Neuropsychopharmacology

ARTICLE

Voluntary oral consumption of Δ^9-tetrahydrocannabinol by adolescent rats impairs reward-predictive cue behaviors in adulthood

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Few preclinical approaches are available to study the health impact of voluntary consumption of edibles containing the psychoactive drug Δ^9-tetrahydrocannabinol (THC). We developed and validated such approach by measuring voluntary oral consumption of THC-containing gelatin by rats and used it to study if and how THC consumption during adolescence impacts adult behavior. We found that adolescent rats of both sexes consumed enough THC to trigger acute hypothermia, analgesic, and locomotor responses, and that 15 days of access to THC-gelatin in adolescence resulted in the down-regulation of cannabinoid 1 receptors (CB_1Rs) in adulthood in a sex and brain area specific manner. Remarkably, THC consumption by adolescent male rats and not female rats led to impaired Pavlovian reward-predictive cue behaviors in adulthood consistent with a male-specific loss of CB_1R-expressing vGlut-1 synaptic terminals in the ventral tegmental area (VTA). Thus, voluntary oral consumption of THC during adolescence is associated with sex-dependent behavioral impairment in adulthood.

Neuropsychopharmacology (2019) 44:1406–1414; https://doi.org/10.1038/s41386-019-0387-7

INTRODUCTION

Evidence suggests that adolescent onset of cannabis use is linked to an increased risk of developing mental health and substance use disorders [1, 2]. Impairments in motivation, decision-making, working memory, problem solving, interpersonal relationships, and educational/job attainment have also been demonstrated [3–5]. However, it is important to note that other studies did not find an association between adolescent cannabis use and cognitive impairments [6, 7], suggesting more research is required to establish a causal relationship. Considering the impact of cannabis on the developing brain, a better understanding of the effects of its main euphoric agent, Δ^9-tetrahydrocannabinol (THC), on brain function and behavior is necessary. The primary route of administration of cannabis-based products in humans has been inhalation through smoking and more recently vaporizing [8]. However, use of edibles infused with cannabis extracts and cannabinoid compounds is also rapidly increasing [9]. Adolescents are particularly drawn to them over traditional methods given their strong appeal due to cues, such as packaging (e.g., brownies, lollipops, gummies, etc.), inconspicuous appearance, and perceived lower risk [10, 11]. Of particular concern, human adolescents classified as frequent users of THC-infused edibles are more likely to have used cannabis-based products in the past 30 days, to use these products more frequently, and report a younger age of first use compared to users who never consumed edibles [11]. Given the increased prevalence of the consumption of THC-containing edibles by adolescents, research on the short-term and long-term consequences of prolonged use is urgently needed.

In preclinical rodent models, the bioactivity of THC has been studied using various routes of administration, including intraperitoneal (i.p.), intravenous, subcutaneous, inhalation, and oral gavage [12–17]. Acute treatment of rodents with THC induces four specific and quantifiable physiological and behavioral responses: hypothermia, analgesia, altered locomotor activity, and catatonia [14, 18–22]. Moderate to high doses (5–30 mg/kg) of THC injected i.p. to rats induces hypothermia by 1–1.5 degrees, reduces sensitivity to thermal pain by 10–80% (1–10 mg/kg), reduces open field locomotion by ~60–85%, and induces catatonia (2–3-fold increase) [19–22]. Rodents that undergo chronic administration of THC (daily i.p. 2.5–10 mg/kg) exhibit increased compulsive-like behaviors and anxiety, as well as impaired attention and object-recognition memory [12, 23–25]. While these studies have established our understanding of the consequences of acute and prolonged THC exposure administered by the experimenter, few studies exist on the effect of freely accessible edibles containing THC on behavior and brain function of rodents, a paradigm that better models an important form of human cannabis use.

Brain development is associated with profound synaptic remodeling that occurs in select regions of the human brain, making these connections critically vulnerable to harmful agents including drugs of abuse [26]. In humans, synaptic remodeling occurs up to 25 years of age in the prefrontal cortex and mesolimbic dopamine (DA) system, two regions involved in higher-order cognitive processes, including reinforcement learning, decision making, and memory [26]. The mesolimbic DA system, which includes the ventral tegmental area (VTA) and nucleus accumbens (NAc), mediates many of the rewarding properties of drugs of abuse including THC [27]. THC predominantly acts through cannabinoid 1 receptors (CB_1Rs) and produces characteristic rewarding responses [28, 29]. CB_1Rs play a critical role in neuronal development, in part due to their ability to modulate the

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Received: 5 December 2018 Revised: 13 March 2019 Accepted: 3 April 2019
Published online: 10 April 2019

release of many neurotransmitters, including DA, and to regulate gene expression and establishment of synaptic connections [29]. Several studies showed that repeated activation of CB_1Rs during adolescence using i.p. administrations significantly affects the normal remodeling of the prefrontal cortex and reduces VTA firing of DA neurons and ensuing DA release in the NAc shell [30, 31].

Here, we developed and validated a novel experimental approach to study THC oral self-administration in adolescent male and female rats in which animals are presented with gelatin containing fixed amounts of THC and given free access to consume during a limited number of hours. We measured voluntary oral intake of THC in adolescent rats and associated acute cannabimimetic responses. We also examined the long-term consequences of oral THC consumption during adolescence on CB_1R expression in adult rats in select brain-regions known to be associated with the rewarding and reinforcing properties of drugs of abuse. Finally, we examined the effect of adolescent THC intake on Pavlovian reinforcement learning in adulthood, a behavior associated with drug craving, seeking, and relapse.

MATERIALS AND METHODS

Detailed descriptions of the methods are presented in the online Supplementary Material section. Male and female Sprague Dawley rats (aged PND 22 at the start of experiments) were used in these experiments. Gelatin preparation was performed as previously described [32], with the exception that the protocol was altered for THC administration. Behavioral assays were generally based off of previously described work: Hypothermia [21], tail flick [22], locomotor activity [21, 22], and Pavlovian conditioned approach [33]. Specific brain processing, immunostaining, and image analysis was performed as previously described [34]. Animal procedures were approved by the University of Washington Institutional Animal Care and Use Committee.

RESULTS

Voluntary consumption of THC-gelatin by adolescent rats

Figure 1a outlines the first experimental design developed for adolescent rats (25–58 days of age) that allows 4 h free access to gelatin (15 ml in a glass jar) containing either gelatin alone or gelatin with escalating amounts of THC (1.0, 1.5, and 2.0 mg/15 ml gelatin) every Monday, Wednesday, and Friday over 33 days (i.e. total of 15 days of THC access). The advantage of this model is the extended time period to consume THC, but a disadvantage is the inability to know when animals consume THC relative to the behavioral measures at the end of 4 h. This experimental design resulted in an average daily amount of THC consumed by female and male rats that ranged from 1 to 5 mg/kg (Fig. 1b), with a comparable average of total THC consumed (45.9 ± 9.7 mg of THC/kg by adolescent females and 42.3 ± 4.5 mg of THC/kg by adolescent males) over the 15 intermittent days of THC access. We found that adolescent rats of both sexes consumed more control-gelatin than THC-gelatin (Supplementary Figure 1a). One plausible explanation for this finding is that animals sense THC in gelatin, making it less palatable. Another possibility is that THC becomes aversive at higher levels, thereby curbing intake. Future studies are required to follow up on these possibilities. Importantly, adolescent rats of both sexes that consumed either control-gelatin or THC-gelatin did not differ in body weight during the 15 intermittent days of gelatin access and the first 15 days of no gelatin access (Supplementary Figure 2).

Our second experimental design for adolescent rats of both sexes used the same doses of THC but allowed shorter access. The advantage of this model is a shorter time period between consumption and the behavioral measures, for a more direct assessment of the effects of consumed THC on hypothermia, analgesia, and locomotor activity. Specifically, the access period was reduced from 4 h on days 1–3, to 2 h on days 4–6, and finally 1 h on days 7–15 (4-2-1 h paradigm; Fig. 1a). We found that adolescent rats of both sexes consumed similar amounts of THC-gelatin under this design and with a similar pattern of 3.5 to 7.0 mg/kg of THC per day the first 2 days of access, and lower and more consistent amounts (from 1.0 to 3.5 mg/kg) during the following 13 days (Fig. 1c). The difference in 4 h intake between Fig. 1b and c on days 1–3 may be due to subtle environmental differences in housing room that we are unable to account for as these experiments were run during different periods of the year. However, we believe this does not impact the interpretation of our findings as control and THC animals were simultaneously

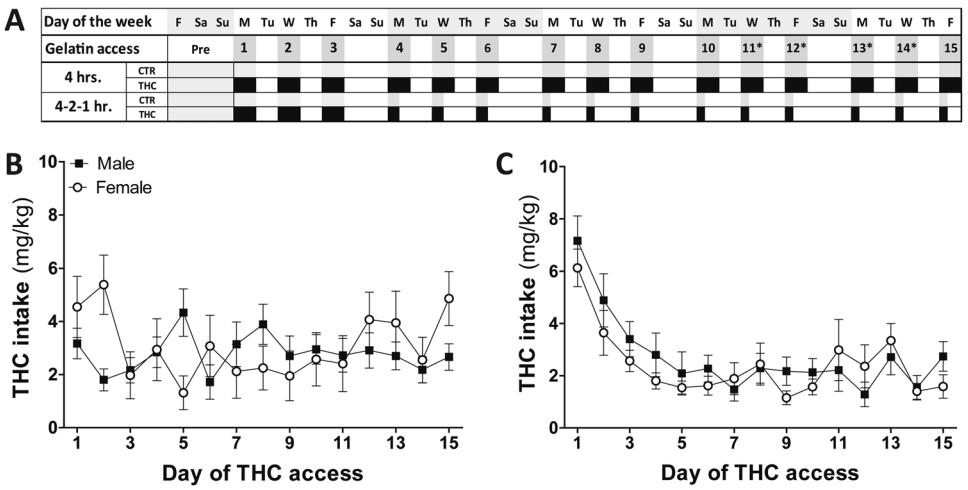

Fig. 1 Voluntary oral THC consumption by adolescent rats and testing schedules. **a** Diagram of the 4 h access to gelatin paradigm. Adolescent rats had access to either control-gelatin (not shown) or THC-gelatin for 4 h every Monday, Wednesday, and Friday for a total of 15 days of gelatin (gray bar). Cannabimimetic responses were measured on days 11, 12, 13, and 14. Diagram of the 4-2-1 h paradigm. Adolescent rats had access to THC every Monday, Wednesday, and Friday for 4 h (dark gray bar), 2 h (light gray bar), and 1 h (white bar), for a total of 15 days of THC access. Hypothermia was measured on day 11, tail flick was measured on day 12, and locomotor activity was measured on days 13 and 14 (indicated by an *). Blood samples were collected on day 15 at the end of the 1 h intake period. **b** THC consumed during the 4 h paradigm by adolescent female rats (open circles, $n = 11$) and male rats (black squares, $n = 13$). **c** Average THC consumed during the 4-2-1 h paradigm by adolescent female rats ($n = 7$) and male rats ($n = 7$). Results are presented as mean ± SEM

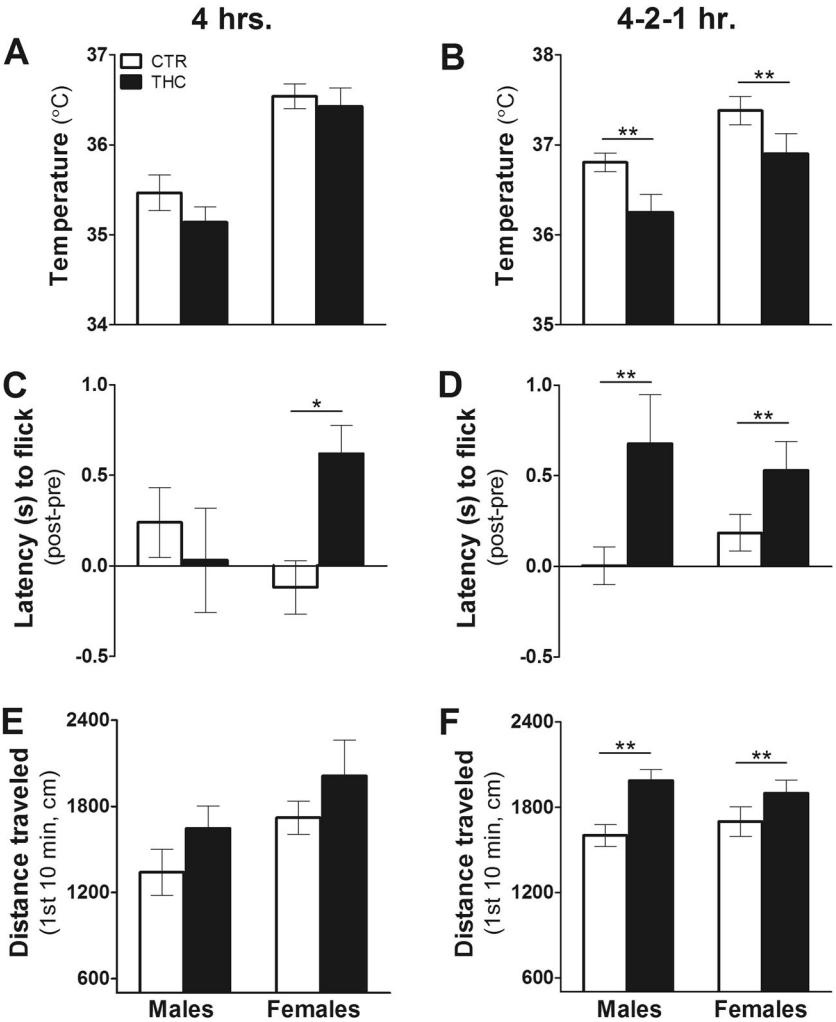

Fig. 2 Voluntary oral consumption of THC triggers hypothermia, analgesia, and increases locomotor activity in adolescent rats. **a** Body temperature measured on day 11 immediately following 4 h. **b** 1 h access to either control-gelatin (open bars) or THC-gelatin (solid bars) ($n = 11–13$ and $n = 6–8$ per treatment and sex, respectively). **c** Analgesia measured on day 12 immediately following 4 h and **d** 1 h access to control-gelatin (open bars) and THC-gelatin (solid bars) ($n = 11–12$ and n = 6–8 per treatment and sex, respectively). **e** Locomotion measured on day 13 immediately following 4 h and **f** 1 h access to control-gelatin (open bars) and THC-gelatin (solid bars) ($n = 3–6$ and $n = 5–7$ per treatment and sex, respectively). Results are mean ± SEM. *$p < 0.05$ and **$p < 0.01$ when THC-gelatin is significantly different from control-gelatin using two-way ANOVA analyses

monitored, and males and females in both paradigms consumed similar total amounts of THC. Total THC consumed during the 15 intermittent access days was 34.5 ± 3.9 mg/kg THC for females and 39.3 ± 7.5 mg/kg THC for males. Here too, adolescent rats of both sexes consumed more control-gelatin than THC-gelatin (Supplementary Figure 1B). To determine the plasma levels of THC reached when using this paradigm, we measured the amount of THC consumed by adolescent rats on the last day of free access to THC-gelatin (day 15) and collected blood samples immediately after the end of the 1 h session. Supplementary Figure 3 shows that consumption of 2–3 mg/kg over 1 h results in 2–3 ng/ml THC in plasma, concentrations of THC known to produce cannabimimetic effects in rodents and humans [35]. Together, these results show that adolescent rats of both sexes will voluntarily and consistently consume THC-gelatin.

Voluntary oral consumption of THC triggers cannabimimetic responses in adolescent rats

Hypothermia. Consistent with previous work [21] we found that consumption of THC-gelatin led to a reduction in body temperature. Body temperature was first measured prior to gelatin access on day 11 and we found no difference in baseline body temperatures between control-gelatin or THC-gelatin animals in either the 4 or 4-2-1 h group (Supplementary Table 2). This result shows that previous consumption of THC-gelatin does not chronically affect baseline body temperature. Interestingly, we found that the 4 h access to THC-gelatin did not affect body temperature in adolescent rats of either sex, but the 1 h access to THC-gelatin significantly reduced body temperature in females by 0.5 °C and in males by 0.6 °C ($F_{(1,24)\ \text{treatment}} = 5.9$; $p = 0.006$; Fig. 2a, b). These results show a significant reduction in body temperature following 1 h of consumption but not 4 h, and that this response is similar in female and male adolescent rats.

Nociception. Latency to withdraw the tail from 52.5 ± 0.5 °C water was used to measure the nociceptive properties of consuming THC-gelatin during either the 4 or 1 h gelatin session on day 12 (Supplementary Table 1). In the 4 h group, THC intake significantly prolonged the tail flick response only in females ($F_{(1,42)\ \text{sex×treatment}} = 5.2$; $p = 0.03$; Bonferroni $p < 0.05$; Fig. 2c). In the 4-2-1 h group, THC intake prolonged the tail flick response in both females and males ($F_{(1,24)\ \text{treatment}} = 10.1$; $p = 0.004$; Fig. 2d).

These data indicate that adolescent rats voluntarily consumed THC-gelatin to levels that produce analgesia.

Locomotor activity. Previous studies showed that low doses of THC (1–2 mg/kg, i.p.) lead to an increase in locomotor activity measured 0–30 min post-injection in an open field, whereas higher doses (5–30 mg/kg, i.p.) lead to a decrease [19, 20, 22, 36]. We found a significant increase in locomotor activity in rats that consumed THC-gelatin in the 4-2-1 h group in the first 10 min of the activity session, a time period during which animals are experiencing the chamber for the first time and is commonly used as a measure of motivated exploration ($F_{(1,20)\ \text{treatment}} = 10.8$; $p = 0.004$; compared to controls: Fig. 2f). Note that there is no difference in locomotor activity when tracking animals at 60 min, a time point when animals are habituated to the open field. By contrast, we measured no difference in locomotor activity in the 4 h group (Fig. 2e). No differences in total distance traveled during the 60 min session in either the 4 or 4-2-1 h groups were found (Supplementary Figure 4). Our results show that male and female rats will voluntarily consume THC to levels the result in an increase in initial locomotor activity reflecting enhanced motivated exploration of a novel environment.

Voluntary oral consumption of THC during adolescence results in sex-region-specific and brain region-specific reductions of CB_1R expression in adult rats

In rats, repeated i.p. injections of THC down-regulates CB_1R expression in select brain regions, including the hippocampus (HPC), amygdala and prefrontal cortex, and induces remarkably little-to-no change in CB_1R expression in the NAc [37]. We examined the effects of voluntary oral consumption of THC-gelatin by adolescent rats on CB_1R expression in select brain areas in adulthood. Adolescent rats of both sexes underwent the 4 h paradigm followed by 37 ± 2 days of abstinence (corresponding to 95 ± 2 days of age) before analyzing CB_1R expression by semi-quantitative immunohistochemistry (sqIHC) analysis. We focused our analysis on the HPC, a brain area particularly sensitive to cannabinoid treatments, and the VTA and NAc core (NAcc), two brain areas with high densities of CB_1R that regulate DA signaling and motivated behaviors [34, 38, 39]. In the HPC, we found a significant down-regulation of CB_1R expression in both males and female adult rats (Supplementary Figure 5). Remarkably, we found a reduction in CB_1R expression in the VTA of male rats that consumed THC-gelatin and no change in female rats, suggesting a sex-specific response ($p < 0.05$; Fig. 3a–d). THC consumption did

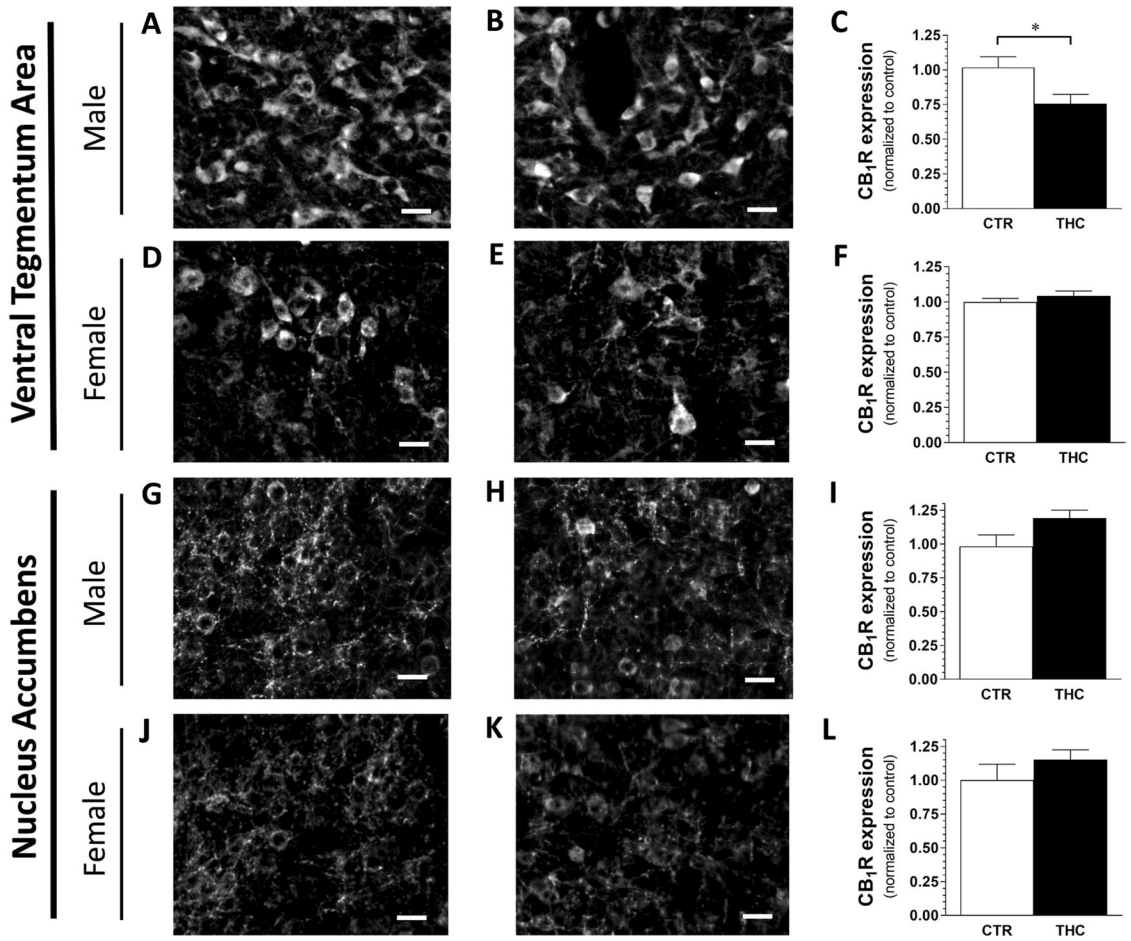

Fig. 3 Voluntary oral consumption of THC during adolescence reduces CB_1R expression in the ventral tegmental area of adult male, but not female rats, with no changes in the NAcc. **a**–**c** CB_1R immunoreactivity in the ventral tegmentum area (VTA) of male adult rats. Representative image from the **a** control-gelatin and **b** THC-gelatin group. **c** Semi-quantitative analysis of CB_1R expression in the VTA of adult males. **d**–**f** CB_1R immunoreactivity in the VTA of female adult rats. Representative image from the **d** control-gelatin and **e** THC-gelatin group. **f** Semi-quantitative analysis of CB_1R expression in the VTA of adult females. **g**–**i** CB_1R immunoreactivity in the nucleus accumbens (NA) of male adult rats. Representative image from **g** control-gelatin and **h** THC-gelatin group. **i** Semi-quantitative analysis of CB_1R expression in the NA of adult males. **j**–**l** CB_1R immunoreactivity in the NA of female adult rats. Representative image from **j** control-gelatin and **k** THC-gelatin group. **l** Semi-quantitative analysis of CB_1R expression in the NA of adult females. Scale bar denotes 25 µm. Results are mean ± SEM of $n = 5$ rats per group using the 4 h paradigm

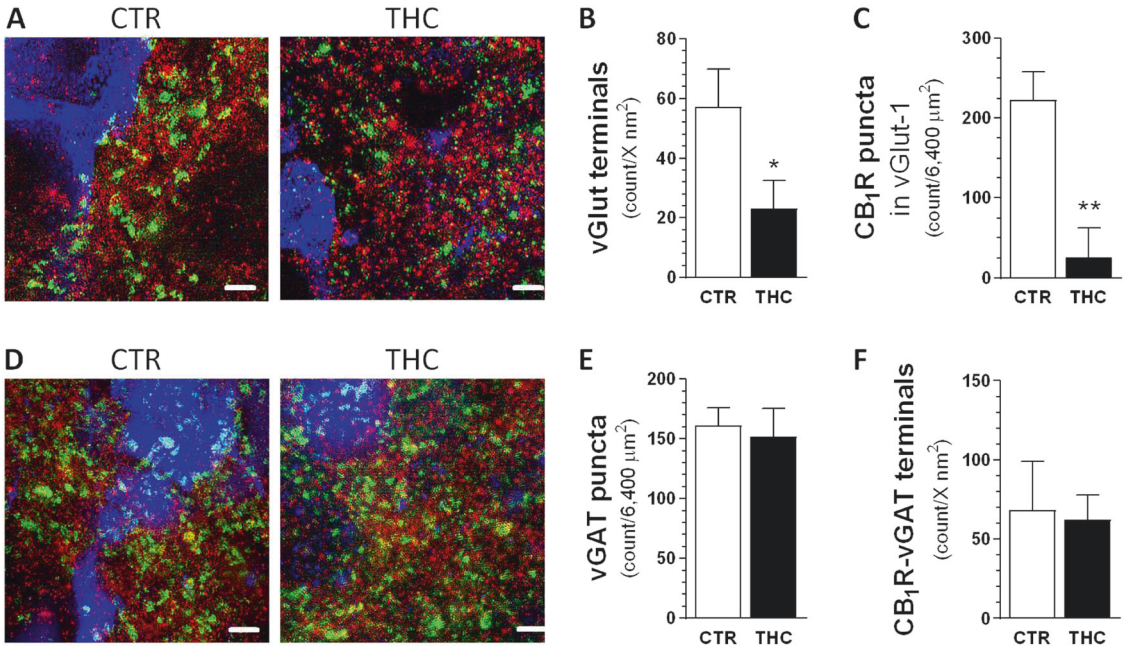

Fig. 4 Voluntary oral consumption of THC during adolescence decreases CB_1R expression in glutamatergic terminals in the ventral tegmental areas of adult male rats. **a** Representative images of CB_1R (red) and tyrosine hydroxylase (blue) and vGlut-1 (green) immunoreactivities in the VTA of adult male rats that consumed either control-gelatin (CTR) or THC-gelatin (THC) during adolescence (4 h paradigm). Scale bar denotes 2 µm. Number of **b** vGlut-1 puncta (particles larger than 250 nm) and **c** CB_1R puncta (particles larger than 25 nm) in vGlut-1 puncta in the VTA of adult males that consumed control-gelatin (open bars) and THC-gelatin (black bars) during adolescence. **d** Representative images of CB_1R (red) and tyrosine hydroxylase (blue) and VGAT (green) immunoreactivities in the VTA of adult male rats that consumed either control-gelatin (CTR) or THC-gelatin (THC) during adolescence. Scale bar denotes 2 µm. Number of **e** vGAT puncta (particles larger than 250 nm) and **f** CB_1R puncta (particles larger than 25 nm) in vGAT puncta in the VTA of adult males that consumed either control-gelatin (open bars) or THC-gelatin (black bars) during adolescence. Results are mean ± SEM from $n = 5$ brain sections, each from different adult rats. *$p < 0.1$ and **$p < 0.01$ different from control using T test analysis

not affect CB_1R expression in NAcc (Fig. 3e–h), nor did it affect GFAP and Iba-1 expression in the VTA of males and females, two markers of astrocyte and microglia activation, respectively (Supplementary Figure 6). Together, these results suggest a selective and male-specific down-regulation of CB_1R in the VTA that is not associated with overt gliotic response indicative of neuroinflammation or cell damage.

In the brain, CB_1Rs are expressed by multiple cells types, including glutamatergic and GABAergic neurons, and the relative expression of CB_1R in these two types of neurons varies between brain regions [40]. Few studies have evaluated CB_1R expression in VTA GABAergic and glutamatergic neurons; however it is known that both VTA glutamatergic and GABAergic neurons express CB_1Rs at similar levels as determined by both electron microscopy and in situ hybridization [41, 42]. To better understand the male-specific change in VTA CB_1R expression, we immunostained sections for tyrosine hydroxylase to locate DA neurons in the VTA, and for CB_1R and vGlut-1 or vGAT to identify CB_1R expressed by GABAergic or glutamatergic neurons, respectively. Immunofluorescence was captured using structured illumination microscopy (SIM), which allows super-resolution imaging that to reduces the diffraction limit to improve resolution in the *X–Y* plane to 120 nm [43]. CB_1R expression was measured using unbiased thresholding of fluorescent intensity to outline vGlut-1 and vGAT puncta (Supplementary Figure 7). Using this approach, we measured in the VTA a 59% reduction in the number of vGlut puncta and an 88% reduction in CB_1R expression measured within vGlut-1 puncta (Fig. 4a–c). By contrast, the number of vGAT-puncta and CB_1R expression within vGAT-puncta was not changed (Fig. 4d–f). These results show that oral consumption of THC by adolescent males leads to a change in the balance of the CB_1R-mediated control of excitatory and inhibitory inputs within the VTA that persist into adulthood.

Voluntary oral consumption of THC during adolescence results in male-specific increase in Pavlovian conditioned approach in adult rats

We determined if adult rats (93 days of age) that consumed THC during adolescence under the 4-2-1 h paradigm, exhibit altered Pavlovian-conditioned approach behavior (conditioned lever presses, head entries, and overall response bias). Importantly, these animals consumed similar total amounts of THC as those used in the CB_1R experiments. Lever presses and head entries were fit with a Weibull function to obtain the best fit parameter for asymptote to examine differences in acquisition (pre-asymptote; first 15 trial bins for females and first 20 trial bins for males) and maintenance (post-asymptote) of behavior during Pavlovian training (Supplementary Table 3) [44, 45]. Results showed that only males that consumed THC during adolescence exhibited a significant alteration in Pavlovian-conditioned approach behavior (Fig. 5). Specifically, adult males exhibited a greater conditioned response to the reward-predicting lever during the pre-asymptotic trials ($F_{(1,209)\ \text{treatment}} = 35.6$, $p < 0.01$; Fig. 5b) compared to controls. Adult males that consumed THC during adolescence also showed reduced conditioned responding to the food tray early on during the pre-asymptotic trials ($F_{(19,209)\ \text{treatment×trial bin}} = 1.8$, $p = 0.03$; $F_{(1,209)\ \text{treatment}} = 4.5$, $p = 0.06$; Fig. 5a). Remarkably, THC and control males did not differ on any of these behavioral measures during the post-asymptotic trials (trial bins 21–35), indicating that THC consumption during adolescence increases the acquisition, but not maintenance, of Pavlovian-conditioned approach behaviors in adulthood. Note that both THC and control males developed a response bias towards sign tracking over the course of 35 trials, but THC males showed a stronger sign-tracking bias during the pre-asymptotic trials compared to controls ($F_{(1,209)\ \text{treatment}} = 6.7$, $p = 0.02$; Fig. 5c). By contrast, adult females that consumed THC during adolescence did not differ from

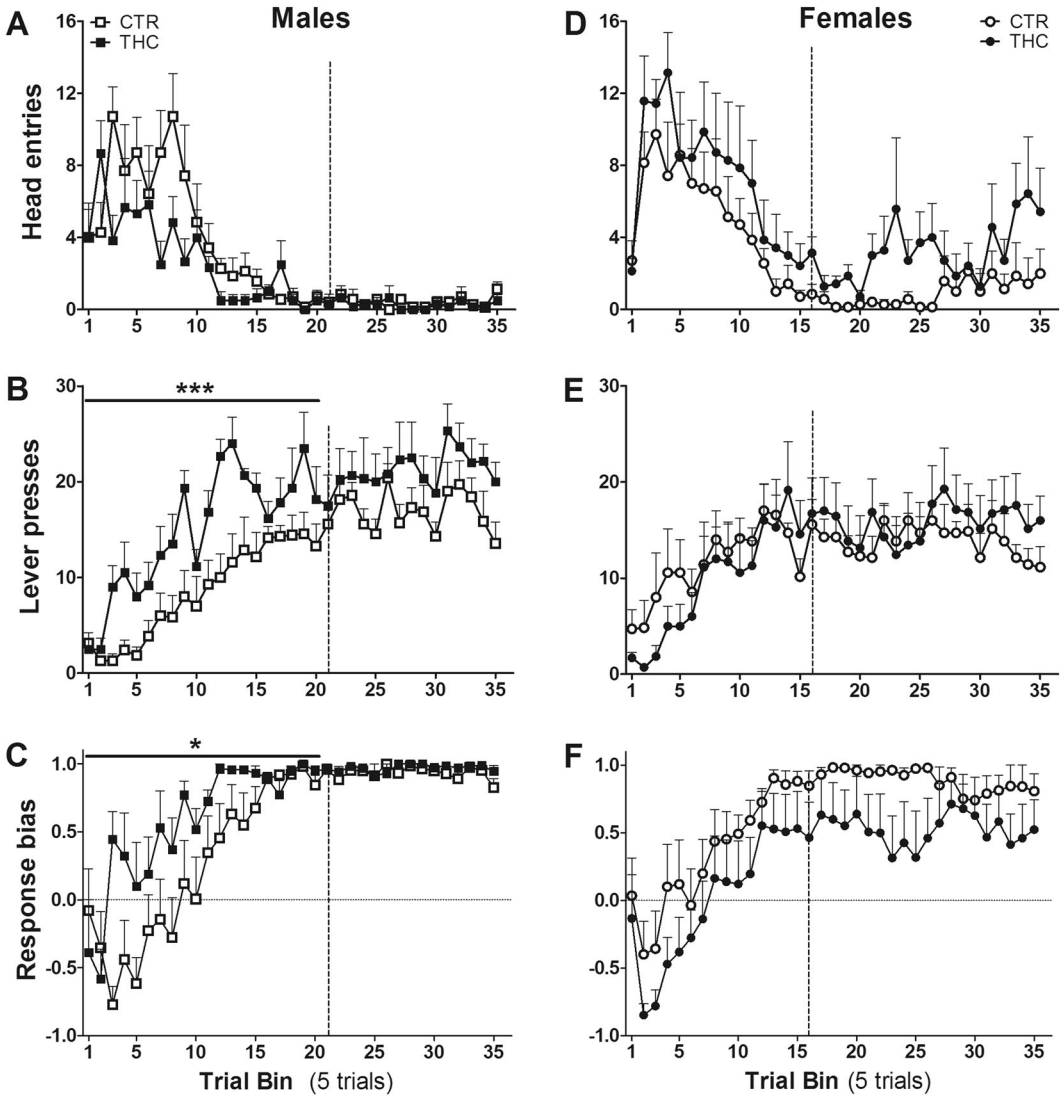

Fig. 5 Voluntary oral THC consumption during adolescence alters Pavlovian-conditioned approach of adult male rats. **a**–**f** Pavlovian conditioned approach in **a**–**c** adult male and **d**–**f** female rats ($n = 6$–7 per treatment and sex) that consumed either control-gelatin or THC-gelatin during adolescence (4-2-1 h paradigm). **a**, **d** Approach to the food tray (head entries) in the pre-acquisition or post-acquisition trials. **b**, **e** Conditioned response to the reward-predicting lever when comparing the pre-acquisition (trial bins 1–20) to the post-acquisition (trial bins 21–35) trials. **c**, **f** Response-bias towards sign tracking over the course of learning. Adult males that consumed THC during adolescence were faster to acquire the sign-tracking response bias during pre-acquisition, as indicated by a response bias score above 0.0, but did not differ from controls post-acquisition. *$p < 0.05$; ***$p < 0.001$ different from control using repeated measures two-way ANOVA analysis. Results are mean ± SEM. Vertical dashed line represents the trial bin that defines the asymptote, which divides performance into pre-acquisition and post-acquisition trials

controls on any of the behavioral measures during the pre-asymptotic or post-asymptotic trials (Fig. 5d–f). These results indicate that THC intake during adolescence increases the assignment of incentive value to reward-predicting cues during the acquisition of conditioned behaviors in adulthood in males but not females.

DISCUSSION

Several rodent models of oral THC self-administration have been reported, although none provided persistent, dose-related, or reliable self-administration under non-food or water-deprived conditions [46–48]. Here we present a novel model of voluntary oral THC consumption that leads to detectable THC levels in the plasma and cannabimimetic responses. Two paradigms (4 and 4-2-1 h) of gelatin availability were implemented to examine THC intake during limited access sessions. Both paradigms resulted in similar intake of ~1.0–5.0 mg of THC/kg by adolescent rats of both sexes but produced different cannabimimetic responses. Such intake levels and plasma THC levels are within the range of human cannabis users who could be classified as regular/semi-regular users that consume cannabis products with low to moderate amounts of THC [35].

In the 4-2-1 h paradigm, but not the 4 h, THC consumption led to the expression of all three classic cannabimimetic responses in both sexes. A likely explanation is that THC bioactivity is evidenced in the cannabimimetic responses in the 4-2-1 h paradigm because of the shorter time between consumption and the behavioral/physiological measurements. It is known that increasing doses of THC triggers a biphasic response on locomotion in rodents typified by an increase in locomotion with low doses of THC and decrease in locomotion with higher doses of THC [36, 49]. We found that oral consumption of an average of

2.0–2.4 mg/kg of THC in 1 h produced an increase in locomotion during the first 10 min of 60 min experimentation sessions in adolescent rats of both sexes, suggesting enhanced exploratory behavior. This result is consistent with an increase in locomotor activity measured during the first 5 min of an activity session following vaporized cannabis exposure [14]. Interestingly, some humans also report stimulating effects associated with cannabis use, suggesting that the experimental approach that we developed should allow the study of the molecular mechanism(s) mediating this stimulatory response [50].

Fifteen intermittent days of voluntary oral consumption of THC during adolescence resulted in the down-regulation of CB_1R expression in adulthood that was sex-region and brain region specific. Consistent with previous work that showed reduced CB_1R expression in the HPC following chronic (i.p.) THC exposure [30], we found reduced CB_1R expression in adult rat brains of both sexes when adolescent rats consumed THC. This is also in agreement with a study demonstrating the differential regulation of 27 proteins in the HPC of adult rats exposed to THC during adolescence, and with human imaging studies showing reduced HPC volume in chronic cannabis users compared to controls [51, 52]. Together, these findings emphasize that the HPC is particularly sensitive to repeated THC consumption and that our model is highly translational to the clinical population.

THC consumption during adolescence also resulted in the down-regulation of CB_1R expression in the VTA of adult males but not females. CB_1R are expressed by presynaptic glutamatergic and GABAergic neurons in the VTA, and their acute activation by THC and potent CB_1R agonist WIN55,212-2 increase DA release in the NAc [21, 53, 54]. Given the retrograde action of eCBs by VTA DA neurons that are excited, depolarization of DA neurons stimulates the post-synaptic synthesis and release of eCBs from VTA DA neurons which activate presynaptic CB_1R on GABA and Glu neurons [55–57]. Thus, considering the role of endocannabinoids (eCB) in the VTA [58], one could hypothesize that CB_1R down-regulation in vGlut-1 terminals reduces the negative feed-back signal provided by eCBs released from VTA DA neurons to reduce glutamate release (thus increasing excitation) while not affecting their ability to control GABA release, the net result of which may be enhanced excitation of VTA DA neurons (Supplementary Figure 8). Future studies are necessary to follow-up on this hypothesis and to measure whether or not there is a persistent change in DA neurotransmission and/or tone in adult male rats that consumed THC during adolescence.

Stimulus-reward (reinforcement) learning is a Pavlovian process through which cues in an environment that repeatedly predict a reward can attain value, and this process is thought to be mediated by increases in phasic NAcc DA release [59]. Drug-associated cues often attain enhanced incentive value and play a critical role in promoting drug craving, seeking, and relapse following periods of abstinence in humans [60–62]. We found that THC consumption during adolescence leads to an increase in acquisition of the attribution of value to reward-predictive cues in adult males but not females. This finding could be explained by our finding of a selective loss of CB_1R glutamatergic terminals in males, which could result in greater VTA DA neuron firing and subsequent DA release in the NAcc, although future studies are required to directly examine this possibility. An important caveat to emphasize is that the behavioral results and the biochemical results were obtained with animals that consumed THC using different paradigms (4-2-1 and 4 h, respectively). However, since both male and female animals consumed similar total amounts of THC across both paradigms, all animals in this study experienced overall similar amounts of THC during adolescence. Furthermore, the pharmacokinetic profile of THC reaching the brain and therefore changes in CB_1R expression as a result of THC-induced CB_1R internalization in both paradigms are also likely to be comparable. Thus, the simplest interpretation is that changes in CB_1R expression occurred in both paradigms and in a likely similar manner.

A potential limitation to our study design is that animals were individually housed during adolescence to allow accurate measurement of gelatin intake by each animal. The model of voluntary oral consumption of THC that we report here was developed based on a commonly used model for voluntary adolescent intake of gelatin containing alcohol [32, 59], which results in increased incentive value of reward predictive cues during Pavlovian conditioning, as well as maladaptive decision making in adulthood [32, 59]. These findings have been replicated with pair-housed [63] and group-housed [64] adolescent rats exposed to alcohol intragastric gavage, respectively, suggesting that our results are likely due to intake of THC during adolescence rather than housing condition. Another limitation is that the estrus cycle of female rats was not tracked in the present study, and thus we are unable to rule out the possibility that estrus cycle stage influenced our results. However, a study examined CB_1R density in various brain regions at each stage of the estrus cycle and found that CB_1R density in the mesencephalon and striatum did not differ by estrus cycle stage [65]. Furthermore, the variability in CB_1R expression in females was consistent and similar to males (Figs. 3c, d, g, h; 2, 5), further suggesting that CB_1R expression was not influenced by estrus cycle.

In conclusion, our study provides the first evidence that voluntary oral consumption of THC during adolescence leads to alterations in reinforcement learning processes that may be sex-specific, producing behaviors often apparent in addiction-prone individuals. These results, coupled with a persistent, localized reduction in CB_1R expression in the VTA of males as a result of THC intake during adolescence, suggest that humans that consume cannabis during adolescence may be more at risk for the development of substance use disorders later in life. Given the recent rise in potency of cannabis-based products due to increased amounts and availability of THC [66], our experimental approach provides a novel and useful preclinical model for studying the biological effects and molecular consequences resulting from oral consumption of THC, a major form of cannabis intake in humans.

FUNDING AND DISCLOSURE

Supported by NIH grants DA014486 (NS), AA021121 (JJC), T32AA007455 (LCK), and by the UW (ADAI 201510 Kruse) (LCK). The authors declare no competing interests.

ACKNOWLEDGEMENTS

We would like to acknowledge Dale Whittington and the School of Pharmacology's Mass Spectrometry Center for their work in analyzing plasma THC levels and writing up the methods for Plasma THC Concentrations.

ADDITIONAL INFORMATION

Supplementary Information accompanies this paper at (https://doi.org/10.1038/s41386-019-0387-7).

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