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Effects of dopamine receptor antagonism and amphetamine sensitization on sign- and goal-tracking after extended training

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Abstract

Rationale. The dopamine system is thought to be important in incentive salience attribution, where motivational value is assigned to a cue that predicts an appetitive reinforcer (sign-tracking), however, dopamine's role may change with extended training.
Objectives. We tested the effects of selective dopamine D1-like and D2-like receptor antagonism on the expression of Pavlovian conditioned approach after extended Pavlovian conditioned approach (PCA) training. We also tested the hypothesis that locomotor sensitization would accelerate the phenotypic shift to sign-tracking.
Methods. 24 male Long-Evans rats were subjected to 20 PCA sessions in which one lever (CS+, 10 s) predicted 0.2 mL sucrose delivery and the other lever (CS−) did not. SCH-23390 or eticlopride were administered prior to behavioral tests at doses of 0, 0.01, and 0.1 mg/kg (s.c.). In a subsequent experiment, rats were exposed to vehicle or 2 mg/kg amphetamine (i.p.) for 7 days (n = 12/group). After a 10-day incubation period, they were subjected to PCA training for 16 sessions.
Results. The D1 antagonist SCH-23390 reduced locomotor activity and port entries during inter-trial intervals, but the D2 antagonist eticlopride selectively reduced CS+ port entries in goal-trackers , i.e. animals motivated towards the reinforcer. Locomotor sensitization had no effect on the acquisition of sign-tracking.
Conclusions. A commonly used dose of SCH-23390 exhibited off-target locomotor effects and D2 receptors were not required for expression of sign-tracking after extended training. Amphetamine-induced locomotor sensitization did not enhance acquisition of sign-tracking behavior, suggesting that the sensitivity of the dopamine system does not drive acquisition of sign-tracking behavior.

Key words: Autoshaping; Pavlovian conditioned approach; sign-tracking; goal-tracking; incentive salience; dopamine; SCH-23390; eticlopride; amphetamine; sensitization; sucrose; reward; learning

Introduction

Dopamine signalling has been heavily implicated in both the acquisition and expression of incentive salience, where motivational value is assigned to a cue that predicts an appetitive reinforcer (Berridge, 2007; Chow et al., 2016; Flagel et al., 2011, 2007; Fraser and Janak, 2017). Incentive salience is typically studied using a Pavlovian conditioned approach (PCA) or 'autoshaping' task, where a lever conditioned stimulus (CS) is paired with an appetitive reinforcer. When the CS is presented, sign-tracking animals approach the CS directly, while goal-tracking animals approach the location where the reinforcer will be presented (Berridge and Robinson, 2003). Both sign- and goal-trackers learn the CS-reinforcer association, but only sign-trackers assign motivational value to the CS.

A number of studies have described a crucial role for dopamine in incentive salience attribution (Table 1). Antagonism of D1-like dopamine receptors (D1 and D5; hereafter 'D1') disrupted acquisition of sign-tracking (Chow et al., 2016; Roughley and Killcross, 2019) and reduced expression of sign-tracking (Clark et al., 2013). Infusing the D1 antagonist SCH-23390 into the nucleus accumbens core immediately after PCA sessions impaired the acquisition of approach behavior towards an appetitive CS (Dalley et al., 2005) and intra-accumbal amphetamine augmented behavioral responses to an appetitive cue during Pavlovian-instrumental transfer (Peciña and Berridge, 2013). Moreover, systemic injections of a non-selective dopamine antagonist impaired expression of sign-

Table 1. Summary of previous studies on the role of dopamine in acquisition and expression of sign- and goal-tracking

Animals	Design	Manipulation	Result	Reference
Male Sprague-Dawley rats	CS+, 8 sessions, 25 trials/session, banana pellet reinforcer	D1&D2: Acb core flupenthixol before test	Impaired expression of sign-tracking	Saunders and Robinson (2012)
Male Sprague-Dawley rats	CS+, 5 sessions, 25 trials/session, food pellet reinforcer	D1&D2: In situ hybridization after session 1 or 5	Session 1: D1 mRNA greater in sign-trackers than goal-trackers Session 5: Tyrosine hydroxylase, dopamine transporter, D2 mRNA greater in goal-trackers than sign-trackers	Flagel et al. (2007)
Male Sprague-Dawley rats	CS+, 15 sessions, 25 trials/session, food pellet reinforcer	DA: Fast scan cyclic voltammetry in sign-trackers	Peak CS-evoked dopamine rises early in training, then diminishes	Clark et al. (2013)
Male Lister rats	CS+/CS-, 3 sessions, 50 trials/session, food pellet reinforcer	D1: Acb Core SCH-23390 after each session	Impaired acquisition	Dalley et al. (2005)
Male Sprague-Dawley rats	Lever-CS+/Tone-CS+, 14 sessions, 16 trials per CS/session, food pellet reinforcer	D1: SCH-23390, i.p. intermittent pretreatment during training	Impaired acquisition of sign-tracking	Chow et al. (2016)
Male Wistar rats	CS+, 7 sessions, 28 trials/session, food pellet reinforcer	D1: SCH-23390, i.p. pretreatment during training	Impaired acquisition of sign- and goal-tracking	Roughley and Killcross (2019)
Male Sprague-Dawley rats	CS+, 5 or 15 sessions, 25 trials/session, food pellet reinforcer	D1: SCH-23390, i.p. before test	Impaired expression of sign-tracking	Clark et al. (2013)
Male Sprague-Dawley rats	CS+/CS-, 9 sessions, 15 trials/session, sucrose pellet reinforcer	D2: Haloperidol or olanzapine, i.p. pretreatment during training	Impaired acquisition of sign-tracking, but not goal-tracking	Danna and Elmer (2010)
Male Wistar rats	CS+, 7 sessions, 28 trials/session, food pellet reinforcer	D2: Eticlopride, i.p. pretreatment during training	Impaired acquisition of sign-tracking and expression of goal-tracking	Roughley and Killcross (2019)
Male Long-Evans rats	CS+, 4 sessions, 25 trials/session, food pellet reinforcer	D2: Eticlopride, i.p. before test sessions	Impaired expression of sign- and goal-tracking	Lopez et al. (2015)
Male Sprague-Dawley rats	Lever-CS+/Tone-CS+, 14 sessions, 16 trials per CS/session, food pellet reinforcer	D2: Eticlopride, i.p. intermittent pretreatment during training	Impaired expression of sign- and goal-tracking	Chow et al. (2016)
Male Sprague-Dawley rats	CS+, 7 sessions, 25 trials/session, banana pellet reinforcer	D2: 7OH-DPAT, pramipexole, or raclopride i.p. before test sessions	Both agonists (7OH-DPAT, pramipexole) and antagonist (raclopride) impaired expression of sign- and goal-tracking	Fraser et al. (2016)

tracking but not goal-tracking (Saunders and Robinson, 2012). Together, these findings suggest that dopamine signalling, particularly at D1 receptors, is important for incentive salience attribution. Several studies have also implicated the D2-like dopamine receptors (D2, D3, and D4; hereafter 'D2') in incentive salience. Systemic pretreatment with antipsychotics that inhibit D2 receptors impaired the acquisition of sign-tracking, but not goal-tracking (Danna and Elmer, 2010), though antipsychotic pretreatment does not change the expression of previously learned sign-tracking behavior (Bédard et al., 2011). Selective D2 receptor antagonists, such as raclopride and eticlopride, impaired performance of previously acquired sign-tracking and goal-tracking (Fraser et al., 2016; Lopez et al., 2015). Although a D2 agonist also impaired performance of sign-tracking, it did not affect goal-tracking (Lopez et al., 2015). These results sit in some tension with other findings that eticlopride administered intermittently during acquisition impaired goal-tracking but not sign-tracking or that eticlopride administered daily during acquisition impaired the expression but not acquisition of goal-tracking (Roughley and Killcross, 2019).

One possible explanation that the role of dopamine varies across training. Sign-trackers show greater expression of D1 receptor mRNA during the first session of PCA training than goal-trackers (Flagel et al., 2007). In contrast, goal-trackers exhibit greater dopamine-related transcriptional activity than sign-trackers in later sessions (Flagel et al., 2007). This may explain why administering eticlopride during a 14-day PCA pro-

Table 2. Materials and Supplier Details

Item	Supplier	Notes
Animals and husbandry		
Long-Evans rats	Charles River, Kingston, NY, USA	Strain code: 006 RRID: RGD_2308852 Area: K72 Kingston
Teklad Sani Chip bedding	Envigo, Lachine, QC, Canada	Cat#: 7090
Nylabone	Bio-Serv, Flemington, NJ, USA	Cat#: K3580
Rat tunnels	Bio-Serv, Flemington, NJ, USA	Cat#: K3245 or K3325
Shredded paper	FiberCore, Cleveland, OH, USA Shepherd Specialty Papers, Watertown, TN, USA	EnviroDri® nesting material
Drugs and Reagents		
SCH-23390	AdooQ Bioscience, Irvine, CA, USA	Cat#: A13066 CAS#: 125941-87-9 Lot#: L13066B001
Eticlopride	Tocris, Abingdon, UK	Cat#: 1847 CAS#: 97612-24-3 Batch#: 1B/21168
Amphetamine	Tocris, Abingdon, UK	Cat#: 2813 CAS#: 51-63-8 Batch#: 7A/214621 Health Canada authorization: #45782.05.18
0.9% sodium chloride	Hospira (Pfizer), Lake Forest, IL, USA	Cat#: 00409-4888-10 CAS#: 7647-14-5 Vehicle solution for SCH-23390, eticlopride, and amphetamine
Sucrose	BioShop Canada, Burlington, ON, Canada	Cat#: SUC600 CAS#: 57-50-1 Dissolved in tap water 100g/L
Behavioral Apparatus		
Modular conditioning chambers	Med Associates, St Albans, VT, USA	Cat#: ENV-009A
White houselight	Med Associates, St Albans, VT, USA	Cat#: ENV-215M
Fluid port	Med Associates, St Albans, VT, USA	Cat#: ENV-200R3AM
Head entry detector for liquid receptacles (rat)	Med Associates, St Albans, VT, USA	Cat#: ENV-254-CB
Syringe pump	Med Associates, St Albans, VT, USA	Cat#: PHM-100 Speed: 3.3 RPM
Retractable lever	Med Associates, St Albans, VT, USA	Cat#: ENV-112M
Rat arena for Tru Scan	Coulbourn Instruments, Holliston, MA, USA	Cat#: E63-20

tocol impaired goal-tracking (Chow et al., 2016), but administering eticlopride after 4 PCA sessions impaired sign-tracking (Lopez et al., 2015). These results suggest that extended training alters the role of the dopamine system in sign-tracking and goal-tracking.

The locomotor sensitization protocol involves exposing animals to a psychostimulant, such as amphetamine, over a period of time and produces long-lasting changes in dopamine signalling rendering the rats more sensitive to dopaminergic manipulations (Downs and Eddy, 1932; Kalivas and Stewart, 1991; Segal and Mandell, 1974). Prior studies have examined the influence of locomotor sensitization on sign- and goal-tracking. Some have found enhanced sign-tracking responses (Robinson et al., 2015; Wyvell and Berridge, 2001) and others found enhanced goal-tracking responses (Simon et al., 2008). These studies may suggest opposing effects on incentive salience, but are difficult to directly compare due to procedural differences. For example, whether sensitization was induced before or after Pavlovian conditioning and whether animals received 7, 8 or 14 PCA sessions (Robinson et al., 2015; Simon et al., 2008; Wyvell and Berridge, 2001). Nonetheless, these studies suggest that locomotor sensitization has the potential to alter the acquisition of incentive salience, especially since dopamine is more important for sign-tracking early in acquisition (Clark et al., 2013; Flagel et al., 2007).

Recent work from our laboratory has shown that a subset of rats will show a phenotypic shift from goal-tracking to sign-tracking (Srey et al., 2015; Villaruel and Chaudhri, 2016). This phenotypic shift typically takes places after 16 or more sessions of training when using an alcohol cue (Srey et al., 2015; Villaruel and Chaudhri, 2016). However, several previous studies have used fewer than 10 PCA sessions (Fraser et al., 2016; Lopez et al., 2015; Robinson et al., 2015). Since the role of dopamine may change with extended training (Clark et al., 2013; Flagel et al., 2007), we studied the effect of selective D1 or D2 antagonism on the expression of sign-tracking and goal-tracking behavior after extended training (20 sessions). We expected that both D1 and D2 antagonism would reduce sign-tracking and that D2 antagonism would reduce sign-tracking and goal-tracking. We then gave a separate cohort of rats a sensitizing regimen of amphetamine and subjected them to PCA training for 16 sessions. We expected this to facilitate acquisition of sign-tracking.

Table 3. Definitions of Key Variables

Variable	Definition
Pavlovian Conditioned Approach	
PCA Score	(Response Bias + Latency Score + Probability Difference) ÷ 3
Response Bias	(Lever Activations − Port Entries) ÷ (Lever Activations + Port Entries)
Probability Difference	(Trials with Lever Activations − Trials with Port Entries) ÷ Number of Trials
Latency Score	(Mean Port Entry Latency - Mean Lever Activation Latency) ÷ CS Duration
Locomotor Behavior	
Distance Travelled	Sum of the coordinate changes during the session (cm) in the floor plane.
Movement Episodes (or moves)	The number of episodes of movement in the floor plane, as defined by continuous coordinate changes without resting for at least one second.
Center Time	The amount of time spent in the center of the chamber, as defined by the animal's coordinates being at least 2.5 beam-widths (6.25 cm) away from the chamber walls.

Methods

Animals

Subjects were 48 experimentally naïve male Long-Evans rats weighing 220-240 g on arrival (Charles River). Rats were initially pair-housed in plastic cages (44.5 × 25.8 × 21.7 cm) containing Teklad Sani Chip bedding, a nylabone, a tunnel, and shredded paper in a climate-controlled (21°C) vivarium on a 12 h:12 h light:dark cycle (lights on at 7am). Rats were allowed to acclimate to the colony room for at least 3 days before being singly-housed and handled for 7 days. Rats had free access to food and water in their home-cage throughout the experiments. All procedures were approved by the Animal Research Ethics Committee at Concordia University and performed in accordance with guidelines from the Canadian Council on Animal Care. See Table 2 for details of animals, reagents, and equipment.

Apparatus

Behavioral training was conducted using a 12 identical conditioning chambers (30.5 x 31.8 x 29.2 cm, Med Associates). Each chamber was contained within a sound-attenuating cubicle with a fan to provide ventilation and background noise (70-75 dB). Each chamber had a white houselight in the center near the ceiling of the left wall (as viewed by the experimenter). The right wall had a fluid port and head entry detector located above the floor. A 20 mL syringe was placed on a syringe pump outside the cubicle and connected to the port with polyethylene tubing. A retractable lever was placed on either side of the port and these would serve as conditioning stimuli. A PC running Med-PC IV controlled presentation of stimuli and recorded responses. For open field locomotor behavior, we used four 39 x 42 x 50 cm arenas (Coulbourn Instruments) housed in sound attenuating boxes and Tru Scan 2.0.

Experiment 1: Effect of dopamine antagonists on expression of sign- and goal-tracking

Home-cage sucrose.
To familiarize rats (n = 24) with sucrose, they were given 48 h of free home-cage access to 10% sucrose. A pre-weighed cylinder with 90 mL sucrose and a regular bottle of water were placed on their home-cage. After 24 h, bottles were re-weighed, refilled, and replaced for another 24 h. Rats consumed all, or nearly all, the sucrose.

Habituation.
Rats were then habituated to the conditioning chambers. On the first day, rats were habituated to transport by being placed on a trolley, taken to the testing room, handled, weighed, and left in the room for 20 min before being returned to the colony room. On the second day, rats were placed in the chambers, and there was a programmed 2 min delay before the houselights were switched on for a 20 min session. Port entries were counted, but had no programmed consequences.

Pavlovian conditioned approach.
Rats then underwent 20 sessions of PCA training. Each PCA session involved 20 trials, with 10 CS+ trials (paired with sucrose delivery) and 10 CS- trials (no sucrose). Each trial consisted of a 10 s Pre-CS interval, a 10 s CS lever presentation, and a 10 s Post-CS interval. One of the two levers was designated as the CS+ lever and the other lever as the CS- lever. These were counterbalanced so that for half of the rats the CS+ lever was on the left of the fluid port and for the other half the CS+ lever was on the right of the fluid port. For a CS+ trial, but not a CS- trial, 6 s of syringe pump operation began at the onset of the Post-CS interval to deliver 0.2 mL of sucrose. The inter-trial interval (ITI), which did not include the Pre-CS, CS, or Post-CS intervals, was set at 60, 120, or 180 s (mean ITI duration = 120 s). The ITI order of CS+ and CS- trials was randomized. Sessions included a 2 min delay before the houselight was switched on for a 52-min PCA test session.

For each session, a Pavlovian Conditioned Approach (PCA) score was calculated from response bias, probability difference, and latency score (Table 3; Meyer et al. (2012)). Rats were classified as sign-trackers if their mean PCA score was ≥ 0.5 for PCA sessions 19 and 20. PCA scores ≤ -0.5 were classified as goal-trackers and PCA scores between -0.5 and 0.5 were intermediates (Ahrens et al., 2016; Meyer et al., 2012).

Dopamine antagonist tests.
The effect of dopamine antagonists on the expression of conditioned responding was tested using a within-subjects design, with dose order counterbalanced using a Latin square design. First, to test the effect of the D1 antagonist SCH-23390, rats were given an injection of saline vehicle, 0.01 mg/kg, or 1 mg/kg SCH-23390 (1 mL/kg, s.c.) 15 min before a test session. Test conditions were identical to training conditions, but no syringes were placed on the pump and no sucrose was delivered. Rats were given at least one day of normal training between tests to allow them to return to baseline levels of responding. After the completion of testing for SCH-23390, the testing procedure was repeated, but rats received saline vehicle, 0.01 mg/kg, or 0.1 mg/kg eticlopride. Previous studies have shown 0.01 mg/kg SCH-23390 or eticlopride to be an effective dose (Chow et al., 2016; Sciascia et al., 2014) and pilot studies using a lower dose (1 µg/kg SCH-23390 or eticlopride) did not show any effect on sign- or goal-tracking.

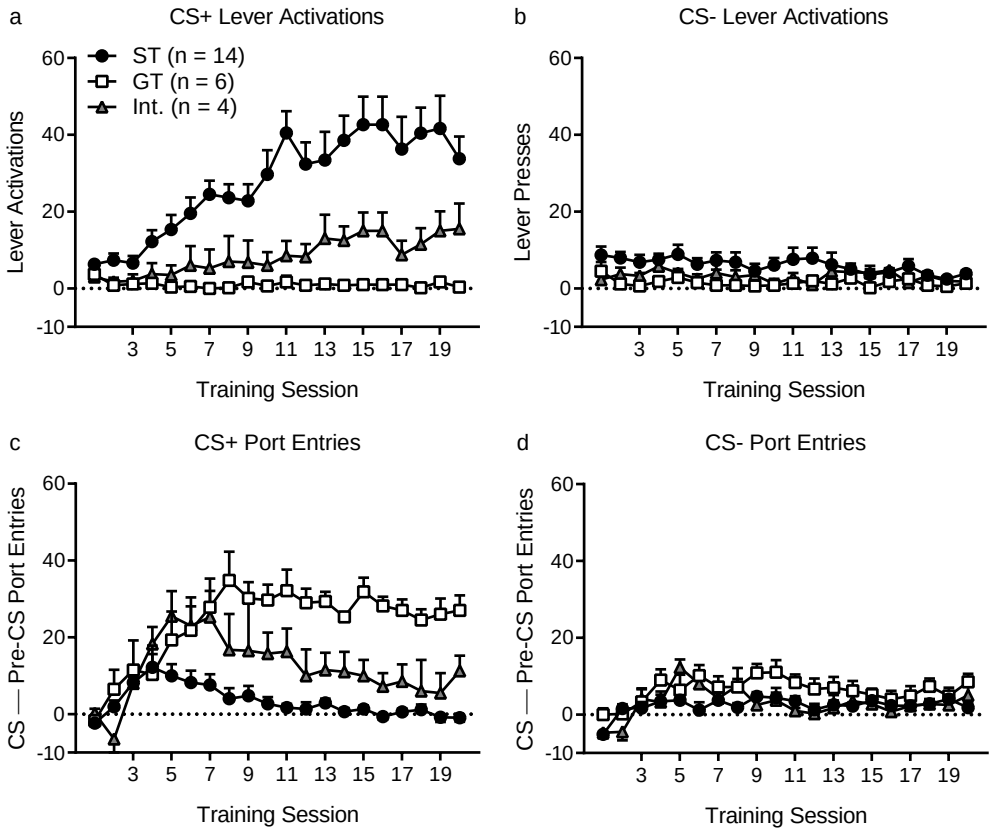

Figure 1. Sign-trackers and goal-trackers acquired distinct behaviors during Pavlovian PCA training sessions. (a) Rats classified as sign-trackers (ST; n = 14) interacted most with a tactile lever cue that predicted 10% sucrose delivery (CS+), while goal-trackers (GT; n = 6) and intermediates (Int.; n = 4) produced fewer CS+ lever activations. (b) The overall level of interaction with a non-predictive lever cue (CS-) remained low throughout 20 sessions of training. (c) Normalized CS+ port entries (CS+ port entries minus Pre-CS+ port entries) were highest in goal-trackers. (d) Normalized CS- port entries (CS- port entries minus Pre-CS- port entries) remained low throughout training. Each cue lever was available for 10 trials for 10 s per trial (total of 100 s/session). Data are means ± SEM.

Open field locomotor testing.

After testing the effects of SCH-23390 and eticlopride on the expression of Pavlovian conditioned approach, we examined the effects of these antagonists on locomotor behavior in an open field (see Table 3 for definition of variables). On the first day, rats were exposed to the locomotor chambers for a 45-min habituation session. The next day, rats were randomly allocated to receive vehicle, 0.01 mg/kg, or 0.1 mg/kg SCH-23390 (1 mL/kg, s.c.) 15 min before the locomotor session (n = 8/dose). On a separate day, rats were randomly allocated to receive vehicle, 0.01 mg/kg, or 0.1 mg/kg eticlopride (1 mL/kg, s.c., n = 8/dose) 15 min before the locomotor session.

Experiment 2: Effect of locomotor sensitization on the acquisition of sign- and goal-tracking

Amphetamine exposure.

Rats (n = 24) were first habituated to experimental procedures. They were given a saline injection (1 mL/kg, i.p.) immediately before being placed in the locomotor chamber for a 30 min open field locomotor session. After the locomotor session, they were immediately placed in conditioning chambers for a 20 min habituation session, which was identical to the habituation session in Experiment 1. Rats were then randomly allocated to vehicle or amphetamine groups (n = 12/group). For the next 7 consecutive days, rats received either 1 mL/kg vehicle or 2 mg/kg amphetamine i.p. immediately before a 30 min locomotor session and 20 min exposure to the conditioning chamber. Doses were based on previous studies (Robinson et al., 2015).

Incubation and home-cage sucrose exposure.

Behavioral training began 10 days after the last amphetamine injection. For the first 7 days, rats were left undisturbed except for normal husbandry activities. On days 8-10, rats received 48 h of home-cage sucrose exposure.

Pavlovian conditioned approach.

After incubation and home-cage sucrose exposure, rats received 14 sessions of PCA training in the conditioning chambers using procedures identical to those described for Experiment 1.

Sensitization test.

After PCA training, rats were tested for locomotor sensitization. All rats were given a 0.75 mg/kg amphetamine challenge (Robinson et al., 2015) immediately before a 30 min open field locomotor session.

Statistical Analysis and Material Availability.

Data were analysed using SPSS 24 (IBM, NY, USA). ANOVA with Bonferroni-corrected post-hoc comparisons and t-tests were used. Greenhouse-Geisser corrections were applied to degrees of freedom following a significant Mauchly's test of sphericity with $\varepsilon < 0.75$. Following violations of ANOVA assumptions for some locomotor measures in Experiment 1, the Kruskal-Wallace test was used. Raw data and Med-PC code will be made available on Figshare.

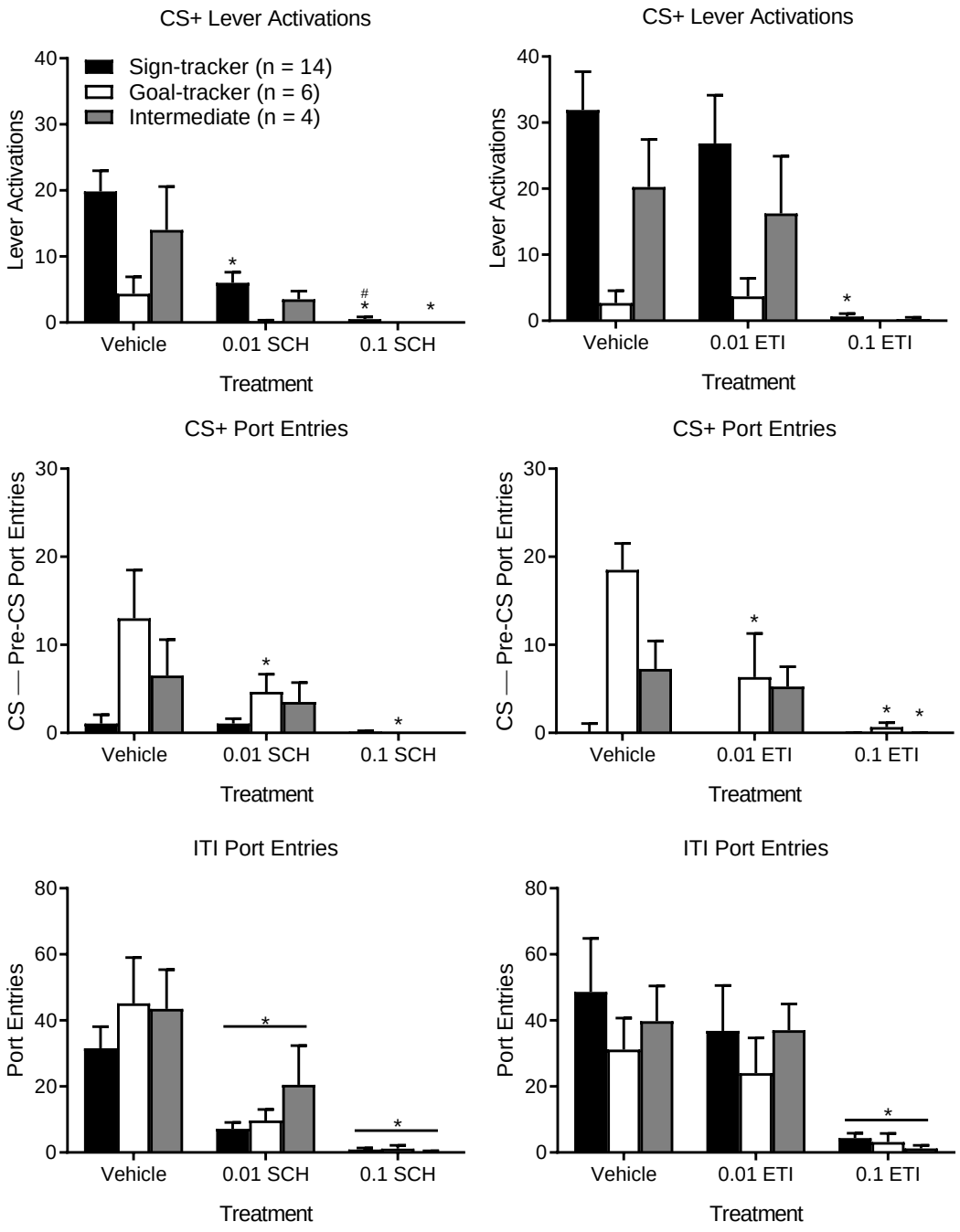

Figure 2. D1 antagonism had off-target effects while D2 antagonism selectively reduced goal-tracking in goal-trackers. (a) Rats received the selective D1 antagonist SCH-23390, produced dose-dependent reductions on CS+ lever activations made by sign-trackers (n = 14). CS+ lever activations by intermediates (n = 4) were reduced at a dose of 0.1 mg/kg. (b) SCH-23390 produced a dose-dependent reduction normalized CS+ port entries in goal-trackers (n = 6). (c) However, SCH-23390 also had dose-dependent off-target effects on ITI port entries. (d) The selective D2 antagonist, eticlopride, reduced CS+ lever activations by sign-trackers at a dose of 0.1 mg/kg. (e) Eticlopride significantly reduced normalized CS+ port entries made by goal-trackers at both doses and reduced CS+ port entries by intermediates at 0.1 mg/kg. (f) Eticlopride did not have significant effects on ITI port entries at a dose of 0.01 mg/kg but did reduce ITI port entries at 0.1 mg/kg. ITIs comprised 42 min of each session. Data are means ± SEM. * $p < 0.05$ for Bonferroni corrected post-hoc comparisons compared to vehicle. # $p < 0.05$ for Bonferroni corrected post-hoc comparison compared to 0.01 mg/kg.

Results

Experiment 1: Effect of Dopamine Antagonists on Expression of Sign- and Goal-tracking

Rats were classified as sign-trackers (n = 14), goal-trackers (n = 6), or intermediates (n = 4), based on their PCA scores in the last two sessions of PCA training (sessions 19 and 20). Across groups, the number of CS+ lever activations increased over the course of training (Figure 1a; session, $F(19,399) = 4.004$, $p < 0.001$). The number of CS+ lever activations differed by phenotype ($F(2,21) = 11.768$, $p < 0.001$) and there was a significant phenotype × session interaction ($F(38,399) = 2.753$, $p < 0.001$). Sign-trackers made more CS+ lever activations than goal track-

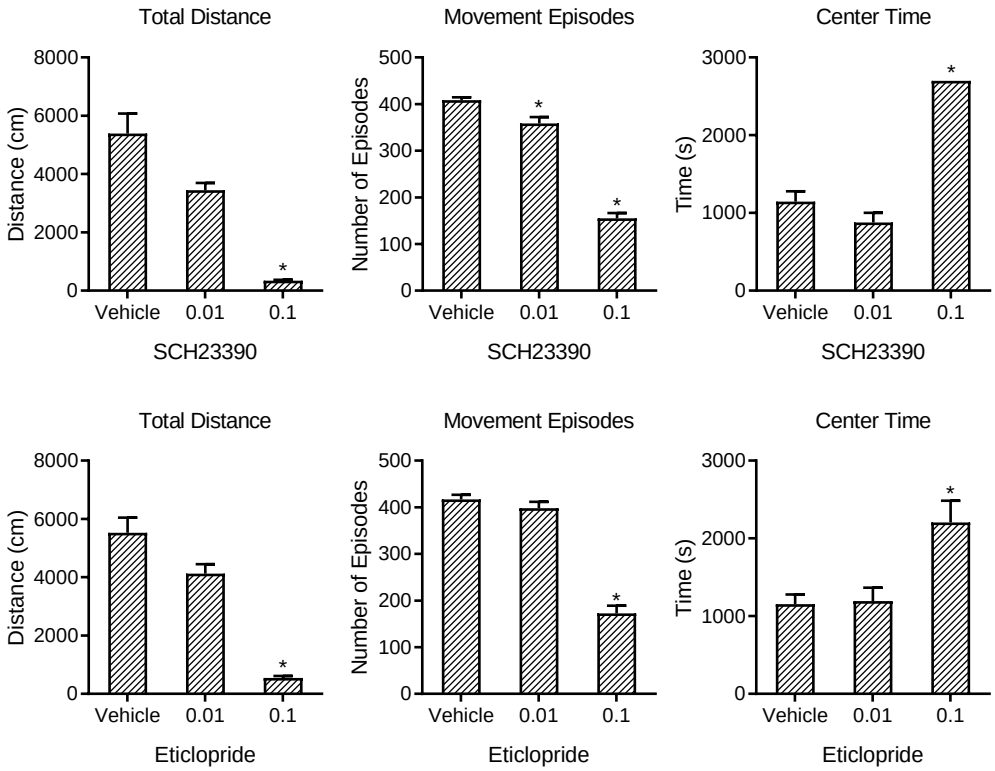

Figure 3. SCH-23390 had greater effects on open field locomotor behavior than eticlopride. (a) Rats received vehicle, 0.01, or 0.1 mg/kg of SCH-23390 prior to a 45 min locomotor test (n = 8/dose). Total distance travelled was significantly reduced at the 0.1 but not 0.01 mg/kg dose. (b) The total number of moves was reduced by SCH-23390 at both the 0.01 and 0.1 mg/kg doses. (c) The amount of time spent in the center of the arena was significantly increased by 0.1 mg/kg SCH-23390, reflecting rats' near-immobility at this dose. (d) Eticlopride was tested using a similar design and reduced total distance travelled at the 0.1 but not 0.01 mg/kg dose. (e) Similarly, eticlopride reduced the total number of moves at the 0.1 but not 0.01 mg/kg dose. (f) Eticlopride only significantly increased center time at the 0.1 mg/kg dose. Data are means ± SEM. * $p < 0.05$ for Bonferroni corrected post-hoc comparisons.

ers for sessions 5-20 ($p \leq 0.036$). Rats with an intermediate phenotype did not significantly differ from sign-trackers or goal-trackers during any sessions.

In contrast, the number of CS- lever activations remained low throughout training (Figure 1b; session, $F(3.383,71.052) = 0.713$, $p = 0.563$, $\varepsilon = 0.178$) for all phenotypes (phenotype × session interaction, $F(6.767,71.052) = 0.501$, $p = 0.826$). Although there was a main effect of phenotype ($F(2,21) = 3.589$, $p = 0.046$), post-hoc comparisons between sign-trackers and goal-trackers or intermediates were not significant ($p \geq 0.054$).

Goal-trackers acquired a port entry response during the CS+. Since there were very few Pre-CS+ port entries throughout training (generally < 5 port entries/session), normalized CS+ port entries are shown in Figure 1c (CS+ port entries minus Pre-CS+ port entries). The number of NormCS+ port entries significantly increased across training sessions ($F(4.776,100.304) = 8.273$, $p < 0.001$, $\varepsilon = 0.251$) and this significantly differed based on phenotype ($F(2,21) = 33.346$, $p < 0.001$; phenotype × session $F(9.553,100.304) = 4.528$, $p < 0.001$). Post-hoc comparisons showed goal-trackers made more NormCS+ port entries than sign-trackers in sessions 7-20 ($p \leq 0.009$). Intermediates made more NormCS+ port entries than sign-trackers on sessions 10-11, 13-14, 16 and 20 ($p \leq 0.03$) and fewer NormCS+ port entries than goal-trackers on sessions 10-20 ($p \leq 0.044$).

Normalized port entries (mean Pre-CS- port entries were generally < 5/session) during CS- trials increased over the course of training (Figure 1d; session, $F(8.864,186.138) = 5.034$, $p < 0.001$, $\varepsilon = 0.467$). This was due to the negative NormCS- scores in sessions 1 and 2 because there were no significant differences between sessions 3-20. NormCS- port entries were affected by phenotype ($F(2,21) = 5.056$, $p = 0.016$), as goal-trackers overall made more NormCS- port entries than sign-trackers ($p = 0.015$). However, there was no significant phenotype × session interaction ($F(17.727,186.138) = 1.292$, $p = 0.121$).

SCH-23390.

The number of CS+ lever activations (Figure 2a) differed by phenotype ($F(2,21) = 5.09$, $p = 0.016$). CS+ lever activations were significantly reduced by SCH-23390, as a function of dose and phenotype (dose, $F(1.347,28.294) = 20.441$, $p < 0.001$, $\varepsilon = 0.674$; dose × phenotype interaction, $F(2.695,28.294) = 3.154$, $p = 0.045$). In sign-trackers, SCH-34490 significantly reduced CS+ lever activations at the 0.01 mg/kg dose, versus vehicle ($p < 0.001$), and the high 0.1 mg/kg dose, relative to vehicle and 0.01 mg/kg SCH-23390 ($p \leq 0.001$). In intermediates, SCH-23390 decreased CS+ lever activations at the 0.1 mg/kg dose ($p = 0.047$), but not 0.01 mg/kg ($p = 0.126$), relative to vehicle.

Normalized CS+ port entries (Figure 2b) varied according to SCH-23390 dose and phenotype (dose, $F(1.271,26.687) = 9.961$, $p = 0.002$, $\varepsilon = 0.635$; phenotype, $F(2,21) = 5.904$, $p = 0.009$) and there was a significant dose × phenotype interaction ($F(2.542,26.687) = 3.802$, $p = 0.027$). In goal-trackers, SCH-23390 decreased CS+ port entries at the 0.01 mg/kg dose, compared to vehicle ($p = 0.041$), and the 0.1 mg/kg dose compared to vehicle ($p = 0.002$) and 0.01 mg/kg ($p = 0.006$). Sign-trackers and intermediates were not significantly affected.

SCH-23390 dose-dependently reduced ITI port entries (Figure 2c). There was a main effect of dose ($F(1.233,25.899) = 28.072$, $p < 0.001$, $\varepsilon = 0.617$), but no effect of phenotype ($F(2,21) = 1.244$, $p = 0.309$) or dose × phenotype interaction

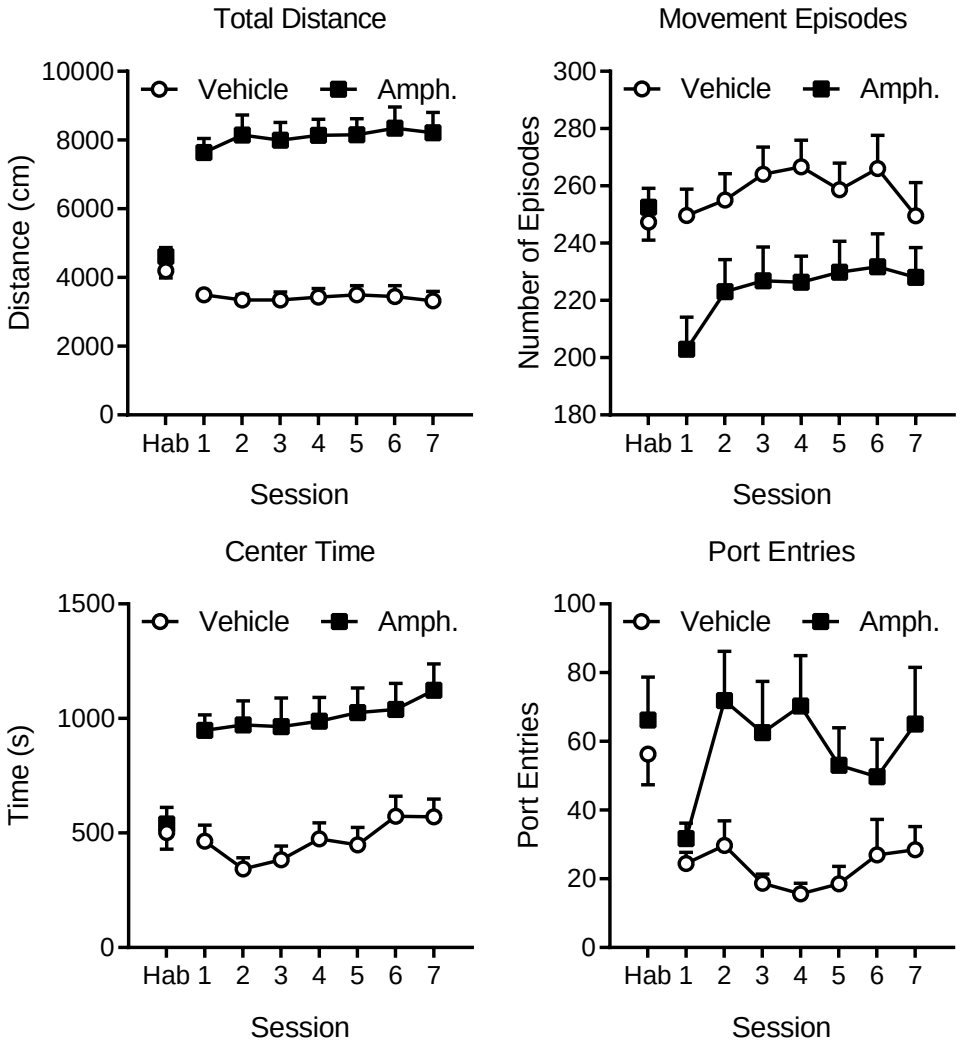

Figure 4. Amphetamine exposure during sensitization induction increased locomotor behavior. (a) Total distance travelled in 30 min was not significantly different between vehicle and amphetamine rats (n = 12/group) during a habituation session when all rats received saline. However, when amphetamine rats began receiving daily 2 mg/kg amphetamine injections, they significantly increased their total distance travelled. (b) Similarly, the number of movement episodes was not different during habituation but was significantly reduced during amphetamine exposure. (c) The amount of center time was also not different between vehicle and amphetamine rats during habituation but was increased during amphetamine exposure. (d) Rats were immediately transferred to behavioral chambers after their locomotor session for 20 min. During habituation, there was no difference in the number of port entries made, but amphetamine rats made significantly more port entries during amphetamine exposure. Data are means ± SEM.

($F(2.467,25.899) = 0.686$, $p = 0.542$). Both SCH-23390 doses reduced ITI port entries (0.01 mg/kg vs. vehicle, $p = 0.001$; 0.1 mg/kg vs vehicle and 0.01 mg/kg, $p < 0.001$).

Eticlopride.

CS+ lever activations (Figure 2d) differed was affected by dose and phenotype (dose, $F(2,42) = 9.982$, $p < 0.001$; phenotype, $F(2,21) = 4.061$, $p = 0.032$) and there was a significant dose × phenotype interaction ($F(4,42) = 2.779$, $p = 0.039$). In sign-trackers, eticlopride reduced CS+ lever activations (0.1 mg/kg vs vehicle, $p < 0.001$; 0.01 mg/kg vs vehicle $p = 0.001$), but had no significant effect on goal-trackers or intermediates.

NormCS+ port entries (Figure 2e) was affected by dose and phenotype (dose, $F(2,42) = 13.676$, $p < 0.001$; phenotype, $F(2,21) = 12.799$, $p < 0.001$) and there was a significant dose × phenotype interaction ($F(4,42) = 7.104$, $p < 0.001$). CS+ port entries were significantly lower for goal-trackers following 0.01 mg/kg ($p = 0.005$) or 0.1 mg/kg ($p < 0.001$) compared to vehicle, with no further reduction from 0.1 mg/kg compared to 0.01 mg/kg ($p = 0.216$) . Intermediates made fewer CS+ port entries following 0.1 mg/kg eticlopride compared to vehicle ($p = 0.026$) but not 0.01 mg/kg ($p = 0.445$).

ITI port entries (Figure 2f) were reduced by high dose eticlopride. There was no effect of phenotype ($F(2,21) = 0.26$, $p = 0.774$) or dose × phenotype interaction ($F(2.591,28.256) = 0.216$, $p = 0.866$). However, there was an effect of dose ($F(1.346,28.256) = 8.6$, $p = 0.003$, $\varepsilon = 0.673$). While 0.01 mg/kg did not differ from vehicle ($p = 0.608$), 0.1 mg/kg eticlopride reduced ITI port entries compared to vehicle ($p = 0.014$) and 0.01 mg/kg ($p = 0.023$).

Open field locomotor behavior.

SCH-23390 significantly reduced locomotor activity at both the high and low dose, compared to vehicle (n = 8/dose). For distance travelled (Figure 3a), a Kruskal-Wallace test found an effect of dose ($H(2) = 18.24$, $p < 0.001$). SCH-23390 reduced

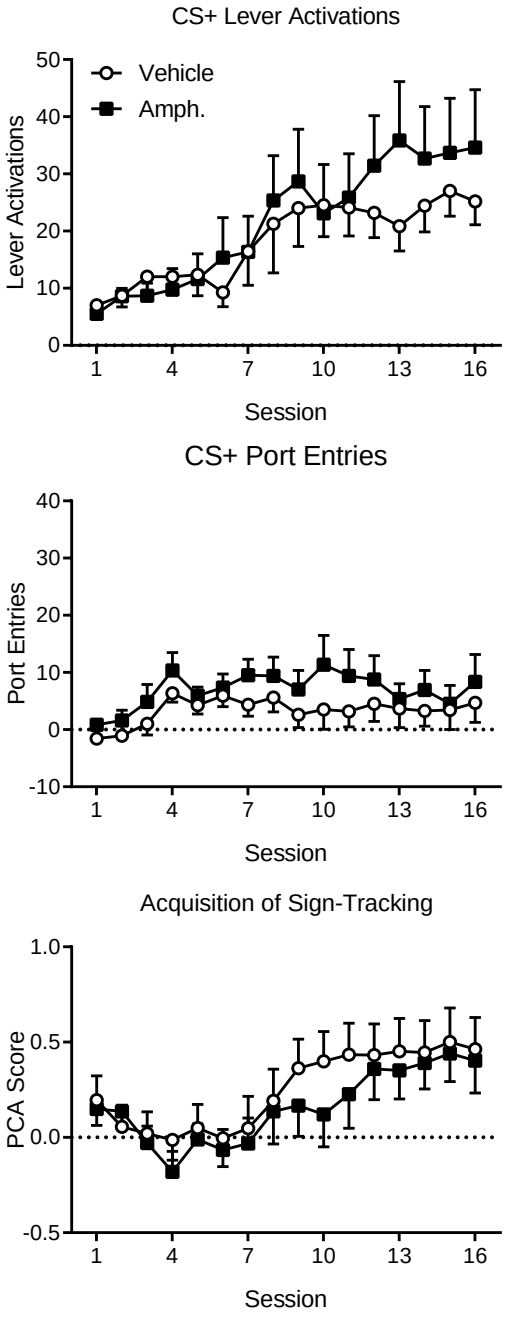

Figure 5. Acquisition of sign-tracking and goal-tracking behaviors did not differ between vehicle and amphetamine-exposed rats. (a) CS+ lever activations did not differ between rats that received vehicle or 2 mg/kg amphetamine during the amphetamine exposure phase (n = 12/group). (b) Normalized CS+ port entries also did not differ between groups during acquisition. (c) PCA scores showed that both cohorts acquired a sign-tracking phenotype overall and there were no significant differences in PCA scores during training. Each cue lever was available for 10 trials for 10 s per trial (total of 100 s/session). Data are means ± SEM.

distance travelled at the 0.1 mg/kg dose ($p < 0.001$ vs vehicle; $p = 0.033$ vs 0.01 mg/kg), but not at 0.01 mg/kg ($p = 0.269$ vs Vehicle). SCH-23390 also dose-dependently reduced the total number of movement episodes (Figure 3b; one-way ANOVA, $F(2,21) = 154.939$, $p < 0.001$). SCH-23390 reduced the number of movement episodes, relative to vehicle, at both doses (0.01 mg/kg, $p = 0.011$; 0.1 mg/kg, $p < 0.001$). The 0.1 mg/kg dose further reduced the movement episodes compared to 0.01 mg/kg ($p < 0.001$). SCH-23390 also increased time spent in the center of the arena (Figure 3c; $H(2) = 16.819$, $p < 0.001$). The 0.1 mg/kg dose significantly increasedcenter time ($p = 0.008$ vs vehicle; $p < 0.001$ vs 0.01 mg/kg), but 0.01 mg/kg had no effect ($p = 1$ vs vehicle).

In a separate test for eticlopride, the high but not lose dose, reduced locomotor behavior (n = 8/dose). Eticlopride reduced total distance travelled (Figure 3d; $H(2) = 17.565$, $p < 0.001$) at the 0.1 mg/kg dose ($p < 0.001$ vs vehicle; $p = 0.024$ vs 0.01 mg/kg), but nobetween at 0.01 mg/kg ($p = 0.413$ vs vehicle). Eticlopride also reduced movement episodes (Figure 3e; one-way ANOVA, $F(2,21) = 100.467$, $p < 0.001$) at the high dose ($p < 0.001$ vs vehicle and 0.01 mg/kg), but nobetween at 0.01 mg/kg ($p = 0.999$ vs vehicle). Eticlopride also increased center time (Figure 3f; $F(2,21) = 8.63$, $p = 0.002$) at the high dose ($p = 0.004$ vs vehicle; $p = 0.006$ vs 0.01 mg/kg, but not at 0.01 mg/kg ($p = 1$ vs vehicle).

Experiment 2: Locomotor sensitization and sign-tracking

Amphetamine exposure.

Rats received vehicle or 2 mg/kg amphetamine before exposure to the locomotor (Figure 4a-c) and behavioral chambers (Figure 4d; n = 12/group). During the habituation session, there were no significant differences in distance travelled (Figure 4a; $t(22) = -1.238$, $p = 0.229$), number of moves (Figure 4b; $t(22) = -0.565$, $p = 0.578$), center time (Figure 4c; $t(22) = -0.365$, $p = 0.719$), or port entries (Figure 4d; equal variances not assumed; $t(19.888) = -0.646$, $p = 0.526$). Amphetamine treatment increased the total distance travelled ($F(1,22) = 89.115$, $p < 0.001$), but there was no effect of session ($F(3.51,77.214) = 0.438$, $p = 0.757$, $\varepsilon = 0.585$) or session × treatment interaction ($F(3.51,77.214) = 0.635$, $p = 0.619$).

Amphetamine treatment decreased the number of movement episodes ($F(1,22) = 9.763$, $p = 0.005$), but there was no effect of session ($F(3.083,67.816) = 2.035$, $p = 0.115$, $\varepsilon = 0.514$), or session × treatment interaction ($F(3.083,67.816) = 0.584$, $p = 0.632$). Amphetamine treatment increased the amount of center time ($F(1,22) = 24.693$, $p < 0.001$). There was an effect of session ($F(3.615,79.527) = 3.582$, $p = 0.012$, $\varepsilon = 0.602$), but no differences between specific sessions or session × treatment interaction ($F(3.615,79.527) = 0.655$, $p = 0.61$). Amphetamine treatment elevated the number of port entries during the 20 min session ($F(1,22) = 11.878$, $p = 0.002$), but there was no effect of session ($F(3.442,75.716) = 1.801$, $p = 0.147$, $\varepsilon = 0.574$) or session × treatment interaction ($F(3.442,75.716) = 1.949$, $p = 0.121$).

Phenotypic shift.

Based on PCA scores from session 15 and 16, vehicle rats were mostly sign-trackers (n = 10) with 2 goal-trackers. Amphetamine rats were also sign-trackers (n = 9) with 1 goal-tracker and 2 intermediates. While there was an effect of session on CS+ lever activations (Figure 5a; $F(3.202,70.437) = 11.309$, $p < 0.001$, $\varepsilon = 0.213$), there was no effect of treatment ($F(1,22) = 0.226$, $p = 0.639$) or session × treatment interaction ($F(3.202,70.437) = 1.024$, $p = 0.391$). For NormCS+ port entries (Figure 5b), there was no effect of treatment ($F(1,22) = 1.593$, $p = 0.22$), session ($F(2.597,57.13) = 2.49$, $p = 0.078$, $\varepsilon = 0.173$), or session × treatment interaction ($F(2.597,57.13) = 0.332$, $p = 0.773$). While PCA scores (Figure 5c; see Supplementary Figure S1 for its components) shifted towards sign-tracking over 16 sessions ($F(3.028,66.624) = 8.259$, $p < 0.001$, $\varepsilon = 0.202$), there was no effect of treatment ($F(1,22) = 0.332$, $p = 0.57$) or session × treatment interaction ($F(3.028,66.624) = 0.4$, $p = 0.756$).

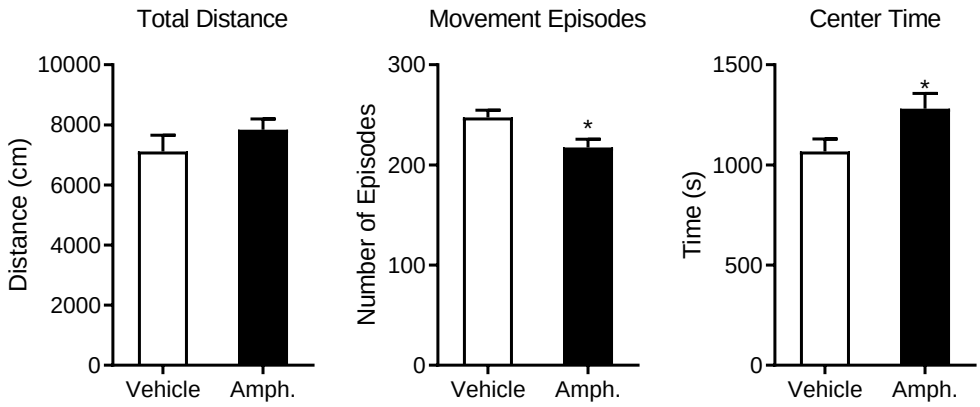

Figure 6. Amphetamine-exposed rats showed evidence of locomotor sensitization. Both vehicle and amphetamine-exposed rats (n = 12/group) received a 0.75 mg/kg amphetamine challenge. (a) Although total distance did not significantly differ between groups during the 30 min test, (b) amphetamine-exposed rats had fewer movement episodes than vehicle rats, suggesting more movement per episode, and (c) spent more time in the center of the arena than vehicle rats. Data are means ± SEM. * $p < 0.05$ for an independent t-test.

Thus, amphetamine exposure had no effect on phenotype over the course of training.

Sensitization test.

After receiving an acute 0.75 mg/kg amphetamine challenge, total distance travelled (Figure 6a) did not differ between vehicle and amphetamine-treated rats ($t(22) = -1.121$, $p = 0.274$). Amphetamine-treated rats had fewer episodes of movement (Figure 6b; $t(22) = 2.783$, $p = 0.011$), and increased center time (Figure 6c; $t(22) = -2.179$, $p = 0.04$), demonstrating locomotor sensitization to amphetamine.

Discussion

We have shown that D1 and D2 antagonists have differential effects after extended PCA training, although locomotor sensitization did not alter the phenotypic shift towards sign-tracking. D1 antagonism using SCH-23390 reduced sign-tracking and goal-tracking, but this was confounded by motor suppressive effects during the ITI and on open field locomotor behavior. The D2 antagonist, eticlopride, selectively reduced goal-tracking in goal-trackers without affecting sign-trackers or intermediates at a dose that did not have significant motor suppressive locomotor effects. These results suggest that dopamine, particularly at D2 receptors, is important for goal-tracking but that these receptors are not required for the expression of a sign-tracking response after extended training.

D1 antagonism using SCH-23390 disrupted both sign-tracking and goal-tracking, however, its effects were confounded by motor suppressive effects. We chose the 0.01 mg/kg dose of SCH-23390 because previous data from our lab showed that it was an effective dose that impaired Pavlovian conditioned responding for an alcohol cue without affecting ITI port entries (Sciascia et al., 2014). Other laboratories have shown this dose disrupted acquisition of sign-tracking (Chow et al., 2016) and expression of sign-tracking (Clark et al., 2013). Although our previous studies did not find effects on ITI port entries (Sciascia et al., 2014) and other laboratories have reported no non-specific effects on food consumption during conditioning (Chow et al., 2016), we found that 0.01 mg/kg SCH-23390 impaired ITI port entries and the number of moves made in an open field locomotor test. While SCH-23390 appeared to reduce sign-tracking in sign-trackers and goal-tracking in goal-trackers, it is difficult to interpret these effects in the presence of these off-target effects.

We found that D2 antagonism using eticlopride was effective at reducing goal-tracking in goal-trackers, but not sign-tracking in sign-trackers at a dose that did not have motor suppressive effects on ITI responses or locomotor behavior. Some studies have found D2 antagonists reduced both sign-tracking and goal-tracking (Fraser et al., 2016; Lopez et al., 2015), which would appear inconsistent with the present findings. However, when administered intermittently prior to training, eticlopride reduced both sign-tracking and goal-tracking responses during training, but left sign-tracking, goal-tracking, and the conditioned reinforcing effects of the CS lever largely intact during a drug-free test (Chow et al., 2016). Chow et al. (2016) suggest that this may be because eticlopride is important for the performance of these behaviors, but not for learning the CS-US association. Consistent with this interpretation and the selective impairment of goal-tracking observed by Roughley and Killcross (2019), the present data shows that D2 antagonism during test disrupts goal-tracking, which is more reliant on the CS-US association, but leaves sign-tracking intact.

We observed a shift to sign-tracking with extended training, replicating previous findings from our laboratory. Previous studies have shown that some rats will shift from goal-tracking to sign-tracking for an alcohol cue after 16 or more training sessions (Srey et al., 2015; Villaruel and Chaudhri, 2016). In the present study, which used a sucrose reinforcer, rats appear to be acquiring a goal-tracking response in sessions 1-4 before PCA scores increase and asymptote around session 10-13. This result suggests a robust shift towards sign-tracking (in our hands) across reinforcers.

Prior amphetamine exposure did not have an effect on the shift towards sign-tracking, despite several previous studies implicating dopamine signalling in the acquisition of sign-tracking. Sign-trackers show greater expression of D1 receptors after the first training session and dopamine signalling remains important for the maintenance of sign-tracking responses (Flagel et al., 2007; Fraser et al., 2016). Moreover, a higher phasic dopamine response was associated with sign-tracking across multiple sessions (Flagel et al., 2011). Previous studies found sensitization augmented CS lever activations (Wyvell and Berridge, 2001), and augmentation may be achieved with a single amphetamine injection (Schuweiler et al., 2018; Wyvell and Berridge, 2000). However, other

studies have shown locomotor sensitization enhanced goal-tracking (Simon et al., 2008). One consideration is these studies used very different protocols, such as inducing sensitization after conditioning (Wyvell and Berridge, 2001) or using food-restricted rats (Simon et al., 2008). However, it has also been shown that the dopamine response to an appetitive CS diminishes with extended training (Clark et al., 2013), suggesting that although dopamine is important for the maintenance of the sign-tracking response (Fraser et al., 2016), additional dopamine signalling may not be required to express a sign-tracking response. Our data suggests that although previous studies have shown the necessity of dopamine for expressing a sign-tracking response, that additional sensitivity to dopamine is not sufficient to enhance the acquisition of sign-tracking.

Our amphetamine exposure regimen produced locomotor sensitization, even if sign-tracking was not affected. We exposed rats to the locomotor chambers and behavioral chambers during amphetamine exposure because sensitization may be context-sensitive (Badiani et al., 1995a,b; Crombag et al., 2001, 2000). Our sensitization test followed previous studies, administering a 0.75 mg/kg amphetamine challenge to all rats (Robinson et al., 2015). Rats previously exposed to amphetamine had fewer movement episodes, suggesting increased movement per episode, and increased center time. While there was no difference in total distance travelled during the sensitization test, total distance travelled during test was similar to the exposure phase. Previous studies have found that differences in locomotor activity emerge later in the sensitization test (Simon et al., 2008), so an effect on total distance travelled may have emerged if our sensitization test was longer. The effect of amphetamine on two of three locomotor activity measures during the sensitization test therefore suggests amphetamine induced locomotor sensitization, but this did not alter the acquisition of sign-tracking behavior.

Conclusion

We found that a commonly used dose of SCH-23390 may have motor suppressive effects at a commonly used dose, necessitating caution in interpreting these results and further studies using D1 antagonists that avoid these confounds. We also found that the D2 antagonism selectively reduced goal-tracking in goal-trackers after extended training, a finding that is consistent with some previous findings (Chow et al., 2016; Roughley and Killcross, 2019) and the view that D2 receptors are required for expressing behaviors related to the CS-US association. While we successfully induced locomotor sensitization, this did not facilitate the acquisition of sign-tracking. Further studies are required to investigate the neurobiological mechanisms underlying the acquisition of sign-tracking following extended Pavlovian conditioned approach training.

Declarations

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Conflict of Interest Declaration

The authors declare no conflict of interest.

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