Dorsomedial Striatal Control of Cue-Directed Versus Goal-Directed Pavlovian Approach Behavior

by

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A Thesis Submitted in Partial Fulfillment of the

Requirements for the Degree of Bachelor of Sciences

With Honors in Biopsychology, Cognition, and Neuroscience from the

University of Michigan

2013

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Abstract

Pavlovian reward cues, while important for normal behavior, under certain conditions may be attributed with exaggerated incentive motivational value, resulting in compulsive behavior, such as addiction. Importantly, there is large variation in the propensity to attribute reward cues with motivational value. These differences are revealed in variation in the expression of Pavlovian conditioned approach behavior: some individuals preferentially approach and engage a discrete, localizable food-predictive cue (i.e., “sign tracking”) while others preferentially approach the location of food delivery, (i.e., “goal-tracking”) upon cue delivery. Sign-tracking is thought to reflect the assignment of “incentive salience” to the cue, and is dependent on dopamine signaling in the nucleus accumbens. Conversely, goal-tracking is less dependent on accumbens dopamine signaling, and has been hypothesized to engage corticostriatal circuitry implicated in act-outcome learning, in which behavior is guided by reward expectations. In the current study we tested the hypothesis that goal-tracking, relative to sign-tracking, is more dependent on activity in a brain region known to mediate act-outcome associations, by a) inactivating neural activity and b) blocking dopamine signaling specifically, in posterior dorsomedial striatum (pDMS). pDMS inactivation significantly *increased* responding in sign-trackers, and marginally decreased responding in goal-trackers, while dopamine antagonism had no effect on either. These results provide preliminary evidence that the neural systems underlying act-outcome responding and goal-tracking could be similar.

Keywords: goal-tracking, sign-tracking, act-outcome behavior, pDMS, reward cues

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An individual is constantly surrounded by a variety of cues in the environment that can play an important role in reward-seeking behavior. When reward-predictive cues are attributed with incentive motivational value ("incentive salience"), and act as incentive stimuli, they have the ability to operate as strong incentives and can even drive motivated behavior, sometimes leading to maladaptive behaviors, such as addiction (Flagel, Akil, & Robinson, 2009; Milton & Everitt, 2010; Robinson & Berridge, 1993; Saunders & Robinson, 2013). Importantly, studies using a Pavlovian conditioned approach (PCA) paradigm have found that there are significant differences in the amount of incentive motivational value individuals attribute to reward cues (Flagel, Watson, Robinson, & Akil, 2007). For example, when a localizable cue (a lever- conditional stimulus, CS) is paired with a reward (delivery of food- unconditional stimulus, US), the cue can gain the properties of an incentive stimulus such that some rats, "sign-trackers" (STs), will approach and engage the lever. For other rats, "goal-trackers" (GTs), however, rather than approaching the cue, these rats, upon cue presentations, go to the food magazine and await the reward delivery. Although both STs and GTs learn conditioned responses (CRs), indicating that the cue is an effective CS for both, the cue acts as a strong incentive stimulus only for STs (Flagel et al., 2009; Robinson & Flagel, 2009). An important step towards understanding this variability in reward-cue responsivity involves parsing the neurobiological mechanisms that govern sign and goal-tracking behavior.

Dopamine Mediates the Attribution of Incentive Salience to Pavlovian Reward Cues

Findings from multiple studies suggest that the incentive salience attribution process thought to underlie sign-tracking is mediated by a "bottom-up" dopamine-dependent mesolimbic

circuit (Saunders & Robinson, 2012, 2013). For example, Flagel et al. (2011) found that dopamine is only necessary for the acquisition of an ST CR, and not for a GT CR, during Pavlovian conditioned approach training. Furthermore, Saunders and Robinson (2012) found that the expression of sign-tracking behavior, but not goal-tracking behavior, is also dependent on dopamine transmission in the nucleus accumbens (NAc) core. These results challenged the widely held view that intact dopamine transmission is necessary for learning stimulus-reward associations through its action as a prediction-error signal (Shultz et al., 1997). Instead, dopamine, particularly within the NAc core, appears to be more important for attributing reward cues with incentive motivational value, and goal-tracking behavior relies on a relatively dopamine-independent process.

While these pharmacological studies have begun to uncover the neural mechanisms underlying sign-tracking, they provide less information about the neural substrates and/or neurotransmitters involved in goal-tracking (Clark, Hollon, & Phillips, 2012; Saunders & Robinson, 2012). Rather than attributing the CS with incentive salience, one hypothesis is that a process similar to act-outcome instrumental learning governs goal-tracking behavior, where presentations of the CS result in an expectation of the rewarding outcome, which guides approach to the location of reward delivery (Balleine & Dickinson, 1998; Dickinson, Smith, & Mirenowicz, 2000; Meyer et al., 2012; Saunders & Robinson, 2012). As opposed to the Pavlovian incentive motivational processes governing sign-tracking, which depend on “bottom-up” mesolimbic dopamine circuitry, act-outcome instrumental learning does not depend on dopamine signaling, and instead is thought to rely on “top-down” corticostriatal circuitry (Balleine & Dickinson, 1998; Balleine & O’Doherty, 2010; Meyer et al., 2012; Yin, Ostlund, & Balleine, 2008). Given that this theory has not been tested under Pavlovian conditions, it is

unclear how well studies examining brain regions involved in act-outcome responding during instrumental tasks can generalize to Pavlovian behavior. Therefore, the primary goal of the current study is to explore the idea that overlapping neural systems mediate the expression of act-outcome responding and goal-tracking.

Multiple Processes Govern Instrumental Action Selection

Having the ability to adapt to the current situation to appropriately select actions and respond to cues is essential for an individual to successfully function in a changing environment (Balleine & Dickinson, 1998). At least two distinguishable psychological processes are involved in selecting instrumental actions. In act-outcome behavior, also known as goal-directed behavior, action selection is guided by an explicit understanding of the associations between different actions and the outcomes they produce. Relying on goal-directed processes allows an individual to flexibly navigate the environment, altering responses as the consequences change. This is in contrast to habitual behavior, where action selection is simply guided by a stimulus-response association (Balleine & O'Doherty, 2010). This process allows an individual to behave efficiently when learned cues present themselves in the environment. Importantly, behavior that was originally goal directed can become habitual, with extended exposure to the same conditions (Yin, Knowlton, & Balleine, 2004).

In actual behaving individuals, both goal- and stimulus-directed processes have important utility for guiding behavior, and they are often used in parallel, but in order to differentiate them in a laboratory setting, outcome devaluation and contingency degradation procedures are used (Balleine & Dickinson, 1998). Since goal-directed behavior is more flexible than habitual behavior, it is sensitive to changes in the response-outcome relationship introduced in these procedures (Balleine & O'Doherty, 2010). Outcome devaluation and contingency degradation

procedures are composed of three parts. First: an initial instrumental training phase where an animal learns the association between specific actions (e.g., pressing a lever) and outcomes (e.g., reward delivery). Second: an experimental manipulation alters some part of the act-outcome relationship. For outcome devaluation, the value of the outcome is diminished, usually through reward satiation or by pairing with an aversive consequence. For contingency degradation, the strength of the act-outcome relationship is changed (the outcome may now be less likely to occur following the response, which reduces the strength of the contingency). Third: a choice test is given to assess whether or not the animal has integrated this new information about the act-outcome association, and can adjust behavior accordingly. If the animal chooses the response that leads to the non-devalued/non-degraded outcome, it demonstrates that he was sensitive to act-outcome contingency changes, and his behavior is considered goal-directed (Balleine & Dickinson, 1998; Balleine & O'Doherty, 2010). It is also important to note that sometimes an extinction model, where the animal should respond less for the devalued/degraded outcome, is used rather than giving a final choice test (Colwill & Rescorla, 1985). The current literature on goal-directed behavior consists of studies that implemented brain manipulations, such as lesions or pharmacological inactivation, in either a pre-training phase, to measure effects on the acquisition of the behavior, or a post-training phase, to measure effects on the expression or performance of the behavior. Three regions in particular have been implicated in goal-directed behavior, including the medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), and dorsomedial striatum (DMS) (Yin, Ostlund, Knowlton, & Balleine, 2005; Balleine & O'Doherty, 2010).

It appears as though the mPFC plays a more active role in the acquisition, rather than the expression of goal-directed behavior. When the prelimbic (PL) area of the mPFC was lesioned

prior to training, rats were impaired in their ability to successfully perform outcome devaluation and contingency degradation tests (Balleine & Dickinson, 1998). A similar finding was observed when pre-training lesions to the PL region of the mPFC, but not the infralimbic region, resulted in impairment in outcome devaluation tests, suggesting there may also be anatomical dissociations within the mPFC (Killcross & Coutureau, 2003). Furthermore, Ostlund and Balleine (2005) found that pre-training lesions, but, importantly, not post-training lesions to the mPFC produce insensitivity to outcome devaluation tests. With these findings combined, it appears as though damage to the mPFC prior to instrumental training prevents the initial learning or encoding of the act-outcome relationship that is needed to properly guide their behavior in these tests, but that the PL is not involved in the long-term storage and/or retrieval of this information.

While the mPFC has been shown to play a specific role in learning information about act-outcome contingencies and the value of outcomes, the OFC seems to be important for updating outcome values. Studies using pre-training and/or post-training OFC lesions have found that this results in insensitivity to the changes that occur in an outcome devaluation test (Gallagher, McMahan, & Schoenbaum, 1999; Pickens et al., 2003). Additionally, using electrophysiology, cell firing between the basolateral amygdala (BLA) and OFC has been examined and it has been found that these two regions must communicate with one another for outcome expectancy to be encoded properly in either region (Holland & Gallagher, 2004). A recent review by Clark, Hollon, & Phillips (2012) suggests that the OFC is a part of a mechanism that helps update the expected outcome value of a cue by integrating information that has already been learned about the cue-reward association and the present incentive value of the outcome before having encountered the cue and the devalued outcome together.

The dorsal striatum is critically involved in action selection, and different subregions mediate different processes. Yin, Knowlton, & Balleine (2004) propose that the DMS and dorsolateral striatum (DLS) mediate two distinct types of learning, and when one of the regions is damaged, the other region can takeover and use its primary type of learning to complete the test. Specifically, the DMS is important for goal-directed behavior, while the DLS is not, instead being more important for habitual behavior. Pre-training lesions to the DMS produce insensitivity to an outcome devaluation test, while lesions to the DLS produce sensitivity to the same outcome devaluation test. This means that without the DMS, the test must be completed using the DLS, which does not guide action control based on the act-outcome association, so the outcome devaluation test is not completed successfully. Alternatively, without the DLS, the test must be completed using the DMS, which does not guide action control based on the stimulus-response association, so the test is completed successfully. Another study looking at the DMS by Yin, Ostlund, Knowlton, & Balleine (2005) found further functional differentiations within the DMS. Both pre-training and post-training lesions to the posterior DMS (pDMS), but not the anterior DMS (aDMS), impaired performance on both outcome devaluation and contingency degradation tests. These results suggest that there are even further dissociations within the DMS where the pDMS is involved in the acquisition and expression of goal-directed behavior and the aDMS is involved in the acquisition and expression of habitual behavior. Finally, in addition to lesion studies, pharmacological manipulations suggest that specific neurotransmitter systems acting in these regions are involved in goal-directed behavior. Lex and Hauber (2010) found that dopamine receptor blockade in the PL had no effect on sensitivity to an outcome devaluation and contingency degradation test, but when dopamine signaling was blocked in the pDMS, however, animals, became insensitive to a contingency degradation test (although they remained sensitive

to a outcome devaluation test). Thus it appears as though the posterior aspect of the DMS is particularly important for goal-directed behavior, and intact dopamine transmission there may be needed for processing some aspects of act-outcome associations.

In the current study I wanted to explore the idea that a brain region involved in instrumental goal-directed behavior may also be more involved in the expression of goal-tracking behavior in response to a Pavlovian cue, relative to sign-tracking behavior. Due to its role in both the acquisition and expression of learning outcome values and contingencies, two key aspects of goal-directed behavior, I chose to look at the effects of 1) reversible inactivation and 2) dopamine receptor blockade in the pDMS on the performance of different types of PCA.

Methods

Subjects

48 male Sprague Dawley rats (Harlan, Indianapolis, IN and Charles River, Portage, Michigan) weighing 275-325 g upon arrival were used. Each rat was singly housed in an 8 x 8 x 9 in hanging acrylic cage where food and water were always available. The colony room consisted of a temperature and humidity regulated climate on a 12-hr light/12-hr dark schedule (lights on at 0800 hr). After arriving, all rats were handled at least three times by the experimenter and given at least one week to familiarize themselves with the colony room before procedures began. The University of Michigan Committee on the Use and Care of Animals (UCUCA) approved all procedures.

Apparatus

All behavioral testing took place in a separate room in sixteen standard (22 x 18 x 13 in) chambers (Med Associates Inc., St. Albans, VT, USA) in sound-attenuating cabinets. A ventilating fan was also used to mask additional noise in the testing room. Each chamber

consisted of a food magazine located in the middle of one wall and 3 cm above the stainless-steel bar floor. An illuminated retractable lever was located either 2.5 cm to the left or right of the food magazine and 6 cm above the floor. The levers' placement was counterbalanced across rats. On the opposite wall was a red house light, centrally located, that was illuminated throughout all testing sessions. Breaks of an infrared photobeam recorded head entries into the food magazine. Med Associates software was used to record beam breaks and lever deflections.

Surgery

A week after arrival, all rats received a bilateral implantation of chronic guide cannulae (22-gauge stainless-steel; Plastics One) 2 mm above the pDMS (relative to Bregma: AP: -0.4 mm, ML: +2.5 mm, and DV: -2.5 mm) using a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). A ketamine hydrochloride (100 mg/kg intraperitoneal [i.p.]) and xylazine (10 mg/kg i.p.) mixture was used for anesthesia. Four skull screws and acrylic dental cement were used to create each head cap to ensure the guide cannulae were secure. A wire stylet (28-gauge; Plastics One) was placed in the cannula to avoid occlusion. Upon completion of surgery, rats received an injection of carprofen (5 mg/kg) for pain relief. Rats were given at least five days to recover before Pavlovian training began.

Pavlovian Training

Two days prior to the commencement of Pavlovian training, rats were given approximately 10 banana-flavored pellets (45 mg, BioServe, #F0059; Frenchtown, NJ) in their home cages to accustom them. The next day they received a pre-training session in the chambers during which the lever was retracted and 25 45-mg pellets banana pellets were delivered on a variable time (VT) 30 s schedule for ~15 minutes. This was to ensure that all rats would obtain pellets from the food magazine. For the next eight days rats went through Pavlovian training.

Rats were placed in the chamber for a daily session, lasting about 45 minutes. Each session consisted of 25 trials where the illuminated lever (CS) was inserted into the chamber for 8 s, retracted, and immediately followed by the delivery of a banana pellet (US) into the food magazine. Lever extensions and pellet deliveries were not contingent on any behavior. Each CS-US pairing occurred on a VT 90 s schedule (time between each CS appearance varied randomly between 30 and 150 s). Lever contacts, food magazine entries during the CS, food magazine entries during the inter-trial interval (ITI), latency to the first lever contact, and latency to the first food magazine entry during the CS were recorded.

Categorizing Rats Using the PCA Index

Upon completion of day eight of Pavlovian training, rats were categorized into one of three groups based on their behavior: (1) Those who were more likely to contact the lever ["sign trackers" (STs)], (2) those who were more likely to enter the food magazine during the CS period ["goal trackers" (GTs)] and (3) those who were not more likely to interact with the lever or the food magazine ['intermediates' (INs)]. These classifications were based on a composite index that quantified the degree to which their behavior was directed at the lever vs. the food magazine during the period of the CS (Lovic et al., 2011; Meyer et al., 2012; Saunders & Robinson, 2011b). Three measures of Pavlovian conditioned approach (PCA) were included in this index: (1) the probability of lever contacts or food magazine contacts during each CS period [$P_{(lever)} - P_{(food\ magazine)}$], (2) the response bias for lever or magazine contacts during each CS period [(#lever contacts - #food magazine entries) / (#lever contacts + # food magazine entries)] and (3) the latency to contact the lever or the magazine during the CS period [(lever contact latency – food magazine contact latency)/ 8]. The final PCA index score included [(probability difference score + response bias score + latency difference score)/ 3]. This calculation resulted in a score of

-1.0 to +1.0 for each rat, where a score closer to -1.0 indicated the rat had entered the food magazine during the CS more and a score closer to +1.0 indicated the rat had contacted the lever more. Rats were classed based on the average PCA index score from days seven and eight of Pavlovian training, at which point performance had stabilized. Rats with an index score of +0.3 or greater were named STs, rats with an index score of -0.3 or less were named GTs, and those with a score that fell somewhere in between -0.3 and +0.3 were named INs.

Microinjections

All drug concentrations were determined based on the findings of previous studies. A combination of baclofen and muscimol (B/M) was used to inactivate the pDMS. Together, baclofen (1.0 mM), a GABA B receptor agonist, and muscimol (0.1 mM), a GABA A receptor agonist, inhibited activity in this region (Corbit & Janak, 2010). Additionally, flupenthixol (Sigma, St. Louis, MO), a non-specific dopamine receptor antagonist, was administered to see if there was a dose-dependent effect of dopamine blockade on the expression of PCA behavior. Flupenthixol was injected in two doses: 10 and 20 μg in 0.9% sterile saline (Saunders & Robinson, 2012). During the microinjections, 28-gauge injector cannulae (Plastics One) extended 2 mm below the ventral tip of the guide cannulae so that the infusion was directly into the pDMS (relative to the skull: -4.5 mm). A syringe pump (Harvard Apparatus, Holliston, MA), connected to the injector cannulae through PE-20 tubing, was used to deliver all infusions bilaterally in a volume of 0.3 μL/side over a 60 s time period. The injectors remained in the pDMS for an additional 60 s to permit drug diffusion, at which point they were removed and the wire stylets were put back in place. During all injections rats were carefully held by the experimenter.

Approximately three hours after the sixth day of Pavlovian training, all rats received a mock injection of saline to habituate them to the injection process. Upon completion of eight days of Pavlovian training, microinjection testing began. Excluding the mock injection, there were a total of five injection days. On the first and fifth injection day, all rats received an infusion of saline that served as the control (vehicle). On the second, third and fourth injection days, in between the vehicle injections, each rat received an infusion of BM and two different doses of flupenthixol (10 and 20 μg) in a counterbalanced fashion. For all BM and vehicle injections, there was a ten-minute delay between completion of the injection and the start of the Pavlovian training session. For all flupenthixol injections, there was a 20-minute delay to account for a delayed onset of drug action (Saunders & Robinson, 2012). In between each injection day, the rats underwent two days of Pavlovian training with no injections to re-stabilize performance.

Histology

At the end of all testing, rats were euthanized with carbon dioxide and their brains were extracted and flash frozen in isopentane. A combination of isopropyl alcohol and dry ice kept the isopentane at about -30°C. Frozen brains were sectioned at a thickness of 60 μm on a cryostat and mounted on slides. The slides were then stained with Cresyl violet and analyzed under a light microscope to determine the center point of the injection damage for each brain. Finally, the location of the damage was mapped onto a rat brain atlas picture (Paxinos & Watson, 2007).

Statistical Analyses

Linear mixed models (LMM) analysis of variance (ANOVA) was used for all repeated-measures data. Significant interactions were followed by post hoc planned comparisons and Bonferroni corrections between vehicle and each drug dose within groups when appropriate.

Spearman's correlations were calculated to assess the relation between drug effects and anatomical placement. Statistical significance was determined when $p < 0.05$.

Results

Individual Variation in Pavlovian Conditioned Approach Behavior

Figure 1A is a picture of a sign-tracking rat (left side) and a goal-tracking rat (right side), illustrating the difference in the topography of their behavior during the CS period. Figure 1B demonstrates the range of PCA index scores among the rats used in this experiment (n = 25). After removing rats that had cannula placements in the wrong location, were classified as an intermediate, or became ill after surgery, the final groups for all statistical analyses consisted of 15 STs and 10 GTs. Rats with negative index scores (left side) directed more of their conditioned responses toward the food magazine, whereas rats with positive index scores (right side of figure) directed more of their conditioned responses toward the lever.

PCA Training

All of the following results were analyzed by grouping rats by phenotype (i.e. ST or GT) based on their PCA index score. Rats with PCA scores between the range of +0.3 and +1.0 were classified as STs and rats with PCA scores between the range of -1.0 and -0.3 were classified as GTs. Rats with PCA ranging from -0.29 to +0.29 were grouped as INs, however, due to an extremely small sample size, they were removed from all statistical analyses. Thus, the data here represent a comparison between two groups who preferentially assign their CR to different locations. Figure 2 shows how each group learned their respective CR over the course of the first eight days of training. As the training sessions progressed, STs demonstrated a higher probability of quickly approaching and contacting the lever, while having few interactions with the food magazine (Figure 2A-C). Likewise, GTs exhibited the opposite pattern where they had a higher

probability of quickly approaching and entering the food magazine, but had few interactions with the lever (Figure 2D-F). Similar to previous studies (Flagel, Watson, Robinson, & Akil, 2007; Meyer et al., 2012), these data indicate that both groups learned a CR as a result of CS-US pairings, and did so at a similar rate.

Pharmacological Inactivation of the pDMS Differentially Affects the Vigor of Approach Behavior in STs and GTs

For each drug comparison, I separately analyzed the effects on probability of approach, number of contacts, and the latency to make a contact with the lever or magazine during the CS period, in STs and GTs.

Probability

A two-way ANOVA revealed that B/M administration did not differentially affect the probability of approach behavior (no phenotype x treatment interaction, $F(1, 8.735) = 2.637$, $p = .140$; Figure 3A). It also revealed that B/M administration did not significantly change the probability of approach behavior in STs or GTs (no effect of treatment, $F(1, 8.735) = 1.017$, $p = .340$).

Contacts

While B/M administration did not have a clear effect on the vigor of conditioned responding, as measured by the number of lever or magazine contacts (no effect of treatment, $F(1, 10.378) = .098$, $p = .760$), B/M administration differentially affected the number of lever or magazine contacts in STs and GTs, as (phenotype x treatment interaction, $F(1, 10.378) = 6.748$, $p = .026$; Figure 3B). Post hoc comparisons showed that B/M significantly increased the number of times STs interacted with the lever ($p = .001$), relative to vehicle. Although B/M qualitatively reduced the number of contacts in GTs, this result did not reach statistical significance.

Latency

B/M administration did not result in an interaction of phenotype and treatment on the latency of approach (no phenotype x treatment interaction, $F(1, 9.688) = 3.308$, $p = .100$; Figure 3C). It also did not have a significant effect on how quickly the lever or food magazine was approached after the CS onset (no effect of treatment, $F(1, 9.688) = .001$, $p = .979$).

Dopamine Receptor Antagonism in the pDMS Does Not Affect the Expression of Approach Behavior in STs and GTs

Probability

Flupenthixol administration did not affect the probability of conditioned approach overall (no effect of treatment, $F(2, 14.403) = 1.759$, $p = .207$; Figure 4A), and this lack of an effect was similar for STs and GTs (no phenotype x treatment interaction, $F(2, 13.403) = 1.611$, $p = .234$).

Contacts

Flupenthixol administration did not affect the number of conditioned responses made (no effect of treatment, $F(2, 16.737) = 3.177$, $p = .068$; Figure 4B), and this lack of effect was similar for STs and GTs (no phenotype x treatment interaction, $F(2, 16.737) = 1.585$, $p = .234$).

Latency

Flupenthixol did not significantly alter how quickly the lever or food magazine was approached after the CS onset (no effect of treatment, $F(2, 12.835) = 2.298$, $p = .140$; Figure 4C), and this lack of and effect was similar for STs and GTs (no phenotype x treatment interaction, $F(2, 12.835) = .664$, $p = .532$).

Inactivation of the pDMS Decreases the Ability of GTs to Discriminate CS and Non-CS Periods

Similar to previous studies (Meyer et al., 2012; Saunders & Robinson, 2012), GTs made more NCS magazine entries – those made during the intertrial interval – relative to STs (effect of phenotype, $F(1, 23.147) = 5.882$, $p = .023$). While B/M administration had no overall effect on the number of NCS magazine entries (no effect of treatment, $F(1, 20.799) = .935$, $p = .345$), this manipulation had a differential effect on NCS entries in STs and GTs (phenotype x treatment interaction, $F(1, 20.799) = 5.317$, $p = .032$; Figure 5). Post hoc comparisons showed that B/M significantly increased the number of magazine entries GTs made during the NCS period ($p = .046$).

Dopamine Receptor Antagonism in the pDMS Does Not Affect Goal-tracking Outside of the CS Period

Flupenthixol also did not significantly influence the number of NCS magazine entries in STs or GTs (no effect of treatment, $F(2, 23.110) = 1.850$, $p = .180$; Figure 6), and produced not differential effect on STs and GTs (no phenotype x treatment interaction, $F(2, 23.110) = .179$, $p = .837$).

Evidence for an Anterior-Posterior Gradient for the Role of the pDMS in Sign-tracking

Figure 7 shows the % of vehicle lever contacts for individual STs plotted against the location of each rat's cannula placement along the anterior-posterior axis within the pDMS for each drug condition. For all drugs, there was a qualitative negative trend as placements became more anterior, but only the correlation for the 10 μg dose of flupenthixol was statistically significant ($r = -0.453$, $p = 0.033$). For B/M, the negative trending line suggests that there is less enhancement of sign tracking at more anterior placements within the pDMS. For flupenthixol, the negative trending lines suggest that at more anterior placements within the pDMS, there is greater suppression of sign tracking.

Dopamine Blockade in the pDMS and Nucleus Accumbens Core Has Dissociable Effects on the Expression of Sign-tracking

Saunders and Robinson (2012) recently found that administration of flupenthixol in the NAc core dose dependently reduces the expression of sign-tracking behavior during Pavlovian testing (Figure 8; data on the left reprinted from Saunders & Robinson, 2012). After blocking dopamine transmission in the current study, however, I so no such reduction in sign tracking (Figure 8, right side), suggesting that dopamine signaling in the pDMS in general is much less important for the expression of sign tracking than dopamine signaling in the NAc core. Together, these studies show a dissociation between ventral and dorsal striatal control of sign tracking behavior.

Histological Verification of Cannulae Placements

Figure 9 shows the location of the injector tips within the pDMS for STs and GTs. The majority of GTs had placements in the anterior portion of the pDMS, while ST placements were distributed throughout the pDMS.

Discussion

The purpose of the current study was to examine the role of the posterior dorsomedial striatum (pDMS), a region implicated in the expression of goal-directed instrumental behavior, in the expression of different forms of Pavlovian conditioned approach behavior – approach directed at the CS itself (sign-tracking) and approach directed at the location of reward delivery (goal-tracking). There were two primary findings. 1) I found that reversible inactivation of the pDMS had differential effects on sign and goal-tracking behavior. Administration of the GABA receptor agonists baclofen and muscimol (B/M), which results in a general inhibition of neuronal activity, significantly *increased* the number of times STs contacted the lever, relative to vehicle

administration. Conversely, B/M produced a *small decrease* in the number of times GTs entered the food magazine, relative to vehicle, though this result did not reach statistical significance, it was trending in the opposite direction as responding in STs. Furthermore, B/M significantly increased the number of non-CS (NCS) food magazine entries, those occurring in between each CS period, made by GTs, but not STs. 2) I also examined the effects of antagonizing dopamine receptors in the pDMS, via administration of flupenthixol, a nonselective dopamine receptor antagonist. Dopamine antagonism had no significant effect on the expression of either sign or goal-tracking. These results suggest that goal-tracking behavior may be more dependent on the functional integrity of the pDMS, relative to sign-tracking, and are consistent with the hypothesis that goal-tracking may be governed by a distinct psychological process, perhaps akin to that which controls goal-directed instrumental behavior. There are several important considerations to make in interpreting these data, particularly in the context of recent findings, which I will discuss below.

An Anterior-Posterior Gradient in the Dorsal Striatum

While inactivation of the pDMS slightly decreased goal-tracking behavior, this effect was not significant, which was unanticipated. It is possible that this can be explained by the location of the final drug injection site along the anterior-posterior axis within the pDMS. Given that Yin, Ostlund, Knowlton, and Balleine (2005) have reported anatomical dissociations for the DMS, where the pDMS mediates the acquisition and expression of *goal-directed* behavior, while the aDMS mediates the acquisition and expression of *habitual* behavior, it is important to keep this in mind when interpreting these results. Like most other studies looking at the pDMS, in the current study I aimed to have the cannula 0.4 mm behind Bregma, but defined any placement within the range of -0.8 mm to +0.2 mm as the posterior region of the DMS (see also Figure 7A).

However, while most studies aim for a cannulae placement approximately 1.0 mm in front of Bregma to target the aDMS, placements within the range of -0.26 to +1.6 mm also tend to be defined as the anterior region of the DMS (Pielock, Lex, & Hauber, 2011; Yin & Knowlton, 2004; Yin et al., 2005). Thus, there is some overlap in the anterior-posterior gradient, where the area just anterior to Bregma is sometimes categorized as either the anterior or posterior DMS.

Figure 9 illustrates that there was a bias in the location of the microinjections given to STs and GTs. Surgeries were completed before PCA training, and thus before a rat's phenotype emerged. By chance, GT cannula placements were clustered in more anterior locations, in terms of the anterior-posterior spread of injections sites, while ST placements were evenly distributed. While the cannula placements for the GTs are still considered to be located in the posterior region of the DMS, they are in the most anterior part of the posterior region and therefore positioned close to the aDMS/pDMS border. Given that the aDMS is known to be less important for goal-directed behavior, the relatively anterior anatomical location of the injection sites in GTs may explain why the effect of inactivation on goal-tracking was not larger. This suggests, therefore, that there would have been a more dramatic effect on goal-tracking if GT cannulae had been positioned in more posterior parts of the pDMS, and additional data is needed to confirm this hypothesis.

To further explore the notion that a functional anterior-posterior gradient within the DMS exists for its role in the expression of conditioned approach behaviors, I examined the more even distribution of cannula placements in the STs, looking at the correlation between the number of lever contacts made, expressed as a % of vehicle, and cannulae placement (organized posterior to anterior), for each rat and drug condition (Figure 7B). While the correlation was statistically significant only for the 10- μ g flupenthixol condition, the negative slope pattern was the same

for all drug conditions. When the pDMS was inactivated, for the B/M inactivation condition, as placements became more anterior within the pDMS, *less enhancement* of sign-tracking was seen. For the flupenthixol conditions, as placements became more anterior, *greater suppression* of sign-tracking was evident. Extrapolating the trend lines in Figure 7A to include more anterior parts of the DMS, I would predict that sign-tracking would be significantly impaired. Together this suggests that there may be an anterior-posterior gradient at play for the role of the DMS in the expression of sign-tracking behavior. Although a similar anatomical-behavioral analysis could not be done for the GTs in this study, if, as hypothesized, pDMS inactivation significantly impairs the expression of goal-tracking, it would suggest the interesting possibility of the existence of opposing gradients in the DMS for different types of *Pavlovian* conditioned approach behavior, similar to what has been seen for goal-directed versus habitual *instrumental* behavior.

It is unclear what accounts for these gradients, but the anterior and posterior portions of the DMS have different connectivity with other structures important for decision making and motivation (Alexander, DeLong, & Strick, 1986; Corbit & Janak, 2010; Haber, Kim, Mailly, & Calzavara, 2006; Parent & Hazrati, 1995; Robbins, & Pennartz, 2004; Voorn, Vanderschuren, Groenewegen). For example, the pDMS receives more inputs from the BLA than the anterior or lateral subregions of the dorsal striatum (Kelley, Domesick, & Nauta, 1982). Additionally, different regions of the PFC are thought to regulate the expression of habits versus goal-directed behavior based on different striatal projections (Balleine & O'Doherty, 2010). The PL area of the mPFC is responsible for sending a large amount of excitatory corticostriatal inputs to the DMS and mediodorsal thalamus (MD) (McGeorge & Faull, 1989). Both the PL and MD appear to be involved in only the acquisition of goal-directed behavior (Balleine & Dickinson, 1998; Corbit,

Muir, & Balleine, 2003; Killcross & Coutureau, 2003; Ostlund & Balleine, 2005). Given that there are connections between the MD and pDMS as well, one possibility is that a corticostriatal circuit between the PL, pDMS and MD may be critical for the acquisition of a goal-directed action, after which point a different circuit would mediate the expression of goal-directed actions (Nauta, 1989; Yin et al., 2005). Since the pDMS also receives inputs from the BLA, which has been implicated in determining the expected outcome value, another possibility is that the BLA and pDMS communicate to share knowledge of the expected outcome value with that of action-outcome contingency to accurately direct behavior (McGeorge & Faull, 1989; Yin et al., 2005). It has also been proposed that the BLA and OFC use reward expectancy information to work together and guide behavior (Holland & Gallagher, 2004), and these regions exhibit different connections with different parts of the dorsal striatum. Thus anatomical divisions within the dorsal striatum exhibit differences in functional connectivity to other regions that may have importance for goal-directed versus habitual behavior (Corbit & Janak, 2010), but much more research is needed to understand these differences.

The Role of Dopamine Signaling in ST and GT behavior

Using a very similar procedure as the one used in the present study, Saunders and Robinson (2012) found that dopamine blockade in the NAc core dose dependently reduced the expression of an ST CR. They proposed that dopamine in the NAc core was needed for the maintenance of incentive salience to the CS, which controls how attractive it is for STs. Alternatively, the results of the current study indicate that dopamine signaling in the pDMS is relatively unnecessary for the expression of a sign-tracking CR. This demonstrates that the effect of dopamine blockade in STs seems to be relatively selective to the NAc core and does not expand to the pDMS, suggesting that a dopamine-dependent mesolimbic circuit is primarily

responsible for this behavior. Figure 8 shows a side-by-side comparison of the effect of flupenthixol on sign-tracking behavior to illustrate the difference in the magnitude of the effect in these two brain regions. Interestingly, a recent paper found that after extended training (presumably when the behavior has become more habitual), sign-tracking behavior was associated with less dopamine release in the NAc core, and dopamine receptor antagonism produced a smaller attenuation of behavior when it was done late versus early in training (Clark, Collins, Sanford, & Phillips, 2013). It is unknown if dopamine signaling somewhere else in the brain is important for sign-tracking expression after extended training, but one possibility is the aDMS. Alternatively, in Saunders and Robinson (2012) the expression of a GT CR was not impaired as a result of dopamine blockade in the NAc core, and my results suggest that this may also be true for the pDMS (although, as noted above, manipulations in more posterior aspects of the pDMS are needed to be sure), consistent with the idea that goal-tracking is a relatively dopamine-independent process and suggesting that distinct psychological processes govern goal-tracking and sign-tracking.

Other differences between STs and GTs in the role of NAc core dopamine have also been found in studies looking at the ability of drug-associated stimuli to reinstate drug-seeking behavior (Saunders & Robinson, 2011a; Saunders, Aurbach, & Robinson, 2012). Under a conflict model of relapse, where rats had to cross an electric barrier in order to nose poke for a cocaine infusion, STs reinstated to noncontingent presentations of a discrete cocaine cue more than GTs (Cooper, Barnea-Ygael, Levy, Shaham, & Zangen, 2007). For STs, this effect was dependent on dopamine signaling in the NAc core (Saunders & Robinson, 2011a). Alternatively, in a separate experiment that examined the ability of a drug-associated *context* to reinstate drug-seeking behavior, the opposite results were found, where GTs renewed drug-seeking in the

context that had been paired with cocaine more than STs. In this case, the effect required dopamine transmission in the NAc core for GTs rather than STs. With a separate group of rats, they also found that GTs displayed a greater cocaine context conditioned hyperactivity than STs. Saunders, Aurbach, and Robinson (2012) propose that these differences in reinstatement are a result of the different types of drug cues (i.e. a discrete cue versus a contextual cue) differentially gaining motivational value in STs and GTs. What remains unclear it whether GTs attribute incentive salience to contextual drug cues, as might be indicated by the effect of NAc core dopamine blockade, similar to how STs attribute incentive salience to discrete drug cues. Thus, the psychological and neurobiological mechanisms responsible for the behavior of STs and GTs outside of the Pavlovian conditioned approach paradigm may be more complicated than previously thought.

The Psychological Processes Underlying Goal-Tracking Behavior

The results of the current study, which revealed that inactivation of the pDMS produced differential effects in the vigor of responding in STs and GTs, provides reason to believe that the pDMS may be more important for the expression of goal-tracking, relative to sign-tracking. This interpretation is consistent with my hypothesis that the expression of goal-tracking in GTs is guided by a process akin to a cognitive expectancy of reward based on an act-outcome relationship, which has been demonstrated in tests of instrumental responding in goal-directed behavior (see introduction). For GTs in the Pavlovian paradigm, this would imply that the presentation of the lever (CS) elicits an explicit representation of the food reward (US), which subsequently leads the rat to approach the food magazine (act) and wait to receive the expected reward (outcome). This is distinct from other hypotheses of GT psychology, such that they attribute the food magazine with incentive salience, similar to how STs attribute the lever with

incentive salience, and it is this process that motivates goal approach (DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009). Although we did not specifically test this theory here, the findings of other studies previously discussed indicate that mesolimbic dopamine does not seem to be necessary for the acquisition or expression of a goal-tracking CR (Flagel et al., 2011; Saunders & Robinson, 2012). This is significant seeing as mesolimbic dopamine has been found to play a central role in the attribution of incentive salience to rewards and reward cues and thus has the ability to make them wanted, which can ultimately lead to motivating behavior in order to obtain them (Berridge, 2007, 2012; Berridge & Robinson, 1998). Given that mesolimbic dopamine does not seem to be involved, it is possible that different psychological processes may govern GT approach behavior, and the behavior that occurs once GTs have arrived at the location of food delivery. While a reward expectancy process, which is not dependent on dopamine, controls GT approach, an incentive salience process could control their interaction with the magazine. Other neurotransmitter systems, such as endogenous opioids, may be involved in the expression of incentive salience in goal-tracking (DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009). Future studies may need to use different paradigms to examine the possible role of incentive salience in goal-tracking behavior.

Although there has yet to be direct evidence to demonstrate this, it is currently hypothesized that a “top-down” corticostriatal circuit mediates goal-tracking behavior during Pavlovian testing (Meyer et al., 2012; Saunders & Robinson, 2012). The results of this study support this idea in that the pDMS, relative to sign-tracking appears to be more involved in the expression of goal-tracking, however, due to the caveats mentioned above, the extent to which it is involved remains to be seen. For example, if goal-tracking is being driven by a cognitive expectancy of reward based on the act-outcome relationship, disentangling which aspect of goal-

directed behavior is controlled by the pDMS will be tricky. Given that the pDMS has been implicated in both outcome devaluation and contingency degradation instrumental studies, it is difficult to know which part(s) would be affected when the pDMS is manipulated during Pavlovian testing (Yin, Ostlund, Knowlton, & Balleine, 2005), and what aspect of goal tracking would be affected. In the current study, I found that the B/M condition resulted in a significant increase in food magazine entries GTs made outside of the CS period. This suggests that one effect of inactivation of the pDMS on GTs was to impair their ability to discriminate CS from non-CS periods, which could be interpreted as a disintegration of the act-outcome association. Thus, while goal-tracking responses to the CS were not significantly affected, the behavior of GTs became less tied to the CS, and thus potentially less goal directed, after inactivation of the pDMS. That pDMS inactivation increased sign-tracking behavior in STs, which could be interpreted as a shift to even less goal-directed behavior and more stimulus-responsive behavior, further supports the idea that the pDMS may regulate goal-directed behavior in a Pavlovian setting.

Future Directions

The next logical step in this line of research would be to replicate the current study, and also include multiple groups to better compare the effects of manipulation of different parts of the DMS along the anterior-posterior gradient. It would be important to have a group with cannula placements specifically targeting the aDMS, pDMS, and the intermediate zone near the anterior-posterior border for STs and GTs to determine whether or not those subregions do actually produce dissociable roles for the expression of sign and goal-tracking behavior. Depending on the results of those experiments, this study could then go in one of two directions. 1) Examining the role of the aDMS and pDMS in the acquisition, rather than the expression, of

sign and goal-tracking could allow stronger conclusions to be drawn. Given that the first eight days of Pavlovian testing are needed to classify each rat as an ST or GT, specifically measuring the acquisition of these behaviors using this procedure would not be possible. Although more involved, designing a study similar to one done by Flagel et al. (2011), using selectively bred rats, who's conditioned approach phenotype is known before any Pavlovian conditioning, would make a study on the role of the DMS in the acquisition of sign and goal-tracking more feasible (Stead et al., 2006). 2) It will also be important to examine potential ST and GT differences in traditional tests of goal-directed versus habitual behavior. For example, after rats are classified as STs or GTs upon completion of Pavlovian training, their behavior on a contingency degradation and/or outcome devaluation procedures could be tested to first test for baseline differences in the tendency for goal-directed behavior in an instrumental setting, and also to see the effects of aDMS and pDMS inactivation on these behaviors.

Conclusion

The overall findings of this study suggest that the pDMS is differentially involved in the performance of different forms of Pavlovian conditioned approach. My results are consistent with the hypothesis that overlapping corticostriatal circuits mediate goal-directed instrumental behavior and goal-tracking Pavlovian approach behavior, suggesting that goal-tracking may be governed by a cognitive reward expectation process, though important future studies are needed to more directly test this prediction. The extent to which these mechanisms guide GT behavior in other testing situations also remains to be known. It is likely that different psychological and neurobiological mechanisms may be underlying sign-tracking and goal-tracking behavior as a result of the specific type of reward cues present and particularly, which ones are attributed with incentive salience. One idea that seems to be consistent across all of the studies previously

discussed is that STs and GTs behavior is driven by two distinct psychological processes. Future studies will need to exploit the known differences between STs and GTs and continue to piece these mechanisms together in hopes of understanding individual differences in reward seeking and how they contribute to impulse control disorders such as addiction.

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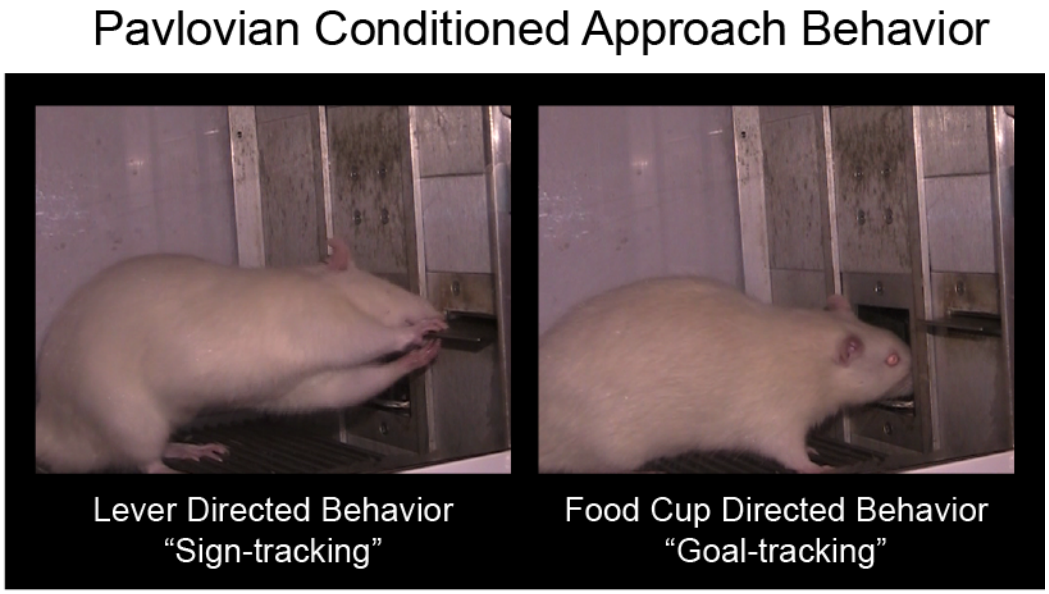
First and foremost, I would like to thank my advisor, Dr. Terry Robinson, for not only allowing me to work in his lab, but also for the opportunity to gain exposure to this world of research, a world that I had not previously discovered. Spending the past two and a half years in his lab has made my experience as an undergraduate at the University of Michigan extremely fulfilling and for that, I am very appreciative. I would also like to thank Dr. Ben Saunders for allowing me to help him with his own research and especially for being my mentor throughout this entire project. He has taught me countless skills and techniques, guided me through each step of the writing process, and provided encouragement whenever it was needed. I would not have been able to accomplish this without him.

I owe a special thanks to Lindsay Yager for her willingness to assist and troubleshoot the various computer programs, especially once I was on my own. Additionally, I would like to thank Lindsay Yager, Liz Cogan, Vedran Lovic, and Kyle Pitchers for their constant support and effort to always make the Robinson lab a productive and welcoming atmosphere. I would also like to thank Curtis Austin for being my writing partner during the past few weeks and for making the late nights in East Hall much more bearable.

Finally, I would like to thank my two best friends, Shannon Hittson and Jacquie Hinds, along with my parents and siblings, for their constant love and support and for their efforts to help me to put things in perspective. I can't even begin to express how grateful I am for them.

A



B

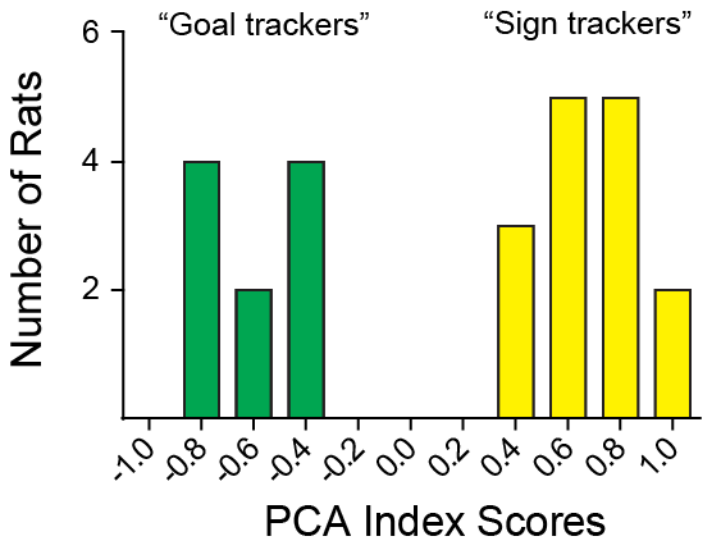


Figure 1. PCA index distribution. 1A shows a rat interacting with the lever (i.e. exhibiting a sign-tracking CR) on the left and a rat entering the food magazine (i.e. exhibiting a goal-tracking CR) on the right. For 1B, moving from left to right, the bars show the individual variation in behavior that was food magazine directed versus behavior that was lever directed based on each rat's PCA index score.

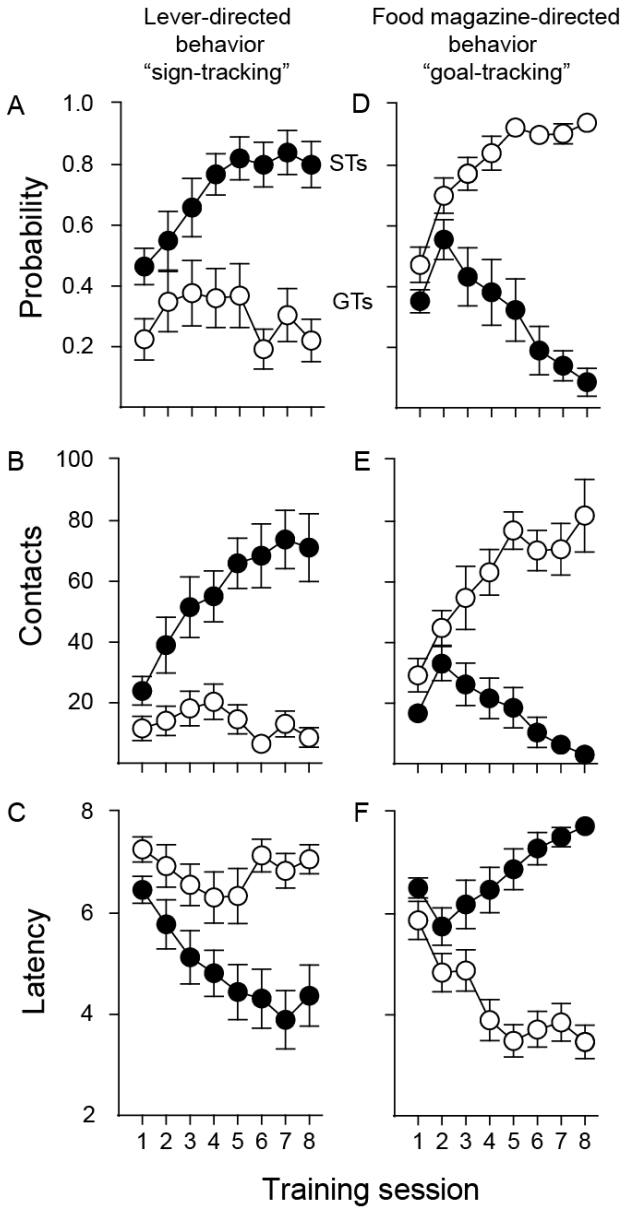


Figure 2. PCA training behavior. Open circles represent GTs and closed circles represent STs. The mean ± SEM is reported for all of the data. Each graph illustrates the behavior of STs and GTs for one of the three measures of PCA behavior across the eight days of training. 2A shows the probability of contacting the lever. 2B shows the number of lever contacts made. 2C shows the latency to the first lever contact. 2D shows the probability of entering the food magazine. 2E shows the number of food magazine entries. 2F shows the latency to the first food magazine entry.

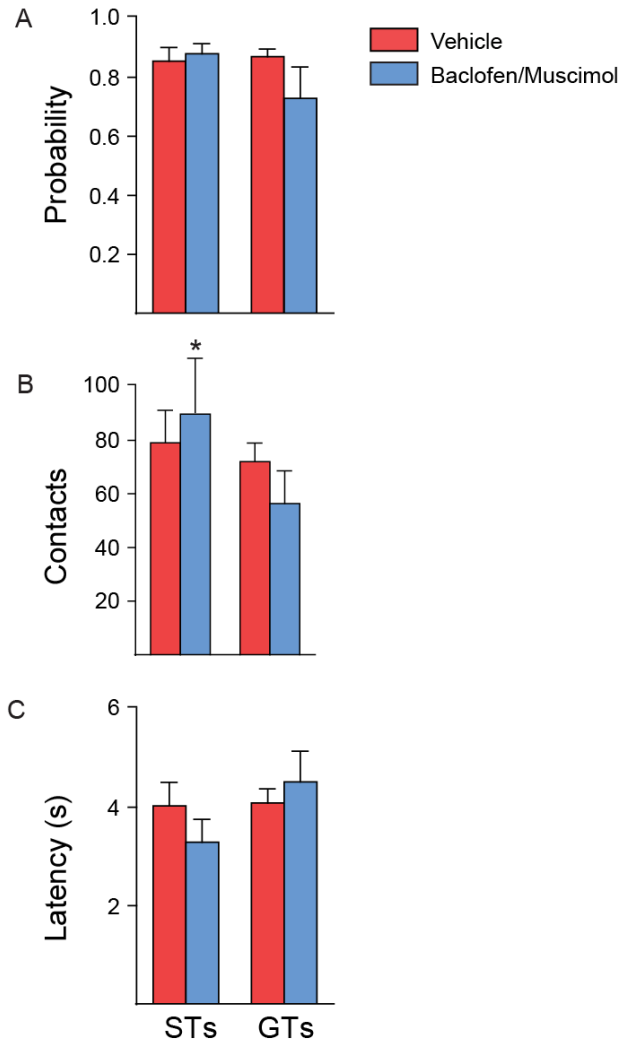


Figure 3. Note. * = $p < 0.05$

Effects of B/M on STs and GTs. The red bars represent the behavior of STs and GTs with a vehicle injection prior to Pavlovian testing and the blue bars represent their behavior with a B/M injection. Each bar represents the mean ± SEM. 3A shows the probability of STs contacting the lever on the left and the probability of GTs entering the food magazine on the right. 3B shows the number of lever contacts made by STs on the left and the number of food magazine entries made by GTs on the right. B/M administration significantly increased the number of contacts in STs. 3C shows the latency to the first lever contact for STs on the left and the latency to the first magazine entry for GTs on the right.

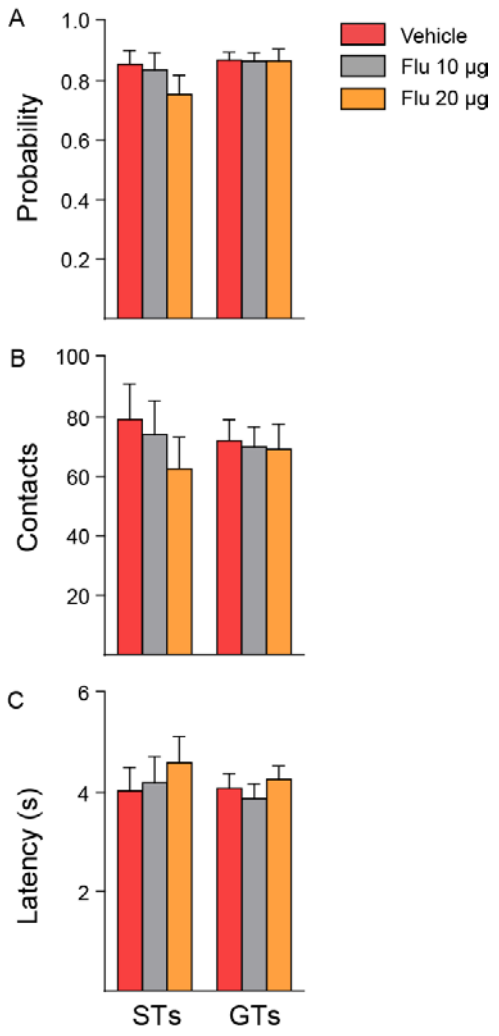


Figure 4. Effects of flupenthixol on STs and GTs. The red bars represent the behavior of STs and GTs when they received a vehicle injection, the grey bars represent their behavior when they received a flupenthixol injection of 10 µg, and the orange bars represent their behavior when they received a flupenthixol injection of 20 µg before the start of testing. Each bar represents the mean ± SEM. 4A shows the probability of STs contacting the lever on the left and the probability of GTs entering the food magazine on the right. 4B shows the number of lever contacts made by STs on the left and the number of food magazine entries made by GTs on the right. 4C shows the latency to the first lever contact for STs on the left and the latency to the first magazine entry for GTs on the right.

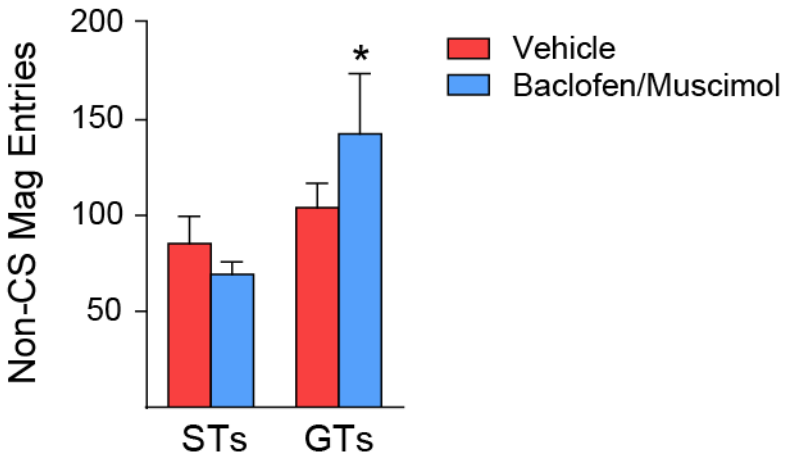


Figure 5. Note. * = $p < 0.05$

Effect of B/M on NCS magazine entries. The red bars represent the number of magazine entries made in between each CS period by STs and GTs after receiving a vehicle microinjection. The blue bars represent the number of magazine entries made in between each CS period by STs and GTs after receiving a microinjection of B/M. Each bar represents the mean ± SEM. B/M administration significantly increased the number of NCS magazine entries in GTs.

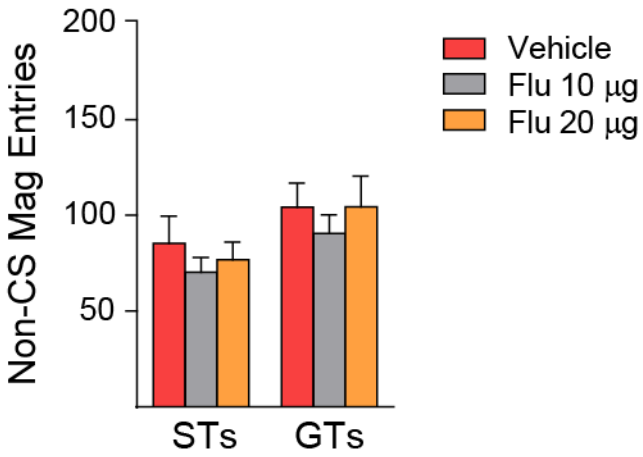


Figure 6. Effect of flupenthixol on NCS magazine entries. The red bars represent the number of magazine entries made in between each CS period by STs and GTs after receiving a vehicle microinjection. The grey bars represent the number of magazine entries made in between each CS period by STs and GTs after receiving a flupenthixol microinjection of 10 µg. The orange bars represent the number of magazine entries made in between each CS period by STs and GTs after receiving a flupenthixol microinjection of 20 µg. Each bar represents the mean ± SEM.

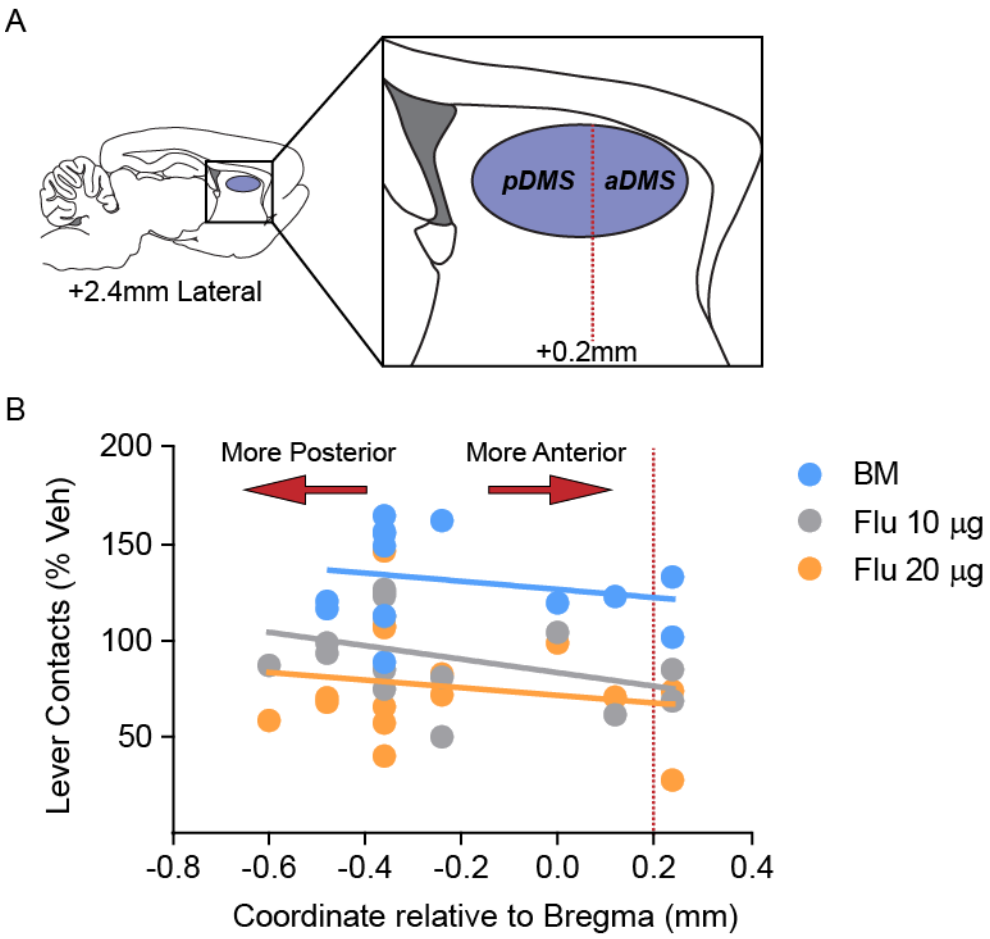


Figure 7. Correlation between coordinates and number of lever contacts in STs. 7A shows a sagittal view of the DMS to highlight the anatomical locations of the anterior-posterior gradient. The line down the middle represents +0.2mm in front of Bregma, an area that is right on the border of the anterior and posterior DMS. 7B shows the correlation between the coordinate of the injection site on the x axis and the number of contacts made expressed as a % of vehicle in STs on the y axis.

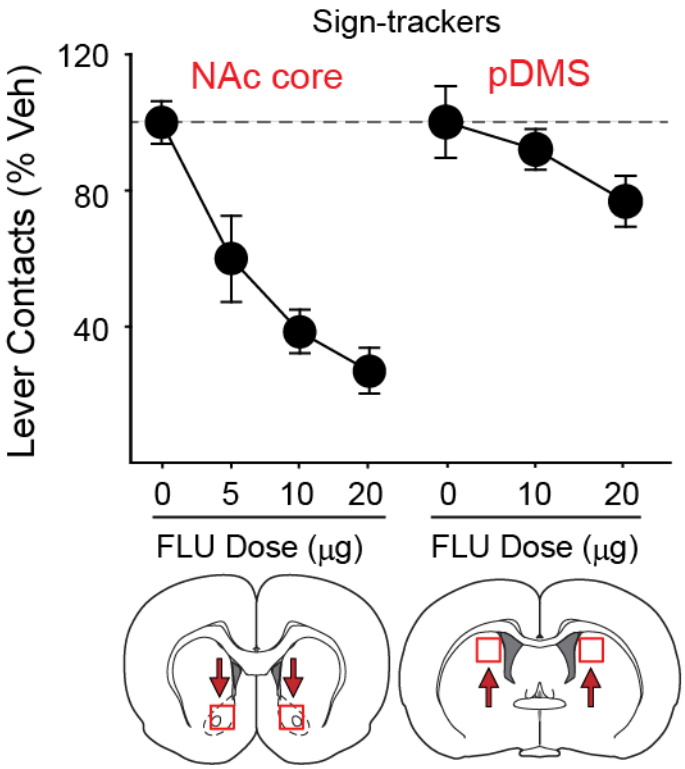


Figure 8. Dopamine signaling in the NAc core (reprinted from Saunders & Robinson, 2012) and pDMS. The filled circles illustrate the effect of dopamine antagonism on the expression of sign-tracking behavior in the NAc core and pDMS, respectively. The red boxes in the coronal sections show the location of each region. While data presented earlier showed the statistical analysis for the raw number of contacts made, here the data are expressed as a % of vehicle. Each circle represents the mean ± SEM.

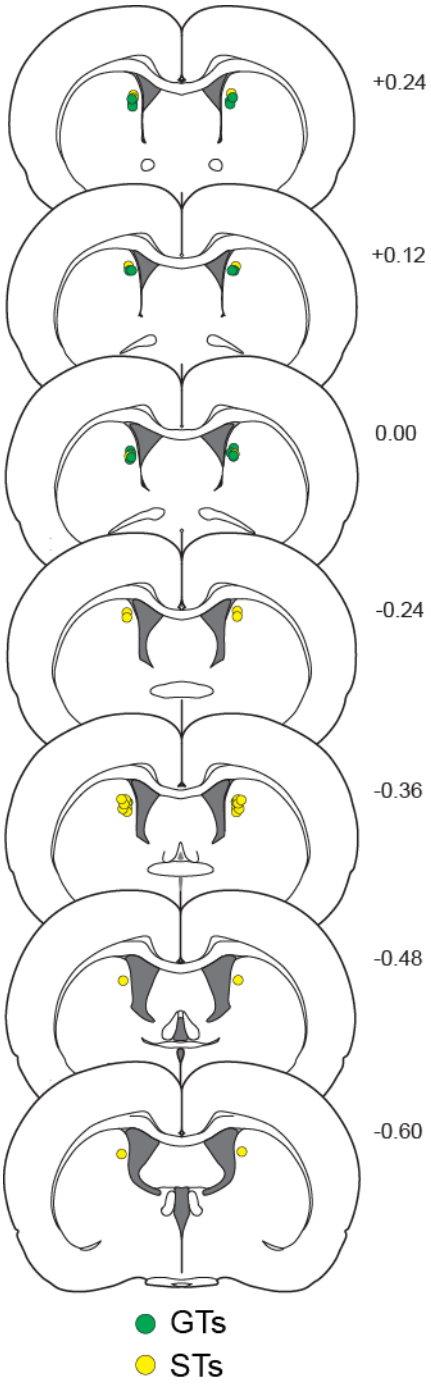


Figure 9. Location of microinjection tips within the pDMS relative to Bregma. Yellow circles represent STs and green circles represent GTs.