MEMORY RETRIEVAL MECHANISMS IN CONTEXT-DEPENDENT AND CATEGORIZATION MEMORY TASKS

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Norma Araceli Hernandez

December 2018

ABSTRACT

The following thesis will present two research projects that provide support for the distinct neural structures involved in memory retrieval, specifically, in tasks that require the use of context and categorization. The first chapter will summarize the two lines of research. In the second chapter, I conclude that the anterior olfactory nucleus and the ventral hippocampus are necessary to support the retrieval of contextually cued memory. In the third chapter, I conclude that the medial prefrontal cortex is necessary in order to resolve interference and to make switches in odor categories.

BIOGRAPHICAL SKETCH

Norma Hernandez received her Bachelor's degree in Neuroscience from Georgia State University. There, she was involved with various science outreach events where she taught neuroscience to elementary-aged students. She continued her education at Cornell University in 2015, joining the laboratory of David M. Smith. While at Cornell, she also continued her outreach efforts by mentoring undergraduate research assistants and participating in yearly outreach events.

Dedicated to my parents: Alfredo and Josefina Hernandez.

ACKNOWLEDGMENTS

I would like to thank my advisor David M. Smith for mentoring me throughout these few years. I have learned so much and will always be thankful for your advice. Thank you to the many undergraduate research assistants who have worked very hard to help me collect the data presented in this thesis.

TABLE OF CONTENTS

Chapter 1: Introduction

Context, briefly defined, is the set of background cues of an environment. Contextual memory involves the ability to differentiate between each set of cues to guide behavior. A number of studies have manipulated the space, sound, odor, color, and even posture of the participants in order to determine if either of these factors influenced memory recall. In a 1975 study by Godden and Baddley, a group of scuba divers is taught a list of words either on land or underwater. They are then asked to recall this list either in the same context in which they learned it or in a new context. They found that memory recall was higher in the same context in which they learned the list. It is well established that context can be used a memory retrieval cue, yet researches are still trying to determine which neural networks are needed to use context as a retrieval cue.

The hippocampus (HPC) is known to play a critical role in supporting the retrieval of contextual memories. Place fields are unique ensembles of hippocampal neurons that are activated in each environment a subject encounters (O'Keefe & Dostrovsky, 1971). Upon visiting a familiar environment, these hippocampal context representations are automatically reactivated and prime context-appropriate behaviors and memories (Smith & Mizumori, 2006; Alme et al., 2014, Bulkin, Law, Smith, 2016). Impairing HPC activity results in an impairment to perform context specific task demands (Butterly, Petroccione, & Smith, 2012; Honey & Good, 1993).

The HPC can keep representations for many different contexts and can rapidly shift between them (Alme et al., 2014). Similar shifts can also occur when rats are trained to remember two different reward locations in a T-maze alternation task. For

example, HPC neurons fire differentially on the stem of a T-maze depending on whether the rat is going to make a left or right turn. These HPC cells have been termed "splitter cells" because they split into two separate representations of left and right trials turns. These representation expressions alternate as the rat remembers left and right reward locations (Wood et al., 2000). Colgin, Moser, and Moser (2008) posit that the ability of hippocampal remapping is important for resolving interference between large numbers of similar experiences that encompass more than just the spatial layout of an environment. With the advent of optogenetics, researchers have been able to manipulate the neurons that are associated with a particular context. This technique not only allows us to activate or inhibit a particular set of neurons but also allows us to have control over when and where we can manipulate neuronal activity. Ramirez and colleagues did just that by attempting to elicit a fear response in mice in a context where they were never fear conditioned. This in-vivo control of neuronal control allows us to have a better understanding of when and where neuronal activity is important for behavior. The ability to quickly and reversibly inhibit neuronal activity provides us with a unique opportunity to control brain structures believed to be involved with memory functions. Human studies have also show that there is a greater degree of ventral HPC (vHPC) activity than in the dorsal hippocampus when processing olfactory information (Cerf-Ducastel & Murphy, 2001). Thus, there is reason to believe that this pathway may play an important role in supporting memory retrieval.

Olfactory cues of any context can aid in the retrieval of memory. Smelling odors that were present during learning can aid recall (Aggleton and Waskett, 1999).

Responses of olfactory bulb cells change depending on the context of task demands in a go-no-go odor discrimination task (Doucette and Restrepo, 2008). This would suggest that context changes can be detected on an electrophysiological level within the olfactory system and that these early sensory responses help shape odor representations. The vHPC and anterior olfactory nucleus (AON) are interconnected (Aqrabawi & Kim, 2018; Brunjes, Illig, & Meyer, 2005; Van Groen & Wyss, 1990). This pathway and its role in supporting contextually cued odor memories are further investigated in Chapter 2 of this thesis.

Memory retrieval may be mediated by top-down control from the medial prefrontal cortex (mPFC). In cases where there is a high degree of interference, such as when there is more than one way to reach a certain outcome, it has been shown that the mPFC plays an important role in mediating the switch between decision-making strategies (Guise & Shapiro, 2017; Peters, David, Marcus, & Smith, 2013; Wu, Peters, Rittner, Cleland, & Smith, 2014). A study by Birrell and Brown (2000) conducted a series of experiments in order to determine whether rodents could shift their attention from certain perceptual domains (odor of digging material, type of digging material, or surface texture) in order to use these cues to determine which bowl contained a food reward. The experimenters used both intra-dimensional shifts within a perceptual domain and extra-dimensional shifts between perceptual domains. Lesions of the mPFC resulted in the rats requiring more trials in order to reach criterion following an extra-dimensional shift, compared to control animals. The mPFC may mediate shifts in other types of tasks that require quick shifts in behaviors. This is further explored in Chapter 3 of this thesis.

Categorization, defined as the sorting of objects and events by their characteristics and set of expectations, has long been studied in humans, yet the studies of animal categorization remain confounded by other psychological phenomena such as discrimination learning and memory. The research presented will discuss a novel categorization task for rodents. The task will also be another way of studying memory interference, specifically, when there is a high degree of similarity between categories. The mPFC may monitor what is important and keep memories at the ready, such as in when changes categorization are necessary. The prefrontal cortex is known to play an important role in executive functioning, working memory, and categorization tasks (Birrell & Brown, 2000; Funahashi, 2017; Keri, 2003; Vogels et al., 2002). There are learning-related changes in the prefrontal cortex when subjects make perseverative errors (Seger et al., 2000; Vogels et al., 2002). For example, these errors occur in (A, not B) tasks, in which subjects are taught that an appealing toy is hidden under box A. Then, while the subject observing, the toy is placed under a new box B. Young babies (< 10 months old) usually reach for the A box to look for the toy in the test trial (Ashby and Casale, 2003). While this change in prefrontal activity has been observed in humans, it's not clear whether the prefrontal cortex is involved in mediating switches between categories.

Chapter 2: Hippocampal Input to the Olfactory System is Needed for Contextually Cued Retrieval of Odor Memories

Introduction

Context is a potent retrieval cue. The background cues of an environment can create a coherent set of cues that prime expectations and appropriate behaviors. It is well known that the hippocampus plays a role in differentiating between different contexts. Hippocampal cells can create place fields that represent each individual context of an environment. The HPC can be segmented into dorsal and ventral parts and these part play distinct roles in memory functions (Moser & Moser, 1998). It has been previously reported that the vHPC, in particular, plays an important role in emotional and affective processes while the dorsal HPC is involved with spatial learning and navigation (Fanselow & Dong, 2010; Gray, 1982). However, more recent studies have suggested that this functional segmentation of the hippocampus is more complex than first thought. There is an increase in place field size from dorsal to ventral HPC, suggesting that the vHPC plays a role in spatial navigation (Kjelstrup et al., 2008). Studies have also found that these vHPC representations encode not just spatial information, but context-specific events that occur across a period of time (Komorowski et al., 2013; McKenzie et al., 2015). Finally, the vHPC has also been shown to support memory retrieval of context-specific fear memories (Hobin, Ji, & Maren, 2006). Together, these studies provide a good framework for the hypothesis that the vHPC might support the contextually cued retrieval of odor memories.

What is less understood is whether the hippocampus works in tandem with other interconnected structures to carry out context-dependent memory retrieval. No

brain structure works in isolation. The anterior olfactory nucleus (AON) receives input and sends information to and from the ventral hippocampus (vHPC) (Aqrabawi & Kim, 2018; Brunjes, Illig, & Meyer, 2005; Van Groen & Wyss, 1990). This pathway may influence processing in the olfactory memories and is ideally positioned to allow contextual information to modulate odor memories.

Communication between the HPC and primary sensory areas may underlie contextual priming of memories. Areas of primary and secondary sensory cortex are reactivated when subjects are asked to recall previously shown visual or auditory cues (Karunanayaka et al., 2015; Wheeler, Petersen, & Buckner, 2000). Simultaneous recordings in the olfactory bulb and vHPC have shown an increase in coherence with beta band oscillations (15-30 Hz) while rats performed an odor discrimination task, suggesting that areas in the olfactory system and hippocampus are important to carry out odor tasks (Martin, Beshel, & Kay, 2007).

One study has investigated the role of hippocampal input to AON. Aqrabawi and colleagues demonstrated that activating vHPC input to the medial AON impairs olfaction-dependent behaviors. Specifically, when this pathway was optogenetically activated, there was a decrease in time spent investigating a familiar conspecific mouse (Aqrabawi et al., 2016). A follow up to this study found that optogenetically inhibiting this pathway impairs contextually cued memories in mice (Aqrabawi et al., 2017). Together, this provides strong evidence for this pathway could mediate top-down context-based modulation of olfactory memory by the vHPC.

This study involved the inactivation of the AON-vHPC pathway before rats on a contextually cued odor discrimination task. Rats learned to dig for a reward in one

cup of odorized digging medium (odor A) and to refrain from digging in a different cup (odor B) when they were presented in one context. The same odor cues were presented in another context in which the reward contingencies of the odors were reversed. Thus, the rats had to use the context in order to guide their choice behavior.

Bilateral inactivation of the AON, vHPC, and crossed inactivation of both structures significantly impaired performance on a contextually cued odor discrimination task. These results indicate that each structure is independently needed and moreover, that communication between the vHPC and AON is critical for contextually cued odor memory. This supports the hypothesis that contextual priming of memories involves hippocampal modulation of primary sensory representations.

Materials and Methods

Subjects

Eighteen adult male Long-Evans rats (Charles River Laboratories, Wilmington, MA) were individually housed and maintained on a 12-h light/dark cycle. Rats were food restricted to 80%–85% of their ad libitum weight and were given free access to water. All experiments were conducted in compliance with guidelines established by the Cornell University Animal Care and Use Committee.

Surgery

Guide cannulae were implanted into either the AON (n = 6), vHPC (n = 6) or both (AON-vHPC; n = 6). Subjects were anesthetized with isoflurane and placed in a stereotaxic device (Kopf Instruments). The skull was exposed, bilateral craniotomies were drilled, and dual (bilateral) guide cannulae (Plastics One) were implanted using standard stereotaxic techniques. Guide cannulae for the AON were positioned 4.7 mm

anterior and 1.5 mm lateral to bregma, and 4.6 mm ventral to the skull surface. Guide cannulae for the vHPC were positioned -5.3 mm anterior and 5 mm lateral to bregma, and 6.6 mm ventral to the skull surface. Infusion injectors protruded 0.5 mm beyond the tip of the AON guide cannulae and 0.5 – 0.7mm beyond the vHPC guide cannula. The guide cannulae were secured to the skull with bone screws and dental acrylic. Rats were allowed to recover for 7–10 days before starting behavioral training

Behavioral Task

Rats were trained on a contextually-cued odor discrimination task. In this task, rats are required to use context cues such as the color of the box, to determine which of the 2 presented odors contains a sucrose pellet reward. All testing and training took place inside a dimly lit room with ambient white noise. There was a removable divider that separated it into two components, a waiting area and a test area (Fig. 1). The test area had two circular compartments attached to the floor in order to hold the cups in place. The conditional discrimination task took place in a three-chamber wooden box. One side of the box painted black and had a rubber black floor mat. The other side was painted white. The intertrial area was in the middle of these two distinct compartments.

Two odor stimuli were produced by mixing two odorants with mineral oil. This mixture was then mixed well into to corncob bedding. First, an amount of odorant was calculated so that when mixed with 50 mL of mineral oil, it created an equivalent vapor phase partial pressure (Cleland et al., 2002; Butterly et al., 2012). 10 mL of this solution was mixed with 2 L of corncob bedding and stored in Tupperware. The odors used for this study were heptanol and ethyl valerate.

Prior to training on the conditional discrimination task, rats were trained to dig for a sucrose pellets in a cup of odorized (octyl aldehyde) bedding using standard shaping techniques. These training sessions were conducted in a smaller, white, Plexiglas box. The shaping process began by placing a pellet on top of the bedding material and allowing the rat to retrieve it. After successive retrievals, the pellets were buried deeper, so that about half of the pellet was sticking out of the digging medium. The rewards were slowly lowered on successive trials until the rats reliably and vigorously dug all the way to the bottom of the cup. Dig training typically required 3-5 sessions.

In addition, rats were acclimated to the contextually cued conditional discrimination box prior to training. This was done while the box was empty with no dividers so that the rat can freely explore the entire environment. Rats were acclimated for one 15-20 minute session.

After dig training and acclimation, the rats began training on the contextually cued conditional discrimination task. Training was split into three sessions: 10 trial blocks, 5 trial blocks, and a set of lists in which context side was randomized. The same two odors were used for the entirety of training. The predictive value of the odors depended on the presence of the context (color of the box, the smell of the odor). For example, in the black context, odor 1 was rewarded and odor 2 was not.

Training began with the 10 trial blocks, which were alternating blocks of 10 trials in each context until 60 trials were completed. At the start of each trial, the rat was placed in the middle holding chamber. For each rat, the predictive value of each odor was assigned for each context, which was maintained throughout testing. For the

first trial, the rewarded odor for that context was baited. The divider was then opened and the rat was allowed to explore the context before any cups were placed in. The ramekins were then placed in the box and the rat was able to dig in the cups. A digging response was recorded if the rat displaced any of the bedding, except incidental displacement (e.g. stepping into the cup while walking over it). After making a digging choice, the rat was returned to the intertrial area. The experimenter recorded this digging response to determine the percent of the total trials that the rat got correct. A correct trial meant that the rat dug in the rewarded odorant for that particular context. A performance of 50% correct, meaning that the rat would be performing at chance and digging in both cups. This would indicate that the rats were not using context to guide their choices.

The rats were given two sessions of the 10 trial blocks before moving onto 5 trial blocks. Rats were then given daily sessions on the 5 trial blocks until they reach a behavioral criterion of 80% correct on two consecutive sessions. Once this was achieved, they were able to begin training on a list that randomized the context for each trial. Again, daily sessions were given until they reach a behavioral criterion of 80% correct on two consecutive sessions.

To ensure that rats that underwent AON cannulae implant surgeries, we needed to ensure that muscimol did not hinder the rats' basic olfactory perception. The AON lies directly posterior to the olfactory bulb. After all of the test infusion and sessions had been run, we had to determine if rats' ability to learn a new odor discrimination was possibly impaired. A simple discrimination test was administered. Four new odors (Odors A-D; furfuryl propionate, n-butyl glycidyl ether, butanol, and n-amyl acetate)

were used for this test. Only five rats participated in this portion of the study, as one rat became ill and was perfused before completion. Rats completed 60 trials of an A vs B and C vs. D discrimination in the white Plexiglas box. On the first day, rats received bilateral saline injections into the AON thirty to sixty minutes prior to testing. They were presented with a pair of odors, where one odor was always rewarded. The position of the rewarded odor was randomized across trials. On the second day, rats received bilateral muscimol injections and repeated the same discrimination task using the other odor pair.

Infusions

Prior to the start of the infusions, a clear-out infusion was performed while the rat was under anesthesia. This was done in order to clear out any dried up blood that may have accumulated in the guide cannulae following surgery. Sterile saline was injected into all guide cannulae during this infusion. The rat was allowed to recover from anesthesia for 1 to 2 days before running the experiment.

In-vivo infusions were performed 30-60 minutes prior to the start of testing. AON-only and vHPC-only rats received bilateral infusions of saline or muscimol, a $GABA_A$ agonist. 0.5 mL of a solution containing 1 mg/mL muscimol or an equivalent volume of saline solution was infused into each hemisphere. The infusion injector was left in place for 1 min after the infusions, to ensure the complete diffusion of the solution into the region of interest.

For AON-vHPC rats, rats received a crossed-hemisphere infusion of muscimol, crossed-hemisphere infusion of saline, and unilateral infusions of muscimol. The crossed-hemisphere infusions of muscimol were to effectively disrupt

communication between the AON and vHPC. The purpose of the unilateral infusion control was to determine if functioning of this pathway in one hemisphere was enough to perform well on this task.

Brain Slicing and Staining

Following the end of the experiments, perfusions were performed with PBS and 4% paraformaldehyde. Brains were frozen and tissue slices were collected at 40 μm. Tissue was stained with a cresyl violet stain in order to identify cell bodies and cellular landmarks to verify cannulae placement (Figs. 2 – 4).

Results

Inactivating the AON impairs performance on a contextually odor discrimination task (Fig. 5). A paired t-test showed that there was a significant difference in performance between the saline ($M = 91.93$, $SD = 5.11$,) and muscimol sessions ($M = 69.53$, $SD = 8.33$, infusion conditions; $t(11) = 7.61$ $p = < .05$). Inactivating the vHPC impairs performance on a contextually cued odor discrimination task (Fig. 6). A paired t-test revealed that there was a significant difference in performance in the saline ($M = 91.15$, $SD = 5.18$) and muscimol sessions ($M = 57.81$, $SD = 11.73$; infusion conditions $t(11) = 10.89$, $p = < .05$).

Inactivating the AON on one hemisphere and the vHPC on the opposite hemisphere impairs performance on a contextually cued odor discrimination task (Fig. 7). A one-way repeated measures ANOVA with infusion condition (crossed saline, unilateral muscimol, and crossed muscimol) as a within-subjects factor was

performed. The ANOVA showed that there was a main effect of infusion condition ($F(2,5) = 64.6$, $p = <.05$).

Post-hoc paired t-tests indicated that there was a significant difference between crossed muscimol performance ($M = 52.60$, $SD = 8.82$) compared to both the crossed saline ($M = 93.17$, $SD = 23.18$; $t(1,5) = 8.83$, $p = < .05$) and unilateral muscimol ($M = 88.99$, $SD = 5.55$; $t(1,5) = -6.43$, $p = < .05$) performance. There is very small significant difference between performance in the crossed saline condition and the unilateral muscimol condition ($t(1,5) = 2.70$, $p = 0.042$). This suggests that leaving one functioning and intact AON and vHPC pathway is sufficient enough to perform well on this task. These findings show that disrupting communication between the AON and vHPC impairs the ability to use context as a retrieval cue.

There was no significant difference in performance when rats were tested under the influence of muscimol versus saline (Fig. 8; $t(4)= -1.27$, $p= 0.27$). This demonstrates that the above result was not due to an inability to smell or to learn a new odor discrimination task.

Discussion

The results of this study support our hypotheses that the vHPC and AON support contextually cued recall. Past studies have shown that the vHPC is needed for contextual fear generalization (Cullen et al., 2015, Xu et al., 2016). The vHPC receives information from the basolateral amygdala and communication between these two structures is important to modulate anxiety-related behaviors (Felix-Ortiz et al., 2013). This present study demonstrates the vHPC plays a role in context-driven recall that is not driven by anxiety-related behaviors and suggests that there may be another

neural network that is supporting contextually cued recall. Additionally, we provide evidence for the theory that the AON is perfectly positioned to modulate top-down information about context to aid in accurate odor memory recall.

The hippocampus generates a representation of context, which could then be sent to the olfactory system via the AON in order to retrieve context-appropriate memories. This theory is supported in these research findings, as cross hemisphere inactivation of both structures results in significant memory impairment. Hypothetically, without a functioning vHPC, task-relevant representations are not able to prime the AON in the service of accurate memory recall. Bilateral inactivation of either the AON or vHPC also significantly impaired performance. In these cases, there was either a representation that was priming an inactive AON or a representation that couldn't be expressed and subsequently, not able to modulate the functioning AON.

While this study provides support that this pathway is important for the contextually-cued recall, it does not provide insight into the direction of communication between the AON and vHPC. Aqrabawi and Kim used anterograde and retrograde tracers in order to better define the connections between the AON and HPC. Anterograde tracers were used to label axonal projections from their source to their point of termination. The vHPC showed a high density of axonal fibers terminating in the medial AON. Retrobead tracers were injected into the medial AON. They are then uptaken at presynaptic axonal terminals, thus labeling the origins of the projections. The CA1 region of the vHPC had the densest labeling of retrobead tracers. These findings suggest that it is indeed communication from the vHPC to the AON that may be responsible to modulate contextually-cued odor memory retrieval.

Figures

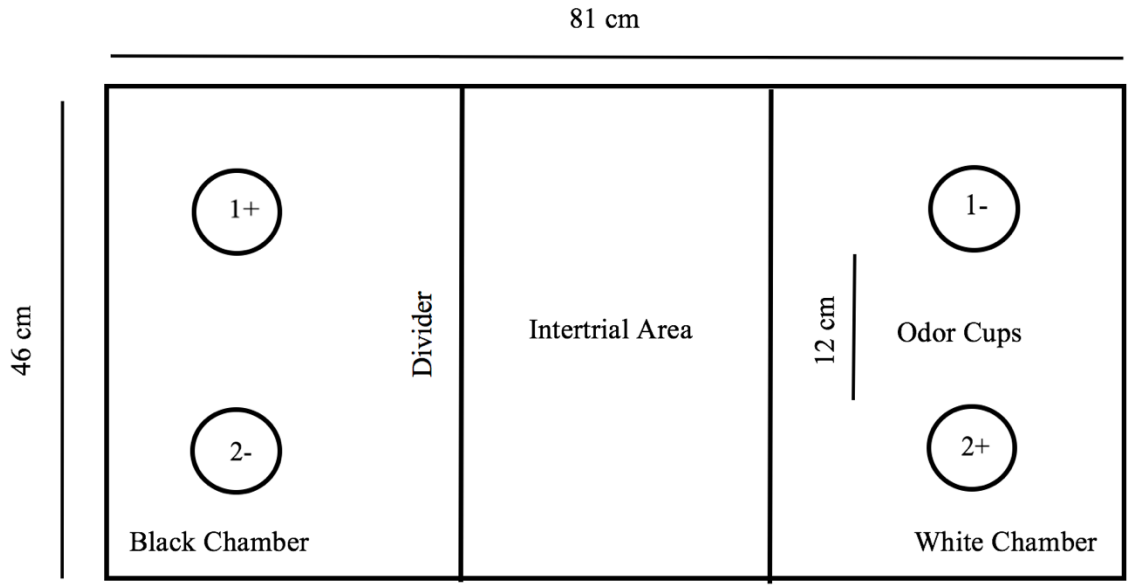

FIGURE 1. A schematic of the training chamber for the contextually-cued conditional discrimination task. The two contexts differed in their wall material (black rubber mat versus wood painted white) and their floor color (black versus white paint). A "+" indicates a rewarded odor, and "-" indicates a non-rewarded odor.

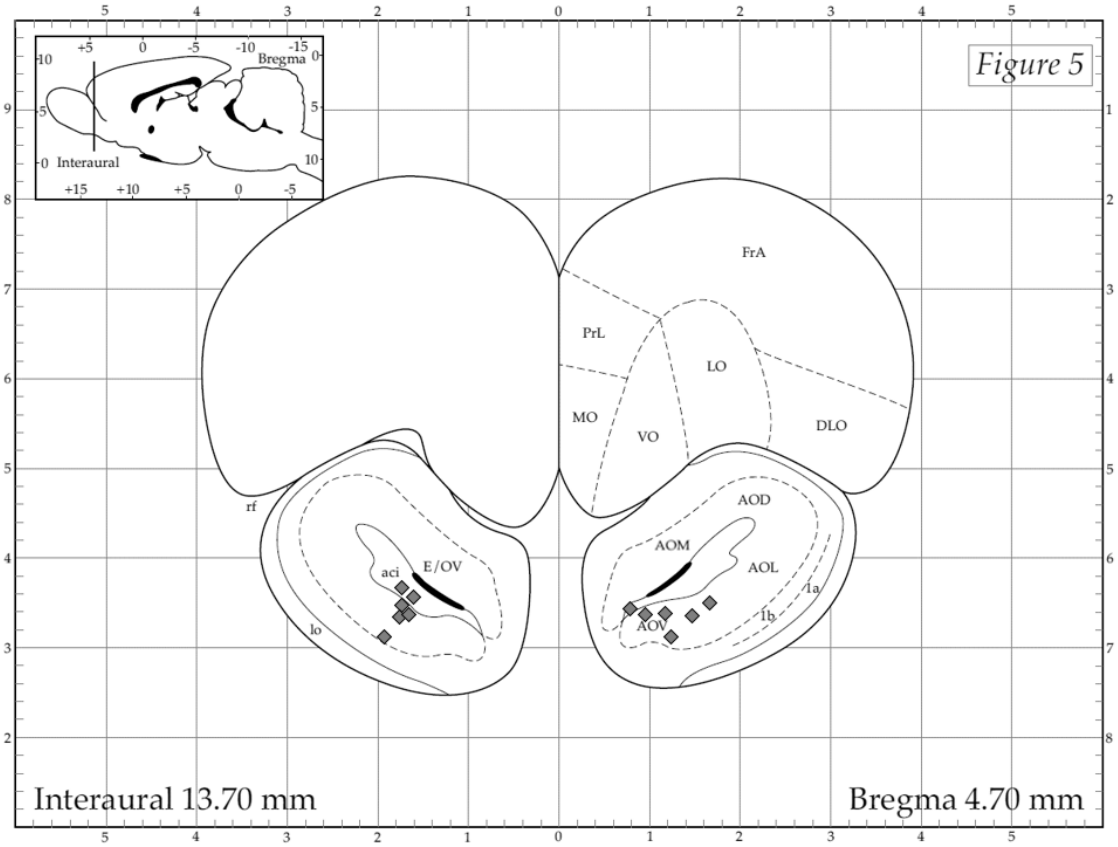

FIGURE 2. AON-only cannula localization.

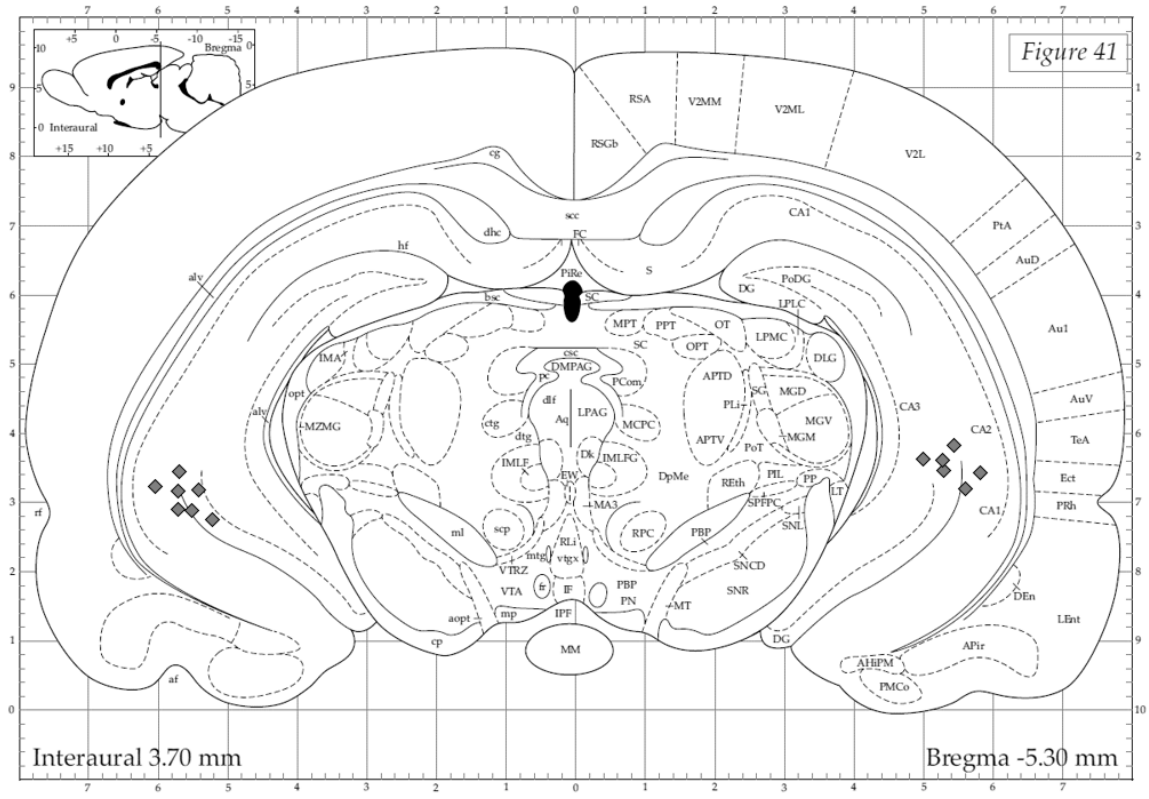

FIGURE 3. vHPC-only cannulae localization.

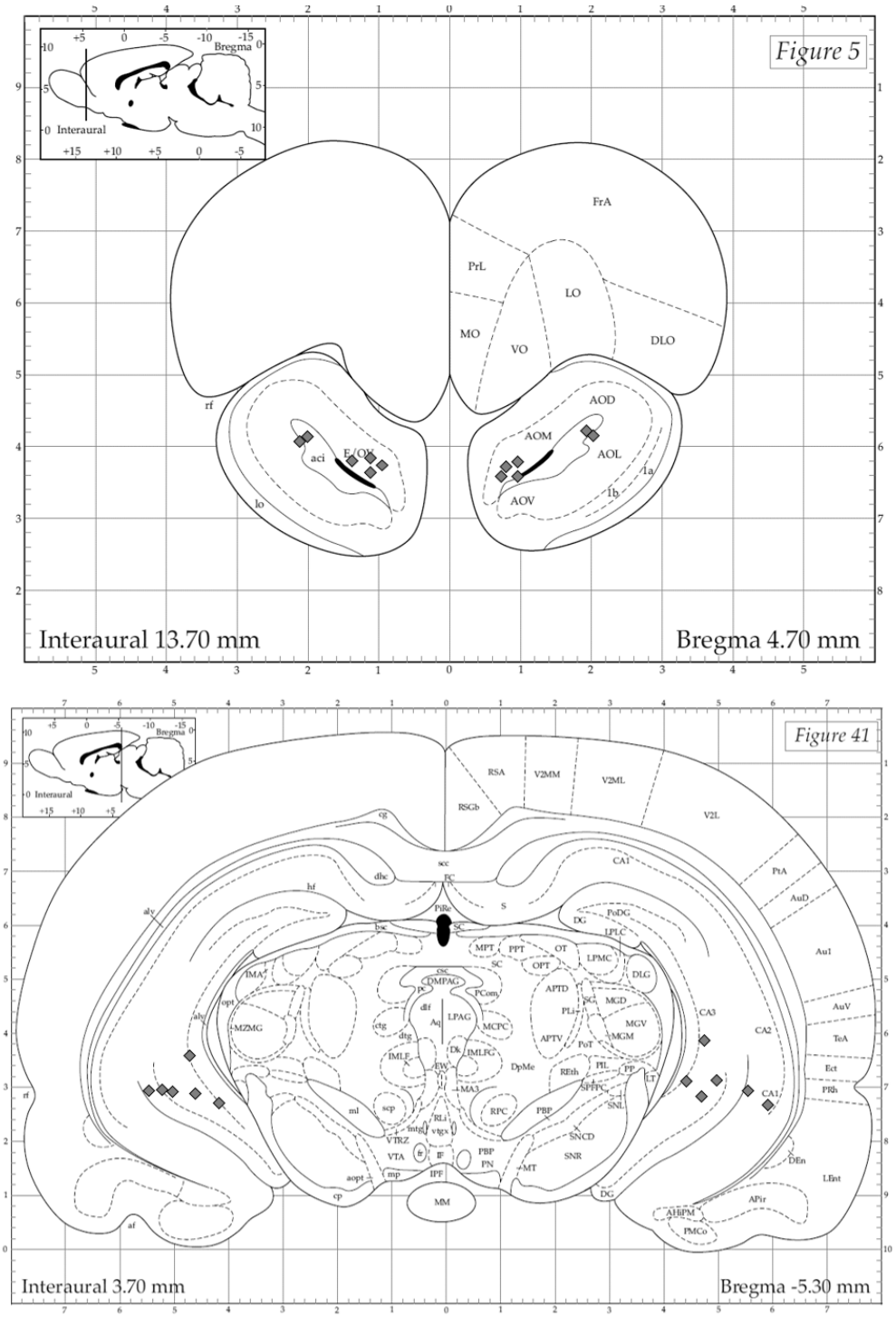

FIGURE 4. AON-vHPC cannulae localization.

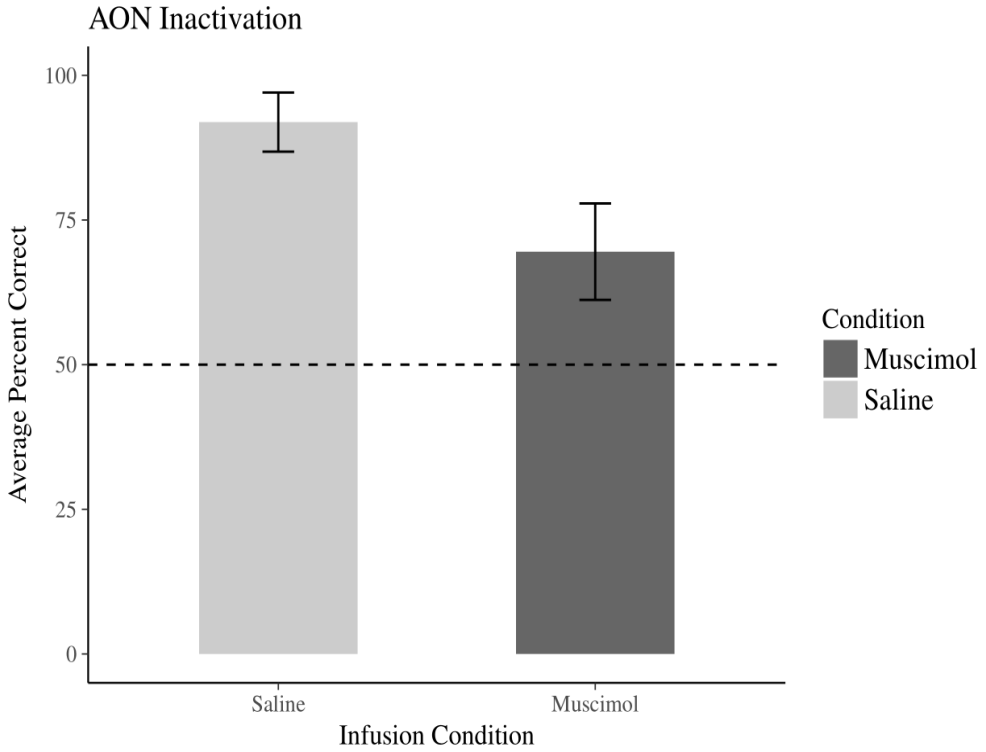

FIGURE 5. AON inactivation impairs contextually cued retrieval of odor memories.

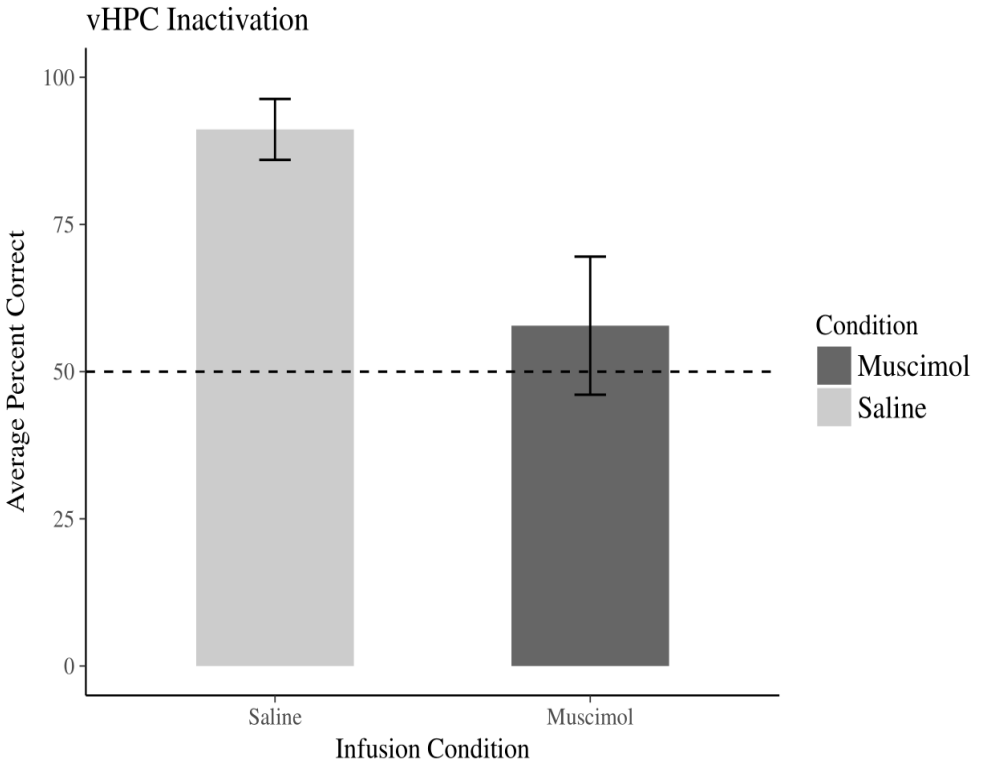

FIGURE 6. vHPC inactivation impairs contextually cued retrieval of odor memories.

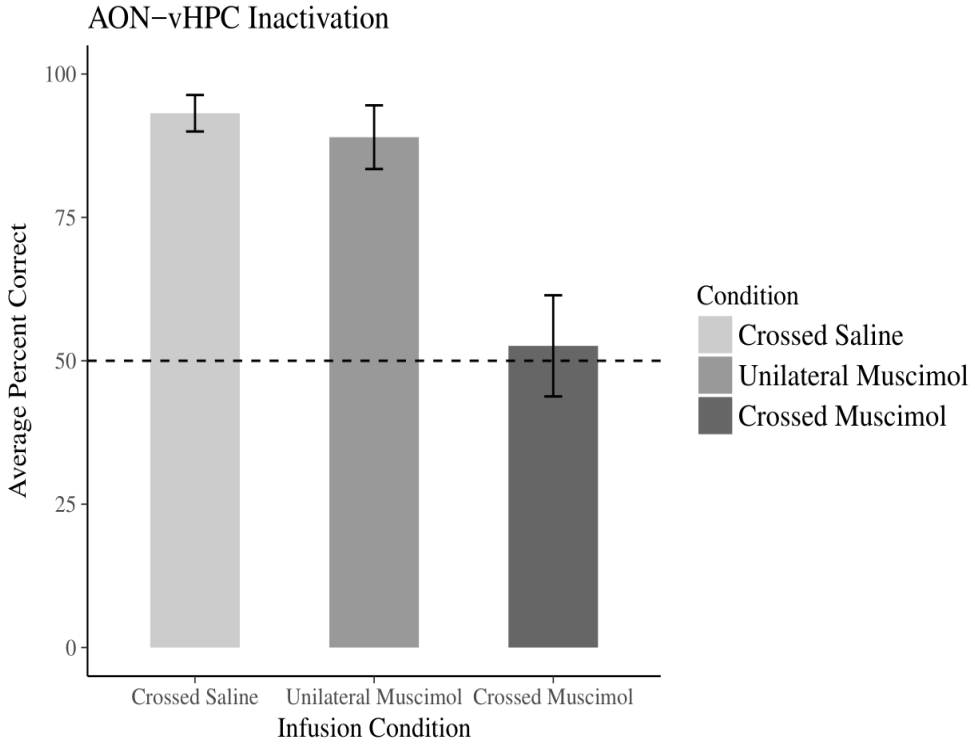

FIGURE 7. Cross-hemisphere inactivation of AON and vHPC significantly impairs contextually-cued retrieval of odor memories.

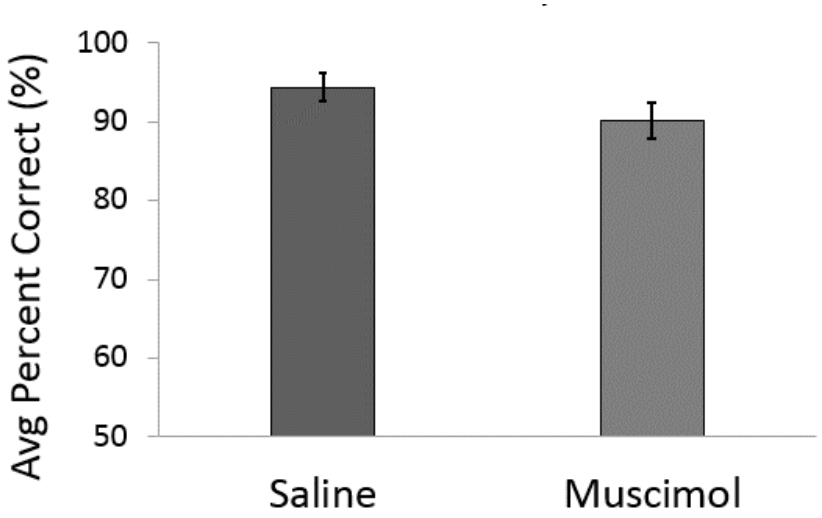

FIGURE 8. AON inactivation does not impair simple odor discrimination learning.

Chapter 3: The Medial Prefrontal Cortex is Needed for Odor Categorization

Introduction

The present study presents a novel odor categorization task for rats in order to study memory interference and switches in task demands. Rats are able to categorize rewarded odors vs. non-rewarded odors and can make switches in categories within the same test session. We found that the medial prefrontal cortex (mPFC) is necessary to make successful switches in categorization. Due to the nature of training, it is possible that this task becomes too easy and even high interference trails do not require mPFC activity.

Categorization is essential in order for animals to determine how to interact with everyday objects and events. Animal categorization has not been as well studied as human categorization, but there is evidence that some animals demonstrate the same categorization techniques as humans (Freedman et al., 2001; Smith et al., 2011). For example, an early study in animal categorization, pigeons were trained to categorize pictures of natural concepts such as trees, bodies of water, and even people (Herrnstein, Loveland, and Cable, 1976). Still, there are few studies in rodent categorization, even though the ability to distinguish between categories is essential for survival (i.e. distinguishing between familiar conspecific vs. unfamiliar rats, edible food vs. poisonous food).

The medial prefrontal cortex (mPFC) may play an important role in differentiating between categories and resolving interference between categories that are very similar. Past studies have shown that the mPFC plays an important role in resolving memory interference, strategy switching and attention set-shifting, and

working memory (Floresco, Block, & Tse, 2008; Funahashi, 2017; Ragozzino et al., 1999; Rich & Shapiro, 2007). In the Wisconsin Card Sorting Task tests cognitive flexibility, specifically, set shifting, or the ability to respond to change appropriately. Subjects taking the test initially have no knowledge of the rule and have to learn the rule-based off of feedback provided by the experimenter. Once that rule is learned and subjects are successfully sorting the cards, the experimenter suddenly changes the rule, without telling the subject, and the experimenter must switch to a new grouping category. Patients with damage to the mPFC are impaired on this task. Lesions of the mPFC result in an impairment to switch between task rules on a rodent model of the WCST (Rich & Shapiro, 2007). We developed a new categorization task, which we hypothesize requires mPFC activity in order to carry out category switches. The results of this study will give us a better understanding of whether or not the mPFC mediates sudden changes in task demands based off previously learned categories.

The mPFC may resolve interference in categorization. Interference can occur when there is a high degree of similarity between objects or events. This can appear when the rules of categorization change and thus, the expectations from an event suddenly change, requiring a change in behavior. Research and reviews have suggested that some form of pattern separation must occur in order to resolve interference (Colgin, Moser, & Moser, 2008). In a study looking at visual categorization in monkeys, researchers found that neurons in the lateral prefrontal cortex respond differentially, depending on which category group that monkeys were viewing (Freedman et al., 2001). The mPFC is also known to exert top-down inhibitory control when subjects have to actively forget one task rule and remember

another (Anderson & Green, 2001). This effect is also observed in rodents, in which inactivation of the mPFC results in an inability to resolve interference (Peters et al., 2013; Wu et al., 2014). An increase in mPFC neuronal activity is observed when rats are faced with a high degree of interference (Bissonette & Roesch, 2015). We propose that in trials with high interference, the mPFC is necessary in order to resolve the sudden change in categorization rules.

Materials and Methods

Subjects

Five adult male Long-Evans rats (Charles River Laboratories, Wilmington, MA) were individually housed and maintained on a 12-h light/dark cycle. Rats were food restricted to 80%–85% of their ad libitum weight and were given free access to water. All experiments were conducted in compliance with guidelines established by the Cornell University Animal Care and Use Committee.

Surgery

Subjects were anesthetized with isoflurane and placed in a stereotaxic device (Kopf Instruments). The skull was exposed, bilateral craniotomies were drilled, and dual (bilateral) 22-gauge guide cannulae (Plastics One) were implanted using standard stereotaxic techniques. The guide cannulae were implanted so that infusion cannulae, protruded 0.5 mm beyond the tip of the guide cannulae, were positioned in the prelimbic/infralimbic cortex (3.2 mm anterior and 0.5 mm lateral to bregma, and 2.7 mm ventral to the cortical surface). The guide cannulae were secured to the skull with bone screws and dental acrylic. Rats were allowed to recover for 7–10 days before beginning behavioral training.

Behavioral Training

Rats were trained in a Plexiglas box that had a removable divider, separating it into two components, a waiting area, and a test area. The test area had two circular compartments attached to the floor in order to hold the test cups in place. Rats were trained to dig for a reward sucrose pellet in a cup of odorized (octyl aldehyde) corn cob bedding using standard shaping techniques. The shaping process began by placing a pellet on top of the bedding material and allowing the rat to retrieve it. After successive retrievals, the pellets were buried deeper, so that about half of the pellet was sticking out of the digging medium. The rewards were slowly lowered on successive trials until the rats reliably and vigorously dug all the way to the bottom of the cup. Dig training typically required 3-5 sessions.

Categorization Task

A novel odor task was created in order to determine if rats could learn how to categorize odors and make switches between these categories. Twelve odors were created by mixing up various ratios (see Fig. 1) of the odors P. Butrayte and 2-Hepatone, diluted into mineral oil, and then mixed into clean corn cobb bedding material. These odors were then grouped into 6 different categories (3 rewarded and 3 non-rewarded). All 12 odors would appear within the same training session. Rats were trained in the same box where they learned how to dig train. Prior to lifting up the divider, two ramekins, each containing one of the 12 odorized bedding material, were placed into the box. For each trial, rats were presented with an odor from the rewarded category and an odor from the unrewarded category.

Upon reaching the criterion of digging in the rewarded cup 85% of the time, the categories were changed. An example of this categorical switch is shown in Figure 1b. Following this switch, some odors remained rewarded while others were now not rewarded. After rats were able to perform at criterion, they were then trained on a within-session switch. In these training sessions, the first 36 trials would be of the first 2 categories they learned. Then, unexpectedly, the experimenter would start rewarding the set of odors for the second set of categories. The goal is to learn that a shift has occurred and to recall the proper category that is now rewarded. Once the rats learned how to categorize and how to make shifts in categories within a single training session, they were ready for infusions.

Trials were classified as high, medium, or low interference. To determine the degree of interference, we first determined the degree of change from the first set of categorization rules to the second set. For example, if a trial includes two odors whose valence did not change at all after the categorization switch, that odor would be classified as a low interference trial. If only one of the odors in a trial had a valence switch, but the other did not, this was classified as a medium interference trial. If the both of the valences' of the odors changed, these trials would be classified as high interference trials.

Finally, for analysis purposes, training sessions were divided up into quarters. The first two quarters were to quantify performance on the first category. The third and fourth quarters quantified performance that occurred after a within-session categorization switch. The third quarter quantified performance at the beginning of the session, and the fourth quarter quantified performance at the end of the session.

Infusions

Prior to the start of the infusions, a clear-out infusion was performed while the rat was under anesthesia. This was done in order to clear out any dried up blood that may have accumulated in the guide cannulae following surgery. Sterile saline was injected into all guide cannulae during this infusion. The rat was allowed to recover from anesthesia for 1 to 2 days before running the experiment.

In-vivo infusions were performed 30-60 minutes prior to the start of behavioral testing. Rats received bilateral infusions of saline or muscimol, a $GABA_A$ agonist. 0.5 mL of a solution containing 1 mg/mL muscimol or an equivalent volume of saline solution was infused into each hemisphere. The infusion injector was left in place for at least 1 min after the proper amount of solution was dispensed in order to ensure the complete diffusion of the solution into the region of interest.

Brain Slicing and Staining

Following the end of the experiments, perfusions were performed with PBS and 4% paraformaldehyde. Brains were frozen and tissue slices were collected at 40 μm. Tissue was stained with a cresyl violet stain in order to identify cell bodies and cellular landmarks to verify cannulae placement (Fig. 3).

Results

First, we determined whether rats could learn how to categorize and then, make a switch in categories across training sessions. Figure 2. shows the average percent correct on each training session and grouped by which the category they were trained on. Rats can learn how to categorize odors and they become proficient in identifying the odors that contain a sugar pellet reward (training session days -3, -2,

and -1). The next days, rats were trained with a new set of categorization rules (training session 1 on graph). Performance drastically drops, however, as the rat receives more training on this list, performance reaches pre-switch levels (days 2, 3, 4).

A two-way repeated measures ANOVA was conducted with infusion condition and quarter as within-subjects factors was conducted. There was a significant main effect of the quarter and condition variables (Fig. 4; quarter: $F(3,4) = 11.37$, $p = .00038$), condition: $F(1,4) = 15.73$, $p = 0.0107$). However, there was no significant interaction between any quarter and condition ($F(3,4) = 0.75$, $p = 0.539$). As expected, rats perform better before a categorization switch occurs and that rats performed better after saline infusion than after muscimol infusion regardless of quarter. This suggests that impairing the mPFC impairs the ability to reach peak performance following a categorical switch.

Performance on each trial following a within-session category switch was then analyzed depending on whether it was a low, medium, or high interference trial (see methods). A 2-way repeated measures ANOVA was conducted with interference type and infusion condition as within-subjects factors. There was no significant difference in performance in any infusion condition (Fig. 5; $F(1,4) = 0.16$, $p = 0.70$). However, there was a significant difference in performance depending on the interference type ($F(2,4) = 4.74$, $p = 0.022$). There was no significant interaction between interference type and infusion condition ($F(3,4) = 1.02$, $p = 0.38$). Together, these results suggest that it might be harder for the rats, in both saline and muscimol conditions, to perform as well as on the low or medium interference trials.

Discussion

We have developed a novel odor categorization task for rats. Rats were able to categorize odors based off of whether they are rewarded or non-rewarded. Not only are rats able to learn odor categories, but they are also able to switch between these categories within a session. These categories are not simple valence reversals. On any trial following the switch, some odors are still rewarded (no change), or not rewarded (complete change). Two odors are presented at the same time. In the trials where rats are presented with two odors that completely changed in valences, we reasoned that these trials had a high degree of interference. Our rats required many trials in order to learn each category. It might be possible that rats are memorizing the specific odors that were used and not relying on the properties that define each category in order to determine which is rewarded. New odors, that contain a novel mixture of the familiar base odorants, could be used in future studies in order to determine if rats are indeed categorizing by the characteristics of the odor and not simply responding to the memory of any odor.

Inactivation of the mPFC resulted in an impaired performance on a categorization task. However, we found that this impairment was not specific to high interference-only trials and that impairment was found across all trial types. The nature of this task required rats to learn how to distinguish between odors that may smell very similar or very different. Because of this, we expected for there to be various types of interference depending on trial type. We analyzed the trials following a within-session switch, however, our results did not show a significant interaction of

muscimol infusions and interference type. We are unable to conclude that mPFC inactivation selectively impairs performance under high interference conditions. It does appear that performance under muscimol does not reach the level of performance under the control conditions.

These results vary in what is currently known about how the mPFC resolves interference. We know that the medial prefrontal cortex resolves cases of high interference (Peters et al., 2013). It is possible that this task might be too easy, even in high interference trials. Even in control infusions, rats are able to perform well above chance levels. Categorization by humans can be divided into different types of categorization, based off of how they are learned. For example, categories can be learned very quickly based off of explicit rules or traits, learned rather slowly based off of rules that are harder to learn and integrate, or learned after much practice. Each of these ways of learning categories requires unique neural pathways (Ashby and Crossley, 2010). The categorization task described here requires a lot of practice sessions to learn but the task is not necessarily procedural because the placement of the odors is randomized. These conflicting results indicated the need to clearly define the type of categorization that is being studied.

Figures

Odor	% of P. Butyrate	% of 2-Hepatone	P. Butyrate	2-Hepatone
A	92%	8%	24 μl	2.3 μl
B	85%	15%	22.2 μl	4.3 μl
C	78%	22%	20.4 μl	6.3 μl
D	71%	29%	18.5 μl	8.3 μl
E	64%	36%	16.7 μl	10.3 μl
F	57%	43%	14.9 μl	12.3 μl
G	8%	92%	2.1 μl	26.4 μl
H	15%	85%	3.9 μl	24.4 μl
I	22%	78%	5.7 μl	22.4 μl
J	29%	71%	7.6 μl	20.4 μl
K	36%	64%	9.4 μl	18.4 μl
L	43%	57%	11.2 μl	16.3 μl

FIGURE 1a. Odors used in categorization task. Categories were: A-F vs. G-L; A,B,C,G,H,I vs. D,E,F,J,K,L; A,B,C,J,K,L vs. D,E,F,G,H,I.

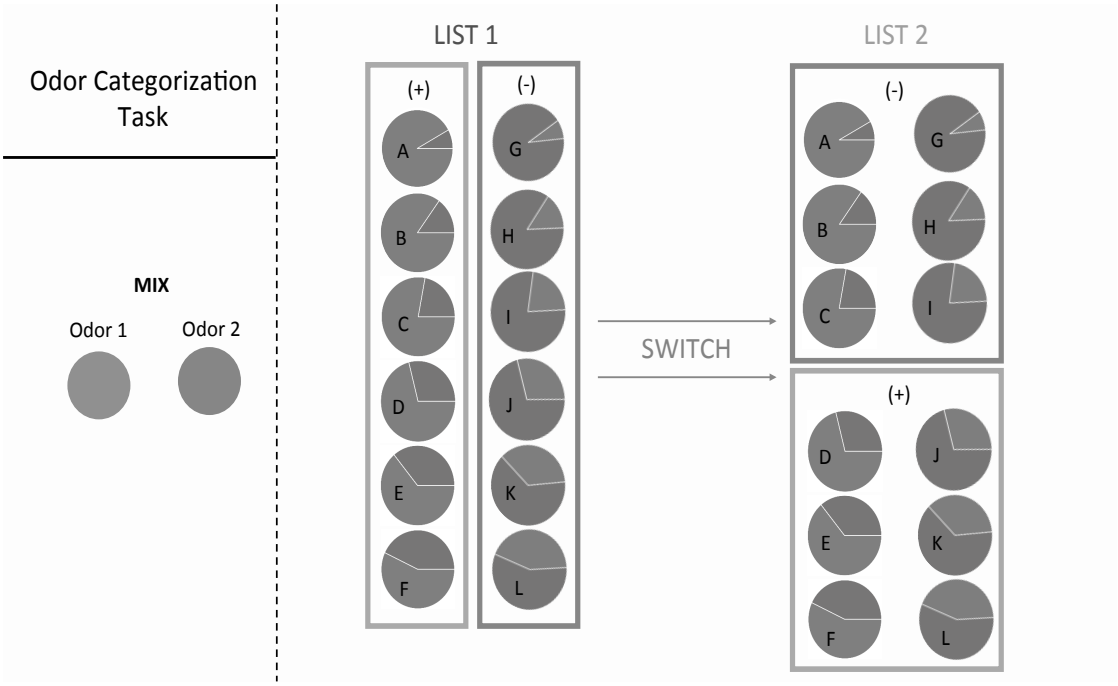

Figure 1b. Example of a categorical shift in rewarded odors.

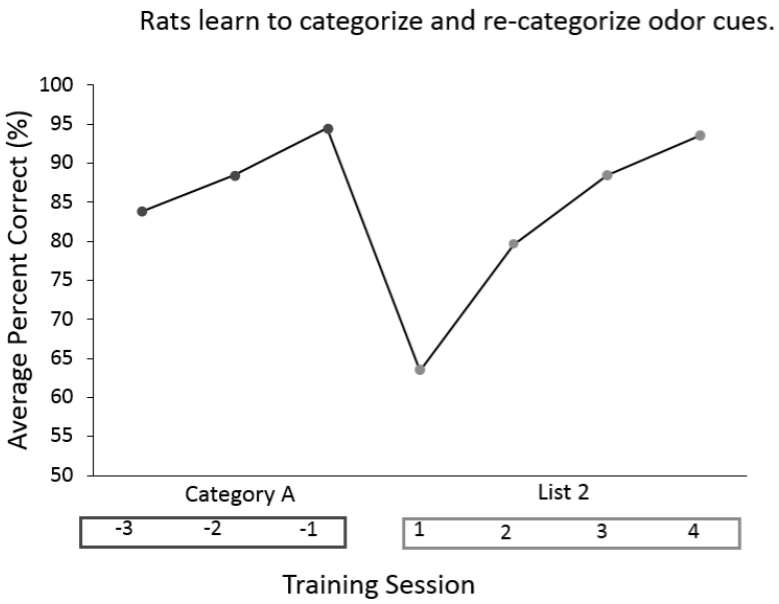

FIGURE 2. Rats can categorize odors and learn to make switches in categories across training days. Performance initially drops following a switch but improves with further training.

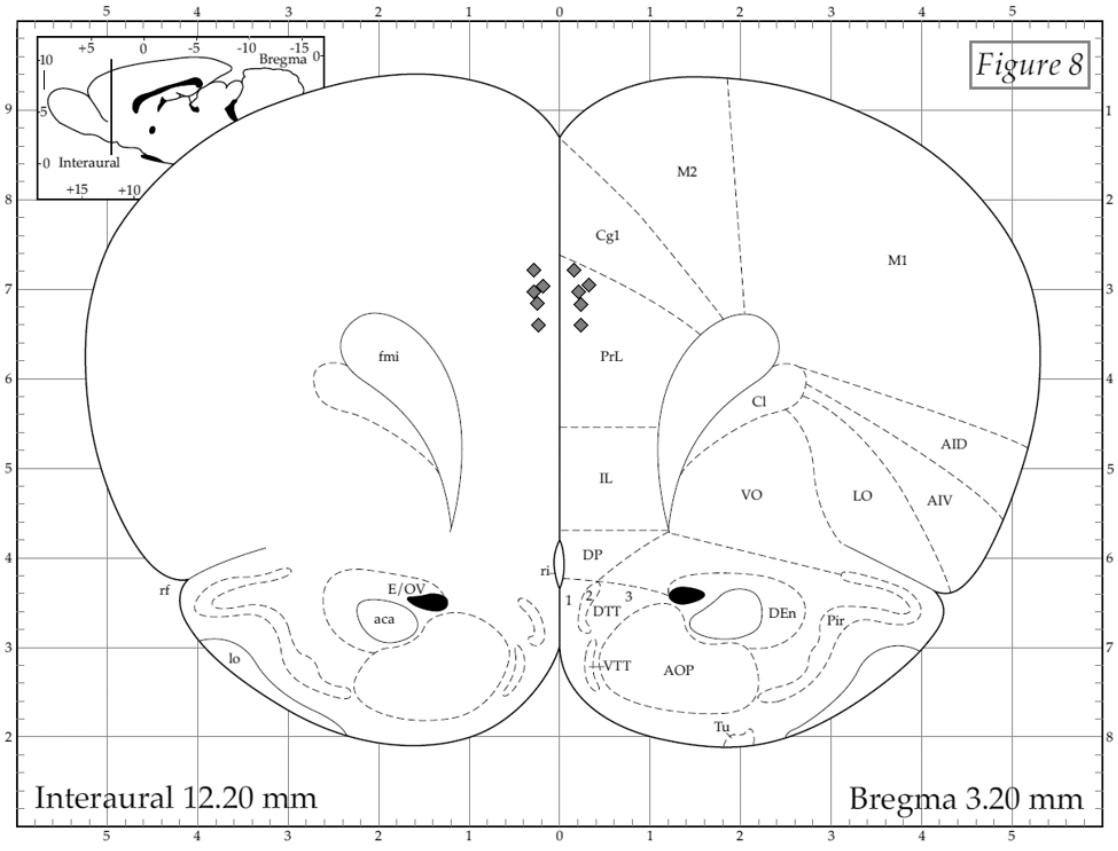

FIGURE 3. Localization of cannulae placement.

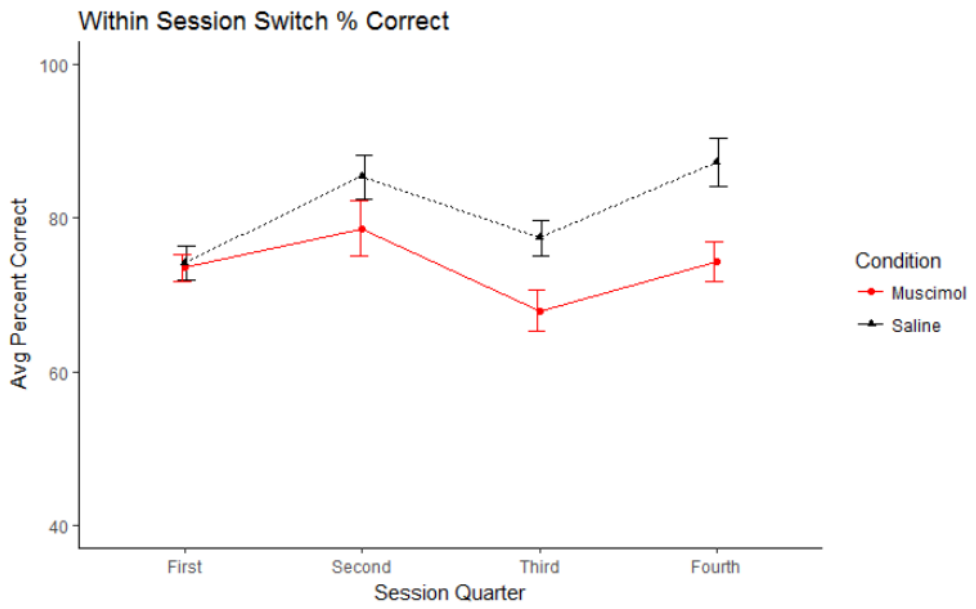

FIGURE 4. Impairing mPFC impairs performance across all quarters in a categorization task.

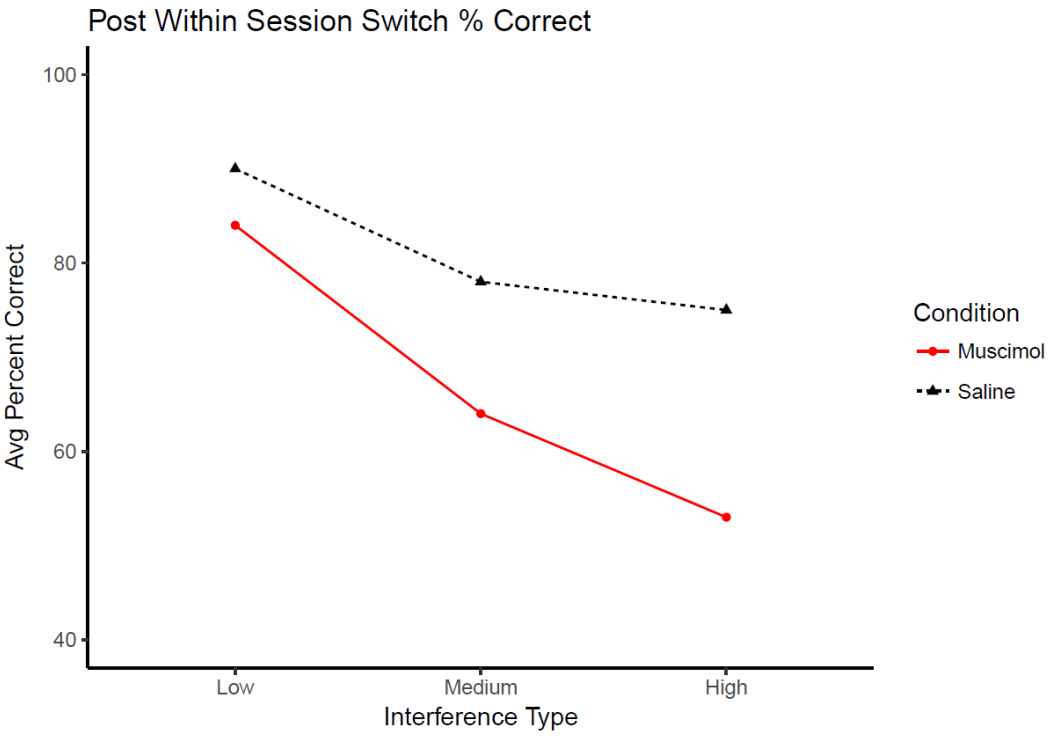

FIGURE 5. Rats differ in performance depending on interference type, regardless of infusion condition.

References

Aggleton, J. P., & Waskett, L. (1999). The ability of odours to serve as state-dependent cues for real-world memories: Can Viking smells aid the recall of Viking experiences? British Journal of Psychology, 90(1), 1–7.

Alme, C. B., Miao, C., Jezek, K., Treves, A., Moser, E. I., & Moser, M.-B. (2014). Place cells in the hippocampus: Eleven maps for eleven rooms. Proceedings of the National Academy of Sciences, 111(52), 18428–18435.

Anderson, M. C., & Green, C. (2001). Suppressing unwanted memories by executive control. Homepage.Psy.Utexas.Edu, 410(March), 366–369.

Aqrabawi, A. J., & Kim, J. (2017). Spatiotemporal information is differentially conveyed by hippocampal projections to the anterior olfactory nucleus during episodic-like odour memory. bioRxiv.

Aqrabawi, A. J., & Kim, J. C. (2018). Topographic Organization of Hippocampal Inputs to the Anterior Olfactory Nucleus. Frontiers in Neuroanatomy, 12(12), 1–7.

Aqrabawi, A. J., Browne, C. J., Dargaei, Z., Garand, D., Khademullah, C. S., Woodin, M. A., & Kim, J. C. (2016). Top-down modulation of olfactory-guided behaviours by the anterior olfactory nucleus pars medialis and ventral hippocampus. Nature Communications, 7, 1–9.

Ashby, F. G., & Casale, M. B. (2003). The cognitive neuroscience of implicit learning in Attention and Implicit Leaning (pp. 132-134). Amsterdam/Philadelphia: John Benjamins Publishing Company.

Ashby, F. G., & Crossley, M. J. (2010). The neurobiology of categorization. The Making of Human Concepts, 75–98.

Birrell, J. M., & Brown, V. J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. Journal of Neuroscience, 20(11), 4320–4324.

Bissonette, G. B., & Roesch, M. R. (2015). Neural correlates of rules and conflict in medial prefrontal cortex during decision and feedback epochs. Frontiers in Behavioral Neuroscience, 9(October), 1–14.

Brunjes, P. C., Illig, K. R., & Meyer, E. A. (2005). A field guide to the anterior olfactory nucleus (cortex). Brain Research Reviews, 50(2), 305–335.

Bulkin, D. A., Law, L. M., & Smith, D. M. (2016). Placing memories in context: Hippocampal representations promote retrieval of appropriate memories. Hippocampus, 26(7), 958–971.

Butterly, D. A., Petroccione, M. A., & Smith, D. M. (2012). Hippocampal context processing is critical for interference free recall of odor memories in rats. Hippocampus, 22(4), 906–913.

Cerf-Ducastel, B., & Murphy, C. (2001). fMRI activation in response to odorants orally delivered in aqueous solutions. Chemical Senses, 26(6), 625–637. doi:10.1093/chemse/26.6.625.

Colgin, L. L., Moser, E. I., & Moser, M.B. (2008). Understanding memory through hippocampal remapping. Trends in Neurosciences, 31(9), 469–477.

Cullen, P. K., Gilman, T. L., Winiecki, P., Riccio, D. C., & Jasnow, A. M. (2015). Activity of the anterior cingulate cortex and ventral hippocampus underlie increases in contextual fear generalization. Neurobiology of Learning and Memory, 124, 19–27.

Doucette, W., & Restrepo, D. (2008). Profound context-dependent plasticity of mitral cell responses in olfactory bulb. PLoS Biology, 6(10), 2266–2285.

Fanselow, M. S., & Dong, H. W. (2010). Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? Neuron, 65(1), 7–19.

Felix-Ortiz, A. C., Beyeler, A., Seo, C., Leppla, C. A., Wildes, C. P., & Tye, K. M. (2013). BLA to vHPC inputs modulate anxiety-related behaviors. Neuron, 79(4), 658–664.

Floresco, S. B., Block, A. E., & Tse, M. T. L. (2008). Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. Behavioural Brain Research, 190(1), 85–96.

Freedman, D. J., Riesenhuber, M., Poggio, T., & Miller, E. K. (2001). Categorical representation of visual stimuli in the primate prefrontal cortex. Science (New York, N.Y.), 291(5502), 312–6.

Funahashi, S. (2017). Working Memory in the Prefrontal Cortex. Brain Sciences, 7(49), 1–22.

Godden, D. R., & Baddeley, A. D. (1975). Context-Dependent Memory in Two Natural Environments: on Land and Underwater. British Journal of Psychology.

Gray, J. A. (1982). The Neuropsychology of Anxiety - an Inquiry into the Functions of the Septo-Hippocampal System. Behavioral and Brain Sciences, 5(1982), 469–534.

Guise, K. G., & Shapiro, M. L. (2017). Medial Prefrontal Cortex Reduces Memory Interference by Modifying Hippocampal Encoding. Neuron, 94(1), 183–192.

Herrnstein, R. J., Loveland, D. H., & Cable, C. (1976). Natural concepts in pigeons. Journal of Experimental Psychology: Animal Behavior Processes, 2(4), 285–302.

Hobin, J. A., Ji, J., & Maren, S. (2006). Ventral hippocampal muscimol disrupts context-specific fear memory retrieval after extinction in rats. Hippocampus, 16(2), 174–182.

Honey, R. C., & Good, M. (1993). Selective Hippocampal Lesions Abolish the Contextual Specificity of Latent Inhibition and Conditioning. Behavioral Neuroscience, 107(1), 23–33.

Karunanayaka, P. R., Wilson, D. A., Vasavada, M., Wang, J., Martinez, B., Tobia, M. J., Yang, Q. X. (2015). Rapidly acquired multisensory association in the olfactory cortex. Brain and Behavior, 5(11).

Keri, S. (2003). The cognitive neuroscience of category learning. Brain Research Reviews, 43, 85–109.

Kjelstrup, K. B., Solstad, T., Brun, V. H., Hafting, T., Leutgeb, S., Witter, M. P., … Moser, M.-B. (2008). Finite Scale of Spatial Representation in the Hippocampus. Science, 321(5885), 140 – 143.

Komorowski, R. W., Garcia, C. G., Wilson, A., Hattori, S., Howard, M. W., & Eichenbaum, H. (2013). Ventral Hippocampal Neurons Are Shaped by Experience to Represent Behaviorally Relevant Contexts. Journal of Neuroscience, 33(18), 8079–8087.

Martin, C., Beshel, J., & Kay, L. M. (2007). An Olfacto-Hippocampal Network Is Dynamically Involved in Odor-Discrimination Learning. Journal of Neurophysiology, 98(4), 2196–2205.

McKenzie, S., Keene, C. S., Farovik, A., Bladon, J., Place, R., Komorowski, R., & Eichenbaum, H. (2016). Representation of memories in the cortical-hippocampal system: Results from the application of population similarity analyses. Neurobiology of Learning and Memory, 134, 178–191.

Moser, M. B., & Moser, E. I. (1998). Functional differentiation in the hippocampus. Hippocampus, 8(6), 608–619.

O'Keefe, J., & Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Research, 34(1), 171–175.

Peters, G. J., David, C. N., Marcus, M. D., & Smith, D. M. (2013). The medial prefrontal cortex is critical for memory retrieval and resolving interference. Learning and Memory, 20(4), 201–209.

Ragozzino, M. E., Wilcox, C., Raso, M., & Kesner, R. P. (1999). Involvement of rodent prefrontal cortex subregions in strategy switching. Behavioral Neuroscience, 113(1), 32–41.

Rich, E. L., & Shapiro, M. L. (2007). Prelimbic/Infralimbic Inactivation Impairs Memory for Multiple Task Switches, But Not Flexible Selection of Familiar Tasks. Journal of Neuroscience, 27(17), 4747–4755.

Seger, C. A., Poldrack, R. A., Prabhakaran, V., Zhao, M., Glover, G. H., & Gabrieli, J. D. (2000). Hemispheric asymmetries and individual differences in visual concept learning as measured by functional MRI. Neuropsychologia, 38(9), 1316–1324.

Smith, D. M., & Mizumori, S. J. Y. (2006). Hippocampal place cells, context, and episodic memory. Hippocampus, 16(9), 716–729.

Smith, J. D., Ashby, F. G., Berg, M. E., Murphy, M. S., Spiering, B., Cook, R. G., & Grace, R. C. (2011). Pigeons' categorization may be exclusively nonanalytic. Psychonomic Bulletin & Review, 18(2), 414–421.

Van Groen, T., & Wyss, M. J. (1990). Extrinsic Projections From Area CA1 of the Rat Hippocampus: Olfactory, Cortical, Subcortical, and Bilateral Hippocampal-Formation Projections. Journal of Comparative Neurology, 302(3), 515–528.

Vogels, R., Sary, G., Dupont, P., & Orban, G. A. (2002). Human brain regions involved in visual categorization. NeuroImage, 16(2), 401–414.

Wheeler, M. E., Petersen, S. E., & Buckner, R. L. (2000). Memory's echo: Vivid remembering reactivates sensory-specific cortex. Proceedings of the National Academy of Sciences, 97(20), 11125–11129.

Wu, J. Q., Peters, G. J., Rittner, P., Cleland, T. A., & Smith, D. M. (2014). The hippocampus, medial prefrontal cortex, and selective memory retrieval: Evidence from

a rodent model of the retrieval-induced forgetting effect. Hippocampus, 24(9), 1070–1084.