Contributions of Dopamine D_2 and D_3 Receptors to Pavlovian Conditioned Approach Behaviors

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Abstract

Dopamine signaling facilitates the attribution of incentive salience, or incentive motivational value, to both food- and drug-associated cues. Understanding the neurobiological mechanisms by which incentive salience is attributed to reward cues may contribute to the development of successful treatments for addiction and related disorders. Previous work has shown that dopamine facilitates the development of a sign-tracking, but not a goal-tracking response. However, it is not yet known which receptors are critical for allowing dopamine to encode the incentive motivational properties of reward cues. Here I studied the impact of different pharmacological agents targeting dopamine D_2 and D_3 receptors on the expression of sign- and goal-tracking behaviors. Following Pavlovian conditioned approach training rats were classified as sign- or goal-trackers. Rats were then treated with the D_2/D_3 agonist 7-OH-DPAT (0.01-0.32 mg/kg), the D_2/D_3 antagonist raclopride (0.1 mg/kg), or the selective D_3 antagonist SB-277011A (6-24 mg/kg). The effects of these agents on the expression of sign- and goal-tracking conditioned responses were examined. Treatment with the D_2/D_3 dopamine receptor agonist 7-OH-DPAT or antagonist raclopride specifically attenuated the performance of the previously acquired conditioned response. Treatment with SB-277011A did not impact the expression of either the sign- or goal-tracking conditioned response. Although dopamine has been shown to encode the incentive motivational properties of reward cues, the present findings suggest that signaling at the dopamine D_2 receptor may be critical for both the incentive and predictive properties of reward cues. That is, both agonism and antagonism at the dopamine D_2 receptor affected the expression of both sign- and goal-tracking behaviors.

Keywords: Incentive Salience, Motivation, Pavlovian conditioning, Autoshaping, Sign-tracking, Goal-tracking, Dopamine, D_2 receptor, D_3 receptor

Introduction

There is general agreement in the literature that mesolimbic dopamine signaling is involved in reward-related processes, but its exact involvement has been controversial. Some have argued that dopamine encodes a reward-prediction error signal, necessary for learning cue-reward relationships (Hollerman & Schultz, 1998; Schultz, Dayan, & Montague, 1997; Steinberg et al., 2013). In contrast, others have argued that mesolimbic dopamine facilitates the attribution of Pavlovian incentive value—or incentive salience—to reward-paired cues (Flagel et al., 2011). Incentive salience is the process by which neutral cues in the environment come to serve as predictors of reward in addition to becoming attractive and rendering cues desirable in their own right (Berridge & Robinson, 1998; 2003). Reward-paired stimuli in the environment that become attributed with incentive salience—incentive stimuli—are able to evoke a motivational drive, or "wanting", that can trigger complex emotional states. Until recently, it was difficult to parse whether cues attain solely a predictive or also an incentive relationship with reward, because Pavlovian conditioned reward cues can simultaneously acquire both incentive and predictive value. However, there now exists an animal model that captures individual variation in the extent to which incentive salience is attributed to reward-paired cues, and this variation can be exploited to elucidate the neural mechanisms underlying stimulus-reward learning.

Using a classical Pavlovian conditioning paradigm termed autoshaping in which presentation of a lever-cue predicts the delivery of a food reward in an adjacent food cup, individuals develop distinct conditioned responses. Some animals, termed sign-trackers (Hearst & Jenkins, 1974), approach and vigorously engage the lever-cue. Others, termed goal-trackers (Boakes, 1977), orient and then enter the food cup upon presentation of the lever-cue. Though both groups of animals readily learn the association between the cue and reward, and consume

all of the food pellets, only for sign-trackers does the cue become imbued with incentive salience. Thus, for sign-trackers, the cue becomes an incentive stimulus (Berridge, 2001; Cardinal, Parkinson, Hall, & Everitt, 2002). That is, only for sign-trackers does the cue become attractive to the extent that it elicits approach (Flagel, Watson, Akil, & Robinson, 2008) and supports the learning of an instrumental response as an effective conditioned reinforcer (Robinson & Flagel, 2009).

The ability to parse the incentive from the predictive qualities of a reward cue facilitates the study of the psychological and neurobiological mechanisms underlying motivated behavior. Indeed, the neurobiology subserving the attribution of incentive salience to cues has only just begun to be elucidated; and thus far, the primary focus has been on the role of dopamine in these behaviors. Using the sign-tracker/goal-tracker animal model, Flagel and colleagues illustrated that non-specific systemic antagonism of dopamine with flupenthixol prevented the development of a sign-tracking conditioned response, thus blocking incentive salience attribution; but flupenthixol administration did not disrupt learning of a goal-tracking conditioned response (Flagel et al., 2011). Also in this study, it was demonstrated that dopamine transmission in the nucleus accumbens core tracked the attribution of incentive, but not predictive, value to a reward-paired stimulus (Flagel et al., 2011). Additionally, nonspecific antagonism of dopamine in the nucleus accumbens core has been shown to selectively attenuate expression of the sign-tracking conditioned response (Saunders & Robinson, 2012). Therefore, sign-tracking is dependent on the actions of dopamine for both its acquisition and expression, whereas the development of a goal-tracking response is dopamine independent.

While previous work has clearly demonstrated a critical role for dopamine in the attribution of incentive salience to reward cues, the mechanisms of these effects are not yet

known. The pharmacological agents that were previously used to target the dopamine system in this animal model were non-specific, antagonizing both dopamine D_1- and D_2-type receptors. Recent work, however, has highlighted a role of the dopamine D_3 receptor in cue-motivated behaviors, especially those pertaining to drug self-administration (for review see Le Foll & Di Ciano, 2014). For example, modulation of dopamine D_3 receptor function by systemic administration of either a dopamine D_3 receptor agonist or antagonist reduces conditioned hyperactivity in response to cues previously associated with cocaine administration (Le Foll, Frances, Diaz, Schwartz, & Sokoloff, 2002). Moreover, both systemic and local injections of a dopamine D_3 receptor antagonist into the nucleus accumbens or central nucleus of the amygdala attenuates the ability of cocaine-cues to produce reinstatement of cocaine seeking behavior (Xi et al., 2012). In addition, antagonism of dopamine D_3 receptors has been shown to decrease self-administration of psychostimulants, and abolish the ability of these substances to elicit conditioned place preference (Song et al., 2014; 2011; 2013).

As the D3 receptors have become a target for the treatment of addictive behaviors, and the sign- and goal-tracking model has preclinical relevance (Robinson, Yager, Cogan, & Saunders, 2014), I sought to explore if signaling at the D_2 and D_3 receptors may mediate the performance of a sign- or goal-tracking conditioned response. In separate cohorts of animals the impact of the D_2/D_3 agonist 7-OH-DPAT and the D_2/D_3 antagonist raclopride on the expression of sign- and goal-tracking conditioned responses was examined. Then, to probe solely the role of D_3 signaling in these behaviors, the selective D_3 antagonist SB-277011A (Reavill et al., 2000) was administered.

Methods

All experiments followed the principles of animal care published in the Guide for the Care and Use of Laboratory Animals: Eighth Edition, revised in 2011, published by the National Academy of Sciences, and all procedures were approved by the University of Michigan's University Committee on the Use and Care of Animals. All rats were housed in a temperature- and humidity-controlled room and maintained on a 12-h light/ dark cycle (lights on at 07:00 hrs). After arrival at our facilities rats were given one week to acclimate before handling and experimental procedures began. Water and food were available *ad libitum* throughout the experiments.

Apparatus

Behavioral testing occurred in standard Med Associates conditioning chambers (20.5 x 24.1 cm floor area, 29.2 cm high; St. Albans, VT) that were located in sound attenuating cabinets equipped with ventilating fans that served as white noise and masked outside noise. For Pavlovian conditioning sessions each chamber had a food cup located in the center of one wall, approximately 3 cm above the grid floor, and was flanked 2.5 cm on the right or left by a retractable lever, located 6 cm above the grid floor. The location of the lever was counterbalanced across rats. Levers required a 10 g force to deflect and each deflection was recorded as a "contact". Located on the opposite wall, near the top of the chamber, was a white house light that was illuminated for the duration of each session. Operation of the pellet dispenser (Med Associates) resulted in the delivery of one 45 mg banana-flavored grain food pellet (Bio-Serv, Frenchtown, NJ) into the food cup.

Pavlovian Conditioned Approach

Pavlovian training procedures were similar to those described previously (Flagel, Watson, Robinson, & Akil, 2007; Meyer et al., 2012). All sessions were conducted between the

hours of 10:00-17:00. For the 2 days prior to training, rats were handled by experimenters and given a small amount of 45-mg banana pellets in their home cage, to acquaint the animals with the food to be used as the unconditioned stimulus (US). Animals then received two sessions of food cup training in the conditioning chambers, for which the food cups were primed with 3 pellets prior to session start. Each pre-training session consisted of the delivery of 25 banana pellets on a variable interval (VI) 30 s schedule, averaging 12.5 minutes per session. All animals retrieved all of the pellets during food cup training and Pavlovian autoshaping procedures began the following day.

During Pavlovian autoshaping sessions a trial consisted of insertion of the illuminated lever (conditioned stimulus, CS) into the chamber for 8 s at which time it was retracted and immediately followed by delivery of a 45-mg food pellet (unconditioned stimulus, US) into the adjacent food cup. Twenty-five trials occurred with an inter-trial interval (ITI) on a VI 90 s schedule (the period between CS presentations could range from 30 s to 150 s), and each session lasted approximately 40 minutes. The following were recorded per trial using Med Associates software: 1) number of lever contacts, 2) latency to first lever contact, 3) number of food cup entries during CS presentation, 4) latency to first food cup entry during CS presentation, and 5) number of food cup entries during the ITI. Using these metrics, sign- and goal-tracking behavior can be quantified to examine an individual's preference for the lever-CS or the food cup. In addition, the number of food pellets consumed was recorded after each session to ensure all animals were consuming their pellets.

Drugs

(±)-7-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (7-OH-DPAT) was received from the National Institutes of Mental Health Chemical Synthesis and Drug Supply Program. S-

(-)-Raclopride was purchased from Sigma-Aldrich (R121; St. Louis, MO). SB-277011A (*trans*-*N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecarboxamide) was provided by Dr. Eliot Gardner (National Institute on Drug Abuse Intramural Research Program). 7-OH-DPAT and raclopride were dissolved in 0.9% sterile physiological saline and administered subcutaneously. SB-277011A was dissolved in 25% w/v hydroxypropyl-β-cyclodextrin in sterile water and was administered intraperitoneally. All solutions were made fresh daily and administered in a volume of 1 ml/kg.

Experiment 1: The Effect of D_2/D_3 Agonism by 7-OH-DPAT on Pavlovian Conditioned Approach Behaviors and Conditioned Reinforcement

Effect of 7-OH-DPAT on Conditioned Responding

Male Sprague-Dawley rats (n=30; Harlan Laboratories, Indianapolis, IN) weighing 250-300 g were triple-housed upon arrival. Rats were classified as sign-trackers (STs) or goal-trackers (GTs) based on their average Pavlovian Conditioned Approach (PCA) index score (Meyer et al., 2012) from sessions 6 and 7 of a 7-day Pavlovian training paradigm. This index score accounts for three measures of approach behavior: the difference in latency to approach the food cup or lever, the difference in probability between approaching the food cup or lever, and the ratio of contacts with the food cup vs. the lever. PCA index scores range from -1.0 to 1.0. Those animals with an average index ranging from -1.0 to -0.3 were classified as GTs (n=8) and those with an index ranging from 0.3 to 1.0 were classified as STs (n=10). On the eighth and ninth sessions all rats received injections of vehicle 15 minutes prior to session start. A within subjects design was then used to examine the effects of 7-OH-DPAT on the expression of sign- and goal-tracking behaviors (see Figure 1a for experimental design). Rats received escalating doses of 7-OH-DPAT in the following order: 0.01, 0.032, 0.1, 0.2, and 0.32 mg/kg fifteen

minutes prior to each session. These doses were selected to examine the effects of increased D_2 relative to D_3 stimulation; and also to avoid nonspecific effects on locomotor activity (G. T. Collins et al., 2007; Li et al., 2010). On the days following each dose rats received vehicle injections to prevent carry-over drug effects. Thus, 7-OH-DPAT was administered prior to sessions 10,12,14,16, and 18 and vehicle was administered prior to sessions 11,13,15 and 17 and an additional 2 days following the last dose tested (i.e. sessions 19 and 20).

Conditioned Reinforcement

The day after the last Pavlovian training session (i.e. Session 20), rats were split into vehicle (GT n=4, ST n=5) or treatment (GT n=4, ST n=5) groups, which were counterbalanced based on their original index score from sessions 6 and 7. Rats in the treatment group received 0.032 mg/kg 7-OH-DPAT. This dose was chosen for its preference for D_3 over D_2 receptors in addition to the effects observed during the expression phase of the experiment (G. T. Collins et al., 2007). The conditioning chambers were rearranged such that the food cup and pellet dispenser were removed and the retractable lever was placed in the center of the chamber, still 6 cm above the grid floor. Two nose ports were located approximately 2.5 cm on either side of the lever and were located with the bottom of the port approximately 4 cm above the grid floor. The nose port placed opposite of the lever's previous position was designated as the active nose port. Pokes in the active port resulted in presentation of the illuminated lever for 2 s on a fixed ratio (FR) 1 schedule, and pokes in the inactive port were without consequence. The session lasted for 40 minutes and the following were recorded using Med Associates software: 1) pokes in the active nose port, 2) pokes in the inactive nose port, and 3) contacts with the lever.

Experiment 2: The Effect of D_2/D_3 Antagonism by Raclopride on the Expression of Sign- and Goal-Tracking Behaviors

Male Sprague-Dawley rats (n=60; Charles River Laboratories, Raleigh, NC) weighing 200-250 g were triple-housed upon arrival. After 7 sessions of autoshaping rats were characterized as sign- or goal-trackers based on the average PCA index score from sessions 6 and 7 as described above. Prior to session 8 all rats received a vehicle injection. A between subjects design was the implemented such that rats received injections of vehicle (ST n=6, GT n=6) or 0.1 mg/kg raclopride (ST n=9,GT n=7) on sessions 9-12 (see Figure 1a for experimental design). This dose was selected as it does not produce locomotor impairment (Chausmer & Ettenberg, 1997). All injections were given 30 minutes prior to session start. Animals then received one additional injection of vehicle prior to the next session (i.e. session 13) to assess possible carry-over effects of treatment. This experimental design allowed for comparing the effects of treatment within subjects to their own vehicle performance on session 8, in addition to comparing between treatment groups on sessions 9 through 12.

Experiment 3: The Effect of D_3 Antagonism by SB-277011A on the Expression of Sign- and Goal-Tracking Behaviors

Male Sprague-Dawley rats (n=60; Charles River Laboratories, Raleigh, NC) weighing 150-200 g were pair-housed upon arrival. Following 5 sessions of autoshaping rats were characterized as sign- or goal-trackers based on the average PCA index score from sessions 4 and 5 as described above. Prior to session 6 all rats received a vehicle injection and then, 30 minutes prior to session 7, rats received either vehicle (ST n=5,GT n=5), 6 mg/kg (ST n=6,GT n=7), or 24 mg/kg SB-277011A (ST n=5,GT n=5). See Figure 1b for a summary of the experimental design. These doses were selected based on previous preclinical studies and do not produce non-specific locomotor effects (Di Ciano, Underwood, Hagan, & Everitt, 2003). This

design allowed for comparisons between treatment groups on session 7, in addition to allowing comparisons of performance within subjects in each treatment group between sessions 6 and 7.

Statistical Analyses

All analyses were performed with SPSS 22.0 (IBM, Armonk, NY). Linear mixed-effects models (LMM) were used to assess the effects of treatment on the expression of Pavlovian conditioned approach behavior (Verbeke & Molenberghs, 2000). Session, phenotype, and/or treatment group were used as independent variables. The best-fit model of covariance was selected by the lowest Akaike information criteria score. Depending on the model selected, degrees of freedom were adjusted to a non-integer value. When significant main effects or interactions were detected, Bonferroni post-hoc comparisons were made. Performance on the conditioned reinforcement test in Experiment 1 was assessed using a three-way analysis of variance (ANOVA) in which phenotype (ST vs. GT), treatment (vehicle vs. drug), and nose port (active vs. inactive) were the independent variables and the number of pokes was the dependent variable. A two-way ANOVA with phenotype and treatment as independent variables and lever contacts as the dependent variable was also used to examine group differences on the conditioned reinforcement test. Planned comparisons were conducted to assess the effect of treatment or nose port within each phenotype. Paired t-tests were used to assess whether treatment in Experiment 2 reduced responding relative to initial training stages and whether treatment had lasting effects on performance following treatment. For all analyses significance levels were set with $p \leq 0.05$.

Results

Experiment 1: Dopamine D_2/D_3 Agonism by 7-OH-DPAT Attenuates Both Sign- and Goal-Tracking Behaviors

Acquisition of Pavlovian Conditioned Approach Behavior

Figure 2 illustrates the differential acquisition of PCA behavior over the initial seven sessions of training for Experiment 1. As described above, rats were classified as GTs or STs using their PCA index scores, with GTs as those ranging from -1.0 to -0.3, and STs as those with scores ranging from 0.3 to 1.0. By the end of this period, STs were reliably approaching (Fig. 2a) and manipulating (Fig. 2b) the lever-CS upon its presentation, and did so with increasing rapidity (Fig. 2c) over the course of training. In contrast, GTs were reliably approaching (Fig. 2d) and entering the food cup (Fig. 2e) upon lever-CS presentation, and did so with increasing rapidity (Fig. 2f) over the course of training. Therefore, as seen in previous studies (Flagel et al., 2007; Meyer et al., 2012) the lever-CS evoked a conditioned response in both sign- and goal-trackers, serving as an equally effective predictor of reward delivery for both groups; yet only for STs did the lever-CS attain incentive qualities to the extent that it was attractive and elicited approach.

Effects of 7-OH-DPAT on Sign- and Goal-tracking Behaviors

Following the initial 7 training sessions, the effects of 7-OH-DPAT on sign- and goal-tracking conditioned responses were assessed. Vehicle was administered on alternate days between drug injections (see Figure 1a for experimental design) and because there were no significant differences in behavior following the repeated vehicle injections, these sessions (i.e. sessions 8,9,11,13,15,17,19,20) were averaged and collapsed into a single datapoint per phenotype. Dose-response curves were then analyzed with respect to this datapoint (i.e. 0 mg/kg in Fig. 3).

7-OH-DPAT Increases Locomotor Activity

Treatment with 7-OH-DPAT impacted the number of food cup entries made by both sign- and goal-trackers as indicated by an overall Effect of Dose ($F_{5,19}$=6.695, p=0.001).

Additionally there was an overall Effect of Phenotype ($F_{1,15}$=5.549, p=0.032). Taken together, and as is evident in Figure 7, treatment increased the number of food cup entries during the inter-trial interval for both sign- and goal-trackers, while goal-trackers made more entries during this period overall.

Sign-Tracking

Treatment with 7-OH-DPAT attenuated lever-directed behavior for rats classified as STs, but not for GTs. There was an overall Effect of Phenotype for the probability to contact the lever ($F_{1,38}$=39.371, p<0.001; Fig. 3a), the number of lever contacts ($F_{1,15}$=15.649, p=0.001; Fig. 3b) and the latency to contact the lever ($F_{1,16}$=17.857, p=0.001; Fig. 3c). There was also an overall Effect of Dose for each of these measures (lever probability, $F_{5,71}$=2.902, p=0.019; lever contacts, $F_{5,15}$=8.769, p<0.001; lever latency, $F_{5,71}$=4.367, p=0.002). As is evident in Figure 3, sign-tracking behavior decreased with increasing doses of the drug. Although there was not a significant Phenotype X Dose interaction, these effects justified further comparisons. When the Effect of Dose was examined for each phenotype separately, there was a significant effect only for STs (lever probability, $F_{5,71}$=4.734, p=0.001; lever contact, $F_{5,15}$=15.817, p<0.001; lever latency, $F_{5,71}$=7.362, p<0.001). In agreement, post-hoc analysis showed that, relative to 0 mg/kg, each dose of 7-OH-DPAT significantly reduced the probability of lever approach (p<0.05), decreased the number of contacts (p<0.05), and increased latency to approach the lever (p<0.05) and for STs. The latter was true for all doses but 0.32 mg/kg. Taken together, these findings demonstrate that 7-OH-DPAT specifically attenuates sign-tracking behavior but appears to have a greater effect in those rats with a predisposition to sign-track.

Goal-Tracking

Interestingly, treatment with 7-OH-DPAT attenuated food cup-directed behaviors for rats classified as GTs, but not for STs. There was an overall Effect of Phenotype for probability to contact the food cup on a given trial ($F_{1,21}$=271.87, p<0.001; Fig. 3d), the number of food cup contacts during the CS period ($F_{1,16}$=72.143, p<0.001; Fig. 3e), and the latency to approach the food cup ($F_{1,18}$=72.482, p<0.001; Fig. 3f). In addition, there was a significant Effect of Dose for each of these measures (food cup probability, $F_{5,62}$=6.252, p<0.001; food cup contacts, $F_{5,23}$=20.451, p<0.001; food cup latency, $F_{5,75}$=9.245, p<0.001). There was also a significant Phenotype X Dose interaction for each of these measures (food cup probability, $F_{5,62}$=3.943, p=0.004; food cup contacts, $F_{5,23}$=15.814, p<0.001; food cup latency, $F_{5,75}$=7.423, p<0.001). As illustrated in Figure 3, goal-tracking behavior decreased with increasing doses of 7-OH-DPAT. Furthermore, examining each phenotype separately revealed a within-group Effect of Dose for all measures in goal-trackers (food cup probability, $F_{5,62}$=8.252, p<0.001; food cup contacts, $F_{5,24}$=30.684, p<0.001; food cup latency, $F_{5,75}$=13.874, p<0.001), but not sign-trackers. Thus, treatment with 7-OH-DPAT attenuated goal-tracking behavior only in rats predisposed to goal-track, and not in those predisposed to sign-track.

7-OH-DPAT Selectively Alters Motivation for Cue Presentation in Sign-Trackers

Figure 4a illustrates the effects of treatment with 7-OH-DPAT (0.032 mg/kg) on the motivation to work for presentation of the lever-CS, or the conditioned reinforcing properties of the lever-CS. Although there was not a significant Nose Port x Phenotype x Treatment interaction, there was a significant Phenotype x Treatment interaction (F=6.957, p=0.013) and a Nose Port X Treatment interaction (F=6.443, p=0.017) that justified further comparisons. As shown in Figure 4a, STs treated with vehicle responded to a much greater degree in the active

port relative to STs treated with 7-OH-DPAT ($p<0.001$) and GTs in either treatment group ($p<0.001$).

Moreover, as expected based on previous studies (Robinson & Flagel, 2009), STs in the vehicle group showed a robust preference for the active nose port over the inactive port ($p<0.001$), but in this instance GTs did not ($p=0.364$). Conversely, STs who received 7-OH-DPAT did not show a preference for the active over the inactive nose port ($p=0.594$), in addition to poking significantly less in the active port compared to STs in the vehicle group ($p<0.001$). That is, STs that received 7-OH-DPAT were indistinguishable from GTs in either treatment group for responding in both the active and inactive ports. There was no effect of treatment with 7-OH-DPAT on inactive nose pokes for either sign-trackers or goal-trackers. The vigor of activity directed towards the lever-CS once it was presented during the conditioned reinforcement task was also analyzed. For lever contacts, there was a significant Effect of Phenotype ($F=10.589$, $p=0.006$) and Treatment ($F=7.332$, $p=0.018$), and a trend towards significance for a Phenotype X Treatment interaction ($F=3.386$, $p=0.089$). Bonferroni post hoc analyses revealed that sign-trackers made more contacts with the lever during its brief presentation compared to goal-trackers ($p=0.006$). Although there was no interaction, the effect of treatment was examined within each phenotype. Administration of 7-OH-DPAT significantly attenuated the number of lever contacts ($p=0.006$) selectively in sign-trackers as seen in Figure 4b, and treatment did not alter performance of goal-trackers. Taken together, these data suggest that treatment with 7-OH-DPAT selectively impeded the ability of the lever-CS to serve as a conditioned reinforcer in sign-trackers, those that attribute incentive salience to the lever-cue.

Experiment 2: Dopamine D_2/D_3 Antagonism by Raclopride Attenuates both Sign- and Goal-Tracking Behaviors

Acquisition of Pavlovian Conditioned Approach Behavior

Similar to Experiment 1, following seven autoshaping sessions, animals were characterized as sign- or goal-trackers based on the conditioned response that emerged. All rats then received a vehicle injection the following day, prior to session 8. For rats assigned to the treatment group (ST n=9,GT n=7), raclopride was administered prior to the following four sessions (i.e. sessions 9-12) at a dose of 0.1 mg/kg, whereas control rats (ST n=6, GT n=6) continued to receive vehicle injections (see Fig. 1a). These five sessions were subjected to statistical analyses in order to assess the effect of D_2/D_3 antagonism on these behaviors. Comparisons were made between treated STs and GTs to those not receiving treatment (i.e vehicle) over sessions 9 through 12. Additionally, this design allowed for comparisons of performance in the treated STs and GTs over sessions 9 through 12 relative to their response to vehicle on session 8 using a within subjects analysis.

Raclopride Does Not Alter Locomotor Activity

As is illustrated in Figure 7, treatment with raclopride failed to alter entries into the food cup during the inter-trial intervals, as there was no Effect of Treatment on this measure ($F_{1,28}$=1.470, p=0.236).

Effect of Raclopride on Sign-Tracking

Raclopride treatment impacted the probability of approaching the lever-CS for both phenotypes as indicated by an overall Effect of Treatment ($F_{1,26}$=20.743, p<0.001; Fig 5a). There was also a Session X Treatment interaction ($F_{4,60}$=5.172, p=0.001), and post hoc analyses identified an Effect of Session ($F_{4,60}$=7.582, p<0.001) was restricted to treated rats. This revealed that raclopride attenuated treated rats probability of lever approach regardless of phenotype across sessions 9 through 12 compared to their probability on session 8. With respect to

contacts with the lever during the CS-period there was a significant Session x Phenotype x Treatment interaction ($F_{4,32}$=3.144 p=0.033; Fig 5b) and post hoc analyses confirmed that raclopride attenuated this behavior only in STs as indicated by a within phenotype Effect of Treatment ($F_{1,24}$=12.172 p=0.002), but treatment did not alter lever contacts for GTs. Between group comparisons within STs identified lever contacts were only attenuated on sessions 10 through 12 (p<0.001),. In addition, an Effect of Session within raclopride treated STs ($F_{4,24}$=16.197 p<0.001) indicated those rats were attenuated across sessions 10 through 12 in comparison to their vehicle performance on session 8 (p<0.001). Finally, raclopride decreased the speed with which treated rats approached the lever-CS as indicated by an overall Effect of Treatment ($F_{1,25}$=18.736, p<0.001). There was also a Session x Phenotype x Treatment interaction ($F_{4,60}$=3.446, p=0.013; Fig 5c) and post hocs identified an Effect of Treatment ($F_{1,25}$=14.187, p=0.001) in STs indicating that on sessions 9-12 treated STs took longer to approach the lever-CS compared to vehicle treated STs. Additionally, there was an Effect of Session only for raclopride treated STs ($F_{4,60}$=17.524, p<0.001) indicating each treatment session increased the time it took to approach the lever-CS compared to their own performance on session 8 (p<0.02) and that the extent of attenuation on sessions 10-12 was greater than on session 9 (p<0.001). Interestingly, there was also an Effect of Treatment ($F_{1,25}$=5.733, p=0.025) in GTs indicating that on sessions 11 and 12 raclopride treatment increased latency compared to vehicle controls (p<0.04).

To determine the extent to which raclopride attenuated performance the final session of treatment, session 12, was compared to the very first session of autoshaping. Treatment with raclopride over four sessions reverted treated sign-trackers to levels equivalent to their performance on the very first session across all measures (paired t test, lever probability, t_8=-

0.211, p=0.838; lever contacts, t_8=-0.094, p=0.927; lever latency, t_8=0.45,p=0.665). Importantly, treatment did not have lasting effects on the asymptotic performance of treated sign-trackers, as performance off drug on session thirteen did not differ from session eight (paired t test, lever probability, t_8=0.0, p=1.0; lever contacts, t_8=-0.165, p=0.873; lever latency, t_8=-0.078, p=0.939). These results suggest that D_2/D_3 antagonism resulted in a decrease in lever-directed behaviors in both sign- and goal-trackers, albeit to a greater extent in sign-trackers.

Effect of Raclopride on Goal-Tracking

Raclopride attenuated responding on measures of goal-tracking behavior, but did so only in rats previously classified as GTs. There was a significant Treatment x Phenotype interaction for both the probability to contact the food cup ($F_{1,24}$=4.175 p=0.05; Fig 5d) and the number of food cup contacts ($F_{1,24}$=4.410, p=0.046; Fig 5e). Further, as is evident in Figure 5 and stated above, raclopride significantly attenuated responding on these measures for GTs as indicated by a within phenotype Effect of Treatment (food cup probability $F_{1,24}$=7.206 p=0.013; food cup contacts $F_{1,24}$=6.727 p=0.016), but not STs, and the effects were consistent across training sessions as there was not an Effect of Session. Finally, raclopride did not alter the time it took to approach the food cup following cue presentation as there was not an overall Effect of Treatment nor a Treatment x Phenotype interaction (Fig 5f). This may be due to the fact that goal-trackers in this study did not approach the food cup as rapidly as previously observed (Meyer et al., 2012; Meyer, Cogan, & Robinson, 2014).

Additionally, treatment had no carryover effects in those goal-trackers receiving raclopride as their performance on session 13, after treatment, was similar to their performance on session 8 (paired t-test, food cup probability t_6=-1.341, p=0.228; food cup contacts t_6=-1.948, p =0.099; food cup latency t_6=1.295, p=0.243). However, although raclopride was able to reduce

food cup-directed behaviors for treated GTs, their performance on the last day of treatement (i.e. session 12) was still greater than their performance on session 1 (paired t-test, food cup probability t_6=-2.567, p=0.043; food cup contacts t_6=-2.885, p =0.028; food cup latency t_6=3.486, p=0.013). Taken together, D_2/D_3 antagonism produced a consistent within-session attenuation of food cup-directed behaviors only in rats classified as goal-trackers, and did not alter these behaviors in rats classified as sign-trackers.

Experiment 3: Selective Antagonism of Dopamine D_3 Receptors by SB-277011A Does Not Alter Sign- or Goal-Tracking Behaviors

Linear mixed-effects models revealed that animals acquired a ST or GT conditioned response and differed in their propensity to approach the lever or food cup, respectively, across sessions as indicated by a Session X Phenotype interaction for all measures (lever probability, $F_{6,69}$=14.432, p<0.001; lever contacts, $F_{6,45}$=12.932, p<0.001; lever latency, $F_{6,101}$=11.615, p<0.001; food cup probability, $F_{6,59}$=16.31, p<0.001; food cup contacts, $F_{6,48}$=19.935, p<0.001; food cup latency, $F_{6,62}$=18,056, p<0.001). However, treatment with either 6 or 24 mg/kg SB-277011A failed to influence either conditioned response, as there was not an effect of D_3 antagonism on any measure of lever- or food cup-directed behavior (Fig. 6). Additionally, SB-277011A did not influence locomotor activity, as there was not an Effect of Treatment (Fig. 7). Thus, treatment with SB-277011A did not alter performance on any behavioral measures when treated sign- and goal-trackers were compared to their vehicle counterparts; nor did SB-277011A administration affect performance relative to baseline (i.e. session 6) of rats within treatment groups.

Discussion

It has recently been shown that dopamine, particularly in the core of the nucleus accumbens, facilitates the attribution and expression of incentive motivational value, or incentive salience, to reward-associated cues (Flagel et al., 2011; Saunders & Robinson, 2012; Saunders, O'Donnell, Aurbach, & Robinson, 2014). The present study aimed to build upon these findings by examining the contributions of the D_2 and D_3 receptors in incentive versus predictive processes. As in previous studies, it was demonstrated that, following repeated pairings of a discrete lever-cue with delivery of food reward in a nearby food cup, two distinct conditioned responses develop (Meyer et al., 2012). For some rats, termed sign-trackers, the lever-CS acquired characteristics of an incentive stimulus, becoming attractive and reliably eliciting approach. In contrast, others developed a goal-tracking response, where the lever-CS served solely as a predictor of reward delivery. Following the acquisition of these conditioned responses, treatment with the D_2/D_3 agonist 7-OH-DPAT or the D_2/D_3 antagonist raclopride attenuated the ability of the lever-CS to evoke a conditioned response in both sign- and goal-trackers. 7-OH-DPAT was also able to abolish the incentive properties of the lever-CS, as it attenuated ability of the lever-CS to serve as a conditioned reinforcer for sign-trackers. Additionally, treatment with the selective D_3 antagonist SB-277011A had no effect on the ability of the lever-CS to elicit either of these conditioned responses.

It has been argued that treatment with dopamine antagonists results in new learning, and produces a gradual reduction in the performance of a learned response (Wise, Spindler, deWit, & Gerber, 1978). However, here it was observed that a D_2/D_3 agonist produced immediate detriments in sign- and goal-tracking behaviors. It is also interesting to note that treatment did not produce extinction-like effects as deficits occurred on the first day of treatment with 7-OH-DPAT (A. G. Phillips, McDonald, & Wilkie, 1981). Raclopride, however, produced immediate

decrements in goal-tracking behaviors, yet sign-tracking was not attenuated until the second treatment session. This would suggest the value of the lever-CS for sign-trackers was not immediately being reduced, possibly due to its properties as an incentive stimulus. Although this was the case for sign-trackers, treated sign-trackers did not exhibit food cup-directed behaviors in response to treatment. This suggests that the value of the CS-US relationship was weakened gradually by raclopride in the absence of the learning of a new conditioned response. Moreover, performance in these studies returned to measures comparable to baseline performance on the session immediately following treatment. These results suggest that treatment with a D_2/D_3 agonist or antagonist altered the predictive and incentive value of the CS without updating prediction-errors or producing new learning (Shiner et al., 2012).

What might then explain why both agonism and antagonism resulted in a similar effect on Pavlovian conditioned approach? Interestingly, the D_3 receptor is located pre- and post-synaptically, like the D_2 receptor (Joseph et al., 2002). It should also be noted that the D_3 receptor is co-expressed in both D_1- and D_2-expressing medium spiny neurons in the nucleus accumbens (Le Moine & Bloch, 1996) in addition to overlapping expression in most regions with the D_2 receptor (Diaz et al., 2000; Mansour & Watson, 1995). Thus, both the D_2 and D_3 receptors can act to inhibit intracellular signaling post-synaptically and reduce release pre-synaptically as autoreceptors (Joseph et al., 2002). Theoretically then, administration of an antagonist, like raclopride, then would impede dopamine's ability to bind post-synaptically, but would in turn increase levels of dopamine in the synapse through autoreceptor blockade. This extra dopamine would possibly then compete with raclopride and ultimately increase dopamine signaling post-synaptically. 7-OH-DPAT, on the other hand, would in turn stimulate autoreceptors, blunting dopamine release, while producing high levels of post-synaptic activity

through agonism in the absence of competition by dopamine. Therefore, it is likely the net effects of agonism and antagonism of D_2/D_3 receptors is paradoxical and results in the same effect in stimulating post-synaptic receptors. Alternatively, at synapses where the post-synaptic target is the D_1 receptor, antagonism of D_2/D_3 receptors would theoretically increase signaling via post-synaptic D_1 receptors, while agonism would reduce post-synaptic D_1 activation.

In addition, although the expression patterns of the D_2 and D_3 receptors mainly overlap, particularly in the striatum, it is interesting to note, though, that the D_3 receptor is the sole dopamine receptor in the paraventricular nucleus of the thalamus, which has been implicated in incentive salience attribution (Haight & Flagel, 2014; Mansour & Watson, 1995). Moreover, dopaminergic signaling in regions expressing the D_2 or D_3 receptor may differ in the extent that this signaling contributes to differences in the effects of D_3 antagonism on food versus drug motivated behaviors. Dopamine D_3 receptor blockade in the ventral striatum and central amygdala are able to inhibit cue-induced cocaine seeking, while microinfusions into the basolateral amygdala or dorsal striatum have no effect (Xi et al., 2012). The ventral tegmental area, the source of mesocorticolimbic dopamine, also appears to be under control via D_3 mediated signaling, yet no current studies have investigated the role of these receptors in behavioral processes (Diaz et al., 2000). Interrogation into the contributions of the D_2 and D_3 receptor in specific nuclei may lead to a better understanding of the neural systems underlying individual differences in Pavlovian conditioned approach.

Nevertheless, the findings with the selective D_3 antagonist SB-277011A suggest a minimal contribution of D_3 receptor signaling at a systemic scale to Pavlovian conditioned approach to a food-cue. These findings are in line with studies using instrumental and second-order scales of reinforcement learning under D_3 receptor blockade. Preclinical animal models of

drug addiction have implicated the D_3 receptor in mediating the ability of drugs and drug-cues to support self-administration and relapse (Di Ciano et al., 2003). Additionally, in these studies employing SB-277011A it has been demonstrated that these attenuations are specific for drugs of abuse, and that D_3 antagonism does not impact responding for food, or food-cue induced responding (Di Ciano et al., 2003; Xi et al., 2012). Thus, it may be that the dopamine D_3 receptor encodes interoceptive effects of reward, particularly important for drugs of abuse as they produce powerful emotional and motivational states that sucrose and grain pellets do not evoke in sated animals. The present study, then, demonstrates for the first time a lack of impact of D_3 receptors alone on conditioned responding for a food-cue in a purely associative paradigm. Future studies should explore the hypothesis that D_3 receptor antagonism will impede the ability of a classically conditioned opioid or cocaine cue to elicit approach in sign-trackers, but not goal-trackers (Yager & Robinson, 2012; Yager, Pitchers, Flagel, & Robinson, 2014).

The results observed here suggest that dopamine's actions at the D_2, but not D_3, receptor may be necessary for the proper execution of a conditioned response. However, the doses of 7-OH-DPAT used in the present studies were selected as they are preferentially selective for D_3 over D_2 receptors. 7-OH-DPAT has been shown *in vivo* to produce yawning behavior, a D_3-mediated behavior, at doses less than 0.1 mg/kg when administered subcutaneously. Additionally, the behavioral correlates of D_2 stimulation *in vivo*, hypothermia, are only produced by 7-OH-DPAT at doses greater than 0.1 mg/kg (G. T. Collins et al., 2007). Raclopride is a pharmacological agent that has almost equal affinities for both the D_2 and D_3 receptor, so interpretations cannot be made as to a possible differential impact at D_2 versus D_3 receptors (Seeman & Van Tol, 1994). Thus, the doses of 7-OH-DPAT used in this study are likely preferentially stimulating D_3 receptors along with low amounts of agonism at D_2 receptors.

It is interesting, then, to consider what underlies the unexpected impairment in both sign- and goal-tracking following systemic manipulations at the D_2/D_3 receptors. Systemic administration of a non-specific dopamine antagonist, flupenthixol, was also able to attenuate the performance of both a sign- and goal-tracking response (Flagel et al., 2011). Interpretations of these effects must be taken with caution, however, as locomotor impairment was produced by flupenthixol. The findings presented here are interesting in this context, suggesting that the findings by Flagel and colleagues may be due to effects primarily at the D_2 and D_3 receptors. Additionally, in the present studies all animals continued to approach and consume all pellets throughout treatment and no decreases in locomotor activity were observed, and in the case of 7-OH-DPAT there was an increase in locomotor activity. However, when administered solely to the core of the nucleus accumbens, non-specific dopamine antagonism only impairs the expression of a sign-tracking response (Saunders & Robinson, 2012). These results are not in conflict with the current findings, as the nucleus accumbens core is simply one target of the mesocorticolimbic dopamine system (Fields, Hjelmstad, Margolis, & Nicola, 2007; Salamone & Correa, 2012; Wise, 2004), and instead point to circuit-level differences in reward learning between sign- and goal-trackers.

One such possibility is that the D_1 receptor contributes to the expression of a conditioned response reliant on incentive salience, and that systemic manipulations of D_1 signaling may differentially impact sign- versus goal-tracking. Dalley and colleagues discovered that D_1 receptors in the nucleus accumbens core were important for the acquisition of a Pavlovian conditioned response, yet their contribution is negligible in later stages (Dalley et al., 2005). Additionally, Clark and colleagues demonstrated similar results in Pavlovian conditioned approach behaviors (Clark, Collins, Sanford, & Phillips, 2013). In accordance with this

argument, following a single session, animals exhibiting sign-tracking behavior have enhanced D_1 mRNA in the core of the nucleus accumbens compared to those exhibiting food cup-directed responses, and following additional training this difference in D_1 mRNA is lost (Flagel et al., 2007). Thus, it would be interesting to examine what contributions, if any, dopamine D_1 receptors have to sign- and goal-tracking behaviors.

Additionally, it should be considered that the experiments presented here occurred following the acquisition of each conditioned response. Although dopamine is involved in the acquisition of a sign-tracking response (Flagel et al., 2011), it appears that following acquisition and during performance of these behaviors dopamine's actions, especially in the core of the nucleus accumbens, are not as important (Clark et al., 2013). This suggests that during so-called "automaintenance" conditioned approach no longer relies on dopaminergic signaling (Hursh, Navarick, & Fantino, 1974). However, the results here are not in line with this interpretation, and instead suggest that for both forms of Pavlovian conditioned approach, signaling at the D_2, possibly in turn with D_3, receptors are required for proper execution of these behaviors. Although goal- and sign-trackers differ in D_2 mRNA expression in the nucleus accumbens following training, it is important to note that for both groups the lever-cue attains predictive value (Flagel et al., 2007). The present findings suggest that manipulations at the D_2/D_3 receptors alter the ability of dopamine to signal a predictive CS-US relationship and elicit approach and that dopamine signaling in places other than the nucleus accumbens core may be vital for the maintenance of conditioned approach.

In conclusion, these findings are the first to investigate the contributions of D_2 and D_3 receptors to Pavlovian conditioned approach behaviors. Following classification as sign- or goal-trackers systemic manipulations of signaling at the D_2 and D_3 receptors combined were able to

attenuate the previously acquired conditioned response. Moreover, systemic D_3 receptor antagonism was not able to alter the performance of either conditioned response. Thus, these results suggest normal signaling at D_2, and to a lesser extent D_3, receptors is necessary for proper execution of a conditioned response irrespective of incentive salience attribution, and it appears that conditioned responding to a food-cue is not reliant on systemic D_3 signaling.

References

Berridge, K. C. (2001). Reward Learning: Reinforcement, Incentives, and Expectations. In D. L. Medin, *The Psychology of Learning and Motivation Advances in Research and Theory.* (Vol. 40, pp. 223–278). San Diego, CA: The Psychology of Learning and Motivation.

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Research Reviews*, *28*, 309–369.

Berridge, K. C., & Robinson, T. E. (2003). Parsing reward. *Trends in Neurosciences*, *26*(9), 507–513. doi:10.1016/S0166-2236(03)00233-9

Boakes, R. A. (1977). Performance on learning to associate a stimulus with positive reinforcement. In H. Davis & H. Hurwitz, *Operant-Pavlovian Interactions* (pp. 67–97). Hillsdale, NJ.

Cardinal, R. N., Parkinson, J. A., Hall, J., & Everitt, B. J. (2002). Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neuroscience and Biobehavioral Reviews*, *26*, 321–352.

Chausmer, A. L., & Ettenberg, A. (1997). A Role for D_2, but not D_1, Dopamine Receptors in the Response-Reinstating Effects of Food Reinforcement. *Pharmacology, Biochemistry and Behavior*, *57*(4), 681–685.

Clark, J. J., Collins, A. L., Sanford, C. A., & Phillips, P. E. M. (2013). Dopamine Encoding of Pavlovian Incentive Stimuli Diminishes with Extended Training. *Journal of Neuroscience*, *33*(8), 3526–3532. doi:10.1523/JNEUROSCI.5119-12.2013

Collins, G. T., Newman, A. H., Grundt, P., Rice, K. C., Husbands, S. M., Chauvignac, C., et al. (2007). Yawning and hypothermia in rats: effects of dopamine D3 and D2 agonists and antagonists. *Psychopharmacology*, *193*(2), 159–170. doi:10.1007/s00213-007-0766-3

Dalley, J. W., Lääne, K., Theobald, D. E. H., Armstrong, H. C., Corlett, P. R., Chudasama, Y., & Robbins, T. W. (2005). Time-limited modulation of appetitive Pavlovianmemory by D1 and NMDA receptors in thenucleus accumbens. *Proceedings of the National Academy of Sciences*, *102*(17), 6189–6194.

Di Ciano, P., Underwood, R. J., Hagan, J. J., & Everitt, B. J. (2003). Attenuation of Cue-Controlled Cocaine-Seeking by a Selective D3 Dopamine Receptor Antagonist SB-277011-A. *Neuropsychopharmacology*, *28*(2), 329–338. doi:10.1038/sj.npp.1300148

Diaz, J., Pilon, C., Le Foll, B., Gros, C., Triller, A., Schwartz, J.-C., & Sokoloff, P. (2000). Dopamine D_3 Receptors Expressed by All Mesencephalic Dopamine Neurons. *Journal of Neuroscience*, *20*(23), 8677–8684.

Fields, H. L., Hjelmstad, G. O., Margolis, E. B., & Nicola, S. M. (2007). Ventral Tegmental Area Neurons in Learned Appetitive Behavior and Positive Reinforcement. *Annual Review of Neuroscience*, *30*(1), 289–316. doi:10.1146/annurev.neuro.30.051606.094341

Flagel, S. B., Clark, J. J., Robinson, T. E., Mayo, L., Czuj, A., Willuhn, I., et al. (2011). A selective role for dopamine in stimulus-reward learning. *Nature*, *469*(7328), 53–57. doi:10.1038/nature09588

Flagel, S. B., Watson, S. J., Akil, H., & Robinson, T. E. (2008). Individual differences in the attribution of incentive salience to a reward-related cue: Influence on cocaine sensitization. *Behavioural Brain Research*, *186*(1), 48–56. doi:10.1016/j.bbr.2007.07.022

Flagel, S. B., Watson, S. J., Robinson, T. E., & Akil, H. (2007). Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats. *Psychopharmacology*, *191*(3), 599–607. doi:10.1007/s00213-006-0535-8

Haight, J. L., & Flagel, S. B. (2014). A potential role for the paraventricular nucleus of the

thalamus in mediating individual variation in Pavlovian conditioned responses. *Frontiers in Behavioral Neuroscience*, *8*, 1–10. doi:10.3389/fnbeh.2014.00079

Hearst, E., & Jenkins, H. (1974). *Sign-tracking: The Stimulus-Reinforcer Relation and Directed Action*. *Monograph of the Pyschonomic Society*. Austin.

Hollerman, J. R., & Schultz, W. (1998). Dopamine neurons report an error in the temporal prediction of reward during learning. *Nature Neuroscience*, *1*(4), 304–309.

Hursh, S. R., Navarick, D. J., & Fantino, E. (1974). "Automaintenance": The Role of Reinforcement. *Journal of the Experimental Analysis of Behavior*, *21*, 117–124.

Joseph, J. D., Wang, Y. M., Miles, P. R., Budygin, E. A., Picetti, R., Gainetdinov, R. R., et al. (2002). Dopamine Autoreceptor Regulation of Release and Uptake in Mouse Brain Slices in the Absence of D_3 Receptors. *Neuroscience*, *112*(1), 39–49.

Le Foll, B., & Di Ciano, P. (2014). Neuronal circuitry underlying the impact of D3 receptor ligands in drug addiction. *European Neuropsychopharmacology*, 1–9. doi:10.1016/j.euroneuro.2014.08.017

Le Foll, B., Frances, H., Diaz, J., Schwartz, J.-C., & Sokoloff, P. (2002). Role of the dopamine D3 receptor in reactivity to cocaine-associated cues in mice. *European Journal of Neuroscience*, *15*(12), 2016–2026. doi:10.1046/j.1460-9568.2002.02049.x

Le Moine, C., & Bloch, B. (1996). Expression of the D_3 Dopamine Receptor in Peptidergic Neurons of the Nucleus Accumbens: Comparison with the D_1 and D_2 Dopamine Receptors. *Neuroscience*, *73*(1), 131–143.

Li, S.-M., Collins, G. T., Paul, N. M., Grundt, P., Newman, A. H., Xu, M., et al. (2010). Yawning and locomotor behavior induced by dopamine receptor agonists in mice and rats. *Behavioural Pharmacology*, *21*(3), 171–181. doi:10.1097/FBP.0b013e32833a5c68

Mansour, A., & Watson, S. J. (1995). Dopamine Receptor Expression in the Central Nervous System. In F. E. Bloom & D. J. Kupfer, *Psychopharmacology: The Fourth Generation of Progress* (pp. 207–219). New York.

Meyer, P. J., Cogan, E. S., & Robinson, T. E. (2014). The Form of a Conditioned Stimulus Can Influence the Degree to Which It Acquires Incentive Motivational Properties. *PLoS ONE*, *9*(6), e98163. doi:10.1371/journal.pone.0098163

Meyer, P. J., Lovic, V., Saunders, B. T., Yager, L. M., Flagel, S. B., Morrow, J. D., & Robinson, T. E. (2012). Quantifying Individual Variation in the Propensity to Attribute Incentive Salience to Reward Cues. *PLoS ONE*, *7*(6), e38987. doi:10.1371/journal.pone.0038987

Phillips, A. G., McDonald, A. C., & Wilkie, D. M. (1981). Disruption of Autoshaped Responding to a Signal of Brain-Stimulation Reward by Neuroleptic Drugs. *Pharmacology, Biochemistry and Behavior*, *14*, 543–548.

Reavill, C., Taylor, S. G., Wood, M. D., Ashmeade, T., Austin, N. E., Avenell, K. Y., et al. (2000). Pharmacological Actions of a Novel, High-Affinity, and Selective Human Dopamine D_3 Receptor Antagonist, SB-277011-A. *Journal of Pharmacology and Experimental Therapeutics*, *294*(3), 1154–1165.

Robinson, T. E., & Flagel, S. B. (2009). Dissociating the Predictive and Incentive Motivational Properties of Reward-Related Cues Through the Study of Individual Differences. *Bps*, *65*(10), 869–873. doi:10.1016/j.biopsych.2008.09.006

Robinson, T. E., Yager, L. M., Cogan, E. S., & Saunders, B. T. (2014). On the motivational properties of reward cues: Individual differences. *Neuropharmacology*, *76*(Part B), 450–459. doi:10.1016/j.neuropharm.2013.05.040

Salamone, J. D., & Correa, M. (2012). The Mysterious Motivational Functions of Mesolimbic

Dopamine. *Neuron*, *76*(3), 470–485. doi:10.1016/j.neuron.2012.10.021

Saunders, B. T., & Robinson, T. E. (2012). The role of dopamine in the accumbens core in the expression of Pavlovian-conditioned responses. *European Journal of Neuroscience*, *36*(4), 2521–2532. doi:10.1111/j.1460-9568.2012.08217.x

Saunders, B. T., O'Donnell, E. G., Aurbach, E. L., & Robinson, T. E. (2014). A Cocaine Context Renews Drug Seeking Preferentially in a Subset of Individuals. *Neuropsychopharmacology*, 1–29. doi:10.1038/npp.2014.131

Schultz, W., Dayan, P., & Montague, P. R. (1997). A Neural Substrate of Prediction and Reward. *Science*, *275*(5306), 1593–1599. doi:10.1126/science.275.5306.1593

Seeman, P., & Van Tol, H. H. M. (1994). Dopamine receptor pharmacology. *Trends in Pharmacological Sciences*, *15*(7), 264–270.

Shiner, T., Seymour, B., Wunderlich, K., Hill, C., Bhatia, K. P., Dayan, P., & Dolan, R. J. (2012). Dopamine and performance in a reinforcement learning task: evidence from Parkinson's disease. *Brain*, *135*(6), 1871–1883. doi:10.1093/brain/aws083

Song, R., Bi, G.-H., Zhang, H.-Y., Yang, R.-F., Gardner, E. L., Li, J., & Xi, Z.-X. (2014). Blockade of D3 receptors by YQA14 inhibits cocaine's rewarding effects and relapse to drug-seeking behavior in rats. *Neuropharmacology*, *77*(C), 398–405. doi:10.1016/j.neuropharm.2013.10.010

Song, R., Yang, R.-F., Wu, N., Su, R.-B., Li, J., Peng, X.-Q., et al. (2011). YQA14: a novel dopamine D3 receptor antagonist that inhibits cocaine self-administration in rats and mice, but not in D3 receptor-knockout mice. *Addiction Biology*, *17*(2), 259–273. doi:10.1111/j.1369-1600.2011.00317.x

Song, R., Zhang, H.-Y., Peng, X.-Q., Su, R.-B., Yang, R.-F., Li, J., et al. (2013). Dopamine D3

receptor deletion or blockade attenuates cocaine-induced conditioned place preference in mice. *Neuropharmacology*, *72*(C), 82–87. doi:10.1016/j.neuropharm.2013.04.042

Steinberg, E. E., Keiflin, R., Boivin, J. R., Witten, I. B., Deisseroth, K., & Janak, P. H. (2013). A causal link between prediction errors, dopamine neurons and learning. *Nature Publishing Group*, *16*(7), 966–973. doi:10.1038/nn.3413

Verbeke, G., & Molenberghs, G. (2000). *Linear mixed models for longitudinal data*. New York: Springer.

Wise, R. A. (2004). Dopamine, learning and motivation. *Nature Reviews Neuroscience*, *5*(6), 483–494. doi:10.1038/nrn1406

Wise, R. A., Spindler, J., deWit, H., & Gerber, G. J. (1978). Neuroleptic-Induced "Anhedonia" in Rats: Pimozide Blocks Reward Quality of Food. *Science*, *201*, 262–264.

Xi, Z.-X., Li, X., Li, J., Peng, X.-Q., Song, R., Gaál, J., & Gardner, E. L. (2012). Blockade of dopamine D3 receptors in the nucleus accumbens and central amygdala inhibits incubation of cocaine craving in rats. *Addiction Biology*, *18*(4), 665–677. doi:10.1111/j.1369-1600.2012.00486.x

Yager, L. M., & Robinson, T. E. (2012). A classically conditioned cocaine cue acquires greater control over motivated behavior in rats prone to attribute incentive salience to a food cue. *Psychopharmacology*, *226*(2), 217–228. doi:10.1007/s00213-012-2890-y

Yager, L. M., Pitchers, K. K., Flagel, S. B., & Robinson, T. E. (2014). Individual Variation in the Motivational and Neurobiological Effects of an Opioid Cue, 1–9. doi:10.1038/npp.2014.314

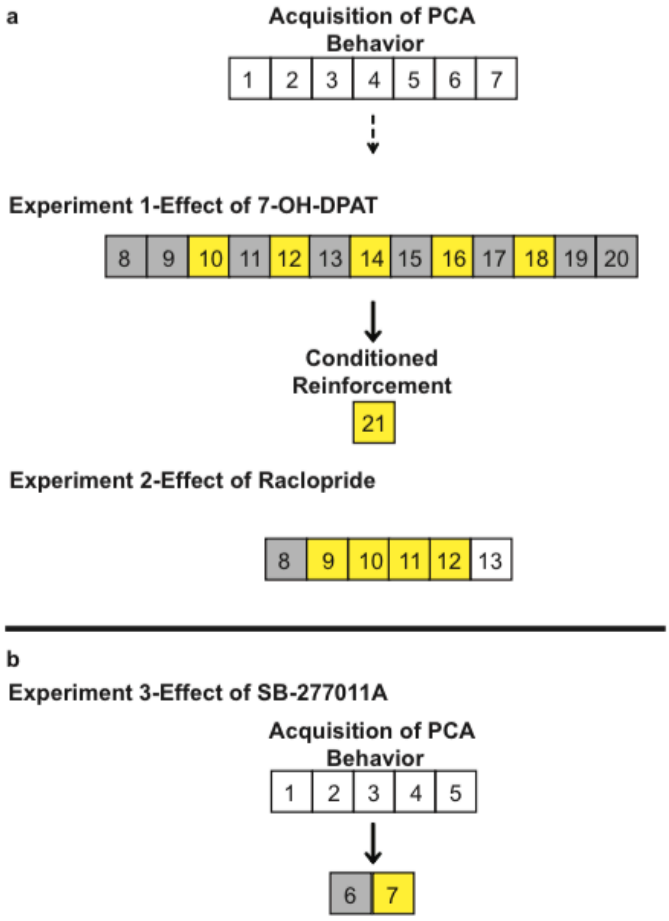

Fig. 1 Schematic illustration of experimental design. Independent groups of rats were used for each experiment. Each numbered box indicates a session of autoshaping. Yellow boxes illustrate sessions that experimental compounds were administered prior to session start. Grey boxes illustrate sessions where all subjects were administered vehicle. a) Following 7 sessions of autoshaping rats were classified as sign- or goal-trackers. For Experiment 1 all animals underwent treatment according to a within subjects design. Following assessing the effects of 7-OH-DPAT rats were split into balanced groups based on their performance in the initial 7 sessions and proceeded to undergo a test of conditioned reinforcement. For Experiment 2 following classification as sign- or goal-trackers rats were split into balanced treatment groups prior to testing with raclopride. b) Following 5 sessions of autoshaping rats were classified as sign- or goal-trackers and were split into balanced treatment groups. All rats then received vehicle prior to session 6, and then their respective treatment prior to session 7.

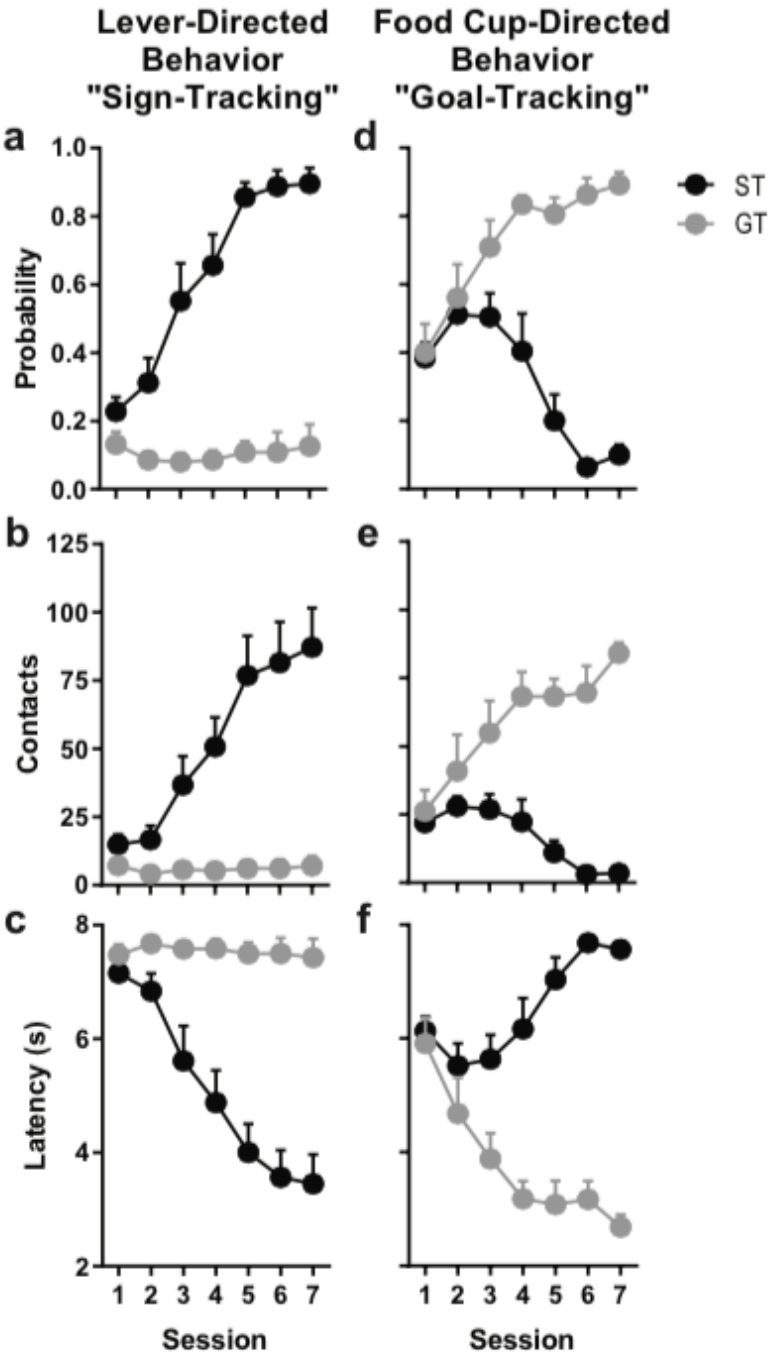

Fig. 2 Individual differences in Pavlovian conditioned approach behavior following autoshaping. Lever-directed behavior (sign-tracking; a-c) and food cup-directed behavior (goal-tracking; d-f) across 7 sessions of training prior to exposure to 7-OH-DPAT for rats classified as STs (n=10) or GTs (n=8). Error bars indicate SEM.

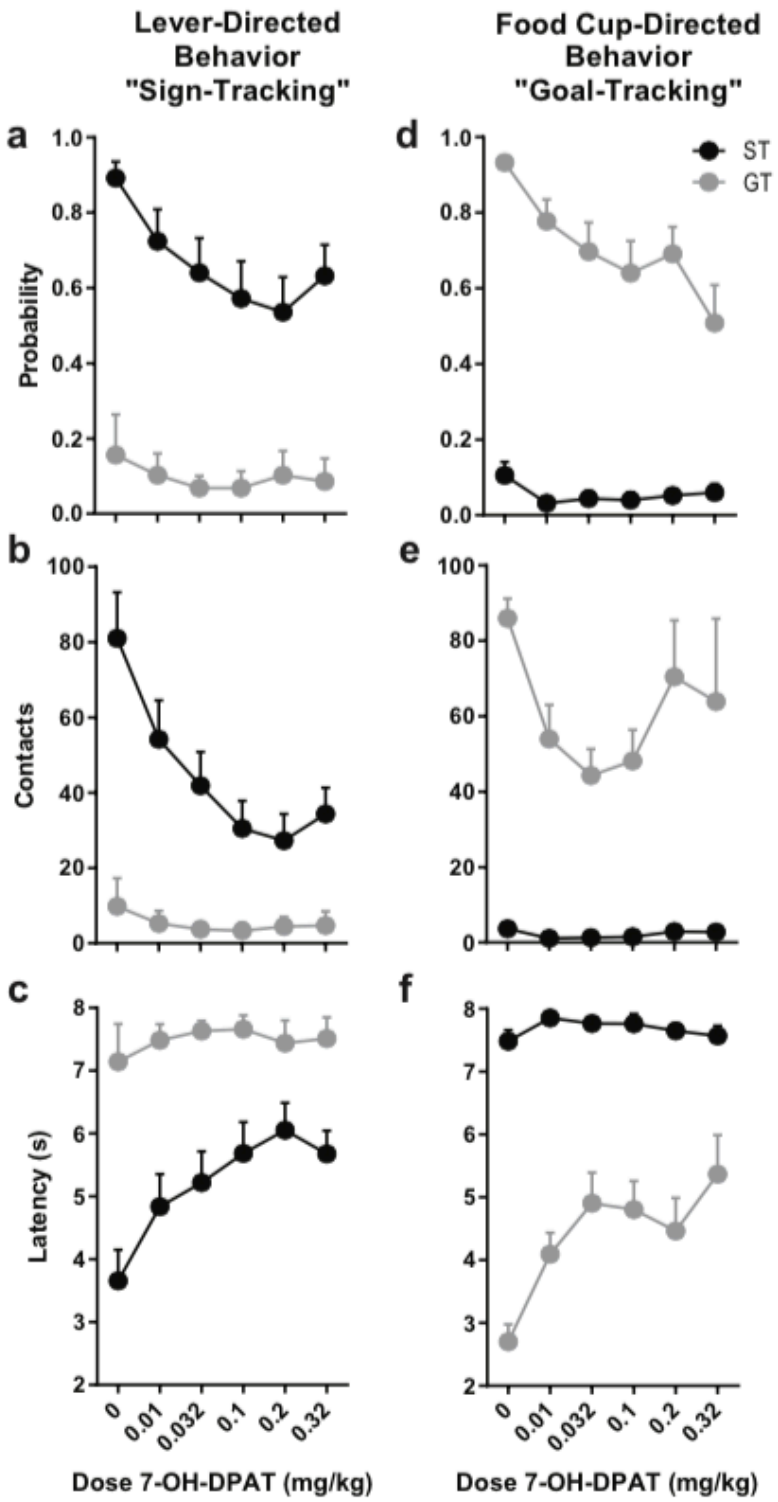

Fig. 3 Effects of 7-OH-DPAT on STs (n=10) or GTs (n=8). Treatment with 7-OH-DPAT selectively attenuated the performance of the previous conditioned response, both in sign-trackers (a-c) and goal-trackers (d-f). Error bars indicate SEM.

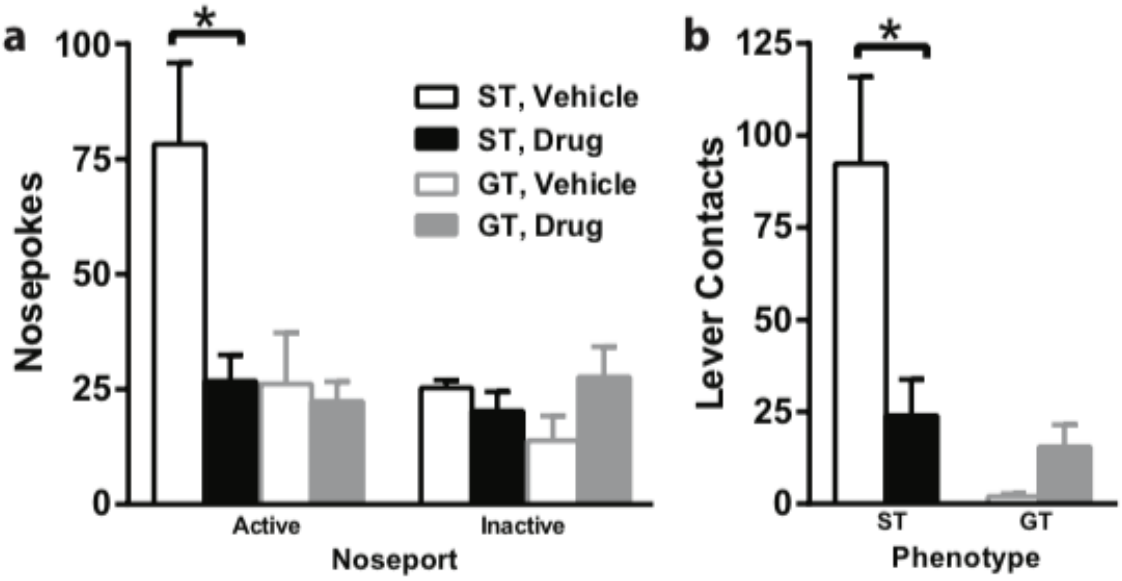

Fig. 4 Effects of 7-OH-DPAT on Conditioned Reinforcement. Treatment with 7-OH-DPAT prevented the acquisition of a new instrumental learning process selectively in sign-trackers. Sign-trackers receiving 7-OH-DPAT (n=5) were significantly attenuated compared to vehicle controls (n=5) on active nose pokes for lever presentation (a) and vigor of responding following lever presentation (b) while goal-trackers were not affected by treatment on either measure. *$p<0.05$ relative to vehicle. Error bars indicate SEM.

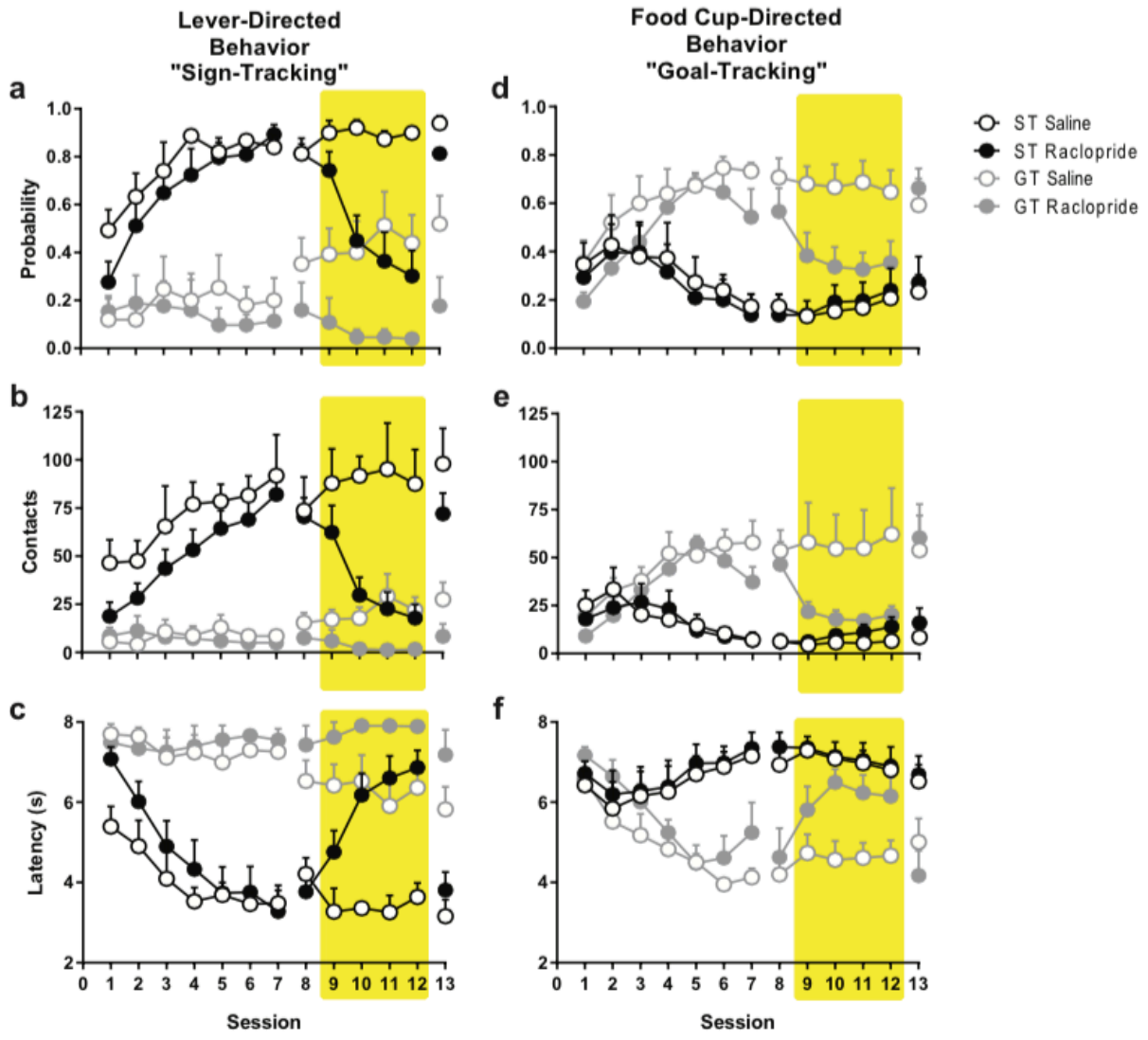

Fig. 5 Effects of Raclopride on Pavlovian conditioned approach behavior. Following seven sessions of autoshaping animals were classified as STs (n=15) or GTs (n=13) based on their propensity to approach the lever or food cup during the CS period. On session 8 all animals received vehicle injections, then were split into treatment groups with ST (n=6) and GT (n=6) receiving only vehicle and the others receiving 0.1 mg/kg raclopride (ST n=9; GT n=7) over the next four sessions (shaded region). On session 13 all animals received vehicle treatment to reassess behavior following raclopride administration. Raclopride attenuated lever-directed behaviors in animals previously classified as STs and GTs (a-c) and food cup-directed behavior only in animals previously classified as GTs (d-f). Shaded region indicates sessions when raclopride was administered. Error bars indicate SEM.

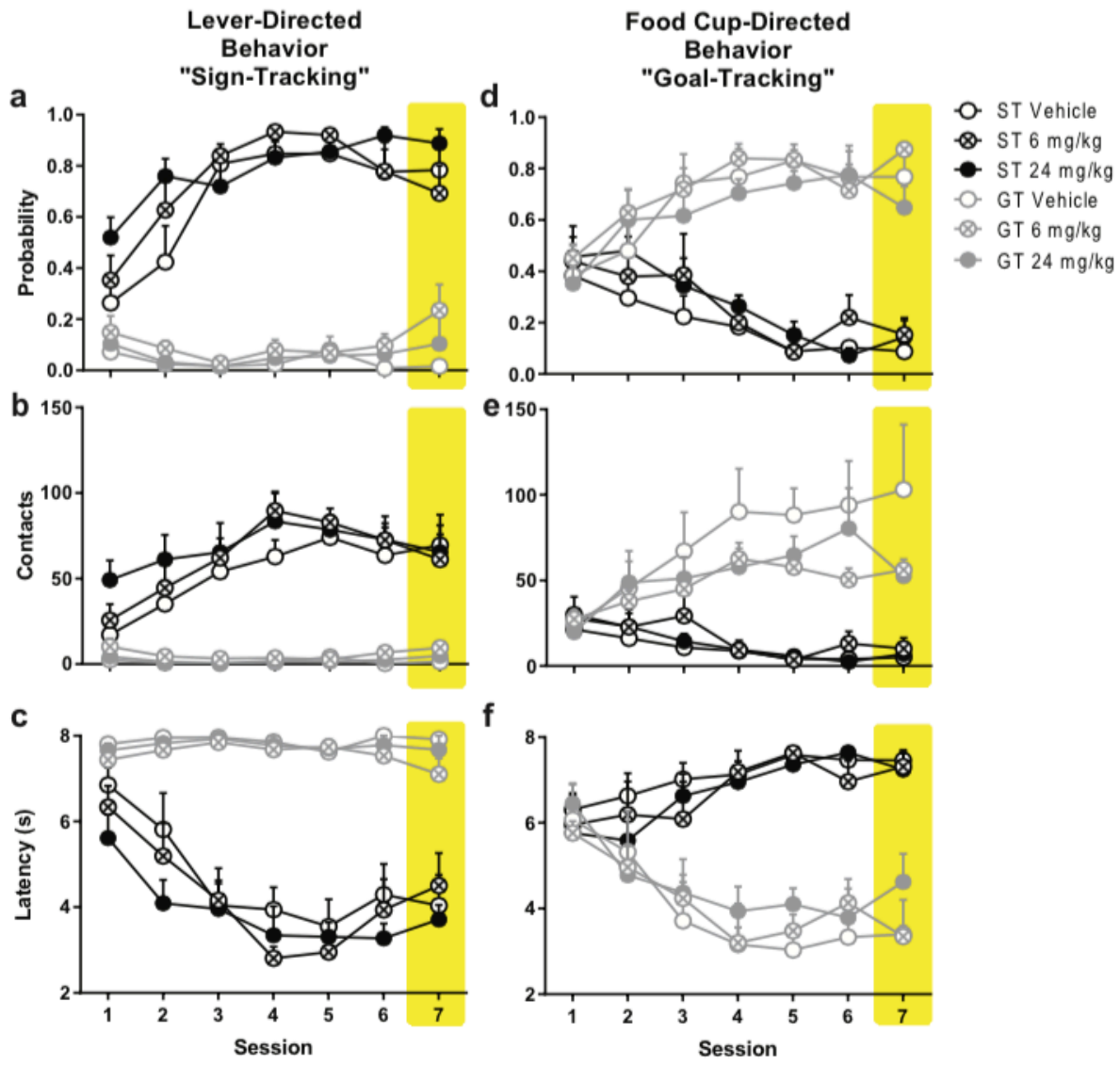

Fig. 6 Effects of SB-277011A on Pavlovian conditioned approach behavior. Following five sessions of autoshaping animals were classified as STs (n=16) or GTs (n=17). All animals received vehicle prior to session six. On session 7 animals received either vehicle (ST n=5;GT n=5), 6 mg/kg (ST n=6;GT n=7), or 24 mg/kg (ST n=5;GT n=5) SB-277011A. Treatment with SB-277011A did not affect sign-tracking (a-c) or goal-tracking (d-f). Shaded region indicates session when SB-277011A was administered. Error bars indicate SEM.

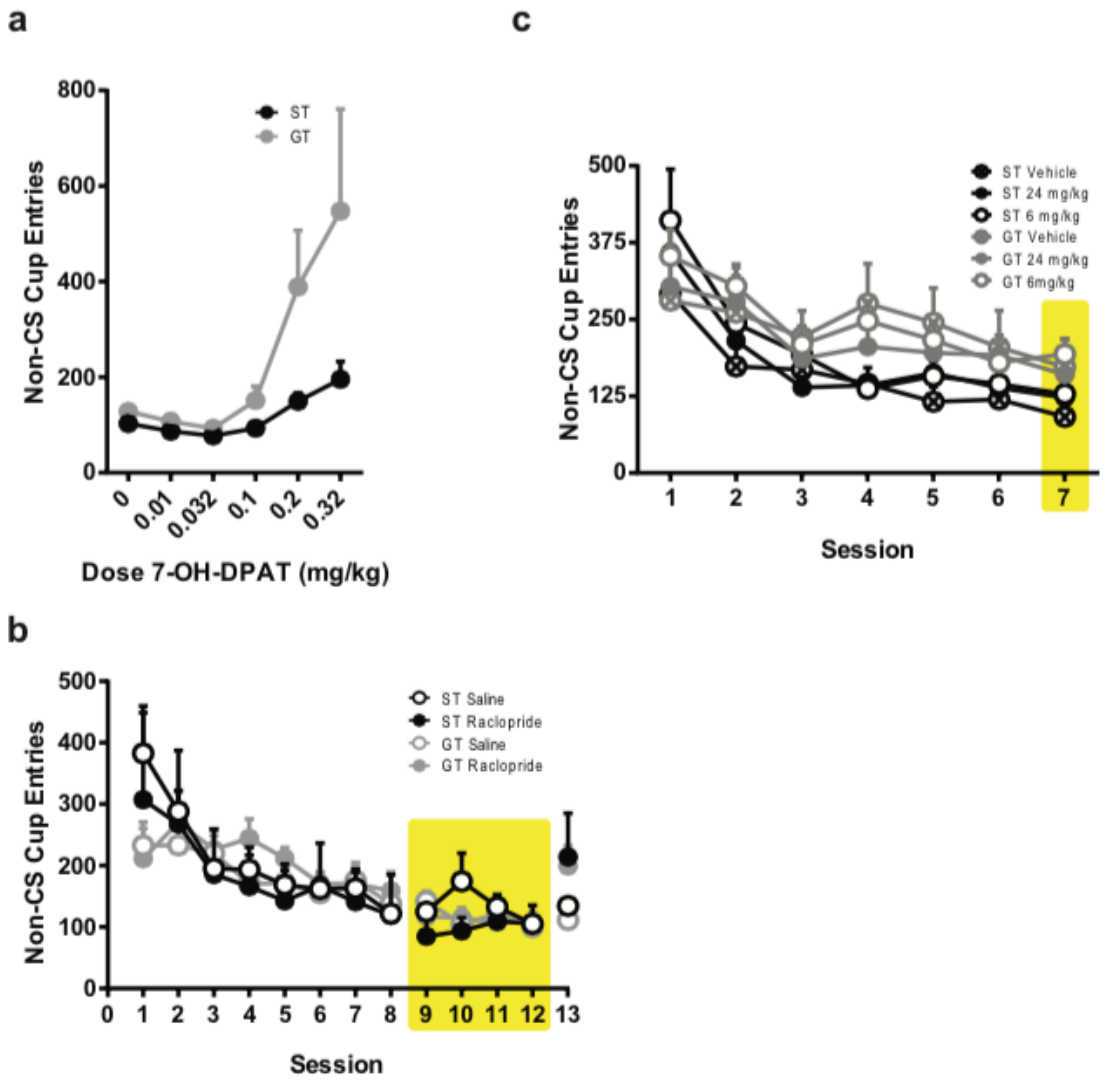

Fig. 7 Impact of treatment on non-specific locomotor activity. Treatment with 7-OH-DPAT increased locomotor activity as indicated by entries into the food cup during the inter-trial intervals for both sign- and goal-trackers as indicated by an Effect of Dose (a). Treatment with either raclopride (b) or SB-277011A (c) failed to alter the number of food cup entries during the inter-trial interval made by animals that received these agents. Shaded regions represent sessions in which treatment was administered for (b) and (c). Error bars indicate SEM.