Adolescent THC impacts on mPFC dopamine-mediated cognitive processes in male and female rats

Martinez*, Maricela X.[1], Alizo Vera, Vanessa[1], Ruiz, Christina M.[1], Floresco, Stan B.[2], Mahler*, Stephen V.[1]

[1]Department of Neurobiology and Behavior, University of California, Irvine. 1203 McGaugh Hall. Irvine, CA 92697
Ph: (949) 824-6128

[2]Department of Psychology, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada

Corresponding authors*: maricexm@uci.edu, mahlers@uci.edu

Abstract

Adolescent use of cannabis is linked to later-life changes in cognition, learning, and memory. Rodent models suggest this could be a causal effect of Δ^9-tetrahydrocannabinol (THC) influencing the development of circuits underlying these processes, especially for the prefrontal cortex (PFC) which undergoes maturation during adolescence. Here we examined how two weeks of daily exposure to THC (5mg/kg IP) during adolescence persistently impacts cognitive processes involving mPFC dopamine. In male and female Long Evans rats treated in adolescence with THC or vehicle, we quantify acquisition of, and stable performance in two mPFC dopamine-dependent cognitive tasks—attentional set shifting and probability-based reward discounting. We also determined how acute pharmacological or chemogenetic augmentation of dopamine signaling impacts cognition in both groups and both sexes. We found that adolescent THC (AdoTHC) sex-dependently impacts acquisition of cue-guided instrumental reward seeking, but has minimal effects on set-shifting or probability discounting in either sex. When we challenged dopamine circuits by acutely potentiating monoamine signaling with amphetamine (0, 0.25, 0.5 mg/kg) during probability discounting, we confirmed prior findings that amphetamine reduced discounting of improbable reward options, and further showed that this amphetamine effect was more robust in rats with a history of adolescent THC. We next asked whether this potentiated effect of amphetamine involves AdoTHC impacts on dopamine circuits in particular, by attempting to recapitulate the amphetamine effect using more specific chemogenetic stimulation of VTA dopamine neurons. In contrast, we found that neither acute chemogenetic stimulation of VTA dopamine neurons (using a Cre-dependent DREADD in tyrosine hydroxylase:Cre rats), nor pathway-specific chemogenetic stimulation of these neurons' projection to mPFC in particular impacted probability discounting, suggesting that AdoTHC's potentiation of amphetamine effects may reflect alterations of non-VTA dopamine neurons influenced by amphetamine. Results confirm the marked specificity in the cognitive processes impacted by adolescent THC exposure, and suggest that some persistent effects of adolescent THC may alter cognitive responses to amphetamine in a manner independent of VTA dopamine projections to mPFC.

Introduction

Cannabis is one of the most widely used drugs among adolescents, and its availability is increasing around the world. Human studies show that early exposure to cannabis, and especially its main psychoactive constituent Δ^9-tetrahydrocannabinol (THC), is associated with later-life cognitive impairments, and increased risk for psychiatric disorders including schizophrenia and addiction (Ehrenreich et al., 1999; Schneider, 2008; Malone et al., 2010; Curran et al., 2016; Rubino and Parolaro, 2016; Volkow et al., 2016; Jenni et al., 2017; Murray et al., 2022). However, in humans it is difficult to dissect whether THC causes these associations, or whether early cannabis use and long-term deficits both result from other underlying comorbidities. Rodent models are thus essential for establishing casual effects of THC on the developing adolescent brain.

Neurodevelopmental disruptions persisting long after adolescent cannabis use are plausible because adolescence is a dynamic critical period for structural and functional brain remodeling, especially in late-developing structures like the prefrontal cortex (PFC) (Casey et al., 2000; Andersen, 2003). Some of this age-dependent plasticity seems to involve the endocannabinoid system, with dynamic changes in cannabinoid receptors (CBRs) and endocannabinoids (ECB) occurring across adolescence (Ellgren et al., 2008; Heng et al., 2011; Bara et al., 2021). Might THC, which also acts via CBRs, disrupt this age-dependent ECB signaling system and thus leave long-lasting consequences on the brain? If so, the adolescent-developing PFC (Spear, 2000; Peters et al., 2021; Scheyer et al., 2023), and its dopaminergic inputs from ventral tegmental area (VTA), which are actively innervating during this period (Manitt et al., 2011; Hoops and Flores, 2017; Reynolds et al., 2018), are a likely candidate for cognition-relevant neurodevelopmental insults caused by adolescent THC (Renard et al., 2016; Renard et al., 2017; Molla and Tseng, 2020).

The adult PFC is crucial for purposeful, goal-directed behaviors driven by the ability to flexibly converge our internal states, like reward motivation, with outside external information about contexts, cues, and rules (Miller, 2000; Miller and Cohen, 2001; Ott and Nieder, 2019). Executive functions like working memory, attention, rule shifting, and decision-making require PFC-dependent cognitive control. Rodent studies show adolescent cannabinoid drug exposure can cause persistent deficits in working memory (Schneider and Koch, 2003; O'shea et al., 2004; De Melo et al., 2005; Quinn et al., 2008; Kirschmann et al., 2017), social cognition (O'shea et al., 2004; Zamberletti et al., 2014; Renard et al., 2016; Renard et al., 2017), and cognitive flexibility (Egerton et al., 2005; Hill et al., 2006; Gomes et al., 2015; Jacobs-Brichford et al., 2019; Szkudlarek et al., 2019) that may depend upon PFC.

Furthermore, dopamine in PFC plays a major role in decision making, working memory, cognitive flexibility, and goal-directed behaviors (Goldman-Rakic, 1995; Seamans and Yang, 2004; Goto et al., 2007; Floresco, 2013). PFC dopamine is also implicated in schizophrenia symptoms (Davis et al., 1991; Howes and Kapur, 2009), and there is a clear link between recent cannabis use and onset of psychosis in humans (Andréasson et al., 1987; Hambrecht and Häfner, 2000; D'Souza et al., 2004), though it is not clear that this link is causal in nature (Fergusson et al., 2005; Henquet et al., 2005; Sewell et al., 2009). Since VTA dopamine neuron axons actively infiltrate mPFC during adolescence and are thus subject to disruption by THC exposure (Behan et al., 2012; Renard et al., 2017), and since THC impacts dopamine signaling at several key nodes in reward and salience circuits (Sakurai-Yamashita et al., 1989; Chen et al., 1990; French et al., 1997; Gessa et al., 1998; Malone and Taylor, 1999; Wu and French, 2000; Gardner, 2002; Pistis et al., 2002; Bossong et al., 2015; Bloomfield et al., 2016; Renard et

al., 2017), it is logical that adolescent THC might exert some of its neurodevelopmental disruptions in cognition-relevant non-VTA dopaminergic inputs to mPFC.

We therefore explored this possibility in a series of experiments characterizing effects of a well-characterized (Torrens et al., 2020; Ruiz et al., 2021a; Ruiz et al., 2021b; Torrens et al., 2022) adolescent THC exposure protocol in rats upon mPFC dopamine-dependent cognition.

Materials and Methods

Subjects

Long Evans rats (*n* = 29 males, 25 females) were used for set shifting and amphetamine experiments, and transgenic TH:Cre (*n* = 19 males, 9 females) and wildtype littermates *(n*= 14 males, 4 females) were used for chemogenetic experiments. Rats were pair or triple-housed in same-sex groups at weaning (postnatal day; PD21), in a temperature, humidity, and pathogen-controlled colony under a 12:12hr reverse light/dark cycle. Water was provided *ad libitum* and food was restricted to ~85% of free-feeding weight during behavioral testing, starting at ~PD70. Experiments were approved by University of California Irvine's Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Drugs

THC was provided in ethanol by the NIDA Drug Supply Program. For injection, THC was prepared fresh each day; ethanol is evaporated under N_2, and THC is reconstituted in 5% Tween-80 in saline, with heat and sonication, to 2 ml/kg for intraperitoneal (IP) injection. D-amphetamine hemisulfate salt (amphetamine) was attained from Sigma and mixed in saline at 0.5 mg/ml for injection. Clozapine-N-oxide (CNO) was obtained from the NIDA Drug Supply Program, stored at 4°C in powder aliquots with desiccant, protected from light. For systemic injection, CNO (5 mg/kg) was dissolved daily for IP injection in 5% dimethyl sulfoxide (DMSO; Sigma Aldrich) in 0.9% saline. For microinjections, CNO (1mM; 0.5 µl/side over 60 s) was dissolved in artificial cerebrospinal fluid (Fisher) with 0.5% DMSO, stored in aliquots at -20°C, and thawed/vortexed just before use.

Adolescent THC and Washout

All rats received daily IP injections of THC (5 mg/kg) or vehicle (VEH; 5% Tween-80 in saline) from postnatal day (PD) 30-43, followed by a washout period of 21+ days, allowing full THC clearance in both sexes (Lee et al., 2022) prior to behavioral testing in adulthood (Fig. 1A).

Experimental Design

Following adolescent THC/VEH treatment, 48 adult (PD 70+) rats (*n* = 29 males, 21 females) underwent attentional set-shifting training, followed by training on probability discounting until a group displayed stable levels of choice for 3 consecutive days, determined with previously established criteria (St. Onge and Floresco, 2009). Thereafter they underwent 3 counterbalanced amphetamine challenge tests, each (0, 0.25, 0.5 mg/kg IP) delivered 5 min before behavioral testing. Following the challenge rats were retrained for at least 2 days before receiving their next challenge.

For chemogenetic experiments, another adolescent THC/VEH-treated group (*n* = 33 males, 13 females; *n* = 28 TH:Cre+,18 wildtype (WT)) underwent stereotaxic VTA virus injection of a Cre-dependent hM3Dq vector at ~PD65, and intra-mPFC bilateral cannulae implantation at least 45 days later, at least 8 days prior to the first microinjection. Following recovery from

surgery, they were trained on the probability discounting task to stability, then subjected to a series of counterbalanced tests, again with re-stabilization training occurring between them. First, rats in the chemogenetic studies were tested 30min after 4 counterbalanced IP injections of the DREADD agonist CNO (5 mg/kg) or vehicle—both CNO and vehicle were administered twice on separate days, and data was averaged between both like tests for analysis. Next, they underwent 4 additional counterbalanced tests of probability discounting, each held 5min after intra-mPFC microinjections of CNO or vehicle. Results from intra-mPFC VEH and CNO tests were again averaged to increase reliability of findings. To average results across veh and CNO test days, data (risky lever responding, latency, omissions, win stay/lose shift probability ratio) were averaged within corresponding probability blocks.

Behavioral Methods

Operant Boxes

Training and testing took place in Med-Associates rat operant conditioning chambers (30.5 x 24 x 21 cm; St Albans, VT) within sound-attenuating boxes, equipped with two retractable levers with white lights above them, and white house light.

Operant Pretraining

Methods for both attentional set shifting and reward probability discounting tasks closely followed prior reports (Floresco et al., 2008; St Onge and Floresco, 2009). Rats were first homecage-habituated to highly palatable, 45 mg banana-flavored reward pellets (Bio-Serv catalogue #: F0059), then given 2d magazine training in the operant boxes, where they received 38 pellets at variable intervals over a 60 min session. They were next sequentially trained to press each of two levers to receive a pellet over 2-5 days. On each lever training day, a lever extended into the chamber (side counterbalanced across rats), and one pellet was delivered on a fixed ratio 1 (FR1) schedule. Once they reliably pressed 50+ times in 30min on a lever, they were transitioned to learning to press the other lever on the subsequent day, and training continued until meeting this criterion. Thereafter, they entered the next phase of the task, in which each lever was periodically extended into the chamber throughout a 30min session. Levers extended in a pseudorandom order such that there were 45 left-lever trials and 45 right-lever trials, but no more than two consecutive trials on which the same lever was extended. Each lever extension was accompanied by illumination of a house light signaling the start of a trial (90 trials/session), and trials occurred every 20s. If the rat pressed the lever within 10s of the start of a trial, the lever was retracted and a pellet was delivered, and the house light remained on for 4s. If the rat failed to press in 10s, the lever retracted and the house light extinguished until the next trial, and the trial was considered an omission. Importantly, cue lights present above each lever were never illuminated during this training phase. Rats were trained in this manner for at least 5 days, or until they omitted less than 5 trials per session. Since individual rats frequently display idiosyncratic lever position biases that can influence interpretation of subsequent behavior (Brady and Floresco, 2015), and to minimize such impacts of stochastic side-preference on subsequent tests, lever side preference was next assessed using a published protocol (Floresco et al., 2008; Brady and Floresco, 2015).

Visual Cue Discrimination Training

Next, rats were trained to respond on only one of the two levers extended on each trial—whichever was signaled as the correct response by illumination of a cue light just above it (Fig. 1B). Sessions began with both levers retracted and the house light off. Every 20s, one of the two stimulus cue lights was illuminated in a randomized order, 3s later both levers extended, and the house light turned on. If the rat pressed the lever that had a cue light illuminated above it within 10s of lever insertion, it received a pellet, the levers retracted, the stimulus light was extinguished and the house light remained illuminated for 4 s. If the rat did not choose a lever in

10s, the trial ended and levers retracted until the next trial, and the trial was scored as an omission. This training proceeded for at least 30 trials, ending when rats either made 10 consecutive correct responses, or after 150 trials had elapsed. If rats did not achieve 10 consecutive correct responses, they received a second identical training session on the following day.

Attentional Set Shifting Test

After learning to follow a cue light to respond for reward in the prior training phase, rats then underwent a single 40min session on which the response rule was suddenly shifted; a procedure analogous to the Wisconsin Card Sorting Task used in humans to aid diagnosis of PFC dysfunction (Owen et al., 1991; Pantelis et al., 1999). On this day, the non-preferred lever (determined in side-bias training described above) became the correct response, and pressing it during trials yielded a pellet and commencement of the next inter-trial interval (Fig. 1H). Light cues above one of the levers were presented just before and during trials as in the prior visual cue discrimination training, but now their location was irrelevant to the receipt of reward. Instead, rats needed to recognize that this old rule (follow the cue light) no longer worked, and that pressing of the previously less-preferred lever, regardless of cue light position, was now the correct strategy for obtaining reward. Trials continued until rats performed 10 consecutive correct responses, or after 150 trials had occurred. Errors during set-shift were categorized into two subtypes as in (Floresco et al., 2008): perseverative errors, where rats responded on the incorrect lever when the previously-relevant visual cue was illuminated above it, and never-reinforced (or non-perseverative) errors, where rats pressed the incorrect lever despite when the visual cue was illuminated above the correct lever.

Probabilistic Discounting

In this task rats must choose between a lever that always delivers a small (1 pellet) “certain” reward, or a second lever that delivers a large (4 pellet) “risky” reward, delivered at various signaled probabilities throughout the 52.5min session (Fig. 2A). The probability of receiving 4 pellets upon pressing the risky lever decreases in each of five sequential choice blocks during each session (100%, 50%, 25%, 12.5%, 6.25%). Each block begins with 8 forced trials (35s apart), in which the houselight was illuminated and 3 s later, one of the two levers extended one at a time for 10s (4 trials for each lever, randomized in pairs). This permitted rats to sample the probability of reward receipt upon pressing of the risky lever in the upcoming certain/risky lever choice phase of the block. During the subsequent choice phase of each block, both levers are extended simultaneously, and rats may choose to press only one of them. Certain lever presses always yielded 1 tasty banana pellet, and risky lever choices delivered 4 or 0 pellets, at a probability that varied across blocks. Failure to respond on a lever within 10s on any trial resulted in lever(s) retraction and house light extinction, and the trial is counted as an omission. Animals were trained on this task for 21 days, when responding had stabilized in all animals. For rats tested with systemic amphetamine during probabilistic discounting, training on this task commenced following attentional set shifting training and testing described above. Rats in chemogenetic experiments did not undergo visual cue discrimination or set shifting tests. Instead, these rats received pellet habituation, magazine training, initial FR1 training, and retractable lever training prior to training on the probability discounting task.

For rats used to test effects of acute amphetamine (0, 0.25, 0.5 mg/kg IP) on risky decision making, 3 counterbalanced tests were conducted on separate days, with re-training between tests to determine stable baseline responding. When rats in chemogenetic experiments achieved stable baseline responding, they similarly received 4 counterbalanced tests, 2 with CNO and 2 with VEH (data from both tests averaged for analysis), all injected IP 30min prior to probability discounting testing, with retraining between tests. After completing

these systemic CNO/VEH tests, rats were implanted with mPFC cannulae, allowed to recover, and re-stabilized on discounting performance for 2+ days. They were then tested on the discounting task in 4 additional counterbalanced tests with intra-mPFC microinjections of CNO (1mM/0.5 µl; 2 tests) and VEH (2 tests).

Chemogenetic Methods

Virus Surgery

Rats were anesthetized with ketamine (56.5 mg/kg) and xylazine (8.7 mg/kg) and given meloxicam (1.0 mg/kg) for pain prophylaxis. An AAV2 vector containing a Cre-dependent, mCherry-tagged hM3Dq excitatory DREADD (hSyn-DIO-hM3Dq-mCherry; titer: 6 x 10^{12} vg/ml; Addgene catalog #: 44361) was injected bilaterally into VTA (relative to bregma (mm): AP: -5.5, ML: ±0.8, DV: -8.1; 0.75 µL/hemisphere) using a Picospritzer and glass micropipette (Martinez et al., 2023). Injections occurred steadily over 1min, and the pipette was left in place for 5min after injection to limit spread. Both TH:Cre and WT rats were injected with the active hM3Dq DREADD virus. Colocalization of mCherry expression to TH+ VTA neurons was verified in each TH:Cre rat, and lack of mCherry expression was confirmed in each WT rat. At least 3 weeks elapsed between virus injections and the first CNO administration. In a second surgery held following behavioral training and systemic CNO tests (to maximize accuracy of placement) guide cannulae (22 ga, 2MM, Plastics One) were implanted bilaterally in the mPFC (relative to bregma (mm): AP: 2.7, ML: ±1, DV: -3.1) to allow intra-mPFC CNO injection upon VTA dopamine neuron axon terminals, and occluded with steel stylets between tests.

Histological Validation

Following behavioral testing, chemogenetic rats were perfused with chilled 0.9% saline and 4% paraformaldehyde, brains were cryoprotected in 20% sucrose-azide, and they were sectioned coronally at 40 µm. VTA virus expression was amplified with mCherry immunohistochemistry, and expression verified to be in dopamine neurons via costaining of tyrosine hydroxylase. VTA sections were then blocked in 3% normal donkey serum PBST, tissue was incubated overnight at RT in rabbit anti-DSred (Clontech; 1:2500) and mouse anti-TH (Immunostar; 1:2000). After washing, sections were incubated in dark at RT for 4hrs in AlexaFluor-donkey anti-Rabbit 594 and donkey anti-Mouse 488 (Thermo Fisher Scientific). Sections were mounted and cover slipped (Fluoromount; Thermo FisherScientific), mCherry/THC expression was imaged at 10X on a Leica DM4000 epifluorescent scope, and expression or lack thereof in VTA was verified in each animal. To verify cannula placement within mPFC, sections were nissl stained with cresyl violet, and cannula tracks were mapped using a rat brain atlas (Paxinos and Watson, 2006).

Data Analysis

Effects of adolescent treatment (AdoTX: THC x VEH) and Sex (M x F) on learning and cognition employed 2 x 2 ANOVA and Sidak posthoc tests. Effects of amphetamine (0 x 0.25 x 0.5mg/kg) on probability discounting were tested with repeated measures ANOVA, and interactions of these acute treatements with AdoTX and Sex were examined by adding these between subjects variables to multivariate General Linear Model ANOVAs. Simple-main effects analyses conducted after observing staticially significant interactions included one-way ANOVA models using the appropriate error term from the overal multifactor analyses. Effects of CNO versus VEH were treated as within-subjects variable, while Genotype (TH:Cre x WT), AdoTX, and sex were treated as between subjects variables in multivariate General Linear Model ANOVAs. Prior to testing, rats were verified to display stable patterns of risky choice by examining discounting performance over at least two consecutive prior training days. Rats of both sexes were tested in chemogenetic experiments, but sample sizes for each sex were insuffecient to allow formal analysis of this variable. Six rats (*n* = 2 males, 4 females; *n* = 1 VEH, 5 THC) were excluded

from set shifting analyses for failure to meet training critera for instrumental or visual cue rule performance, and nine rats (*n* = 9 females; *n* = 2 VEH, 7 THC) tested with amphetamine were excluded for failing to achieve stable performance on the probabilistic discounting task. Nine rats (*n* = 3 males, 6 females; *n*= 4 VEH, 5 THC) were excluded from the DREADD experiments for failure to stabilize on the probabilistic discounting, death, or inability to confirm virus expression.

Results

Effects of Adolescent THC on Attentional Set Shifting in Each Sex

Initial Instrumental Training: AdoTHC did not affect initial acquisition of instrumental food seeking behavior during initial training (no main effect of AdoTX: $Fs_{(1, 45)} < 0.396$, *ps* > 0.532), and acquisition was similar in both sexes (No main effect of sex: $Fs_{(1, 45)} < 3.604$, *ps* > 0.064; no interaction of Sex and AdoTX ($Fs_{(1, 45)} < 1.122$, *ps* > 0.295).

Visual Cue Discrimination: Rats were next trained to press whichever lever that had a cue light illuminated above it. AdoTHC exposed rats were more likely to learn the visual cue rule in one session rather than two, relative to AdoVEH rats (X^2: *p* = 0.039). This was driven by AdoTHC females, as evidenced by these animals requiring fewer trials and making fewer errors to reach criterion performance of 10 consecutive correct choices (Fig. 1C-G, AdoTX x Sex interaction; trials to criterion: $F_{(1,44)} = 5.308$, *p* = 0.026; errors to criterion $F_{(1,44)} = 5.032$, *p* = 0.03; (Šídák's posthoc for trials *p* = 0.022), errors *p* = 0.024. In addition, relative to controls, AdoTHC females were slower to respond ($F_{(1, 44)} =4.546$, *p* = 0.039) but also made fewer omissions: ($F_{(1, 44)} =$ 8.460, *p* = 0.006). In contrast, no differences were observed on these measures between AdoTHC males and controls (trial to criterion: *p* = 0.873; errors: *p* = 0.892; latency: *p* = 0.684 omissions: *p* = 0.719).

Attentional Set Shifting: Following training on the initial visual cue discrimination, rats were then trained to use an egocentric spatial rule (always press the left/right lever regardless of cue location), and tested in a single-session (Brady and Floresco, 2015)(Fig. 1I-M). AdoTHC had few effects on the ability to learn this new response rule with no change in either sex seen on the number of trials to reach criterion on the new rule (No main effect of AdoTX: $F_{(1,44)} = 0.416$, *p* = 0.522; or Sex: $F_{(1,44)} = 0.011$, *p* = 0.916; or interaction: $F_{(1,44)} = 0.796$, *p* = 0.377). Total errors to criterion were also unaffected (AdoTX: $F_{(1,44)} = 0.971$, *p* = 0.33; Sex: $F_{(1,44)} =0.323$, *p* = 0.573; AdoTX x Sex interaction: $F_{(1,44)} = 0.192$, *p* = 0.664), as were errors of either a perseverative or never-reinforced subtype (AdoTX: $F_{(1,44)} =1.556$, *p* = 0.219; Sex: $F_{(1,44)} = 0.134$, *p* = 0.716; AdoTX x Sex interaction: $F_{(1,44)} = 0.583 \times 10^3$, *p* = 0.981). Additionally, we saw no effect of AdoTX on response latency or on omissions (AdoTX: $Fs_{(1,44)} < 2.620$, *ps* > 0.113; AdoTX x Sex interaction: $Fs_{(1,44)} < 0.006$, *ps* > 0.937).

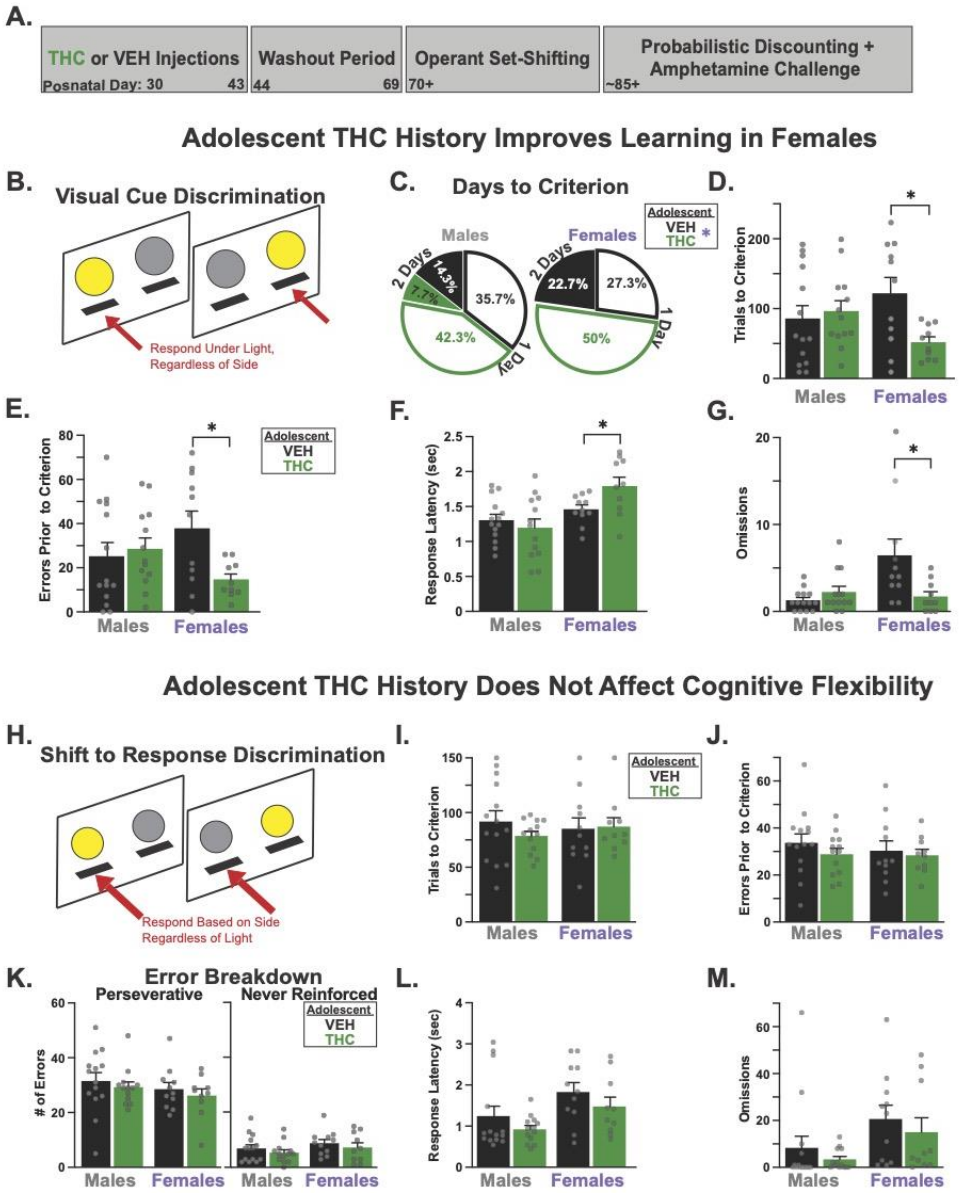

Figure 1. Adolescent THC history selectively impacts adulthood learning and cognition A) Experimental timeline is shown. **B)** Schematic of the visual cue discrimination task. **C)** AdoTHC rats (green wedges) were more likely than AdoVEH rats (black wedges) to acquire the visual cue discrimination task to criterion in only one training session (unfilled wedges), rather than requiring 2 sessions to acquire (filled wedges). Both sexes showed similar patterns. **D, E)** AdoTHC females took fewer trials to meet criterion, and made fewer errors when learning visual cue discrimination than AdoVeh females, no such effects were seen in males. **F, G)** AdoTHC females took longer to respond, and made fewer errors than AdoVEH females during cue discrimination training, without effects in males. **H)** Schematic of the subsequently tested attentional set-shifting task. **I)** AdoTHC did not alter the number of trials to learn the new rule to criterion in either sex. Likewise, AdoTHC did not alter in either sex **J)** the number of errors, **K)** the types of errors, **L)** latency to respond, or **M)** omitted trials during set shifting training.
Individual rats shown as grey dots in each graph: AdoVEH (n = 14 males, 11 females), AdoTHC (n = 13 males, 10 females). X^2 $p^* < 0.05$ and repeated measure two-way ANOVA, Sidak post hoc: $p^* < 0.05$. Data presented as mean + SEM.

Effects of Adolescent THC on Probability Discounting in Each Sex:

Acquisition of Probability Discounting: The same rats were next trained on a probabilistic discounting task, and all rats had acquired stable performance by the last three days of training (no block X day interaction: $F_{(5.498,241.89)} = 1.695$, $p = 0.130$). No effect of AdoTX ($F_{(1, 44)} = 0.817$, $p = 0.37$), Sex ($F_{(1,44)} = 0.116$, $p = 0.735$), or AdoTX x Sex interactions ($F_{(1, 44)} = 0.585$, $p = 0.448$) were found, suggesting that rats of both sexes and AdoTX histories acquired the discounting task similarly.

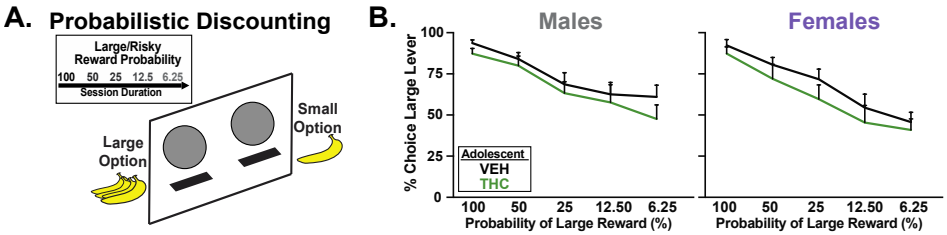

Figure 2. AdoTHC history does not affect baseline probability discounting. A) Schematic of the probability discounting task. Sessions consisted of 5 training blocks, in which 2 levers are presented, and rats must choose either a small reward lever that always delivers one pellet, or a large reward-delivering lever which becomes increasingly unlikely to deliver any reward as the session progresses. **B)** Data is shown for males and females, indicating that AdoTHC rats did not differ from their AdoVEH counterparts in probability discounting, with both shifting from nearly exclusively preferring the large reward lever, but appropriately shifting away from it as it became less likely to deliver reward. AdoVEH (*n* = 14 males, 11 females), AdoTHC (*n* = 15 males, 8 females). Data represented as average within each probability block from three consecutive days of stable performance. Mean ± SEM.

Stable Probability Discounting: Following training, all rats exhibited stable and comparable performance on the task by the last three days, with decreasing choice of the high-reward lever as delivery of this reward became increasingly unlikely or "risky" (main effect of Block: $F_{(4,176)} = 75.01$, $p < 0.001$). No effect of AdoTX ($F_{(1,44)} = 0.854$, $p = 0.361$), Sex ($F_{(1,44)} = 0.103$, $p = 0.75$), or AdoTX x Sex interactions ($F_{(1, 44)} = 0.616$, $p = 0.437$) were found, suggesting that rats of both sexes and AdoTX histories performed comparably (Fig. 2B). Likewise, neither AdoTX nor Sex affected win stay or lose shift choice strategies (no main effect of AdoTX: $Fs_{(1,44)} < 1.642$, $ps > 0.207$; no main effect of Sex $Fs_{(1,44)} < 0.549$, $ps > 0.463$; no AdoTX x Sex $Fs_{(1,44)} < 1.51$, $ps > 0.226$; data not shown). AdoTX also did not affect choice latencies (AdoTx: $F_{(1, 44)} = 0.161$, $p = 0.69$; no AdoTX X Sex interaction: $F_{(1, 44)} = 0.1.294$, $p = 0.262$). Finally, we found that AdoVEH rats omitted more on the "riskier" probability blocks compared to AdoTHC animals (AdoTX x Block interaction: $F_{(4, 176)} = 4.391$, $p = 0.002$; no AdoTx: $F_{(1, 44)} = 0.939$, $p = 0.338$), an effect that was particularly apparent in females (AdoTX x Sex x Block: $F_{(4, 176)} = 3.949$, $p = 0.004$; no AdoTX x Sex interaction: $F_{(1, 44)} = 0.804$,$p = 0.375$; data not shown).

Effects of Acute Amphetamine on Probability Discounting: As previously reported (St. Onge et al., 2010), amphetamine increased choice of the large/risky option in a dose dependent manner when analyzed across both groups and sexes in both groups (Fig. 3A, amphetamine x Block interaction: $F_{(8,328)} = 10.02$, $p < 0.0001$; main effect of amphetamine: $F_{(2,82)} = 9.88$, $p < 0.0001$). However, the effects of different doses of amphetamine varied as a function of AdoTX treatment and sex. Analysis of the choice data also revealed significant amphetamine x AdoTX ($F_{(2,82)} = 3.33$, $p = 0.041$) and amphetamine x AdoTX x Sex interactions ($F_{(2,82)} = 3.20$, $p = 0.045$). Partitioning this latter interaction by AdoTX group revealed that for control rats, amphetamine exerted different effects on choice in males vs females (amphetamine x Sex, ($F_{(2,44} = 3.73$, $p = 0.032$). Post-hoc comparisons showed that, in males, amphetamine increased risky choice following treatment with the 0.5 mg/kg dose ($p < 0.016$) but not the lower, 0.25 mg/kg dose ($p = 0.19$). In contrast, the 0.5 mg/kg dose had more deleterious effects on choice in females, reducing risky choice in the high probability blocks and increasing it in the lower ones, while the 0.25 mg/kg dose induced a modest increase in risky choice in the latter block. This yielded an overall lack of effect of amphetamine in control females (Fig. 3B, main effect of amphetamine; $F_{(2,18)} = 0.568$, $p = 0.57$). On the other hand, amphetamine induced a more reliable and

pronounced increases in risky choice in both males and females AdoTHC animals, with analysis of these data producing significant main effects of amphetamine treatment (Fig. 3A-B, $F_{(2,38)}$ = 12.07, p<0.0001) in the absence of an interaction with the sex factor ($F_{(2,38)}$ = 1.56, p=0.22).

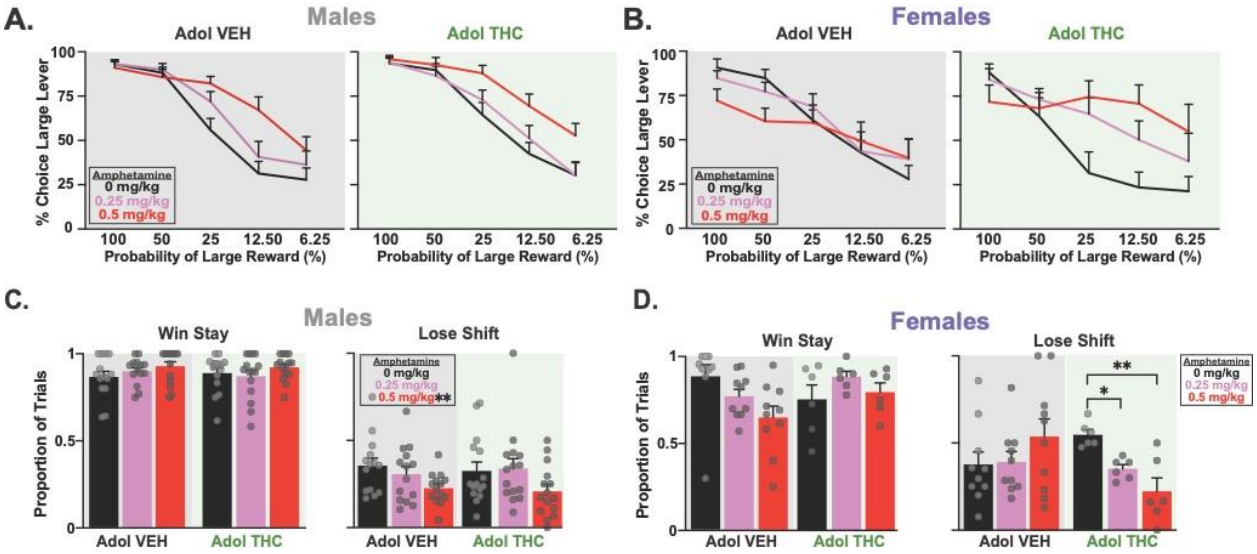

Figure 3. Amphetamine-induced 'risky' responding is potentiated after adolescent THC. A) Data from saline (black line/bar), low dose (0.25mg/kg; pink line/bar) and high dose (0.5mg/kg; red line/bar) amphetamine tests in each sex are shown. When performance patterns were further interrogated, we found that high dose of AMPH in Males increased inflexibility across both AdoTX groups, however in **B)** females, the high dose of AMPH in AdoVEH rats reduced risky responding at high probability blocks not seen in AdoTHC females. **C)** AMPH did not alter win-stay,but reduced lose-shift in AdoTX males. **D)** In AdoVEH females, AMPH decreased win-stay and increased lose-shift, while in AdoTHC AMPH had no effect on win-stay but decreased lose-shift. AMPH = amphetamine. AdoVEH (n = 14 males, 10 females), AdoTHC (n = 15 males, 6 females). Repeated measure three-way ANOVA; Sidak post hoc: p^* < 0.05, p^{**} < 0.01. Data represented as mean + SEM, individual animals shown as grey dots.

When collapsed across sex, post hoc comparisons showed that both the 0.25 and 0.5 mg/kg dose increase risky choice (both ps < 0.05), although inspection of Fig. 3B indicates that the effect of the 0.25 mg/kg dose was driven primarily by females. From these data, we conclude that AdoTHC treatment makes rats more sensitive to the ability of amphetamine to increase risky choice, and this effect appears to be more prominent in females.

Subsequent analyses examined how amphetamine alters sensitivity to recent rewarded or non-rewarded choices by comparing win-stay and lose shift ratios. Analysis of the win-stay data yielded a significant amphetamine x AdoTX x Sex interaction (Fig. 3C-D, $F_{(2,82)}$ = 4.34, p = 0.016). This was driven by the fact that in control rats, the 0.5 mg/kg dose resulted in lower win-stay values in females vs males (p < 0.01), although neither group showed significant changes in these values relative to saline (both Fs < 2.5, both ps > 0.10). Win-stay behavior was unaltered in AdoTHC rats (all Fs < 2.4, all p > 0.10). In contrast, amphetamine had more pronounced effects on sensitivity to reward omissions, as indexed by changes in lose shift behavior. The analyses here revealed a significant amphetamine x AdoTX interaction ($F_{(2,38)}$ = 4.84, p = 0.013) and a three-way interaction with the sex factor ($F_{(2,38)}$ = 5.15, p = 0.007). In controls, amphetamine reduced lose shift behavior in males, but actually increased it in females (Fig. 3C-D, amphetamine x sex interaction; $F_{(2,44)}$ = 3.86, p = 0.028 , whereas in AdoTHC rats, these treatments uniformly reduced lose-shift behavior across sexes (main effect of amphetamine: $F_{(2,38)}$ = 8.87, p < 0.001; amphetamine x sex interaction $F_{(2,38)}$ = 2.61, p = 0.08). From these data, we conclude that AdoTHC treatment makes rats more sensitive to the ability of amphetamine to increase risky choice and reduce sensitivity to losses, and this effect appears to be more prominent in females.

With respect to other performance measures, amphetamine increased choice latency and number of omissions across all probability blocks (main effect of amphetamine: $Fs_{(2,82)} < 15.19$, *ps* < 0.001, no amphetamine x Block interaction: $F_{(8,328)} < 0.98$, *ps* > 0.454). Analysis of the latency data revealed a significant amphetamine x AdoTX x Block ($F_{(8,328)} = 2.27$, *p*= 0.023) and amphetamine x AdoTX x Sex x Block interaction ($F_{(2,82)} = 2.67$, *p* = 0.008). Partitioning this latter interaction by AdoTX group revealed that for control rats, females took longer to respond than males (Sex x Block: $F_{(4,176} = 3.02$, *p* = 0.022). Irrespective of amphetamine and AdoTX, we saw that females took longer to respond and omitted more compared to males (Fig. 6Q-R, Sex x Block interaction: $Fs_{(4,164)} < 12.93$, *ps* < 0.001; main effect of Sex: $Fs_{(1,41)} < 71.39$, *ps* < 0.001).

Chemogenetic Dopamine Neuron Stimulation During Probability Discounting

The above experiments showed that AdoTHC increases visual cue learning in females but had few other effects on cognitive flexibility or probability discounting under basal conditions. However, when treated with the monoamine-enhancing drug amphetamine, we found evidence for stronger enhancement of "risky" responding in AdoTHC rats, relative to AdoVEH controls. We therefore next asked whether this effect relates to changes in the functions of VTA dopamine neurons in particular, by using Gq-coupled DREADDs to acutely stimulate VTA dopamine neurons or VTA dopamine neuron projections to mPFC in rats with both AdoTX histories.

Initial Training: Rats in this experiment did not undergo attentional set shifting training prior to probability discounting training, so we confirmed that AdoTX again did not affect initial acquisition of instrumental food seeking behavior during initial training (no main effect of AdoTX: $Fs_{(1, 46)} < 0.78$, *ps* > 0.381). We also confirmed that genotype (Geno: TH:Cre+ or WT littermate) did not alter instrumental training ($Fs_{(1,46)} < 2.55$, *ps* > 0.117; No AdoTX x Geno interaction: $Fs_{(1,46)} < 0.629$, *ps* > 0.432).

Probability Discounting Training: Likewise, all included rats (TH:Cre and WT) successfully learned the discounting task (Main effect of Block: $F_{(4,184)} = 129.26$, *p* < 0.001, no main effects of, or interactions involving AdoTX or Geno: $Fs_{(1,46)} < 0.884$, *ps* > 0.352). Additionally, AdoTX nor genotype did not affect latency, omissions, or win-stay/lose-shift choice strategies (no main effects of, or interactions involving AdoTX or Geno: $Fs_{(1,46)} < 3.34$, *ps* > 0.074).

Impact of Acutely Activating VTA Dopamine Neurons on Probability Discounting

Despite the robust changes in locomotion and reward seeking that is seen following chemogenetic VTA dopamine neuron manipulations in TH:Cre rats (Boekhoudt et al., 2016; Boekhoudt et al., 2018; Runegaard et al., 2018; Halbout et al., 2019; Mahler et al., 2019; Lawson et al., 2023), we found few effects of VTA dopamine neuron stimulation on the probability discounting task. First, we looked at VTA dopamine neuron stimulation in only TH:Cre rats. All rats displayed normal discounting profiles (TH:Cre+; main effect of Block: $F_{(4, 84)} = 105.934$, *p* < 0.001), and as in the prior experiment, no effect of AdoTX alone was seen on discounting (AdoTX x Block: $Fs_{(4, 84)} < 0.908$, *ps* > 0.463). Moreover, when we tested the effects of systemic CNO to activate DA neurons, we did not observe any effects of this treatment in either control or AdoTX groups (Fig. 4E no main effect of, or interactions involving AdoTX or CNO: Fs < 2.27, *ps* > 0.068). Additionally, we did not see any effects of CNO, AdoTX, nor interactions therein on choice latency, omissions, or win-stay/lose-shift choice strategy ($Fs_{(1,21)} < 3.00$, *ps* > 0.098; data not shown). These findings are inconsistent with our hypothesis that amphetamine's ability to increase risky/perseverative responding preferentially in AdoTHC-experienced rats is due to actions of the drug to increase dopamine signaling in mesolimbic pathways in particular.

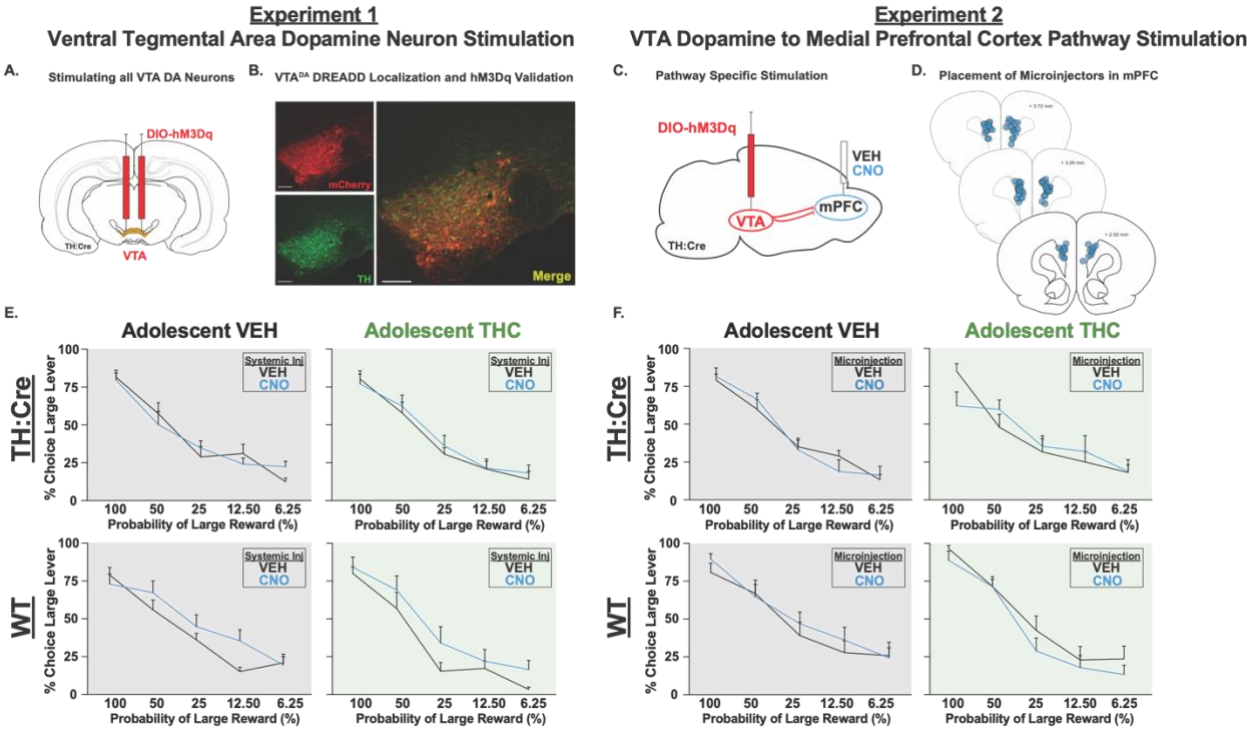

Figure 4. Stimulation of VTA dopamine neurons, or VTA dopamine projections to mPFC does not affect probability discounting. **A)** Bilateral Cre-dependent hM3Dq DREADD AAV injections were made in VTA of TH:Cre rats, and of wildtype (WT) littermates. **B)** Example hM3Dq DREADD expression (red) is localized to tyrosine hydroxylase+ (TH; green) neurons within VTA (yellow=merge). Scale bar, 300 µm. **C)** For pathway-specific stimulation of VTA dopamine projections to mPFC, Cre-dependent hM3Dq DREADDs were injected into VTA as in Experiment 1, and cannulae targeting mPFC allowed CNO microinjection (1mM, 0.5 µl) upon DREADD-expressing dopamine neuron axons in this pathway. **D)** Cannula placements of each rat in pathway stimulation Experiment 2 is shown. **E)** Neither VTA dopamine neuron stimulation induced by systemic CNO in TH:Cre rats, nor **F)** stimulation of the VTA dopamine projection to mPFC induced by mPFC CNO microinjections in TH:Cre rats altered probability discounting in AdoTHC or AdoVeh rats. Likewise, lower panels of **E&F)** indicate that CNO did not have robust effects on WT rats without DREADDs. Data is represented as average % choice of the large reward lever in each probability block across the two CNO, and two VEH tests conducted in each rat, M±SEM. TH:Cre+: VTA dopamine stimulation; AdoVEH (*n* = 8M, 3F) AdoTHC (*n* = 8M, 4F); VTA dopamine to mPFC: AdoVEH (*n* = 10M, 4F) AdoTHC (*n* = 9M, 5F). Wildtype: VTA dopamine stimulation; AdoVEH (*n* = 5M, 3F) AdoTHC (*n* = 5M); VTA dopamine to mPFC: AdoVEH (*n* = 9M, 4F) AdoTHC (*n* = 8M).

Impact of Selectively Activating VTA Dopamine Projections to mPFC

We next asked whether selectively stimulating VTA dopamine neuron projections to mPFC would recapitulate the potentiation of amphetamine effects in AdoTHC rats. We did so by locally applying CNO upon DREADD-expressing axons of VTA dopamine neurons in mPFC. We have shown that this pathway-specific stimulation approach is capable of potentiating both axonal dopamine release and motivated reward seeking (Mahler and Aston-Jones, 2012; Halbout et al., 2019; Mahler et al., 2019). We found few effects of this manipulation on probability discounting. On the probability discounting task, "risky" responding across the session varied across CNO and AdoTX (Fig. 4; AdoTX x CNO x Block interaction: $F_{(4,104)} = 2.991$, $p = 0.022$; no main effect of AdoTX: $F_{(1,26)} = 0.153$, $p = 0.699$; no main effect of CNO: $F_{(1,26)} = 0.000$ $p = 0.997$), in AdoVEH animals there were no measurable effects of or interactions with CNO (all Fs < 1.80, *ps* > 0.143). However, in AdoTHC rats CNO modestly reduced risky responding at the 100% probability block (CNO x Block: $F_{(4,52)} = 2.99$, $p = 0.027$).Rats performed similarly on choice latency, omissions, and win-stay/lose-shift regardless of AdoTX history, CNO, or interactions therein with block (All Fs < 1.996, *p*s > 0.101).

Minimal DREADD-independent Effects of CNO

Neither systemic nor intra-mPFC CNO had major behavioral effects in WT rats lacking DREADD expression (Fig. 4E,F). Systemic CNO in WT rats seemed to promote a more risk-prone phenotype, based on increased preference for the risky lever across all blocks (Main effect of CNO in WT rats: $F_{(1, 11)} = 9.424$, $p = 0.011$; no interactions of CNO, AdoTX, or Block: $Fs_{(4, 44)} < 1.936$, $ps > 0.121$.), but did not affect response latency, omissions, or win-stay/lose-

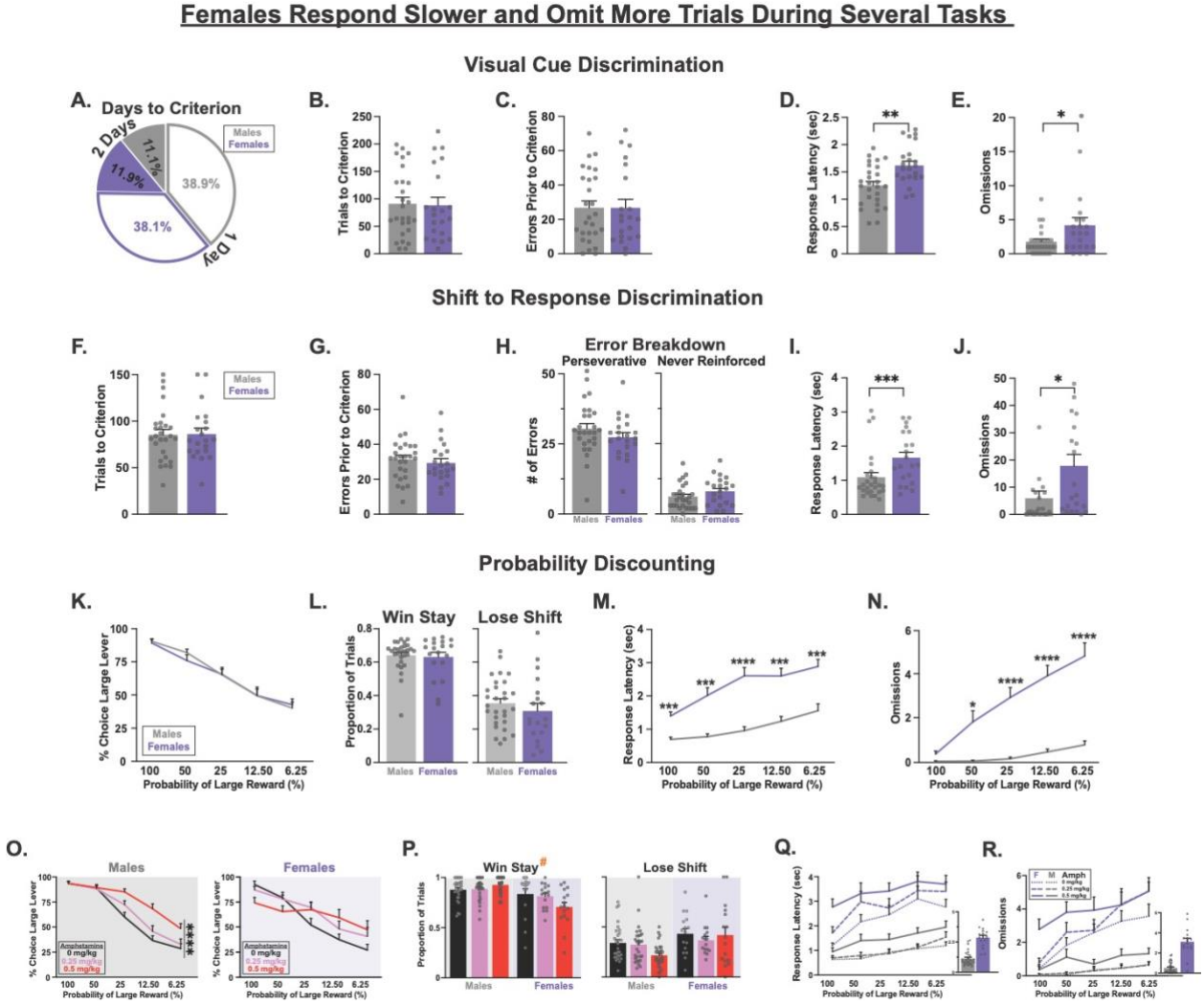

Figure 6. Summary of sex differences observed in operant set-shifting and probability discounting. For the initially learned visual cue discrimination rule, there were no sex differences seen on **A)** training days to criterion, **B)** trials to criterion, or **C)** number of errors prior to reaching criterion. However, females (purple bar/line) **D)** took longer to make choices and **E)** omitted more trials compared to males (grey bar/line) on the cue discrimination task. For the subsequent attentional set shifting task, sexes likewise did not differ on **F)** trials to criterion, **G)** errors made, or **H)** the types of errors made, but again **I)** females took longer than males to respond, and **J)** omitted more trials. For the probability discounting task, males and females **K)** did not differ in their overall performance, or **L)** on the Win-stay, Lose-Shift strategies employed. However, females again **M)** took longer to make decisions in this task, and **N)** omitted more trials than males, especially later in the session when probabilities of receiving the large reward were low. When effects of AMPH on probability discounting were examined by sex, we found that **O)** AMPH increased perseverative responding in males, but did not do so as robustly in females. **P)** AMPH caused females to decrease win-stay, but there were no major sex differences in wlose-shift patterns were seen, however lose-shift decreases were apparent in males, not females. Finally, as in the other tasks, **Q, R)** we again saw that females had longer choice latencies, and omitted more trials than males after AMPH as well as after saline responding. Hashtags represent significant AMPH by sex interaction. Bar graphs in inset represent: probability blocks collapsed across AMPH for each sex. Operant Visual Cue Discrimination and Attentional Set-Shifting: Males *(n* = 27) Females (*n* = 21); Probability Discounting: Males *(n* = 29) Females (*n* = 19); AMPH Challenge during Probability Discounting: Males *(n* = 29) Females (*n* = 16). AMPH = amphetamine.

shift choice strategies ($Fs_{(1,11)} < 2.962$, *ps* > 0.113). Similarly, intra-mPFC CNO in WT rats did not alter, risk responding, response latency, omissions or win-stay/lose-shift behaviors ($Fs_{(1,16)} < 3.742$, *ps* > 0.071).

A Focused Analysis of Behavioral Sex Differences: The primary objective of these experiments was to determine impacts of AdoTHC on later-life performance of mPFC-dependent behaviors, both normally and in the presence of acute stimulations of dopamine signaling. Yet we were also able to ascertain sex differences that persisted across these manipulated variables (i.e. main effects of sex), some of which replicate prior findings (St. Onge and Floresco, 2009; St. Onge et al., 2010; Islas-Preciado et al., 2020), and some of which are novel (Fig.5). *Visual Cue Discrimination & Set Shifting*: Relative to males, females were slower to respond during both visual-cue (Sex main effect: $F_{(1,44)} = 13.02$, $p < 0.001$) and spatial rule-based training ($F_{(1,44)} = 7.523$, $p = 0.009$), and omitted more trials (visual-cue: $F_{(1,44)} = 5.602$, $p = 0.022$); set-shift: $F_{(1,44)} = 6.201$, $p = 0.017$). *Probability Discounting*: As previously reported in this task (Islas-Preciado et al., 2020), females took longer to respond than males (Sex: $F_{(1,44)} = 40.03$, $p < 0.0001$) and omitted more trials ($F_{(1,44)} = 63.83$, $p < 0.0001$), especially in low-probability training blocks at the end of the session (Latency: Sex x Block interaction: $F_{(4,176)} = 4.572$, $p = 0.0015$; Omissions: $F_{(4,176)} = 26.57$, $p < 0.0001$). *Amphetamine Effects on Probability Discounting:* There was a significant effect of amphetamine in males and females (Fig. 5O; Amphetamine x Sex x Block: $F_{(8, 328)} = 7.13$, $p < 0.001$; main effect of sex: $F_{(1,41)} = 8.47$, $p = 0.006$) Amphetamine in males increased 'risky' responding (amphetamine x Block: $F_{(8,224)} = 6.53$, $p < 0.001$; main effect of amph: $_{(2,56)} = 12.97$, $p < 0.001$), however in females the high dose of amphetamine lead to a decrease in responding at the highest probability block (amphetamine X Block: $F_{(8,120)} = 3.624$, $p < 0.001$, Sidak's: $p < 0.02$), but did not exert an overall effect on response (no main effect of amph: $_{(2,30)} = 1.01$, $p = 0.378$). Amphetamine also had sex-dependent effects on response latency (Sex: $F_{(1,41)} = 61.51$, $p < 0.001$) and omissions ($F_{(1,41)} = 71.39$, $p < 0.001$), with the drug slowing responding and increasing omissions in females without altering these behaviors in males. Additionally, amphetamine sex-dependently altered win-stay (WinStay: Sex x amphetamine interaction: $F_{(2,82)} = 4.08$, $p = 0.021$) with the high dose of amphetamine causing females to shift to the certain lever following a 'risky' win compared to males (Sex: $F_{(1,41)} = 20.11$, $p < 0.001$). Amphetamine did not sex-dependently alter lose-shift (LoseShift: Sex x amphetamine interaction: $F_{(2,82)} = 1.10$, $p = 0.338$), however males tended to stick with the 'risky' lever following a risky loss compared to females (Sex: : $F_{(1,41)} = 8.51$, $p = 0.006$). *Additional Analysis of Sex Effects:* To further verify effects of sex on behavior and potentially reveal details that were obscured by other tested variables in the sex main effects analysis above, we employed a complementary analysis in which effects of sex were determined on tests following control injections prior to probability discounting (i.e. saline day for amphetamine-tested rats and VEH day from chemogenetic rats). We again observed that relative to males, females took longer to respond on trials (Sex X Block interaction: $F_{(4, 340)} = 12.30$, $p < 0.001$; main effect: $F_{(1, 85)} = 112.47$, $p < 0.001$), and omitted more trials (Sex X Block interaction: $F_{(4, 340)} = 20.56$, $p < 0.001$; main effect: $F_{(1, 85)} = 97.45$, $p < 0.001$) as probabilities decreased across the session. We did not observe any sex effects on 'risky' lever responding (main effect: $F_{(1, 85)} = 1.48$, $p = 0.228$) or on win-stay/lose-shift behaviors (Sex: $Fs_{(1, 85)} < 3.58$, *ps* > 0.62).

Discussion

Here we show that administration of a well-characterized, human-relevant dose of THC (Torrens et al., 2020; Ruiz et al., 2021b; Torrens et al., 2022) during adolescence has subtle effects on behavioral tests of instrumental learning, without measurably altering performance on mPFC dopamine-dependent attentional set shifting or probability-based discounting decision

making tasks. However, when monoamine signaling was acutely enhanced with systemic amphetamine, an underlying effect of Adolescent THC (AdoTHC) history was revealed—relative to AdoVeh controls, amphetamine in AdoTHC rats caused greater increases in perseverative responding for a large reward option when this choice was unlikely to result in reward. However, this potentiation of amphetamine effects in AdoTHC rats was not recapitulated by more specific chemogenetic stimulation of VTA dopamine neurons, or of their projections to mPFC in particular. This could suggest non-VTA dopaminergic mechanisms underlying potentiation of this cognitive effect of amphetamine in AdoTHC rats, suggesting potential impacts of THC on adolescent development of other systems upon which amphetamine acts. Alternatively, the enhanced effect of amphetamine reported here may be driven by alterations at the level of the dopamine terminal rather than changes in dopamine cell excitability as dopamine receptor antagonism is able to block this amphetamine-induced risky responding (St Onge and Floresco, 2009). Further, we thoroughly characterize sex differences in decision-making across these behavioral tasks, some of which mediate the persistent impacts of AdoTHC on behavior. Results open new directions for investigating long-term impacts of AdoTHC on non-VTA dopaminergic modulation of cognition, and may inform associational studies of the long-term impacts of adolescent cannabis use in humans.

Adolescent THC Exposure Enhances Initial Discrimination Learning

AdoTHC rats learned a visually cued instrumental response rule quicker than AdoVEH-treated controls, and this effect was most robust in females. This finding adds to the growing literature on potentially pro-learning/cognitive effects of AdoTHC (Bilkei-Gorzo et al., 2017; Sarne, 2019; Hernandez et al., 2021; Stringfield and Torregrossa, 2021a). This said, previous studies using other adolescent cannabinoid exposure models have not found analogous increases in initial rule discrimination learning (Gomes et al., 2015; Hernandez et al., 2021; Freels et al., 2024), though this might be due to the fact that few studies included female subjects, and the THC administration protocols employed were quite different.

We found no clear effects of AdoTHC across our attentional set shifting performance metrics, including trials to criterion, number of errors made, or error types on the set shift day. These findings are consistent with others that found no major AdoTHC-induced changes in cognitive flexibility, as quantified in an attentional set shifting task (Gomes et al., 2015; Hernandez et al., 2021; Poulia et al., 2021). However, in a study utilizing the same attentional set-shifting task as used here, females exposed to self-administered cannabis extract vapor during adolescence took longer to learn the new rule, and made more errors on the set-shift day, though this deficit was not seen after experimenter-administered cannabis vapor (Freels et al., 2024). It is presently unclear whether differences in patterns of results reflect differences in route of THC administration or dose, the specific timing of THC exposure during adolescence, or other experimental details.

Though untested here, it is possible that AdoTHC altered other related cognitive processes such as transitioning between tasks, mental sets, or rule structures, which depend upon more lateral PFC subregions such as OFC (Birrell and Brown, 2000; McAlonan and Brown, 2003; Floresco et al., 2008). For example, adolescent pubertal administration of the potent CBR agonist WIN 55,212 alters OFC-dependent reversal learning in the attentional set-shifting task (Gomes et al., 2015), though adolescent vaporized cannabis or cannabis smoke did not impact this same type of reversal learning (Hernandez et al., 2021; Freels et al., 2024). Again, discrepancies between studies may reflect differences in effects of cannabinoid drugs, doses, exposure timing, and washout period; further underscoring the need for a consistent, rationally-designed AdoTHC exposure model in the field—we argue that the model used here is the best-characterized to date in the field (Torrens et al., 2020; Ruiz et al., 2021b; Lee et al., 2022; Torrens et al., 2022;

Halbout et al., 2023; Lin et al., 2023; Lee et al., 2024). This said, the possibility that OFC is even more sensitive to disruption by AdoTHC than mPFC should be directly tested in future studies.

Adolescent THC Does Not Alter Basal Probability Discounting

In the mPFC-dependent probability discounting task (St. Onge and Floresco, 2009), rats choose between a small reward that is always delivered when chosen (1 palatable banana pellet), and a larger reward that becomes increasingly unlikely to be delivered over the course of the ~1h session (providing either 4 or 0 pellets). Efficient performance on this task demands evaluation of both risk and opportunity, and is dependent on intact functioning of both the mPFC and mesocortical and mesoaccumbens dopamine transmission (St. Onge et al., 2010; Onge et al., 2012; Stopper et al., 2013; Jenni et al., 2017). Impaired PFC activity results in deficits in adjusting choice in response to changes in reward probabilities, loss assessment, and a diminished ability to appropriately compare and favor larger rewards even when the probability of receiving them is higher (St. Onge and Floresco, 2009; Bercovici et al., 2023).

We found no major impacts of AdoTHC history on acquisition of, or stable performance on this task, as measured by choices of the 'risky' lever, choice strategies following rewarded vs unrewarded trials, latency to decide, and decisions to omit trials. One prior study (Jacobs-Brichford et al., 2019) found that administration of WIN 55,212 in both sexes during adolescence elevated preference for the 'risky' lever at lower reward probabilities, which we did not see in either sex as a result of our AdoTHC exposure model. Though several other differences exist between this study and the present one, we note that several prior reports have also shown more severe lasting effects of synthetic CBR agonists versus THC when administered in adolescents (Renard et al., 2014; Higuera-Matas et al., 2015; Stringfield and Torregrossa, 2021b).

Adolescent THC History Potentiates Amphetamine Effects on Probability Discounting

Dopamine markedly influences mPFC-dependent cognition (Goldman-Rakic, 1995; Seamans and Yang, 2004; Floresco and Magyar, 2006; Goto et al., 2007), including in the probability discounting task employed here (Floresco and Whelan, 2009; St Onge and Floresco, 2009; St Onge et al., 2010; Onge et al., 2012; Jenni et al., 2017; Islas-Preciado et al., 2020). In the present study we replicated prior findings that pharmacologically challenging monoamine systems with amphetamine increased perseveration of responding for a large reward in both males and females (St Onge and Floresco, 2009; Islas-Preciado et al., 2020). This was apparent in that amphetamine increased 'risky' choices selectively, and this change was accompanied by a reduction in lose-shift behaviors following 'risky' losses. Interestingly, we saw that amphetamine led to an overall increase in choice latency, and omitted trials.

Moreover, we found that this effect of amphetamine was more potent in AdoTHC, relative to AdoVEH rats, especially in females. At the highest dose of amphetamine, AdoTHC rats chose the 'risky' lever more when probabilities of that reward were low. These effects were also markedly sex-dependent. In males, amphetamine reduced overall lose-shift behavior. In females, the highest dose of amphetamine reduced win-stay behaviors only in AdoVEH females and reduced lose-shift behaviors only in AdoTHC females, contributing to the heightened increased 'risky' responding observed in the AdoTHC groups. These results suggest that although probability discounting under basal conditions is not altered by AdoTHC, underlying differences were nonetheless revealed upon acute activation of monoaminergic signaling. Amphetamine blocks and reverses the transporter for dopamine, NE, 5HT, and changes in one or more of these systems could be responsible for the potentiated response to amphetamine we see in this task in AdoTHC rats (especially females).

Effects of Chemogenetic VTA Dopamine Neuron and mPFC Dopamine Projection Stimulation on Probability Discounting

In our next experiments, we sought to determine whether, as predicted, changes in VTA dopamine neuron signaling is responsible for the potentiated effect of amphetamine in AdoTHC rats. We therefore prepared transgenic TH:Cre rats with excitatory DREADDs specifically in either dopamine neurons, or in dopamine neuron projections to mPFC in particular. Surprisingly, no major effects of chemogenetically stimulating all VTA dopamine neurons, were found on probability discounting behavior. We found a small effect on risky choice when stimulating VTA dopamine neuron projections to mPFC that varied by probability block in AdoTHC rats, however the lack of effect on pathway-specific stimulation in AdoVEH is similar to a prior finding examining manipulations of dopamine projections during probability discounting (Verharen et al., 2018). Moreover, no differential effects of DREADD stimulations were seen in AdoTHC versus AdoVEH groups. This result is unlikely to be due to a lack of efficacy of the chemogenetic manipulations employed, since our prior work and that of others' shows that such stimulation in TH:Cre rats has pronounced effects on locomotion, reward intake, and motivation (Boekhoudt et al., 2016; Boekhoudt et al., 2018; Runegaard et al., 2018; Halbout et al., 2019; Mahler et al., 2019; Lawson et al., 2023). This may imply that alterations of VTA and non-VTA dopamine caused by amphetamine might simply be qualitatively distinct from manipulations of VTA dopamine neuron activity and dopamine release, as were conducted here chemogenetically (Mahler and Aston-Jones, 2012; Mahler et al., 2019). Alternatively, amphetamine-induced potentiation seen in AdoTHC rats may not solely depend upon VTA dopamine neurons themselves, but may instead depend upon other neural systems targeted by amphetamine, and/or non-mPFC targets of dopamine neurons. Regardless, this intriguing finding refines our understanding of the neural substrates of probability-based decision making and underscores the need for further exploration to identify the specific mechanisms involved.

Sex Differences

We conducted these studies in female as well as male rats, and found several consistent sex differences, both overall and in interaction with other experimental variables. Consistent with existing literature (Islas-Preciado et al., 2020; Gargiulo et al., 2022), we saw sex differences in response characteristics during operant set-shifting as well as probabilistic discounting, with females showing longer deliberation times (response latency), and more omitted trials than males. Strikingly, these specific sex differences consistently surfaced in the probability task across various experiments and cohorts, underscoring the robustness of the response latency and trial omission effects in females. Some of these sex differences may derive from females attaining satiety on the palatable reward quicker than males, but since both effects at least partially emerged early in sessions during otherwise apparently vigorous reward seeking (e.g. omissions and latencies), this finding may also reflect different strategies taken by females relative to males (Orsini and Setlow, 2017; Chen et al., 2021). Females, which have previously been shown to be more risk-averse than males on average (Orsini et al., 2016), may exhibit prolonged response times and higher omission rates due to aversion to the risk of losing a reward, as demonstrated by their heightened sensitivity to loss (van den Bos et al., 2012). Alternatively, extended choice latencies and omissions may signify females' tendency to take more time in learning about the probability distribution of the outcomes. This aligns with both rodent and human findings, indicating that females take longer to develop a preference for the more advantageous option when learning about probability distributions of reward versus punishment (van den Bos et al., 2012; van den Bos et al., 2013). Both possibilities warrant further exploration in future research.

Limitations:

The present report has a number of limitations that should be considered. A single dose of THC (5mg/kg IP) was administered to adolescents, and persistent effects of THC are known to be dose-dependent (Amal et al., 2010; Irimia et al., 2015; Freeman-Striegel et al., 2023), and dependent upon the specific developmental stage at which it is experienced (Cha et al., 2006; Schramm-Sapyta et al., 2007; Mokrysz et al., 2016; Gorey et al., 2019; Torrens et al., 2020; Murray et al., 2022; Torrens et al., 2022). Also relevant to dose, blood and brain levels of the THC active metabolite 11-OH-THC are seen in adolescent and adult rodents (Tseng et al., 2004; Wiley and Burston, 2014; Craft et al., 2019; Torrens et al., 2020; Baglot et al., 2021; Ruiz et al., 2021a; Ruiz et al., 2021b; Torrens et al., 2022), as well as humans (Matheson et al., 2020; Sholler et al., 2021; Arkell et al., 2022) —which may at least in part account for the more robust persistent effects of adolescent THC in females than males, as seen here and in numerous prior reports (Tseng et al., 2004; Cha et al., 2007; Rubino et al., 2009; Rubino and Parolaro, 2011; Le et al., 2022). Another limitation of this report is the lack of data on complementary cognitive tasks dependent upon other cortical regions like OFC, which is notably sensitive to adolescent cannabinoid exposure (Egerton et al., 2005; Klugmann et al., 2011; Gomes et al., 2015), and mediated by ECB-dependent processes, and therefore may be sensitive to AdoTHC's long-lasting impacts.

Summary:

In sum, this paper provides novel information about the impact of AdoTHC on the developing brain of males, and especially females. Results point to long-lasting changes that influence cue learning, and the ability of acute amphetamine to alter risky decision making. A key finding is the pervasive nature of sex-differences—both in behavioral strategies employed by rats in these tasks, and in the severity of long-lasting consequences of AdoTHC.

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