Psychopharmacology
https://doi.org/10.1007/s00213-018-4993-6

ORIGINAL INVESTIGATION

Cannabinoid receptor-1 signaling contributions to sign-tracking and conditioned reinforcement in rats

Sam Z. Bacharach[1,2] · Helen M. Nasser[1] · Natalie E. Zlebnik[1] · Hannah M. Dantrassy[1] · Daniel E. Kochli[1] · Utsav Gyawali[1,2] · Joseph F. Cheer[1,2,3] · Donna J. Calu[1,2]

Received: 30 January 2018 / Accepted: 1 August 2018

Abstract

Rationale Endocannabinoids (eCBs) are critical gatekeepers of dopaminergic signaling, and disrupting cannabinoid receptor-1 (CB1) signaling alters DA dynamics to attenuate cue-motivated behaviors. Prior studies suggest that dopamine (DA) release plays a critical role in driving sign-tracking.

Objectives Here, we determine whether systemic injections of rimonabant, a CB1 receptor inverse agonist, during Pavlovian lever autoshaping impair the expression of sign-tracking. We next examine whether rimonabant blocks the reinforcing properties of the Pavlovian lever cue in a conditioned reinforcement test.

Methods In Exp. 1, we trained rats in Pavlovian lever autoshaping prior to systemic rimonabant injections (0, 1, 3 mg/kg) during early and late Pavlovian lever autoshaping sessions. In Exp. 2, we trained rats in Pavlovian lever autoshaping prior to systemic rimonabant injections (0, 1 mg/kg) during a conditioned reinforcement test.

Results Rimonabant dose-dependently decreased lever contact and probability, and increased sign-tracker's latency to approach the lever cue early in Pavlovian training. With extended training, many previously goal-tracking and intermediate rats shifted to lever approach, which remained dose-dependently sensitive to rimonabant. Rimonabant attenuated cue-evoked food cup approach early, but not late, in conditioning, and did not affect pellet retrieval or consumption. The inserted lever cue served as a robust conditioned reinforcer after Pavlovian lever autoshaping, and 1 mg/kg rimonabant blocked conditioned reinforcement.

Conclusions Together, our results suggest that CB1 signaling mediates two critical properties of incentive stimuli; their ability to attract (Exp. 1) and their ability to reinforce (Exp. 2) behavior.

Keywords Sign-tracking · Endocannabinoids · CB1 receptor · Pavlovian · Conditioned reinforcement · Appetitive · Approach · Cue-motivated · Incentive

Introduction

Endocannabinoids (eCBs) are critical gatekeepers of the dopamine (DA) system and influence cue-motivated behaviors (Lupica and Riegel 2005; Cheer et al. 2007; Solinas et al. 2008; Hernandez and Cheer 2012; Oleson et al. 2012). Disrupting cannabinoid receptor-1 (CB1) signaling attenuates cue-evoked accumbal DA release and interferes with cue-motivated behaviors (Oleson et al. 2012). Cue-evoked dopamine release in the accumbens is a neurobiological signature of sign-tracking, but not goal-tracking (Flagel et al. 2011). Sign-tracking and goal-tracking are behaviors that emerge during a Pavlovian lever autoshaping (PLA) paradigm. Sign-tracking rats interact with an inserted lever cue that predicts reward, while goal-trackers interact with the food cup where

Sam Z. Bacharach and Helen M. Nasser contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00213-018-4993-6) contains supplementary material, which is available to authorized users.

✉ Donna J. Calu
dcalu@som.umaryland.edu

[1] Department of Anatomy and Neurobiology, University of Maryland School of Medicine, 20 Penn Street – HSFII Room S263, Baltimore, MD 21201, USA

[2] Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD, USA

[3] Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, USA

Published online: 14 August 2018

food is delivered (Hearst and Jenkins 1974; Boakes 1977; Flagel et al. 2007). Sign-tracking to lever cues has been posited to reflect an incentive motivational process in which the appetitive motivational properties of the reward are transferred to the conditioned lever cue, such that the lever cue attracts, invigorates, and reinforces behavior (Tomie 1996; Flagel et al. 2009; Robinson and Flagel 2009; Beckmann and Chow 2015). While DA plays a role in driving approach behaviors of both sign- and goal-trackers (Lopez et al. 2015; Fraser et al. 2016), DA action in the nucleus accumbens (NAc) mediates sign-tracking, but not goal-tracking (Flagel et al. 2011; Saunders and Robinson 2012; Clark et al. 2013; Saddoris et al. 2016; Fraser and Janak 2017). More specifically, sign- but not goal-trackers show phasic fluctuations in cue-evoked DA in the NAc during PLA, and DA antagonists block sign-tracking, but not goal-tracking (Flagel et al. 2011; Clark et al. 2013). Since CB1 receptors modulate cue-evoked phasic DA fluctuations (Cheer et al. 2004; Oleson et al. 2012), we predict that their activation is critical for sign-tracking approach in Pavlovian lever autoshaping.

Here, we first determine whether systemic injections of the CB1 receptor inverse agonist, rimonabant, mimic the reduction in sign-tracking observed by antagonizing DA receptors (Flagel et al. 2011; Saunders and Robinson 2012; Clark et al. 2013; Chow et al. 2016; Fraser and Janak 2017). The endogenous mechanism of eCB modulation of dopamine release occurs in the ventral tegmental area (VTA), via CB1 receptor-mediated inhibition of GABAergic neurotransmission onto DA neurons. Decreased GABA release consequently disinhibits VTA DA cell firing and increases dopamine release in striatal targets (Szabo et al. 2002; Riegel and Lupica 2004; Lupica and Riegel 2005; Covey et al. 2017). Inverse agonists such as rimonabant counteract endogenous CB1 receptor activation and reduce striatal DA release (Cheer et al. 2004; Oleson et al. 2012). A prior study testing the effects of systemic CB1 receptor blockade in PLA observed modest changes in lever-directed behavior only at the highest rimonabant dose tested (Thornton-Jones et al. 2005), but concluded that there was *no effect* of CB1 receptor blockade on Pavlovian approach. However, that study did not consider individual differences in approach during lever autoshaping (i.e., sign- and goal-tracking), which are associated with neurobiological variability in dopamine system involvement (Flagel et al. 2011; Saunders and Robinson 2012). In the present study, we address this critical knowledge gap about eCB involvement in PLA by examining individual differences in approach behaviors across the entire tracking distribution to determine the role of CB1 receptor signaling in driving sign-tracking during Pavlovian lever autoshaping.

In Experiment 1, we trained male and female rats in PLA to determine their sign-tracking (ST), goal-tracking (GT), or intermediate (INT) group, prior to injections of rimonabant (0, 1, 3 mg/kg, i.p.) during early (5–7) and late (15–17) reinforced PLA sessions. We examined the eCB system involvement early versus late in PLA because of conflicting evidence that DA plays a time-limited role in supporting sign-tracking (Clark et al. 2013; Fraser and Janak 2017). Here, we aim to understand the involvement of CB1 signaling after limited and extended training in PLA. To narrow in on the role of eCB in mediating the motivational significance of the lever cues used in PLA, we next examined the effect of disrupting CB1 signaling during conditioned reinforcement. Conditioned reinforcement, which is also influenced by NAc DA manipulations (Taylor and Robbins 1984; Wolterink et al. 1993), specifically probes whether a Pavlovian cue acquires motivational significance such that it can serve as a reinforcer itself. Specifically, in Experiment 2, we over-trained male and female rats in PLA prior to examining the effect of rimonabant (0 or 1 mg/kg, i.p.) on conditioned reinforcement, in which nosepoke responding is reinforced with the previously conditioned lever cue alone. Together, the present study directly probes the extent to which CB1 signaling mediates two critical properties of incentive stimuli, their ability to attract (Exp. 1) and their ability to reinforce (Exp. 2) behavior.

Materials and methods

Animals

In Exp. 1, $n = 40$ (male: $n = 20$, female: $n = 20$), and Exp. 2, $n = 25$ (male: $n = 12$, female: $n = 13$), Long-Evans rats (Charles River Laboratories, Wilmington, MA) weighing 216–243 g at experimental onset were single-housed and maintained on a 12:12 h light-dark cycle (lights on at 6:30 a.m.). For Exp. 1 and 2, rats had ad libitum access to standard laboratory chow and tap water before food deprivation to 90% of their baseline weight, which was maintained throughout the experiment. Chow was provided after daily behavioral sessions. In both Exp. 1 and Exp. 2, one male rat was removed from each experiment due to aggressive behavior towards experimenters; thus, the final number of rats used for analysis was Exp. 1: 39 (male ($n = 19$) and female ($n = 20$), Exp. 2: $n = 24$ (male: $n = 11$, female: $n = 13$) All procedures were performed in accordance with the "Guide for the care and use of laboratory animals" (8th edition, 2011, US National Research Council) and were approved by the University of Maryland, School of Medicine Institutional Animal Care and Use Committee (IACUC).

Apparatus

Experiments were conducted in individual sound-isolated standard experimental chambers (25 × 27 × 30 cm; Med Associates). For Exp. 1 and 2, during Pavlovian lever autoshaping, each chamber had one red house light (6 W)

located at the top of a wall that was illuminated for the duration of each session. During PLA, the opposite wall in the chamber had a recessed food cup (with photo beam detectors) located 2 cm above the floor grid. The food cup was attached to a programmed pellet dispenser that delivered 45-mg food pellets (catalog no. 1811155; Test Diet 5TUL; protein 20.6%, fat 12.7%, carbohydrate 66.7%). One retractable lever was positioned on either side of the food cup, counterbalanced, 6 cm above the floor. In Exp. 2, for conditioned reinforcement testing, the food cup was removed and the lever was positioned 6 cm above the floor in the center panel of the chamber wall. Each chamber had one red house light (6 W) located at the top of a wall that was illuminated for the duration of the conditioned reinforcement test session. One active and one inactive nosepoke port were positioned 6 cm above the floor on either side of the centrally located lever. The active nosepoke location was counterbalanced such that for half the rats, the active nosepoke was on the opposite side relative to former PLA lever location, and half the rats had active nosepoke on the same side relative to former PLA lever location. Sessions began with the illumination of a red houselight and ended 30 min later. For motor tests, the chamber walls were bare (no levers, food cups, lights, or nosepokes).

Drugs

Rimonabant (SR141716A, 5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide, NIDA Drug Supply Program) was dissolved in a 1:1:18 solution of ethyl alcohol (Sigma), emulphor (Alkamuls EL-620, Solvay Chemicals, Princeton, NJ), and saline (Hospira) and sonicated for 15–30 min. Drug solutions were prepared immediately before each test session. Injections of rimonabant were administered i.p. in a volume of 1 ml/kg at doses of 0, 1, and 3 mg/kg.

Statistical analyses

Data were analyzed using SPSS statistical software (IBM) with mixed-design repeated-measures ANOVA. Significant main and interaction effects ($p < 0.05$) were followed by post hoc within-tracking group repeated-measures ANOVA or Bonferroni tests (reported in figure legends). For PLA training data, we used mixed repeated measures ANOVA of lever and food cup measures (contact, latency, and probability), using between-subject factors of Tracking group (ST, GT, INT) and within-subject factor of Session (1–4 or 8–14) to analyze lever- and food cup-directed behaviors. For PLA rimonabant test data, we used mixed repeated measures ANOVA including within-subject factor of drug (0, 1, 3 mg/kg) and between-subject factors of Tracking. For conditioned reinforcement data, we used a repeated-measures ANOVA of nosepoke measures, using between-subject measures of drug (vehicle or 1 mg/kg rimonabant) and within-subject factor of response (active or inactive poke). We recognize the importance of using both males and females in our study (McCarthy et al. 2017; Miller et al. 2017) and begin exploring the possibility of sex-differences by reporting sex effect sizes (Miller et al. 2017). This approach allows reporting of observed differences that are independent of sample size. Sex effect sizes are expressed as Cohen's d ($d = (M_1 - M_2) / \mathrm{SD}_{\mathrm{pooled}}$), where M_1 is mean of group 1, M_2 is mean of group 2, and $\mathrm{SD}_{\mathrm{pooled}} = \sqrt{(s_1^2 + s_2^2) / 2}$, which is the pooled standard deviation of the two groups (Cohen 1988). We follow general guidance for interpreting effect sizes where small effect $d = 0.2$, medium effect $d = 0.5$, and large effect $d = 0.8$ or larger (Cohen 1988), and note instances that future studies should be powered to explore sex as a biological variable.

Experimental procedures

Experiment I: early and late Pavlovian lever autoshaping training and testing

Training We gave rats a single 38-min magazine training session during which one food pellet was delivered into the food cup on a variable interval (VI) 120 s schedule (60–180 s) for 20 trials. Exp. 1 timeline appears in Fig. 1a. We trained rats in four daily Pavlovian lever autoshaping (PLA) sessions, which consisted of 25 reinforced lever conditioned stimulus (CS+) presentations occurring on a VI 90 s schedule (60–120 s). Trials consisted of the insertion of a retractable lever for 10 s, after which the lever was retracted and two food pellets were delivered to the food cup regardless of whether a lever or food cup response was made.

Measurements and difference scores Measurements were collected during the 10-s CS period, and the 2.5-s post-CS reward delivery period. An automated measurement of the latency to first contact the lever and/or food cup during the cue for each trial was recorded. On trials in which a contact did not occur, a latency of 10 s was recorded. For each session, the lever or food cup probabilities were calculated by determining the number of trials that the lever or food cup response was made, divided by total number of trials in the session. We used a Pavlovian Conditioned Approach (PCA) analysis (Meyer et al. 2012) of day 4 performance in PLA to determine sign-, goal-, and intermediate tracking groups. The PCA score quantifies the difference between lever-directed and food cup-directed behaviors, and ranges from − 1.0 to + 1.0. An individual rat's PCA score is the average of three difference score measures (each ranging from − 1.0 to 1.0) including (1) preference score, (2) latency score, and (3) probability score. The preference score is the number of lever presses during the CS, minus the number of food cup responses during the CS, divided by the sum of these two measures. The latency score is

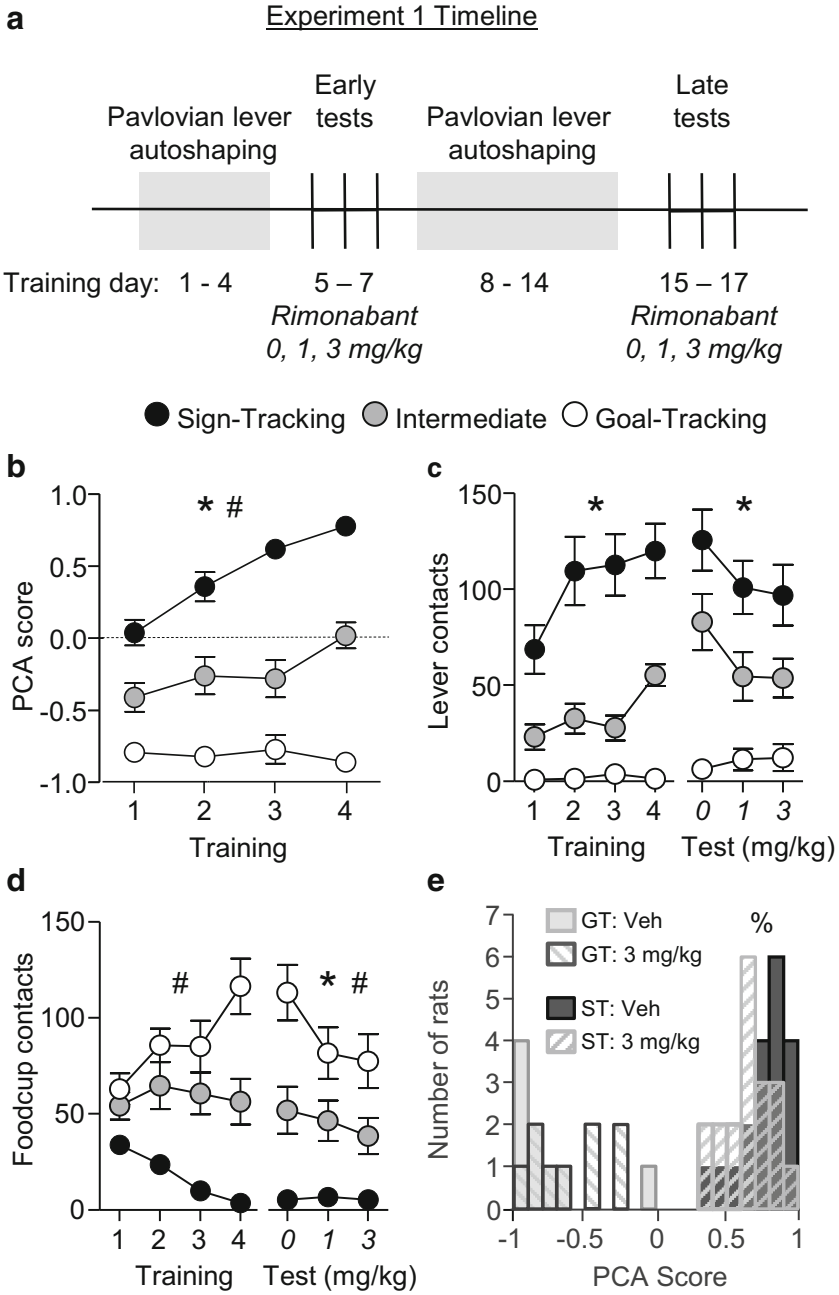

Fig. 1 Lever and food cup approach is attenuated by rimonabant treatment early in PLA. **a** Exp. 1 timeline. We trained male and female rats in four daily sessions (1–4) of PLA to determine their ST, GT, or INT group assignments. We next tested rats with systemic injections of the CB1 inverse agonist, rimonabant (0, 1, 3 mg/kg), during early reinforced PLA sessions (5–7). Rats were retrained in daily autoshaping sessions (8–14) and again tested with systemic injections of rimonabant in late reinforced PLA sessions (15–17). **b**–**d** Data are mean ± standard error of the mean (SEM) for PCA scores (**b**), lever contacts (**c**), and food cup contacts (**d**) for training sessions 1–4 (left) and three counterbalanced early test sessions for each rimonabant dose (right). **e** Population distribution of PCA scores under vehicle and 3 mg/kg rimonabant. *Significant main effect of Session or Drug; #significant Session × Tracking or Drug × Tracking interaction; %significant shift in population. Main effects of Tracking are not indicated in the figure

the average latency to make a food cup response during the CS, minus the latency to lever press during the CS, divided by the duration of the CS (10 s). The probability score is the probability of lever press minus the probability of food cup response observed across the session. Sign-tracking PCA scores range from + 0.5 to + 1.0, goal-tracking PCA scores range from − 0.5 to − 1.0, and intermediate group PCA scores range from − 0.49 to + 0.49. In Exp. 1, of 39 rats, 19 were ST (10 male, 9 female), 9 were GT (4 male, 5 female), and 11 were INT (5 male, 6 female). After PLA training (sessions 1–4) and testing (sessions 5–7 described below), we retrained rats in seven drug-free PLA sessions (8–14) followed by the late rimonabant testing (sessions 15–17; Fig. 1). To explore possible sex differences in response to rimonabant, we calculated behavioral difference scores and examined sex effect sizes between males and females under drug and vehicle conditions. For each rat, we calculated difference scores for each rimonabant dose that compared contact behaviors under drug and vehicle conditions. The lever and food cup difference scores were equal to the number of contacts after rimonabant injections minus number of contacts after vehicle injections (1 mg/kg − vehicle) and (3 mg/kg − vehicle).

Testing We habituated rats to i.p. vehicle injections before the start of the fourth PLA session. For early PLA testing (sessions 5–7), we gave each rat an injection of rimonabant (0, 1,

and 3 mg/kg) in three separate counterbalanced test sessions that occurred 48–72 h apart. To be consistent with a prior rimonabant PLA study (Thornton-Jones et al. 2005) immediately after injections, we placed rats in their homecage for 30 min, then to behavioral chambers for testing. After a 10-min acclimation period in the behavioral chambers, we started the PLA test session. Injection procedures were identical for late PLA testing (sessions 15–17). The order of injections was counterbalanced across tracking groups and sex.

Experiment 2: Pavlovian lever autoshaping training and conditioned reinforcement

Training We trained a separate cohort of $n = 24$ (male $n = 11$, female $n = 13$) rats in 22 drug-free, PLA sessions as described in training section of Exp. 1 above, prior to conditioned reinforcement testing.

Conditioned reinforcement testing We gave rats a 30-min conditioned reinforcement test 1 day after the last PLA session. In this task, rats learn to make an instrumental nosepoke response to gain brief access to the Pavlovian lever cue alone. Importantly, no food reinforcers are delivered during conditioned reinforcement. A single poke in the active port resulted in a 2-s extension and retraction of the lever cue. A poke in the inactive port had no programmed consequences. The number of active pokes and inactive pokes was recorded. We gave half of the rats i.p. vehicle injections and the other half i.p. rimonabant (1 mg/kg) injections. Immediately after injections, we placed rats in their homecage for 30 min, then to behavioral chambers for testing. After a 10-min acclimation period in the behavioral chambers, we started the conditioned reinforcement test session. We counterbalanced tracking group, sex, and active nosepoke location between treatment groups.

Measurements and difference scores Measurements and analyses for PLA were collected and analyzed as described above. For conditioned reinforcement testing, measurements were collected continuously through the entire 30-min test session. Automated measurements included the total number of active and inactive nosepoke beambreaks. To examine the time-course of nosepoke responding during conditioned reinforcement, video was recorded for 16 (male = 8; four veh, four rimonabant, female = 8; three vehicle, four rimonabant) out of the 24 rats. To explore possible sex differences in conditioned reinforcement and response to rimonabant, we calculated nosepoke discrimination scores and examined sex effect sizes between males and females under drug and vehicle conditions. For each rat, we calculated discrimination scores that were equal to active nosepokes minus inactive nosepokes. For time-course data, we scored videos counting the number of active and inactive nosepokes per minute and summing in 5-min bins. Total active and inactive pokes scored from the videos correlated with the automated nosepoke totals collected by the computer ($r^2 = 0.98$ vehicle group, $r^2 = 0.91$ rimonabant group).

Motor and consumption testing We maintained rats from Exp. 2 at 90% food deprivation and gave them a 30-min motor activity test followed immediately by a 30-min pellet consumption test. We gave $n = 8$ rats per group i.p. vehicle, 1 or 3 mg/kg rimonabant injections. Immediately after injections, we placed rats in their homecage for 30 min, then to empty behavioral chambers where we video recorded motor activity for 30 min (EthoVision XT v9; Noldus, Wageningen, the Netherlands). To calculate % time motile in chamber, we divided the cumulative time motile by 30 min. Time motile is defined as time spent walking, grooming, or rearing. Immediately after the session, we placed rats in the homecage with 50 pellets (weighing a total of 2.25 g) in a pre-habituated white, ceramic ramekin dish (diameter = 10.5 cm, height = 4.2 cm) for 30 min. Pellets (45 mg pellets; catalog no. 1811155; Test Diet 5TUL; protein 20.6%, fat 12.7%, carbohydrate 66.7%) were identical to those used in autoshaping. We quantified consumption by subtracting the number of pellets remaining after 30 min from 50 pellets given at the start of the test.

Results

Experiment 1: effect of rimonabant on approach in Pavlovian lever autoshaping

In Experiment 1, we examine the role of CB1 signaling in mediating sign-tracking. Here, we sought to understand the involvement of CB1 signaling after limited and extended conditioning in PLA. Exp. 1 timeline appears in Fig. 1a. The tracking group was determined from performance in the fourth PLA training session using each rats' Pavlovian conditioned approach (PCA) score (Fig. 1b), which are a comprehensive measure of individual differences in PLA that account for contact, latency, and probability differences between lever- and food cup-directed behaviors (Meyer et al. 2012). Table 1 summarizes main effects and interactions from analyses of lever and food cup contact (Fig. 1c, d, left), latency (Fig. S1B–C left), and probability data (Fig. S1D–E left) from the first four training sessions. Additionally, there were no differences in acquisition of the preferred conditioned response (lever contact for ST and food cup contact for GT) between sign- and goal-trackers (supplemental information, Fig. S1A). Looking within tracking groups, there were no notable sex effect sizes during the fourth PLA training session.

In the next three consecutive sessions, we gave rats rimonabant (0, 1, or 3 mg/kg, counterbalanced) before each PLA session. Consistent with our prediction that rimonabant

Table 1 ANOVA for phase I: early Pavlovian lever autoshaping: training sessions 1–4

Effect	Degrees of freedom	Lever						Food cup						PCA	
		Contact		Latency		Probability		Contact		Latency		Probability			
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Session	(3, 108)	4.16	0.008	15.96	< 0.001	12.18	< 0.001	1.91	0.132	3.86	0.012	.154	< 0.001	16.07	< 0.001
Tracking group	(2, 36)	24.05	< 0.001	57.11	< 0.001	82.58	< 0.001	33.79	< 0.001	34.52	< 0.001	23.48	< 0.001	85.60	< 0.001
Session × Tracking	(6, 108)	1.91	0.09	4.24	= 0.001	3.70	0.002	11.20	< 0.001	10.94	< 0.001	12.22	< 0.001	8.01	< 0.001

would suppress sign-tracking (Fig. 1c right), in our analysis of lever contacts, we observed main effects of Drug ($F(2,72) = 5.035$, $p = 0.009$) and Tracking ($F(2,36) = 12.88$, $p = 0.001$) but surprisingly no Drug × Tracking interaction, suggesting that rimonabant similarly reduced the number of lever contacts across all rats. Exploring Drug × Tracking interactions (supplementary information) for other lever measurements revealed that rimonabant dose-dependently increased lever latency in ST, but not GT or INT (supplementary information, Fig. S1B right) and decreased probability of lever contact exclusively in ST rats (supplementary information, Fig. S1C right). Thus, only in ST rats were all three measures of sign-tracking (contact, latency, and probability) affected by rimonabant treatment. We observed a small sex effect size for the effect of rimonabant on lever contact difference scores at 1 mg/kg that was amplified when looking specifically in ST rats (supplementary information Fig. S1F left; all rats Cohen's $d = 0.36$; Fig. S1F right; ST rats Cohen's $d = 0.53$).

Surprisingly, rimonabant also affected food cup contacts early in lever autoshaping (Fig. 1d right). We observed main effects of Drug ($F(2,72) = 17.94$, $p < 0.001$) and Tracking ($F(2,36) = 32.67$, $p < 0.001$) and a Drug × Tracking interaction ($F(4,72) = 8.63$, $p < 0.001$), suggesting that rimonabant differentially affected food cup behavior between groups. Separated by tracking, there was a main effect of Drug for GT and INT, but not ST (GT: $F(2,16) = 6.89$, $p = 0.007$; INT: $F(2,20) = 5.91$, $p = 0.01$). Rimonabant dose-dependently increased food cup latency in GT, but not ST or INT (supplementary information, Fig. S1C right). The observation that rimonabant treatment did not slow either lever or food cup responding in INT rats that perform both responses suggests that rimonabant did not have general motor suppressive effects that could impair responding at these stimulus locations. Consistent with reduced number of food cup contacts, there was also a reduced probability of food cup contact in GT and was marginally significant for INT (supplementary information, Fig. S1E right). There were no notable sex effect sizes that would indicate potential sex differences in sensitivity of food cup approach to rimonabant.

To test the possibility that rimonabant affected reward collection or consumption, we analyzed food cup contacts during the 2.5-s period after lever retraction when pellets were delivered. There were no significant main effects of Drug, Tracking, nor Drug × Tracking interactions (Fig. S1G). Rats consumed all of their pellets during PLA test sessions (data not shown). Altogether, the effects of rimonabant were specific to approach behaviors during the Pavlovian cue period and did not affect reward collection or consumption once pellets were delivered.

We examined the effect of rimonabant on the population distribution of PCA scores during early tests (Fig. 1e). We performed Wilcoxon signed-rank tests to compare PCA scores under vehicle versus rimonabant conditions (Fig. 1e). PCA scores for the ST population were significantly left-shifted by 3 mg/kg rimonabant treatment (Wilcoxon signed rank: $Z = -2.98$, $p < 0.01$), but there was no shift for GT (Fig. 1e) or INT (data not shown). Notably, there may have been limited power to detect PCA shifts in the considerably smaller GT and INT groups, yet even when combined, there was no evidence for a positive shift in the GT/INT population. Altogether, analyses of individual data and population level analyses of comprehensive PCA scores reveal that rimonabant treatment significantly reduces sign-tracking early in Pavlovian lever autoshaping.

Next, we trained rats in seven additional PLA sessions. Notably, for PCA scores during extended training (Fig. 2a left), there were main effects of Session and Tracking and a Session × Tracking interaction (Table 2). Separated by tracking group, there were main effects of session for all three groups, with previously identified GT rats showing the most pronounced shift in PCA scores during extended training (GT: $F(6,48) = 8.77$, $p < 0.001$; INT: $F(6,60) = 5.49$, $p < 0.001$; ST: $F(6,108) = 2.63$, $p = 0.02$). The population distribution of PCA scores on day 14 differed from day 4 PCA scores (supplementary information; Fig. S2D). Rats' lever and food cup contact data are shown in Fig. 2b, c left. There were main effects of Session and Tracking and Session × Tracking interactions for nearly all lever and food cup contact measures, which are reported in Table 2. We observed large sex effect sizes for lever-directed behavior that emerged during retraining phase of PLA, such that on the last 3 days of training, females made fewer lever contacts ($d = 1.03$) at a slower

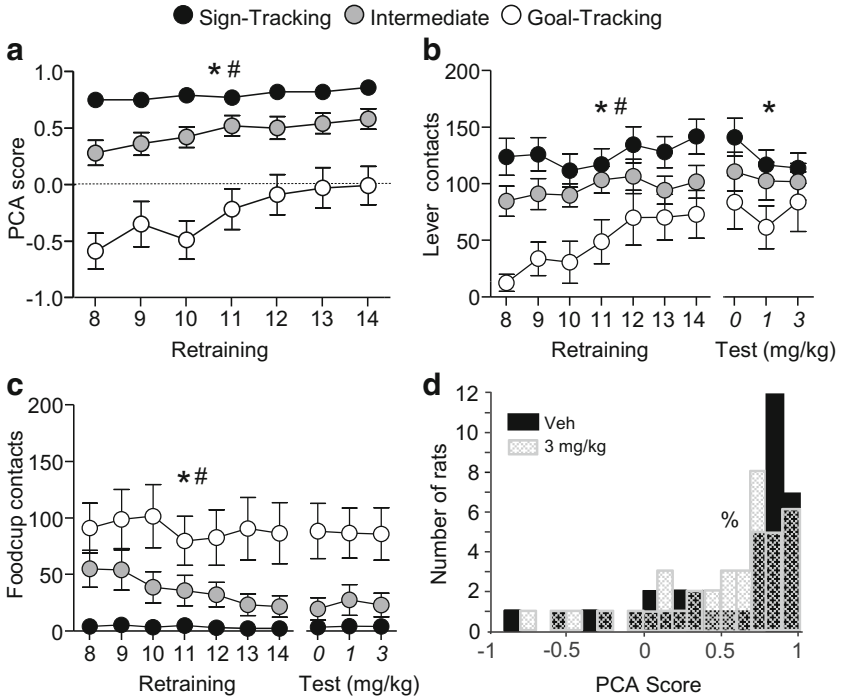

Fig. 2 Extended training shifts behavior towards sign-tracking, which continues to be sensitive to rimonabant treatment. **a**–**c** Data are mean ± standard error of the mean (SEM) for PCA scores (**a**), lever contacts (**b**), and food cup contacts (**c**). Data are shown for retraining sessions 8–14 (left) and the three late test sessions for each rimonabant dose (right). **a** Extended training shifts behavior towards sign-tracking. **b** Lever contacts increase with extended training across all groups (left), and lever-directed behavior across all rats continues to be sensitive to the effects of rimonabant (right). **c** Food cup contacts are not affected by extended training and are not affected by rimonabant treatment. **d** Population distribution of PCA scores are significantly left-shifted by 3 mg/kg rimonabant compared to vehicle. *Significant main effect of Session or Drug; #significant Session × Tracking or Drug × Tracking interaction; %significant shift in population. Main effects of Tracking are not indicated in the figure

latency ($d = 0.99$) than males (supplementary information Fig. S2A–C).

Next, we examined the effect of rimonabant on lever-directed behavior after extended Pavlovian training. For lever contact data (Fig. 2b right), there were main effects of Drug ($F(2,72) = 5.68$, $p = 0.005$) but no main effect of Tracking nor any interactions. This suggests an overall drug effect that reduced lever contacts in all tracking groups. Lever latency and probability data are reported in Fig. S2B–C right and supplementary information. The sex differences observed during training were maintained in testing, in that females made fewer lever contacts than males (Fig. S2A). We observed a small sex effect size for the effect of rimonabant on lever contact difference scores at 3 mg/kg (supplementary information; Fig. S2C left; Cohen's $d = 0.244$) that was similar when looking only at behavior of ST rats (Fig. S2C right; Cohen's $d = 0.210$). For food cup contacts (Fig. 2c), there was a main effect of Tracking ($F(2,36) = 14.90$, $p < 0.001$) but no main effect or interactions with Drug. Similar to earlier phases of rimonabant testing, there were no effects of rimonabant on food pellet collection or consumption during the post-cue period (Fig. S2F). Finally, we performed a single Wilcoxon

Table 2 ANOVA for phase II: late Pavlovian lever autoshaping: retraining sessions 8–14

Effect	Degrees of freedom	Lever						Food cup						PCA	
		Contact		Latency		Probability		Contact		Latency		Probability			
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Session	(6, 216)	7.659	< 0.001	17.947	< 0.001	10.57	< 0.001	6.032	< 0.001	20.369	< 0.001	7.667	< 0.001	21.33	< 0.001
Tracking group	(2, 36)	6.766	0.003	9.379	.001	15.012	< 0.001	13.94	< 0.001	29.154	< 0.001	26.250	< 0.001	40.22	< 0.001
Session × Tracking	(12, 216)	1.951	0.03	7.207	< 0.001	8.283	< 0.001	3.067	0.001	4.522	< 0.001	1.981	0.027	5.01	< 0.001

signed-rank test of the PCA scores of the entire population to compare PCA scores with vehicle versus 3 mg/kg rimonabant (Fig. 2d). There was a significant negative shift of PCA scores with 3 mg/kg compared to vehicle conditions ($Z = -2.04$, $p < 0.05$) reconfirming that predominantly lever-directed behavior in late Pavlovian conditioning is reduced by rimonabant.

Experiment 2: effect of rimonabant on conditioned reinforcement

In Experiment 1, rimonabant's attenuating effects on lever-directed behaviors were consistent after both limited and extended training in PLA. This suggests that CB1 signaling mediates the "attracting" properties of lever cues, which are postulated to accrue reinforcing value through pairings with the reward. However, from testing rimonabant's effects in reinforced lever autoshaping sessions, it is unclear whether CB1 signaling is critical for representing the reinforcing value of the lever cue itself. In Experiment 2, we examined the effect of rimonabant on conditioned reinforcement in rats with extended training in PLA. Conditioned reinforcement is a procedure that specifically probes whether the Pavlovian lever cue can serve as a reinforcer itself.

Exp. 2 timeline appears in Fig. 3a. We first gave rats extended training in PLA. Similar to Exp. 1, we observed that rats with GT and INT PCA scores early in lever autoshaping shifted towards ST PCA scores with extended training (Fig. 3b). Importantly, on the last 3 days of conditioning prior to the conditioned reinforcement test, all rats engaged in lever-directed behavior prior to conditioned reinforcement (Fig. S3A right) and there were no notable sex effect sizes across all rats (but see supplementary information for large sex effect sizes in ST rats).

Behavior during the conditioned reinforcement test is shown in Fig. 3c. During conditioned reinforcement, we observed a main effect of Response ($F(1,20) = 100.069$, $p < .0001$), indicating that rats discriminated between active and inactive ports. Thus, the Pavlovian lever cue served as a robust conditioned reinforcer after Pavlovian lever autoshaping. We did not observe any notable sex effect sizes under vehicle conditions, suggesting that males and females showed similar discrimination of the active and inactive nosepokes (Fig. S3B–C left). During conditioned reinforcement, we observed a main effect of Drug ($F(1,22) = 6.808$, $p = 0.016$) and a Response × Drug interaction ($F(1,22) = 8.080$, $p = 0.009$) demonstrating that rimonabant reduces active nosepoke responding that resulted in the lever cue insertion. The time course of conditioned reinforcement is shown in Fig. 3d. Broken up into blocks of 5 min, there are main effects of Drug ($F(1,14) = 5.406$, $p = 0.036$) and Block ($F(5,$

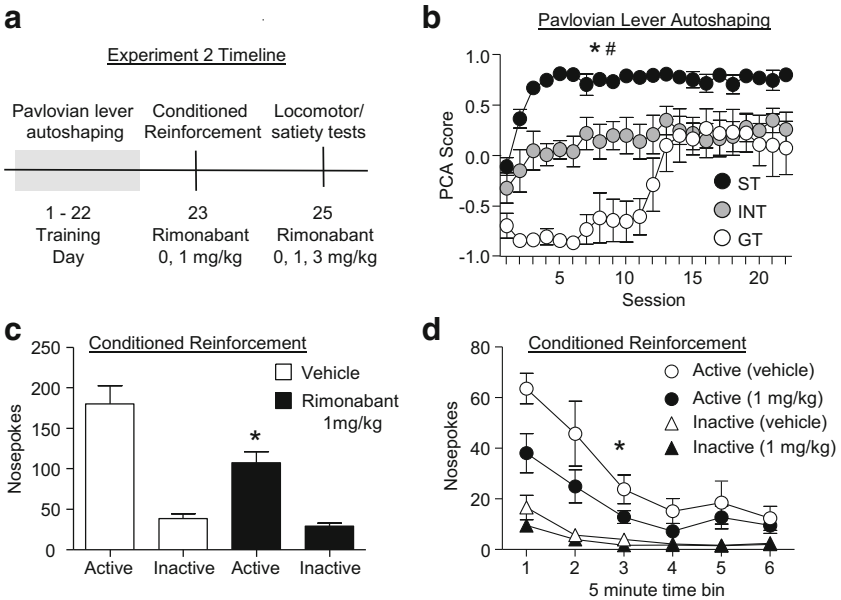

Fig. 3 After extended training in PLA, rimonabant attenuates conditioned reinforcement for Pavlovian lever cue presentations. **a** Exp. 2 timeline. We trained rats in 22 daily PLA sessions. We tested rats with systemic injection of rimonabant (0 and 1 mg/kg) during a conditioned reinforcement test. We gave rats rimonabant (0, 1, or 3 mg/kg) before a motor activity and satiety test. **b** Rats with GT and INT PCA scores early in lever autoshaping shifted towards ST PCA scores with extended PLA training. **c** During conditioned reinforcement, rats discriminated between active and inactive ports, suggesting the inserted lever cue served as a robust conditioned reinforcer. Rimonabant decreased the number of active, but not inactive pokes suggesting that rimonabant attenuates the conditioned reinforcing properties of the Pavlovian lever cue. **d** Time course of nosepokes (binned in 5-min blocks) during conditioned reinforcement test. Rimonabant blunted the number of active nosepokes, while extinction curves were unaffected. *Significant main effect of Session, Response, or Drug; #significant Session × Tracking, Drug × Response interaction

70) = 29.523, $p < 0.001$), but the Drug × Block interaction was not significant. This suggests that rimonabant blunted nosepoke responding for the lever cue presentation, while the rate of extinction was similar in both groups. That is, when CB1 signaling is disrupted, the lever cue is a less effective conditioned reinforcer. We observed a very large sex effect size for rimonabant's attenuation of nosepoking, such that conditioned reinforcement in females may be more sensitive to rimonabant treatment compared to males (Fig. S3C; Cohen's $d = 1.057$). Thus, future studies examining CB1-mediated effects on conditioned reinforcement should be powered to examine sex as a biological factor.

To determine whether rimonabant had non-specific effects on motor activity, we measured rats' percent time motile in the experimental chamber after vehicle, 1 or 3 mg/kg rimonabant (Fig. S4A; supplementary information). There was no evidence for sedative effects of rimonabant. To determine whether rimonabant had sating effects, we measured pellet consumption in these three dose conditions. Rats in all three groups consumed all of their pellets (Fig. S4B). These two results suggest that rimonabant (1 mg/kg or 3 mg/kg) did not induce satiety or sedation that could account for our observations in Exp. 1 or 2. Thus, rimonabant specifically attenuates the motivational properties of the lever cue, both in the presence (Exp. 1) and absence (Exp. 2) of primary reinforcement.

Discussion

Here, we first examined whether disrupting CB1 receptor signaling in Pavlovian lever autoshaping would reduce sign-tracking behavior in Pavlovian lever autoshaping. We found that systemic rimonabant injections dose-dependently attenuated the cue-driven lever approach in sign-trackers early in lever autoshaping. With extended training, many previously goal-tracking and intermediate rats shifted towards lever-directed behaviors, which remained dose-dependently sensitive to the effects of rimonabant. A separate cohort of rats also showed an extended training-dependent shift towards lever-directed behavior, in the absence of any rimonabant treatment during training. During conditioned reinforcement tests, rats receiving vehicle injections acquired a novel instrumental nosepoke response for the lever cue alone, but rats given rimonabant failed to acquire this conditioned response. Together, these results suggest that CB1 signaling is critical for mediating the attracting and conditioned reinforcing properties of Pavlovian lever cues.

Sign-tracking to lever cues has been posited to reflect an incentive motivational process in which the appetitive motivational properties of the reward are transferred to the conditioned lever cue, such that the lever cue attracts, invigorates, and reinforces behavior (Tomie 1996; Flagel et al. 2009; Robinson and Flagel 2009; Beckmann and Chow 2015). While CB1 signaling is involved in the attracting (Exp. 1) and reinforcing properties (Exp. 2) of lever cues, we also find that CB1 signaling supports late lever-directed behaviors regardless of rats' initial tracking group. Rimonabant's attenuation of lever-directed behavior late in training when sign-tracking has been shown to be less dependent on dopaminergic activity might suggest a non-specific effect of rimonabant (Clark et al. 2013). However, recent work has shown sustained dopamine dependency for lever-directed behavior after extended training (Fraser and Janak 2017). Rimonabant also attenuated cue-evoked food cup approach early, but not late, in conditioning. Importantly, rimonabant did not affect pellet retrieval during PLA (Exp. 1) or ad libitum pellet consumption (Exp. 2), demonstrating that CB1 signaling is uniquely involved early in learning to drive *cue-evoked* food cup and lever-directed approach. Thus, CB1-dependent early food cup approach may in part reflect a cue-specific, but response-independent, motivational process common to both sign- and goal-trackers. Consistent with this interpretation, only GT and ST rats showed dose-dependent reductions in all three measures of approach behavior (contact, latency, and probability), while INT rats did not. In Exp. 1, the latency to approach the lever and food cup in INT rats did not change significantly with rimonabant treatment, and in Exp. 2, there were no motor suppressive effects of rimonabant, limiting the possibility that rimonabant had sedative effects. Notably, by examining multiple measures of approach behaviors across the entire continuum of PCA scores, we have elucidated a critical role of CB1 signaling in supporting both sign- and goal-tracking early and learning that may have previously been overlooked (Thornton-Jones et al. 2005). Whether CB1-mediated approach in sign- and goal-trackers is driven by common or divergent brain systems remains an open question, as does the specific brain region mediating CB1-signaling of incentive and reinforcing properties of Pavlovian lever cues.

Notably, the eCB system is involved in the regulation of food intake as well as the sensory and hedonic processing of food (Mahler et al. 2007; Soria-Gomez et al. 2014; Lau et al. 2017). CB1 agonists generally induce hyperphagia and locally modulate neurotransmission in the VTA and NAc to influence dopamine release in response to the consumption of palatable foods (Mahler et al. 2007). Conversely, blocking eCB signaling has anorectic effects and decreases ad libitum feeding behavior (Tallett et al. 2007). In the present study, we observed that rats consumed all of the pellets delivered during Pavlovian lever autoshaping (Exp. 1) and during an ad libitum homecage pellet consumption test (Exp. 2). The food deprivation conditions used in our study enhance motivation for food, which likely masked any anorectic effects of rimonabant, particularly in PLA during which only 50 pellets were delivered. Together, these results limit the possibility that rimonabant had sating effects that lead to the blunted

motivation to engage in the PLA task. In further support of this conclusion, in Exp. 2, we found that rimonabant specifically attenuated the reinforcing properties of the lever cue, in the absence of any pellet reinforcers. Under reinforced testing conditions in Exp. 1, cue-reward associations could rapidly develop in competing brain systems in order to compensate for loss of function due to CB1 signaling disruption. In Exp. 2 when we behaviorally isolate the conditioned reinforcing properties of the lever cue in the absence of primary reinforcement, we observe a stronger effect of disrupting CB1 signaling.

While we did not observe evidence for rimonabant-induced satiety or motor deficits, the possibility remains that rimonabant may have increased anxiety, thereby disrupting behavior in PLA and conditioned reinforcement. However, it is unlikely that rimonabant increased anxiety, as prior studies have found that blocking CB1 receptors has anxiolytic effects (Zador et al. 2015) and reverses stress-induced anxiety (Di et al. 2016). There is some evidence that chronic, intermittent exposure to highly palatable diets induces anxiogenic effects of rimonabant, which are not observed in chow fed conditions like those used in our study (Blasio et al. 2014).

Similar to a prior PLA study using alcohol as a reinforcer (Villaruel and Chaudhri 2016), we observed GT and INT rats, in two separate cohorts that shifted towards lever-directed behavior with extended training in PLA. It is unlikely that GT and INT rats are simply slower to learn than ST rats in PLA, as we have previously reported that sign- and non-sign trackers (made up of GT and INT rats) similarly acquire discrimination of reinforced cues (CS+) from non-reinforced cues (CS−) (Nasser et al. 2015). Consistent with this, in the present study, we observe similar acquisition of the preferred conditioned response in sign- and goal-tracking rats. Thus, the shift from goal-tracking to sign-tracking that we observed in both experiments is likely the result of extended training, which in instrumental settings has been associated with a shift from goal-directed to habitual behaviors (Everitt and Robbins 2016). Further work is needed to determine whether the shift from GT to ST reflects a similar psychological transition in a Pavlovian setting.

By examining sex effect sizes, which are independent of sample size, we identified two measures for which females may be more sensitive than males to the effects of rimonabant. For both early lever-directed behavior and conditioned reinforcement, females showed stronger behavioral suppression to 1 mg/kg rimonabant. While the present study was not powered to analyze sex as a biological variable, these larger sex effect sizes in females suggest that future studies should be powered to explore sex differences in sensitivity of cue-motivated behaviors to manipulations of CB1 receptor signaling (Wagner 2016). Consistent with prior PLA studies (Pitchers et al. 2015; Madayag et al. 2017), with limited training, we did not observe notable sex effect sizes in sign- or goal-tracking behaviors. However, in contrast to previous findings showing enhanced sign-tracking in female rats (Madayag et al. 2017) we found in both Exp. 1 and Exp. 2 with extended training, females made fewer lever contacts at a slower latency than males—effects that were carried largely by ST rats. There are a number of methodological differences used in our study and the Madayag et al. study, including strain differences (Long Evans vs. Sprague Dawley), PLA cue duration (10 vs. 30 s), cue type (lever vs. illuminated lever), reinforcer type (food pellet vs. sucrose solution), and number of trials per session (25 vs. 15), which may result in divergent sex effects during Pavlovian lever autoshaping.

There are several other methodological and mechanistic considerations for the current study. Our a priori hypothesis, predictions and interpretational focus are on endocannabinoid contributions to appetitive behaviors with regard to dopaminergic transmission (Day et al. 2007; Flagel et al. 2011; Hernandez and Cheer 2012; Oleson et al. 2012; Clark et al. 2013; Fraser and Janak 2017). Prior work has shown that cue-evoked phasic DA fluctuations mediate sign-tracking but not goal-tracking (Flagel et al. 2011; Saunders and Robinson 2012; Clark et al. 2013; Saddoris et al. 2016; Fraser and Janak 2017). Since CB1 receptors modulate cue-evoked phasic DA fluctuations (Cheer et al. 2004; Oleson et al. 2012), we predicted and observed that CB1 receptor activation is critical for sign-tracking approach in PLA. Endocannabinoid gating of the mesocorticolimbic dopamine system occurs via inhibition of GABAergic and glutamatergic neurotransmission onto dopamine neurons in the ventral tegmental area (Szabo et al. 2002; Melis et al. 2004; Riegel and Lupica 2004; Covey et al. 2017), by which CB1 receptor activation enhances dopamine release in the striatum (Cheer et al. 2004; Oleson et al. 2012). Inverse agonists such as rimonabant counteract endogenous CB1 receptor activation and reduce striatal DA release and cue-motivated behavior (Cheer et al. 2004; Oleson et al. 2012), the latter of which is reversed by optogenetic activation of VTA DA neurons (Wenzel et al. 2018). Further studies are necessary to determine whether VTA CB1 signaling mediates the reported effects on sign-tracking and conditioned reinforcement.

Yet certainly, other candidate CB1 receptor mechanisms may have contributed to the behavioral effects reported here. Route of administration (systemic vs. intracranial) is an important methodological consideration for interpreting our findings. The seemingly broader role for CB1 signaling in supporting early approach behaviors of both sign- and goal-trackers is similar to that seen with systemic dopamine antagonists (Lopez et al. 2015; Fraser et al. 2016). Systemic rimonabant effects in the present study likely result from eCB modulation of multiple neurotransmitter/neuromodulator systems, including but not limited to the dopamine system, in a broader circuitry than has been previously targeted in PLA. A number of appetitive conditioning studies

implicate other putative CB1-mediated targets. For example, cholinergic modulation of striatal dopamine release also mediates the expression of cue-motivated behaviors (Collins et al. 2016). Endocannabinoid regulation of striatal glutamate release drives striatal cholinergic interneurons, which in turn drive impulse-independent DA release (Exley et al. 2008; Cachope et al. 2012; Threlfell et al. 2012; Mateo et al. 2017). Others have shown that endocannabinoid attenuation of cortical glutamate release in the dorsal medial striatum mediates the transition between goal-directed and habitual behaviors, which are defined by their sensitivity to changes in outcome value (Gremel et al. 2016). Given the differential sensitivity of sign- and goal-trackers to changes in outcome value (Anselme et al. 2013; Ahrens et al. 2015; Morrison et al. 2015; Nasser et al. 2015; Smedley and Smith 2018), this may be another viable target for exploring the contributions of CB1 signaling on sign- and goal-tracking behaviors. Notably, the motivational properties of natural rewards depend on endocannabinoid and opiate system interactions within mesocorticolimbic circuitry. CB1-mediated mu- and/or kappa-opiate receptor-dependent motivational effects may contribute to the behavioral effects we observed in the present study (Solinas and Goldberg 2005; Ahmad et al. 2013; Ahmad and Laviolette 2017). Endocannabinoid signaling in the amygdala as also been implicated in relevant behaviors including positive and negative reinforcement, aversive learning, and affective memory processes (Campolongo et al. 2009; Tan et al. 2011; Trezza et al. 2012; Ahmad and Laviolette 2017; Ahmad et al. 2017). The current study highlights the important role CB1 signaling plays in representing the attracting and reinforcing properties of Pavlovian lever cues, and serves as a foundation for exploring a variety of CB1 mechanisms mediating neurotransmission in VTA, NAc, dorsal striatum, and beyond.

Together, our results suggest that CB1 signaling supports sign-tracking, through the expression of the incentive motivational and conditioned reinforcing properties of Pavlovian lever cues. Our comprehensive analyses of individual differences across the entire continuum of tracking behaviors have elucidated a critical role of CB1 signaling in supporting Pavlovian approach that was previously overlooked. Future studies targeting CB1 signaling in specific brain circuitry will determine whether these individual differences in approach and conditioned reinforcement are neurobiologically dissociable.

Funding information This work was supported by a McKnight Memory and Cognitive Disorders Award (DJC), a NARSAD Young Investigator Grant No. 24950 (DJC), NIDA grants R01DA043533 (DJC), R01DA042595 (JFC) and R01DA022340 (JFC), and the Department of Anatomy and Neurobiology at the University of Maryland, School of Medicine. The authors declare that they do not have any conflicts of interest (financial or otherwise) related to the data presented in this manuscript.

Compliance with ethical standards

All procedures were performed in accordance with the "Guide for the care and use of laboratory animals" (8th edition, 2011, US National Research Council) and were approved by the University of Maryland, School of Medicine Institutional Animal Care and Use Committee (IACUC).

Conflict of interest The authors declare that they have no conflict of interest.

References

Ahmad T, Laviolette SR (2017) Cannabinoid reward and aversion effects in the posterior ventral tegmental area are mediated through dissociable opiate receptor subtypes and separate amygdalar and accumbal dopamine receptor substrates. Psychopharmacology 234(15):2325–2336

Ahmad T, Lauzon NM, de Jaeger X, Laviolette SR (2013) Cannabinoid transmission in the prelimbic cortex bidirectionally controls opiate reward and aversion signaling through dissociable kappa versus mu-opiate receptor dependent mechanisms. J Neurosci 33(39):15642–15651

Ahmad T, Sun N, Lyons D, Laviolette SR (2017) Bi-directional cannabinoid signalling in the basolateral amygdala controls rewarding and aversive emotional processing via functional regulation of the nucleus accumbens. Addict Biol 22(5):1218–1231

Ahrens AM, Singer BF, Fitzpatrick CJ, Morrow JD, Robinson TE (2015) Rats that sign-track are resistant to Pavlovian but not instrumental extinction. Behav Brain Res 296:418–430

Anselme P, Robinson MJ, Berridge KC (2013) Reward uncertainty enhances incentive salience attribution as sign-tracking. Behav Brain Res 238:53–61

Beckmann JS, Chow JJ (2015) Isolating the incentive salience of reward-associated stimuli: value, choice, and persistence. Learn Mem 22(2):116–127

Blasio A, Rice KC, Sabino V, Cottone P (2014) Characterization of a shortened model of diet alternation in female rats: effects of the CB1 receptor antagonist rimonabant on food intake and anxiety-like behavior. Behav Pharmacol 25(7):609–617

Boakes R (1977) Operant pavlovian interactions. Erlbaum, Hillsdale

Cachope R, Mateo Y, Mathur BN, Irving J, Wang HL, Morales M, Lovinger DM, Cheer JF (2012) Selective activation of cholinergic interneurons enhances accumbal phasic dopamine release: setting the tone for reward processing. Cell Rep 2(1):33–41

Campolongo P, Roozendaal B, Trezza V, Hauer D, Schelling G, McGaugh JL, Cuomo V (2009) Endocannabinoids in the rat basolateral amygdala enhance memory consolidation and enable glucocorticoid modulation of memory. Proc Natl Acad Sci U S A 106(12):4888–4893

Cheer JF, Wassum KM, Heien ML, Phillips PE, Wightman RM (2004) Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. J Neurosci 24(18):4393–4400

Cheer JF, Wassum KM, Sombers LA, Heien ML, Ariansen JL, Aragona BJ, Phillips PE, Wightman RM (2007) Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. J Neurosci 27(4):791–795

Chow JJ, Nickell JR, Darna M, Beckmann JS (2016) Toward isolating the role of dopamine in the acquisition of incentive salience attribution. Neuropharmacology 109:320–331

Clark JJ, Collins AL, Sanford CA, Phillips PE (2013) Dopamine encoding of Pavlovian incentive stimuli diminishes with extended training. J Neurosci 33(8):3526–3532

Cohen J (1988) Statistical power analysis for the behavioral sciences. Lawrence Erlbaum Associates, Hillsdale

Collins AL, Aitken TJ, Greenfield VY, Ostlund SB, Wassum KM (2016) Nucleus Accumbens acetylcholine receptors modulate dopamine and motivation. Neuropsychopharmacology 41(12):2830–2838

Covey DP, Mateo Y, Sulzer D, Cheer JF, Lovinger DM (2017) Endocannabinoid modulation of dopamine neurotransmission. Neuropharmacology 124:52–61

Day JJ, Roitman MF, Wightman RM, Carelli RM (2007) Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. Nat Neurosci 10(8):1020–1028

Di S, Itoga CA, Fisher MO, Solomonow J, Roltsch EA, Gilpin NW, Tasker JG (2016) Acute stress suppresses synaptic inhibition and increases anxiety via endocannabinoid release in the basolateral amygdala. J Neurosci 36(32):8461–8470

Everitt BJ, Robbins TW (2016) Drug addiction: updating actions to habits to compulsions ten years on. Annu Rev Psychol 67:23–50

Exley R, Clements MA, Hartung H, McIntosh JM, Cragg SJ (2008) Alpha6-containing nicotinic acetylcholine receptors dominate the nicotine control of dopamine neurotransmission in nucleus accumbens. Neuropsychopharmacology 33(9):2158–2166

Flagel SB, Watson SJ, Robinson TE, Akil H (2007) Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats. Psychopharmacology 191(3):599–607

Flagel SB, Akil H, Robinson TE (2009) Individual differences in the attribution of incentive salience to reward-related cues: implications for addiction. Neuropharmacology 56(Suppl 1):139–148

Flagel SB, Clark JJ, Robinson TE, Mayo L, Czuj A, Willuhn I, Akers CA, Clinton SM, Phillips PE, Akil H (2011) A selective role for dopamine in stimulus-reward learning. Nature 469(7328):53–57

Fraser KM, Janak PH (2017) Long-lasting contribution of dopamine in the nucleus accumbens core, but not dorsal lateral striatum, to sign-tracking. Eur J Neurosci 46(4):2047–2055

Fraser KM, Haight JL, Gardner EL, Flagel SB (2016) Examining the role of dopamine D2 and D3 receptors in Pavlovian conditioned approach behaviors. Behav Brain Res 305:87–99

Gremel CM, Chancey JH, Atwood BK, Luo G, Neve R, Ramakrishnan C, Deisseroth K, Lovinger DM, Costa RM (2016) Endocannabinoid modulation of Orbitostriatal circuits gates habit formation. Neuron 90(6):1312–1324

Hearst E, Jenkins H (1974) Sign-tracking: the stimulus–reinforcer relation and directed action. Monograph of the Psychonomic Society, Austin

Hernandez G, Cheer JF (2012) Effect of CB1 receptor blockade on food-reinforced responding and associated nucleus accumbens neuronal activity in rats. J Neurosci 32(33):11467–11477

Lau BK, Cota D, Cristino L, Borgland SL (2017) Endocannabinoid modulation of homeostatic and non-homeostatic feeding circuits. Neuropharmacology 124:38–51

Lopez JC, Karlsson RM, O'Donnell P (2015) Dopamine D2 modulation of sign and goal tracking in rats. Neuropsychopharmacology 40(9): 2096–2102

Lupica CR, Riegel AC (2005) Endocannabinoid release from midbrain dopamine neurons: a potential substrate for cannabinoid receptor antagonist treatment of addiction. Neuropharmacology 48(8): 1105–1116

Madayag AC, Stringfield SJ, Reissner KJ, Boettiger CA, Robinson DL (2017) Sex and adolescent ethanol exposure influence Pavlovian conditioned approach. Alcohol Clin Exp Res 41:846–856

Mahler SV, Smith KS, Berridge KC (2007) Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances 'liking' of a sweet reward. Neuropsychopharmacology 32(11):2267–2278

Mateo Y, Johnson KA, Covey DP, Atwood BK, Wang HL, Zhang S, Gildish I, Cachope R, Bellocchio L, Guzman M, Morales M, Cheer JF, Lovinger DM (2017) Endocannabinoid actions on cortical terminals orchestrate local modulation of dopamine release in the nucleus Accumbens. Neuron 96(5):1112–1126 e1115

McCarthy MM, Woolley CS, Arnold AP (2017) Incorporating sex as a biological variable in neuroscience: what do we gain? Nat Rev Neurosci 18(12):707–708

Melis M, Pistis M, Perra S, Muntoni AL, Pillolla G, Gessa GL (2004) Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. J Neurosci 24(1):53–62

Meyer PJ, Lovic V, Saunders BT, Yager LM, Flagel SB, Morrow JD, Robinson TE (2012) Quantifying individual variation in the propensity to attribute incentive salience to reward cues. PLoS One 7(6): e38987

Miller LR, Marks C, Becker JB, Hurn PD, Chen WJ, Woodruff T, McCarthy MM, Sohrabji F, Schiebinger L, Wetherington CL, Makris S, Arnold AP, Einstein G, Miller VM, Sandberg K, Maier S, Cornelison TL, Clayton JA (2017) Considering sex as a biological variable in preclinical research. FASEB J 31(1):29–34

Morrison SE, Bamkole MA, Nicola SM (2015) Sign tracking, but not goal tracking, is resistant to outcome devaluation. Front Neurosci 9: 468

Nasser HM, Chen YW, Fiscella K, Calu DJ (2015) Individual variability in behavioral flexibility predicts sign-tracking tendency. Front Behav Neurosci 9:289

Oleson EB, Beckert MV, Morra JT, Lansink CS, Cachope R, Abdullah RA, Loriaux AL, Schetters D, Pattij T, Roitman MF, Lichtman AH, Cheer JF (2012) Endocannabinoids shape accumbal encoding of cue-motivated behavior via CB1 receptor activation in the ventral tegmentum. Neuron 73(2):360–373

Pitchers KK, Flagel SB, O'Donnell EG, Woods LC, Sarter M, Robinson TE (2015) Individual variation in the propensity to attribute incentive salience to a food cue: influence of sex. Behav Brain Res 278: 462–469

Riegel AC, Lupica CR (2004) Independent presynaptic and postsynaptic mechanisms regulate endocannabinoid signaling at multiple synapses in the ventral tegmental area. J Neurosci 24(49):11070–11078

Robinson TE, Flagel SB (2009) Dissociating the predictive and incentive motivational properties of reward-related cues through the study of individual differences. Biol Psychiatry 65(10):869–873

Saddoris MP, Wang X, Sugam JA, Carelli RM (2016) Cocaine self-administration experience induces pathological phasic Accumbens dopamine signals and abnormal incentive behaviors in drug-abstinent rats. J Neurosci 36(1):235–250

Saunders BT, Robinson TE (2012) The role of dopamine in the accumbens core in the expression of Pavlovian-conditioned responses. Eur J Neurosci 36(4):2521–2532

Smedley EB, Smith KS (2018) Evidence of structure and persistence in motivational attraction to serial Pavlovian cues. Learn Mem 25(2): 78–89

Solinas M, Goldberg SR (2005) Motivational effects of cannabinoids and opioids on food reinforcement depend on simultaneous activation of cannabinoid and opioid systems. Neuropsychopharmacology 30(11):2035–2045

Solinas M, Goldberg SR, Piomelli D (2008) The endocannabinoid system in brain reward processes. Br J Pharmacol 154(2):369–383

Soria-Gomez E, Bellocchio L, Reguero L, Lepousez G, Martin C, Bendahmane M, Ruehle S, Remmers F, Desprez T, Matias I, Wiesner T, Cannich A, Nissant A, Wadleigh A, Pape HC, Chiarlone AP, Quarta C, Verrier D, Vincent P, Massa F, Lutz B, Guzman M, Gurden H, Ferreira G, Lledo PM, Grandes P, Marsicano G (2014) The endocannabinoid system controls food intake via olfactory processes. Nat Neurosci 17(3):407–415

Szabo B, Siemes S, Wallmichrath I (2002) Inhibition of GABAergic neurotransmission in the ventral tegmental area by cannabinoids. Eur J Neurosci 15(12):2057–2061

Tallett AJ, Blundell JE, Rodgers RJ (2007) Grooming, scratching and feeding: role of response competition in acute anorectic response to rimonabant in male rats. Psychopharmacology 195(1):27–39

Tan H, Lauzon NM, Bishop SF, Chi N, Bechard M, Laviolette SR (2011) Cannabinoid transmission in the basolateral amygdala modulates fear memory formation via functional inputs to the prelimbic cortex. J Neurosci 31(14):5300–5312

Taylor JR, Robbins TW (1984) Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. Psychopharmacology 84(3):405–412

Thornton-Jones ZD, Vickers SP, Clifton PG (2005) The cannabinoid CB1 receptor antagonist SR141716A reduces appetitive and consummatory responses for food. Psychopharmacology 179(2):452–460

Threlfell S, Lalic T, Platt NJ, Jennings KA, Deisseroth K, Cragg SJ (2012) Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. Neuron 75(1):58–64

Tomie A (1996) Locating reward cue at response manipulandum (CAM) induces symptoms of drug abuse. Neurosci Biobehav Rev 20(3): 505–535

Trezza V, Damsteegt R, Manduca A, Petrosino S, Van Kerkhof LW, Pasterkamp RJ, Zhou Y, Campolongo P, Cuomo V, Di Marzo V, Vanderschuren LJ (2012) Endocannabinoids in amygdala and nucleus accumbens mediate social play reward in adolescent rats. J Neurosci 32(43):14899–14908

Villaruel FR, Chaudhri N (2016) Individual differences in the attribution of incentive salience to a Pavlovian alcohol cue. Front Behav Neurosci 10:238

Wagner EJ (2016) Sex differences in cannabinoid-regulated biology: a focus on energy homeostasis. Front Neuroendocrinol 40:101–109

Wenzel JM, Oleson EB, Gove WN, Cole AB, Gyawali U, Dantrassy HM, Bluett RJ, Dryanovski DI, Stuber GD, Deisseroth K, Mathur BN, Patel S, Lupica CR, Cheer JF (2018) Phasic dopamine signals in the nucleus Accumbens that cause active avoidance require endocannabinoid mobilization in the midbrain. Curr Biol 28(9): 1392–1404 e1395

Wolterink G, Phillips G, Cador M, Donselaar-Wolterink I, Robbins TW, Everitt BJ (1993) Relative roles of ventral striatal D1 and D2 dopamine receptors in responding with conditioned reinforcement. Psychopharmacology 110(3):355–364

Zador F, Lenart N, Csibrany B, Santha M, Molnar M, Tuka B, Samavati R, Klivenyi P, Vecsei L, Marton A, Vizler C, Nagy GM, Borsodi A, Benyhe S, Paldy E (2015) Low dosage of rimonabant leads to anxiolytic-like behavior via inhibiting expression levels and G-protein activity of kappa opioid receptors in a cannabinoid receptor independent manner. Neuropharmacology 89:298–307