Sex specific effects of dorsomedial striatal cannabinoid receptor-1 signaling on Pavlovian outcome devaluation.

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ABSTRACT

Cannabinoid-1 receptor (CB1R) signaling in the dorsal striatum regulates the shift from flexible to habitual behavior in instrumental outcome devaluation. Based on prior work establishing individual, sex, and experience-dependent differences in Pavlovian behaviors, we predicted a role for dorsomedial striatum CB1R signaling in driving sign-tracking and rigid responding in Pavlovian outcome devaluation. We trained male and female rats in Pavlovian Lever Autoshaping to determine sign-, or goal- or intermediate tracking groups. After extended Pavlovian training, we gave intra-DMS infusions of the CB1R inverse agonist, rimonabant, before satiety-induced outcome devaluation test sessions, in which we sated rats on training pellets (devalued) or home cage chow (valued) and examined responding to cues in brief nonreinforced Pavlovian Lever Autoshaping sessions. Overall, DMS CB1R signaling inhibition blocked Pavlovian outcome devaluation. After extended training, male rats were sensitive to devaluation while female rats were not. Inhibition of DMS CB1R signaling impaired Pavlovian outcome devaluation in male sign-tracking rats making their behavior more rigid but had no effects in female sign-tracking rats. Intra-DMS rimonabant infusions before reinforced sessions had no effects on Pavlovian sign- or goal-tracking in either sex. The sex-specific and CB1R-dependent effects were specific to outcome devaluation and were consistent between sign- and goal-tracking groups. Our results demonstrate that DMS endocannabinoid receptor signaling regulates behavioral flexibility in a sex-specific manner, suggesting differences in CB1R signaling in DMS between male and female rats.

INTRODUCTION

Impairments in behavioral flexibility occur across a range of mental health disorders including substance use disorder, schizophrenia, obsessive-compulsive disorder, and depression [1–6]. Preclinical studies suggest that sex and individual differences influence behavioral control when environmental conditions change from what is expected [7–11]. Specifically, the pre-clinical sign-tracking model suggests more rigid response strategies in females compared to males [12,13]. Understanding the neurobiological underpinnings of individual and sex differences in behavioral flexibility may help to identify novel therapeutic targets for disorders of behavioral control.

Instrumental conditioning procedures in rats identified dorsal striatal regulation of behavioral flexibility, which involves dorsomedial and dorsolateral striatal (DMS, DLS) subdivisions. After limited training in an instrumental task in which an action leads to a food outcome, the DMS is critical for decreasing actions when the associated food outcome is devalued [14–16]. After extended training, actions become insensitive to outcome devaluation, and this rigid responding requires DLS activity [15,17–19]. The shift from goal-directed to habitual behavior that occurs with instrumental experience is mediated by a shift from DMS to DLS control. An instrumental study in mice established that CB1Rs on glutamatergic synapses projecting from the orbitofrontal cortex (OFC) to the dorsal striatum (DS) gate behavioral flexibility in outcome devaluation [20]. CB1R deletion in the OFC-DS projection promotes devaluation sensitivity even during schedules of reinforcement that ordinarily drive rigid responding [20]. While these studies are not region specific, they suggest that CB1R-mediated inhibition of synaptic inputs in the outcome devaluation sensitive DMS shift behavior towards rigid, devaluation insensitive actions. We hypothesize that DMS CB1R signaling also biases behavior towards devaluation insensitive Pavlovian approach. Here we use intracranial CB1R inverse agonist, rimonabant, to determine the role of DMS CB1R in mediating Pavlovian devaluation sensitivity in male and female rats.

While far less is known about the role of dorsal striatum in driving behavioral flexibility in Pavlovian outcome devaluation, Pavlovian procedures are ideal for identifying individual and sex differences in behavioral flexibility. The sign-tracking model has uncovered considerable individual, sex and, experience-dependent differences in Pavlovian devaluation sensitivity [9,12,13,21,22].

After limited training (<10 sessions) in Pavlovian lever autoshaping (PLA), in which an insertable lever cue predicts a food outcome, goal-tracking (GT) rats show sensitivity to outcome devaluation while sign-tracking (ST) rats do not [9,11,23,24]. After extended training (>10 session), both GT and ST rats show sensitivity to satiety-induced outcome devaluation [9], an effect established in male rats. Female rats show increased levels of sign-tracking, or lever-directed approach during PLA compared to males [12,13,22,25–27], suggesting they may be less sensitive to outcome devaluation even after extended training. Here we evaluate sex differences in behavioral flexibility using the sign-tracking model and determine whether DMS CB1R signaling contributes to individual and sex differences in devaluation sensitivity after extended Pavlovian training.

METHODS

Subjects

We used 68 Long Evans rats (33 Male, 35 Female; run as 5 cohorts) in the age range of 7-9 weeks old at the start of training for this study. All rats were double-housed upon arrival and then single-housed 24-48 hours after arrival. We maintained rats on a reverse 12hr:12hr light-dark cycle (lights off at 1000). We performed all behavioral procedures during the dark phase of the light cycle. All rats had *ad libitum* access to standard laboratory chow and water before we food deprived them to maintain 90% of their baseline weight. We surgerized one cohort prior to any behavioral training and testing and surgerized the remaining cohorts after three days of training. There were no pre- or post-surgery differences in behavior between groups. We performed all procedures in accordance with the "Guide for the Care and Use of Laboratory Animals" (8th edition, 2011, US National Research Council) and with approval by the University of Maryland, School of Medicine Institutional Animal Care and use Committee (IACUC).

Apparatus

We conduct behavioral experiments in identical operant chambers (25 X 27 X 30 cm; Med Associates) located in a separate room from the animal colony. An individual sound-attenuating cubicle with a ventilation fan surrounds each chamber. One wall contains a red house light and the opposing wall contains a food cup with photobeam detectors that rests 2 cm above the grid floor. A programmed pellet dispenser attached to the foodcup and dispensed 45 mg food pellets (catalog #1811155; Test Diet Purified rodent Tablet [5TUL]; protein 20.6%, fat 12.7%, carbohydrate 66.7%). We installed one retractable lever at 6cm above the grid floor on either side of the foodcup and we counterbalanced the lever side between subjects.

Surgical Procedures

After three days of Pavlovian Lever Autoshaping training, we gave ad libitum access to food before we performed intracranial cannula placement surgery. We anesthetized 8-week-old rats with isoflurane (VetOne, Boise, ID, USA; 5% induction, 2-3% maintenance) then administered the pre-operative analgesic carprofen (5mg/kg, s.c.) and lidocaine (10mg/mL subdermal at incision site). We placed them in a stereotaxic frame (model 900, David Kopf Instruments, Tujunga, CA, USA) over a heating pad to maintain stable body temperature throughout surgery.

We implanted guide cannula (23G; PlasticsOne INC, Roanoke, VA, USA) bilaterally at an 8 degree angle and 1mm above the injection site into the DMS (coordinates from bregma -0.24 mm AP, ± 2.6 mm ML and -4.5 mm DV). We determined distance from bregma using the Paxinos and Watson rat brain atlas [28]. Cannula were secured to the skull with jeweler's screws and dental cement. At the end of surgery, we inserted dummy cannula into the guide cannula, which we only removed during infusion habituation and infusion test procedures. We moved rats to a recovery cage over a heating pad, administered carprofen analgesic at 24 hr, 48 hr and 72 hr post-surgery. We gave animals 1 week of recovery before resuming behavioral procedures.

Pavlovian Lever Autoshaping Training

Prior to training, we exposed all rats to the food pellets in their home cage to reduce novelty to the food. Then we trained them in daily Pavlovian lever autoshaping sessions which lasted ~ 26 minutes and included 25 trials of non-contingent lever presentations (conditioned stimulus; CS) and occurred on a VI 60 s schedule (50-70s). At the start of the session, the houselight turned on and remained on for the duration of the session. Each trial consisted of a 10 s lever presentation and retraction of the lever followed immediately by delivery of two 45 mg food pellets into the foodcup. At the end of the session, we returned rats to their cage and colony room. We trained rats in PLA first for 5 days to determine their tracking group, then continued training through 12 days following PLA testing.

Pavlovian Lever Autoshaping Testing

We tested the effects of blocking DMS CB1R during reinforced Pavlovian Lever Autoshaping. We infused rimonabant (SR141716 1 µg/µl or 2 µg/µl; dissolved in 1:1:18 ethanol: emulphor: saline solution) or vehicle bilaterally into DMS at a rate of 0.5 µl/min over the span of 1 minute. We left the infusion cannula in place for an additional minute before slowly removing them and replacing the dummy cannula. We waited 10 min after infusion before placing rats into the behavioral chamber and running the lever autoshaping test. We infused all rats with vehicle, low (1 µg/µl) or high (2 µg/µl) dose of rimonabant across three days and we counterbalanced the dose across days.

Satiety-Induced Outcome Devaluation Testing

After the 12th training session, we gave rats two sessions of satiety-induced outcome devaluation. Rats had one hour of *ad libitum* access to 30 g of either their homecage chow (valued condition) or food pellets used during PLA training (devalued condition) in a ceramic ramekin. Within 15 min of the end of the satiation hour, we performed intra-DMS rimonabant infusions as described in the previous section. We waited 10 min after the infusion before placing rats into the behavioral chamber and running the lever autoshaping test. Tests consisted of 10 non-rewarded lever presentations on VI 60s schedule (50-70s). Immediately after each test, we gave rats a 30 min food choice test in their homecage which included 10 g of homecage chow and 10 g of food pellets in separate ceramic ramekins to confirm satiety was specific to the outcome they had been fed before the test session. We retrained rats on 25 reinforced trials on a separate day between devaluation probe tests.

Measurements

For training and devaluation probe tests, we recorded the number and duration of foodcup and lever contacts, the latency to contact, and the probability during the 10 s CS (lever) period. On trials with no contacts, a latency of 10s was recorded. To determine tracking group, we used a Pavlovian Conditioned Approach (PavCA) analysis [29] which quantifies behavior along a continuum where +1.00 indicates behavior is primarily lever directed (sign-tracking) and -1.00 indicates behavior is primarily foodcup directed (goal-tracking). PavCA scores are the average of three separate scores: the preference score (lever contacts minus foodcup contacts divided by the sum of these measures), the latency score (time to contact foodcup minus the time to contact lever divided by 10 s (duration of the cue)) and the probability score (probability to make a lever contact minus

the probability to make a foodcup contact across the session). We use the PavCA score from the 5th day of training to determine an individual's tracking group as follows: sign-trackers (ST) have a PavCA sore +0.33 to +1.00, goal-trackers (GT) have a PavCA score -1.00 to -0.33, intermediates (INT) have scores ranging from -0.32 to +0.32. Rats in goal- and intermediate tracking groups were combined into a single GT/INT group as in our prior studies [9]. On day 6, we were unable to record latency data for 6 rats and only retained lever and foodcup contacts for these rats. Preference score was used in place of PavCA for rats on this day.

For devaluation probe tests, we also report total approach (the sum of food cup and lever contacts during the 10 s CS period) and individual contact measurements. We recorded consumption on the test days and calculated the amount of pellet or chow consumed in grams during the satiety hour and during the 30 min choice test.

Histology

At the end of experiments, we deeply anesthetized rats with isoflurane and transcardially perfused 100ml of 0.1M sodium phosphate buffer (PBS), followed by 200ml of 4% paraformaldehyde (PFA) in PBS. We removed brains and post-fixed them in 4% PFA over night before we transferred them to 30% sucrose in dH2O for 48-72 hr at 4 °C. We rapidly froze brains in dry ice before storing them in -20 °C until slicing. We sliced brains with the Leica Microsystems 1850 cryostat to collect 40 µm coronal sections in three series through the cannula placements in the DMS. We mounted sections onto gel-coated slides and then stained with cresyl violet before coverslipping with Permount. We examined placements under a light microscope for confirmation of cannula placement in the DMS (Fig. 2D). We excluded 11 rats due to cannula placements being outside the region of interest.

Experimental Design and Statistical Analysis

We analyzed behavioral data using SPSS statistical software (IBM) with mixed-design repeated measures analysis of variance (ANOVA). Significant main and interaction effects ($p<0.05$) were followed by post-hoc repeated-measures ANOVA or paired t-tests. Analyses included between subject factors of Tracking (ST, GT/INT) Sex (male, female) and Treatment (vehicle, rimonabant) and within-subject factors of Session (1-12), Outcome Value (Valued, Devalued), or Outcome (Nonsated, Sated).

RESULTS

Acquisition of Pavlovian Lever Autoshaping

We trained rats for 12 days in Pavlovian Lever autoshaping before outcome devaluation testing. We used the Pavlovian Conditioned Approach Index (PavCA) on the 5th session of training to determine tracking groups (Fig. 1A). Consistent with our prior studies [9,30] showing that GT and intermediate rats shift away from food-cup approach and towards lever approach with extended training, we observe a main effect of Session for PavCA Index, $F(11,583)=106.292$, $p<0.001$, and a Session x Tracking (ST, GT/INT) interaction, $F(11,583)=13.909$, $p<0.001$. Next, we examined whether there were sex differences in the acquisition and expression of Pavlovian lever autoshaping (Fig. 1B). We used a mixed design repeated measures ANOVA with between-subject factors of Sex (Male, Female) and within subject factor of Session (1-12) and found a Session x Sex interaction for PavCA Index, $F(11,605)=1.823$, $p=0.047$). While males and females have similar approach during initial acquisition, female rats showed more sign-tracking, via a higher PavCa Index, than males with extended training (day 8, $t(55)=-1.754$, $p=0.043$; day 9, $t(55)=-2.007$, $p=0.025$). However, there were no sex differences in responding on the last day of training (PavCa Index; $t(55)=-1.099$, $p=0.277$). Thus, we observe no sex differences in rats' autoshaping behavior on the day prior to testing in outcome devaluation.

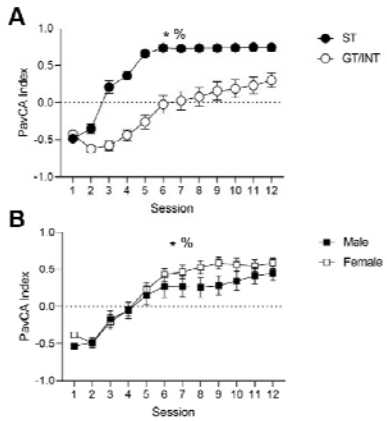

Figure 1. Pavlovian Conditioned Approach during training split by Tracking and Sex ***A,*** PavCA Index mean± SEM for ST and GT/INT (collapsed on sex) that acquire individual differences in conditioned responding in PLA task. *Main effect of Session. %Significant Session X Tracking interaction ***B,*** PavCA Index mean± SEM for Male and Female rats (collapsed on tracking) that acquire conditioned responding in a PLA task. *Main effect of Session. %Significant Session X Sex interaction.

Effects of intra-DMS blockade of CB1R signaling on Pavlovian Approach during outcome devaluation.

We tested rats using within-subject satiety-induced outcome devaluation. To determine the effects of intra-DMS rimonabant injections on devaluation sensitivity of Pavlovian approach, we analyzed total approach which is the sum of lever and foodcup contacts. First, to examine treatment effects on devaluation sensitivity across all rats, we compared responding during the valued (chow sated) versus devalued (pellet sated) conditions. Figure 2A shows the performance of all rats that received either intra-DMS vehicle or rimonabant infusions during the outcome devaluation probe test. We found a main effect of Outcome Value ($F(1,49)=5.558$, $p=0.022$) and an Outcome Value x Treatment interaction ($F(1,49)=6.663$, $p=0.013$), indicating that intra-DMS rimonabant blocked Pavlovian devaluation sensitivity across all rats. Under vehicle conditions, rats

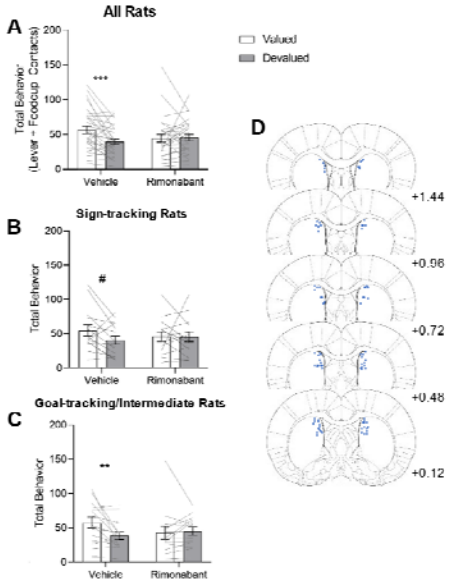

Figure 2. Effect of Intra-DMS CB1R Inhibition on responding in Outcome Devaluation in Sign-and Goal-tracking Rats. Data are represented as within-subject individual data (lines) and group data (bars; mean± SEM). Rats received intraDMS injections of either vehicle (left) or rimonabant (right) 10 minutes prior to probe test. ***A,*** Total behavior (sum of lever and food cup contacts) in outcome devaluation across all rats. We observed a main effect of Outcome Value and a significant Outcome Value X Treatment interaction ***B,*** In ST rats, we did not observe any significant main effects or interactions, when collapsed across sex. ***C,*** In GT/INT rats, we observed a significant Outcome Value X Treatment interaction. ***D,*** Coronal sections (in mm) depicting the location of DMS injector tips for rimonabant infusion. #p=0.051 **p<0.025 ***p<0.01

decreased total approach when sated on the training pellet (devalued state) compared to when they were sated on the homecage chow (valued state). In contrast, with intra-DMS rimonabant, rats showed a similar amount of Pavlovian approach in the valued and devalued state. These results suggest a divergent mechanism for endocannabinoids in mediating Pavlovian behavioral flexibility where CB1R promote flexibility, in contrast to prior studies employing instrumental outcome devaluation [31,32,20].

Considering the established individual differences in devaluation sensitivity during Pavlovian autoshaping, we also added Tracking and Sex as factors in this analysis. We observed an Outcome Value x Treatment x Tracking x Sex interaction ($F(1,49)=4.545$, $p=0.038$) which points to differences in the effects of treatment on devaluation sensitivity due to Sex and/or Tracking. In ST rats, we did not observe any significant main effects or interactions, when collapsed across Sex. (Fig. 2B). In GT/INT rats**,** we observed a significant Outcome Value x Treatment interaction (Fig. 2C, $F(1,27)=5.377$, $p=0.028$) collapsed across Sex. Collapsed across Tracking, in male rats we observed a main effect of Outcome Value and an Outcome Value x Treatment interaction (Fig. 3A, Value: $F(1,25)=6.084$, $p=0.021$; Value X Treatment: $F(1,25)=6.440$, $p=0.018$), while in female rats, we did not observe any significant main effects or interactions (Fig. 3B). We confirm that under vehicle conditions, male rats are sensitive to outcome devaluation ($t(13)=4.670$, $p<0.001$), while female rats are not. Altogether, these results point to sex differences in the effects of treatment on devaluation sensitivity within Tracking groups.

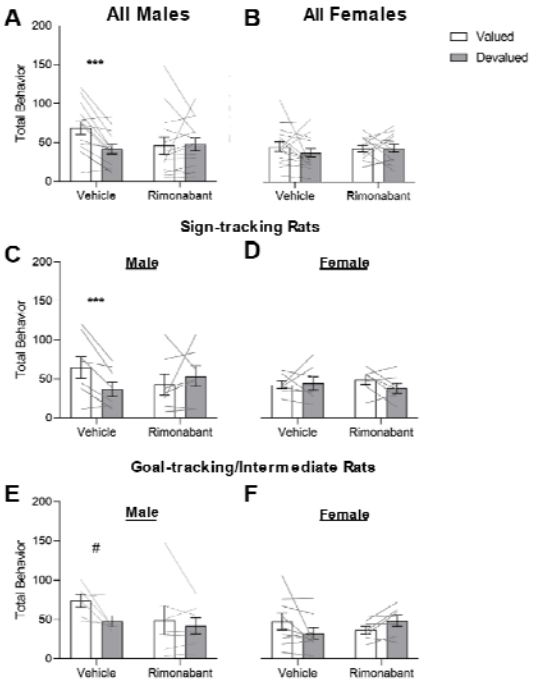

Figure 3. Effects of intraDMS CB1R inhibition on specific satietyinduced outcome devaluation split within Tracking and by Sex. Data are represented as withinsubject individual data (lines) and group data (bars; mean±SEM). Rats received intraDMS injections of either vehicle (left) or rimonabant (right) 10 minutes prior to probe tests. In Male rats, we observed a significant main effect of Outcome Value and a significant Outcome Value X Treatment interaction (***B***). In Female rats, we did not observe any significant main effects or interactions (***C, D***). In ST rats, we observe a significant Outcome Value X Treatment X Sex interaction on total behavior (***C***). In Male ST rats we observed a significant Outcome Value X Treatment interaction (***D***). In ST Female rats there were no main effects or interactions. We then performed a parallel analysis in our GT/INT rats (***E, F***). In GT/INT rats, we observe an Outcome Value X Treatment interaction, but no interaction with Sex. #p=0.055 ***p<0.01

In a prior study using male rats we established that initially devaluation insensitive ST rats become devaluation sensitive after extended training [33]. Here we use both sexes and identify an Outcome Value x Treatment x Sex interaction in ST rats ($F(1,24)=6.210$, $p=0.020$), suggesting potential sex differences in devaluation sensitivity and/or effects of CB1R signaling inhibition (Fig. 3). We replicate our prior finding under vehicle conditions that male ST rats are sensitive to outcome devaluation after extended training (Fig. 3C, $t(7)=3.910$, $p=0.006$). We also observe a similar Outcome Value x Treatment interaction in male ST rats ($F(1,12)=5.063$, $p=0.044$) that was observed across all rats (Fig. 2A), indicating that CB1R signaling inhibition blocks devaluation sensitivity in male ST rats. Post-hoc analyses confirmed that intra-DMS rimonabant injections impaired devaluation sensitivity with similar levels of Pavlovian approach for valued and devalued conditions ($t(5)=-0.556$, $p=0.602$). In contrast to males, female ST

rats were rigid on all measures in our devaluation probe tests and intra-DMS rimonabant had no effects (Fig. 3D; Fs<1.236, ps>0.288).

Consistent with our prior study, male GT/INT rats were sensitive to outcome devaluation after extended training (main effect of Outcome Value (Fig. 3E; F(1,11)=5.203, p=0.043)). In contrast to our sign-tracker data, we observed no main effects or interactions with Sex during devaluation. Despite this, we performed parallel analysis and first looked across male GT/INT rats and found marginal devaluation effect for vehicle treatment (t(5)=2.490, p=0.055), and no differences between value conditions for intra-DMS rimonabant treatment (t(6)=0.723, p=0.497). Female GT/INT rats showed a significant Outcome Value X Treatment interaction (Fig. 3F; F(1,14)=5.100, p=0.040) that was driven by opposite patterns of behavior for the two treatments, however differences between value conditions did not reach significance for either treatment (vehicle, valued vs. devalued, t(8)=1.582, p=0.152, rimonabant, valued vs. devalued (t(6)=-2.113, p=0.079).

Altogether our data indicate that male rats are sensitive to devaluation after extended training, while female rats are not. The effects of intra-DMS CB1R blockade to impair devaluation sensitivity are driven by strongest treatment effects in male sign-tracking rats.

These effects of DMS CB1R signaling inhibition were specific to the satiety-specific outcome devaluation test. We found no difference in responding between vehicle and rimonabant groups during a non-sated, non-reinforced lever autoshaping test of the same duration (10 trials, Fig. 4A; Sex x Treatment x Response (lever, foodcup), Fs<0.479, ps>0.493). This suggests that sex-specific intra-DMS rimonabant treatment effects on Pavlovian approach emerge only after outcome-specific satiety. The observed effects are also not due to differences in consumption between male and female rats during the 1-hour satiation period. We normalized the amount (g) of food consumed during either satiation period or post-probe choice test to the average body weight of each rat across both days of outcome devaluation tests to account for the difference in body weight between male and female rats of the same strain and age [34,35]. We found no difference in the amount of food consumed during the satiation period prior to the probe test (g/bw chow Mean: Male, 0.032, SEM 0.002; Female, 0.031, SEM 0.002; g/bw pellet Mean: Male, 0.039, SEM 0.002; Female, 0.036, SEM 0.002; Fs<1.153, ps>0.288). To confirm devaluation of the sated food, we gave rats a post-probe choice test between the chow and training pellets (Fig. 4B) immediately after the end of the outcome devaluation probe test. Rats consumed less of the food when they were sated on and more of the alternative food, verified by a main effect of Outcome (F(1,45)=8.134, p<0.007) and this did not differ by sex or treatment (Fs<1.790, ps>0.187).

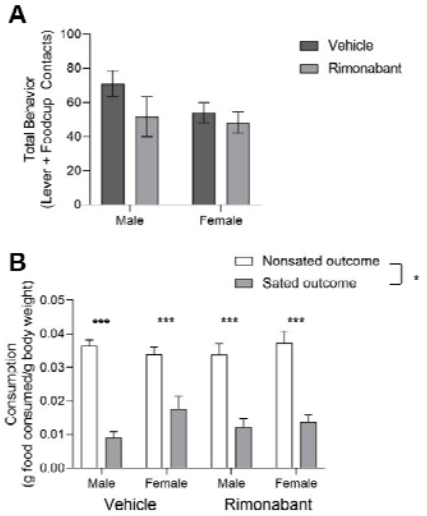

Figure 4. ***Responding during nonsated probe tests and consumption data during food choice period*** Data are represented as group data (bars; mean ± SEM) ***A,*** In a non-sated extinction test identical in duration to the sated tests, we observed no effects of CB1R inhibition on total behavior for male or female rats. ***B,*** In a post-session choice test, we gave rats 30 minutes of access to both outcomes and they consumed less of the outcome they were stated on, regardless of Sex or Treatment. ***p<0.01

The observed effects of blockade of CB1R signaling were also not evident during non-sated, reinforced Pavlovian lever autoshaping sessions. We tested the effect of intra-DMS rimonabant infusion on a subset of rats (N=12) during non-sated, reinforced PLA sessions and found no significant difference between vehicle, low (1µg/µl) or high dose (2µg/µl) of rimonabant on lever presses across sex or tracking (Fig. S1A; Fs<1.972, ps>0.198) or on foodcup contacts (Fig. S1B; Fs<1.078, ps>0.329). We did observe a significant main effect of Sex for lever contacts (F(1,10)=5.395, p=0.043), which is in line with acquisition data, during which females showed more sign-tracking early in training. Overall, rimonabant inhibition of DMS CB1R signaling did not impact conditioned approach under reinforced conditions.

DISCUSSION

In the current study, we examined the influence of sex on the acquisition of Pavlovian conditioned approach and tested the effects of blocking DMS CB1R signaling on Pavlovian satiety-specific outcome devaluation. Consistent with previous studies [13,26,12], we found that female rats showed more lever-directed behaviors than males during extended training, but this difference diminished before testing in outcome devaluation. Under vehicle conditions, we replicated previous findings that male ST rats show devaluation sensitivity after extended training in PLA [9] but found that female ST rats persist in being insensitive to devaluation after extended training. Similarly, female GT/INT rats also show insensitivity to devaluation in this study. These results echo the findings of other studies that indicate females are less sensitive to instrumental and Pavlovian devaluation [36–38]. Surprisingly, we found that DMS CB1R signaling promotes devaluation sensitivity in male rats, without affecting the behavior of female rats.

At first, we predicted that dorsal striatal CB1R signaling would promote rigid, or habitual, behaviors as has been shown for instrumental outcome devaluation [20]. However, our study suggests that CB1Rs in DMS promote behavioral flexibility in male rats, running counter to this established understanding. This prior study trained CB1R flox mice in both random-ratio (RR) and random interval schedules of instrumental reinforcement and generated an OFC-DS specific CB1R knockout. CB1R deletion in the OFC-DS projection promoted “goal-directed” devaluation sensitivity even during schedules of reinforcement that ordinarily drive “habitual” devaluation insensitivity. There are several factors that contribute to the divergence of results including species differences, the use of Pavlovian versus instrumental devaluation procedures and the subregion-specific effects of experimental manipulations. Competing action-outcome and stimulus-response associations mediate instrumental devaluation, and studies show that goal-directed behaviors shift to habit with extended training or with RR schedules of reinforcement [39,40,20]. This is not the case with Pavlovian behaviors that are sensitive to devaluation even after extended training [9,41], suggesting stimulus-outcome associations support adaptive reward seeking despite overtraining. Never-the-less, the present study suggests that Pavlovian behaviors are insensitive to devaluation in females after extended training, suggesting that female rats rely on stimulus-response associations to drive behavior. This is in line with other studies where females more readily acquire a Pavlovian approach and remain insensitive to devaluation [25,13,22,42,12,26]. Additionally, the CB1R deletion study in mice targeted the dorsal striatum without subregion specificity. Prior work has shown that systemic activation of CB1Rs promotes rigid responding [32,43] and while both DLS and DMS express CB1Rs [44–46],

more of the CB1R work has focused on the DLS. The current targeting of DMS may explain why our results diverge from the canonical view that dorsal striatal CB1Rs support rigid responding.

Prior work reveals that the DMS supports flexible, goal-directed conditioned responding. Reducing the activity of the DMS through lesion or pharmacological inhibition impairs flexible responding in a variety of tasks [47,16,48,19,49]. To be interpreted in this conceptual framework, the present results suggest that CB1R signaling promotes DMS activation and that inhibiting CB1Rs signaling decreases DMS output to impair Pavlovian devaluation sensitivity. A primary prediction stemming from this interpretation is that DMS CB1R signaling at GABAergic inputs to MSNs reduces inhibitory tone in the area, allowing for DMS activation to promote flexible responding in Pavlovian devaluation. While the current study does not identify the cell-type affected by DMS CB1R manipulations, parvalbumin-expressing fast spiking interneurons (PV-FSIs) are a prime candidate mechanism to explain the observed results here with intra-DMS CB1R during Pavlovian devaluation. While PV-FSIs make up only 1-5% of striatal neurons [50], each cell is estimated to project onto ~100 MSNs and receive glutamatergic cortical projections, forming a strong feedforward inhibitory circuit [51–54]. Direct manipulation of DLS PV-FSIs shows that their activity is critical to supporting habitual responding [55,56] but much less is known about DMS PV-FSIs and their contribution to habitual or goal-directed responding. CB1Rs are expressed on striatal PV-FSIs and mediate a form of inhibitory LTD that disinhibits MSNs, a mechanism that is associated with striatal regulation of behavioral flexibility [57,58]. CB1Rs are also expressed on cortical inputs that target MSNs [59–63], but it has not yet been established whether cortical projections targeting PV-FSIs also contain CB1Rs. CB1R signaling at cortical-striatal FSI synapses would be expected to reduce inhibitory tone and increase DMS MSN activation, a similar result to CB1R signaling at FSI-MSN synapses. Thus, these two hypotheses must be tested further to discover the mechanism of DMS CB1R regulation of Pavlovian devaluation sensitivity. Finally, it is possible that off-target effects impacted DLS function, an area with high CB1R density [44–46], though this is unlikely given the volume of rimonabant injected (0.5 µL per hemisphere).

Overall, the current studies shows that DMS CB1Rs promote sensitivity to Pavlovian devaluation for male rats, regardless of tracking. Female rats show insensitivity to Pavlovian devaluation and CB1R manipulation. More work must be done to identify the population of DMS CB1Rs that support flexible responding in males and the underlying neurobiology that guides devaluation insensitivity in females.

AUTHOR CONTRIBUTIONS

DJC supervised the project. CAS, SEK, and JMM acquired the data. CAS analyzed the data. CAS and DJC designed the experiments, interpreted the data, and wrote the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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COMPETING INTERESTS

The authors declare no competing interests.

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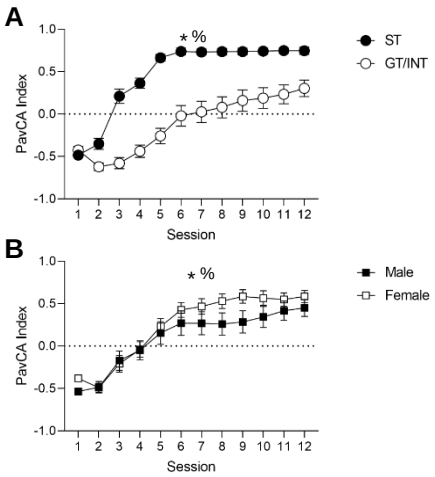

Figure 1. Pavlovian Conditioned Approach during training split by Tracking and Sex. ***A,*** PavCA Index mean ± SEM for ST and GT/INT (collapsed on sex) that acquire individual differences in conditioned responding in PLA task. *Main effect of Session. %Significant Session X Tracking interaction. ***B,*** PavCA Index mean ± SEM for Male and Female rats (collapsed on tracking) that acquire conditioned responding in a PLA task. *Main effect of Session. %Significant Session X Sex interaction.

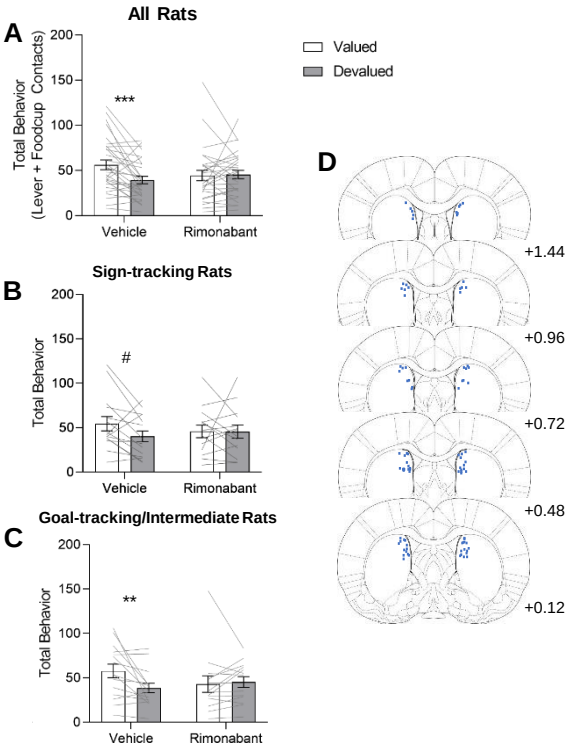

Figure 2. Effect of intra-DMS CB1R inhibition on responding in Outcome Devaluation in Sign-and Goal-tracking Rats. Data are represented as within-subject individual data (lines) and group data (bars; mean ± SEM). Rats received intra-DMS injections of either vehicle (left) or rimonabant (right) 10 minutes prior to probe test. ***A,*** Total behavior (sum of lever and food cup contacts) in outcome devaluation across all rats. We observed a main effect of Outcome Value and a significant Outcome Value X Treatment interaction. ***B,*** In ST rats, we did not observe any significant main effects or interactions, when collapsed across sex. ***C,*** In GT/INT rats**,** we observed a significant Outcome Value X Treatment interaction. ***D,*** Coronal sections (in mm) depicting the location of DMS injector tips for rimonabant infusion. #p=0.051 **p<0.025 ***p<0.01

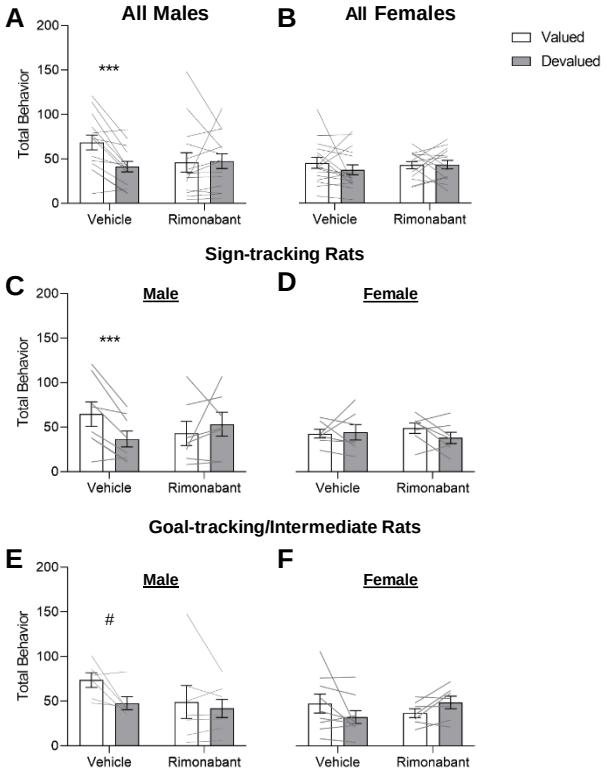

Figure 3. Effects of intra-DMS CB1R inhibition on specific satiety-induced outcome devaluation split within Tracking and by Sex. Data are represented as within-subject individual data (lines) and group data (bars; mean ± SEM). Rats received intra-DMS injections of either vehicle (left) or rimonabant (right) 10 minutes prior to probe test. ***A,*** In Male rats, we observed a significant main effect of Outcome Value and a significant Outcome Value X Treatment interaction. ***B,*** In Female rats, we did not observe any significant main effects or interactions. ***C, D,*** In ST rats, we observe a significant Outcome Value X Treatment X Sex interaction on total behavior. ***C,*** In Male ST rats we observed a significant Outcome Value X Treatment interaction. ***D,*** In ST Female rats there were no main effects or interactions. We then performed a parallel analysis in our GT/INT rats. ***E, F*** In GT/INT rats, we observe an Outcome Value X Treatment interaction, but no interaction with Sex. #p=0.055 ***p<0.01

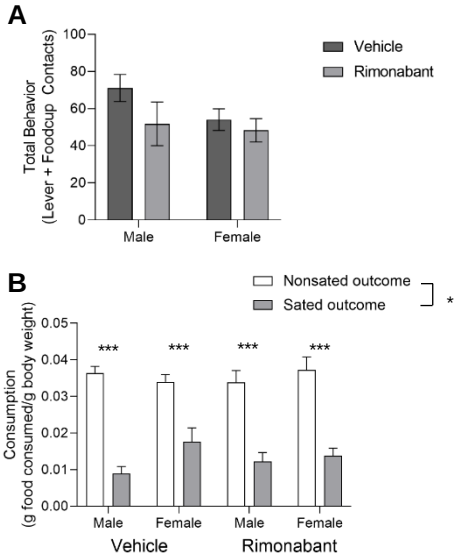

Figure 4. ***Responding during nonsated probe tests and consumption data during food choice period.*** Data are represented as group data (bars; mean ± SEM) ***A,*** In a non-sated extinction test identical in duration to the sated tests, we observed no effects of CB1R inhibition on total behavior for male or female rats. ***B,*** In a post-session choice test, we gave rats 30 minutes of access to both outcomes and they consumed less of the outcome they were stated on, regardless of Sex or Treatment. ***p<0.01