by causing malformation in the system, reduce D2 gene expression, down regulate CB1 receptor proteins and mRNA expression, increase MAG lipase, decrease diacylglycerol lipase enzymes, over activation at CB1 growth cones, decreased glutamine synthetase, Glu1 and Glu2/3 down-regulation, glutamatergic down regulation, GABAergic alterations, and alterations in cannabinoid genes and neurotrophins [77-91]. Prenatal marijuana exposure is also known to cause impairments in cognition, visual-motor coordination, social behaviour, attention deficits and anxiety and depression. Some of these symptoms are similar to those of autistic individuals [62-69].

Since some of the symptoms and endocannabinoid alterations of autism and prenatal marijuana exposure are the same, it is possible that these are related such that prenatal marijuana exposure increases the risk of developing autism spectrum disorders (Figure 1) [92,93]. Additionally, glutamatergic signalling is important for cognition and learning [94]. When the glutamatergic signalling is altered, cognition and learning can be affected. Since glutamatergic signalling has been found to be altered in those exposed to marijuana prenatally, it is likely the cognitive and learning symptoms related to prenatal marijuana exposure are caused by these alterations [83-85]. This is particularly relevant because individuals with autism also have decreased glutamatergic signalling [95,50]. It is, therefore, possible that prenatal marijuana exposure causes a decrease in glutamatergic signalling similar to the decrease seen in autistic individuals. This finding does not suggest a directional relationship, but it is possible that marijuana exposure causes decreased glutamate signalling, increasing the risk for autism spectrum disorder.

Similarly, GABAergic signalling has been identified as being down-regulated in both individuals with autism and prenatal marijuana exposure [49,86-88]. These findings together suggest a potential link between autism and prenatal marijuana exposure because prenatal marijuana exposure causes over excitation of these neurons due to unnatural stimulation of CB1 and CB2, these neurons may

down regulate and never correct themselves. This could cause the pathology seen in individuals with autism.

Marijuana exposure in utero is also believed to decrease immune response [13]. Since autism has been linked to both bacterial and viral infections it is possible that the decreased immune responses caused by exposure to marijuana could increase the risk of infection leading to development of autism spectrum disorders [31,96-98]. This idea is supported by the Quantitative Threshold Exposure (QTE) hypothesis which suggests that there is a relationship between critical neural developmental stages and the immune system that is influenced by environmental and genetic risk factors. Further, the greater the number of risk factors an individual possesses, the greater the likelihood of developing autism spectrum disorder. It is, therefore, likely that the decreased immune response produced by prenatal marijuana exposure is a risk factor for developing autism spectrum disorder, and that the occurrence of this disorder is more likely when paired with other risk factors [99].

LIMITATIONS

There are some limitations to understanding the relationship between prenatal marijuana exposure and autism. The first limitation is that the disease of autism is uniquely human [100]. To date, there are no reported cases of autistic animals occurring naturally. Further, there are very few accepted animal models for this disease. Animal models that produce autism-like behaviours as a result of autism risk factors such as fragile x mental retardation and infection are useful but there is no true autism animal model. Similarly, the work that is produced from these animal models may be relevant to human patients, but also may not. The animal nervous system does differ from the human and these results may not generalize.

FURTHER DIRECTIONS

While it is possible that marijuana exposure in utero can increase risk of developing autism, much more work needs to be done in order to understand this relationship [92,93,101-105]. In particular,

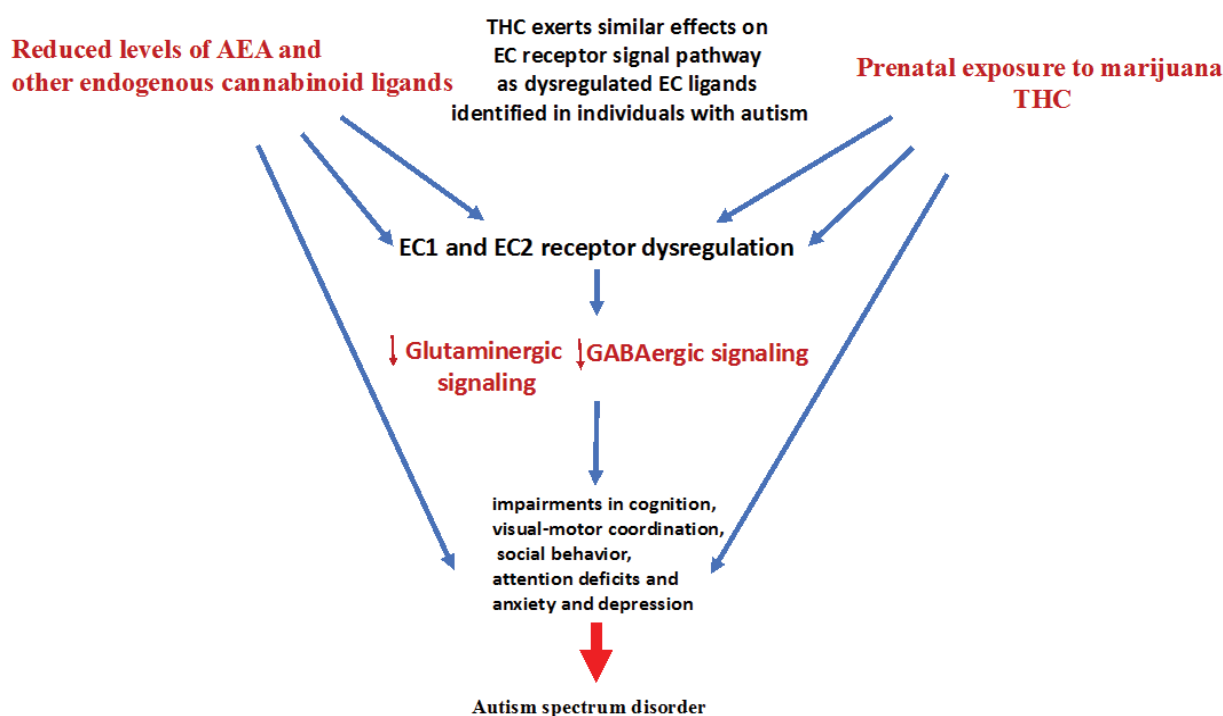


Figure 1: Proposed role of THC in the causation of impairments of the endocannabinoid system identified in Autism Spectrum Disorder (See text for details).

there is a gap in the literature in regard to what is happening at a cellular and molecular layer of the endocannabinoid system in children who have been prenatally exposed to marijuana [70,71,106-113]. Great work has been done on these children from a longitudinal behavioural, cognitive and social standpoint but these studies fail to address the underlying biological mechanisms causing these changes. Similar work should be conducted which also addresses biomarker changes. Further, post-mortem studies should be conducted to evaluate the specific neural alternations caused this prenatal exposure, specifically, to explore further the link between autism, prenatal marijuana and GABAergic and glutamatergic down regulation. Future work should focus on further understanding this interaction.

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