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What Can Rats Tell Us about Adolescent Cannabis Exposure? Insights from Preclinical Research

Que peuvent nous dire les rats sur l'exposition des adolescents au cannabis ? Aperçus de la recherche préclinique

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Abstract

Marijuana is the most widely used drug of abuse among adolescents. Adolescence is a vulnerable period for brain development, during which time various neurotransmitter systems such as the glutamatergic, GABAergic, dopaminergic, and endocannabinoid systems undergo extensive reorganization to support the maturation of the central nervous system (CNS). Δ-9-tetrahydrocannabinol (THC), the psychoactive component of marijuana, acts as a partial agonist of CB1 cannabinoid receptors (CB1Rs). CB1Rs are abundant in the CNS and are central components of the neurodevelopmental changes that occur during adolescence. Thus, overactivation of CB1Rs by cannabinoid exposure during adolescence has the ability to dramatically alter brain maturation, leading to persistent and enduring changes in adult cerebral function. Increasing preclinical evidence lends support to clinical evidence suggesting that chronic adolescent marijuana exposure may be associated with a higher risk for neuropsychiatric diseases, including schizophrenia. In this review, we present a broad overview of current neurobiological evidence regarding the long-term consequences of adolescent cannabinoid exposure on adult neuropsychiatric-like disorders.

Abrégé

La marijuana est la drogue d'abus la plus largement utilisée chez les adolescents. L'adolescence est une période vulnérable pour le développement du cerveau, où divers systèmes neurotransmetteurs comme le système glutamatergique, GABAergique, dopaminergique, et endocannabinoïde subissent une réorganisation importante afin de soutenir la maturation du système nerveux central (SNC). Le Δ-9-tétrahydrocannabinol (THC), le composant psychoactif, sert d'agoniste partiel des récepteurs cannabinoïdes CB1 (RCB1). Les RCB1 sont abondants dans le SNC et sont des composantes centrales des changements neurodéveloppementaux qui surviennent à l'adolescence. Ainsi, la sur-activation des RCB1 par l'exposition aux cannabinoïdes durant l'adolescence a la capacité de radicalement altérer la maturation du cerveau, entraînant des changements persistants et durables dans la fonction cérébrale adulte. De plus en plus de données probantes précliniques soutiennent les données probantes cliniques qui suggèrent que l'exposition chronique des adolescents à la marijuana peut être associée à un risque plus élevé de maladies neuropsychiatriques, dont la schizophrénie. Dans cette revue, nous présentons un vaste aperçu des données probantes neurobiologiques des connaissances actuelles en ce qui concerne les conséquences à long terme de l'exposition des adolescents aux cannabinoïdes sur les troubles adultes de nature neuropsychiatrique.

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Consistent with findings in human clinical studies, evidence from preclinical, basic neuroscience research has demonstrated that exposure to cannabinoids during vulnerable periods of adolescent neurodevelopment may increase the risk of neuropsychiatric-like disturbances in later adulthood.[1] Indeed, using animal models to study adolescent vulnerability to cannabinoids has several advantages over retrospective clinical reports. First, experimentally designed cannabinoid exposure protocols can precisely control the age and amount of exposure to specific cannabis-related phytochemicals, such as tetrahydrocannabinol (THC). Second, we can choose specific time points after exposure to measure neuropsychiatric-like behavioural, cognitive, or neural adaptations caused by the exposure. Third, once the specific effects of cannabinoid exposure on these neural and behavioural phenomena are identified, we can mechanistically investigate how experimental manipulations of these neuroadaptations may prevent and/or reverse the potential deleterious effects of adolescent cannabinoid exposure.

In rodents, the adolescent period is defined as starting around postnatal day 28 (PND28)[2] and is finished when animals reach full sexual maturity at PND60. Thus, in rodents, adolescence ranges from PND28 to PND60 and can be subdivided into specific phases such as early adolescence (beginning around PND28), middle adolescence (beginning around PND38), and late adolescence (beginning around PND49). For studying the long-term effects of adolescent cannabinoid exposure in rodents, 3 different agonists for the cannabinoid receptor type 1 (CB1R) are generally used: Δ9-THC (THC), the main psychoactive component of marijuana, and the synthetic cannabinoids WIN55,212-2 (WIN) and CP55,940 (CP). THC acts as a partial agonist for CB1R, while CP and WIN are full CB1R agonists.[3]

Modelling Psychotic-like Symptoms in Preclinical Exposure Protocols

In terms of using preclinical animal models of schizophrenia-like behavioural, cognitive, and neuronal abnormalities, animal studies have focused on assays such as prepulse inhibition (PPI) and hyperlocomotor activity. PPI is a classic preclinical model of sensorimotor gating that measures the ability to filter out insignificant sensory information, a cognitive abnormality also seen in schizophrenia. Indeed, PPI impairment and hyperagitation are endophenotypes of psychotic disorders with high translational validity between humans and rodents.[4-6] Chronic WIN or THC exposure during middle (PND35 to PND45) or late adolescence (PND40 to PND65), but not during adulthood, induced persistent PPI deficits in adult rats.[7-9] This PPI deficit may be associated with hyperdopaminergic (hyper-DAergic) activity in the ventral tegmental area (VTA) observed in adult rats following adolescent THC exposure.[7] Indeed, PPI is thought to be regulated by cortico-limbic-striatopallidal circuitry in which dopamine (DA) transmission plays a central role.[10,11] Furthermore, drugs that stimulate DA release, such as amphetamine (AMPH), cause profound disruptions in PPI.[12] Interestingly, PPI deficits can be reversed by an acute systemic injection with the typical antipsychotic haloperidol, supporting the idea of a dysregulation of the dopaminergic (DAergic) system in adolescent cannabinoid-treated rats.[8] Conversely, other groups reported no long-term changes in PPI following chronic adolescent treatment with THC from PND35 to PND48 in the Lewis strain of rats.[13] This discrepancy may be due to differences in the adolescent cannabinoid period treatment, the cannabinoid agonists, and/or the genetic background of rats used in the different studies.

Hyperlocomotor activity is another classic preclinical model of schizophrenia-like abnormalities that is believed to model dysregulation of mesocorticolimbic DAergic transmission. This can be induced pharmacologically by chronic treatment with DAergic activating drugs such as AMPH and can be modelled in both rodents and humans.[14,15] With respect to locomotor hyperactivity abnormalities related to cannabinoids, results obtained from studies examining the spontaneous locomotor activity of rodents following chronic treatment with cannabinoids during adolescence have generally been inconsistent. For example, using the open field test, investigators have reported either locomotor hyperactivity following adolescent WIN treatment,[9] hypoactivity following adolescent THC treatment,[7,16] or no effects after adolescent THC or CP treatments.[17,18] However, results seem to be more consistent when researchers measure locomotor activity induced by psychoactive drugs such as AMPH or phencyclidine (PCP). Indeed, adolescent THC exposure has been found to increase both PCP-induced locomotor activity[19] and the locomotor response to AMPH challenge in adulthood.[20]

Given the well-established role of DAergic transmission abnormalities in schizophrenia-related symptoms,[21-23] such findings are consistent with the hypothesis that adolescent cannabinoid exposure may dysregulate the mesocorticolimbic DA system, leading to disturbances in DAergic transmission consistent with a schizophrenia-like phenotype.

Effects of Adolescent Cannabinoid Exposure on Measures of Cognition and Memory

In addition to behavioural paradigms that measure sensorimotor gating (PPI) to model neuropsychiatric-like cognitive

filtering disturbances, other preclinical cognitive models have been examined following neurodevelopmental cannabinoid exposure. For example, the object recognition task is based on the natural tendency for rats to explore a novel object more than a familiar one. A reduction of this behaviour is indicative of impairments in working memory. Chronic exposure to various cannabinoid agonists (THC, CP, or WIN) during adolescence (early, middle, and late) in rats of either gender was shown to induce long-term impairments in working memory as evaluated using the object recognition task.[8,19,24-27] Interestingly, similar treatments during adulthood did not produce such long-term deleterious effects.[8,26]

When investigators tested spatial working memory using either the spatial version of the object recognition task (i.e., the object location task), the radial maze, or the Y-maze, deficits in spatial working memory were observed following chronic exposure to different cannabinoids during all stages of adolescence (early, middle, and late) in rats.[26,27,28,29] Similar treatment in adult rats did not induce long-term deficits in spatial working memory.[26] The active place avoidance task has been used to analyze associative learning and memory. In this task, rodents must learn to avoid an environment in which an aversive stimulus (such as a foot-shock) was previously delivered. During the reversal learning of the task, which consists of measuring the flexibility of learning (e.g., a switch in the shock zone location), THC exposure during early adolescence (PND22 to PND40) but not late adolescence (PND41 to PND60) impaired performance in both male and female adult rats.[16] Finally, when investigators used the attentional set-shifting task (a rodent analog of the Wisconsin Card Sort Test in Humans[30]), which allows for the measurement of attention and cognitive flexibility in rats, chronic WIN exposure during late adolescence (PND40 to PND65) induced persistent impairments in cognitive flexibility.[20] Conversely, when the Morris water maze (MWM) has been used to analyze pure spatial learning, results have been less consistent. The MWM is used as a test of spatial learning for rodents where animals have to escape from a large circular pool of water onto a submerged escape platform. The location of the escape platform can be identified using spatial memory. In the spatial version of the task, WIN exposure during middle adolescence (PND27 to PND47) induced impairments in spatial learning and memory in adult male rats tested after a 20-day drug-free period,[31] while similar exposure to WIN during late adolescence (PND45 to PND60) impaired spatial learning only after a 1-day drug-free period.[27] However, no significant persistent alteration in spatial learning was observed following chronic adolescent treatment with either CP or THC in rats of either gender.[32,33]

Overall, these data indicate a greater vulnerability of the adolescent brain to the deleterious cognitive effects of cannabinoids, especially with regard to working memory, spatial working memory, and cognitive flexibility. However, pure spatial learning seems to be less affected by chronic adolescent cannabinoid exposure in the rat, although more studies are required to draw such conclusions.

With respect to the potential neurobiological substrates that may underlie these cognitive deficits, reduced synaptic plasticity has been found in the hippocampus and prefrontal cortex (PFC), 2 brain regions that play a crucial role in learning and memory processes. For example, expression levels of the synaptic plasticity markers VAMP2, synaptophysin, PSD95, β-catenin, mTOR, and P70S6 K were found to be decreased in the hippocampus and/or the frontal cortex of adult rats following adolescent THC or CP exposure.[7,34-37] Reduction in total dendritic length, arborisation, and spine number in the dentate gyrus and/or in the PFC of adult rats treated with THC or CP during adolescence was also observed.[28,34,36] In addition, long-term potentiation (LTP), a process necessary for learning and memory-related synaptic plasticity, was impaired in the hippocampus-PFC pathway of adult rats treated with CP during adolescence.[34] These data strongly suggest that synaptic structure and function in the hippocampus-PFC pathway are dysregulated following adolescent cannabinoid exposure. Finally, a dysregulation in the excitation-inhibition balance (i.e., between glutamatergic and GABAergic signaling), which is crucial for regulating and maintaining proper synaptic activity, is also evident in the hippocampus-PFC pathway. Indeed, chronic adolescent cannabinoid treatment decreased the expression of the GABA transporter 1 gene (GAT-1) and glutamate NMDA receptor levels in the hippocampus,[28,36,38] while reductions in parvalbumin and cholecystokinin-positive GABAergic cells and levels of the GABA synthetic enzyme (GAD67) have been observed in the PFC.[19] Finally, adolescent cannabinoid exposure abolished endocannabinoid-mediated long-term depression and cortical oscillations in the PFC[36,39] and impaired the long-term potentiation of the ventral subicular-nucleus accumbens (NAcc) pathway.[27]

Taken together, these data provide strong evidence for impaired structural and synaptic plasticity in brain regions that play a crucial role in learning and memory processing following adolescent exposure to cannabinoids. Such alterations may be related to the well-established long-term cognitive deficits that may endure well into adulthood following long-term cannabis use.

Adolescent Cannabinoid Exposure and Emotional Dysregulation

Considerable evidence has shown that adolescent exposure to cannabinoids can lead to dysregulation of emotional processing. For example, chronic exposure to various cannabinoid agonists (THC, CP, or WIN) during early to late adolescence increases social anxiety in adulthood measured using the social interaction task.[24,25,40,41] In addition, chronic exposure to high doses of WIN from PND30 to PND50 or THC from PND35 to PND45 induced anxiety-like effects at adulthood measured using the novelty-

suppressed feeding test[42] or the light-dark box test.[7] However, when other anxiety assays are used, such as the open-field and the elevated plus-maze tests (EPM), the data obtained are less consistent, demonstrating either anxiolysis,[9,17,43] anxiogenesis,[37,44] or no effects following adolescent treatment with different cannabinoids. In these latter tests, the anxiolytic effects were more often observed following late-adolescent exposure (PND40 to PND65) whereas anxiogenic effects were observed more often following early to middle adolescence exposure (PND28 to PND45) and were independent of the specific cannabinoid agonist used in the study. The genetic backgrounds of experimental animals can also influence these differential effects. For example, whereas in Lewis strain rats adolescent THC exposure decreased anxiety levels measured using the EPM at adulthood, no anxiety changes were found in the Fisher 344 strain of rats.[43]

While it is difficult to draw firm conclusions regarding the potential long-term effects of adolescent cannabinoid exposure on anxiety levels, research that has examined depressive-like effects of adolescent cannabinoid exposure has generally been more consistent. For example, previous studies have demonstrated that hedonic responses are affected following adolescent cannabinoid exposure. Anhedonia is a core feature of depression and is defined as a generalized loss of interest in normally rewarding stimuli or activities. In preclinical animal models, anhedonia can be measured using assays such as the sucrose preference test, which assesses the animal's interest in seeking out a rewarding drink (sucrose solution) relative to plain water. THC or WIN exposure during adolescence was shown to induced anhedonia-like effects (i.e., decreased sucrose preference) in both male and female rats when measured in adulthood.[18,24,42]

The Forced Swim Test (FST) is a well-established preclinical model used to examine depressive-like phenotypes. It is based on the observation that when rats are exposed to water, after initial intense escape-directed behavior such as swimming, climbing, or diving, they will eventually stop struggling and show passive, immobile behavior.[45] This immobile behavior is believed to reflect resignation/depressive-like behaviour. Adolescent exposure to WIN (PND30 to PND50) or THC (PND35 to PND45) increased immobility times in adult rats.[18,24,42,46] However, chronic WIN exposure in rats during late adolescence (PND45 to PND60) did not induce any long-term deficits in the FST,[47] suggesting once again the important role that the specific cannabinoid exposure period (early and middle adolescence vs. late-adolescence) may play in causing later neuropsychiatric-like symptoms.

With respect to the neurobiological mechanisms that may underlie the effects of emotional dysregulation following adolescent cannabinoid exposure, several candidates have been proposed. For example, hypoactivity of serotoninergic neurons in dorsal raphe nucleus, concomitant with hyperactivity of noradrenergic neurons in the locus coeruleus, was observed in adult rats treated with WIN during adolescence.[42] In addition, following adolescent cannabinoid exposure, other neuroadaptations were found in brain regions involved in the regulation of emotional processing. For example, higher dopamine D1 receptor levels were observed in the NAcc, a brain region central to reward and motivated behaviors, of adult female rats treated with THC during adolescence[46] or adult male rats treated with CP during adolescence.[48] In contrast, higher D2 receptor levels were found in the PFC of adult male rats and in the NAcc of either gender[46] treated with THC during adolescence. In addition, we have recently found evidence for a hyper-DAergic D2 state directly in the PFC following adolescent THC exposure in rats, characterized by alterations in the Akt and GSK-3 signaling pathways and hyperactivity of subcortical DAergic neurons.[7] CB1R expression levels were also found to be reduced in the amygdala, VTA, and NAcc of adult female rats treated with THC during adolescence.[18] The decrease in CB1R expression was accompanied by changes in the activity of the cellular transcription factor CREB in the hippocampus, PFC, and NAcc.[18] Thus, while future studies are required to explore the mechanistic roles associated with these neuroadaptations, these molecular alterations may underlie the dysregulation in emotional processing observed following chronic adolescent cannabinoid exposure.

Adolescent Cannabinoid Exposure and Increased Addiction Vulnerability

The gateway drug hypothesis postulates that adolescent cannabis use can predispose individuals to abuse other illicit drugs (such as cocaine, heroin, amphetamines, and LSD) later in life, thereby increasing the risk for drug addiction in general.[49] The intravenous drug self-administration paradigms, in which subjects voluntarily self-administer drugs on various schedules of reinforcement, have been used to model addiction in nonhuman animals. Even though sex and/or strain differences have been observed in drug self-administration paradigms, there is evidence that adolescent cannabinoid exposure may increase the vulnerability and reward sensitivity to at least some psychoactive drugs when tested in adulthood. For example, chronic THC exposure during adolescence increased opiate self-administration in adult male Long Evans rats,[50,51] an effect correlated with increased μ-opioid receptor function in the VTA and substantia nigra[50] and increases in the proenkephalin peptide[50,51] in the adult NAcc. In addition, an increase in morphine self-administration was observed following adolescent chronic CP treatment in adult male Wistar rats,[52] an effect correlated with a decrease in μ-opioid receptor activity in the shell part of the NAcc.

Increased vulnerability to the rewarding effects of opiates following adolescent THC exposure has been demonstrated in adult male Fisher 344 rats[43] using the conditioned place preference (CPP) test, a Pavlovian conditioning model of

drug-seeking behaviours. Finally, chronic administration of CP during adolescence increased cocaine self-administration in adult female Wistar rats but produced no such effects in adult male Wistar rats.[53] In a follow-up study, the authors identified different long-term alterations in the DAergic system of adult male versus female rats that may underlie the enhanced cocaine self-administration observed selectively in adult females.[48] Indeed, it was reported that only adult female rats that were exposed to CP during adolescence expressed up-regulated DA transporter (DAT) levels in the dorsal striatum, whereas adult males showed only D1 receptor upregulation. Conversely, adolescent CP treatment down-regulated the expression of D2 receptors in the CA1 region of hippocampus, irrespective of gender.[48]

The above-described preclinical evidence suggests that chronic cannabinoid exposure during adolescence may increase sensitivity to certain drug classes (e.g., opiates) when tested in later adulthood. Nevertheless, more research is required to more closely determine the possible causal mechanisms between adolescent cannabinoid exposure and long-term alterations in drug-reward neural pathways. In addition, preclinical investigations are required to more closely examine the potential effects of adolescent cannabinoid exposure on reward sensitivity to other drugs of abuse.

Conclusions

A growing body of evidence from preclinical studies suggests that adolescent cannabis use may increase the risk of developing neuropsychiatric disorders later in life. Such disturbances may include emotional dysregulation, schizophrenia-related psychosis, and increased vulnerability to certain classes of other addictive drugs. While future studies are required to more closely examine the underlying molecular mechanisms and neuroadaptations caused by adolescent cannabinoid exposure, overactivation of the cannabinoid system during adolescence may potentially interfere with neural maturational processes occurring during this critical window of brain development.

An important question relates to the identification of the specific neuroanatomical pathways that may be vulnerable to dysregulation following adolescent cannabinoid exposure. For example, adolescence represents a critical period wherein cortical regions necessary for executive control and cognitive regulation are forming functional connections with subcortical, emotional processing regions such as the mesolimbic DA system.[54] Disruptions of this cortical-subcortical regulation may in turn lead to general dysregulation of DAergic function, which may underlie a plethora of potential neuropsychiatric disturbances in later life. The exponential rise in cannabis use among adolescents, particularly within Canada,[55] points to the need for preventive methods and the development of effective public health policy and education aimed at regulating and/or reducing adolescent exposure to cannabinoid drugs.

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