Trajectory of Adolescent Cannabis Use on Addiction Vulnerability

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Abstract

The adolescent brain is a period of dynamic development making it vulnerable to environmental factors such as drug exposure. Of the illicit drugs, cannabis is most used by teenagers since it is perceived by many to be of little harm. This perception has led to a growing number of states approving its legalization and increased accessibility. Most of the debates and ensuing policies regarding cannabis were done without consideration of its impact on one of the most vulnerable population, namely teens, or without consideration of scientific data. We provide an overview of the endocannabinoid system in relation to adolescent cannabis exposure and provide insights regarding factors such as genetics and behavioral traits that confer risk for subsequent addiction. While it is clear that more systematic scientific studies are needed to understand the long-term impact of adolescent cannabis exposure on brain and behavior, the current evidence suggests that it has a far-reaching influence on adult addictive behaviors particularly for certain subsets of vulnerable individuals.

Keywords: marijuana, cannabinoid, opioid neuropeptide, nucleus accumbens, prefrontal cortex

1. Introduction

Adolescence is an important stage of behavioral maturation and brain development during which the high degree of neuroplasticity that occurs in this ontogenetic period places the adolescent brain at particular risk to environmental factors such as drug exposure. Marijuana (*Cannabis sativa*) continues to be the illicit drug most commonly used by teenagers in the United States as well as in other Western societies (Johnston et al., 2012; SAMHSA, 2011). Although cannabis is not as highly addictive as other substances, such as heroin and cocaine, cannabis-dependent individuals still greatly outnumber those reporting dependence on other illicit drugs and the number of people seeking treatment for cannabis dependence continues to increase yearly (SAMHSA, 2011).

Despite these facts, there is a growing perception, particularly in adolescents and young adults (Kilmer et al., 2007; Lopez-Quintero and Neumark, 2010), that cannabis is 'harmless' especially when compared to other abused substances like nicotine (tobacco) and alcohol that are legal. Reasons cited for this perception include the consideration that cannabis-associated mortality is lower than tobacco and alcohol, which are associated with cancer and overdose/vehicular accidents, respectively. In addition, cannabinoids provide medicinal benefits (Hermanson and Marnett, 2011; Hill et al., 2012) in contrast to tobacco and alcohol, which have no medical indications. These and other considerations have contributed to the decriminalization, or even legalization, of cannabis in a number of states within the USA. Economic factors have also been suggested as a rationalization for legalization as a potential source of tax revenue for state governments. Despite some cogent arguments in the current debates regarding legalization and increased availability of cannabis, most of the discussion and policies have been made without significant consideration of scientific data.

Growing evidence suggests a differential effect of cannabis exposure on the human brain based on the age of exposure, but the question remains as to the potential long-term mental health consequences of cannabis exposure in teens. Few scientific studies have systematically investigated the long-term impact of cannabis use in relation to the developing teenage brain, the population most crucial to the current debates. Nevertheless, the available data to date, as discussed in this review, suggest that adolescent cannabis exposure induces significant protracted effects suggestive of

enhanced vulnerability to addiction and psychiatric disorders in later life, at least in certain subsets of individuals.

2. Neurobiology of the endocannabinoid system

The main psychoactive component of cannabis, Δ^9-tetrahydrocannabinol (THC), acts primarily via cannabinoid receptors (CBRs) — CB_1R and CB_2R (Gerard et al., 1991; Griffin et al., 2000; Matsuda et al., 1990; Munro et al., 1993). The CB_1R is one of the most abundant G-protein-coupled receptor in the brain (Herkenham et al., 1990; Herkenham et al., 1991a) and is $G_{i/o}$-coupled, suppressing neurotransmitter release (Howlett et al., 2002). The expression of CB_1R is most pronounced within the basal ganglia, cerebellum, cerebral cortex, hippocampus and amygdala (Biegon and Kerman, 2001; Glass et al., 1997; Herkenham et al., 1990; Herkenham et al., 1991b; Mailleux et al., 1992; Pettit et al., 1998; Wang et al., 2003) (**Fig. 1**), consistent with cannabis exerting significant effects on motor function, cognition, and emotional regulation. Recent evidence, though initially controversial, suggests that CB_2R is also expressed within the central nervous system in immune cells as well as glia and potentially neurons (Gong et al., 2006; Lanciego et al., 2011; Onaivi et al., 2006; Van Sickle et al., 2005). Nevertheless, the broad and abundant expression of CB_1R in neuronal circuits relevant to addiction and psychiatric disorders still place a prominent emphasis on cannabis' modulation of this CBR subtype in relation to psychiatric vulnerability.

Imaging studies of rodents (Verdurand et al., 2011) and human subjects (Mato et al., 2003) suggest global increases in CB_1R throughout early life into adolescence, at which period adult levels are generally maintained (Belue et al., 1995; McLaughlin et al., 1994; Rodriguez de Fonseca et al., 1993), but there are also reports of reduced CB_1R expression from juvenile to adulthood that mirrors developmental changes in CB_1R-mediated signaling (Heng et al., 2011). Some of the inconsistencies regarding the ontogenic pattern of the CB_1R may be due to regional, as opposed to global, developmental differences in the receptor development in addition to differences in mRNA, receptor protein or receptor binding being studied. Most preclinical investigations to date that have examined the neurodevelopment of CB_1R have focused on the striatum and prefrontal cortical brain regions which are key components of neuronal circuits implicated in addiction and related psychiatric disorders. Reward, motivated behavior, decision-making, habit formation and motor function are mediated by the prefrontal

cortex as well as components of the dorsal (associative- and sensorimotor-related) and ventral (limbic-related) striatal areas making these brains regions relevant to adolescent cannabis exposure.

With respect to cortical development, remodeling of excitatory connections in the prefrontal cortex is a key feature of adolescent neurodevelopment. For instance, there is significant pruning of excitatory synapses that parallels the delayed maturity of cognitive behaviors such as inhibitory control and working memory. The CB_1R is highly abundant in the prefrontal cortex, primarily localized on large cholecystokinin interneurons (Marsicano and Lutz, 1999), but molecular and functional evidence suggest that CB_1R is also present in a subset of pyramidal neurons (Hill et al., 2007; Marsicano and Lutz, 1999; Matsuda et al., 1993). Interestingly, the most pronounced and progressive cortical alteration observed on CB_1R expression and CB_1R-mediated functional signaling evident during adolescent development in rodents is within the medial prefrontal and other limbic/associative cortices as compared to sensorimotor cortices (Heng et al., 2011). Whether such developmental fluctuations of the CB_1R directly relate to plasticity and the synaptic remodeling that occurs in the prefrontal cortex during adolescence is unknown.

In the striatum, CB_1R are localized in the medium spiny GABAergic neurons that constitute the major output pathways — striatonigral and striatopallidal (**Fig. 2**). These receptors are expressed on both striatonigral 'direct' and striatopallidal 'indirect' pathways which mediate 'Go' facilitatory (positive reward/choice) and 'NoGo' (avoidance learning/inhibitory control) behaviors, respectively, relevant to motor function and decision-making processes (Durieux et al., 2009; Frank et al., 2007; Klein et al., 2007; Sano et al., 2003). While no study thus far has characterized the pattern and abundance of CB_1R in the different output pathways during development, it is known that CB_1R expression is dynamic during the course of adolescent development in different brain regions. For example, *in vivo* (Verdurand et al., 2011) and *in vitro* (Belue et al., 1995) imaging of the rat brain that revealed global enhanced CB_1R in the cortex, also showed increased CB_1R in other brain structures including the striatum during the transition from early adolescence to adulthood. However, other investigators have provided significant evidence for reduced CB_1R expression and mRNA levels from juvenile to adulthood (Van Waes et al., 2012). Moreover, examination of CB_1R protein expression restricted to the adolescent developmental window suggests significant CB_1R differences even within

distinct compartments of the nucleus accumbens (Ellgren et al., 2008). During adolescence, CB_1R protein decreases in the nucleus accumbens shell yet concomitantly increases in the core compartment. This suggests that distinct time periods during adolescence may have different sensitivity to cannabis exposure relevant to mesolimbic striatal function.

Given anatomical and functional relationships between the prefrontal cortex and the striatum, it is not surprising that these regions are coordinated in regard to development of the endocannabinoid (eCB) system. There appears to be a direct correlation between the developmental trajectory of CB_1R expression in specific cortical regions with their projection to distinct striatal subregions. For example, striatal subregions with high levels of CB_1R expression (dorsolateral sensorimotor regions) receive input primarily from cortical areas with relatively low CB_1R levels (motor, somatosensory) (Van Waes et al., 2012). In contrast, striatal subregions with low mRNA expression of *Cnr1*, such as the associative ventromedial dorsal striatum and limbic nucleus accumbens, receive afferents from cortical areas with greater CB_1R expression (cingulate, insular). These findings emphasize a strong inverse relationship between cortical and striatal CB_1R expression in frontostriatal circuits, suggesting a functional orchestration vulnerable to adolescent cannabis exposure.

In addition to CBRs, other components of the eCB system, such as the endogenous cannabinoids anandamide and 2-arachidonoylglycerol (2-AG), have significant developmental implications. Indeed, eCB signaling plays a key role in hardwiring of the brain during prenatal ontogeny, regulating synaptogenesis and target selection for the development of neuronal pathways (Harkany et al., 2008; Mulder et al., 2008). During later developmental stages, the eCBs are well-documented regulators of synaptic plasticity (Katona and Freund, 2008). Within adolescent ontogeny, anandamide and 2-AG are dynamically altered in the striatum and prefrontal cortex, as for instance, 2-AG is reduced from early to late adolescence in these regions (Ellgren et al., 2008). There is also a continuous increase of anandamide in the prefrontal cortex over the course of adolescence. The fact that the eCB system is dynamically altered during adolescence in brain areas central to reward, decision-making and motivation suggests that cannabis exposure during this critical developmental phase may have long-term influence on behaviors linked to the mesocorticolimbic system. Clearly, however, the limited studies

to date are incomplete, so there still remains a large gap of knowledge regarding the adolescent ontogeny of the eCB system.

3. Cannabis and 'gateway' effects

A major aspect of the debate regarding adolescent cannabis use is whether it increases the use of other addictive substances such as heroin and cocaine later in life, a phenomenon known as the gateway hypothesis. Clinical and epidemiological studies have documented a significant link between repeated early cannabis exposure and an increased risk of other illicit drug use (Agrawal et al., 2004; Brook et al., 1999a; Fergusson and Boden, 2008; Fergusson and Horwood, 2000; Hall and Lynskey, 2005; Kandel, 1975; Yamaguchi and Kandel, 1984). Altogether, the data suggest that use of 'heavy' drugs is almost systematically preceded by cannabis use, and that risk is correlated with the intensity of cannabis exposure. Moreover, cannabis use also appears more deleterious when its onset occurs in younger versus older adolescents in regard to adjustment in transition from adolescence to young adulthood, education attainment, employment, delinquency and ability to conform to adult role (Brook et al., 1999a; Brook et al., 1999b; Fergusson and Boden, 2008; Fergusson et al., 2002; Lynskey et al., 2003; Tucker et al., 2006). Despite these findings, a major caveat of human studies is the difficulty of demonstrating a *causal* relationship between adolescent cannabis use and subsequent behavioral disturbances, especially when considering the influence of genetic and environmental factors alongside other aspects such as polysubstance use (Cleveland and Wiebe, 2008; Fergusson et al., 2006; Kandel et al., 2006; Lessem et al., 2006; Maccoun, 2006; Tarter et al., 2006). Given these complexities, animal models are a valuable tool to obtain direct insights about the relationship between early cannabis exposure and behavioral disruptions.

Many rodent investigations exploring the potential gateway effects of cannabis have primarily studied synthetic cannabinoid agonists that differ in pharmacological properties to THC. Nevertheless, studies examining adolescent exposure to cannabinoid agonists or THC provide evidence of enhanced intake and sensitivity later in life to opiate drugs (Biscaia et al., 2008; Ellgren et al., 2007; Tomasiewicz et al., 2012). In our experimental rat model that mimics the more periodic use of most adolescent cannabis users, adult male rats with low-to-moderate THC exposure during adolescence exhibit enhanced heroin self-administration behavior (Ellgren et al., 2007). Cannabinoid-opioid interactions

have also been documented by studies showing that developmental exposure to cannabinoid agonists increases heroin-induced conditioned place preference (Biscaia et al., 2008; Singh et al., 2006). Short adolescent exposure to cannabinoid agonist, WIN 55,212-2, has also been reported to instead induce tolerance to morphine (Pistis et al., 2004).

Animal models make it possible to identify neuroadaptations that may contribute to the behavioral vulnerability related to adolescent cannabis. Intriguingly, many experimental animal studies to date have implicated the striatopallidal circuit in association with developmental cannabinoid exposure (Corchero et al., 1998; Corchero et al., 1999; Ellgren et al., 2007; Morel et al., 2009; Perez-Rosado et al., 2000; Spano et al., 2007; Valverde et al., 2001). This theory is based on consistent alterations of striatal dopamine D2 receptors (*Drd2*) and proenkephalin (*Penk*) mRNA expression both of which are preferentially co-expressed within striatopallidal medium spiny neurons (Gerfen et al., 1990; Gerfen and Young III, 1988; Le Moine et al., 1990). In our model of adolescent THC exposure, reduced *Drd2* and *Penk* (Ellgren et al., 2007; Tomasiewicz et al., 2012) mRNA levels were observed within the nucleus accumbens of adult animals (**Fig. 3**). Reduced D_2R, the protein encoded by *Drd2*, has long been a characteristic neurobiological feature of addiction vulnerability. *In vivo* positron emission tomography (PET) evidence has consistently demonstrated that subjects with substance abuse have less available D_2R in the striatum (Heinz et al., 2004; Volkow et al., 2001; Volkow et al., 2004; Volkow et al., 1999; Wang et al., 1997), findings that animal models have shown to be linked to enhanced drug self-administration vulnerability (Morgan et al., 2002; Nader et al., 2006). Over-expression of *Drd2* in the ventral striatum attenuates cocaine intake (Thanos et al., 2008), and D_2R binding in this region in cocaine-naïve rats negatively predicts future cocaine-seeking behavior (Michaelides et al., 2012). In addition to adolescent THC exposure, prenatal THC also leads to dysregulation of the *Drd2* gene in adulthood (DiNieri et al., 2011). That developmental THC exposure reduces *Drd2* mRNA expression in the striatum, and affects related behavioral traits, support the hypothesis that developmental cannabis may induce a neurobiological state of addiction vulnerability.

The finding of impaired *Penk* gene expression in cannabis-exposed subjects is perhaps not surprising given the tight neurobiological interactions between the opioid and eCB

systems. Opioid neuropeptide receptors and CB_1R are coexpressed on similar neurons in the striatum, share similar G-protein coupled signaling mechanisms and appear functionally interdependent (Blume et al., 2013; Canals and Milligan, 2008). Of the opioid neuropeptides, enkephalin, encoded by the *Penk* gene, directly regulates hedonic states (Kelley et al., 2002; Skoubis et al., 2005). We recently documented a direct causal link between regulation of ventral striatal *Penk* mRNA expression and heroin self-administration behavior. Overexpression of the *Penk* gene in the nucleus accumbens shell by use of viral-mediated manipulation enhanced heroin self-administration and heroin-seeking behavior in animals naïve to THC, whereas in contrast, knocking down the *Penk* gene in THC-exposed rats reduced heroin intake behavior (Tomasiewicz et al., 2012). Altogether, these and other findings (Spano et al., 2010; Vigano et al., 2005) suggest the tight interaction between cannabinoids and the opioid system could contribute to the development of opiate abuse in adults with previous exposure to THC during adolescence.

In contrast to the effects noted for opiates, the impact of early cannabinoid exposure on the subsequent sensitivity to stimulant drugs have yielded inconsistent findings. While some studies failed to find significant behavioral differences in response to amphetamine later in life (Ellgren et al., 2004), others report that adolescent cannabinoids enhance cocaine-induced motor behavior (Dow-Edwards and Izenwasser, 2012). Moreover, both increased (Higuera-Matas et al., 2008) and deceased self-administration of cocaine (Panlilio et al., 2007) have been reported in adult animals with adolescent cannabinoid exposure. Differences in experimental factors, such as the duration and frequency of exposure, dose and formulation of the cannabinoid, and even gender likely contribute to these inconsistencies. For example, adolescent administration of the CB_1R agonist CP 55,940 was reported to increase cocaine self-administration primarily in females, not males (Higuera-Matas et al., 2008). These findings emphasize the need to systematically probe factors such as the magnitude and duration of cannabinoid exposure, adolescent period of exposure and gender in order to help expand insights about individual risk factors contributing to the gateway effects of adolescent cannabis.

4. Genetic and behavioral traits contribute to individual vulnerability

Although animal studies demonstrate protracted behavioral and neurobiological effects of adolescent THC exposure into adulthood, there remains the fact that not all teenage

cannabis users develop future addictions or psychiatric disorders. In fact, despite its common use, only a subset of teens (~25%) and young adults (~19%) using cannabis progress to abuse or dependence (SAMHSA, 2011). Indeed, for most teenagers, cannabis is a terminus with no further use of that or other illicit drugs as they mature into full adulthood, suggesting that there are differences in individual vulnerability. Humans vary tremendously for instance in regards to environment, behavioral traits, genetics, and cultural norms. While these and other factors play significant roles in complex disorders as addiction, understanding the contribution of each factor is as much a challenge as determining their interactions to risk..

4.1 Behavioral traits and personality

Cannabis users are generally characterized by apathy, loss of goal-motivated behavior and negative mood states, and dependent subjects report more negative affect, neuroticism, aggressivity and impulsivity (Dorard et al., 2008; Hyman and Sinha, 2009; Jutras-Aswad et al., 2012; Zvolensky et al., 2007). Negative affect in cannabis-dependent subjects is also related to the severity of dependence insomuch that this personality trait correlates with years of cannabis use, implying that long-term use of cannabis also worsens negative affect. Such findings are consistent with the hypothesis that in attempts to 'self-medicate', a user's continued consumption of cannabis itself exacerbates underlying negative traits (Arendt et al., 2007). Rat studies also substantiate these human findings by documenting that exposure to CB1R agonists during adolescence induces long-term increases in anxiety- and depression-like behaviors as adults (Biscaia et al., 2003; Ciccocioppo et al., 2002). Indeed, a growing body of evidence suggests that cannabis exposure in humans during adolescence is linked to the development of symptoms characteristic of mood and anxiety disorders (Fergusson et al., 2002; Hayatbakhsh et al., 2007; Patton et al., 2002).

While the use of cannabis itself appears to lead to negative affect, which could contribute to subsequent drug abuse as individuals try to self-medicate, even young cannabis dependent subjects without a long history of drug use show high neuroticism/anxiety and depression traits, implying a preexisting negative emotional affect in these users (Dorard et al., 2008). As such, there may be subsets of individuals with at-risk behavioral traits that contribute to self-medication ultimately leading to dependence. Self-medication due to a preexisting vulnerable state is also evident in

regard to psychosis risk. Cannabis use is high among people with psychosis (Koskinen et al., 2010; van Gastel et al., 2013) and it has been documented that psychotic symptoms are evident in subjects who have never used cannabis before the onset of psychotic symptoms, which also predicts future cannabis use (Ferdinand et al., 2005). This suggests that current cannabis-dependent subjects may have underlying psychiatric disorders that contributed to self-medication and that through repeated use, led to dependence. Thus, while cannabis may itself increase drug addiction and psychiatric vulnerability, pre-existing prodromal states (or disease vulnerability) may initially promote the initiation and continuation of cannabis use.

4.2 Heritable genetic factors

A growing number of family, twin and adoption studies have shown that cannabis use disorder is strongly heritable (30-80%) (Agrawal and Lynskey, 2009; Kendler et al., 2000; Kendler and Prescott, 1998; Maes et al., 1999; McGue et al., 2000; Miles et al., 2001; Rhee et al., 2003; Tsuang et al., 1998; van den Bree et al., 1998). Most studies examining cannabis dependence and genetic risk have used a candidate gene approach. Thus far, genes encoding CB_1R (*CNR1*) and the fatty acid amide hydrolase (*FAAH*), an enzyme responsible for the hydrolysis of the eCB anadamide, have been shown to modulate cannabis dependence risk (Agrawal et al., 2009; Hopfer et al., 2006; Tyndale et al., 2007).

Given the effects of adolescent THC on striatopallidal-related genes, namely *Drd2* and *Penk* expression, in the rodent models, (Corchero et al., 1998; Corchero et al., 1999; Ellgren et al., 2007; Morel et al., 2009; Perez-Rosado et al., 2000; Spano et al., 2007; Valverde et al., 2001; Wang et al., 2006), it was of interest to explore DRD2 and PENK SNPs in humans in relation to cannabis dependence. The *DRD2* Taq1A polymorphism, which has been studied in multiple addiction disorders, did not associate with cannabis use (Creemers et al., 2011; Jutras-Aswad et al., 2012; Sakai et al., 2007). However, considering the link between *DRD2* and inhibitory control, we probed their interacting association with cannabis dependence risk. A number of neurocognitive studies have documented that *DRD2* SNPs predict avoidance-based decisions in healthy subjects, in line with this gene's association with the striatopallidal NoGo pathway (Frank and Hutchison, 2009; Frank et al., 2007; Klein et al., 2007). Our results confirmed that negative reinforcement learning, linked with the ability to avoid maladaptative choices in

a probabilistic learning task, was indeed associated with the *DRD2 rs6277* SNP (Jutras-Aswad et al., 2012). For this *DRD2* SNP, however, both cannabis users and controls exhibited the same association with negative reinforcement. Interestingly, negative affect (high anxiety/neuroticism trait) which was prominent in cannabis users significantly modulated the association between *DRD2* and *PENK* genotypes with cannabis dependence. This interaction was most apparent for *PENK* SNPs (rs2609997 and rs2576573), with neuroticism/anxiety trait explaining approximately 15% to 20% of the association between genotype and cannabis dependence (Jutras-Aswad et al., 2012).

Recent evidence from animal models have demonstrated a direct role of the nucleus accumbens *Penk* striatopallidal pathway in mediating behavioral responses associated with aversive behavior (Hikida et al., 2010). In the human brain, there is a significant association of *PENK* SNPs (rs2609997 and rs2576573) with mRNA expression of this gene in the nucleus accumbens and dorsal striatum as well as with striatal met-enkephalin peptide levels (Jutras-Aswad et al., 2012). These findings emphasize the transcriptional and translational functional relationships of polymorphisms of the PENK gene (**Fig. 4A**). In addition to the striatum, *PENK* mRNA amygdala expression is also related to *PENK* genotype which is interesting given that the amygdala plays a prominent role in negative mood states and enkephalinergic neurons in the central amygdala are critically involved in anxiety and stress responsivity (Kang et al., 2000; Kung et al., 2010) (**Fig. 4B**).

4.3 Interactions between genetics and behavioral traits

It is clear that multiple factors can converge to contribute to vulnerability. For example, while PENK genotype and anxiety/neuroticism trait are individually associated with cannabis dependence, there was a strong synergism between high-risk genotypes and the negative affect trait that enhanced cannabis dependence risk 8-9-fold (Jutras-Aswad et al., 2012) **(Fig. 5**). . This synergistic interaction was also evident in another population (homogenous Caucasian Greek army conscripts) in which aspects of cigarette use could be explored separately from cannabis use (Jutras-Aswad et al., 2012). The finding that cannabis dependence is significantly enhanced in individuals with both high neuroticism/anxiety and risk genotypes emphasizes the important synergistic contribution of negative emotional traits and genetics to vulnerability. Thus, developmental cannabis exposure may confer susceptibility primarily in those individuals

with underlying genetic and behavioral trait (**Fig. 6**). Both clinical reports and research studies suggest that coping with stress and negative mood states is a common motive for use among heavy abusers (Hyman and Sinha, 2009), which would be consistent with self-medicating even subthreshold anxiety and negative affect induced by *PENK* dysfunction. Cannabis exposure and negative affect may thus interact in a complex way such that cannabis is used to cope with subthreshold symptoms, but paradoxically further increases these symptoms in the long term.

5. Summary

Different lines of evidence suggest a link between adolescent THC and subsequent vulnerability to addiction and psychiatric risk. Yet, it is clear that more scientific evidence is critically needed to fully understand this relationship considering the multiple factors that appear to influence this trajectory. While some neurobiological insights have been obtained, it is clear that additional information is needed to fully understand the dynamic neurodevelopment of distinct components of the eCB and related neuronal systems, and the impact of cannabis upon these systems during adolescent ontogeny. The mechanisms by which cannabis may disrupt the functional organization of brain structures such as the striatum and prefrontal cortex during adolescent development as well as specific behavioral phenotypes are still unknown. Aside from the direct pharmacological effects of the drug on brain development, individual factors contribute tremendously to the complexity of the relationship between adolescent cannabis exposure and addiction risk (**Fig. 6**). The apparent synergistic interactions of genetics and negative affective trait suggest that genetic screens should begin to consider behavioral endophenotypes since the gene-addiction risk relationship is not direct. The possibility to identify vulnerable adolescents is essential for early intervention of cannabis dependence and related psychiatric disorders. Overall, it is impossible to ignore the evidence that cannabis/THC is not harmless to the developing brain, but there remain large gaps of knowledge that need to be filled in order to help inform public policy, thereby enhancing teenagers' well-being and their mental health later in life.

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Figure 1. Cannabinoid receptor mRNA (*CNR1*) expression in the human brain emphasizes this gene's abundant expression in cerebral cortex – such as insular cortex (I) and prefrontal cortex (PFC) – as well as the caudate nucleus (CN), putamen (Pu), nucleus accumbens (NAc), hippocampus (Hipp), amygdala (Amy), and cerebellum (CB). Absent-to-low mRNA expression is notable in the thalamus (T), basal forebrain (BF), globus pallidus (GP), and midbrain (Ms).

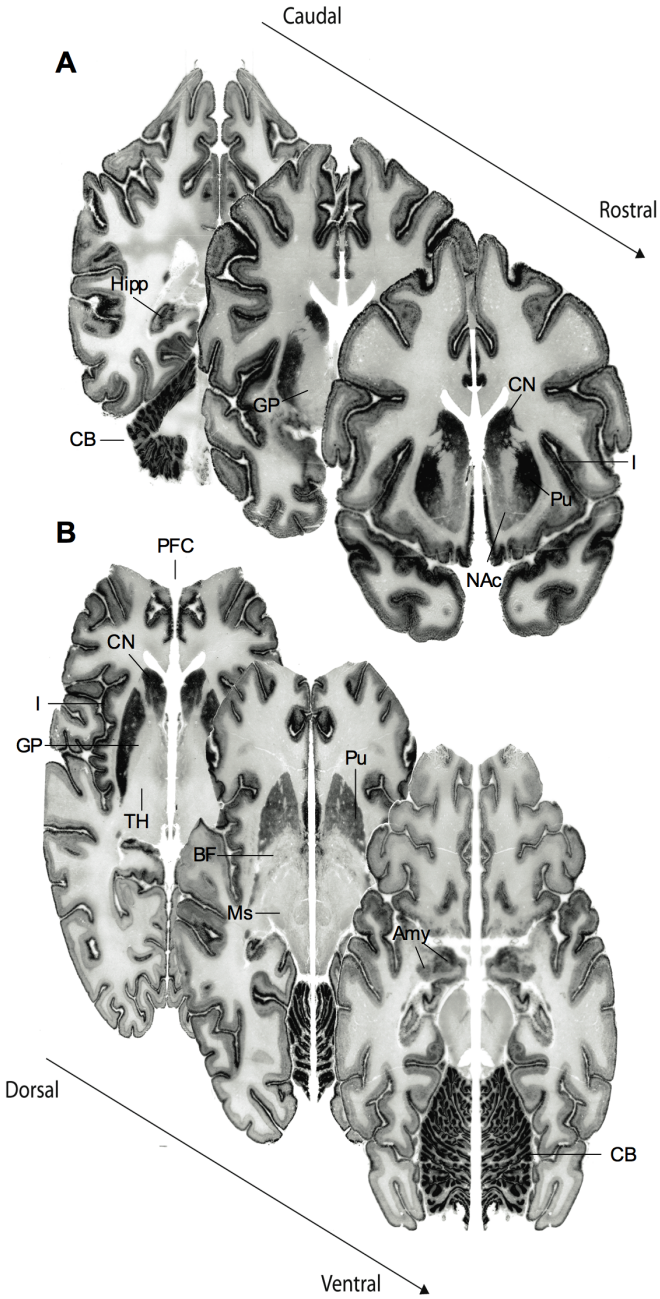


Figure 2. Schematic illustration of the striatonigral 'Go' andstriatopallidal 'NoGo' pathways. These medium spiny output neurons are distinguishable based on their targets and subcellular markers, namely the expression of D_1R (purple) and D_2R (brown), respectively. Both cell-types, however, express CB_1R (orange). This dissociation is based mainly on the dorsal striatal circuit, but a similar organization, particularly with respect to the 'NoGo' pathway, exists for the ventral striatal circuit.

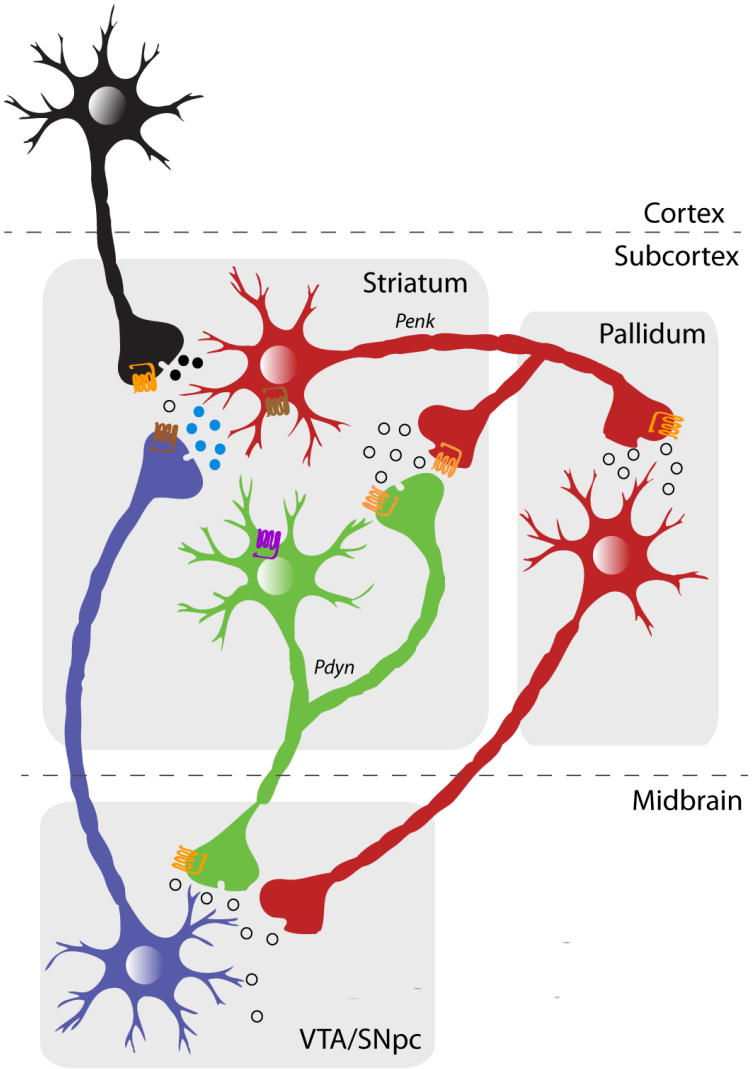


Figure 3. Periodic low-to-moderate THC exposure during adolescence (1.5 mg/kg every third day between postnatal days 28 and 49) alters *Drd2* and *Penk* mRNA expression in the adult nucleus accumbens. These genes are strongly enriched in striatopallidal neurons of the nucleus accumbens (*$p < 0.05$).

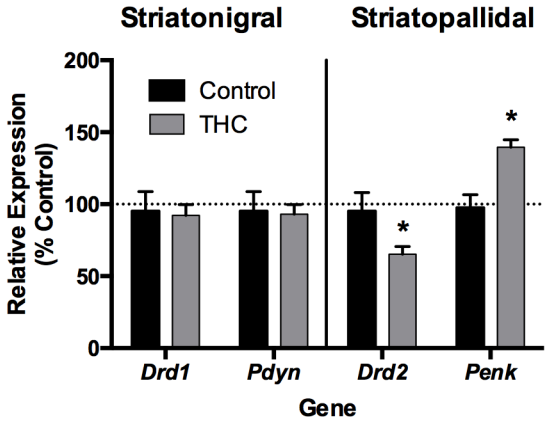


Figure 4. *PENK* SNPs (rs2609997 and rs2576573) are associated with *PENK* expression in the human brain. High-risk alleles for cannabis dependence of the rs2609997 and rs2576573 *PENK* SNPs associate with elevated mRNA expression and met-enkephalin peptide (met-enk) levels in the human striatum (A). Similarly, these alleles associate with elevated mRNA expression in central amygdala nucleus (B). (High-risk genotypes = C/C + C/T for rs2609997 or A/A + A/G for rs2576573; low-risk genotypes = T/T for rs2609997 or G/G for rs2576573. *$p < 0.05$.)

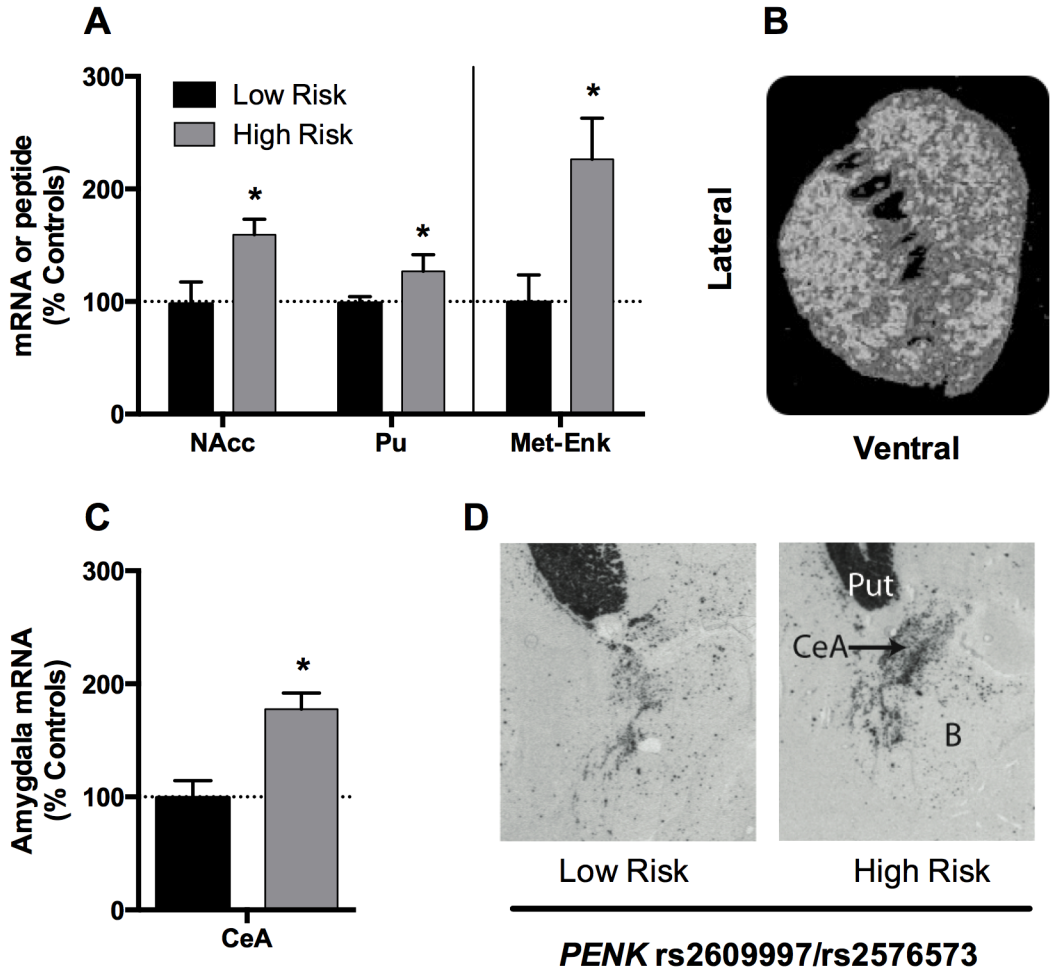


Figure 5. Synergistic Contribution of negative affect trait and *PENK* variants to cannabis dependence vulnerability. (High-risk genotype = C/C + C/T for rs2609997 or A/A + A/G for rs2576573; low-risk genotype = T/T for rs2609997 or G/G for rs2576573. ****p* < 0.01; *****p* < 0.001. Modified from Jutras-Aswad et al., 2012.)

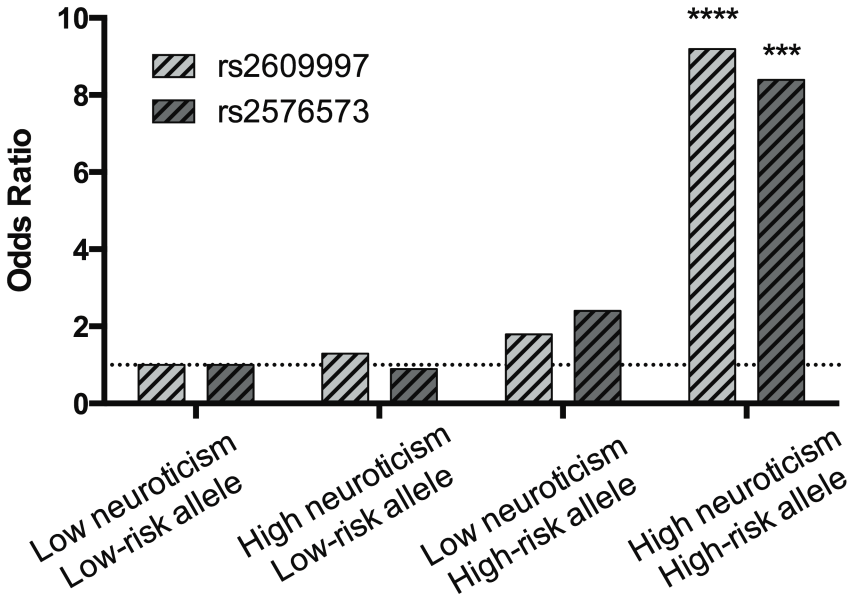
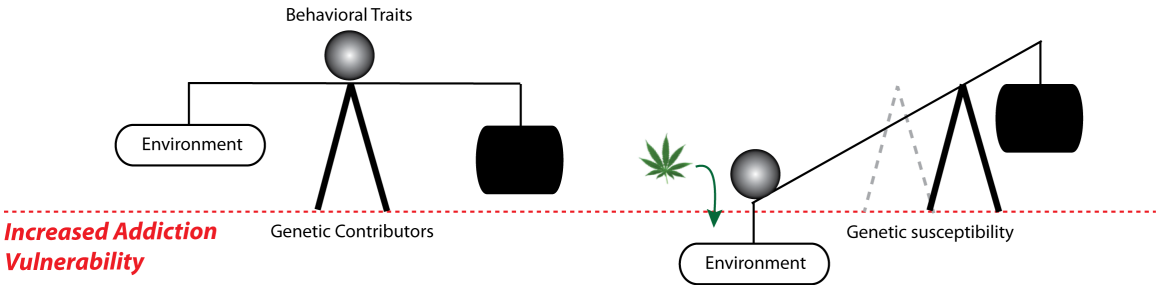


Figure 6. Schematic overview of the interaction between environmental factors, genetics and behavioral traits that together contribute to complex neuropsychiatric disorders like addiction. Vulnerability involves a delicate balance between factors that promote and protect against disease, and adolescent THC, an environmental factor, may tip this balance in teens with high-risk genotypes and behavioral traits.



Agrawal, A., Lynskey, M. T., 2009. Candidate genes for cannabis use disorders: findings, challenges and directions. Addiction 104, 518-532.

Agrawal, A., Neale, M. C., Prescott, C. A., Kendler, K. S., 2004. Cannabis and other illicit drugs: comorbid use and abuse/dependence in males and females. Behav Genet 34, 217-228.

Agrawal, A., Wetherill, L., Dick, D. M., Xuei, X., Hinrichs, A., Hesselbrock, V., Kramer, J., Nurnberger, J. I., Jr., Schuckit, M., Bierut, L. J., Edenberg, H. J., Foroud, T., 2009. Evidence for association between polymorphisms in the cannabinoid receptor 1 (CNR1) gene and cannabis dependence. Am J Med Genet B Neuropsychiatr Genet 150B, 736-740.

Arendt, M., Rosenberg, R., Fjordback, L., Brandholdt, J., Foldager, L., Sher, L., Munk-Jorgensen, P., 2007. Testing the self-medication hypothesis of depression and aggression in cannabis-dependent subjects. Psychol Med 37, 935-945.

Belue, R. C., Howlett, A. C., Westlake, T. M., Hutchings, D. E., 1995. The ontogeny of cannabinoid receptors in the brain of postnatal and aging rats. Neurotoxicol Teratol 17, 25-30.

Biegon, A., Kerman, I. A., 2001. Autoradiographic study of pre- and postnatal distribution of cannabinoid receptors in human brain. Neuroimage 14, 1463-1468.

Biscaia, M., Fernandez, B., Higuera-Matas, A., Miguens, M., Viveros, M. P., Garcia-Lecumberri, C., Ambrosio, E., 2008. Sex-dependent effects of periadolescent exposure to the cannabinoid agonist CP-55,940 on morphine self-administration behaviour and the endogenous opioid system. Neuropharmacology 54, 863-873.

Biscaia, M., Marin, S., Fernandez, B., Marco, E. M., Rubio, M., Guaza, C., Ambrosio, E., Viveros, M. P., 2003. Chronic treatment with CP 55,940 during the peri-adolescent period differentially affects the behavioural responses of male and female rats in adulthood. Psychopharmacology (Berl) 170, 301-308.

Blume, L. C., Bass, C. E., Childers, S. R., Dalton, G. D., Roberts, D. C., Richardson, J. M., Xiao, R., Selley, D. E., Howlett, A. C., 2013. Striatal CB1 and D2 receptors regulate expression of each other, CRIP1A and delta opioid systems. J Neurochem 124, 808- 820.

Brook, J. S., Balka, E. B., Whiteman, M., 1999a. The risks for late adolescence of early adolescent marijuana use. Am J Public Health 89, 1549-1554.

Brook, J. S., Richter, L., Whiteman, M., Cohen, P., 1999b. Consequences of adolescent marijuana use: incompatibility with the assumption of adult roles. Genet Soc Gen Psychol Monogr 125, 193-207.

Canals, M., Milligan, G., 2008. Constitutive activity of the cannabinoid CB1 receptor regulates the function of co-expressed Mu opioid receptors. J Biol Chem 283, 11424- 11434.

Ciccocioppo, R., Antonelli, L., Biondini, M., Perfumi, M., Pompei, P., Massi, M., 2002. Memory impairment following combined exposure to delta(9)-tetrahydrocannabinol and ethanol in rats. Eur J Pharmacol 449, 245-252.

Cleveland, H. H., Wiebe, R. P., 2008. Understanding the association between adolescent marijuana use and later serious drug use: gateway effect or developmental trajectory? Dev Psychopathol 20, 615-632.

Corchero, J., Garcia-Gil, L., Manzanares, J., Fernandez-Ruiz, J. J., Fuentes, J. A., Ramos, J. A., 1998. Perinatal delta9-tetrahydrocannabinol exposure reduces proenkephalin gene expression in the caudate-putamen of adult female rats. Life Sci 63, 843-850.

Corchero, J., Romero, J., Berrendero, F., Fernandez-Ruiz, J., Ramos, J. A., Fuentes, J. A., Manzanares, J., 1999. Time-dependent differences of repeated administration with Delta9-tetrahydrocannabinol in proenkephalin and cannabinoid receptor gene expression and G-protein activation by mu-opioid and CB1-cannabinoid receptors in the caudate-putamen. Brain Res Mol Brain Res 67, 148-157.

Creemers, H. E., Harakeh, Z., Dick, D. M., Meyers, J., Vollebergh, W. A., Ormel, J., Verhulst, F. C., Huizink, A. C., 2011. DRD2 and DRD4 in relation to regular alcohol and cannabis use among adolescents: does parenting modify the impact of genetic vulnerability? The TRAILS study. Drug Alcohol Depend 115, 35-42.

DiNieri, J. A., Wang, X., Szutorisz, H., Spano, S. M., Kaur, J., Casaccia, P., Dow-Edwards, D., Hurd, Y. L., 2011. Maternal cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring. Biological psychiatry 70, 763-769.

Dorard, G., Berthoz, S., Phan, O., Corcos, M., Bungener, C., 2008. Affect dysregulation in cannabis abusers: a study in adolescents and young adults. European child & adolescent psychiatry 17, 274-282.

Dow-Edwards, D., Izenwasser, S., 2012. Pretreatment with Delta9- tetrahydrocannabinol (THC) increases cocaine-stimulated activity in adolescent but not adult male rats. Pharmacology, biochemistry, and behavior 100, 587-591.

Durieux, P. F., Bearzatto, B., Guiducci, S., Buch, T., Waisman, A., Zoli, M., Schiffmann, S. N., de Kerchove d'Exaerde, A., 2009. D2R striatopallidal neurons inhibit both locomotor and drug reward processes. Nat Neurosci 12, 393-395.

Ellgren, M., Artmann, A., Tkalych, O., Gupta, A., Hansen, H. S., Hansen, S. H., Devi, L. A., Hurd, Y. L., 2008. Dynamic changes of the endogenous cannabinoid and opioid mesocorticolimbic systems during adolescence: THC effects. Eur Neuropsychopharmacol 18, 826-834.

Ellgren, M., Hurd, Y. L., Franck, J., 2004. Amphetamine effects on dopamine levels and behavior following cannabinoid exposure during adolescence. Eur J Pharmacol 497, 205-213.

Ellgren, M., Spano, S. M., Hurd, Y. L., 2007. Adolescent cannabis exposure alters opiate intake and opioid limbic neuronal populations in adult rats. Neuropsychopharmacology 32, 607-615.

Ferdinand, R. F., Sondeijker, F., van der Ende, J., Selten, J. P., Huizink, A., Verhulst, F. C., 2005. Cannabis use predicts future psychotic symptoms, and vice versa. Addiction 100, 612-618.

Fergusson, D. M., Boden, J. M., 2008. Cannabis use and later life outcomes. Addiction 103, 969-976; discussion 977-968.

Fergusson, D. M., Boden, J. M., Horwood, L. J., 2006. Cannabis use and other illicit drug use: testing the cannabis gateway hypothesis. Addiction 101, 556-569.
Fergusson, D. M., Horwood, L. J., 2000. Does cannabis use encourage other forms of illicit drug use? Addiction 95, 505-520.
Fergusson, D. M., Horwood, L. J., Swain-Campbell, N., 2002. Cannabis use and psychosocial adjustment in adolescence and young adulthood. Addiction 97, 1123- 1135.
Frank, M. J., Hutchison, K., 2009. Genetic contributions to avoidance-based decisions: striatal D2 receptor polymorphisms. Neuroscience 164, 131-140.
Frank, M. J., Moustafa, A. A., Haughey, H. M., Curran, T., Hutchison, K. E., 2007. Genetic triple dissociation reveals multiple roles for dopamine in reinforcement learning. Proceedings of the National Academy of Sciences of the United States of America 104, 16311-16316.
Gerard, C. M., Mollereau, C., Vassart, G., Parmentier, M., 1991. Molecular cloning of a human cannabinoid receptor which is also expressed in testis. Biochem J 279 (Pt 1), 129-134.
Gerfen, C. R., Enber, T. M., Susel, Z., Chase, T. N., Monsma, F. J., Mahan, L. C., Sibley, D. R., 1990. D1 and D2 dopamine receptor regulated gene expression of striatonigral and striatopallidal neurons. Science 250, 1429-1432.
Gerfen, C. R., Young III, W. S., 1988. Distributution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. Brain Res 460, 161-167.
Glass, M., Dragnunow, M., Faull, R. L., 1997. Cannabinoid receptors in the human brain: a detailed anatomical quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neurosci. 77, 299-318.
Gong, J. P., Onaivi, E. S., Ishiguro, H., Liu, Q. R., Tagliaferro, P. A., Brusco, A., Uhl, G. R., 2006. Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. Brain Res 1071, 10-23.
Griffin, G., Tao, Q., Abood, M. E., 2000. Cloning and pharmacological characterization of the rat CB(2) cannabinoid receptor. J Pharmacol Exp Ther 292, 886-894.
Hall, W. D., Lynskey, M., 2005. Is cannabis a gateway drug? Testing hypotheses about the relationship between cannabis use and the use of other illicit drugs. Drug Alcohol Rev 24, 39-48.
Harkany, T., Keimpema, E., Barabas, K., Mulder, J., 2008. Endocannabinoid functions controlling neuronal specification during brain development. Mol Cell Endocrinol 286, S84-90.
Hayatbakhsh, M. R., Najman, J. M., Jamrozik, K., Mamun, A. A., Alati, R., Bor, W., 2007. Cannabis and anxiety and depression in young adults: a large prospective study. J Am Acad Child Adolesc Psychiatry 46, 408-417.
Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grusser, S. M., Flor, H., Braus, D. F., Buchholz, H. G., Grunder, G., Schreckenberger, M., Smolka, M. N., Rosch, F., Mann, K., Bartenstein, P., 2004. Correlation between dopamine D(2)

receptors in the ventral striatum and central processing of alcohol cues and craving. Am J Psychiatry 161, 1783-1789.
Heng, L., Beverley, J. A., Steiner, H., Tseng, K. Y., 2011. Differential developmental trajectories for CB1 cannabinoid receptor expression in limbic/associative and sensorimotor cortical areas. Synapse 65, 278-286.
Herkenham, M., Lynn, A., Little, M., Johnson, M., Melvin, L., De Costa, B., Rice, K., 1990. Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. USA 87, 1932.
Herkenham, M., Lynn, A. B., de Costa, B. R., Richfield, E. K., 1991a. Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. Brain Res 547, 267-274.
Herkenham, M., Lynn, A. B., Johnson, M. R., Melvin, L. S., de Costa, B. R., Rice, K. C., 1991b. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. The Journal of neuroscience : the official journal of the Society for Neuroscience 11, 563-583.
Hermanson, D. J., Marnett, L. J., 2011. Cannabinoids, endocannabinoids, and cancer. Cancer Metastasis Rev 30, 599-612.
Higuera-Matas, A., Soto-Montenegro, M. L., del Olmo, N., Miguens, M., Torres, I., Vaquero, J. J., Sanchez, J., Garcia-Lecumberri, C., Desco, M., Ambrosio, E., 2008. Augmented acquisition of cocaine self-administration and altered brain glucose metabolism in adult female but not male rats exposed to a cannabinoid agonist during adolescence. Neuropsychopharmacology 33, 806-813.
Hikida, T., Kimura, K., Wada, N., Funabiki, K., Nakanishi, S., 2010. Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior. Neuron 66, 896-907.
Hill, A. J., Williams, C. M., Whalley, B. J., Stephens, G. J., 2012. Phytocannabinoids as novel therapeutic agents in CNS disorders. Pharmacol Ther 133, 79-97.
Hill, E. L., Gallopin, T., Ferezou, I., Cauli, B., Rossier, J., Schweitzer, P., Lambolez, B., 2007. Functional CB1 receptors are broadly expressed in neocortical GABAergic and glutamatergic neurons. J Neurophysiol 97, 2580-2589.
Hopfer, C. J., Young, S. E., Purcell, S., Crowley, T. J., Stallings, M. C., Corley, R. P., Rhee, S. H., Smolen, A., Krauter, K., Hewitt, J. K., Ehringer, M. A., 2006. Cannabis receptor haplotype associated with fewer cannabis dependence symptoms in adolescents. Am J Med Genet B Neuropsychiatr Genet 141B, 895-901.
Howlett, A. C., Barth, F., Bonner, T. I., Cabral, G., Casellas, P., Devane, W. A., Felder, C. C., Herkenham, M., Mackie, K., Martin, B. R., Mechoulam, R., Pertwee, R. G., 2002. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 54, 161-202.
Hyman, S. M., Sinha, R., 2009. Stress-related factors in cannabis use and misuse: implications for prevention and treatment. J Subst Abuse Treat 36, 400-413.
Johnston, L. D., O'Malley, P. M., Bachman, J. G., Schulenberg, J. E., 2012. Monitoring the Future national results on adolescent drug use: Overview of key findings, 2011. Institute for Social Research, The University of Michigan., Ann Arbor.
Jutras-Aswad, D., Jacobs, M. M., Yiannoulos, G., Roussos, P., Bitsios, P., Nomura, Y., Liu, X., Hurd, Y. L., 2012. Cannabis-dependence risk relates to synergism between

neuroticism and proenkephalin SNPs associated with amygdala gene expression: case-control study. PloS one 7, e39243.
Kandel, D., 1975. Stages in adolescent involvement in drug use. Science 190, 912- 914.
Kandel, D. B., Yamaguchi, K., Klein, L. C., 2006. Testing the Gateway Hypothesis. Addiction 101, 470-472; discussion 474-476.
Kang, W., Wilson, S. P., Wilson, M. A., 2000. Overexpression of proenkephalin in the amygdala potentiates the anxiolytic effects of benzodiazepines. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 22, 77-88.
Katona, I., Freund, T. F., 2008. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. Nat Med 14, 923-930.
Kelley, A. E., Bakshi, V. P., Haber, S. N., Steininger, T. L., Will, M. J., Zhang, M., 2002. Opioid modulation of taste hedonics within the ventral striatum. Physiol Behav 76, 365-377.
Kendler, K. S., Karkowski, L. M., Neale, M. C., Prescott, C. A., 2000. Illicit psychoactive substance use, heavy use, abuse, and dependence in a US population-based sample of male twins. Arch Gen Psychiatry 57, 261-269.
Kendler, K. S., Prescott, C. A., 1998. Cannabis use, abuse, and dependence in a population-based sample of female twins. Am J Psychiatry 155, 1016-1022.
Kilmer, J. R., Hunt, S. B., Lee, C. M., Neighbors, C., 2007. Marijuana use, risk perception, and consequences: is perceived risk congruent with reality? Addict Behav 32, 3026-3033.
Klein, T. A., Neumann, J., Reuter, M., Hennig, J., von Cramon, D. Y., Ullsperger, M., 2007. Genetically determined differences in learning from errors. Science 318, 1642-1645.
Koskinen, J., Lohonen, J., Koponen, H., Isohanni, M., Miettunen, J., 2010. Rate of cannabis use disorders in clinical samples of patients with schizophrenia: a meta-analysis. Schizophr Bull 36, 1115-1130.
Kung, J. C., Chen, T. C., Shyu, B. C., Hsiao, S., Huang, A. C., 2010. Anxiety- and depressive-like responses and c-fos activity in preproenkephalin knockout mice: oversensitivity hypothesis of enkephalin deficit-induced posttraumatic stress disorder. J Biomed Sci 17, 29.
Lanciego, J. L., Barroso-Chinea, P., Rico, A. J., Conte-Perales, L., Callen, L., Roda, E., Gomez-Bautista, V., Lopez, I. P., Lluis, C., Labandeira-Garcia, J. L., Franco, R., 2011. Expression of the mRNA coding the cannabinoid receptor 2 in the pallidal complex of Macaca fascicularis. J Psychopharmacol 25, 97-104.
Le Moine, C., Normand, E., Guitteny, A. F., Fouque, B., Teoule, R., Bloch, B., 1990. Dopamine receptor gene expression by enkephalin neurons in rat forebrain. Proc. Natl. Acad. Sci. USA 87, 230-234.
Lessem, J. M., Hopfer, C. J., Haberstick, B. C., Timberlake, D., Ehringer, M. A., Smolen, A., Hewitt, J. K., 2006. Relationship between adolescent marijuana use and young adult illicit drug use. Behav Genet 36, 498-506.

Lopez-Quintero, C., Neumark, Y., 2010. Effects of risk perception of marijuana use on marijuana use and intentions to use among adolescents in Bogota, Colombia. Drug Alcohol Depend 109, 65-72.

Lynskey, M. T., Coffey, C., Degenhardt, L., Carlin, J. B., Patton, G., 2003. A longitudinal study of the effects of adolescent cannabis use on high school completion. Addiction 98, 685-692.

Maccoun, R. J., 2006. Competing accounts of the gateway effect: the field thins, but still no clear winner. Addiction 101, 473-474; discussion 474-476.

Maes, H. H., Woodard, C. E., Murrelle, L., Meyer, J. M., Silberg, J. L., Hewitt, J. K., Rutter, M., Simonoff, E., Pickles, A., Carbonneau, R., Neale, M. C., Eaves, L. J., 1999. Tobacco, alcohol and drug use in eight- to sixteen-year-old twins: the Virginia Twin Study of Adolescent Behavioral Development. J Stud Alcohol 60, 293-305.

Mailleux, P., Parmentier, M., Vanderhaeghen, J. J., 1992. Distribution of cannabinoid receptor messenger RNA in the human brain: an in situ hybridization histochemistry with oligonucleotides. Neurosci Lett 143, 200-204.

Marsicano, G., Lutz, B., 1999. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. The European journal of neuroscience 11, 4213-4225.

Mato, S., Del Olmo, E., Pazos, A., 2003. Ontogenetic development of cannabinoid receptor expression and signal transduction functionality in the human brain. The European journal of neuroscience 17, 1747-1754.

Matsuda, L. A., Bonner, T. I., Lolait, S. J., 1993. Localization of cannabinoid receptor mRNA in rat brain. J Comp Neurol 327, 535-550.

Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., Bonner, T. I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561-564.

McGue, M., Elkins, I., Iacono, W. G., 2000. Genetic and environmental influences on adolescent substance use and abuse. Am J Med Genet 96, 671-677.

McLaughlin, C. R., Martin, B. R., Compton, D. R., Abood, M. E., 1994. Cannabinoid receptors in developing rats: detection of mRNA and receptor binding. Drug and Alcohol Dependence 36, 27-31.

Michaelides, M., Thanos, P. K., Kim, R., Cho, J., Ananth, M., Wang, G. J., Volkow, N. D., 2012. PET imaging predicts future body weight and cocaine preference. Neuroimage 59, 1508-1513.

Miles, D. R., van den Bree, M. B., Gupman, A. E., Newlin, D. B., Glantz, M. D., Pickens, R. W., 2001. A twin study on sensation seeking, risk taking behavior and marijuana use. Drug Alcohol Depend 62, 57-68.

Morel, L. J., Giros, B., Dauge, V., 2009. Adolescent exposure to chronic delta-9-tetrahydrocannabinol blocks opiate dependence in maternally deprived rats. Neuropsychopharmacology 34, 2469-2476.

Morgan, D., Grant, K. A., Gage, H. D., Mach, R. H., Kaplan, J. R., Prioleau, O., Nader, S. H., Buchheimer, N., Ehrenkaufer, R. L., Nader, M. A., 2002. Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. Nat Neurosci 5, 169-174.

Mulder, J., Aguado, T., Keimpema, E., Barabas, K., Ballester Rosado, C. J., Nguyen, L., Monory, K., Marsicano, G., Di Marzo, V., Hurd, Y. L., Guillemot, F., Mackie, K., Lutz, B., Guzman, M., Lu, H. C., Galve-Roperh, I., Harkany, T., 2008. Endocannabinoid signaling controls pyramidal cell specification and long-range axon patterning. Proceedings of the National Academy of Sciences of the United States of America 105, 8760-8765.

Munro, S., Thomas, K. L., Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. Nature 365, 61-65.

Nader, M. A., Morgan, D., Gage, H. D., Nader, S. H., Calhoun, T. L., Buchheimer, N., Ehrenkaufer, R., Mach, R. H., 2006. PET imaging of dopamine D2 receptors during chronic cocaine self-administration in monkeys. Nat Neurosci.

Onaivi, E. S., Ishiguro, H., Gong, J. P., Patel, S., Perchuk, A., Meozzi, P. A., Myers, L., Mora, Z., Tagliaferro, P., Gardner, E., Brusco, A., Akinshola, B. E., Liu, Q. R., Hope, B., Iwasaki, S., Arinami, T., Teasenfitz, L., Uhl, G. R., 2006. Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. Annals of the New York Academy of Sciences 1074, 514-536.

Panlilio, L. V., Solinas, M., Matthews, S. A., Goldberg, S. R., 2007. Previous exposure to THC alters the reinforcing efficacy and anxiety-related effects of cocaine in rats. Neuropsychopharmacology 32, 646-657.

Patton, G. C., Coffey, C., Carlin, J. B., Degenhardt, L., Lynskey, M., Hall, W., 2002. Cannabis use and mental health in young people: cohort study. Bmj 325, 1195-1198.

Perez-Rosado, A., Manzanares, J., Fernandez-Ruiz, J., Ramos, J. A., 2000. Prenatal Delta(9)-tetrahydrocannabinol exposure modifies proenkephalin gene expression in the fetal rat brain: sex-dependent differences. Brain Res Dev Brain Res 120, 77-81.

Pettit, D. A., Harrison, M. P., Olson, J. M., Spencer, R. F., Cabral, G. A., 1998. Immunohistochemical localization of the neural cannabinoid receptor in rat brain. J Neurosci Res 51, 391-402.

Pistis, M., Perra, S., Pillolla, G., Melis, M., Muntoni, A. L., Gessa, G. L., 2004. Adolescent exposure to cannabinoids induces long-lasting changes in the response to drugs of abuse of rat midbrain dopamine neurons. Biological psychiatry 56, 86-94.

Rhee, S. H., Hewitt, J. K., Young, S. E., Corley, R. P., Crowley, T. J., Stallings, M. C., 2003. Genetic and environmental influences on substance initiation, use, and problem use in adolescents. Arch Gen Psychiatry 60, 1256-1264.

Rodriguez de Fonseca, F., Ramos, J. A., Bonnin, A., Fernández-Ruiz, J. J., 1993. Presence of cannabinoid binding sites in the brain from early postnatal ages. Neuroreport 4, 135-138.

Sakai, J. T., Hopfer, C. J., Hartman, C., Haberstick, B. C., Smolen, A., Corley, R. P., Stallings, M. C., Young, S. E., Timberlake, D., Hewitt, J. K., Crowley, T. J., 2007. Test of association between TaqIA A1 allele and alcohol use disorder phenotypes in a sample of adolescent patients with serious substance and behavioral problems. Drug Alcohol Depend 88, 130-137.

SAMHSA, 2011. Results from the 2010 National Survey on Drug Use and Health: National Findings. Office of Applied Studies, NSDUH Series H-41, HHS Publication No. (SMA) 11-4658, Rockville, MD.

Sano, H., Yasoshima, Y., Matsushita, N., Kaneko, T., Kohno, K., Pastan, I., Kobayashi, K., 2003. Conditional ablation of striatal neuronal types containing dopamine D2 receptor disturbs coordination of basal ganglia function. J Neurosci 23, 9078-9088.

Singh, M. E., McGregor, I. S., Mallet, P. E., 2006. Perinatal exposure to delta(9)-tetrahydrocannabinol alters heroin-induced place conditioning and fos-immunoreactivity. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 31, 58-69.

Skoubis, P. D., Lam, H. A., Shoblock, J., Narayanan, S., Maidment, N. T., 2005. Endogenous enkephalins, not endorphins, modulate basal hedonic state in mice. The European journal of neuroscience 21, 1379-1384.

Spano, M. S., Ellgren, M., Wang, X., Hurd, Y. L., 2007. Prenatal cannabis exposure increases heroin seeking with allostatic changes in limbic enkephalin systems in adulthood. Biological psychiatry 61, 554-563.

Spano, M. S., Fadda, P., Fratta, W., Fattore, L., 2010. Cannabinoid-opioid interactions in drug discrimination and self-administration: effect of maternal, postnatal, adolescent and adult exposure to the drugs. Curr Drug Targets 11, 450-461.

Tarter, R. E., Vanyukov, M., Kirisci, L., Reynolds, M., Clark, D. B., 2006. Predictors of marijuana use in adolescents before and after licit drug use: examination of the gateway hypothesis. Am J Psychiatry 163, 2134-2140.

Thanos, P. K., Michaelides, M., Benveniste, H., Wang, G. J., Volkow, N. D., 2008. The effects of cocaine on regional brain glucose metabolism is attenuated in dopamine transporter knockout mice. Synapse 62, 319-324.

Tomasiewicz, H. C., Jacobs, M. M., Wilkinson, M. B., Wilson, S. P., Nestler, E. J., Hurd, Y. L., 2012. Proenkephalin mediates the enduring effects of adolescent cannabis exposure associated with adult opiate vulnerability. Biological psychiatry 72, 803- 810.

Tsuang, M. T., Lyons, M. J., Meyer, J. M., Doyle, T., Eisen, S. A., Goldberg, J., True, W., Lin, N., Toomey, R., Eaves, L., 1998. Co-occurrence of abuse of different drugs in men: the role of drug-specific and shared vulnerabilities. Arch Gen Psychiatry 55, 967-972.

Tucker, J. S., Ellickson, P. L., Collins, R. L., Klein, D. J., 2006. Are drug experimenters better adjusted than abstainers and users?: a longitudinal study of adolescent marijuana use. J Adolesc Health 39, 488-494.

Tyndale, R. F., Payne, J. I., Gerber, A. L., Sipe, J. C., 2007. The fatty acid amide hydrolase C385A (P129T) missense variant in cannabis users: studies of drug use and dependence in Caucasians. Am J Med Genet B Neuropsychiatr Genet 144B, 660-666.

Valverde, O., Noble, F., Beslot, F., Dauge, V., Fournie-Zaluski, M. C., Roques, B. P., 2001. Delta9-tetrahydrocannabinol releases and facilitates the effects of endogenous

enkephalins: reduction in morphine withdrawal syndrome without change in rewarding effect. Eur J Neurosci 13, 1816-1824.

van den Bree, M. B., Johnson, E. O., Neale, M. C., Pickens, R. W., 1998. Genetic and environmental influences on drug use and abuse/dependence in male and female twins. Drug Alcohol Depend 52, 231-241.

van Gastel, W. A., Maccabe, J. H., Schubart, C. D., Vreeker, A., Tempelaar, W., Kahn, R. S., Boks, M. P., 2013. Cigarette smoking and cannabis use are equally strongly associated with psychotic-like experiences: a cross-sectional study in 1929 young adults. Psychol Med, 1-9.

Van Sickle, M. D., Duncan, M., Kingsley, P. J., Mouihate, A., Urbani, P., Mackie, K., Stella, N., Makriyannis, A., Piomelli, D., Davison, J. S., Marnett, L. J., Di Marzo, V., Pittman, Q. J., Patel, K. D., Sharkey, K. A., 2005. Identification and functional characterization of brainstem cannabinoid CB2 receptors. Science 310, 329-332.

Van Waes, V., Beverley, J. A., Siman, H., Tseng, K. Y., Steiner, H., 2012. CB1 Cannabinoid Receptor Expression in the Striatum: Association with Corticostriatal Circuits and Developmental Regulation. Front Pharmacol 3, 21.

Verdurand, M., Nguyen, V., Stark, D., Zahra, D., Gregoire, M. C., Greguric, I., Zavitsanou, K., 2011. Comparison of Cannabinoid CB(1) Receptor Binding in Adolescent and Adult Rats: A Positron Emission Tomography Study Using [F]MK- 9470. Int J Mol Imaging 2011, 548123.

Vigano, D., Rubino, T., Parolaro, D., 2005. Molecular and cellular basis of cannabinoid and opioid interactions. Pharmacology, biochemistry, and behavior 81, 360-368.

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Ding, Y. S., Sedler, M., Logan, J., Franceschi, D., Gatley, J., Hitzemann, R., Gifford, A., Wong, C., Pappas, N., 2001. Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. Am J Psychiatry 158, 2015-2021.

Volkow, N. D., Fowler, J. S., Wang, G. J., Swanson, J. M., 2004. Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. Molecular psychiatry 9, 557-569.

Volkow, N. D., Wang, G. J., Fowler, J. S., Logan, J., Gatley, S. J., Gifford, A., Hitzemann, R., Ding, Y. S., Pappas, N., 1999. Prediction of reinforcing responses to psychostimulants in humans by brain dopamine D2 receptor levels. Am J Psychiatry 156, 1440-1443.

Wang, G. J., Volkow, N. D., Fowler, J. S., Logan, J., Abumrad, N. N., Hitzemann, R. J., Pappas, N. S., Pascani, K., 1997. Dopamine D2 receptor availability in opiate-dependent subjects before and after naloxone-precipitated withdrawal. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 16, 174-182.

Wang, X., Dow-Edwards, D., Andersen, V., Minkoff, H., Hurd, Y. L., 2006. Discrete opioid gene expression impairment in the human fetal brain associated with maternal marijuana use. Pharmacogenomics J 6.

Wang, X., Dow-Edwards, D., Keller, E., Hurd, Y. L., 2003. Preferential limbic expression of the cannabinoid receptor mRNA in the human fetal brain. Neuroscience 118, 681-694.

Yamaguchi, K., Kandel, D. B., 1984. Patterns of drug use from adolescence to young adulthood: III. Predictors of progression. Am J Public Health 74, 673-681.

Zvolensky, M. J., Vujanovic, A. A., Bernstein, A., Bonn-Miller, M. O., Marshall, E. C., Leyro, T. M., 2007. Marijuana use motives: A confirmatory test and evaluation among young adult marijuana users. Addict Behav 32, 3122-3130.