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Review article

Prenatal cannabis exposure - The "first hit" to the endocannabinoid system

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ABSTRACT

As more states and countries legalize medical and/or adult recreational marijuana use, the incidences of prenatal cannabis exposure (PCE) will likely increase. While young people increasingly view marijuana as innocuous, marijuana preparations have been growing in potency in recent years, potentially creating global clinical, public health, and workforce concerns. Unlike fetal alcohol spectrum disorder, there is no phenotypic syndrome associated with PCE. There is also no preponderance of evidence that PCE causes lifelong cognitive, behavioral, or functional abnormalities, and/or susceptibility to subsequent addiction. However, there is compelling circumstantial evidence, based on the principles of teratology and fetal malprogramming, suggesting that pregnant women should refrain from smoking marijuana. The usage of marijuana during pregnancy perturbs the fetal endogenous cannabinoid signaling system (ECSS), which is present and active from the early embryonic stage, modulating neurodevelopment and continuing this role into adulthood. The ECSS is present in virtually every brain structure and organ system, and there is also evidence that this system is important in the regulation of cardiovascular processes. Endocannabinoids (eCBs) undergird a broad spectrum of processes, including the early stages of fetal neurodevelopment and uterine implantation. Delta-9-tetrahydrocannabinol (THC), the psychoactive chemical in cannabis, enters maternal circulation, and readily crosses the placental membrane. THC binds to CB receptors of the fetal ECSS, altering neurodevelopment and possibly rewiring ECSS circuitry. In this review, we discuss the Double-Hit Hypothesis as it relates to PCE. We contend that PCE, similar to a neurodevelopmental teratogen, delivers the first hit to the ECSS, which is compromised in such a way that a second hit (i.e., postnatal stressors) will precipitate the emergence of a specific phenotype. In summary, we conclude that perturbations of the intrauterine milieu via the introduction of exogenous CBs alter the fetal ECSS, predisposing the offspring to abnormalities in cognition and altered emotionality. Based on recent experimental evidence that we will review here, we argue that young women who become pregnant should immediately take a "pregnant pause" from using marijuana.
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Contents

1. Introduction . . . 6
1.1. Significance of PCE research models . . . 6
1.2. Prenatal cannabis exposure and the endogenous cannabinoid signaling system. . . . 7
1.3. Teratology, "double hit hypothesis", and the concept of fetal malprogramming . . . 7
1.4. Epigenetic inheritance and increased susceptibility to neuropsychiatric disorders. . . . 9
1.5. PCE and cognitive impairment, emotional dysregulation, and enhanced sensitivity to drugs of abuse or schizophrenia in offspring . . . 10
1.6. Effect of ECSS on the cardiovascular system and PCE-related cardiovascular disease. . . . 10
1.7. Concluding remarks . . . 11
Transparency document . . . 12
Acknowledgements . . . 12
References . . . 12

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1. Introduction

Marijuana refers to the desiccated leaves, flowers, stems, and seeds from the hemp plant, *Cannabis sativa*, which contains 85 plant cannabinoids (CBs) or phytocannabinoids, and 525 natural compounds belonging to several classes of chemicals (Gaoni and Mechoulam, 1964; Ahmed et al., 2008a, 2008b; Elsohly and Slade, 2005; Radwan et al., 2009; Radwan et al., 2008a, 2008b). For >4000 years, preparations of cannabis, containing the main psychotomimetic component, D9-tetrahydrocannabinol (THC), have been used for utilitarian (hemp cord), recreational, and medicinal purposes, and in religious rites (Gaoni and Mechoulam, 1964; Breivogel et al., 1998). In the U.S., marijuana is the most pervasively used illicit substance among individuals who are 12 years of age or older. Data from the U.S. National Survey on Drug Use and Health (NSDUH) shows that in individuals who are 18 to 25 years of age, 52% have never used marijuana, 32% have used marijuana within the past year, and 19% have used in the past month (Hedden et al., 2015). According to an annual survey of middle and high school students, the number of young people who believe marijuana use is hazardous to their health is dwindling (Johnston et al., 2015); which may, in part, be due to the ever-growing number of states that have legalized marijuana for medical and/or adult recreational use (State Medical Marijuana Laws, 2016). Currently, 23 states, Washington DC, and Guam have comprehensive public medical marijuana/cannabis programs, and 15 additional states have approved low THC/high cannabidiol (a non-psychoactive CB) products for medical use in limited situations (State Medical Marijuana Laws, 2016). Not surprisingly, increasing rates of marijuana use have been documented in states where medical marijuana has been legalized. Marijuana usage peaks in the early twenties coinciding with the average age of first birth, which is approximately twenty-three years of age (Martinez et al., 2012). In 2015, >1 in 10 pregnant women reported using marijuana within the past year, and most of these women were daily users, met abuse and/or dependence criteria, and were polysubstance users (Ko et al., 2015). In the U.S., marijuana is notorious for being the most extensively used illicit drug during pregnancy (McCabe and Arndt, 2012; Ebrahim and Gfroerer, 2003; Campolongo et al., 2011) with approximately 5% of pregnant women reporting marijuana use in the past month (Anon, 2014; Calvigioni et al., 2014). In addition, data over the last 50 years show a steady increase in the potency of marijuana (Calvigioni et al., 2014; Mehmedic et al., 2010). This may mean exposure to higher THC levels with an increased risk of adverse effects, which may also explain the recent rise in marijuana-related emergency room visits (State Medical Marijuana Laws, 2016).The U.S. appears to be in the midst of the perfect storm of marijuana use during pregnancy: the recent increase in marijuana use in women of reproductive age (Hedden et al., 2015; Anon, 2014), the increase in marijuana potency (Calvigioni et al., 2014; Mehmedic et al., 2010), and the legalization of medical and/or adult recreational marijuana use in several states. These factors could further elevate marijuana use during pregnancy, which could pose a global clinical, public health, and workforce concern. Therefore, it is prudent to investigate the frequency and characteristics of marijuana use during pregnancy and in women of childbearing age (Ko et al., 2015). Reports in the literature are conflicting as to whether or not PCE, in humans, induces fetal developmental abnormalities (Huizink, 2014; Jutras-Aswad et al., 2009; Fried, 2002; Morris et al., 2011). There are links reported between PCE and undesirable birth outcomes, including decreased birth weight, preterm labor, abnormal size to gestational age ratios, increased admission to NICUs (Hayatbakhsh et al., 2012), and increased incidence of stillbirths (Varner et al., 2014). Other investigators have found no link between undesirable birth outcomes and PCE (Huizink, 2014; Fergusson et al., 2002; Mark et al., 2016; van Gelder et al., 2010; Bada et al., 2005). One challenge faced by researchers studying PCE is that marijuana usage does not always result in an *overt* neurodevelopmental phenotype (as seen in Fetal Alcohol Spectrum Disorder), but may result in *covert* functional abnormalities that may become overt when other factors/stressors are present. This phenomenon forms the basis of the "Double Hit Hypothesis", which has been used to describe the effects of neurodevelopmental teratogens (Calvigioni et al., 2014; Maccarrone et al., 2014). We contend that PCE, similar to other neurodevelopmental teratogens, delivers a "first hit" to the endogenous cannabinoid signaling system (ECSS), which is compromised in such a way that a "second hit" (i.e., postnatal environmental stressors) may precipitate the emergence of a specific phenotype. This model may also be used to explain the results observed in clinical studies of fetal outcomes in the offspring of marijuana users.

In this review, we will discuss the primary human studies of PCE and offspring outcomes, the function of the ECSS and its importance during fetal neurodevelopment, and the Double-Hit Hypothesis as it relates to teratology principles, epigenetic inheritance, and increased susceptibility to neuropsychiatric disorders. We will also discuss the relationship between the ECSS and the developing cardiovascular system and subsequent cardiovascular diseases in offspring exposed to cannabis.

1.1. Significance of PCE research models

There are three prospective longitudinal human cohort studies that follow and assess offspring exposed to cannabis from the fetal period through young adulthood: the Ottawa Prenatal Prospective Study (OPPS) (Fried, 1980), the Maternal Health Practices and Child Development Study (MHPCD) (Day and Richardson, 1991), and the Generation R Study (Calvigioni et al., 2014; Huizink, 2014; Hofman et al., 2004; Diaz-Alonso et al., 2012). These three studies have provided invaluable information regarding PCE and its effects on development. We have previously summarized the major findings of these studies (McLemore and Richardson, submitted for publication).

The Ottawa Prenatal Prospective Study (OPPS) started by Fried and colleagues in 1978 (Fried, 1980) was a prospective study of approximately 300 white, middle-class, low-risk women who self-reported using at least six marijuana joints/week (i.e., heavy users) before and/or during pregnancy. A strength of the OPPS study is that it followed a relatively low-risk population who self-reported as heavy users, allowing for focus on the maternal effects of marijuana smoking in relative isolation. In neonates born to marijuana users, the researchers reported a decrease in fetal gestational age, and neonates presented with neuromuscular tremors, and abnormal startle and visual responses. Infants with PCE were followed throughout childhood, and presented with a range of developmental issues at various ages (summarized in (McLemore and Richardson, submitted for publication)). Young adults (18–22 year olds) presented with alterations in response inhibition as demonstrated by increased activity in the prefrontal cortex (PFC) and motor cortex, and reduced activity in the cerebellum as measured via functional magnetic resonance imaging (fMRI). Moreover, fMRI scans revealed changes in working memory as demonstrated by decreased activity in the medial, dorsolateral, and ventrolateral PFC, and increased activity in the left medial PFC, frontal gyrus, and cerebellum (Calvigioni et al., 2014; Huizink, 2014; Fried, 1980; Diaz-Alonso et al., 2012; Fried et al., 1998).

One weakness of the OPPS stems from the use of a relatively low-risk pregnancy population, and thus does not take into account many of the related stressors that are often present in an environment that includes cannabis usage. In addition, factors such as maternal tobacco smoking and alcohol drinking were not controlled. These factors, especially the effects of second-hand smoke if continued postnatally, could cause significant skew of the results.

In contrast to the OPPS, the Maternal Health Practices and Child Development Study (MHPCD), started by Day and colleagues in 1982, did focus on a high-risk pregnancy population. The MHPCD was a longitudinal study of over 500 mixed-race, mostly single, low socioeconomic status, high-risk pregnant women, who used marijuana during pregnancy. The MHPCD used the following categories of marijuana use: heavy use (>1 joint/day), moderate use (3–7 joints/week), and light use

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(<3 joints/week). The infants were followed throughout childhood into early adulthood and presented with a range of developmental abnormalities as summarized in Table 1 (Calvigioni et al., 2014; Huizink, 2014; Day and Richardson, 1991; Diaz-Alonso et al., 2012). A strength of the MPHCD study is that some of the results provide evidence for PCE as a "first hit" in the "Double Hit Hypothesis". However, the multiple environmental stressors (including polysubstance abuse) that were present apart from marijuana usage can make controlling for confounding factors, such as ethnicity and socioeconomic status, very challenging. Another issue in analyzing results from both the OPPS and the MPHCD is that THC levels have significantly increased in the last 20 years; thus, effects of PCE can be presumed to have been less in these populations than in current users of marijuana.

The more recent Generation R Study was started by Hofman and colleagues in 2001 (Hofman et al., 2004). A strength of the Generation R study is that it is a large-scale, prospective longitudinal population-based cohort study, following nearly 10,000 multi-ethnic urban children from the fetal stage to young adulthood, whereas the OPPS and MPHCD followed approximately 300 and 500 participants, respectively. This study examines four areas: (1) growth and development; (2) cognitive and behavioral development; (3) childhood diseases; and (4) healthcare for pregnant women and children. The authors of the Generation R Study reported that the offspring of women who used marijuana from the second trimester through parturition had reduced birth weight and slower growth (Hofman et al., 2004). The researchers reported a decrease in head circumference of infants born to mothers that used marijuana during early pregnancy or regularly throughout pregnancy (Hofman et al., 2004).

The Generation R study also analyzed maternal determinants for marijuana smoking, which include childhood delinquency and trauma, paternal smoking, and being single; thus helping to control for marijuana usage in isolation from multi-drug use and other risk factors. In contrast to the earlier studies, the Generation R study also followed prenatal fetal growth via ultrasound. This study is currently in progress and has yet to follow offspring into early adulthood.

One difficulty when performing any large-scale study on the effects of behavior on health, particularly those that rely on self-reporting, is that genetic, environmental, and socioeconomic factors can be complex, and individual factors cannot easily be considered in isolation. These factors can influence the observed effects of PCE, increasing the challenge of interpreting experimental data and in establishing cause-and-effect relationships between PCE and neurodevelopmental abnormalities in adulthood (Calvigioni et al., 2014).

Thus, well-designed animal models can allow the investigation of hypothesized causal links by holding constant genetic and environmental factors to allow the systematic exploration of neurophysiological mechanisms that may undergird cognitive and behavioral abnormalities observed in humans (Jutras-Aswad et al., 2009; Navarro et al., 1995; Rice and Barone, 2000; Schneider, 2009; Ferraro et al., 2009; Dinieri and Hurd, 2012; Trezza et al., 2012; Silva et al., 2012). Animal studies (particularly in knock-out mice) have allowed elucidation of the role of specific components of the ECSS in neurodevelopment (Maccarrone et al., 2014; Zimmer et al., 1999; Berghuis et al., 2007; Leterrier et al., 2006; Mulder et al., 2008; Li et al., 2009). Manipulation of endocannabinoid (eCB) metabolism in mice has also been shown to alter cortical circuitry development, a result which has particular significance when interpreting results of longitudinal human studies in children with compulsive/depressive behaviors and memory deficits after PCE (Huizink and Mulder, 2006; Goldschmidt et al., 2004).

In summary, animal models can be used in concert with human longitudinal cohort studies to probe observed phenotypes in the offspring of cannabis users, and to test proposed hypotheses regarding PCE in humans. Animal studies, particularly those that disrupt the ECSS or introduce exogenous CBs, could be especially valuable in determining isolated factors when exploring the "Double Hit Hypothesis". For example, one study has shown that in rodent progeny, exposure in utero to low doses of cannabis-like drugs results in increased susceptibility to drugs of abuse as adults (Campolongo et al., 2011). Thus, a sub-threshold "first hit" of PCE can be exacerbated by subsequent postnatal "second hits", and the responses of offspring can be examined and correlated with known teratologic effects in humans.

1.2. Prenatal cannabis exposure and the endogenous cannabinoid signaling system

The ECSS is a multifaceted communication network that is present and functional in early pregnancy (Berghuis et al., 2007; Fernandez-Ruiz et al., 2000; Fride et al., 2009) and exists in virtually every brain structure from the earliest embryonic stage through the postnatal stage, playing an essential modulatory role in early embryonic and prenatal brain development (Fride et al., 2009).

This system is crucial to a broad spectrum of processes, including the early stages of fetal development and uterine implantation (Paria et al., 2001), neurodevelopment (Mulder et al., 2008), neural stem cell proliferation and differentiation (Galve-Roperh et al., 2013), creation of functional and efficacious synapses (Diaz-Alonso et al., 2012; Sonon et al., 2015; Gaffuri et al., 2012), orchestration of axonal migration and connectivity, synaptogenesis (Fride et al., 2009), and modulation of excitatory and inhibitory synaptic neurotransmission in the postnatal brain (Kano et al., 2009) and spinal cord (Mulder et al., 2008; Pernia-Andrade et al., 2009). In the adult, the ECSS modulates intercellular communication (Kano et al., 2009; Regehr et al., 2009) and neurogenesis (Goncalves et al., 2008; Jiang et al., 2005). The ECSS contains numerous endocannabinoids (eCBs), including arachidonoylethanolamine (anandamide; AEA), and 2-arachidonoylglycerol (2-AG) (Mechoulam et al., 1995; Sugiura et al., 1995; Tan et al., 2006; Hanus et al., 2014). The eCB transporters include intracellular membrane transport proteins that facilitate eCB uptake, and are purported to ferry eCBs from the plasma membrane to their catabolic enzymes (Zubrzycki et al., 2014; Fowler, 2012).

Two distinct members of the family of G protein-coupled receptors (GPCR), designated cannabinoid 1 receptor (CB1R) and cannabinoid 2 receptor (CB2R) have been identified and characterized (Maccarrone et al., 2014; Galve-Roperh et al., 2013; Pertwee et al., 2010; DiPatrizio and Piomelli, 2012; Howlett and Fleming, 1984; Mechoulam et al., 1988; Devane et al., 1988; Matsuda et al., 1990; Munro et al., 1993; Devane et al., 1992). CB1R is found in abundance in the CNS as well as in the peripheral tissues of the cardiovascular, respiratory, immune, reproductive, hepatic, gastrointestinal, muscular, skeletal, and integumentary systems (Matias and Di Marzo, 2007; Maccarrone et al., 2015). CB2Rs are found peripherally in the cells/organs of the immune system where it modulates pro- and anti-inflammatory cytokine activities, and are also present at low levels in certain areas of the brain (Pandey et al., 2009; Pacher and Mechoulam, 2011; Di Marzo, 2009; Gong et al., 2006).

In the mammalian ECSS, THC, which mimics the actions of eCBs, has been found to bind to CB1Rs and CB2Rs (Campolongo et al., 2011). Lipophilic THC enters the maternal bloodstream, readily crosses the placenta (Grotenhermen, 2003; Hurd et al., 2005; Sundram, 2006) and is associated with physiological effects, which include human fetal distress and growth retardation (Day and Richardson, 1991; Fried and O'Connell, 1987). It is likely that THC disrupts the modulation of the ECSS in early development (i.e., the "first hit" in the "Double Hit Hypothesis"), resulting in offspring that are more vulnerable to a "second hit" delivered postnatally, which could result in a distinct neurological phenotype, including emotional control issues, cognitive impairment, or depression (Jutras-Aswad et al., 2009).

1.3. Teratology, "double hit hypothesis", and the concept of fetal malprogramming

Increasingly, the scientific community recognizes the importance of the prenatal and early postnatal developmental periods in chronic and psychiatric disease (Gross and Hen, 2004; Lyon et al., 1989). Nutritional

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Table 1
Three human prospective longitudinal cohorts of prenatal marijuana exposed offspring from the fetal period through young adulthood.

Study	Fetus	Neonate	Infant - Child	Adolescence - Young Adult
Ottawa Prenatal Prospective Study (OPPS) (Fried PA, 1980; Fried PA, Watkinson B, 1988; Fried PA et al., 1998)	↓ Gestational age	↓ Response to light ↑ Startle response ↑ Tremors	**3 years:** ↑ Motor skills **4 years:** ↓ Memory ↓ Verbal scores **6 years:** ↓ Attention ↑ Impulsivity ↑ Hyperactivity	**9-12 years:** ↓ Visual perception ↑ Impulsivity **13-16 years:** ↓ Concentration ↓ Visual memory ↓ Verbal reasoning **18-22years:** ↓ Response inhibition **Response inhibition as measured via fMRI:** ↓ Cerebellum activity ↑ Bilateral PFC activity ↑ Premotor cortex activity **Working memory as measured via fMRI:** ↓ Medial PFC activity ↓ Dorsolateral PFC activity ↓ Ventrolateral PFC activity ↑ Left medial PFC activity ↑ Inferior frontal gyrus activity ↑ Left cerebellum activity
Maternal Health Practices and Child Development Study (MHPCD) (Day NL, Richardson GA, 1991; Day N et al., 1991)	↓ Birth length *(after 1st trimester exposure only)* ↑ Birth weight *(after 3rd trimester exposure)*	↓ Body length **Subpopulation:** *slight* changes in EEG traces	**9 months:** ↓ Mental development ↓ BSID scores **3 years:** ↓ Short-term memory ↓ Verbal reasoning *(African Americans only)* **3 years:** ↓ Sleep efficiency ↑ Nocturnal arousals ↑ Wake-time after sleep onset **6 years:** ↓ Concentration ↓ Overall IQ score ↓ Verbal reasoning ↓ Quantitative reasoning ↓ Short-term memory ↑ Impulsivity ↑ Hyperactivity ↑ Delinquency	**10 years:** ↓ Abstract reasoning ↓ Visual reasoning ↓ Concentration ↓ Internalization (Implosion) ↓Learning and memory ↓ IQ score ↑ Externalization (Expolsion) ↑ Depression ↑ Impulsivity ↑ Hyperactivity ↑ Delinquency **14 years:** ↑ Delinquency **16 years:** *slight* ↓ in fine motor coordination *slight* ↑ in visual-motor coordination
Subsample of the Generation R Study, the Generation R Focus Study (El Marroun H et al., 2009; El Marroun H et al., 2010; Hofman A et al., 2004; Jaddoe VW et al., 2010; Jaddoe VW et al., 2012)	↓ Birth weight ↑ Growth (from 2nd trimester to parturition)		**18 months:** ↓ Attention ↑ Aggression (for girls only)	

Adapted from ***Calvigioni D et al., 2015 [1], Huizink AC, 2014 [10], and Wu CS et al., 2011 [15]***
↑ - Increased; ↓ - Decreased; BSID - Bayley Scales of Infant Development; fMRI - Functional Magnetic Resonance Imaging; Response inhibition - an indicator of executive control, refers to one's ability to suppress inappropriate actions or impulses, which undergird goal-oriented and adaptable responses to dynamic surroundings ***(Verbruggen F and Logan GD, 2008) [14]***.

status, stress hormone levels, or exogenous drugs can adversely affect embryonic signaling systems (e.g., ECSS), resulting in long-lasting alterations in neurocircuitry, forcing the fetus to adapt to a hostile in utero milieu in anticipation of a challenging environment at birth (Calvigioni et al., 2014). These external stimuli-induced aberrant signaling events can lead to physiological complications and neuropsychiatric disorders (Jutras-Aswad et al., 2009; Driscoll et al., 1990; Thompson et al., 2009). Adverse in utero stimuli can result in overt structural or functional abnormalities visible at birth (e.g., microcephaly in fetal alcohol exposure) (Bell et al., 2010) or covert asymptomatic perturbations of fetal neurocircuitry (Calvigioni et al., 2014), in which a sub-threshold "second hit" to the already compromised brain circuitry (i.e.,

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asymptomatic developmental "first hit") may precipitate neurodevelopmental disease later in life (Calvigioni et al., 2014; Maccarrone et al., 2014). The "first hit" landed by PCE may cause only subtle *buckling* of the developing nervous system, perhaps due to perturbation of the ECSS. The "second hit" is the *power punch*, which cripples the developing nervous system, and is manifested as neurodevelopmental and behavioral abnormalities that may last into adulthood (see Fig. 1).

Teratogenesis is characterized by embryonic/fetal death, structural malformations, functional defects, or prenatal growth retardation in the developing embryo/fetus (Steg et al., 2012; Vorhees, 1989). Both clinical trials and basic research studies have shown that PCE can have teratogenic effects (Campolongo et al., 2011; Fried, 2002; Navarro et al., 1995; Schneider, 2009; Huizink and Mulder, 2006; Campolongo et al., 2009; Fried and Smith, 2001). The "fetal programming hypothesis" asserts that organ structures and functions will be programmed via prenatal exposure to external stimuli to the fetus, which will determine the set points of physiological and metabolic responses that will continue into adulthood (Calvigioni et al., 2014). Fetal malprogramming of the multifaceted ECSS may be initiated via maternal marijuana use during pregnancy, contributing to structural, functional, and behavioral abnormalities in the adult offspring (Barker, 1998; Alwasel et al., 2012). Animal and clinical studies demonstrate that cannabis-like drugs can precipitate long-lasting neurobehavioral abnormalities (e.g., cognitive impairments, hyperactivity, and altered emotionality) in exposed offspring (Campolongo et al., 2011; Goldschmidt et al., 2004; Gong et al., 2006; Fried and Smith, 2001; Fried et al., 1992; Goldschmidt et al., 2000; Smith et al., 2006). Moreover, in adult rodent progeny, in utero exposure to low doses of cannabis-like drugs results in cognitive deficits, altered emotionality, abnormal locomotor activity, and increased susceptibility to drugs of abuse (Campolongo et al., 2011). These studies bring to light the possibility that PCE can act as a "*second hit" trigger*, which will manifest as a psychiatric disorder in adulthood, as well as an asymptomatic "*first hit stressor"*, the damage of which will be manifested after subsequent "maternal distress" (Dipietro, 2012). In humans, genetic or epigenetic factors could conspire with PCE to influence neurobiological effects. Therefore, CB use during the critical stages of fetal brain development should be viewed as a potential neuroteratogen targeting the multidimensional ECSS (Yang et al., 2011).

1.4. Epigenetic inheritance and increased susceptibility to neuropsychiatric disorders

Epigenetic processes are understood to be essential links between the genetic code and environmental factors, in which the environment may permanently alter the genome (Bohacek and Mansuy, 2013; Bird, 2007). Thus, epigenetic information acquired during the lifetime of the parent can be inherited by subsequent generations (Hackett et al., 2013). DNA methylation in conjunction with histone posttranslational modifications (PTMs) can dynamically remodel chromatin structure, allowing regulatory proteins access to uncoiled DNA, thereby controlling the genes that will be expressed (Bohacek and Mansuy, 2013; Bird, 2007). Evidence for the role of epigenetic processes in susceptibility of offspring to psychiatric disease, obesity, alcoholism, and heart disease has been well documented (Morris et al., 2011; Bird, 2007). The

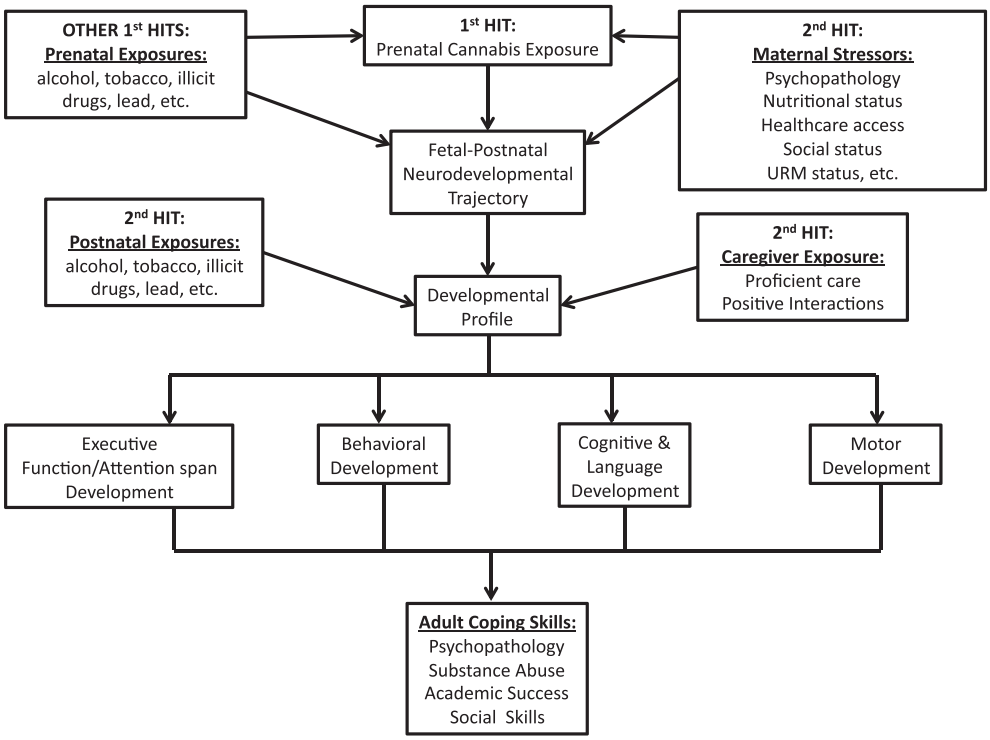

Fig. 1. Double hit hypothesis of prenatal cannabis exposure (PCE). The first hit is landed by prenatal cannabis exposure (PCE), which leads to asymptomatic changes in the trajectory of fetal-postnatal neurodevelopment (e.g., Endocannabinoid signaling system, ECSS, alterations). Other first hits could come in the form of prenatal exposure to alcohol, other illicit drugs, lead, etc. The second hit is landed in the form of maternal stressors (e.g., maternal psychopathology, poor nutritional/healthcare access, low social/minority status, etc.), which also will impact the trajectory of fetal-postnatal neurodevelopment. Postnatal exposure to alcohol, other illicit drugs, lead, etc. or exposure to an incompetent or abusive caregiver will land the second hit, which will impact the developmental profile. The second hit is the *power punch* that cripples the developing nervous system, and is manifested as deficits in executive function/attention, and behavioral, cognitive, language, and motor development. In adulthood, these neurodevelopmental deficits may manifest as psychopathology, substance abuse, and/or poor academic or social skills. This latter maternal programming outcome forms the basis of the double hit hypothesis in which a sub-threshold first hit to the brain circuitry (i.e., asymptomatic developmental first hit) precipitates neurodevelopmental disorders by an otherwise sub-threshold stimuli (second hit) later in life (Calvigioni et al., 2014; Maccarrone et al., 2014). Adapted from Morris et al., 2011. URM – underrepresented minority.

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relationship between PCE and susceptibility to psychiatric disorders (Calvigioni et al., 2014), and the role of the ECSS in gamete function (Maccarrone et al., 2015), raises the possibility that transmission of cannabis-induced epigenetic changes are contributory factors in the development of neuropsychiatric disorders and/or increased vulnerability to drug abuse in subsequent generations (Morris et al., 2011; Szutorisz et al., 2014). Human fetuses that have been exposed to marijuana have lower mRNA levels of the dopamine receptor D2 (DRD2) in the brain ventral striatum, a key reward region (Dinieri et al., 2011). Down-regulation of DRD2 expression continues into adulthood in rodent progeny prenatally exposed to THC, accompanied by increased drug-seeking behavior, indicating that epigenetic processes are likely a factor (Dinieri and Hurd, 2012; Dinieri et al., 2011). Histochemistry using brain tissue from rats that had been exposed to THC prenatally demonstrated that down-regulation of the DRD2 gene was due to histone PTMs (Dinieri et al., 2011). Thus, it is likely that disruption of DRD2 expression, due to epigenetic factors, plays a key role in the effects of PCE, which may continue into adulthood.

Transgenerational epigenetic effects occur when an internal (e.g., mental health disease) or external (e.g., drug abuse, low socioeconomic status, underrepresented minority status, and/or being a descendant of slavery) environmental stressor, or "maternal distress" (Dipietro, 2012), triggers epigenetic alterations that are transmitted to the subsequent generation. Three different routes of transgenerational epigenetic information have been described, including fetal programming, behavioral/social transfer, and germline transmission (Jirtle and Skinner, 2007; Youngson and Whitelaw, 2008). Briefly, fetal programming (which could constitute the "first hit" in the Double-Hit Hypothesis) occurs when internal or external maternal stressors experienced during pregnancy induce epigenetic changes in fetal somatic cells, which may lead to a specific postnatal, adolescent, and/or adult phenotype. Behavioral/social transfer occurs when epigenetic alterations are transmitted from generation to generation via social interaction between parents and offspring. In the context of PCE, behavioral/social transfer may constitute a "second hit" if offspring have previously received a "first hit" from PCE in utero. Since behavioral/social epigenetic transmission does not involve Mendelian inheritance, the environmentally-induced epigenetic triggers must be present in each successive generation in order for transmission to occur. The third route of epigenetic information transmission involves germline epigenetic (Mendelian) inheritance; thus, the environmental triggers do not need to be repeated for epigenetic effects on the offspring to be observed (Bohacek and Mansuy, 2013). As an example of germline transmission, Szutorisz and colleagues demonstrated that not only does marijuana exposure during adolescence in rats have an effect on somatic cells (Szutorisz et al., 2014), but that these effects are inherited by marijuana-naïve offspring, as manifested by distinct behavioral characteristics and aberrant molecular and neurophysiological processes in neuronal systems associated with purposeful and habit-forming behaviors (Szutorisz et al., 2014). These researchers demonstrated that parental germline exposure to THC alters the striatal molecular regulatory machinery, thereby increasing the susceptibility of THC-naive offspring to compulsive drug use (Szutorisz et al., 2014).

Other studies have demonstrated decreased immune function in offspring with PCE, an effect that continues into adulthood, operating via epigenetic mechanisms, including microRNA regulation of T cell proliferation (Zumbrun et al., 2015). Thus, while the use of marijuana as an immunosuppressant drug is well-established as therapeutic in treating cancer-related inflammatory responses, it could also cause profound and long-lasting epigenetic insult to the developing immune system of a fetus (Zumbrun et al., 2015).

In summary, epigenetic factors as a result of PCE in utero could act as a "first hit" in the Double Hit scenario, and may potentially operate transgenerationally (Zumbrun et al., 2015), so that descendants (even those without PCE) are more susceptible to a "second hit", which may result in an associated phenotypic syndrome.

1.5. PCE and cognitive impairment, emotional dysregulation, and enhanced sensitivity to drugs of abuse or schizophrenia in offspring

Despite recognition of the impact of perinatal development on subsequent psychiatric disease and the increasing acceptance of the "Double Hit Hypothesis", the specific mechanisms leading to neuronal impairment as a result of PCE have not been entirely elucidated. Recently, it has been demonstrated that repeated THC exposure disrupts CB1R signaling; thereby rewiring fetal cortical circuitry (Tortoriello et al., 2014). Using a THC-sensitive neuronal proteome, in mice, investigators have identified a microtubule-binding protein in axons, superior cervical ganglion 10 (SCG10)/stathmin-2, as a substrate of altered neuronal connectivity (Tortoriello et al., 2014). Moreover, SCG10 mRNA and protein were reduced in the hippocampus of mid-gestational human cannabis-exposed fetuses; thereby defining SCG10 as the first cannabis-mediated molecular effector in the developing brain (Tortoriello et al., 2014).

Both clinical and animal studies have demonstrated that prenatal and postnatal (i.e., during lactation) cannabis exposure can induce neurobehavioral teratogenic effects (Fried, 2002; Navarro et al., 1995; Schneider, 2009; Huizink and Mulder, 2006; Vorhees, 1989; Campolongo et al., 2009; Fried and Smith, 2001; Coyle et al., 1976; Trezza et al., 2008). THC readily crosses the placenta during gestation (Grotenhermen, 2003; Hurd et al., 2005; Sundram, 2006), and is secreted in maternal milk during lactation (Hutchings et al., 1989; Jakubovic and McGeer, 1977).

Low doses of PCE have been shown to cause cognitive dysfunction, locomotor activity abnormalities, emotional dysregulation, and increased vulnerability to drugs of abuse in adult rodent offspring (Campolongo et al., 2011). Chronic cannabis use before the age of 17 is associated with deficits in working memory, attention span, decision-making, visual search, overall/verbal IQ, executive functioning, visual-spatial memory, cognitive inhibition, and impulsivity; and the magnitude of these deficits is proportional to the age at onset of use, frequency, and dosage (Renard et al., 2014). Moreover, studies support the contention that PCE is a contributory factor for increased vulnerability to neuropsychiatric disorders (e.g., schizophrenia) (Jutras-Aswad et al., 2009; Burns, 2013; Weiser and Noy, 2005), which reinforces the hypothesis that cannabis can act as an indirect "second hit" stressor to trigger otherwise unrevealed diseases in adulthood (Calvigioni et al., 2014).

1.6. Effect of ECSS on the cardiovascular system and PCE-related cardiovascular disease

Cardiovascular disease is the primary cause for deaths worldwide (Steg et al., 2012), and CBs have been associated with pathologic cardiovascular conditions (Batkai and Pacher, 2009; Jouanjus et al., 2014). Therefore, understanding the mechanisms that underlie CB-induced cardiovascular complications and the impact that these diseases have on public health is essential. Cannabis use can affect heart rate, cardiac contractility, blood volume, and blood pressure regulation; all of which culminate in decreased myocardial function (Gorelick et al., 2006; Mittleman et al., 2001; Jouanjus et al., 2011). Young cannabis users have reported being hospitalized for serious heart complications such as cerebral strokes, myocardial infarctions, and thromboses (Jouanjus et al., 2011).

While adolescent and adult exposure has been the focus of many studies, there have been limited studies on PCE and its impact on heart defects. Since congenital heart defects are the most prevalent birth defect that contributes to infant mortality, it is essential to understand the risk factors for heart defects (Centers for Disease, C. and Prevention, 1998; Watkins and Botto, 2001; Hoffman and Kaplan, 2002). Investigation of the effects of illicit drug use on the risk of ventricular septal defects (VSD) revealed that increased maternal and paternal marijuana use correlated positively with a greater risk of VSD (Williams

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et al., 2004; Ewing et al., 1997), the most common type of congenital cardiac anomaly (Tikkanen and Heinonen, 1991). Maternal marijuana use was also found to increase the risk of Ebstein's anomaly, a congenital heart defect in which the tricuspid valve leaflet is displaced, affecting cardiac blood flow, which can lead to an increased incidence of heart failure or enlargement of the heart (Lurie and Ferencz, 1997).

Research studies (in vivo and in vitro) have been used to determine effects of the ECSS on cardiovascular function (Montecucco et al., 2012; Montecucco and Di Marzo, 2012). Both CB1Rs and CB2Rs are located on the myocardium (Zubrzycki et al., 2014; Weis et al., 2010), coronary and cerebral vasculature (Wagner et al., 2001; Mukhopadhyay et al., 2008; Randall et al., 2004; Pacher et al., 2005), and on cardiomyocytes (Mukhopadhyay et al., 2010). The onset and development of cardiac arrhythmias are influenced by the ECSS (Zubrzycki et al., 2014). Ischemia-related arrhythmias (Walsh et al., 2010) and atrial fibrillation in young healthy individuals (Korantzopoulos et al., 2008) have been linked to CBs either from marijuana smoking or pharmacological manipulation of the ECSS. Cardiomyopathies are regulated by the ECSS as evidenced by CB1R mediating cardiomyocyte damage and CB2R preventing that damage (Di Marzo and De Petrocellis, 2012). Several studies have shown that activation of CB1Rs can exacerbate cardiovascular complications (Batkai and Pacher, 2009; Jouanjus et al., 2014). Activation of CB1Rs in an in vitro model using human primary cardiomyocytes treated with doxorubicin resulted in oxidative stress and increased cell death (Mukhopadhyay et al., 2010). Individuals with unstable angina, cardiovascular plaques, with high lipid and macrophage content, have high CB1R expression (Sugamura et al., 2009). The beneficial role of the CB2R in protection of the heart by decreasing atherosclerosis and inflammation has been confirmed in several studies (Joyeux et al., 2002; Montecucco et al., 2009; Defer et al., 2009; Steffens and Pacher, 2012). There are very few studies to indicate any deleterious effects of CB2R activation; however, one study indicates possible tissue damage after CB2R activation (Gaffuri et al., 2012). One study of an ex vivo preparation demonstrated that the extent of a myocardial infarction can be influenced by antagonism of the CB2R (Joyeux et al., 2002). This data revealed that CB2Rs are cardioprotective during ischemia-reperfusion injury (Joyeux et al., 2002). In particular, both chemokine receptor expression and tumor necrosis factor (TNF)a-mediated endothelial cell activation are reduced with CB2R stimulation (Montecucco et al., 2012; Montecucco et al., 2008; Rajesh et al., 2007). In vivo studies have confirmed the protective role of CB2R activation on myocardial infarction using a mouse model (Montecucco et al., 2009; Defer et al., 2009). Although adult animals were used in these studies, these findings indicate potential pre- and postnatal cardiac-associated complications due to consistent maternal marijuana use during pregnancy. Our studies have established the localization of CB1Rs in peripheral arterial chemoreceptors (PAC) during postnatal development (McLemore et al., 2004). Exposure to marijuana during early development could potentially modify cardiorespiratory responses via PAC, and abnormalities in PAC responses during sleep may be a factor in infants at risk for SIDS (McLemore et al., 2004).

In addition to cardiovascular effects, PCE use has been associated with a decrease in fetal oxygenated hemoglobin (McTiernan et al., 1988). In an animal model of PCE, a pregnant ewe and her fetus showed a lower level of oxygenated hemoglobin after marijuana inhalation versus tobacco-exposed or placebo groups (McTiernan et al., 1988). This decreased level of oxygenated hemoglobin persisted for six hours after marijuana exposure (McTiernan et al., 1988). The prolonged depression of fetal oxygenated hemoglobin after marijuana inhalation may have been due to impaired placental blood flow after drug exposure. Due to the increased inhalation associated with smoking marijuana, there is greater exposure per breath to tar and other pollutants (Wu et al., 1988).

In light of the importance of the ECSS in early cardiovascular development as well as the central regulatory role of CB1Rs, CB2Rs, and PAC in these processes, it is likely that PCE serves as a “first hit” to compromise the modulation of these receptors early in gestation. These receptors could be compromised in such a way that a “second hit” (possibly in utero) would precipitate the emergence of a specific phenotype, such as VCD or Ebstein's anomaly, as well as increased risk for SIDS.

1.7. Concluding remarks

The medicinal properties of marijuana have long been recognized (Lamarine, 2012), and marijuana usage has significantly improved the quality of life for numerous individuals. Today, medicinal marijuana is used in the management of seizures, glaucoma, multiple sclerosis, HIV wasting, and other conditions associated with chronic pain (Leung, 2011). The general population increasingly views marijuana as safe (Johnston et al., 2015), and there is mounting political pressure to reclassify it in the Diagnostic and Statistical Manual of Mental Disorders.

While positive aspects of marijuana are clear, concerns about its widespread acceptance and use cannot be ignored, and the harmful effects associated with marijuana usage must also be considered in the debate concerning its legalization.

The short-term effects of marijuana use include altered senses and mood, impaired body movement, and deficits in cognition, memory, and executive thinking. Meier and colleagues investigated the effects of PCE and marijuana use during adolescence, contending that young people who use marijuana heavily during adolescence lose a significant number of IQ points, which may not be recovered after cessation of drug use in adulthood (Meier et al., 2012). The mental effects that develop after long-term marijuana use include paranoia, hallucinations, and exacerbation of other mental health issues like depression and schizophrenia (Marijuana, 2015). The cardiovascular risks and respiratory effects can potentially exacerbate existing medical conditions.

In this review, we have made a case that the Double-Hit Hypothesis may provide an effective model with which to view the results of PCE clinical studies, particularly when considering effects on the ECSS, the development of the neuronal circuitry undergirding cognition, and vulnerability to drugs of abuse and neuropsychiatric disorders. Marijuana use during pregnancy may constitute a “first hit”, which may cause buckling of the developing nervous system, possibly due to perturbation of the ECSS. Postnatal distress or other environmental triggers, which may be related to a lifestyle in which marijuana use is prevalent, are the fetal nervous system cripplers, which manifest as cognitive and behavioral abnormalities that may continue into adulthood.

Until there is more evidence that unequivocally demonstrates that PCE poses a significant threat to the unborn fetus, marijuana will continue to be perceived as harmless by the general population. Thus, further studies on PCE are of significant interest. Animal models using prenatal exposure to low or moderate doses of CB compounds have demonstrated abnormal locomotor activity, cognitive impairments, altered emotional behavior, and an enhanced sensitivity to drugs of abuse in the progeny of adult rodents (Campolongo et al., 2011; Tortoriello et al., 2014). While studies investigating PCE and other CB exposures to the human fetus are limited, those studies which have directly examined the effects of marijuana in utero have demonstrated that repeated THC exposure disrupts CB1R signaling in the fetal brain, thus affecting development of cortical neurons, especially axonal elongation processes (Tortoriello et al., 2014). Furthermore, analysis of the hippocampus of mid-gestational cannabis-exposed human fetal brain tissue revealed that SCG10 mRNA and protein levels were reduced (Tortoriello et al., 2014). Since SCG10 is a protein that modulates cytoskeletal dynamics and microtubule disassembly (Manna et al., 2007), an imbalance of this protein due to cannabis use would certainly disrupt formation of neuronal circuitries in the fetal brain (Tortoriello et al., 2014).

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Further research is needed to gain insight into the following areas: (1) the impact of THC on placental integrity; (2) the effects of PCE on cardiovascular and respiratory systems with respect to increased vulnerability to cardiorespiratory diseases; (3) the long-term effects of PCE on organ system development; and (4) the role that epigenetic alterations (particularly germline effects) play in susceptibility to drugs of abuse and psychiatric disorders. These future studies will help to inform researchers, physicians, social workers, and educators as to how more adequately to care for future generations of marijuana smokers. Most importantly, these new and compelling studies will equip parents and healthcare workers with unequivocal evidence with which to encourage young women to take a pregnant pause from smoking marijuana during pregnancy.

Transparency document

The Transparency document associated with this article can be found, in online version.

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