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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 important for incentive salience attribution, where motivational value is assigned to conditioned cues that predict appetitive reinforcers. However, the role of dopamine in this process may change with extended training. *Objectives*. We tested the effects of dopamine D1-like and D2-like receptor antagonism on the expression of sign-tracking and goal-tracking conditioned responses following extended Pavlovian conditioned approach (PCA) training. We also tested if amphetamine sensitization would accelerate the phenotypic shift to sign-tracking that is observed with extended training. *Methods*. In experiment 1, 24 male Long-Evans rats received 20 PCA sessions in which one lever (CS+, 10 s) predicted 0.2 mL sucrose (10%, w/v) delivery and the other lever (CS−) did not. SCH-23390 (D1-like antagonist) or eticlopride (D2-like antagonist) were administered before non-reinforced behavioural tests at doses of 0, 0.01, and 0.1 mg/kg (s.c.). In experiment 2, 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 received 16 PCA training sessions. *Results*. Both doses of SCH-23390 reduced sign- and goal-tracking, but also reduced locomotor behaviour. A low dose of eticlopride (0.01 mg/kg) selectively reduced goal-tracking, without affecting sign-tracking or locomotor behaviour. Amphetamine sensitization did not affect the acquisition of sign- or goal-tracking. *Conclusions*. Following extended PCA training, dopamine D2-like receptor activity is required for the expression of goal-tracking but not sign-tracking. Amphetamine sensitization did not impact incentive salience attribution; however, more selective manipulations of the dopamine system may be needed.

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

Introduction

Environmental cues that predict appetitive reinforcers can acquire incentive salience, whereby motivational value is assigned to the cue Flagel et al. (2007, 2011); Chow et al. (2016); Fraser and Janak (2017); Berridge (2007). Incentive salience has been studied using a Pavlovian conditioned approach (PCA) or 'autoshaping' task, where the brief insertion and then retraction of a lever conditioned stimulus (CS) is paired with an appetitive, unconditioned stimulus (US). When the CS is presented, sign-tracking animals preferentially approach and interact with the CS, while goal-tracking animals preferentially approach the location where the US will be presented Berridge and Robinson (2003). Both sign- and goal-trackers learn the CS-US association, but only sign-trackers assign motivational value to the CS.

While robust sign- and goal-tracking phenotypes emerge rapidly in subsets of rats Meyer et al. (2012); Robinson and Berridge (2008), we have reported that extended PCA training can produce a shift from goal-tracking to sign-tracking. In one study, goal-tracking responses to an alcohol-associated CS in rats peaked between sessions 7 and 10 of PCA training before decreasing, while the number of sign-tracking responses continued to increase over 27 PCA sessions Srey et al. (2015). In a subsequent, large-scale analysis of data from 5 different experiments, this shift from goal- to sign-tracking behaviour emerged as a significant behavioural phenotype Villaruel and Chaudhri (2016). However, it is currently unclear which neural systems could play a role in mediating this shift from goal- to sign-tracking behaviour with extended PCA training.

Dopamine signalling has been heavily implicated in the acquisition and expression of incentive salience in studies us-

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 bred to respond to novelty	CS+, 7 sessions, 25 trials/session, food reinforcer	D1&D2: Flupenthixol before training DA: Fast scan cyclic voltammetry	Flupenthixol prevented expression of sign and goal-tracking. Only acquisition of sign-tracking was blocked.	Flagel et al. (2011)
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)

ing the PCA task (see Table 1). Systemic injections of a non-selective dopamine antagonist during PCA training impaired the expression of both sign-tracking and goal-tracking, while blocking the acquisition of sign-tracking but not goal-tracking Flagel et al. (2011). Moreover, several studies suggest that dopamine signalling at D1-like receptors (D1 and D5; hereafter, D1) in the nucleus accumbens core is particularly important in the attribution of incentive salience to an appetitive CS Flagel et al. (2011); Chow et al. (2016); Roughley and Killcross (2019); Clark et al. (2013); Dalley et al. (2005); Saunders and Robinson (2012).

However, it has also been shown that extended PCA training can reduce the extent to which sign-tracking relies on dopamine signalling. Sign-trackers show greater expression of D1 receptor mRNA during the first session of PCA training than goal-trackers, while goal-trackers exhibit greater tyrosine hydroxylase, dopamine transporter and D2 receptor mRNA expression than sign-trackers in later sessions Flagel et al.

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
Behavioural 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

(2007). Moreover, measurement of CS-elicited dopamine release over the course of extended training has revealed that, in sign-trackers, dopamine release peaks early in training and then diminishes Clark et al. (2013). This change in dopamine signalling over time may explain why administering eticlopride during a 14-day PCA protocol 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.

Based on this literature, we sought to examine the involvement of dopamine D1-like and D2-like receptors in the expression of sign- and goal-tracking behaviour in rats that had received extended PCA training. Specifically, in Experiment 1 we gave rats extended PCA training and classified them as sign-trackers, goal-trackers or intermediates using criteria developed by Meyer et al. (2012). We then tested the dose-dependent effects of the selective D1-like and D2-like receptor antagonists, SCH-23390 and eticlopride, on the expression of sign- and goal-tracking behaviour to verify whether the dopamine system was necessary for the expression of incentive salience after extended PCA training. As an additional behavioural control, we examined the effects of these antagonists on locomotor activity tested in an open field arena.

A second, parallel objective was to determine if amphetamine sensitization would accelerate the shift from goal-tracking to sign-tracking. The sensitization protocol involves exposing animals to a psychostimulant, such as amphetamine, over a period of time, which produces long-lasting changes in dopamine signalling and renders animals more sensitive to dopaminergic manipulations Downs and Eddy (1932); Kalivas and Stewart (1991); Segal and Mandell (1974). Prior studies have examined the influence of amphetamine sensitization on sign- and goal-tracking. Some found enhanced sign-tracking responses Wyvell and Berridge (2001); Robinson et al. (2015) 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, protocols differed in whether sensitization was induced before or after Pavlovian conditioning and animals variously received 7, 8 or 14 PCA sessions in different studies Wyvell and Berridge (2001); Robinson et al. (2015); Simon et al. (2008). Nonetheless, these studies suggest that amphetamine sensitization has the potential to accelerate a shift from goal-tracking to sign-tracking.

In Experiment 2, we therefore examined the effect of am-

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 Behaviour	
Distance Travelled	Sum of the coordinate changes during the session (cm) in the floor plane.
Movement Episodes	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.

phetamine sensitization on the acquisition of sign-tracking over time. Prior to PCA training, rats received a series of vehicle or amphetamine injections. We hypothesised that, over the course of 16 PCA sessions, rats that had previously received amphetamine injections would shift towards sign-tracking more rapidly than control animals.

Materials and 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 nylon bone chew toy, a plastic 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 acclimated to the colony room over at least 3 days before being singly-housed and handled for 7 days. Rats had free access to food and water in their home-cages throughout the experiments. All procedures were approved by the Animal Research Ethics Committee at Concordia University and accorded with guidelines from the Canadian Council on Animal Care. See Table 2 for details of animals, reagents, and equipment.

Apparatus

Behavioural training was conducted using 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 with infrared detectors 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 on either side of the port served as conditioning stimuli. A PC running Med-PC IV controlled stimulus presentations and recorded responses. For locomotor behaviour, we used four 39 x 42 x 50 cm open field arenas (Coulbourn Instruments) housed in sound attenuating boxes and Tru Scan 2.0.

Experiment 1: Effect of dopamine receptor antagonism on the expression of sign- and goal-tracking

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

Habituation.
Rats were then habituated to the conditioning chambers. On day 1, they were exposed to the testing room for 20 min where they were handled and weighed. On day 2, rats were placed in the conditioning chambers, where after a programmed 2 min delay the houselights were switched on for a 20 min session in which port entries were counted, but had no programmed consequences.

Pavlovian conditioned approach.
Rats then underwent 20 PCA sessions. Each session began with a 2 min delay, after which the houselight was switched on to indicate session onset. Each session involved 20 trials, with 10 CS+ trials (paired with sucrose) 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, while the other was the CS– lever. Levers were counterbalanced so that for half of the rats the CS+ lever was on the left of the fluid port and for the remainder 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 (i.e., immediately after retraction of the CS+ lever) to deliver 0.2 mL of sucrose into the fluid port for oral consumption. The inter-trial intervals (ITI), which did not include the Pre-CS, CS, or Post-CS intervals, were set at 60, 120, or 180 s (mean ITI duration = 120 s). The ITI durations and order of CS+ and CS– trials were randomized. For each session, a 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 Meyer et al. (2012); Ahrens et al. (2016).

Dopamine antagonist tests.
We tested the effect of dopamine receptor antagonists on the expression of conditioned responding, with dose order counterbalanced using a, within-subjects, Latin square design. First, to test the effect of the D1 antagonist SCH-23390, rats received an injection of saline vehicle, 0.01 mg/kg, or 0.1 mg/kg SCH-23390 (1 mL/kg, s.c.) 15 min before a test session. Test sessions were identical to training sessions, but no syringes were placed on the pump and no sucrose was delivered. Rats received at least one day of PCA training between tests to allow them to return to baseline levels of responding. After test-

ing SCH-23390, the same 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 is a behaviourally effective dose for SCH-23390 or eticlopride Chow et al. (2016); Sciascia et al. (2014) and in a pilot test conducted in the same rats we found that a lower dose (0.001 mg/kg SCH-23390 or eticlopride) had no effect on sign- or goal-tracking.

Locomotor activity test in an open field arena.
In the same rats, we examined the effects of SCH 23390 and eticlopride on locomotor behaviour in an open field arena (see Table 3 for definition of variables). On day 1, rats were exposed to the open field arena 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 a test in which we measured locomotor activity in the open field arena (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 a locomotor activity test.

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

Amphetamine exposure.
We gave rats repeated exposure to amphetamine in order to induce psychomotor sensitization. Since amphetamine sensitization can be context-specific Crombag et al. (2000, 2001); Badiani et al. (1995b,a), we exposed rats to both the open field arena as well as the conditioning chamber after each amphetamine injection. On day 1, all rats (n = 24) were habituated to the procedure with a saline injection (1 mL/kg, i.p.) immediately before placement into an open field arena for 30 min, followed by placement into a conditioning chamber for 20 min. When in the conditioning chamber, entries into the fluid port were recorded, but no stimuli were presented. Rats were then randomly allocated (n = 12 /group) to receive saline vehicle (1 mL/kg) or amphetamine (2 mg/kg, 1 mL/kg, i.p.) for the next 7 consecutive days, which were otherwise identical to day 1. Doses were based on previous studies Robinson et al. (2015).

Incubation of amphetamine sensitization and home-cage sucrose exposure.
For the first 7 days after amphetamine or vehicle exposure, rats were left undisturbed in their home cages except for normal husbandry activities. On days 8-10, rats received 48 h of home-cage sucrose exposure, as described in Experiment 1.

Pavlovian conditioned approach.
Behavioural training began on the 11th day after the last amphetamine/vehicle injection. Rats received 14 PCA sessions in the conditioning chambers using procedures that were identical to those described for Experiment 1.

Sensitization test.
After PCA training, rats were tested for amphetamine sensitization. All rats received a 0.75 mg/kg, 1 mL/kg, i.p. amphetamine challenge Robinson et al. (2015) immediately before a 30 min locomotor activity test in an open field arena.

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 available on Figshare.

Results

Experiment 1: Effect of dopamine receptor antagonism on the expression of sign- and goal-tracking

Following the acquisition of PCA, we identified the behavioural phenotypes of all rats based on PCA scores averaged across sessions 19 and 20 Villaruel and Chaudhri (2016). Fourteen rats were identified as sign-trackers, 6 as goal-trackers and 4 as intermediates. Conditioned responses obtained across 20 PCA sessions in these rats are shown in Figure S1.

SCH-23390.
Systemic administration of a dopamine D1-like receptor antagonist following extended PCA training reduced the expression of sign-tracking in sign-trackers and intermediates, reduced the expression of goal-tracking in goal-trackers, and reduced ITI port entries in all phenotypes. CS+ lever activations (Figure 1a) was our behavioural index of sign-tracking. CS+ lever activations differed by phenotype [$F(2,21) = 5.09$, $p = 0.016$] and dose [$F(1.347,28.294) = 20.441$, $p < 0.001$, $\varepsilon = 0.674$] with a significant interaction [dose × phenotype, $F(2.695,28.294) = 3.154$, $p = 0.045$]. In sign-trackers, both 0.01 and 0.1 mg/kg of SCH-23390 reduced CS+ lever activations compared to vehicle (p's < 0.001) and 0.1 mg/kg of SCH 23390 further reduced CS+ lever activations relative to 0.01 mg/kg SCH-23390 ($p = 0.001$). In intermediates, SCH-23390 reduced 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. There were no differences between doses for goal-trackers (p's ≥ 0.914). ΔCS+ port entries (Figure 1b) was our behavioural index of goal-tracking. This measure considers differences in baseline levels of port entry behaviour by subtracting PreCS+ port entries from CS+ port entries Panayi and Killcross (2018); Khoo et al. (2019); Chaudhri et al. (2013). ΔCS+ port entries varied according to SCH-23390 dose [$F(1.271,26.687) = 9.961$, $p = 0.002$, $\varepsilon = 0.635$] and 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 ΔCS+ 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 ($p = 0.006$). ΔCS+ port entries in sign-trackers and intermediates was not significantly affected by SCH 23390. SCH-23390 dose-dependently reduced ITI port entries (Figure 1c). 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 [$F(2.467,25.899) = 0.686$, $p = 0.542$]. 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.
Systemic administration of a low dose of a dopamine D2-like receptor antagonist (0.01 mg/kg) following extended PCA training reduced the expression of goal-tracking in goal-trackers, without affecting sign-tracking or ITI port entries. However, at a higher dose (0.1 mg/kg), dopamine D2-like receptor antagonism reduced sign-tracking, goal-tracking and ITI port entries. CS+ lever activations (Figure 1d) differed by dose [$F(2,42) = 9.982$, $p < 0.001$] and phenotype ($F(2,21) = 4.061$, $p = 0.032$) with a significant dose × phenotype interaction [$F(4,42) = 2.779$, $p = 0.039$]. A dose of 0.1 mg/kg of eticlopride re-

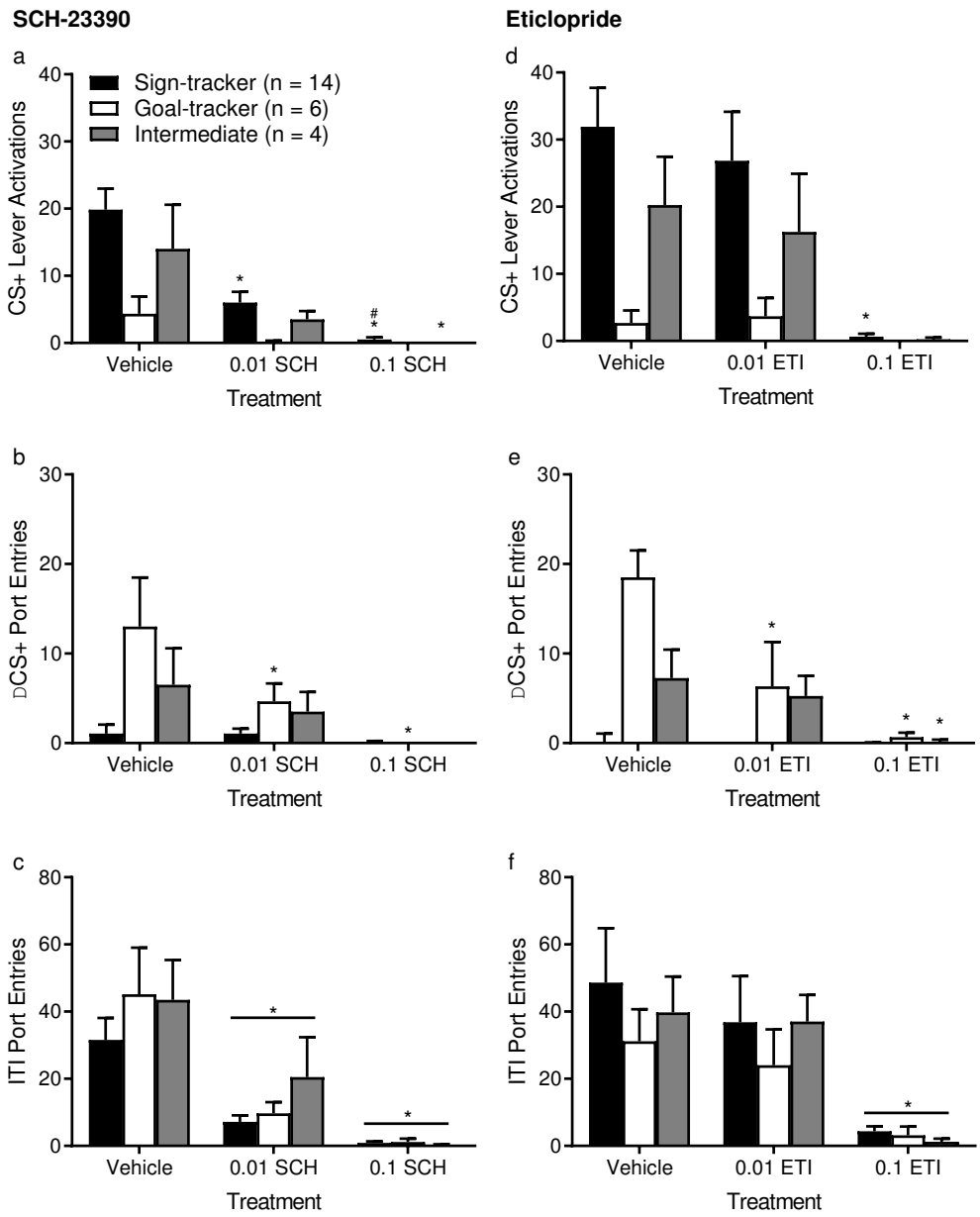

Figure 1. Antagonism of dopamine D1-like receptors reduced goal-tracking, sign-tracking and port entries during the intertrial interval (ITI), while dopamine D2-like receptor antagonism selectively reduced goal-tracking by goal-trackers. (a) The selective dopamine D1 receptor antagonist, SCH-23390, dose-dependently reduced CS+ lever activations 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 dose-dependently reduced ΔCS+ port entries (CS+ port entries – Pre-CS+ port entries) in goal-trackers (n = 6). (c) SCH-23390 dose-dependently reduced ITI port entries across phenotypes. (d) The selective dopamine D2 receptor antagonist, eticlopride, reduced CS+ lever activations by sign-trackers at a dose of 0.1 mg/kg. (e) Eticlopride significantly reduced ΔCS+ port entries made by goal-trackers at both doses and reduced ΔCS+ 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 reduced ITI port entries across phenotypes 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.

duced CS+ lever activations in sign-trackers (0.1 mg/kg vs vehicle, $p < 0.001$; 0.1 mg/kg vs 0.01 mg/kg $p = 0.001$). However, 0.01 mg/kg of eticlopride had no effect on CS+ lever activations in sign-trackers, goal-trackers or intermediates (all p's = 1). ΔCS+ port entries (Figure 1e) were affected by eticlopride dose [$F(2,42) = 13.676$, $p < 0.001$] and phenotype [$F(2,21) = 12.799$, $p < 0.001$], with a significant dose × phenotype interaction [$F(4,42) = 7.104$, $p < 0.001$]. Compared to vehicle, ΔCS+ port entries were significantly lower in goal-trackers following 0.01 mg/kg ($p = 0.005$) or 0.1 mg/kg ($p < 0.001$) eticlopride, with no further reduction from 0.1 mg/kg to 0.01 mg/kg ($p = 0.216$). Intermediates made fewer ΔCS+ port entries following 0.1 mg/kg eticlopride compared to vehicle ($p = 0.026$), but not 0.01 mg/kg ($p = 0.445$). Eticlopride reduced ITI port entries (Figure 1f), but only at the highest tested dose. 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 ($p = 0.023$).

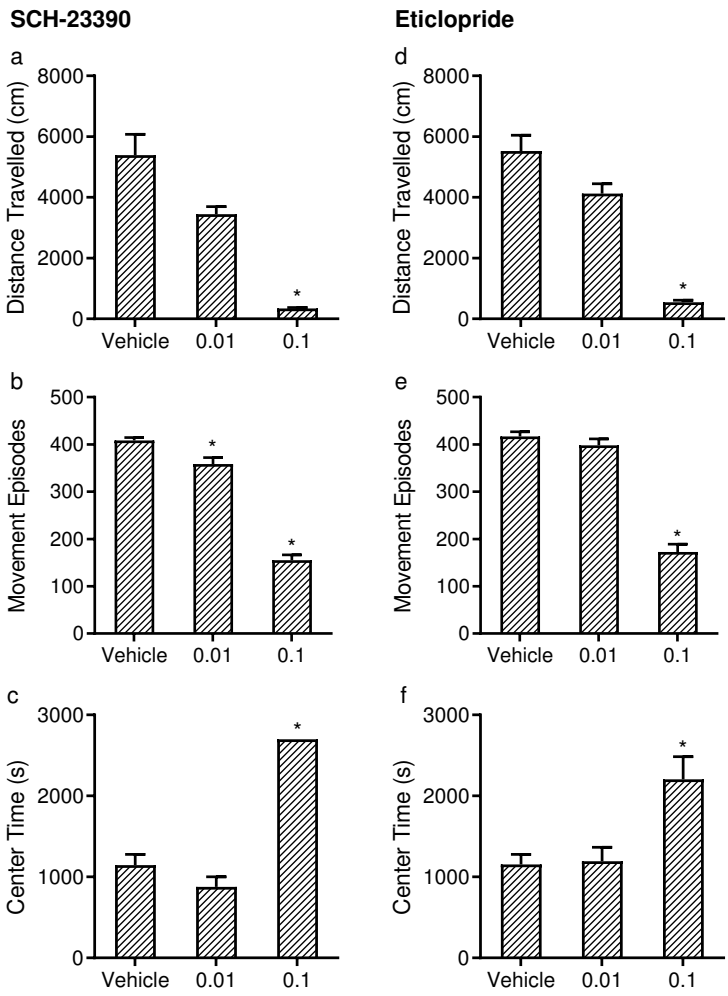

Figure 2. A reduction in locomotor behaviour by dopamine receptor antagonists. (a) Rats received vehicle, 0.01, or 0.1 mg/kg of SCH-23390 prior to a 45 min locomotor test in an open field arena (n = 8/dose). Total distance travelled was significantly reduced at the 0.1 mg/kg but not 0.01 mg/kg dose. (b) The total number of movement episodes 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 open field 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 mg/kg but not 0.01 mg/kg dose. (e) Similarly, eticlopride reduced the total number of movement episodes at the 0.1 mg/kg 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.

Locomotor activity test in an open field arena.

Compared to vehicle, SCH-23390 significantly reduced locomotor activity at both the high (0.1 mg/kg) and low (0.01 mg/kg) dose (n = 8/dose). A Kruskal-Wallace test found an effect of dose [H(2) = 18.24, $p < 0.001$] on total distance travelled (Figure 2a). SCH-23390 reduced total distance travelled at 0.1 mg/kg ($p < 0.001$ 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 2b; one-way ANOVA, F(2,21) = 154.939, $p < 0.001$]. The number of movement episodes was reduced, 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 movement episodes compared to 0.01 mg/kg ($p < 0.001$). SCH-23390 also increased time spent in the center of the open field arena [Figure 2c; H(2) = 16.819, $p < 0.001$]. The 0.1 mg/kg dose significantly increased center 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). Compared to vehicle, the high but not low dose of eticlopride reduced locomotor activity (n = 8/dose). Eticlopride reduced total distance travelled (Figure 2d; 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 not at 0.01 mg/kg ($p = 0.413$ vs vehicle). Eticlopride also reduced movement episodes (Figure 2e; one-way ANOVA, F(2,21) = 100.467, $p < 0.001$) at the 0.1 mg/kg dose ($p < 0.001$ vs vehicle and 0.01 mg/kg), but not at 0.01 mg/kg ($p = 0.999$ vs vehicle). Finally, eticlopride increased center time (Figure 2f; F(2,21) = 8.63, $p = 0.002$) at the 0.1 mg/kg 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).

Effect of amphetamine sensitization on the acquisition of sign- and goal-tracking

Amphetamine exposure.

We conducted amphetamine sensitization by giving rats either vehicle or 2 mg/kg amphetamine (n = 12/group) before exposure to the open field arena for 30 minutes (Figures 3a-c), followed immediately by placement into the conditioning chambers for 20 minutes (Figure 3d). During an initial, drug-free habituation session, there were no significant differences between groups in total distance travelled [Figure 3a; t(22) = -1.238, $p = 0.229$], number of movement episodes [Figure 3b; t(22) = -0.565, $p = 0.578$], center time [Figure 3c; t(22) = -

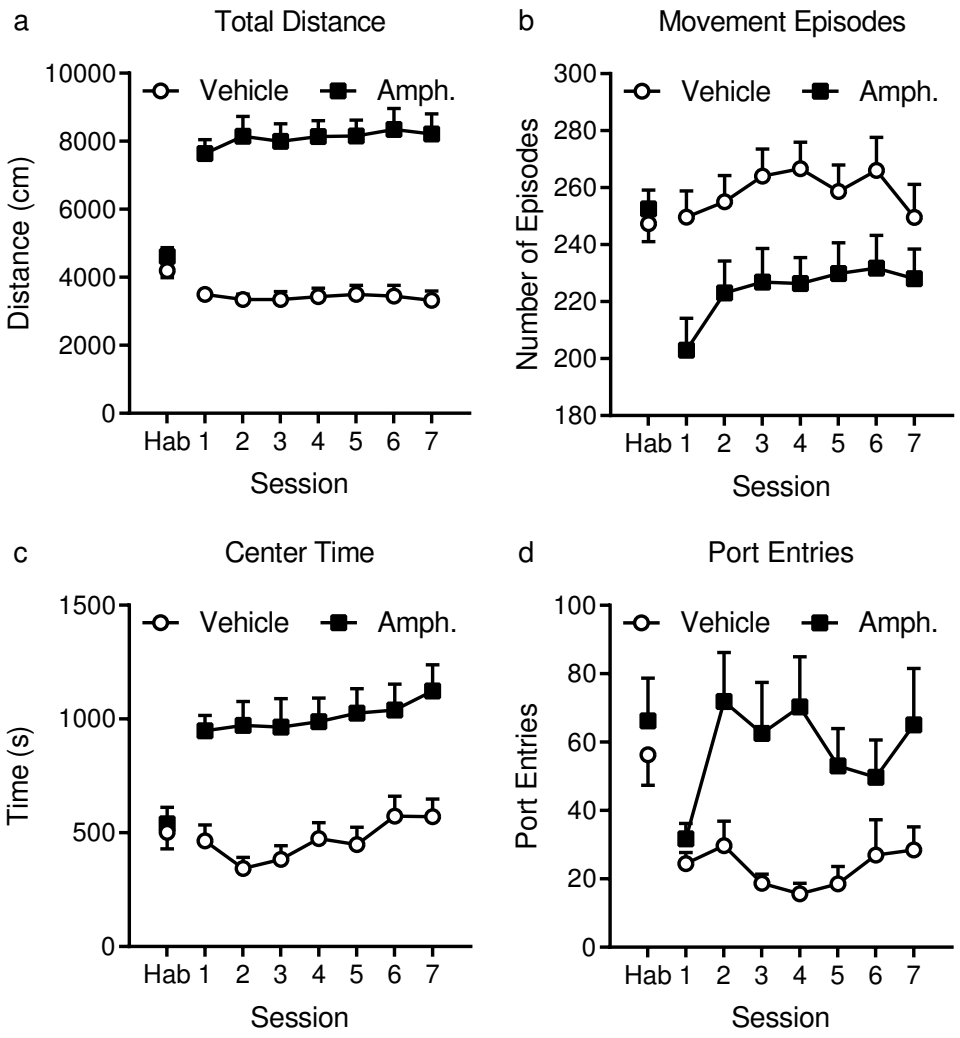

Figure 3. Amphetamine exposure during the induction of sensitization increased locomotor acitivty. (a) Total distance travelled in 30 min was not significantly different between vehicle and amphetamine rats (n = 12/group) during a habituation session when all rats received saline. However, daily, 2 mg/kg amphetamine injections significantly increased total distance travelled. (b) Similarly, the number of movement episodes was not different during habituation, but was significantly reduced by amphetamine. (c) The amount of center time was not different between vehicle and amphetamine rats during habituation, but was increased by amphetamine. (d) Rats were immediately transferred to conditioning 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 the amphetamine exposure phase. Data are means ± SEM.

0.365, p = 0.719], or port entries [Figure 3d; equal variances not assumed; t(19.888) = -0.646, p = 0.526]. Amphetamine treatment increased the total distance travelled [Figure 3a; 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, ε= 0.585] or session × treatment interaction [F(3.51,77.214) = 0.635, p = 0.619]. Amphetamine treatment decreased the number of movement episodes [Figure 3b; 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, ε= 0.514], or session × treatment interaction [F(3.083,67.816) = 0.584, p = 0.632]. Amphetamine treatment increased the amount of center time [Figure 3c; F(1,22) = 24.693, p < 0.001]. There was an effect of session [F(3.615,79.527) = 3.582, p = 0.012, ε= 0.602], but no differences between specific sessions or session × treatment interaction [F(3.615,79.527) = 0.655, p = 0.61]. Finally, amphetamine treatment elevated the number of port entries made during the 20 min session [Figure 3d; F(1,22) = 11.878, p = 0.002] in the conditioning chamber, but there was no effect of session [F(3.442,75.716) = 1.801, p = 0.147, ε= 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 mostly sign-trackers (n = 9) with 1 goal-tracker and 2 intermediates. CS+ lever activations increased as a function of session [Figure 4a; F(3.202,70.437) = 11.309, p < 0.001, ε= 0.213], but there was no effect of prior amphetamine exposure on the acquisition of this behaviour [treatment: F(1,22) = 0.226, p = 0.639]. There was also no session × treatment interaction (F(3.202,70.437) = 1.024, p = 0.391). For ΔCS+ port entries (Figure 4b), 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, ε= 0.173), or session × treatment interaction (F(2.597,57.13) = 0.332, p = 0.773). While PCA scores (Figure 4c; see Figure S2 for its components) shifted towards sign-tracking over 16 sessions (F(3.028,66.624) = 8.259, p < 0.001,

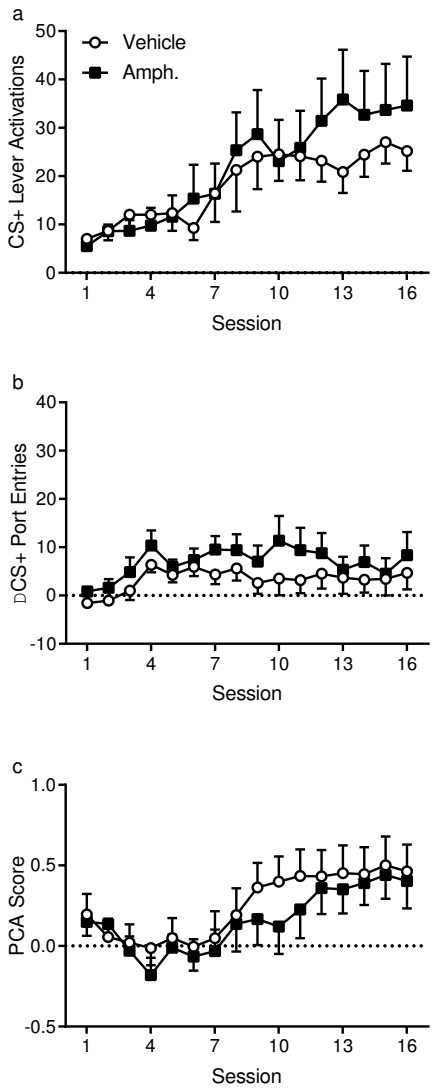

Figure 4. Acquisition of sign-tracking and goal-tracking did not differ between vehicle and amphetamine-exposed rats. (a) CS+ lever activations did not differ between rats that received vehicle or 2 mg/kg amphetamine during the amphetamine exposure phase (n = 12/group). (b) ΔCS+ port entries (CS+ port entries – Pre-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. Data are means ± SEM.

ε= 0.202), there was no effect of treatment ($F(1,22) = 0.332$, $p = 0.57$) or session × treatment interaction ($F(3.028,66.624) = 0.4$, $p = 0.756$). Thus, contrary to our hypothesis, prior, repeated amphetamine exposure had no effect on the development of sign-tracking and goal-tracking phenotypes over the course of training.

Sensitization test.

After receiving an acute 0.75 mg/kg amphetamine challenge, total distance travelled (Figure 5a) did not differ between vehicle and amphetamine-treated rats ($t(22) = -1.121$, $p = 0.274$). However, amphetamine-treated rats initiated fewer movement episodes (Figure 5b; $t(22) = 2.783$, $p = 0.011$), and had increased center time (Figure 5c; $t(22) = -2.179$, $p = 0.04$), demonstrating amphetamine sensitization.

Discussion

We found that antagonism of dopamine D1-like receptors using SCH 23390 following 20 sessions of PCA training reduced the expression of sign-tracking and goal-tracking conditioned responses. However, SCH 23390 also reduced ITI port entries and locomotor activity in an open field arena. The D2-like receptor antagonist, eticlopride, reduced sign- and goal-tracking behaviour, ITI port entries and locomotor activity at the 0.1 mg/kg dose. However, 0.01 mbg/kg of eticlopride selectively reduced goal-tracking behaviour in goal-trackers, without affecting sign-trackers or intermediates, and this dose did not reduce ITI port entries or locomotor activity in an open field arena. We also found that repeated amphetamine pretreatment did not alter the subsequent acquisition of sign-tracking behaviour. These results suggest that after extended PCA training, dopaminergic activity at D2 receptors is important for the expression of goal-tracking, but not for the expression of sign-tracking.

Following extended PCA training in Experiment 1, D1 antagonism using SCH-23390 disrupted both sign-tracking and goal-tracking; however, the interpretation of these effects is confounded by data suggesting that SCH 23390 pre-treatment produced a motor suppressive effect. We chose the 0.01 mg/kg dose of SCH-23390 because this dose impaired Pavlovian conditioned responding elicited by an auditory alcohol-predictive CS without affecting ITI port entries Sciascia et al. (2014); Sparks et al. (2014). Other laboratories have shown that this dose disrupted the acquisition of sign-tracking Chow et al. (2016) and expression of sign-tracking Clark et al. (2013). Moreover, 0.01 mg/kg SCH-23390 has been shown to increase cocaine self-administration Koob et al. (1987), reduce saccharin seeking Aoyama et al. (2016) and alter risky decision-making in rats Smith et al. (2018). Although our previous studies did not find effects of SCH-23390 on ITI port entries Sciascia et al. (2014) and other laboratories have reported no effect of SCH-23390 on food consumption during Pavlovian conditioning Chow et al. (2016) or on Pre-CS locomotor activity Palmatier et al. (2014), we found that 0.01 mg/kg SCH-23390 impaired ITI port entries and the number of movement episodes in a locomotor activity test. This dose is even lower than doses that produced non-specific effects reported by Smith et al. (2018), who observed rats stopped eating pellets after 0.017 and 0.03 mg/kg. 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. There may be effects that are specific to sign-tracking or goal-tracking, but further studies are required using lower doses of a D1 antagonist that do not produce motor suppressive effects.

We found that following extended PCA training, D2 antagonism using eticlopride reduced goal-tracking in goal-trackers, but not sign-tracking in sign-trackers, at a dose that did not affect ITI responses or locomotor behaviour in an open field arena. Some studies have found that D2 antagonists reduced both sign-tracking and goal-tracking Lopez et al. (2015); Fraser et al. (2016), which would appear inconsistent with the present findings. However, when administered intermittently prior to training, eticlopride reduced both sign-tracking and goal-tracking responses, but left sign-tracking, goal-tracking, and the conditioned reinforcing effects of the CS lever largely intact during drug-free tests Chow et al. (2016). Chow et al. (2016) suggest that this may be because D2-like receptors are important for the performance of these behaviours, but not for learning the CS-US association. Consistent with this interpretation Roughley and Killcross (2019) observed an impairment of sign-tracking, but not goal-tracking, in drug-free sessions that followed one week of daily pre-training eticlopride. The

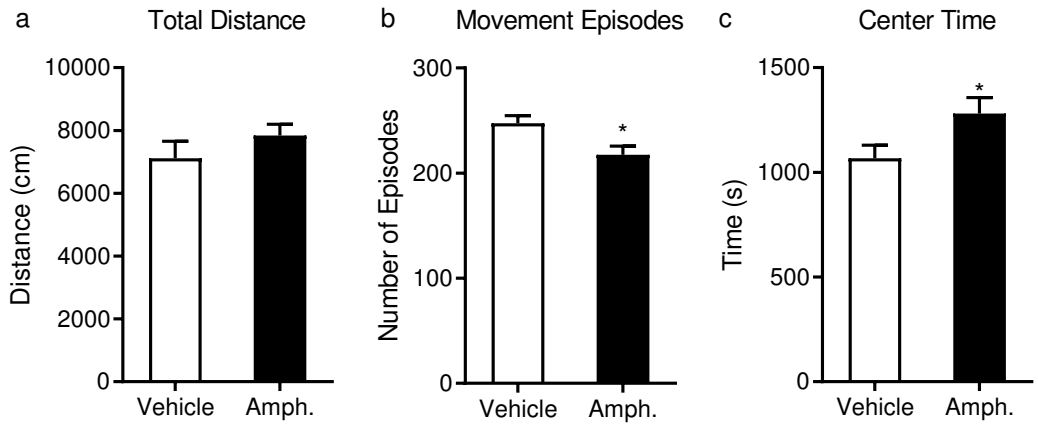

Figure 5. Amphetamine-exposed rats showed evidence of sensitization. Both vehicle and amphetamine-exposed rats (n = 12/group) received a 0.75 mg/kg amphetamine challenge dose before a locomotor activity test in an open field arena. (a) Although total distance did not significantly differ between groups 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 open field arena than vehicle rats. Data are means ± SEM. * $p < 0.05$ for an independent t-test.

present data show that D2 antagonism at test disrupts goal-tracking, which is entirely reliant on the CS-US association but leaves sign-tracking, which involves phasic dopamine responses to the CS rather than the US Flagel et al. (2011), intact. Moreover, it is consistent with previous studies showing that antipsychotic treatment did not affect the expression of established sign-tracking Bédard et al. (2011).

One limitation of our design is that we always tested rats with eticlopride after they had received tests with SCH-23390. We did all of our tests within-subjects to minimise the number of animals used and maximise statistical power, however, if administration of SCH-23390 had long-term effects on PCA behaviour it could confound the eticlopride results. However, we did provide a day of normal PCA training between drug tests to allow rats to return to baseline and dopamine supersensitivity is typically associated with chronic, continuous administration of dopamine antagonists in animals Servonnet and Samaha (2020). There was also no significant difference in performance in the vehicle tests for SCH-23390 and eticlopride with respect to CS+ or CS− lever activations, ΔCS+ or ΔCS− port entries and ITI port entries (paired t-tests, $p > 0.05$). Therefore, a confound from drug testing order is not obvious in our data, but remains a possibility.

In Experiment 2, we observed a shift from goal-tracking to sign-tracking with extended PCA training. This finding replicates our previous studies, where some rats shifted from goal-tracking to sign-tracking with an alcohol cue after 16 or more PCA sessions Srey et al. (2015); Villaruel and Chaudhri (2016). In the present study, which used a sucrose reinforcer, rats appeared to acquire a goal-tracking response in sessions 1-4 before PCA scores increased and asymptote around session 10-13. This result suggests a robust shift towards sign-tracking (in our hands) across reinforcers.

Contrary to our expectations, prior amphetamine exposure did not affect 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 that amphetamine 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 that amphetamine 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.

Even though the acquisition of sign-tracking was not affected, our amphetamine exposure regimen produced sensitization. We exposed rats to first the open field arena and then to the conditioning chambers during the amphetamine exposure regimen because sensitization may be context-sensitive Crombag et al. (2000, 2001); Badiani et al. (1995b,a). Our sensitization test followed previous studies, administering a 0.75 mg/kg amphetamine challenge to all rats Robinson et al. (2015). Rats that were previously exposed to amphetamine had fewer movement episodes, suggesting increased movement per episode, and increased center time, consistent with anxiolytic effects. While there was no difference in total distance travelled during the sensitization test, total distance travelled at 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 that repeated amphetamine exposure induced locomotor sensitization, but that this did not alter the acquisition of sign-tracking behaviour.

It is possible that other methods for intervening in the dopamine system could still alter the acquisition of incentive salience. For example, it has recently been shown that inhibition of prelimbic cortex projections to the paraventricular nucleus of the thalamus can shift goal-trackers towards sign-

tracking Campus et al. (2019). It is therefore possible that more temporally or anatomically precise dopamine manipulations using opto- or chemogenetics may facilitate a shift in incentive salience acquisition. Nonetheless, our data suggest that increasing the overall sensitivity of the dopamine system through repeated psychostimulant administration does not predispose animals towards incentive salience acquisition.

Conclusion

The present studies sought to evaluate the role of the dopamine system in incentive salience attribution resulting from extended PCA training. Antagonism of dopamine D1-like receptors reduced the expression of both sign- and goal-tracking conditioned responses, but these results should be interpreted with caution in the face of reduced ITI port entries and general locomotor behaviour. Interestingly, dopamine D2-like receptor signally was specifically required for the expression of goal-tracking in goal-trackers, a finding that is consistent with several previous studies Chow et al. (2016); Roughley and Killcross (2019); Bédard et al. (2011). A second, parallel objective was to investigate whether the dopamine system is involved in the shift from goal- to sign-tracking behaviour resulting from extended PCA training. Although we observed amphetamine sensitization, it did not affect the rate at which rats shifted from goal-tracking to sign-tracking. However, it is possible that more temporally or anatomically precise dopaminergic manipulations could alter the trajectory of sign- and goal-tracking across extended PCA training. Further studies are therefore required to investigate the neurobiological mechanisms underlying the acquisition of sign-tracking following extended PCA training.

Declarations

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Conflict of Interest Declaration

The authors declare no conflict of interest.

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

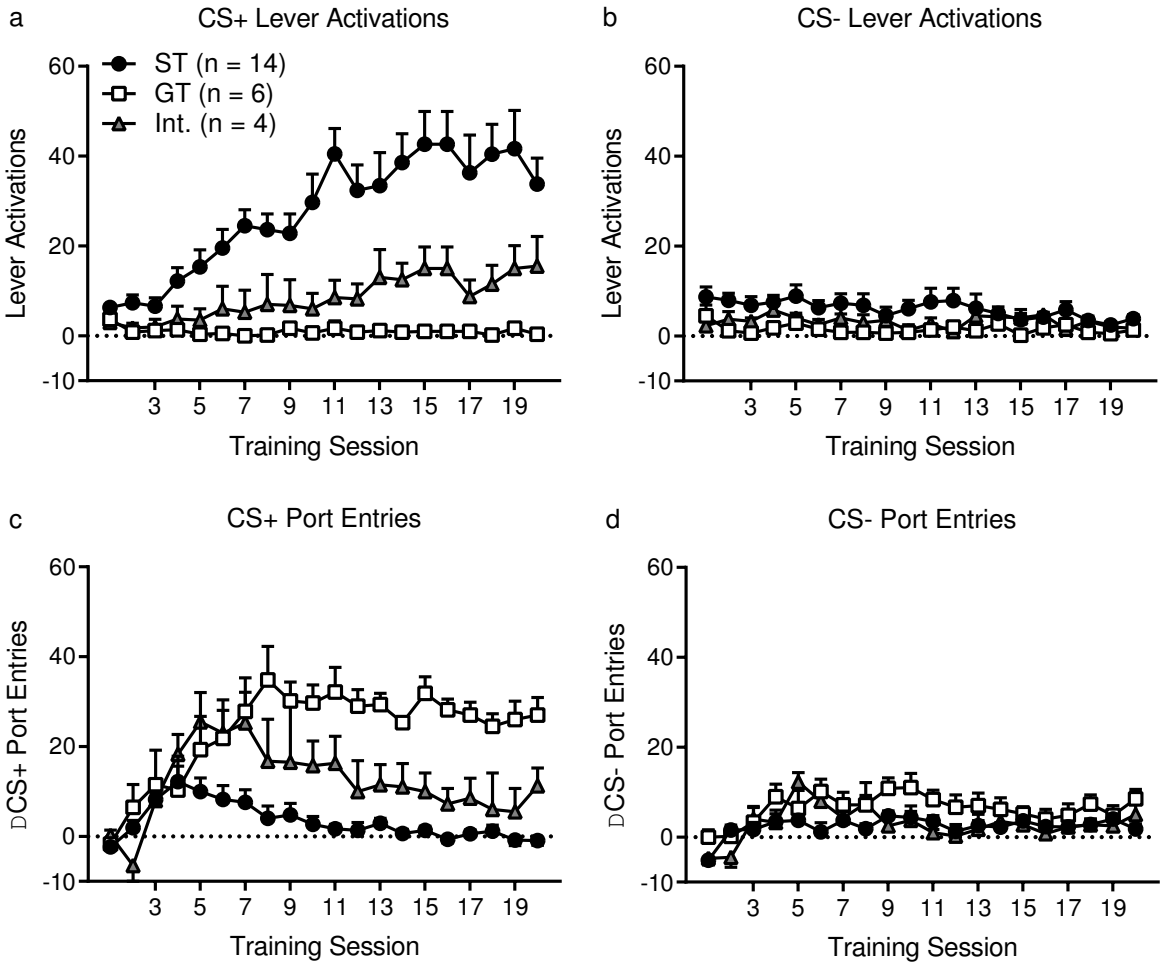

Figure S1. Sign-trackers and goal-trackers acquired distinct behaviours during Pavlovian PCA training sessions. (a) Rats classified as sign-trackers (ST; n = 14) interacted most with a tactile lever cue that predicted 10% sucrose delivery (CS+), while goal-trackers (GT; n = 6) and intermediates (Int.; 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) ΔCS+ port entries (CS+ port entries minus Pre-CS+ port entries) were highest in goal-trackers. (d) ΔCS− 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.

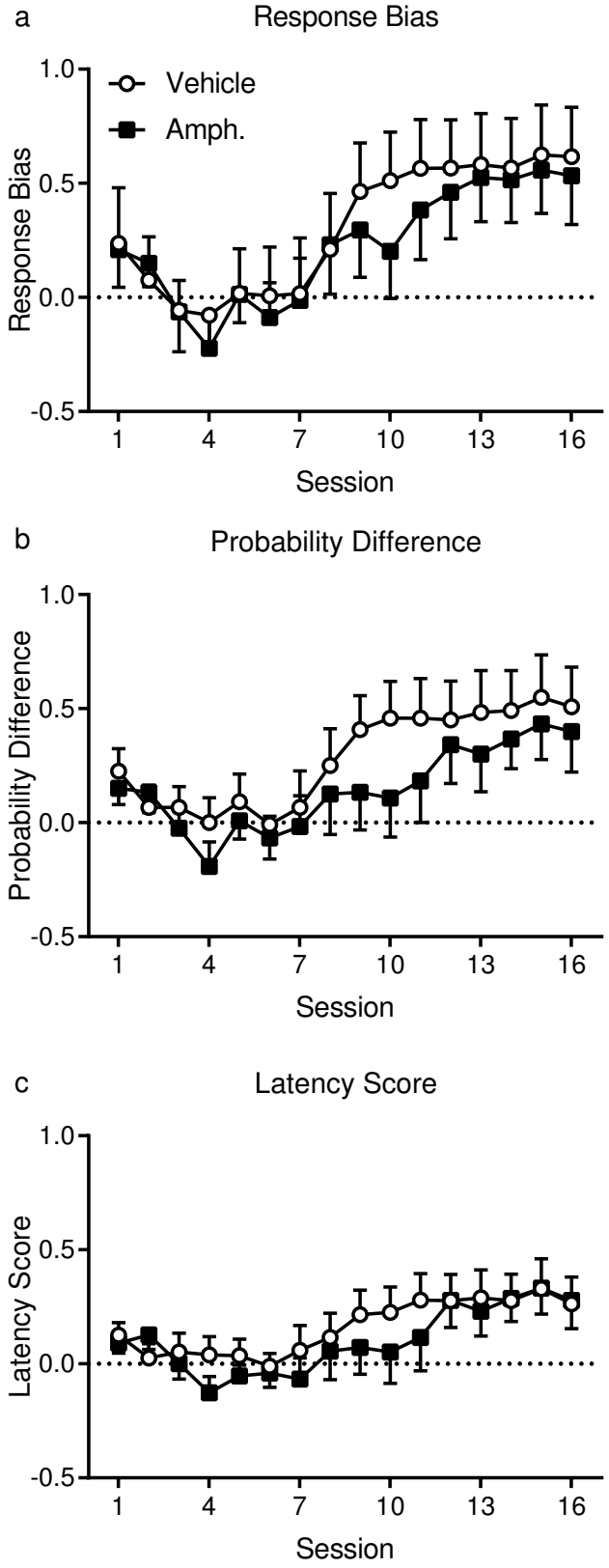

Figure S2. Component measures in the Pavlovian conditioned approach score. Prior amphetamine exposure did not alter (a) response bias, (b) probability difference or (c) latency score. Data are means ± SEM.