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Authors	Tubridy, Abby E.V.
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**The Role of the Mediodorsal Nucleus of the Thalamus in Context-dependent Learning**

Abby E. V. Tubridy

CAS Honors Thesis

Department of Psychological Science

University of Vermont

## **ABSTRACT**

Context has an important influence on voluntary behaviors. For example, drug use is more likely in certain settings due to the associations that can be made between the environment and drug taking behaviors. Our lab's previous research has shown that the medial prefrontal cortex (mPFC) supports voluntary behavior in the context in which it is first learned. In rats, pharmacological inactivation of the mPFC attenuates responding for a reward only in the context in which the behavior was first learned. Preliminary data from another experiment in our lab suggests that the nucleus reuniens (RE), a thalamic nucleus strongly and reciprocally connected with the mPFC, plays a similar role in modulating context-dependent learning. In the current experiment, we examined the mediodorsal (MD) nucleus of the thalamus, a second thalamic nucleus strongly and reciprocally connected with the mPFC. Rats were conditioned to lever press for a sucrose pellet reward in Context A and exposed to Context B for familiarization. Animals were then tested in both contexts after intra-MD infusion of either muscimol (a GABA<sub>A</sub> receptor agonist) that temporarily inactivates neurons in the region, or a saline vehicle. Rats were then extinguished in Context B and again tested (using the same design as the first test) in both Context A and B. Inactivation of the MD failed to affect responding in either test. Our results suggest that the MD may not be involved in the contextual modulation of operant learning but further study is needed.

## INTRODUCTION

Associative learning plays a significant role in the behaviors we see in everyday life. Through associative learning, individuals are able to form connections between stimuli, events, and behaviors, which can guide their actions. This learning occurs in two primary forms: Pavlovian learning, where a stimulus becomes associated with an outcome, and operant (instrumental) learning, where a behavior is linked with an outcome. Operant behaviors, in particular, are voluntary actions that are influenced by their outcomes, such as performing a behavior because it leads to a reward.

Operant behaviors can also be further categorized as goal-directed actions, where performance of the action depends on the organism's knowledge of the reward outcome's value, or habits, where the behavior is completed regardless of the outcome's value (Thraillkill et al., 2018). An action becomes a habit when the reinforcer becomes highly predictable by its preceding stimuli (Thraillkill et al., 2018). A distinction between an action and a habit can be made when the reward is devalued (e.g., made less appealing). If devaluing the reward leads to a reduction in a behavior, this indicates a goal-directed action. However, if the behavior continues despite the reward losing value, it suggests a habit has formed (Thraillkill et al., 2018). Finally, a relationship between Pavlovian and operant conditioning can be shown through Pavlovian-Instrumental Transfer (PIT), which demonstrates how Pavlovian cues can influence operant (instrumental) responses, such as drug seeking behaviors (Pearce & Hall 1979).

Drug taking is a prime example of associative learning. If a person frequently uses a drug in a particular location, that location and all the environmental cues (stimuli) it provides can become associated with both the drug's effects (outcomes) and the drug taking behavior. Put simply, the background stimuli can become a "context" for drug taking behavior. As a result, this

context can have a significant influence on an individual's behavior, which is demonstrated most clearly in rodent models. Similarly, the transformation of an action to a habit can be seen in drug use, where the individuals' drug taking is triggered automatically by stimuli in the environment. When in the drug taking context, environmental cues can trigger a strong desire for drug taking and reminders of the actions associated with this behavior, in the complete absence of the drug itself.

When a voluntary behavior is no longer reinforced, a decline in behavior is often observed in what the literature calls "extinction". In operant conditioning, both the initial learning and subsequent extinction are context-dependent (for a review, see Bouton, Maren, & McNally, 2021). An extinguished behavior can return when the organism is not in the extinction context, a process referred to as "renewal". Renewal is often seen in drug use as relapse. For example, an individual who has learned to abstain from drug use in a therapy setting may later encounter the original drug-taking context or associated cues, where the previously extinguished behavior has a high risk of returning. Understanding these context-dependent responses is crucial for developing more effective relapse prevention measures for individuals that misuse drugs. To further explore this field of study, the purpose of this project is to extend our knowledge of the brain circuits controlling contextual (background stimulus) effects on voluntary behavior.

When examining the neural networks involved in associative learning, a key area of focus is the medial prefrontal cortex (mPFC). The mPFC contains a distinct sub-region involved in contextual modulation of associative learning, the prelimbic cortex (PL). The PL plays a significant role in operant responding in the original learning context (called Context A in the literature) (for a review, see Green & Bouton, 2021). For example, in an experiment conducted by Trask and colleagues, rats underwent operant conditioning (lever-pressing for food pellet

reward) in one context (Context A). When the PL was pharmacologically inactivated prior to test sessions (assessing retention), responding decreased only in Context A, the context where the original learning occurred, and not in a second, untrained context (Context B) (Trask et al., 2017). In a subsequent experiment, the same rats were trained to respond in Context A, and then the behavior was extinguished in Context B. When the PL was pharmacologically inactivated prior to testing, responding was again reduced in Context A and again unaffected in Context B (Trask et al., 2017). Both experiments demonstrate a context-dependent effect on responding, suggesting a role of the PL specifically in the modulation of operant behavior by the original context of learning.

The PL supports the effects of other types of contexts on operant behavior as well. In a series of studies conducted by Thomas and colleagues, contexts such as interoceptive (internal) states were examined. For example, one experiment examined hunger state as the context (Thomas, Bouton, & Green, 2023a). Rats learned that lever-presses yielded food pellets when they were sated (Context A) but not when they were food-deprived (Context B) (Thomas et al., 2023a). When the PL was inactivated prior to tests while either sated or hungry, responding was reduced when rats were in a sated state (Context A) but was unaffected when rats were in a hungry state (Context B) (Thomas et al., 2023a). In a follow-up study, stress level functioned as the context (Thomas, Bouton, & Green, 2023b). Rats learned to lever-press for a reward, following pre-training exposure to a stressor (e.g., oscillation, foot shock, pedestal, and restraint) (Context A). In subsequent sessions, rats underwent extinction when there was an absence of a stressor (Context B). Prior to test, the PL was inactivated, and responding was reduced when rats received a stressor prior to testing (Context A) but was unaffected when they did not receive a stressor prior to testing (Context B) (Thomas, Bouton, & Green, 2023b). These studies further

support the idea that the PL can treat a context as external (e.g., physical environment, visual or auditory stimuli) or internal factors (e.g., hunger level, stress) that provide information.

The PL does not function alone in learning and memory. The PL operates within a complex neural network that involves limbic structures as well as other cortical and subcortical regions (Green & Bouton, 2021, Vertes 2004). The extensive connections between these regions and the mPFC are critical to learning and memory circuits, facilitating information flow between the hippocampus, amygdala, thalamus and other cortical areas (Dixsaut & Gräff, 2022, Georgescu et al., 2020).

With roughly 60 nuclei, the thalamus is vitally involved in regulating cortical function (Herrero et al., 2002). Occupying a central position in the brain, the human thalamus is a symmetrical pair of structures that are separated medially by the third ventricle (Burt et al., 1993). The human thalamus is divided into 6 major anatomical regions: medial, lateral, anterior, posterior, midline, and intralaminar, all of which host a number of distinct nuclei (Burt et al., 1993). These nuclei support the functioning of the limbic system, assisting in learning, emotional processing, memory and behavior (Georgescu et al., 2020, Torrico & Abdijadid, 2023).

Playing a significant role in corticothalamic and thalamocortical communication, all thalamic nuclei receive afferent projection fibers from at least one extrathalamic source, and all nuclei have efferent projections to the cerebral cortex (Burt et al., 1993, Guillery, 1995). Across species, the primate and rodent thalamus have relatively similar cortical inputs. The bidirectional relationship between the cortex and thalamus is supported by the extensive inputs from the thalamus to cortical layer IV and outputs from cortical layer V and VI to the thalamus (Bertero et al., 2022, Georegescu et al., 2020). Layer IV neurons project to the upper cortical layers II and III, allowing for the relay and integration of thalamic input to higher cortical structures (Bertero

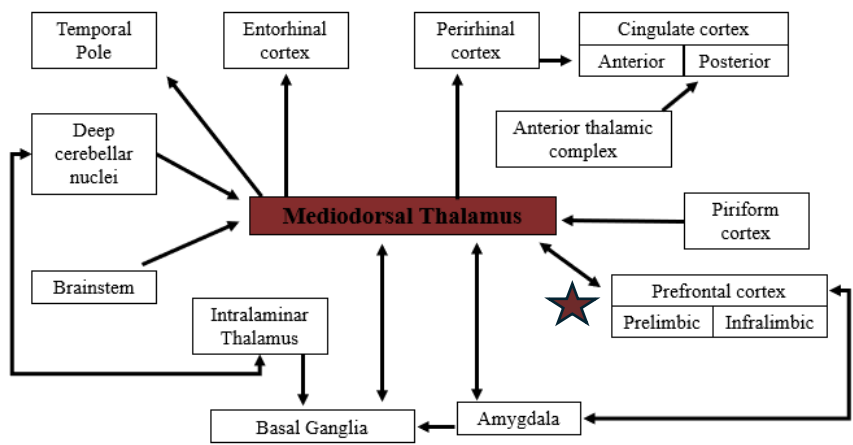
et al., 2022). Layer VI is the deepest layer of the cortex, distinguished by the robust presence of excitatory glutamatergic pyramidal cells (Georgescu et al., 2020). The function of pyramidal cells in cortico-thalamic communication is essential. In layer VI, almost 50% of these neurons play a role in the thalamus's ability to regulate neural activity (Briggs, 2010), receiving afferents directly from the thalamus and all cortical layers simultaneously.

Of the numerous thalamic nuclei, we have chosen to examine the MD (also referred to as: *medial dorsal nucleus, dorsomedial nucleus and dorsal medial nucleus*). The MD is strongly and reciprocally connected with the PL region of the prefrontal cortex in both humans and rodents (Herrero et al., 2002, Vertes et al., 2015), making it a key region of interest for learning and memory systems. The MD belongs to the medial or limbic division of thalamic nuclei (depending on classification system) and is most developed amongst the human species, parallel to the difference in development of the prefrontal cortex across species (Mitchell & Chakraborty, 2013, Vertes et al., 2015). In humans, the MD of the thalamus is located between the midline nuclei and the internal medullary lamina, a thin layer of white matter that separates the thalamus's gray matter (Burt et al., 1993). In a review of the functional anatomy of the thalamus, patients that had infarcts involving the medial region of the thalamus and found a loss of memory processes and disruption of cognition, suggesting that damage to this region impacts learning and memory (Herrero et al., 2002).

The Golgi stain technique, which stains the structure of neurons (e.g., cell bodies, axons, dendrites), can be used to decipher the cytoarchitecture of various brain regions. Georgescu and colleagues examined the anatomy of thalamic neurons using the Golgi technique and found two distinct types of relay neurons in the MD: stellate and fusiform (Georgescu et al., 2020). Stellate neurons are primarily involved in local processing in sensory regions, while fusiform neurons

project farther and serve as output neurons to distant brain areas (Chauhan et al., 2021). The distribution of these two types of neurons provides further evidence for the division of the MD into distinguishable sub regions. In rats, stellate neurons can be found in all subdivisions of the MD, but significantly populate the central region, while fusiform neurons are rarer and located more laterally (Georgescu et al., 2020). Stellate neurons are GABAergic, whereas fusiform neurons are primarily glutamatergic (Yamanaka et al., 2004; Yang et al., 2021). Additionally, pyramidal neurons, which are glutamatergic and excitatory and whose cell bodies are in the cortex, project to thalamic neurons and also receive projections from thalamic neurons (Georgescu et al., 2020).

In rats, the MD is subdivided into four main groups. These groups include the medial, central, lateral and paralamellar nuclei (Mitchell & Chakraborty, 2013). Considered one of the largest thalamic nuclei, the MD has a central role in corticothalamic and thalamocortical pathways. Of focus, the MD has distinct input-output projections with rostral regions of the prefrontal cortex, in addition to connections with many other brain regions (Figure 1).



**Figure 1. MD pathways.** The MD has numerous connections within the central nervous system. The reciprocal projection between MD and mPFC is noted. (adapted from Georgescu et al., 2020).

Prior research on the function of the MD supports the nucleus's role in associative learning. Mitchell and Chakraborty conducted a meta-analysis on cognitive and memory impairments following MD lesions, concluding that the MD may play a role in actively processing and integrating novel information to support the acquisition and retrieval stages of learning (Mitchell & Chakraborty, 2013). In a review of the function of the MD, rats with MD lesions (or MD inactivation) were unable to switch from one response to another amidst changing task demands, suggesting a role for the MD in new learning of information (Vertes et al., 2015). The ability of the rats to adjust their behavior to new environmental demands illustrates a decrease in behavioral flexibility when the MD has been inactivated and suggests the region's role in new learning. However, little is known about the role of the MD in support of contextual effects on operant conditioning.

There is some evidence that the MD may contribute to the formation of response-outcome associations (which support operant "actions") in operant conditioning (Corbit et al., 2003). Corbit et al. (2003) examined the effect of an MD lesion on operant learning. Rats had surgical lesions of either the MD region, the anterior thalamic (ANT) region or a sham lesion (SHAM) prior to conditioning. They were then trained to lever-press on two different levers: one yielding food pellets and the other a sucrose solution. After training, rats experienced a satiety-induced devaluation of one of the two outcomes (pellets or sucrose). At test, only the SHAM and ANT groups showed a selective reduction in responding to the devalued outcome, whereas the MD rats failed to demonstrate a goal-directed "action" (Corbit et al., 2003). However, the effects of context were not examined.

My goal for this experiment was to study whether inactivation of the MD, a brain region strongly and reciprocally connected with the PL, would produce a similar effect to inactivation of

the PL, reducing retention of Context A operant learning. I predicted that when the MD is inactivated, operant responding in Context A (where a lever-press produces food pellets) would decrease (similar to the effects of PL inactivation observed by Trask et al., 2017). Muscimol, a GABA<sub>A</sub> receptor agonist, served as the pharmacological agent. When muscimol is administered, it binds to GABA<sub>A</sub> receptors and activates them, causing an influx of the negatively charged ion, chloride, which hyperpolarizes the neuron, and silences the region.

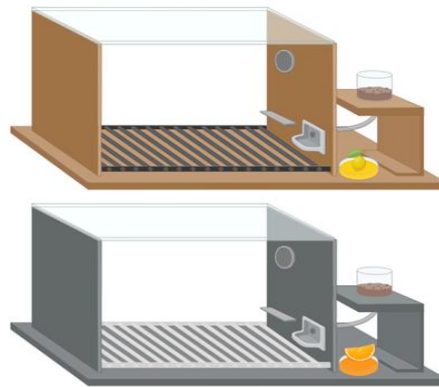
## **METHODS**

***Subjects.*** The subjects were 16 male Wistar rats purchased from Charels River Laboratory (Wilmington, MA, USA). Rats were individually housed in a room maintained on a 12:12 h light: dark cycle. Experimentation took place during the light period of the cycle. Rats were approximately 75 days old at the start of the experiment. All procedures were approved by the UVM IACUC protocol PROTO202000068 (John Green).

***Surgery.*** We performed stereotaxic surgery on the rats after at least 6 days of allowing them to habituate to the colony room. Rats were anesthetized with isoflurane and stereotaxic surgery was performed to bilaterally implant guide cannulae (22 gauge, Plastics One) into the MD nucleus of the thalamus (anterior-posterior: -2.52mm from bregma, medial-lateral: +/- 1.6mm, dorsal-ventral: -4.5mm). Cannulae were lowered to the target coordinates at a 15° angle. Lactated Ringer's solution (1 mL) was administered for hydration and carprofen (5.0 mg/kg) was administered for analgesia. Dummy cannulae were inserted into guide cannulae to keep guide cannulae debris-free. Following surgery, rats were given 5-6 days of recovery. After recovery, a

new baseline weight was taken, and rats were then food restricted and maintained at 90% of their baseline body weight.

*Apparatus.* Operant chambers consisted of two sets of four conditioning chambers (Med Associates; model ENV-008-VP) that were housed in separate rooms of the laboratory (*Figure 2*).



**Figure 2.** *Operant conditioning chambers.* Chambers are distinguished by visual, tactile and odor cues.

Each operant chamber was housed in a sound attenuating chamber. The operant chambers, which measured 30.5 X 24.1 X 21.0 cm (l X W X H), contained a recessed 5.1 X 5.1 cm food cup (magazine) centered on the front wall, which was approximately 2.5 cm above the stainless-steel grid floor. The front and back walls were brushed aluminum, and the side walls and ceiling were acrylic plastic. Each chamber contained a pellet hopper, which delivered the reinforcer, a 45-mg sucrose pellet (TestDiet 1811251), into the food cup. A retractable lever positioned to the left of the food cup protruded into the chamber. The two sets of chambers were distinguished by a distinct odor (Vicks VapoRub or Heinz white vinegar) that was continuously present outside of each operant chamber by placing a small quantity into a glass dish near the chamber. The apparatuses were controlled by a single computer, running MedPC, located in an adjacent room.

**Procedure: Magazine Training.** On the first day of the experiment, all rats were assigned to a box within each set of chambers. They then received one 30-minute session of magazine training in one context. Approximately 10-20 minutes after, rats received a second 30-minute session of magazine training in a box within the other set of chambers. Half the animals were trained first in the box later used as Context A, and half were trained first in the box used as Context B. Once all the animals were placed in their respective chambers, a 2-minute delay was imposed before the start of the session. Pellets were delivered on a variable interval (VI) 60 s schedule during magazine training. The levers were not present during this training.

**Procedure: Acquisition.** On each of the following 6 days, all rats received one 30-minute session of operant training in Context A. Following a 2-minute delay after the rat was placed in the chamber, sessions were initiated by the insertion of the lever into the chamber. A VI 30 s schedule of reinforcement was used to reinforce lever presses.

**Procedure: Test 1.** Test 1 took place on the day after the final session of acquisition. Prior to the test, rats were given an infusion of either saline vehicle (control) or muscimol (0.03 $\mu$ g), a GABA<sub>A</sub> receptor agonist, to temporarily inactivate the MD region. Dummy cannulae were removed and internal cannulae inserted into the guide cannulae. An infusion of 0.3 $\mu$ L of solution per side was delivered at a rate of 0.15 $\mu$ L per minute using a micro infusion pump. Following completion of the infusion, the internal cannulae were left in place for an additional 1 minute to allow diffusion of the infusate away from the cannulae tips. Internal cannulae were then removed and dummy cannulae were replaced.

After infusions of either muscimol or saline-vehicle, rats were given two 10-minute tests: one in Context A and one in Context B. Time between the end of infusion and the start of testing was ~10-30 minutes. Following a 2-minute delay, each test session began with the insertion of

the lever and ended with the retraction of the lever. Responding on the lever was not reinforced. Testing order was counterbalanced such that half the animals were tested first in Context A, and half were tested in Context B. Sessions were separated by 30 minutes.

***Procedure: Extinction.*** Extinction began on the day after Test 1. Animals received one Context A session and one Context B session per day for four days; these sessions were separated by approximately 40 minutes, and the order of the context presentations followed a double alternating sequence. Rats received four 30-minute sessions of extinction training in Context B. Following a 2-minute delay, the lever was inserted and available for 30 minutes. During this phase, responding on the lever produced no sucrose-pellet reward. Rats were exposed to Context A for 30 minutes, where no lever was present.

***Procedure: Test 2.*** Test 2 took place the day after the final session of extinction. Prior to test, rats were given an infusion of either saline vehicle (control) or muscimol (0.03 $\mu$ g), the GABA<sub>A</sub> receptor agonist, to temporarily inactivate the MD region, following the same procedure as Test 1.

Drug group was counterbalanced such that rats that received intra-MD infusion of muscimol in Test 1 now received the saline vehicle, and rats that received the saline vehicle now received muscimol. All rats were again given two 10-minute tests: one in Context A and one in Context B, following the same procedure as Test 1.

***Histology.*** Following the completion of the experiment, rats were deeply anesthetized with isoflurane and transcardially perfused with 0.9% saline followed by 10% buffered formalin. Electrodes were inserted into guide cannulae and a marking lesion (10 s, 0.1 mA) was made extending 1 mm below the guide cannulae (i.e., where the internal cannulae tips would have been

during infusions). Brains were then removed and stored in 10% buffered formalin. Prior to embedding in the optimal cutting temperature (OCT) compound, brains were stored in a 30% sucrose/10% buffered formalin solution for 3 days. Brains were then sectioned coronally at 50 $\mu$ m. The tissue was stained with cresyl violet and Prussian blue (for marking lesions) before being coverslipped with Permount.

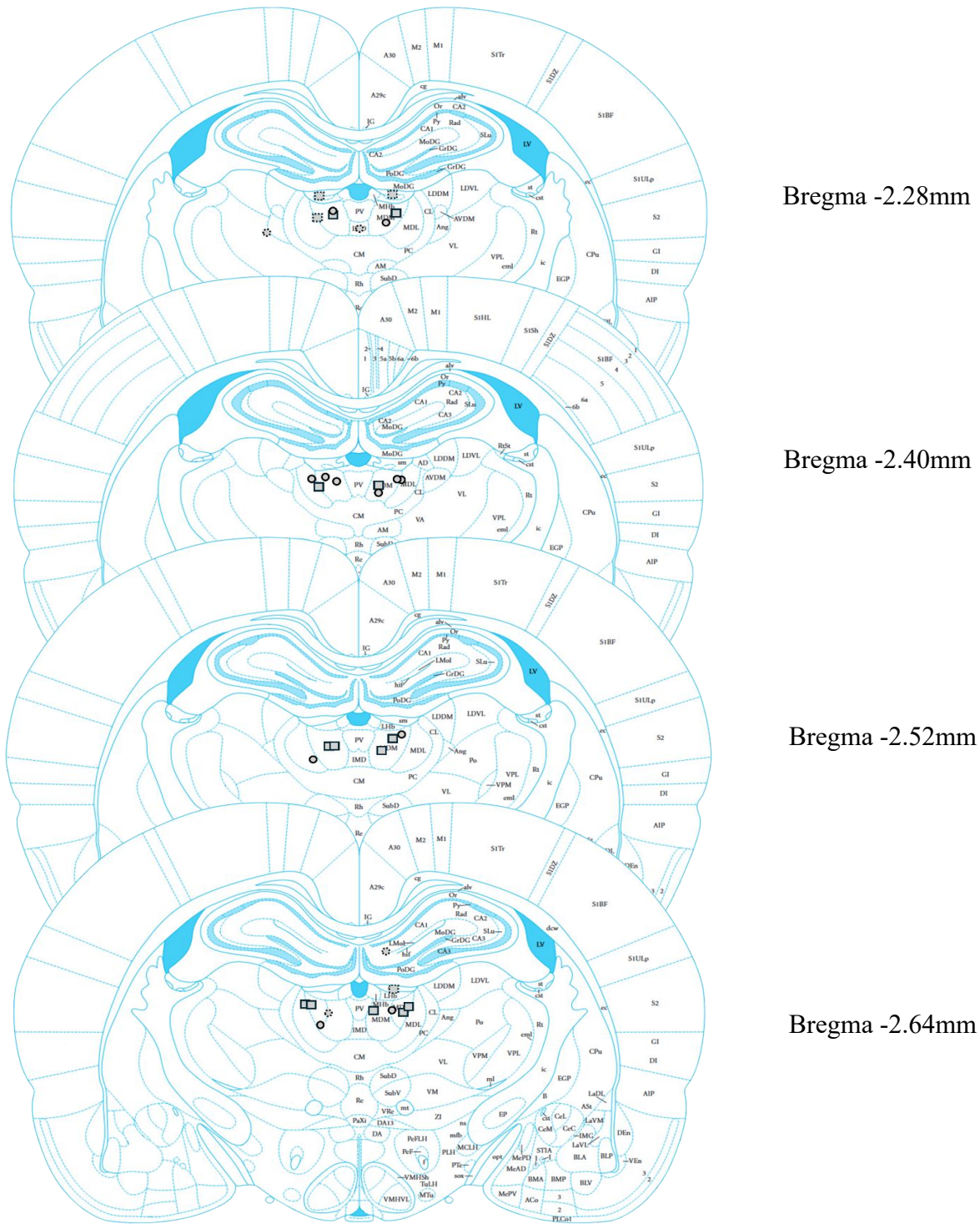
**Data Analysis.** To assess learning, the primary dependent measure was lever-pressing. During acquisition and extinction training, lever-pressing was analyzed across sessions (6 acquisition days and 4 extinction days) to track the progression of learning over time. During both tests, lever-pressing was analyzed, as a proportion of responding in the final session of acquisition in two different contexts (Context A and Context B). The main statistical test used was a repeated measures ANOVA, which was conducted on all data sets (i.e., acquisition training, acquisition test, extinction training, and extinction test). The within subjects (repeated measures) factor was Session (for acquisition and extinction training) or Context (for tests). The between-subjects factor was Group, as data was analyzed between the muscimol and the saline vehicle groups.

Throughout the experiment, we also examined a secondary dependent measure of magazine entries, to assess whether motivation to obtain a food pellet was affected by inactivation of the MD. Magazine entries were obtained by recording the number of head entries into the food port during all sessions. During acquisition and extinction training, magazine entries were analyzed across sessions (6 acquisition days and 4 extinction days). During both tests, magazine entries were analyzed, as a proportion of magazine entries in the final session of acquisition in two different contexts (Context A and Context B). The main statistical test used was a repeated measures ANOVA, which was conducted on all data sets (i.e., acquisition

training, acquisition test, extinction training, and extinction test). The within subjects (repeated measures) factor was Session (for acquisition and extinction training) or Context (for tests). The between-subjects factor was Group, as data was analyzed between the muscimol and the saline vehicle groups.

## **RESULTS**

Four rats were removed from the final data set (2 MUS-VEH, 2 VEH-MUS). Three of these rats were excluded due to bilateral or unilateral cannulae misses, and one rat lost its headcap. Therefore, the final data set consisted of 12 rats, with 6 in the MUS-VEH group and 6 in the VEH-MUS group. Cannula placements were verified and compared to a rat brain atlas (Paxinos & Watson, 2014) (see Figure 3).



**Figure 3.** *MD Cannula Placement.* Circles indicate cannula placement for rats that received VEH during the extinction test and squares indicate cannula placement for rats that received MUS during extinction test. Solid outlines represent rats that are included in the final dataset and dotted outlines represent rats that were excluded. Atlas panel adapted from Paxinos and Watson (2014).

## Lever Pressing

The main dependent measure of learning was lever-pressing: lever-presses per minute (acquisition training; extinction training) or proportion lever-pressing (acquisition test; extinction test), the latter calculated by dividing each rat's responding during the test by their responding during the first 10 mins of the last day of acquisition training. Proportion scores accounted for individual differences in lever-presses per minute (Figure 4).

**Acquisition training.** Animals in both groups (MUS-VEH and VEH-MUS; a dummy variable in acquisition training) increased lever-pressing in Context A as conditioning progressed.

A 2 (Group) x 6 (Session) repeated measures ANOVA was conducted on the number of lever presses per minute. There was a significant effect of session on lever pressing,  $F(5,50) = 30.2$ ,  $p < .001$ , indicating that lever pressing differed across sessions. There was no group effect or interaction between group and session,  $F's < 1$ , suggesting that lever pressing did not differ between groups.

**Acquisition test.** Rats in Group MUS-VEH received an intra-MD muscimol infusion and rats in Group VEH-MUS received an intra-MD vehicle infusion prior to the acquisition test. Animals in both groups responded more in Context A compared to Context B, but responding did not differ between groups in either context.

A 2 (Group) x 2 (Context) repeated measures ANOVA was conducted on lever-pressing proportion scores. There was a significant effect of context on proportion scores,  $F(1,10) = 29.5$ ,  $p < .001$ , indicating that proportion scores differed across contexts. There was no group effect or interaction between group and context,  $F's < 1$ .

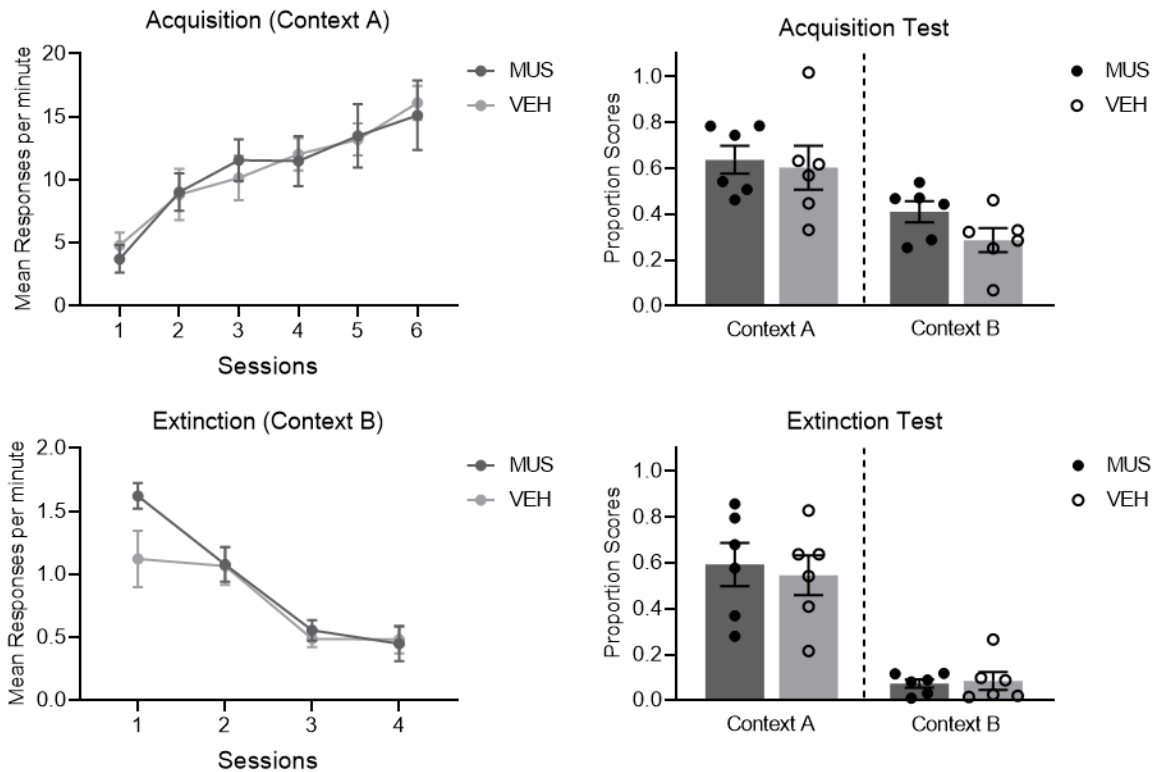
**Extinction training.** Animals in both groups (MUS-VEH and VEH-MUS; a dummy variable in extinction training) decreased lever-pressing in Context B as extinction progressed.

A 2 (Group) x 4 (Session) repeated measures ANOVA was conducted on the number of lever presses per minute. There was a significant effect of session on lever pressing,  $F(3,30) = 19.7$ ,  $p < .001$ , indicating that lever pressing differed across sessions. There was no group effect or interaction between group and session,  $F's < 2.8$ , suggesting that lever pressing did not differ between groups.

**Extinction test.** Rats in Group VEH-MUS received an intra-MD muscimol infusion and rats in Group MUS-VEH received an intra-MD vehicle infusion prior to the extinction test.

Animals in both groups responded more in Context A compared to Context B, but responding did not differ between groups in either context.

A 2 (Group) x 2 (Context) repeated measures ANOVA was conducted on lever-pressing proportion scores. There was a significant effect of context on proportion scores,  $F(1, 10) = 54.0$ ,  $p < .001$ , indicating that proportion scores differed between contexts. There was no group effect or interaction between group and context,  $F's < 1$ .



**Figure 4.** Mean lever-presses per minute during acquisition and extinction training and mean proportion scores during the acquisition and extinction tests. Proportion scores are calculated by dividing each rat’s responding during the test by their responding during the first 10 mins of the last day of acquisition training. Individual points during the acquisition and extinction test represent individual rats’ scores. Error bars are standard error of the mean (SEM).

### Magazine Entries

A secondary dependent measure was magazine entries, which may reflect the motivation to obtain food pellets: magazine entries per minute (acquisition training; extinction training) or proportion magazine entries (acquisition test; extinction test), the latter calculated by dividing each rat’s responding during the test by their responding during the first 10 mins of the last day of acquisition extinction training (Figure 5).

**Acquisition training.** Animals in both groups (MUS-VEH and VEH-MUS; a dummy variable in acquisition) showed no difference in magazine entries in Context A as conditioning progressed.

A 2 (Group) x 6 (Session) repeated measures ANOVA was conducted on the number of magazine entries per minute. There was no effect of session on magazine entries,  $F < 1$ , indicating that magazine entries did not differ across sessions. There was no group effect or interaction between group and session,  $F$ 's  $< 1$ , suggesting that magazine entries did not differ between groups.

**Acquisition test.** Rats in Group MUS-VEH received an intra-MD muscimol infusion and rats in Group VEH-MUS received an intra-MD vehicle infusion prior to the acquisition test. Animals in both groups showed more magazine entries in Context A compared to Context B, but responding did not differ between groups in either context.

A 2 (Group) x 2 (Context) repeated measures ANOVA was conducted on magazine entry proportion scores. There was a significant effect of context on proportion scores,  $F(1,10) = 9.20$ ,  $p < .05$ , indicating that proportion scores differed across contexts. There was no group effect or interaction between group and context,  $F$ 's  $< 1$ .

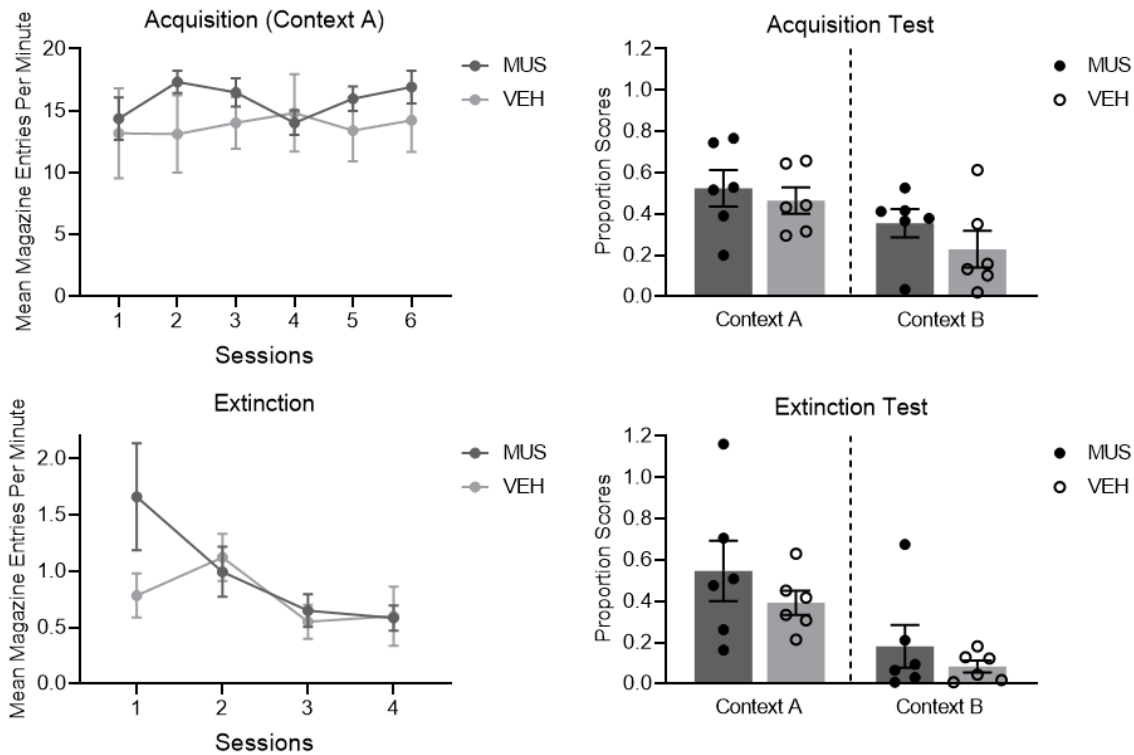
**Extinction training.** Animals in both groups (MUS-VEH and VEH-MUS; a dummy variable in extinction) decreased magazine entries in Context B as extinction progressed.

A 2 (Group) x 4 (Session) repeated measures ANOVA was conducted on the number of magazine entries per minute. There was a significant effect of session on magazine entries,  $F(3,30) = 5.66$ ,  $p < .05$ , indicating that number of magazine entries differed across sessions. There was no group effect or interaction between group and session,  $F$ 's  $< 2.9$ , suggesting that magazine

entries did not differ between groups, although the Session x Group interaction did approach significance ( $p = 0.054$ ).

**Extinction test.** Rats in Group VEH-MUS received an intra-MD muscimol infusion and rats in Group MUS-VEH received an intra-MD vehicle infusion prior to the extinction test. Animals in both groups showed more magazine entries in Context A compared to Context B, but responding did not differ between groups in either context.

A 2 (Group) x 2 (Context) repeated measures ANOVA was conducted on magazine entry proportion scores. There was a significant effect of context on proportion scores,  $F(1, 10) = 57.80$ ,  $p < .05$ ., indicating that proportion scores differed across contexts. There was no group effect or interaction between group and context,  $F$ 's  $< 1$ .



**Figure 5.** Mean magazine entries per minute during acquisition and extinction training and mean proportion scores during the acquisition and extinction tests. Proportion scores are calculated by dividing each rat’s magazine entries during the test by their magazine entries during the first 10 mins of the last day of acquisition training. Individual points during the acquisition and extinction test represent individual rat’s scores. Error bars are SEM.

## DISCUSSION

The present experiment tested whether inactivation of the Mediodorsal nucleus of the thalamus (MD) would affect context-dependent operant learning. In the acquisition phase of this experiment, rats were trained to lever press for a food pellet in one context (Context A) and exposed to a second context (Context B). Over 6 sessions, animals increased responding in Context A. Rats were then tested (without reinforcement) in both Context A and Context B following either an intra-MD infusion of muscimol or a saline-vehicle. Animals in both groups responded more in Context A compared to Context B, but responding did not differ between the

muscimol and saline-vehicle group in either context. Following the first test, rats underwent 4 sessions of extinction training in Context B (lever presses no longer produced a reward) and were exposed to Context A. Responding decreased in Context B. Rats were then tested again in both Context A and Context B following either an intra-MD infusion of muscimol or a saline-vehicle. Similar to the first test, animals in both groups responded more in Context A compared to Context B, and this did not differ between groups. Overall, the results suggest that inactivation of the MD did not affect operant responding in either context. Therefore, this research suggests that the MD may not be involved in the contextual modulation of operant learning.

We chose to study the MD in context-dependent learning due to its extensive connections with the mPFC (Vertes et al., 2015). Unlike the results of our previous studies on the PL, a region of the mPFC, when we tested the pharmacological inactivation of the MD in a similar experimental design, we did not see any effect on Context A responding. Our PL studies suggested that the PL may play a role in modulating operant responding in the original learning context as suggested by the weakening of responding in the acquisition context after inactivation at test. This same pattern of results occurred using both exteroceptive contexts (Eddy et al., 2016; Trask et al., 2017) and interoceptive contexts (Thomas et al., 2023a, 2023b).

A previous experiment conducted in our lab examined the effects of the inactivation of another thalamic nucleus, the nucleus reuniens (RE) on context-dependent operant responding. The RE is a ventral midline nucleus of the thalamus that has strong and reciprocal projections with the mPFC (Vertes et al., 2016) making it a target for research on learning and memory. Our lab examined the RE using the exact same operant conditioning experimental design as used here. The RE was inactivated prior to both an acquisition and extinction test using muscimol (0.03 $\mu$ g). Unlike the current results with the MD, responding (lever-pressing) decreased in

Context A after RE inactivation (unpublished results). The difference between the MD and RE results suggests that certain thalamic nuclei may play a role in the contextual modulation of operant learning while others do not.

While one explanation for our results is that the MD is simply not involved in context-dependent operant learning, alternative explanations are crucial to rule out. Our experiment used muscimol (a GABA<sub>A</sub> receptor agonist) to inactivate the MD. While we used the same dose that produced behavioral effects when infused into RE, it may not have been the right dose for the MD. Likewise, the fact that we did not use baclofen (a GABA<sub>B</sub> receptor agonist) in our study could have been a potential reason for null results. Further study into the density of both GABA<sub>A</sub> and GABA<sub>B</sub> receptors in the MD demonstrated a similar density of both of these receptor types in the MD, therefore, this suggests our null results may not have been the result of only inactivating GABA<sub>A</sub> receptors (Churchill et al., 1996). A different experiment examined the role of the MD in an odor span task, showing that pharmacological inactivation reduced the total number of approaches to odors and increased choice latency, suggesting an impact on working memory (Scott et al., 2020). Notably, their study used a 1:1 baclofen-muscimol concentration. These studies found an effect of pharmacological inactivation of the MD using baclofen and a different dosage of muscimol, therefore, we can rule out this explanation as a reason for our null results.

Another area of interest is examining whether the timing of the intra-MD infusion of muscimol could produce different results. Our experiment found no effect of inactivating the MD prior to test, suggesting that the MD is not involved in memory retention or retrieval of operant conditioning. It may be valuable to examine inactivating the MD at other stages of the experiment to clarify its role in learning. If the MD is inactivated prior to acquisition training,

any decrease in operant responding may suggest that the MD is important in the initial learning of an operant behavior. If any decrement in responding was also seen in a subsequent drug-free test, then it suggests that a muscimol-produced decrement during acquisition represents a learning impairment; if not, it would suggest that inactivation during acquisition produced a performance decrement, such as muscimol interfering with sensorimotor processes or motivation. Similarly, if the MD is inactivated prior to extinction training, any increase in operant responding may suggest that the MD is important in the updating/altering of the initial learning as well as the suppression of that learning. If the increase in responding is still seen in a subsequent drug-free test, then it suggests that the muscimol-produced increase in extinction was due to a learning impairment; if not, it would suggest that the increase in responding during extinction could have been due to a performance effect. Overall, if the MD is necessary for learning but not the retention of learning, we might see impairment of performance when inactivation occurs during learning stages but not when it occurs prior to testing.

Several other limitations should be acknowledged. First, our sample size was only 6 rats per group so it's possible that we did not have enough statistical power to detect an effect of inactivation. We also only used male rats as prior work in our lab utilized this sex for similar experiments. Future research should examine whether responding following the inactivation of the MD differs in female rats. While there is very little research on sex differences in MD in context-dependent learning, there is evidence of sex differences in other subfields of learning and memory which may be useful for future study. For example, one study in humans examined the functional connectivity (the dynamic coordination of activity between regions) of the MD in memory tasks (Spets & Slotnick, 2020). Researchers examined these connections using fMRI to study the activation of specific brain regions during memory tasks (e.g., memorizing a series of

shapes and their spatial location on a screen). An increase in activation in both the MD and hippocampus suggested that the ability of the MD to regulate hippocampal activation (as there is no direct link between the MD and the hippocampus) differs between females and males (Spets & Slotnick, 2020). However, these results were correlational, and it is unclear whether the MD is directly involved in this task.

Another study, using rats, examined whether there was sex-specific recruitment of the mPFC-hippocampal-thalamic system during context-dependent appetitive renewal (Anderson & Petrovich, 2016). It is important to note that the thalamic nucleus examined in this study was the paraventricular nucleus (PVT) not the MD, and that it was a Pavlovian experiment (where a cue, the conditioned stimulus predicted the delivery of food, the unconditioned stimulus) rather than an operant design (where a behavior would produce the food delivery). Likewise, this study found that male rats exhibited a renewal of responding to food cues while female rats did not, which makes these results an exception in the field, as renewal has been observed across both sexes (Anderson & Petrovich, 2016, Bouton et al., 2011). However, this study utilized the neuronal activation marker Fos to examine the neural circuitry involved in learning and memory. Male rats showed a significant increase in Fos induction within the mPFC-hippocampal-thalamic system while female rats showed greater activation in the amygdala (Anderson & Petrovich, 2016). While behaviorally, there has been evidence of a renewal effect across both sexes, this study helps expand the idea that there may be differences in the anatomical pathways that drive context-dependent learning across sexes.

In conclusion, pharmacological inactivation of the MD failed to affect retention of lever-press behavior. Thus, the present experiment suggests that the MD may not play a role in the contextual modulation of operant learning. However, the presence of a significant renewal effect

following a context switch supports the idea that learning can be contextually dependent. Research on the brain circuits that support the contextual modulation of operant learning is important to address the ongoing need for more effective relapse-prevention strategies and drug misuse treatment.

## References

- Anderson, L. C., & Petrovich, G. D. (2017). Sex specific recruitment of a medial prefrontal cortex-hippocampal-thalamic system during context-dependent renewal of responding to food cues in rats. *Neurobiology of learning and memory*, *139*, 11–21.  
<https://doi.org/10.1016/j.nlm.2016.12.004>
- Bertero, A., Verrillo, L., & Apicella, A. J. (2022). A Novel Layer 4 Corticofugal Cell Type/Projection Involved in Thalamo-Cortico-Striatal Sensory Processing. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *42*(8), 1383–1405.  
<https://doi.org/10.1523/JNEUROSCI.1738-21.2021>
- Bouton, M. E., Todd, T. P., Vurbic, D., & Winterbauer, N. E. (2011). Renewal after the extinction of free operant behavior. *Learning & behavior*, *39*(1), 57–67.  
<https://doi.org/10.3758/s13420-011-0018-6>
- Briggs F. (2010). Organizing principles of cortical layer 6. *Frontiers in neural circuits*, *4*, 3.  
<https://doi.org/10.3389/neuro.04.003.2010>
- Burt, A. M (1993). Textbook of neuroanatomy. Philadelphia: W. B. Saunders Company.
- Chauhan P, Rathawa A, Jethwa K, et al. The Anatomy of the Cerebral Cortex. In: Pluta R, editor. Cerebral Ischemia. Brisbane (AU): Exon Publications; 2021 Nov 6. Chapter 1. <https://www.ncbi.nlm.nih.gov/books/NBK575742/> doi: 10.36255/exonpublications.cerebralischemia.2021.cerebralcortex
- Churchill, L., Zahm, D. S., Duffy, P., & Kalivas, P. W. (1996). The mediodorsal nucleus of the thalamus in rats--II. Behavioral and neurochemical effects of GABA agonists. *Neuroscience*, *70*(1), 103–112. [https://doi.org/10.1016/0306-4522\(95\)00352-j](https://doi.org/10.1016/0306-4522(95)00352-j)
- Corbit, L. H., Muir, J. L., & Balleine, B. W. (2003). Lesions of mediodorsal thalamus and anterior thalamic nuclei produce dissociable effects on instrumental conditioning in rats. *The European journal of neuroscience*, *18*(5), 1286–1294.  
<https://doi.org/10.1046/j.1460-9568.2003.02833.x>
- Corcoran, K. A., & Quirk, G. J. (2007). Activity in prelimbic cortex is necessary for the expression of learned, but not innate, fears. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *27*(4), 840–844.  
<https://doi.org/10.1523/JNEUROSCI.5327-06.2007>
- Dixsaut, L., & Gräff, J. (2022). Brain-wide screen of prelimbic cortex inputs reveals a functional shift during early fear memory consolidation. *eLife*, *11*, e78542.  
<https://doi.org/10.7554/eLife.78542>

- Green, J. T., & Bouton, M. E. (2021). New functions of the rodent prelimbic and infralimbic cortex in instrumental behavior. *Neurobiology of learning and memory*, 185, 107533. <https://doi.org/10.1016/j.nlm.2021.107533>
- Guillery R. W. (1995). Anatomical evidence concerning the role of the thalamus in corticocortical communication: a brief review. *Journal of anatomy*, 187 ( Pt 3)(Pt 3), 583–592.
- Herrero, M. T., Barcia, C., & Navarro, J. M. (2002). Functional anatomy of thalamus and basal ganglia. *Child's nervous system : ChNS : official journal of the International Society for Pediatric Neurosurgery*, 18(8), 386–404. <https://doi.org/10.1007/s00381-002-0604-1>
- Mitchell AS and Chakraborty S (2013) What does the mediodorsal thalamus do? *Frontiers in System Neuroscience*, 7:37. <https://doi.org/10.3389/fnsys.2013.00037>
- Paxinos, G. & Watson, C. (2014). *The rat brain in stereotaxic coordinates* (7th ed.). San Diego: Elsevier Academic Press
- Pearce, J.M., Hall, G. The influence of context-reinforcer associations on instrumental performance. *Animal Learning & Behavior* 7, 504–508 (1979). <https://doi.org/10.3758/BF03209710>
- Scott, G. A., Liu, M. C., Tahir, N. B., Zabder, N. K., Song, Y., Greba, Q., & Howland, J. G. (2020). Roles of the medial prefrontal cortex, mediodorsal thalamus, and their combined circuit for performance of the odor span task in rats: analysis of memory capacity and foraging behavior. *Learning & memory (Cold Spring Harbor, N.Y.)*, 27(2), 67–77. <https://doi.org/10.1101/lm.050195.119>
- Snell, R. S. (2010). *Clinical neuroanatomy* (7th ed.). Philadelphia: Wolters Kluwer
- Spets, D. S., & Slotnick, S. D. (2020). Thalamic Functional Connectivity during Spatial Long-Term Memory and the Role of Sex. *Brain Sciences*, 10(12), 898. <https://doi.org/10.3390/brainsci10120898>
- Thomas, C. M. P., Bouton, M. E., & Green, J. T. (2023a). Prelimbic cortex inactivation prevents ABA renewal based on satiety state. *Neurobiology of learning and memory*, 202, 107759. <https://doi.org/10.1016/j.nlm.2023.107759>
- Thomas, C. M. P., Bouton, M. E., & Green, J. T. (2023b). Prelimbic cortex inactivation prevents ABA renewal based on stress state. *Behavioral neuroscience*, 137(6), 373–379. <https://doi.org/10.1037/bne0000570>
- Thraillkill, E. A., Trask, S., Vidal, P., Alcalá, J. A., & Bouton, M. E. (2018). Stimulus control of actions and habits: A role for reinforcer predictability and attention in the development of habitual behavior. *Journal of experimental psychology. Animal learning and cognition*, 44(4), 370–384.

- Torrice TJ, Abdijadid S. (2023) Neuroanatomy, Limbic System. National Library of Medicine. <https://www.ncbi.nlm.nih.gov/books/NBK538491/>
- Trask, S., Shipman, M. L., Green, J. T., & Bouton, M. E. (2017). Inactivation of the Prelimbic Cortex Attenuates Context-Dependent Operant Responding. *The Journal of Neuroscience: the official journal of the Society for Neuroscience*, 37(9), 2317–2324. <https://doi.org/10.1523/JNEUROSCI.3361-16.2017>
- Vertes, R. P., Linley, S. B., & Hoover, W. B. (2015). Limbic circuitry of the midline thalamus. *Neuroscience and Biobehavioral Reviews*, 54, 89–107. <https://doi.org/10.1016/j.neubiorev.2015.01.014>
- Vertes, R. P. (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse*, 51(1), 32–58. <https://doi.org/10.1002/syn.10279>
- Yamanaka, H., Yanagawa, Y., & Obata, K. (2004). Development of stellate and basket cells and their apoptosis in mouse cerebellar cortex. *Neuroscience research*, 50(1), 13–22. <https://doi.org/10.1016/j.neures.2004.06.008>
- Yang, S.-S., Mack, N. R., Shu, Y., & Gao, W.-J. (2021, June 14). Prefrontal GABAergic interneurons gate long-range afferents to regulate prefrontal cortex-associated complex behaviors. *Frontiers*. <https://www.frontiersin.org/journals/neural-circuits/articles/10.3389/fncir.2021.716408/full>