Dorsolateral striatum implicated in the acquisition, but not expression, of immediate response learning in rodent submerged T-maze

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ABSTRACT

Animals can use multiple strategies when learning about, and navigating within, their environment. Typically, in the frequently-studied food-rewarded T-maze, rats initially adopt a flexible, hippocampal-dependent place strategy. However, as learning progresses, rats switch to an automatic, striatal-dependent response strategy (Packard & McGaugh, 1996). Interestingly, in a similar but aversively motivating water-submerged T-maze, rats exhibit the opposite behavioral pattern, initially adopting a response strategy but switching to a place strategy with extended training (Asem & Holland, 2013). Here, we examined the effects of transient lidocaine inactivation of the dorsolateral striatum (DLS) on rats' acquisition and expression of place and response strategies in the submerged T-maze. DLS inactivation prior to probe tests had no effect on rats' initial expression of a response strategy nor on their transition to the use of a place strategy with further training. Nevertheless, in a second experiment using the same rats, identical inactivation parameters significantly affected performance in an appetitively motivating positive control task, which required a response strategy. Furthermore, in a third experiment, DLS inactivation prior to early learning trials interfered with the acquisition of the response strategy in the submerged T-maze. These differences in DLS inactivation effects across appetitive and aversive tasks support the view that task motivation plays crucial roles in guiding learning, memory, and behavior. Additionally, differences in DLS inactivation effects between tests of acquisition and expression suggest that the DLS is required during early acquisition but not expression of the response learning strategy.

Keywords: spatial; place; response; striatum; habit

For decades, scientists have investigated different types of learning, such as flexible relational memory and less flexible stimulus-response (S-R) learning. These differing types of learning have been thoroughly investigated in the field of spatial navigation as “place” and “response” strategies; the place strategy has been discussed as relational and spatial learning, whereas the response strategy as being symptomatic of S-R and habit learning. For example, in the “dual-solution” maze paradigm, rats are trained to find either a food reward or an escape platform in one arm of a dry or submerged T-maze, respectively (Tolman & Gleitman, 1949; Tolman, Ritchie, & Kalish, 1946; Asem & Holland, 2013). The animal can solve the task by employing either a place or response strategy, the relative usage of each assessed by interspersed one-trial probe tests (see Asem & Holland, 2013, for details).

In the dry T-maze, rats switch from a hippocampal-dependent place strategy to a striatal-dependent response strategy (Packard & McGaugh, 1996). However, in the submerged T-maze, we observed the opposite behavioral pattern—rats initially used a response strategy and gradually switched to a place strategy (Asem & Holland, 2013). Due to a complete inversion of previous behavioral results, the underlying neural circuitry and the characterization of strategy shifts over training in the submerged T-maze warrants investigation.

There are a number of ways by which the hippocampal and striatal systems may be dissociated. First, perhaps they are dissociable by the type of learning—the hippocampus subserves place learning (Packard & McGaugh, 1996; McDonald & White, 1993; 1994) and the striatum subserves response learning (Packard & McGaugh, 1996), regardless of the amount or stage of learning. Second, they may be

dissociable by the amount of learning—the hippocampus is recruited for early or immediate learning (Camarota et al., 2000; Chang & Gold, 2004; Izquierdo et al., 2000; Rutishauser et al., 2006), followed by a gradual recruitment of cortical and subcortical structures, regardless of the behavioral phenotype. Third, and finally, there may be differences in the stage of learning: many studies indicate the critical role of the hippocampus and striatum in the expression of these strategies (McDonald & White, 1993; Packard & McGaugh, 1996), but fewer have tested such roles in their acquisition.

Here, we investigated the role of the dorsolateral striatum (DLS) in the submerged T-maze. In Experiment 1, we used a within-subject design to examine the effects of DLS lidocaine or saline infusions on performance in probe tests, interspersed after varying amounts of drug-free acquisition training. We observed no effect of drug condition on the behavior exhibited during probe tests, either early or late in training, suggesting that DLS function was not required for the expression of either response or place strategies. In Experiment 2, we confirmed the effectiveness of the infusion protocol in a positive control task with the same subjects. In Experiment 3, we used a between-subject design to examine the effects of DLS lidocaine or saline infusions made prior to training sessions in the submerged T-maze. Lidocaine infusions slowed acquisition very early in training, and had marginally significant effects on performance in some of the drug-free probe tests. In combination, these experiments suggest that the DLS is involved in the initial acquisition of the immediate response strategy, but is not critical to the expression of that strategy once it is acquired.

EXPERIMENT 1

1.1 Methods and Materials

1.1.1 Subjects

Twenty-nine male Long-Evans rats (Charles River Laboratories; Raleigh, NC) were housed individually in a temperature-controlled room, with lights on 7 A.M. to 7 P.M, and with free access to food (Teklad Chow 2018, Harlan Laboratories; Madison, WI) and water. At the beginning of the experiment, weights of the rats ranged from 395 to 450 g.

1.1.2 Apparatus

The water tank (h = 57 cm, d = 173 cm) was a circular, galvanized steel tank whose innards were painted an opaque white. A suspended circular black curtain (h = 180 cm) with salient white extra-maze cues completely surrounded the tank and protruded 3 cm from the side of the tank. The T-maze consisted of a 4-arm Plexiglas plus maze placed inside the water tank. The four arms (North, South, East, and West) were each 72.5 cm long and 20 cm wide. Removable Plexiglas inserts were used to block access to individual arms when necessary.

One day prior to animal exposure, the tank was filled with water (depth = 51.5 cm) and maintained at a temperature of 27 ± 1 °C (80.6 ± 1.8 °F). Soluble, non-toxic, white Premium Tempura paint (Blick Art Materials; Galesburg, IL) was added to the water in order to obscure the location of the escape platform (h = 50 cm, d = 12 cm). The foremost edge of the platform was positioned 5 cm from the side of the tank and 1.5 cm below the surface of the water.

1.1.3 Surgery

Subjects were anesthetized with 2-3% isoflurane (Piramal Critical Care, Inc., Bethlehem, PA, USA) mixed with oxygen and placed into the stereotaxic apparatus (Model 902, Kopf, Tijuana, CA, USA). After incision and craniotomy, two 1/8-inch self-tapping mounting screws were installed into the skull. The dura was punctured with a 27-gauge needle, and 26-gauge stainless steel guide cannulae (PlasticsOne, Roanoke, VA, USA) were implanted bilaterally into the dorsolateral striatum. Coordinates for these placements were AP = - .26 mm, ML = $\pm$ 4.2 mm, DV = - 2.0 mm.

The guide cannulae were anchored with dental acrylic and fitted with dummy injectors, to ensure cannulae patency, which were cut to match the length of the guide cannulae. The incision was closed with surgical staples and topical antibiotic ointment was applied to the wound. A single subcutaneous injection (0.03 mg/kg) of sterile buprenorphine HCL (Sigma, St. Louis, MO, USA) was administered to ameliorate pain. Rats were weighed and handled during post-operative care, including removal and replacement of dummy cannulae to reduce stress during later infusions. Behavioral procedures began approximately 10 days after surgery.

1.1.4 Infusions

Dummy injectors were removed and reinserted before each training session, to familiarize them with manipulation of their headsets. Two 33-gauge injector cannulae that extended 2 mm below the tip of the guides (to a depth of 4.0 mm below the brain surface) were connected by polyethylene (PE50) tubing to separate 10 μ l Hamilton microsyringes in a multiple-syringe pump (KD Scientific, Holliston, MA, USA). The pump simultaneously administered 0.5 μ l of either 2% lidocaine hydrochloride solution (Vedco, Inc., St. Joseph, MO, USA) or 0.9% saline to the DLS in each hemisphere, over 1 min.

The volume of 2% lidocaine solution used was chosen on the basis of previous evidence indicating that this volume produces functional inactivation of the striatum sufficient to cause memory impairment (Perez-Ruiz & Prado-Alcala, 1989; Packard & McGaugh, 1996). After infusion, the injector was left in place for an additional 1 min to allow for diffusion of the solution away from the needle tip. The dummy injectors were reinserted after removal of the injectors.

1.1.5 Histology

Histological procedures designed to identify the injector sites were conducted after completion of Experiment 2. The rats were deeply anesthetized with isoflurane and perfused intracardially with 0.9% saline followed by a 3.7% formalin solution. After removal of the headset, brains were removed and stored at 4°C in a 3.7% formalin + 12% sucrose solution. Brains were sliced on a freezing microtome and 40-µm coronal sections were taken in series.

To confirm cannulae tip placements, every third section was mounted on glass slides, dehydrated in ascending concentrations of alcohol, defatted in xylene, and stained with thionin. Slides were coverslipped using Permount, thinned with xylene, and examined with a light microscope.

1.2 Behavioral Testing

1.2.1 Habituation

Access to the North arm was blocked with a Plexiglas insert during habituation and subsequent training trials. Rats were placed in the South (Start) arm and allowed to explore the maze, without the escape platform, for 2 min. All rats received two consecutive trials and were placed facing the interior of the maze and then facing the

wall of the maze, respectively. The experimenter recorded the initial turning preference of each rat as well as whether it explored both goal arms; this information was used to counterbalance rats for training sessions.

1.2.2 Training

Training sessions began 24 hours after habituation. The escape platform was placed in either the East or West (Goal) arm, counterbalanced by habituation performance, such that the training platform was placed in the preferred arm for half of the rats and the non-preferred arm for the other half. On each trial, rats were placed in the Start arm, facing the wall, and allowed to traverse the maze to locate the escape platform in the Goal arm. A correction procedure was applied such that rats making errors were allowed to subsequently enter the trained arm and find the escape platform. Entries into the untrained arm and re-entries into the Start arm were scored as errors. Each rat received two trials per day, for 24 total training sessions.

Rats were required to remain on the escape platform for 10 sec before removal from the maze. Immediately after removal, each rat was towel-dried and placed in a holding cage. The rats were run in squads of 6, such that each rat was given its first trial before any rat received its second trial (McDonald & White, 1994). Thus, the intertrial interval (ITI) depended on the performance of all rats in a squad; these intervals ranged from approximately 2 min to 8 min during early training (Sessions 1-4) and 1 min to 3 min during later training (Sessions 5-24).

1.2.3 Probe Tests

After each of four stages of training (early, intermediate, late, and final), rats received one probe test after lidocaine and one after saline infusion. Thus, each rat

could serve as its own control for the evaluation of the effects of the drug. We limited our probes to these samples to minimize the number of infusions needed, and to minimize potential permanent damage to the DLS. Rats in Group 1 received lidocaine on the first of each pair of tests and saline on the second, whereas rats in Group 2 received the infusions in the opposite order within each probe pair. Rats in these two groups were matched for mean latency and number of errors over the first two training sessions. Probe tests were given after training sessions 2 and 4 (early), 10 and 12 (intermediate), 18 and 20 (late), and 22 and 24 (final).

As in training, the rats were run in squads of 6, such that all rats in a squad were infused before maze exposure. Each squad contained rats from both Groups 1 and 2. The order of infusions (lidocaine or saline) was counterbalanced across squads. In the first replication, the delays between infusion and exposure to the maze ranged from 2 to 12 min, and in the second replication, an additional 10-min delay was inserted between infusion and testing (12-22 min delay). This additional 10-min delay was introduced in the second replication to better match the infusion-performance delays in Experiment 2, and to explore the infusion parameter space.

Each probe test consisted of a single trial. On this trial, no escape platform was available in either arm and access to the South (Start) arm was blocked using a Plexiglas insert. Rats were placed in the North arm (opposite the Start arm) and were allowed to choose between the previously trained or untrained arm. We reported the first arm entered by each rat. Rats approaching the reinforced extra-maze cues and entering the previously trained arm were designated as “place learners,” whereas rats producing the reinforced response and entering the previously untrained arm were

designated as “response learners.” Upon reaching the end of the chosen arm, rats were removed from the maze, towel-dried, and returned to their home cages.

1.2.4 Data Analysis

An overhead camera and computer-assisted tracking system recorded each rat's position in the maze. An experimenter recorded errors accrued and latency to reach the platform (training sessions) or the goal location, the area that the platform typically occupies (probe tests). These measures were analyzed with mixed-design 2 x 2 x 2 x 24 ANOVAs, with between-subject variables Replication (no delay, delay), and the two counterbalancing variables, Group (1 or 2) and Goal Arm (East or West), with repeated measures on Training Day. Similar ANOVAs were used to analyze latency on Probe Days. All ANOVAs used the Greenhouse-Geisser correction for violations of sphericity.

For each probe test, we determined the probability of observing the recorded number of “response learners” in each group, based on the binomial distribution. We used a Chi-squared test to compare the proportions of response learners in rats tested under lidocaine versus saline conditions on individual probe tests, and a sign test to compare these proportions within-subjects on pairs of probe tests. We also analyzed the change in probe test performance over the entire course of testing using mixed-design 2 x 2 x 2 x 2 x 4 ANOVAs, with between-subject variables Replication (no delay, delay), and the two counterbalancing variables, Group (1 or 2) and Goal Arm (East or West), with repeated measures on Drug (lidocaine or saline) and Probe Pair (early, intermediate, late, final).

1.3 Results

Five of the rats lost their cannulae headsets prior to completing the experiment and were dropped from the study, leaving 12 rats in each of Groups 1 and 2, equal representation of the two goal arm locations in each group, and 16 rats with no delay and 8 rats with a delay. Figure 1 shows the cannulae tip locations; they ranged between +0.20 mm and -0.40 mm AP. Five subjects showed small unilateral lesions near the site of the guide cannula; data from these rats were included in our data analysis.

1.3.1 Acquisition

During training, we observed rapid acquisition, with substantial improvement over the first three sessions and asymptotic mean number of errors and latency to the platform after six training sessions (Figures 2A and 2B). A repeated measures ANOVA across Training Day reported a significant decline in the mean number of errors ($F(23,368) = 5.304, p = .002$) and mean latency to reach the platform ($F(23,368) = 3.926, p = .023$).

There was no effect or interaction of Replication or any counterbalancing variable (Group, Goal Arm) across Training Day or with each other for either mean number of errors ($F(23,368) < 2.039, p > .114, F(1,16) < 1.979, p > .179$) or mean latency ($F(23,368) < 2.234, p > .114, F(1,16) < 2.563, p > .129$) except a Replication x Goal Arm interaction on mean number of errors ($F(1,16) = 7.137, p = .017$).

1.3.2 Probe Tests (Strategy Selection)

Across the interspersed probe tests, we observed the behavioral pattern expected on the basis of Asem and Holland's (2013) findings, with rats switching from response learning to place learning. However, this pattern was not affected by lidocaine infusions.

On the first probe test, 18 of 24 rats ($p = .011$) entered the previously untrained arm and were designated as response learners. Of these 24 rats, 10 of 12 rats that had received lidocaine ($p = .019$) and 8 of 12 rats that had received saline ($p = .194$) prior to this probe test were response learners. There was no significant difference between these proportions ($X^2(1, n = 24) = .889, p = .346$). Similarly, within the Early probe pair, 18 of the 24 rats ($p = .011$) showed the response strategy on their saline test, and 17 of the 24 rats ($p = .032$) showed this strategy on their lidocaine test. There was no significant difference between these proportions (sign test, $z = 0, p = 1$).

On the final probe test, 16 of 24 rats ($p = .076$) entered the previously trained arm and were designated as place learners. Eight of the 12 rats in each drug condition ($ps = .194$) were place learners. There was no significant difference between these proportions ($X^2(1, n = 24) = 0, p = 1$). Similarly, within the Final probe pair, 12 of the 24 rats ($p = .581$) showed the place strategy on their saline test, and 16 of the 24 rats ($p = .076$) showed this strategy on their lidocaine test. There was no significant difference between these proportions (sign test, $z = .617, p = .537$).

This gradual switch from a response strategy to an (albeit nonsignificant) place strategy was confirmed by a repeated measures ANOVA. Although ANOVA is often considered inappropriate for dichotomous data, D'Agostino (1971) found that, with adequate degrees of freedom, and when mean cell proportions range between .25 and .75 (as was the case here), the assumptions of ANOVA are not violated. This ANOVA showed a significant effect of Probe Pair ($F(3,48) = 6.276, p = .003$), but no effect of Drug ($F(1,48) = .574, p = .460$) nor a Drug x Probe Pair interaction ($F(3,48) = .992, p = .399$). Furthermore, there was a significant linear trend over Probe Pairs ($F(1,16) =$

10.070, $p = .006$) (Figure 3A). Additional analyses showed that the simple main effects of Probe Pair were significant for both lidocaine ($F(3,48) = 3.636$, $p = .028$) and saline ($F(3,42) = 5.920$, $p = .002$) tests (Figure 3A). Likewise, there was a significant linear trend over Probe Pairs for both Drug ($F(1,16) = 7.376$, $p = .015$) and Saline ($F(1,16) = 8.540$, $p = .010$) tests, suggesting a similar decrease in the use of a response strategy when tested under either condition. There were no effects or interactions involving Replication or any counterbalancing variables (Group, Goal Arm) on performance ($ps > .079$).

1.3.3 Probe Tests (Latency)

Not only was there no effect of Drug on strategy choice during probe tests, a repeated measures ANOVA reported no effect of Drug on latency to reach the goal location ($F(1,48) = .262$, $p = .616$), no effect of Probe Pair ($F(3, 48) = 1.530$, $p = .236$), and no Drug x Probe Pair interaction ($F(3,48) = .359$, $p = .700$) (Figure 3B). Likewise, there was no significant linear trend across Probe Pair ($F(1,16) = 2.196$, $p = .158$), nor of the effect of Drug ($F(1,16) = .262$, $p = .616$), nor of the Drug x Probe Pair interaction ($F(1,16) = .011$, $p = .919$). Additional separate analyses confirmed that there was no change in latency to the goal location by Probe Pair when tested with either lidocaine ($F(3,48) = 1.399$, $p = .262$) or saline ($F(3,48) = .758$, $p = .457$). Finally, there were no significant effects or interactions involving any counterbalancing variables ($ps > .235$).

These probe test latencies were comparable to those observed on contemporaneous training sessions (Figure 2A). The lack of a drug effect on the mean latency to the goal location during probe tests indicates that the lidocaine infusions did not affect general motor movement.

The probe test data are consistent with Asem and Holland's (2013) observation that, in the submerged T-maze, rats exhibit a response strategy at the beginning of training and switch to a place strategy with extended training. Importantly, we found that, unlike previous results from appetitive tasks (McDonald & White, 1994; Packard & McGaugh, 1996), the expression of a response strategy was unaffected by temporary bilateral DLS inactivation by lidocaine infusion.

EXPERIMENT 2

Because we observed no effect of lidocaine infusions in Experiment 1, proper interpretation of the results requires demonstrating disruption by the same drug in a task known to depend on DLS function. In principle, we could have simply replicated Packard and McGaugh's (1996) experiment with the rats of Experiment 1; however, this approach is unreasonable because we might expect substantial transfer between submerged and dry versions of the task used in Experiment 1. Instead, we examined the effects of identical infusions on performance of these same rats in another spatial task previously found to require DLS for acquisition of a response strategy (Chang & Gold, 2004).

In this task, rats placed in a plus maze are given food reward for making the same response (turning right or turning left, counterbalanced) when started from various maze arms. Rats appear to be intrinsically biased toward using spatial cues in this task; even control rats in Chang and Gold's (2004) study were extremely poor at solving this task unless the environment had minimal spatial cues and dim lighting (cue-available vs. cue-poor conditions). However, rats that received DLS lidocaine infusions were much slower to acquire correct performance, even in the cue-poor condition (Chang & Gold, 2004).

2.1 Materials and Methods

2.1.1 Subjects

The subjects were the rats used in Experiment 1. After 2-3 days rest, they were deprived to 85% of their maximum weight; their 85% weights ranged from 390 to 490

grams. Next, rats were pre-exposed to sucrose pellets (45 mg, Formula 5TUT, Test Diets, Richmond, IN, USA) in their home cage until consumption was verified.

2.1.2 Apparatus

The plus maze was removed from the tank and a Plexiglas floor was added, giving it walls that were 12 cm high. An aluminum reward cup ($h = 2$ cm, $d = 6$ cm) was secured at the end of each arm. The Plexiglas maze was placed in a different experimental room, stripped of as many extra-maze cues as possible. Due to poor acquisition in the first replication, the second replication included black cloth hung from the ceiling to obscure any immovable cues present in the room, producing a more cue-poor environment as suggested by Chang and Gold (2004).

The room was dimly lit, illuminated only by a small lamp with a single red lightbulb, which was initially located behind the North arm. In the first replication, this lamp was moved to the South arm after every block of 10 trials of training so as to not provide a predictive cue. In the second replication, the lamp was moved from the SouthWest quadrant to the SouthEast quadrant after every block of 10 trials. The experimenter and cart (containing the holding cage and sucrose pellets) were located directly behind the Start arm for each trial (first replication) or moved behind North and South arms after every block of 10 trials (second replication).

2.1.3 Infusions

Infusions were administered as in Experiment 1 except that rats were trained one at a time, with the single training session beginning immediately after the infusion. Training sessions lasted 50-75 min, depending on the rat's performance in attaining criterion.

2.2 Behavioral Testing

2.2.1 Habituation

All rats were habituated to the experimental conditions on the same day. Rats were started from the South arm and exposed to the maze for 2 min. Four sucrose pellets were spaced apart in each arm as well as four pellets in each aluminum cup, secured at the end of each arm. The experimenter recorded the order of explored arms. Matched by previous trained arm and drug experience from Experiment 1, as well as their Experiment 2 habituation performance, rats were divided into four conditions, determining their rewarded response (turning left or right) and new drug condition (lidocaine or saline).

2.2.2 Training

Training began 24 hours after habituation. Each rat received an infusion (lidocaine or saline) immediately prior to its single maze training session. Rats were started from each of the four arms, in a random order, for 60 trials, divided into six 10-trial blocks. The rewarded arm was always the left or right (counterbalanced) arm from the Start arm and was baited with four sucrose pellets, placed inside the aluminum cup. Approximately 2-4 rats were run each day until all rats were run (~7 days).

There was no correction procedure: rats were removed from the maze after producing an incorrect response. If correct, rats were allowed 10 sec to consume the reward; any remaining pellets were placed in the holding cage for the rat to consume during the ITI, which was 30-60 sec. A rat “timed out” if it did not leave the Start arm after 2 min and was removed from the maze. Similar procedures have been shown to produce striatal-dependent learning (Chang & Gold, 2004).

2.2.3 Data Analysis

An experimenter recorded the number of correct trials as well as the rat's latency to reach the reward cup on correct trials. These measures were analyzed with mixed-design 2 x 2 x 2 x 6 ANOVAs, with between-subject variables Replication (curtains or no curtains), Drug (saline, lidocaine), and one counterbalancing variable, Response (right, left), with repeated measures on Block (1-6, consisting of 10 trials each). All ANOVAs used the Greenhouse-Geisser correction for violations of sphericity.

Analyses involving the latency to reach the reward cup on correct trials included fewer subjects ($n = 13$) because of subject elimination due to incomplete data. Several subjects had a lack of a value for mean latency on correct trials in one or more blocks due to a lack of any correct trials in that block. In these analyses, there are Lidocaine rats from the second replication ($n = 4$) and Saline rats from both replications ($n = 5$, $n = 4$, respectively). Although unfortunate, these eliminations provided a conservative comparison between drug conditions.

2.4 Results

Lidocaine rats showed significantly impaired acquisition of the response strategy compared to saline controls. Notably, even the control subjects performed poorly, supporting the contention that rats are predisposed to spatial solutions of this task.

2.4.1 Acquisition (Trials to Criterion)

A simple approach to determine the acquisition of the task is to compare the subject's performance to a standard, such as the number of successful trials performed consecutively. We selected a liberal criterion of 3 or more consecutive trials performed correctly in a block of 10 trials.

By this standard, 3 of 12 lidocaine rats and 11 of 12 saline rats acquired the task. These proportions are significantly different from each other ($\chi^2(1, n = 24) = 10.971, p < .001$), suggesting that, even with a liberal standard for acquisition, rats receiving lidocaine infusions to the DLS were severely impaired in solving the task. This conclusion was corroborated by a univariate analysis showing a significant effect of Drug on the highest number of correct consecutive trials per subject across all blocks ($F(1,17) = 39.099, p < .001$) (lidocaine: mean = 1.988, SEM = .207; saline: mean = 3.842, SEM = .213) and on the total number of correct trials ($F(1,17) = 28.106, p < .001$) (lidocaine: mean = 10.363, SEM = 1.287; saline: mean = 20.150, SEM = 1.324).

2.42 Acquisition (Number of Correct Trials)

There was no effect of Drug on the mean number of correct trials in Block 1 ($F(1,17) = .023, p = .882$), suggesting that all rats were matched for performance at the beginning of training. A repeated measures ANOVA showed that, although the main effect of Block was not significant ($F(5,85) = 1.502, p = .227$), there was a significant main effect of Drug ($F(1,17) = 28.106, p < .001$) and a Drug x Block interaction ($F(5,85) = 3.217, p = .033$), such that saline rats showed an increase in the number of correct trials per block, whereas lidocaine rats showed no such improvement (Figure 4A).

2.4.3 Acquisition (Mean Latency on Correct Trials)

A repeated measures ANOVA showed a decrease in the mean latency on correct trials across Blocks ($F(5,30) = 28.562, p < .001$), but there was no main effect of Drug ($F(1,6) = 1.141, p = .327$), and the Drug x Block interaction was only marginally significant ($F(5,30) = 3.181, p = .055$) (Figure 4B).

2.4.4 Acquisition (Replication Effects)

The poor acquisition observed in the first replication prompted the addition of black curtains in the second replication, further reducing the salience of potential spatial cues. The addition of the curtains in the second replication is confounded by the added infusion-test delay that these rats received in Experiment 1, but the latter is unlikely to be critical.

Consistent with Chang and Gold's (2004) observation of better acquisition by control rats in cue-poor versus cue-available conditions, the addition of curtains (presumably reducing the salience of spatial cues) resulted in better acquisition overall, but did not significantly alter the drug effect. In particular, for the number of correct trials, we observed a main effect of Replication ($F(1,17) = 53.373$, $p < .001$) and a Replication x Block interaction ($F(5,85) = 2.856$, $p = .049$), but no Replication x Drug ($F(1,17) = .431$, $p = .520$) nor Replication x Drug x Block ($F(5,85) = 2.285$, $p = .093$) interaction.

Likewise, ANOVAs of the number of consecutive correct trials and the mean latency on all correct trials revealed significant effects of Replication ($F(1,16) = 5.852$, $p = .028$ and $F(1,17) = 15.591$, $p = .001$, respectively), such that there were more consecutive correct trials and shorter latencies when curtains were introduced. Finally, there were no significant effects or interactions involving the Response counterbalancing variable ($ps > .134$).

The addition of curtains in Replication 2 primarily affected learning: performance in the initial block of trials was unaffected. ANOVAs of performance in Block 1 alone showed there was no effect of Replication on either the mean number of correct trials ($F(1,17) = 2.434$, $p = .137$) or the mean latency on those trials ($F(1,12) = 1.152$, $p = .304$).

In summary, we used this paradigm to verify the effectiveness of the infusions used in the submerged T-maze task in Experiment 1. Although lidocaine infusions produced no effect in the submerged T-maze, the same lidocaine infusion protocol in the same rats produced a significant impairment in learning in another task requiring the use of a response strategy. Notably, the learning deficit in Experiment 2 was observed as early as the second block of trials (that is, 10-20 min post-infusion), comparable to the infusion-test intervals used in Experiment 1 (2-22 min).

EXPERIMENT 3

Although the DLS appears to be unnecessary for the expression of a previously-acquired response strategy (Experiment 1), it is possible that it is required for the acquisition of that strategy. In Experiment 3, we examined the effects of DLS lidocaine infusions on the acquisition of performance in the submerged T-maze: rats received infusions before training sessions, but not before probe tests. Because, in the previous experiment, the response strategy was evident only during initial acquisition, we only trained the rats for eight sessions, and performed only four probe tests, which permitted a reasonably small number of infusions per animal.

3.1 Methods and Materials

3.1.1 Subjects and Apparatus

Forty-six male Long-Evans rats (Charles River Laboratories) were housed and maintained as in Experiment 1. They received bilateral DLS cannulations as in Experiment 1. Their weights at the start of behavioral experimentation ranged from 385 to 485 grams. The apparatus was that used in Experiment 1.

3.1.2 Surgery, Infusions, and Histology

The surgical, infusion, and histological procedures were identical to those of Experiment 1, except that infusions were given before training sessions rather than probe tests, and the Drug variable was evaluated between separate groups of subjects. That is, as a between-subject drug manipulation, each rat received either lidocaine or saline infusions throughout the entire experiment.

3.2 Behavioral Testing

3.2.1 Habituation, Training, and Probe Tests

The habituation procedure was identical to that used in Experiment 1. Training procedures were the same as those used in Experiment 1, except that rats received infusions prior to training sessions and not probe tests.

Subjects were trained for eight days and received infusions of either lidocaine or saline prior to each training session, with one drug-free probe test after every two training sessions (for a total of four probe tests). Rats were constant in their drug condition throughout the experiment. The experiment was conducted in three replications.

Subjects were run in squads of 3-5 rats, such that all rats in a squad were infused before maze exposure. Each squad contained both lidocaine and saline rats. The order of infusions (lidocaine or saline) was counterbalanced across squads. The intervals between infusion and the first daily trial ranged from 2 to 10 min in the first replication, and between 12 to 20 min in the second and third replications (similar to Experiment 1). The interval between the two daily trials ranged from approximately 2 min to 8 min during early training (Sessions 1-4) and 1 min to 3 min during later training (Sessions 5-8) (similar to Experiment 1).

The probe test procedures were the same as those used in Experiment 1 except that no infusions were given; rats were drug-free for probe tests.

3.2.2 Data Analysis

As in Experiment 1, an overhead camera and computer-assisted tracking system recorded each rat's position in the maze. During training, an experimenter recorded each rat's latency to reach the platform as well as errors accrued. These measures were analyzed with mixed-design 3 x 2 x 2 x 8 ANOVAs, with between-subject variables

Replication, Drug (saline, lidocaine), and one counterbalancing variable of Goal Arm (East, West), with repeated measures on Training Day. All ANOVAs used the Greenhouse-Geisser correction for violations of sphericity. Another mixed-design ANOVA investigated the effects of the aforementioned between-subject variables with repeated measures on the first four trials of training (Training Days 1 and 2).

For each probe test in the submerged T-maze, we determined the probability of observing the recorded number of “response learners” in each group, based on the binomial distribution. We compared the proportions of response learners across groups with a Chi-squared test. We also analyzed the change in probe test performance over the course of testing using repeated measures ANOVAs, as previously described.

3.3 Results

Two rats lost their cannulae headsets after the first probe test. Because they showed no adverse symptoms prior to the loss, these subjects were included in analyses involving the first two training days and the first probe day (lidocaine: $n = 24$, saline: $n = 22$). However, we removed their data from all analyses involving extended training and testing (lidocaine: $n = 23$, saline: $n = 21$). Figure 4 shows the cannulae tip locations; they ranged between $+0.20$ mm and -0.40 mm AP. Four subjects showed small unilateral lesions near the site of the guide cannula; data from these rats were included in our data analysis.

3.3.1 Acquisition (All Training Days)

Prior to each training session, rats received either saline or lidocaine infusions to the DLS. As in Experiment 1, we observed rapid acquisition of task performance. However, acquisition of rats that received lidocaine infusions was initially impaired

(Figures 6A and 6B). A repeated measures ANOVA that included all training sessions confirmed a significant effect of Sessions for both mean number of errors ($F(7,224) = 30.305, p < .001$) and mean latency to the platform ($F(7,224) = 42.837, p < .001$). Importantly, the main effect of Drug on mean number of errors was also significant ($F(1,32) = 6.306, p = .017$), although the main effect of Drug on mean latency to the platform was not ($F(1,32) = 2.383, p = .132$).

There were no main effects of Replication or of the counterbalancing variable for either the mean error or latency measures. For mean number of errors, only the Replication x Training Day interaction ($F(7,224) = 2.508, p = .042$) was significant, and for mean latency, only the Replication x Drug x Goal Arm ($F(2,32) = 12.553, p > .001$) and Replication x Drug x Goal Arm x Training Day ($F(7,224) = 7.350, p < .001$) interactions were significant.

3.3.2 Acquisition (Early Training Trials)

To further investigate the main effect of Drug on training performance, we examined the mean number of errors and mean latency on a trial-by-trial basis over the first two training days (Trials 1-4). All subjects were included in these analyses. A repeated measures ANOVA reported a significant decline in number of errors ($F(3,102) = 11.850, p < .001$) and latency ($F(3,102) = 11.709, p < .001$) across trials (Figures 6A and 6B).

Critically, there was a significant main effect of Drug on errors ($F(1,34) = 5.412, p = .026$), although that effect was not significant for latency ($F(1,34) = 2.457, p = .126$). There were no significant interactions for either errors ($F(3 \text{ or } 6, 102) < 2.093, p > .090$) or latency ($F(3 \text{ or } 6) < 1.685, p > .197$) across Trials 1-4 except a Replication x Drug x

Goal Arm x Trial interaction for latency ($F(6,102) = 2.802, p = .039$). Additionally, we observed a marginally significant main effect of our Goal Arm counterbalancing variable on errors ($F(1,34) = 3.973, p = .054$), such that rats swimming to the West arm had more errors than those swimming to the East arm.

Because performance of lidocaine and saline rats started at the same level and reached similar asymptotes on the fourth training trial, we contrasted the quadratic trends of these two groups of rats over the first four trials. This contrast was significant for errors ($F(1,34) = 6.778, p = .014$) and marginally significant for latency ($F(1,34) = 3.989, p = .054$), supporting our assertion that lidocaine infusions slowed the initial acquisition of maze performance.

3.3.3 Probe Tests (Strategy Selection)

All rats were trained for two days before receiving the first probe test. On the first probe, the majority of saline rats entered the previously untrained arm and were designated response learners (19/22, $p < .001$), marginally greater ($X^2(1, n = 46) = 3.390, p = .066$) than the chance performance observed in the lidocaine group (15/24, $p = .154$).

Both saline and lidocaine rats displayed a marginally significant response strategy on Probe 2 (15/22, $p = .067$ and 16/24, $p = .076$, respectively). However, saline rats continued to display a significant response strategy on Probes 3 and 4 (16/22, $ps = .026$), whereas lidocaine rats did not (13/24, $p = .419$ and 11/24, $p = .729$, respectively). The proportions of rats exhibiting a response strategy did not differ between lidocaine- and saline-trained rats on Probe 2 ($X^2(1, n = 46) = .012, p = .913$) or 3 ($X^2(1, n = 46) =$

1.697, $p = .193$), but there was a marginally significant superiority of saline rats on Probe 4 ($X^2(1, n = 46) = 3.424, p = .064$).

3.3.4 Probe Tests (Latency)

Unlike in Experiment 1, a Replication x Drug x Goal Arm x Probe Day ANOVA showed that response latency differed across Probe Days ($F(3,96) = 3.532, p = .029$) (Figure 7B). Furthermore, there was a significant main effect of Drug ($F(1,32) = 11.528, p = .002$), such that lidocaine rats were faster to the goal location. This difference was observed on the first probe day and did not change; there was no Drug x Probe Day interaction ($F(3,96) = 1.428, p = .246$). Although we have no account for this apparent speed-accuracy trade-off, it at least supports our contention that lidocaine did not generally impair motor performance.

Finally, response latency during probe tests was affected by a number of the counterbalancing variables. There were significant main effects of both Replication and Goal Arm ($F(1 \text{ or } 2, 32) > 7.237, p < .011$) such that rats in the second replication and rats swimming to the West arm were slower to reach the goal location. In addition, the Replication x Probe Day interaction ($F(6,96) = 2.963, p = .021$), and all between-subject interactions (Replication x Goal Arm, Replication x Drug, Goal Arm x Drug, Replication x Drug x Goal Arm) were significant ($F(1 \text{ or } 2, 32) > 3.993, p < .028$).

Thus, we successfully replicated our previous observations that, in the submerged T-maze, control rats exhibit a response strategy at the beginning of training. Additionally, we saw that early acquisition of maze performance was impaired by bilateral DLS inactivation by lidocaine infusion. This effect was transient; the impairment

observed in lidocaine rats dissipated after two training sessions, but resulted in the disruption of response strategy selection on subsequent drug-free probe tests.

DISCUSSION

Many discussions of animal learning and behavior stipulate that, over extended use, animals switch to automatic, inflexible responses, termed “habits.” These habits are fixed routines—acquired as a result of repeated presentations to static task demands—thought to accrue slowly, over the course of many iterations (Graybiel, 2008; Lally et al., 2010). In spatial tasks, the response strategy has often been characterized as a habit based on the above attributes. However, previous observations (Asem & Holland, 2013) suggest that this division may not always be a useful characterization. In the aversive maze, this same strategy is not inflexible; instead, subjects later switch to a different (place) strategy. Nor is this strategy acquired gradually; it is exhibited immediately, after as few as two days of training, with two trials per day.

Furthermore, the response strategy specifically and habit formation generally are thought to require the striatum (Barnes et al., 2005; Faure et al., 2005; Yin et al., 2004). In particular, striatal projection neurons and interneurons show activity patterns that modify gradually during the slow acquisition of stimulus-response (S-R) learning, producing “action chunks” for future efficient performance (Graybiel, 1998). However, the role of the DLS in the production of a response strategy may not be universal.

In Experiment 1, bilateral lidocaine inactivation of the dorsolateral striatum (DLS) prior to probe tests had no effect on the expression of an immediate response strategy, nor that of a later place strategy. Nevertheless, in Experiment 2, similar inactivation in the same rats disrupted performance in a dry maze task that required use of a response strategy. Notably, in Experiment 3, bilateral lidocaine inactivation prior to training sessions resulted in disrupted early acquisition of the task and prevented the

subsequent display of any solution strategy on drug-free probe tests. Thus, these data suggest differences in the role of striatal systems in spatial strategy selection in various maze tasks, depending on the motivational system involved in that task.

The role of the striatum might be dissociated based on the type of learning (place vs. response strategy), amount of learning (early vs. late in training), or stage of learning (acquisition vs. expression). Our results suggest that the DLS is implicated in the response strategy, but its role may be specific to acquisition during early training in the “dual-solution” submerged T-maze. Supporting evidence comes from Experiment 2, in which subjects receiving lidocaine to the DLS were impaired in the acquisition of a response strategy compared to matched controls in a “single-solution” dry plus maze, even after extended training. This result corroborates our conclusion that the DLS is implicated during acquisition.

On the other hand, interestingly, the DLS is necessary in the expression of the response strategy after extended training in the “dual-solution” dry T-maze (Packard & McGaugh, 1996). Indeed, several experiments using food reward suggest a role of the DLS in the performance of learned S-R associations, but not in relational “place” learning. For example, post-acquisition lesions of the DLS affect simple discrimination learning (Adams et al., 2001; Broadbent et al., 2007; Featherstone et al., 2005) and egocentric spatial learning (Cook & Kesner, 1988), but do not affect performance in a conditioned place preference task (Featherstone et al., 2005) or allocentric spatial learning (Cook & Kesner, 1988). Furthermore, using a two-choice discrimination task, Broadbent et al. (2007) showed the olfactory version was acquired more quickly than either objects or patterns and was also most affected by post-acquisition DLS lesions.

This role in simple S-R associations extends to habitual performance, which is insensitive to changes in reward. This insensitivity is commonly assessed using outcome devaluation. In operant tasks, reducing the value of the reinforcer yields predictable behavioral changes. Habitual behavior, on the other hand, shows less variation after outcome devaluation.

This characteristic has proved fruitful in discerning the profile of several substances. For example, consumption of a high-fat diet and drugs of abuse, e.g. ethanol, yields S-R performance that is insensitive to outcome devaluation (Corbit et al., 2012; Furlong et al., 2014; Corbit et al., 2014). This insensitivity is correlated with increased Fos positive nuclei in the DLS (Furlong et al., 2014) and is mediated by AMPA and D2 receptor signaling in the DLS (Corbit et al., 2014).

Many distinctions have been proposed between the control of initial learning and later—supposedly striatal-based—automaticity (see Ashby et al., 2010, for a review). For example, neurons in the sensorimotor (dorsolateral) striatum tend to respond strongly after over-learning of a motor sequence and temporary inactivation does not interfere with the learning of new motor sequences, but disrupts the execution of previously acquired ones (Miyachi et al., 2002), whereas the opposite effect was observed after temporarily inactivating the associative (dorsomedial) striatum (Miyachi et al., 1997).

However, this effect of extended training may be task-dependent, mediated by requiring a single action or sequenced responding. For example, the DLS is active in monkeys when learning a sequence of motor responses, such as a series of successive button presses (Miyachi et al., 2002), and shows task-related ensemble firing that

correlated with performance in the dry T-maze (Barnes et al., 2005). On the other hand, after extended instrumental training with reinforced vertical head movements, head movement-related neurons in the DLS decreased their firing rate (Tang et al., 2007). Similarly, lateral striatal neurons decreased responding to reinforced lever-pressing after extended training (Carelli et al., 1997).

As mentioned, our observations are in stark contrast to others (i.e., Packard & McGaugh, 1996), in which extended training in the “dual-solution” dry T-maze produced a switch to a response strategy, the expression of which required the DLS. It is worth noting that Packard and McGaugh’s (1996) coordinates were significantly more ventral to those targeted here, raising the possibility that the differences in the effects of lidocaine inactivation in the submerged and dry T-maze reflects differences in subregion functions, rather than task differences. Recently, Jonkman et al. (2012) separately targeted the dorsolateral striatum, using coordinates comparable to ours, and a region they described as the midlateral striatum, comparable to Packard & McGaugh’s (1996) DLS coordinates. They found different effects of inactivating these two regions on punished and unpunished drug-seeking behavior. Although inactivation of midlateral striatum affected responding throughout training, DLS inactivation only interfered with rigid responding after overtraining. However, it is notable that, in Experiment 2, we found that our DLS inactivation indeed interfered with the ongoing acquisition of a food-based maze task that required a response strategy. Critically, these results replicate Chang and Gold’s (2004) observation, whose cannulae placements are intermediate to ours and Packard and McGaugh’s (1996).

A popular framework suggests that the DMS is critical for flexible learning, whereas the DLS is critical for performance (e.g., Balleine et al., 2007, Liljeholm & O'Doherty, 2012). It is also likely that the clusters of nuclei within the striatum are differentially critical for different components of behavior. For example, the medium spiny neurons of the corticostriatal pathway can be divided into two subtypes, based on their dopamine receptor types and projections (Paton & Louie, 2012). The direct pathway striatonigral MSNs (dMSNs) express D1 dopamine receptors and project directly to the basal ganglia output nuclei: the internal segment of the globus pallidus and/or the substantia nigra pars reticulata. The indirect pathway striatopallidal MSNs (iMSNs) express D2 dopamine receptors and project indirectly to basal ganglia output; they initially terminate in the external segment of the globus pallidus.

Interestingly, these two subtypes of MSNs appear to differentially code reward and punishment (Kravitz & Kreitzer, 2012): optogenetic stimulation of dMSNs acted as a reward, but stimulation of iMSNs elicited escape responses and served as a punisher of operant behavior (Kravitz et al., 2012). This division is one clear example that volitional actions, based on the type of reinforcement, can be mediated by different pathways within the same brain regions. This reinforcement-based modulation might be similar to the mediation that we suggest might occur from task-based motivation, which also includes differing reinforcers. Thus, a more directed, specific approach might be fruitful in discerning the role of striatal subregions.

The submerged T-maze is inherently an escape-avoidance task, in which subjects are motivated to escape a negative experience via learned behaviors. Schwabe and colleagues have examined various effects of stress on instrumental

behavior in rodents and declarative memory in humans. Stress disrupts spatial learning, but not early stimulus-response learning (Schwabe et al., 2010), and appears to modulate the use of each, respectively, in humans (Schwabe et al., 2007; Schwabe et al., 2008) and rodents (Schwabe et al., 2010). Notably, stress affects instrumental behavior during both goal-directed and habitual control (Schwabe et al., 2011) and prompts habit behavior (Schwabe & Wolf, 2009). Additionally, we see that the effects of stress are pervasive, disrupting not only spatial learning, but context-dependent memory (Schwabe et al., 2009) and declarative memory as well.

We might imagine similar effects in an aversive task in which the environment and task demands themselves elicit a stress response. It is likely that the aversive task results in a fight-or-flight response, producing automatic motor movements and the release of stress hormones. These actions are subsequently reinforced by escape and, therefore, conditioned. Packard and colleagues found that administration of anxiogenic drugs biased rats towards using a response strategy in the “dual-solution” submerged T-maze: after training that yielded a place strategy in control rats, drug-treated rats showed significantly greater use of a response strategy (Elliot & Packard, 2008; Packard & Wingard, 2004).

Critically, there appears to be a difference between the dry T-maze and the submerged T-maze, such that the aversive motivation results in the opposite behavioral pattern (Asem & Holland, 2013). However, this alteration in motivation alone cannot explain our present results. For example, Hamilton et al. (2009) found a place-to-response strategy switch in the Morris water maze, using water that was colder (22°C) than ours (27°C), and, hence, likely to have been even more aversively motivating.

Notably, the submerged T-maze differs from the Morris water maze in its constraint upon behavior; subjects are forced to make a discrete left or right turn, whereas subjects in the Morris water maze are free to take more flexible routes to the goal location.

These constraints might bias the production of more response-based strategies compared to arbitrary paths and might have an additional effect in altering the perception of the extramaze cues. The discrete turn results in all-or-none exposure to the cue at the end of the goal arm, possibly making it akin to a salient cue-based landmark as opposed to broader relational information. A more parametric experiment may be necessary to evaluate the dissimilarities between these paradigms that result in such variances in behavioral strategy selection and neural recruitment.

Another variable that may have influenced our results is the intertrial interval (ITI). Packard and Goodman (2013) noted that short ITIs promote place learning and long ITIs promote response learning. Because, in our study, the ITIs were determined in part by the rats' performances, our rats received longer ITIs over the first few training sessions than over the later sessions. It might be argued that our observation in Experiments 1 and 3 of initial place learning followed later by response learning reflected this shift in ITIs.

However, this possibility seems unlikely for several reasons. First, our rats received only two trials during each training session, so variations in the within-session ITI were small compared to the 24-hr interval between each pair of trials. Experiments that have found these ITI effects used multiple trials in each session, and so within-session ITIs constituted a greater proportion of the experienced intervals. Second,

although the ITIs in Experiment 2 were consistently short, the rats were heavily biased to use a response strategy. Third, and perhaps most critically, using identical training procedures in the dry T-maze, Asem and Holland (2013) found that rats used a place strategy early in training (when the ITIs were longer) and switched to a response strategy later in training (when the ITIs were shorter).

Our observations regarding the behavior and role of the DLS in the rodent submerged T-maze have important implications across a broader framework of habit formation in animal behavior. Although the response strategy might be symptomatic of habit learning in appetitive tasks, this assumption may be deceptive for aversive ones. Furthermore, the role of the DLS in such motor-based movements is not universal and may be modified by task-based parameters such as motivation and reinforcement via amygdalar modulation.

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FIGURE CAPTIONS

Figure 1. Dorsolateral striatum cannulae placements showing the anterior/posterior extent of needle tip locations at 0.4mm sections. Experiment 1 placements ranged from +0.20 to -0.40 mm from bregma. Plates adapted from atlas of Paxinos and Watson (1998), used by permission of Elsevier.

Figure 2. (A) Mean number of errors and (B) mean latency (s) to the escape platform on correct trials during acquisition in Experiment 1. Rats received two training trials per day. Error bars denote standard error of the mean.

Figure 3. (A) Percentage of response learners in Experiment 1 based on initial response. Rats received an infusion prior to each one-trial probe test after Early (Days 2 and 4), Intermediate (Days 10 and 12), Late (Days 18 and 20), and Final (Days 22 and 24) training. Trendlines are based on relevant data points. (B) Mean latency (s) to the goal location. Error bars denote standard error of the mean.

Figure 4. (A) Mean number of correct trials and (B) mean latency (s) to Goal Arm on correct trials across trial blocks (each block consisted of 10 trials) in Experiment 2. Rats received infusions immediately prior to the first training block. Error bars denote standard error of the mean.

Figure 5. Dorsolateral striatum cannulae placements showing the anterior/posterior extent of needle tip locations at 0.4mm sections. Experiment 3 placements ranged from +0.20 to -0.30 mm from bregma. Plates adapted from atlas of Paxinos and Watson (1998), used by permission of Elsevier.

Figure 6. (A) Mean number of errors and (B) mean latency (s) to the escape platform on correct trials during acquisition in Experiment 3. Rats received infusions

immediately prior to the first of two training trials per day. Error bars denote standard error of the mean.

Figure 7. (A) Percentage of response learners in Experiment 3 based on initial response. Trendlines are based on relevant data points. (B) Mean latency (sec) to the goal location. Error bars denote standard error of the mean.

Figure1

Figure2

Figure3

Figure 4

Figure5

Figure6

Figure 7