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Molecular mechanisms of maternal cannabis and cigarette use on human neurodevelopment

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Abstract

Prenatal development is highly sensitive to maternal drug use due to the vulnerability for disruption of the fetal brain where the ongoing neurodevelopmental, resulting in lifelong consequences that can enhance risk for psychiatric disorders. Cannabis and cigarettes are the most commonly used illicit and licit substances, respectively, among pregnant women. While the behavioral consequences of prenatal cannabis and cigarette exposure have been well-documented in epidemiological and clinical studies, only recently have investigations into the molecular mechanisms associated with the developmental impact of early drug exposure been addressed. This article reviews the literature relevant to long-term gene expression disturbances in the human fetal brain in relation to maternal cannabis and cigarette use. To provide translational insights, we discuss animal models in which protracted molecular consequences of prenatal cannabis and cigarette exposure can be better explored and enable future evaluation of epigenetic pathways such as DNA methylation and histone modification that could potentially maintain abnormal gene regulation and related behavioral disturbances. Altogether, this information may help to address the current gaps of knowledge regarding the impact of early drug exposure that set in motion lifelong molecular disturbances that underlie vulnerability to psychiatric disorders.

Keywords

Prenatal; fetal; marijuana; nicotine; epigenetics

Introduction

The insidious intractable nature of drug abuse, which is characterized by the inability to stop drug use despite its negative consequences, has far-reaching implications for pregnant women. The prenatal period is particularly sensitive to the effects of drugs due to the dynamic neurobiological events that occur during gestation to ensure proper patterning of the nervous sy stem. These processes can be disrupted by maternal drug use and can have lifelong consequences. In North America, marijuana (*Cannabis sativa*) is the most commonly abused illicit drug among pregnant women (~4%), while cigarettes are the most frequently used licit substance (~16%) (SAMHSA, 2006). Similar prevalence rates are noted in European countries for prenatal cannabis exposure (~2–5%) (Fergusson *et al.*, 2002; Lozano *et al.*, 2007; El Marroun *et al.*, 2008), reaching even up to 13% in high-risk populations (Williamson *et al.*, 2006), but there is greater cigarette smoking (~30%) by pregnant women (Troe *et al.*, 2008). While significant epidemiological studies have established that these substances can be harmful to the developing fetus (Richardson *et al.*,

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1995; Fried & Smith, 2001; Fried & Watkinson, 2001; Porath & Fried, 2005; Huizink & Mulder, 2006b), there is still relatively little information as to what extent such early developmental drug exposure can have long-lasting effects on the adult brain. Such information is critical given that it is now acknowledged that most mental disorders are developmental in nature, thus insults such as drug exposure during early development could alter neurobiological processes, inducing molecular disturbances that enhance neuropsychiatric susceptibility later in life.

In this review, we outline behavioral and molecular brain alterations documented in the offspring of women with cannabis and cigarette use during pregnancy. Furthermore, we explore results obtained from animal models using the main psychoactive component of cannabis and cigarette smoke, Δ^9-tetrahydrocannabidiol (THC) and nicotine, respectively, to provide greater neurobiological insights into the long-lasting effects of early developmental drug insult.

Behavioral consequences of maternal cannabis and cigarette use on human offspring

Various studies have evaluated the behavioral effects of prenatal exposure to drugs in offspring of women who smoked cannabis and/or cigarettes when pregnant. Since multiple review articles have already addressed phenotypic effects (Fried & Smith, 2001; Langley *et al.*, 2005; Huizink & Mulder, 2006a; Pauly & Slotkin, 2008; Shea & Steiner, 2008; Jutras-Aswad *et al.*, 2009), we provide only a general overview of key findings. The long-term consequences of prenatal cannabis and cigarette exposure on neurodevelopmental outcome have been primarily assessed by two ongoing longitudinal investigations (Figure 1). The Ottawa Prenatal Prospective Study, which was initiated in 1978, collects neurobehavioral data from offspring of a low-risk, middle-class population of women who smoked cigarettes and marijuana during pregnancy (Fried *et al.*, 2003). The Maternal Health Practices and Child Development Project, which began in 1982, focuses on the long-term behavioral consequences of prenatal marijuana and alcohol exposure in a low-income African-American population in Pittsburgh, Pennsylvania (Goldschmidt *et al.*, 2008).

Data from these two cohorts suggest that cannabis-induced neurobehavioral alterations can be detected in newborns (<1 week old) as well as during later stages of child development. Offspring exposed to cannabis *in utero* show impairments in specific functional domains including cognitive deficits, impairments in inhibitory control, as well as increased sensitivity to drugs of abuse later in life as detailed in Figure 1 (Day *et al.*, 1994; Fried, 1996; Fried *et al.*, 2002; Day *et al.*, 2006; Willford *et al.*, 2010; Day *et al.*, 2011). It has been well documented that prenatal cigarette exposure also leads to developmental impairments from infancy through adolescence. Offspring exposed to tobacco *in utero* score lower on intelligence tests, have impaired cognitive functioning, and reduced auditory processing. Moreover, multiple studies have shown that prenatal cigarette exposure leads to increased impulsivity, increased incidence of conduct disorders, and serves as a risk factor for developing multiple neuropsychiatric diseases including anxiety, depression, attention deficit disorder, and addiction (Fried *et al.*, 1983; Fried *et al.*, 1992; Fried, 1993; 1996; Fried *et al.*, 1997; Fried, 1998; Cornelius *et al.*, 2000; Ernst *et al.*, 2001; Fried, 2002; Langley *et al.*, 2005; Cornelius *et al.*, 2011a; Cornelius *et al.*, 2011b).

Overall, these epidemiological and clinical studies clearly emphasize that prenatal cannabis and cigarette exposure leads to long-term behavioral disturbances associated with increased risk of neuropsychiatric disorders in the offspring. Such findings naturally raise the question as to what are the molecular pathophysiological events that underlie the disturbances.

Molecular impairments due to maternal cannabis and cigarette use in the human fetal brain

Unfortunately few investigations have directly examined the human fetal brain given the obvious challenges of conducting such studies. In an attempt to fill this significant gap of knowledge, we developed a post-mortem human fetal brain collection of midgestational subjects (17–22 weeks) with maternal cannabis and cigarette exposure (Hurd *et al.*, 2005). Using this resource, insights into the molecular and biochemical alterations associated with *in utero* drug exposure on human neurodevelopment could be explored. While our studies have primarily focused on cannabis exposure in this cohort of subjects, some women in the cannabis and control groups also smoked cigarettes, making it possible to begin to evaluate neurobiological patterns potentially related to cigarettes as compared to cannabis. Neurobiological systems related to the above behavioral phenotypes are components of the dopamine, opioid neuropeptide and cannabinoid systems within the striatal and amygdala brain regions. Development of these circuits plays an important role in regulatory processes relevant to the behavioral consequences of prenatal cannabis and cigarette exposure.

Endocannabinoid System

The cannabinoid receptor (CB_1R) is the primary molecular target of THC, the psychoactive component of cannabis (Pertwee, 2008). Signaling within the endocannabinoid system dynamically controls neuronal hardwiring during prenatal ontogeny relevant to the development of neural pathways such as the corticostriatothalamic circuit and numerous cortical regions (Berghuis *et al.*, 2007; Harkany *et al.*, 2008; Mulder *et al.*, 2008), which are implicated in addiction (Schmidt *et al.*, 2005; Centonze *et al.*, 2006; Kalivas *et al.*, 2006) and psychiatric disorders (Schwartz *et al.*, 1996; Foerde *et al.*, 2008; Killgore *et al.*, 2008; Harrison *et al.*, 2009). The CB_1R mRNA is detected early in gestation and in contrast to the adult brain in which the CB_1R is widely distributed, CB_1R expression has a heterogenous distribution during early development with expression evident in mesocorticolimbic structures such as the amygdaloid complex, hippocampus, and ventral striatum (Wang *et al.*, 2003). Moreover, the CB_1R sites expressed in the human fetal brain are also functional during prenatal development (Mato *et al.*, 2003; Wang *et al.*, 2003; Dow-Edwards *et al.*, 2006). Despite significant disturbances noted in other neuronal systems described below, no alteration was detected on CB_1R mRNA expression in relation to prenatal cannabis exposure in multiple brain areas (Wang *et al.*, 2004). However, potential disturbance of receptor function or in other components of the endocannabinoid signaling cannot be excluded.

Dopamine

Dopaminergic genes are of particular interest in relation to prenatal cannabis and cigarette exposure since dopamine impairment has been highly implicated in the neurobiology of addiction disorders (Volkow *et al.*, 2004; Pierce & Kumaresan, 2006; Le Foll *et al.*, 2009). Moreover, dopaminergic neurons are expressed early in life. Dopaminergic cell bodies in the ventral tegmental area and substantia nigra pars compacta have been detected in the human fetal brain as early as the 5^{th} embryonic week (Verney *et al.*, 1991). In the human fetus, dopamine receptor 1 (DRD1) and dopamine receptor 2 (DRD2) mRNA, as well as dopamine 1 (D_1R) and dopamine 2 (D_2R) protein and binding sites have been documented by week 12 in the striatum (Kumar & Sastry, 1992). In addition, components of the dopamine system are anatomically linked to the CB_1R. For example, CB_1Rs are co-expressed with dopaminergic $D_1R$and D_2R on medium spiny neurons in the dorsal striatum, known to play a role in motor function and habit formation, and the ventral striatum (nucleus accumbens; NAc), which regulates reward-related behaviors (Haber, 2003). Dopaminergic receptors, particularly the D_2R subtype, have been linked with addiction risk in humans. For example, imaging studies of drug abusers consistently report reduced levels of striatal D_2Rs (Volkow et al., 2004).

Similarly, human genetic studies link variants of the D_2R gene to drug addiction phenotypes related to nicotine, alcohol, opiates and psychostimulant abuse (Le Foll *et al.*, 2009). Interestingly, studies of our human fetal specimens with maternal cannabis exposure also revealed a decrease of DRD2 mRNA expression (Dinieri *et al.*, 2011) (Figure 2). This DRD2 impairment was specific to the mesolimbic NAc since no alterations were evident in the dorsal striatum. The DRD2 mRNA levels in the NAc negatively correlated with maternal report of cannabis use. These effects were selective since mRNA levels of the dopamine DRD1 receptor subtype was not altered in the striatum of subjects exposed to cannabis *in utero*.

Intriguingly, abnormal DRD2 mRNA expression was also observed in the amygdala of the human fetal subjects with *in utero* cannabis exposure (Wang *et al.*, 2004). Given that the amygdala and NAc are key mesolimbic structures important for emotional regulation, cannabis-induced disturbances in D2-related mesocorticolimbic neuronal populations that could be highly relevant to vulnerability to addiction and psychiatric disorders.

Opioid neuropeptides

There is significant evidence in support of a strong interaction between the cannabinoid and opioid systems, especially in relation to reward and addictive behaviors (Cossu *et al.*, 2001; Valverde *et al.*, 2001; Ghozland *et al.*, 2002). The endogenous opioid system consists of three opioid peptide precursor genes encoding enkephalins (preproENK, PENK), dynorphins (preproDYN, PDYN) and β-endorphin, as well as three receptor genes encoding mu-opiod receptor (μOR), delta-opioid receptor (∂OR), and kappa-opioid receptor (κOR) (Akil *et al.*, 1984). Dynorphin peptides are primary targets for κORs that are linked to dysphoria and negative mood states (Pfeiffer *et al.*, 1986; Bals-Kubik *et al.*, 1993), whereas enkephalins mainly target μOR and ∂OR that are associated with reward (Shippenberg *et al.*, 1987; Bals-Kubik *et al.*, 1993).

In the human brain, PDYN- and PENK-positive neurons are present in the striatum from at least 12 weeks of development (Brana *et al.*, 1995), and opioid receptors are apparent by at least midgestation (Magnan & Tiberi, 1989; Wang *et al.*, 2006; Tripathi *et al.*, 2008). Studies of human fetal subjects exposed to cannabis revealed that PENK-containing neurons appeared to be more sensitive to prenatal cannabis exposure than cells containing PDYN. PENK mRNA levels were decreased in the striatum in association with cannabis exposure and was directly correlated to the amount of maternal use, whereas PDYN levels were not significantly related to *in utero* cannabis exposure (Wang *et al.*, 2006). It is important to note that striatal PENK mRNA expression, particularly in the rostral region, was also associated with maternal alcohol use (Dinieri *et al.*, 2011) which is an important consideration when interpreting the alterations ascribed to cannabis-related disruption of PENK expression. However, in contrast to the specific disturbances noted for cannabis, alcohol exposure generally lead to more global changes on multiple genes. For example, prenatal alcohol exposure was also significantly associated with decreased expression of DRD1 and DRD2 mRNA levels in the dorsal striatum (Dinieri *et al.*, 2011). Maternal alcohol use also had a broader neurobiological impact particularly on the PDYN/kOR system (see(Wang *et al.*, 2006).

Interestingly, although PDYN gene expression in the human fetal striatum is not related to maternal cannabis use, its expression in the NAc appears to be associated with cigarette exposure (Dinieri *et al.*, 2011). There is a negative correlation observed with the amount of reported maternal cigarette smoking and NAc PDYN mRNA expression levels. PDYN striatal neurons are highly implicated in facilitating motivated behavior and in regulating mood (Akil *et al.*, 1984). Aside from the PDYN disturbances, maternal cigarette use was not associated with expression levels of the other opioid or dopamine markers examined, thus

far suggesting that the effect of maternal cannabis is highly significant and affects specific genes in our human fetal postmortem cohort.

Opioid receptors

Of the opioid receptor genes studied, κOR had the highest striatal levels in the midgestational human brain as assessed by *in situ* hybridization histochemistry. However, cannabis exposure was not associated with alterations of κOR mRNA expression in the striatum but its expression was most affected by maternal alcohol use (Wang *et al.*, 2006). Examination of opioid expression throughout other forebrain structures revealed increased μOR mRNA expression in the amygdala of cannabis-exposed fetuses as well as reduced κOR mRNA in the mediodorsal subdivision of the thalamus, the most limbic-related nucleus of that structure (Wang *et al.*, 2006). No other opioid receptor alterations were detected in relation to maternal cannabis intake and none showed significant relationship to cigarette exposure.

Other neural substrates

It is expected that cannabis and cigarettes could influence multiple neural systems, not only those examined above. In particular, the cholinergic system may be influenced by prenatal cigarette and cannabis exposure, which has known roles in differentiation, axonal guidance, synaptic formation, and autonomic control (Role & Berg, 1996; Kenny *et al.*, 2000). Nicotine exerts its pharmacological action via stimulation of nicotinic acetylcholinergic receptors (nAChRs) that are widely distributed in the brain. Abnormal levels of nicotinic receptors subunits have been detected in the cerebellum, medulla, and pons of cigarette-exposed first trimester human fetuses (Falk *et al.*, 2005). Dysfunction of these brain stem regions resulting in sudden infant death syndrome is strongly correlated with maternal cigarette use during pregnancy and altered gene expression of receptor subunits may be one of the contributing factor to brainstem failure in these infants (Matturri *et al.*, 2004; Huizink & Mulder, 2006a). Specifically, in the first trimester fetal brain, the level of nAChR subunit α4 is increased in the pons and, α4 and α7 receptors are reduced in the fetal cerebellum. Within the medulla, there was an age related increase of nicotinic α4 that was not found in cigarette-exposed fetal tissue. In addition to nAChRs, muscarinic receptor expression m_1, m_2, and m_3 were also altered in the pons and cerebellum, during early fetal development as well (Falk *et al.*, 2005).

To our knowledge, there is a gap in the scientific literature regarding the impact of prenatal cannabis exposure on the cholingeric system. There is though one report of a reduction of choline acetyltransferase, which catalyzes the synthesis of acetylcholine, as a consequence of THC exposure in an *in vitro* fetal rat telencephalon mixed primary cell culture model. However, much is still unknown about potential consequences of *in vivo* prenatal exposure.

Overall, the findings observed to date emphasize the discrete dysfunction of mesocorticolimbic (D_2R, μOR, and κOR) and striatal (D_2R and PENK) neuronal populations with prenatal cannabis exposure and mesolimbic opioid (PDYN) disturbance with cigarette exposure. Within the cholinergic system, tobacco exposure alters both nicotinic (α4 and α7) and muscarinic receptors (m_1, m_2, and m_3) within brainstem and cerebellum regions. Clearly, multiple neuronal systems need to be studied in more depth to better understand the molecular pathophysiology of maternal cannabis and cigarette use on the human fetal brain especially for neuronal systems related to neurocognitive aspects, impulsivity, and addiction vulnerability. Such studies would provide neurobiological insights underlying the behavioral disturbances observed in human offspring exposed to these drugs during prenatal development.

Molecular consequences of early developmental cannabis exposure - animal models

Despite the apparent associations of cannabis exposure to discrete neurobiological alterations in the human fetal brain, the specificity of such disturbances attributed to cannabis must be verified especially in light of potential polysubstance exposure in humans. Moreover, cannabis consists of many compounds including over 60 cannabinoids one of which is THC (Taura *et al.*, 2007). Additionally, cannabis preparations contain differing amounts of these various cannabinoids, confound the psychoactive effects of the drug (Van der Kooy *et al.*, 2008). Thus, interpretation of the human data is significantly enhanced by ascertaining the relevance of the drug changes specifically to THC, the main psychoactive component of cannabis. Considering the noted enduring effects of prenatal drug exposure on behavior, the question is also raised as to what the long-lasting neuronal effects associated with the behavioral disturbances are. Animal models are extremely useful tools to help resolve such issues. We and others have shown that prenatal and perinatal exposure to cannabinoid compounds lead to long-lasting effects into adulthood on neuronal systems linked to dopamine and opioid neuropeptide disturbances observed in the human fetuses (Table 1). For example, rats with prenatal THC treatment have a similar reduction of D_2R mRNA expression in the NAc (Figure 2), but not dorsal striatum, at the same developmental time period as that examined in the human fetus and the mesolimbic D_2R gene impairment persists long-term into adulthood (Dinieri *et al.*, 2011). Moreover, a behavioral study showed that adult rats exposed to doses of THC relevant to human consumption during the perinatal period exhibit altered D_2R dopamine autoreceptor sensitivity as evidenced by an enhanced reduction of locomotor activity in response to dopamine receptor agonists targeting the presynaptic receptor (Moreno *et al.*, 2003). These behavioral effects were most prominent in males.

In addition, the alteration of PENK observed in human cannabis-exposed fetuses is also reproduced by prenatal THC exposure in neonatal rats and an allostatic upregulation, again specifically localized to the NAc, is observed in adulthood (Spano *et al.*, 2007). Adult animals with prenatal cannabinoid drug exposure self-administer more heroin, particularly when stressed, show greater opiate reward and also exhibit enhanced emotional reactivity (Trezza *et al.*, 2008). Other studies have also demonstrated dopamine, opioid and behavioral impairments with early exposure to CB_1 agonists that are in line with the above observations (Walters & Carr, 1986; Rodriguez de Fonseca *et al.*, 1991; Navarro *et al.*, 1994; Corchero *et al.*, 1998; Perez-Rosado *et al.*, 2000; Perez-Rosado *et al.*, 2002; Schneider, 2009).

In addition to specific mesolimbic disturbances in association with perinatal THC exposure, there also appears to be continued specificity in regard to the striatal populations affected by prenatal drug exposure. For example, striatonigral cells specifically express D_1R and PDYN whereas striatopallidal neurons express D_2R and PENK (Heiman *et al.*, 2008) and in contrast to PENK and D_2R, no significant alterations were found on PDYN or D_1R mRNA expression levels, in rats with prenatal THC exposure (Spano *et al.*, 2007; DiNieri *et al.*, 2011). Similar to the human fetuses, no significant alterations were detected in relation to the CB_1R level of receptor binding, or GTP coupling, although there was a tendency for increased CB_1R agonist (WIN 55,2212)-stimulated [35S]GTPγS binding in the core subregion of the NAc (Spano *et al.*, 2007). Overall, a number of the animal studies confirm findings in the human cannabis-exposed fetuses such that prenatal exposure to THC is associated with specific mesolimbic and striatopallidal disturbances that persist into adulthood. Indeed, prenatal cannabis exposure disrupts the expression of dopamine and opioid related genes and the resulting impairments persist into adolescence and adulthood (Garcia-Gil *et al.*, 1999; Perez-Rosado *et al.*, 2000; Perez-Rosado *et al.*, 2002; Spano *et al.*, 2007).

Molecular consequences of early developmental nicotine exposure - animal models

Although various animal models have examined the effects of nicotine directly in adult animals, few studies have evaluated the long-term impact of prenatal nicotine exposure on gene expression. Numerous groups have identified behavioral differences in perinatally nicotine treated animals that present later in life. Briefly, elevated plus maze behavioral testing demonstrated that nicotine-treated adolescent (PND40) and young adult (PND60) rats showed an increase in anxiety-related behavior. Furthermore, prenatal nicotine treated animals yielded lower scores in cognitive and memory testing (Vaglenova *et al.*, 2008). Compared to saline exposed rats, prenatal nicotine-treated adolescent rats demonstrated an increase in both drug (cocaine self-administration) and natural reward (sucrose pellets) behaviors (Franke *et al.*, 2008). Phenotypic changes correlate well with human epidemiological studies (Figure 1), thus strengthening the findings identified in animal models.

Of the developmental nicotine studies that focus on molecular events, the majority of investigations were conducted during the perinatal period of the rodent and the results have emphasized significant early developmental disturbances of a number of neuronal systems (Miao *et al.*, 1998; Zhu *et al.*, 1998; Xu *et al.*, 2001). Of the studies addressing the long-term consequences, a few have directly examined gene expression in the brain induced by prenatal nicotine exposure (Table 1). Chen *et al.* demonstrated that prenatal nicotine treatment leads to reduced mRNA levels of the nAChR subunits α3, α4, α5, and β4 in the ventral tegmental area (VTA) of adolescent rats and increased α3 mRNA in the NAc; both the VTA and NAc are highly implicated in reward and addiction (Chen *et al.*, 2005). Although Miao *et al.* failed to observe long-term alteration of nicotinic acetylcholine receptor mRNA expression in striatal and mesocorticolimbic brain regions in adult animals following perinatal nicotine exposure (equivalent to the third trimester in human gestation), there were nevertheless noted alterations in the receptor activity in these regions (Miao *et al.*, 1998).

Long-term impairments in other neuronal systems have also been reported. For example, a recent study observed an increase in dopamine receptor 5 mRNA expression in the striatum of adult rats with prenatal nicotine suggesting long-term dopaminergic disturbance with early nicotine exposure (Schneider *et al.*, 2011). In addition, Cao *et al* (Cao *et al.*, 2011), provided evidence that prenatal nicotine exposure leads to long-term gene expression alterations of neural cell adhesion 1 and Neuroligin1 cell adhesion molecules that modulate synapse development and function in the brain (Dalva *et al.*, 2007; Hines *et al.*, 2008; Xiao *et al.*, 2009; Jung *et al.*, 2010). Interestingly, dysfunction of several of these cell adhesion molecules has been implicated in neuropsychiatric disorders (Vawter, 2000; Fujita *et al.*, 2010; Cichon *et al.*, 2011). Although we focus on investigations that have examined the long-term impact on gene expression, it is important to note that several studies have also observed long-term disturbances following prenatal nicotine on protein or receptor function (Seidler *et al.*, 1992; Xu *et al.*, 2001; Kane *et al.*, 2004; Vaglenova *et al.*, 2008) and it is predicted that some of these disturbances could relate to dysregulation of gene expression. Such reports emphasize impairment of dopamine (Xu *et al.*, 2001; Schneider *et al.*, 2011), cholinergic (Navarro *et al.*, 1989; Nordberg *et al.*, 1991; van de Kamp & Collins, 1994; Tizabi *et al.*, 1997), and serotonergic (Muneoka *et al.*, 2001; Xu *et al.*, 2001) systems. No animal studies to our knowledge have thus far examined opioid neuronal system in relation to prenatal nicotine exposure.

Epigenetic mechanisms underlying the long-term effects of prenatal drug exposure

A fundamental question is how prenatal THC or nicotine exposure causes alterations in gene expression that are maintained into adulthood, long after the initial drug exposure period *in utero*. As discussed above, both THC and nicotine causes substantial changes in gene expression levels with abnormalities persisting long into the life of the individual. One potential mechanism by which prenatal drug exposure could lead to long-lasting changes in behavior is through inducing alterations in epigenetic gene regulation mechanisms in the brain, which would be propagated throughout development stably enough to cause enduring phenotypical abnormalities. 'Epigenetic' refers to mechanisms that modulate gene expression without altering the genetic code. The epigenome is influenced by the environment and thus is a highly relevant biological candidate to maintain aberrant neuronal processing as a result of drug exposure during prenatal development. Despite the vulnerability of the developing brain to drugs, most epigenetic addiction studies have focused on the adult brain so limited information currently exists as to epigenetic effects associated with developmental drug exposure. In this section, we discuss several epigenetic processes that will merit future investigation as the most likely candidate mechanisms involved in mediating the long-term effects of prenatal cannabis and cigarette exposure.

Epigenetic modifications that can regulate gene expression levels include microRNAs, DNA methylation and post-translational modifications of nucleosomal histones (Figure 3) (Saetrom *et al.*, 2007; Ooi & Wood, 2008; Guil & Esteller, 2009). The role of microRNAs in neuronal development has been widely documented and a number of observations have indicated, for example, that cocaine addiction can change the normal composition of striatal microRNAs in adult animals (Eipper-Mains *et al.*, 2011). Interestingly, ethanol treatment during the prenatal period has been associated with teratogenesis and changes in microRNA composition in the mouse fetal brain, leading to mental retardation later in the life of the offspring (Wang *et al.*, 2009). It will be interesting to assess whether prenatal cannabis and nicotine exposure affects neuronal microRNA populations and related gene regulatory processes.

DNA methylation is an epigenetic mark that is known to be particularly stable throughout development. Maternal stress or nutritional deficiencies have been reported to cause long-lasting impairments in DNA methylation (Ronald *et al.*, 2010; Jousse *et al.*, 2011; Tarantino *et al.*, 2011), and it is conceivable that exposure to psychoactive drugs during prenatal development could induce similar abnormalities. In fact, maternal cocaine administration has indeed been shown to trigger changes in DNA methylation that are detectable in hippocampal neurons of neonatal and adolescent mice (Novikova *et al.*, 2008). Several groups have identified atypical DNA methylation patterns within the placenta (Suter *et al.*, 2010) of cigarette smoke-exposed infants and altered methylation of CpG islands at specific gene loci has been reported as long-term effects associated with prenatal nicotine exposure in somatic tissues (Toro *et al.*, 2008; Breton *et al.*, 2009; Suter *et al.*, 2010; Toledo-Rodriguez *et al.*, 2010). These observations raise the important question whether similar epigenetic disturbances occur in the brain that could possibly explain the development of abnormal behavioral tendencies.

Most studies related to drug abuse in the adult brain have focused on histone modification, which is broadly implicated in the dynamic regulation of transcriptionally repressive (inactive) and permissive (active) states (Figure 3). Covalent modifications of histones play a major part in epigenetic regulation and histone acetylation, methylation and phosphorylation have been implicated in gene regulation and neurobiological disturbances related to drug abuse (Li *et al.*, 2007; Nestler, 2009). Published data thus far has indicated

that increases in acetylation and phosphorylation are transient and appear to be associated with the quick activation of genes in response to drug exposure rather than the maintenance of an altered transcription state (Kumar *et al.*, 2005). Histone methylation is known to maintain stable gene expression alterations. In addition, the regulation of histone H3 modification is unique because methylation of distinct residues can have the opposite effect on transcription (Bannister & Kouzarides, 2005). Especially interesting is the developmental role of H3K9 dimethylation and H3K27 trimethylation in the maintenance of life-long tissue-specific gene silencing patterns (Horn & Peterson, 2006; Swigut & Wysocka, 2007). This raises the possibility of histone methylation playing a role in the propagation of perturbed gene transcription states induced by developmental cannabis and cigarette exposure.

Our studies of offspring from the prenatal THC rat model have begun to uncover such disturbances in histone modification in the adult brain. The reduction of Drd2 mRNA transcript levels in the NAc of cannabis-exposed human fetuses and in neonatal rats with prenatal THC exposure, together with the persistence of the change into adulthood in rats, indicated that the observed downregulation of Drd2 gene expression may be achieved via epigenetic processes. Examining various marks with antagonistic roles in histone H3 regulation revealed increased levels of dimethylation of lysine 9 on histone H3 (2meH3K9), a repressive mark, as well as decreased RNA polymerase II association with the gene in the NAc which are consistent with reduction of the Drd2 gene expression (Figure 2). The methylation profile of histone H3 on the Drd2 gene was specific since there was no alteration of 2meH3K9 observed at the Drd1 gene (Dinieri *et al.*, 2011). These findings suggest that maternal cannabis use alters the developmental regulation of mesolimbic D_2R in offspring through epigenetic mechanisms, specifically histone lysine methylation, and the ensuing reduction of D_2R may contribute to increased vulnerability to drug abuse later in life. To date, no other epigenetic studies to our knowledge have been published regarding the effects of prenatal cannabis exposure on the brain.

Summary and future perspectives

The studies reviewed here emphasize the sensitive nature of the prenatal developmental period, during which cannabis and cigarette exposure can set into motion epigenetic alterations that contribute to long-term disturbances in mesocorticolimbic gene regulation, thereby laying a foundation for increased vulnerability to addiction and potentially other psychiatric disorders. While a large number of human longitudinal studies have shown significant behavioral disturbances with *in utero* cannabis or cigarette exposure, the lack of molecular investigations in the human brain as well as the limited knowledge regarding their long-term impact significantly hinders insights about the neurobiology underlying risk for psychiatric disorders. This, however, provides an important opportunity to expand our current knowledge regarding the molecular mechanisms underlying the long-term consequences of prenatal cannabis and cigarette exposure. Current state-of-the-art approaches to explore in-depth epigenetic mechanisms will no doubt provide significant advances within this field.

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Abbreviations

Actn1 Actinin, alpha 1

AMY	Amygdala:
AT_1R	Angiotension Receptor 1
AT_2R	Angiotension Receptor 2
Bai3	Brain-specific angiogenesis inhibitor 3
Cdh13	Cadherin 13
CB1R	Cannabinoid receptor
Ctnna1	Catenin (cadherin associated protein), alpha 1
Ctnna2	Catenin (cadherin associated protein), alpha 2
Ctnnb1b	Catenin (cadherin associated protein), beta 1
Ctnnd2	Catenin (cadherin associated protein), delta 2
CP	Caudate-Putamen
CAMs	Cell Adhesion Molecules
Cntn4	Contactin 4
Cntn5	Contactin 5
Cntn6	Contactin 6
CTX	Cortex
Csmd1a	CUB and Sushi multiple domains 1
THC	Δ^9-tetrahydrocannabinol
∂OR	Delta-opioid receptor
Dat1	Dopamine transporter 1
D1R	Dopamine receptor 1 protein
Drd1	Dopamine Receptor 1 gene
D_2R	Dopamine Receptor 2 protein
Drd2	Dopamine Receptor 2 gene
Drd4	Dopamine Receptor 4
Drd5	Dopamine Receptor 5
Dscam	Down syndrome cell adhesion molecule
Fyn	Fyn proto-oncogene
GD	Gestational Day
HIP	Hippocampus
kOR	Kappa Opioid Receptor
μOR	Mu Opioid Receptor
NAc	Nucleus Accumbens
Nr4a2	Nuclear Receptor subfamily 4, group A, member 2:
Lphn3	Latrophilin 3
nAchR	Nicotinic Cholinergic Receptor

Ncam1	Neural cell adhesion molecule1
Nrxn3	Neurexin3
Nlgn1	Neuroligin1
Postn	Periostin, osteoblast specific factor
Pecam1	Platelet/endothelial cell adhesion molecule 1, PND, Postnatal day
PFC	Prefrontal cortex
Pdyn	Prodynorphin
PENK	Proenkephalin
Ptprd	Receptor-type protein tyrosine phophatse D
Sgcz	Sarcoglycan zeta
TH	Tyrosine hydroxylase
VTA	Ventral Tegmental Area

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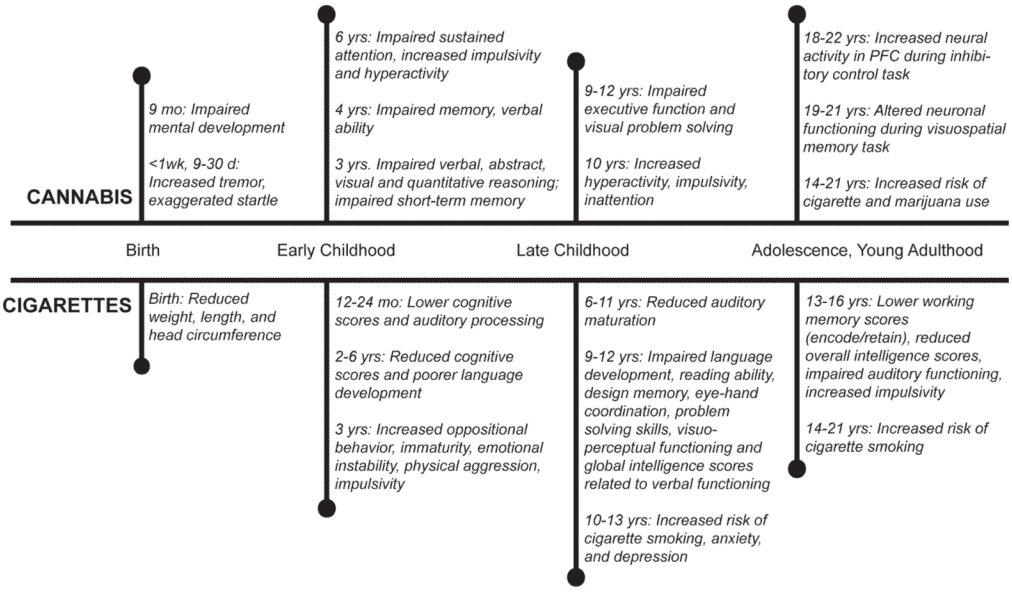

Figure 1.

Overview of developmental effects associated with prenatal cannabis and cigarette exposure in human subjects. Data compiled from two longitudinal studies Ottawa Prenatal Prospective Study and Maternal Health Practices and Childhood Development Project that followed offspring from mothers who used marijuana or cigarettes during pregnancy that are representative of other epidemiological studies. Maternal cannabis and cigarette use is clearly associated with neurobehavioral disturbances that persist into adulthood. Overall, *in utero* cannabis and cigarette exposure is characterized by impaired cognitive functioning, impulsivity, hyperactivity, and increased risk of developing an addiction disorder.

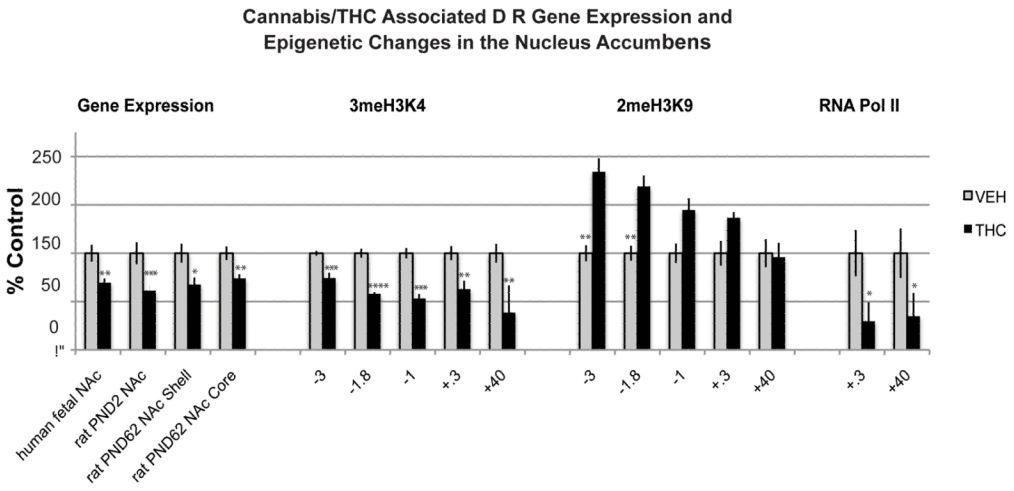

Figure 2. Mesolimbic dopamine D_2R gene is vulnerable to prenatal cannabis exposure

Prenatal cannabis affects dopamine D_2R gene expression in the nucleus accumbens of the human midgestational fetus exposed to cannabis *in utero*. The effects are mimicked in the prenatal THC animal model studied at postnatal day 2 (PND2; comparable to the midgestation human fetal period) and the early developmental disturbances on the Drd2 mRNA expression persist into adulthood (PND62) and are mediated by epigenetic modifications at the Drd2 promoter. Data modified from (Dinieri *et al.*, 2011) 2meH3K9, di-methylation of lysine 9 on histone H3; 3meH3K4, tri-methylation of lysine 4 on histone H3; KB, kilobases; RNA Poly II, RNA Polymerase II; TSS, transcription start site. Values are expressed as mean ± SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$ vs control subjects. Black bars, THC exposed group; white bars, vehicle-exposed group; kb, kilobases; TSS, transcription start site.

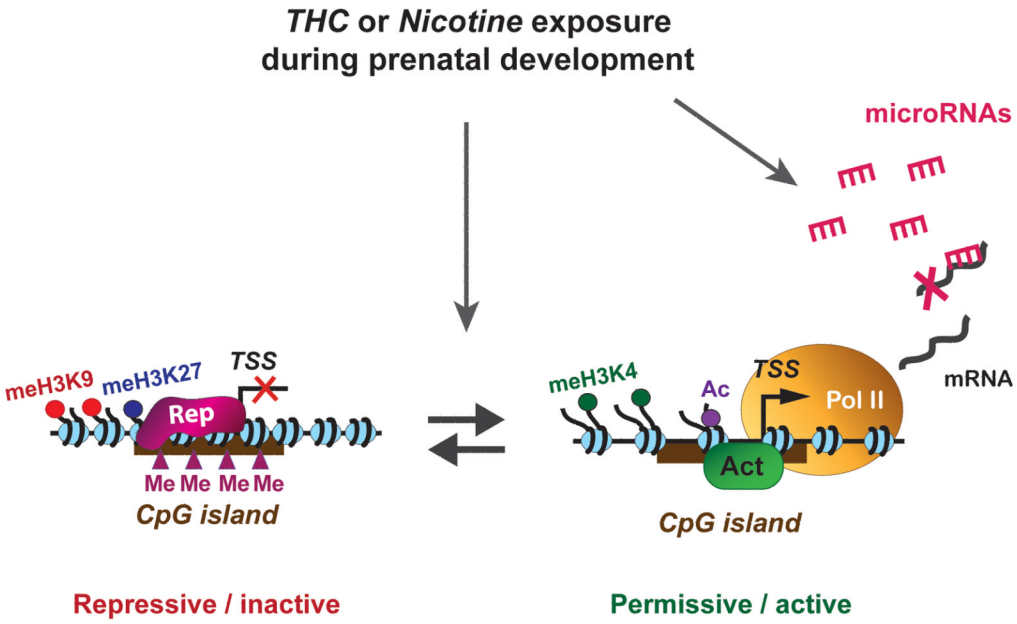

Figure 3. A few possible epigenetic regulatory mechanisms that can be disrupted by maternal cannabis and nicotine u se, leading to persistent abnormal gene expression levels

Changes in DNA methylation at CpG islands, along with post-translational modification of histones, can create 'repressive' (transcriptionally silent) and 'permissive' (transcriptionally active) chromatin states. These states are dynamically regulated by the activities of DNA or histone modifying and chromatin remodeling enzymes and exposure to drugs during prenatal development is likely to disrupt the balance of repressive and permissive mechanisms. In silent chromatin, methylation of H3 lysine 9 (meK9) and lysine 27 (meK27) is known to be associated with the binding of repressor complexes (Rep) and this leads to chromatin condensation and decreased accessibility of the gene. In the permissive state, methylation of H3 lysine 4 (meK4) and acetylation (ac) is often associated with the binding of activator proteins (Act), generating increased accessibility for the recruitment of the RNA polymerase II transcription machinery (Pol II). Prenatal THC exposure was recently shown to enhance dimethylation of H3K9 of the dopamine D_2 R gene promoter in adult animals relevant to the THC-induced long-term impairment on gene expression (see Figure 3). MicroRNAs can regulate mRNA levels post-transciptionally and changes in the neuronal microRNA populations due to developmental drug exposure may lead to disturbances in protein function. TSS, transcription start site.

Table 1

Long Term Gene Expression Related to Prenatal THC or Nicotine Exposure in Experimental Animal Models

Overview of studies examining the effects of exposure to prenatal THC and nicotine, the psychoactive components of cannabis and cigarettes, respectively, on mRNA expression during adolescence (PND35) and in adulthood (PND62-150).

Treatment	Subject	Age at testing	Effects on mRNA expression	Reference
Δ^9-THC 5 mg/kg Daily oral GD5-PND24	Wistar rat ♂/♀	PND70	♂ Cnr1: ↑ in HIP ♀ Cnr1: no Δ in CTX, CP, NAc, HIP, AMG	(Garcia-Gil *et al.*, 1999)
Δ^9-THC 0.15 mg/kg Daily i.v., GD5-PND2	Long Evans rat ♂	PND62	♂ Drd2: ↓ in NAc core and shell	(Dinieri *et al.*, 2011)
Δ^9-THC 0.15 mg/kg Daily i.v. GD5-PND2	Long Evans rat ♂	PND62	♂ Penk: no Δ in CP, ↑ in NAc core, ↑ in NAc shell, ↑ in AMG ♂ Pdyn: no Δ in DS, NAc core, NAc shell	(Spano *et al.*, 2007)
Nicotine 3 mg/kg Oral GD4-GD15	Sprague- Dawley rat ♂	PND35	*mRNA expression changes in CAMs* ↓ in CP: Actn1[b], Bai3[a], Cdh13[a] , Cntn4[a], Cntn5[a] Cntn6[a] Ctnna1[b] Ctnna2[b] Ctnnb1[b] Ctnnd2[ab] Csmd1a, Dscam, Fyn[b], Lphn3[a], Nlgn1, Postn ↑ in NAc: Postn ↓ in NAc: Ptprd[a] ↑ in PFC: Postn ↓ in PFC: Dscam Nlgn1 Nrxn3 ↓ in Amy: Ncam1, Pecam1, Sgcz[a]	(Cao *et al.*, 2011)
Nicotine 2mg/kg Daily oral GD1-PND14	Sprague- Dawley rat ♂/♀	PND35	nAchR subunit a3, a4, a5, b4 in ↓ VTA nAchR subunit a3 in ↑ NAc	(Chen *et al.*, 2005)
Nicotine 100 mg/l oral GD7-PND28	Wistar rats	PND35	M1 & M2 muscarinic receptor: no Δ PFC, midbrain, HIP, cerebellum, brainstem	(Zhu *et al.*, 1998)
Nicotine 0.1 mg/kg oral PND1-21 and PND8- P21	Sprague- Dawley ♂/♀	PND35	α2, α3, α4, α7, α2 nAchR: no Δ PFC, HIP, striatum, brainstem, thalamus	(Miao *et al.*, 1998)
Nicotine 0.06/ml Oral	Lister hooded rats ♂	PND150	↑Drd5: Striatum TH, NR4A2, DAT1, DRD4: no Δ striatum, PFC	(Schneider *et al.*, 2011)
Nicotine 2.1 mg/day Daily i.v. GD4-PND1	Sprague- Dawley ♂/♀	PND150	♀↑ AT_1R: total brain tissue ♀♂↓ AT_2R: total brain tissue	(Mao *et al.*, 2008)