Transient Inactivation of the Thalamic Nucleus Reuniens Produces Deficits of a Delayed Spatial Alternation Task

by

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ABSTRACT

Electrophysiological evidence has shown that the hippocampus and prefrontal cortex functionally synchronize during working memory tasks in rodents, indicating that the two brain structures form a neural circuit that is important for working memory performance (Hallock et al., 2013; Gordon, 2011). Current hypotheses propose a possible time-dependent functional relationship between HPC and PFC, with results of functional inactivation studies suggesting that HPC and PFC act together during performance of tasks that require working memory over long delays, and operate in parallel or have dissociable functions over short delays (Churchwell & Kesner 2011). The nucleus reuniens of the thalamus (RE) is anatomically well positioned to gate the flow of information between HPC and PFC, and has been shown to be necessary for the performance of spatial working memory tasks (Hallock et al., *in preparation*; Hembrook & Mair 2010); however, the extent to which RE is necessary for the maintenance of information over both long and short delays remains unclear. Our lab first collected behavioral data from rats on a delayed-alternation T-maze task (DA30) that requires spatial working memory over a 30-second delay (Hallock et al., 2013). DA30 requires rats to alternate between a left and a right goal arm to obtain a reward, relying on their previous choice of direction to determine the location of the next reward. We found that RE inactivation impaired DA30 performance (Hallock et al., 2013). To better understand the importance of RE in working memory during a delay, we inactivated RE prior to the performance of the same DA task without a delay – continuous alternation (CA). We found that

performance on DA30 was significantly more impaired when compared to the performance on CA. This suggests that the dependence on RE for working memory tasks increases as the delay period for the tasks increases, providing evidence for a time-dependent component of RE activity during working memory tasks.

Chapter 1

INTRODUCTION

1.1 The Medial Prefrontal Cortex

The rodent medial prefrontal cortex (mPFC) is important for a variety of processes including conflict monitoring, error detection, executive control, reward-guided learning, and decision-making (Botvinick et al., 2004; Holroyd et al., 2002; Posner et al., 2007; Rushworth et al., 2011). The mPFC has also become an area of interest in the study of memory. Euston et al. (2012) proposed a role for the mPFC in learning associations between context, locations, events, and forming emotional responses to these associations. The authors suggest that the ability of the mPFC to regulate emotional responses is utilized when recalling the best action or emotional response to use in a specific situation.

The mPFC has been broken down into three main anatomical divisions: the anterior cingulate (ACC), the prelimbic (PL), and the infralimbic (IL) cortices (Berendse and Groenewegen, 1991; Ray and Price, 1992; Ongur and Price, 2000; Heidbreder and Groenewegen, 2003). Furthermore, Heidbreder and Groenwegen (1991) have shown evidence for a dorso-ventral functional distinction between these areas. The more dorsal region of the mPFC, the ACC, is important for motor behaviors, while the more ventral regions of the mPFC, the IL and PL, are implicated

in cognitive and mnemonic processes. More specifically, the IL has been shown to be important for emotional processes, such as fear extinction, and the PL may be important for executive functions, such as spatial working memory (Vertes, 2006). In humans, the prefrontal cortex (PFC) has been divided into orbital, medial, and lateral parts (Fuster, 2002), and some hypothesize that the orbitomedial prefrontal cortex (OMPFC) and dorsolateral prefrontal cortex (DLPFC) in humans may be functionally homologous to the IL and PL in rodents, respectively (Vertes, 2006).

Lesion and electrophysiological recording studies have both shown that the mPFC is involved in behaviors that require the use of previously acquired information to guide further responses (Seamans et al., 1995; Rainer et al., 1999). Furthermore, lesions of the rat mPFC have produced behavioral deficits in mnemonically dependent tasks, such as spatial delayed response tasks (Kolb, Nonneman, & Singh, 1974; Eichenbaum, Clegg, & Feely, 1983). Anatomically, afferents to the mPFC have been identified that originate from the hippocampus, a limbic structure known for its role in memory (Swanson, 1981). Taken together, these findings provide evidence for mPFC involvement in mnemonic processes, and highlight the need to study this area in memory research.

1.2 The Hippocampus

The hippocampus (HPC supports many types of memory systems, including allocentric spatial memory (Morris, Garrud, Rawlins, & O'Keefe, 1982; O'Keefe and

Nadel, 1978), conscious declarative memory (Squire, 1992; Tulving & Markowitsch, 1988), and the formation of conjunctive representations (Rudy & Sutherland 1989, 1995). The HPC contains three cortical regions, the hippocampus proper (consisting of the CA1, CA2, and CA3 subfields), the subicular complex, and the entorhinal cortex, which, together, are known as the "hippocampal formation" (Amaral and Witter, 1989). For purposes of simplicity, our study uses the term "hippocampus" to refer to the hippocampus proper, specifically the CA1 subfield. It is generally believed that the entorhinal cortex projects back to the denate gyrus, which projects unilaterally to the hippocampus proper (Blackstad 1956, 1958). It is also known that the hippocampus proper projects back to the entorhinal cortex, as well as the subiculum.

Similar to the anatomical distinction seen in the mPFC, evidence exists for a dorso-ventral distinction within the hippocampus, as well. In an anatomical study, Swanson and Cowan (1977) showed distinct, separate input and output connections for the dorsal hippocampus (DH) and ventral hippocampus (VH). In rodents, the DH receives sensory information from the primary and secondary sensory cortices via the entorhinal cortex (Czerniawski et al., 2009). The ventral CA1, however, is prominently connected, unidirectionally, with the amygdala, known for emotion regulation, and with the infralimbic and prelimbic cortices in the mPFC (Chiba, 2000; Hoover & Vertes, 2007; Thierry et al., 2000). In one study, Pothuizen et al. (2004) used a radial arm maze to attempt to separate the roles of the DH and VH in rodents. Radial arm mazes mandate that subjects visit a spatial location that has not previously been visited in order to obtain a reward, and are thus known to be reliable in testing

spatial memory (Fanselow & Dong, 2010). Pothuizen et al. (2004) found that lesions to the rodent DH produced significant performance deficits on the task, while lesions to the VH did not. Furthermore, lesions to the DH reduced the number of returns to an arm that was previously associated with a food reward, while lesions to the VH increased the number of returns, demonstrating opposite roles for the DH and VH. Additionally, Czerniawski et al. (2009) demonstrated dissociable roles for the DH and VH, with inactivation of VH but not DH producing deficits in a trace fear conditioning task, and the inactivation of DH but not VH producing deficits in a delayed reinforced alternation task, which tests spatial memory. Together with anatomical studies, these results provide evidence for a clear dorsal/ventral distinction in the hippocampus, with the DH being involved in spatial memory processing, and the VH being involved in emotional memory.

1.3 Working Memory and Hippocampal-prefrontal Connectivity

Working memory is a specific type of memory that requires the active maintenance of information that is pertinent to ongoing behavior (Kandel, Schwartz & Jessell, 2000). According to Baddley and Logie (1992), working memory is important for activities that require moment-to-moment monitoring and maintenance of task-relevant information. Working memory may be further categorized by the modality of the information, such as spatial or auditory, that must be "held in mind" (Baddley, 1983). Cognitive activities such as reasoning, language comprehension, and even

activities as simple as counting or reading a story, all require the use of working memory. In rodents, spatial working memory is commonly measured using delayed non-match to sample tasks (DNMS); the animal generally must visit a specific location on a maze during one trial and keep the location of that visit in mind during the next trial so it can correctly choose to visit a different (non-matching) location.

Previous literature has shown that the mPFC and HPC are both involved in a certain type of working memory known as spatial working memory, which refers to the ability to hold spatially-relevant information in mind in order to navigate an environment (Hampson et al. 2000). Theta rhythms, oscillations ranging from 5-10 Hz, are believed to be important for learning and memory, and are commonly examined in mnemonic studies (Buzsaki 2005). During the choice phase of a spatial working memory task, Jones and Wilson (2005) observed coherence, or alignment, in theta oscillations between the DH and the mPFC, providing evidence that the DH and mPFC may act together over spatial working memory tasks.

It is known that the mPFC and HPC have an excitatory, monosynaptic connection. The projections that make up this connection, beginning in ventral CA1 in rodents and ending in the ventral mPFC, are direct, with considerable plasticity in the synapses (Thierry et al., 2000). These projections are widely accepted in the literature, as several other reports demonstrate this CA1-mPFC connectivity (Swanson, 1981; Irle and Markowitsch, 1982; Cavada et al., 1983; Goldman-Rakic et al., 1984; Ferino et al., 1987; Jay et al., 1989; van Groen and Wyss, 1990; Jay and Witter, 1991; cited in Vertes, 2006). With mounting evidence to support hippocampal efferents to the

mPFC, however, it is important to note that no direct return projections have been identified from the mPFC to CA1 (Laroche et al., 2000; Beckstead, 1979; Goldman-Rakic et al., 1984; Room et al., 1985; Reep et al., 1987; Takagishi and Chiba, 1991; Buchanan et al., 1994; cited in Vertes, 2006).

In an anatomical study using an anterograde tracer *Phaseolus vulgaris-leucoagglutinin*, Jay and Witter (1991) demonstrated that hippocampal efferents primarily innervate the ipsilateral, or same-side, mPFC. One may infer, then, that if the connection between the left CA1 and left mPFC, for example, were lesioned, communication between the two areas would still exist between the right CA1 and right mPFC. If this communication necessitates proper spatial working memory performance, then silencing the communication between CA1 and the mPFC in rodents on a spatial working memory task and observing their performance would test for CA1-mPFC involvement in spatial working memory. Indeed, several studies have shown that bilateral, or two-sided, lesioning or silencing of either the HPC or mPFC can impair performance on spatial working memory tasks, while unilateral, or one-sided, lesioning or silencing of either of the areas has no effect (Brito et al., 1982; Seamans et al., 1995; Lee and Kesner, 2003; Kellendonk et al., 2006; Floresco et al., 1997). Furthermore, spatial working memory task performance has been disrupted by a combination of unilateral hippocampal lesions and contralateral, or opposite side, mPFC lesions (Floresco et al., 1997; Jay and Witter, 1991). Taken together, these results provide support for the idea that CA1 and ventral mPFC communicate ipsilateraly, and that this communication is required for spatial working memory.

One area of debate in working memory research concerns how the length of the temporal delay over which information must be held is impacted by the integrity of the CA1-mPFC circuit. For example, Churchwell and Kesner (2011) argue that the ventral/intermediate HPC and mPFC may act together during "long" time spans, or delays, which they defined as lasting for five minutes, but may act in parallel or be completely dissociable over "short" delays, which they defined as lasting for ten seconds. Thus, it appears as though a time-dependent component does exist for CA1-mPFC communication over working memory tasks; however, with a large temporal gap between their "short" and "long" delay conditions, conclusions have not yet been made about CA1-mPFC communication over intermediate delays.

1.4 The Thalamic Nucleus Reuniens (Re) and Rhomboid Nuclei (Rh)

While there is extensive research on the rodent mPFC and its projections from the VH, as well as research on time-dependent components of working memory studying mPFC afferents from the intermediate hippocampus, the anatomical connections that mediate neural communication between the DH and the mPFC are not well understood. The electrophysiological studies (Jones and Wilson, 2005; Hyman et al., 2010) demonstrating theta coherence between the DH and mPFC during spatial working memory tasks highlight the importance of studying the communication between the DH and mPFC in working memory. Although the rodent mPFC only receives direct input from the VH and does not directly project back to CA1, it does

connect with other brain regions to send indirect projections to the hippocampus. One of these regions is the nucleus reuniens (RE). RE is a ventral midline thalamic nucleus that is reciprocally connected with both the DH and the mPFC, making it a strong candidate to gate the flow of information between the two areas (Vertes, 2006). Hembrook et al. (2012) supported this idea by temporarily inactivating RE with muscimol on a DNMS bar-pressing task, performance on which is also compromised following either HPC or mPFC lesions, and observing significant performance deficits. In the same study, low doses of muscimol did not have an effect on performance on a DNMS radial arm maze task, which is only disrupted by HPC and not mPFC lesions, supporting the idea that RE may be important for tasks that require the use of both the HPC and mPFC.

While RE inactivation impairs working memory performance, several inconsistencies remain in the literature pertaining to RE involvement in working memory. In the same study, Hembrook and his colleagues also found that the observed impairments following RE inactivation were not delay-dependent. Furthermore, in a separate study, Churchwell and Kesner (2011) found that communication between HPC and mPFC is necessary over long delays on a DNMS radial arm maze task, contradicting the findings from Hembrook et al. (2012). It is believed that the RE does have an important role in tasks involving communication between the HPC and mPFC; however, more research is needed to address the inconsistencies in existing literature on the subject.

1.5 Purpose

The purpose of this study is to better understand how the length of the working memory task delay period impacts the involvement of the RE in task performance, while attempting to account for any inconsistencies observed in the existing literature. Our hypothesis is that as the length of the delay period increases, RE becomes more critical for working memory task performance. We predict that inactivation of RE will produce delay length-specific impairments that parallel those following hippocampal-prefrontal disconnection in working memory tasks. Specifically, no performance deficit is expected on the CA task, and performance deficits are expected to increase as the delay length increases over the DA30 task. We have previously collected behavioral data from rodents on a delayed-alternation T-maze task with a 30-second delay (DA30). DA requires rodents to alternate between a left and a right goal arm to obtain a reward, relying on their previous choice of direction to determine the location of the next reward. On DA30, RE inactivation, via fluorescent GABA-A receptor agonist muscimol, significantly impaired performance (Hallock et al., 2013). The current study expands on our previous findings by inactivating RE prior to the performance of the task with no delay, continuous alternation (CA). Using tasks that differ solely in their working memory demand will help to control for some of the inconsistencies seen in previous work. Additionally, the use of fluorescent muscimol

will allow for better visualization of its spread to ensure its localization to RE, something that was not done in previous studies.

Chapter 2

MATERIALS AND METHODS

2.1 Subjects

Subjects were 16 male, adult Long-Evans hooded rats weighing between 300-500 g. Subjects were individually housed in a temperature and humidity-controlled colony room on a 12 h light/dark cycle. All experiments took place during the light portion of the cycle. During the handling, pre-training, and testing portions of the experiment, each subject was food restricted in order to maintain him at 80-90% of his ad libitum body weight. All animal procedures were carried out in accordance with the University of Delaware Institutional Animal Care and Use Committee.

2.2 Behavioral Apparatus

Behavioral tasks were performed on a modified wooden T-maze, which consisted of a central stem (116 x 10 cm), two return arms (112 x 10 cm each), and two goal arms (56.5 x 10 cm each) in which plastic bottle caps were located that contained a chocolate sprinkle reward. Each maze section was surrounded by 6 cm high wooden walls. Between trials for the DA tasks, and at the beginning and end of each session for the CA task, animals were required to wait in a start box located at the base of the maze. The start box was blocked off from the maze by a large, removable wooden blocker.

2.3 Handling and Pre-Training

The handling and pre-training procedures were identical to those described previously by our laboratory (see Hallock and Griffin 2013; Hallock et al. 2013). Briefly, rats were handled by the experimenter for 5 days, at which time they were introduced to the chocolate sprinkle reward, which was scattered directly into their homecages. Each rat was then shaped to consume the chocolate sprinkle reward from plastic bottle caps located in the goal zone of the T-maze. During these "goal box" sessions, rats were confined to the maze goal zones and given a time limit of 3 minutes to consume the chocolate sprinkles. Once rats successfully consumed the chocolate sprinkles in under 90 seconds on each trial for two consecutive goal box sessions, they were advanced to the "forced run" phase of pre-training, in which they were shaped to run down the maze stem, go down a non-blocked goal arm (either the left or right goal arm was blocked according to a pseudorandom sequence), eat the chocolate sprinkle reward, and return to the start box via the return arm. Once rats successfully ran through the maze and consumed the chocolate sprinkle reward on at least 10/12 trials for two consecutive sessions, they were advanced to behavioral training.

2.4 Behavioral Training

For behavioral training, rats were assigned to learn one of two tasks – continuous alternation (CA), or delayed alternation with a thirty-second delay (DA30) and trained until they reached proficiency.

Delayed alternation: 10 rats learned a delayed spatial alternation task. Each session began with a chocolate sprinkle reward in both goal arms. The rat ran down the central stem, chose one goal arm, ate the reward, and returned down the return arm. This first trial was not recorded. In the next 24 recorded trials, the rat was required to alternate between the two goal arms, choosing the opposite goal arm from the previous trial to get a reward. Incorrect choices resulted in returning to the start box, unrewarded. In between each trial, the rat had a 30-second delay period (DA30) on the start box. The criterion for DA was 80% correct for two consecutive sessions before the rats proceeded to surgery.

Continuous alternation: 6 rats learned a continuous spatial alternation task. Each session began identically to the session described in DA. After the first sprinkle reward was obtained, the rat was required to return down the return arm, but continue back up the central stem and to the goal arm opposite that used in the previous session, instead of returning to the start box. The rat continuously alternated between reward locations in this fashion for 24 recorded trials. Incorrect choices resulted in returning to the beginning of the central stem, unrewarded. The rat returned to the start box only at the end of the session. The criterion for CA was 80% correct for two consecutive sessions before the rats proceeded to surgery.

2.5 Surgical Procedures

After rats reached performance criterion on either the DA or CA task, guide cannulae targeting the nucleus reuniens (RE) of the thalamus were surgically implanted. Prior to surgery, each rat was given a subcutaneous injection of atropine (0.05 mg/kg), followed by isoflurane (1.5 – 3.0% in oxygen) in a Plexiglas induction chamber. Once the rat was anesthetized, his head was shaved and he was placed into a stereotaxic instrument (Kopf Instruments) that was fitted with a specialized nose cone for continuous flow of isoflurane throughout the surgery. A subcutaneous injection of lidocaine was given into the scalp, and the incision site was sterilized with Novalsan. An incision was made, the skull was leveled and cleaned, and bregma was identified. Four small bone screws (Fine Science Tools) were placed into four burr holes that were made with a stereotaxic-mounted drill (Fine Science Tools). The bone screws were cemented to the skull with dental acrylic (Lang Dental). A circular hole was drilled 1.8 mm posterior to bregma and 2.0 mm lateral to the midline. Dura mater was removed, and an 8.0 mm stainless steel guide cannula (PlasticsOne) was lowered 6.5 mm ventral to the surface of the brain at a 15º angle. The cannula was cemented to the skull and the bone screws with dental acrylic, and a dummy cannula made to fit the guide cannula with a 1.0 mm projection was inserted. Since RE is a midline structure, only one cannula was necessary for temporary inactivation of the nucleus. Fluoxetine was injected subcutaneously approximately one hour prior to the end of surgery for pain relief. Following surgery, children's Ibuprofen (20 mg/ml) was mixed into each rat's drinking water for two days.

2.6 Infusion Protocol

The dummy cannula was removed, and an internal cannula made to fit the guide cannula with a 1 mm projection was inserted. The internal cannula was attached to a plastic tube that contained either PBS or muscimol. The plastic tube was attached to a microinfusion syringe (Hamilton), which was placed into an automated infusion pump (World Precision Instruments) that controlled the infusion rate and volume (0.25 µl/min and 0.5 µl, respectively). The position of the infusate was monitored by marking the position of an air bubble that separated the infusate from distilled water within the plastic tubing. Internal cannulae were allowed to sit in the brain for 2 minutes post-infusion. Behavioral testing took place Twenty minutes after infusions were given.

2.7 Behavioral Testing

Following a 5 day post-surgery recovery period, rats were re-trained on their designated tasks until they reached a pre-infusion performance criterion of >80% correct choices for three consecutive sessions. Prior to the following session, 0.5 µl of a vehicle (phosphate-buffered saline (PBS)) was infused, and task performance was measured. A 0.5 µg/µl concentration of muscimol (a $GABA_A$ receptor agonist) was infused and task performance was measured. The rats trained with no infusions the following day, or until reaching performance criterion again. A 0.25 µg/µl concentration of muscimol was then infused the next day and task performance was measured. The rats trained with no infusions the following day, or until reaching performance criterion again. A 0.125 µg/µl concentration of muscimol was then delivered the following day and task performance was measured for a final time.

2.8 Histology

At the conclusion of behavioral testing, rats were given an infusion (0.5 µl volume) of a fluorophore-conjugated BODIPY TMR-X muscimol (Life Technologies, Carlsbad, CA) in order to visualize the spread of the drug in brain, and then anesthetized with isoflurane (Allen et al. 2008). The fluorescent muscimol was diluted to a concentration of 0.25 µg/µl by placing the powder into a solution made of half PBS and half DMSO in order to aid in dissolution. Twenty minutes following infusion of the fluorescent muscimol, rats were transcardially perfused with 0.9% saline followed by 10% buffered formalin. Brains were removed and allowed to sit in 10% buffered formalin for 2 days, and were then transferred to a 30% sucrose solution (30 mg sucrose/100 ml PBS). After sinking, the brains were sectioned (40 µm) with a cryostat and mounted on slides. The slides were stained with ProLong Gold with DAPI (Life Technologies, Carlsbad, CA), and visualized with a confocal microscope. Cannula placement and spread of the fluorescent muscimol was characterized by placing digital plates from the Paxinos and Watson (2005) rat brain atlas over pictures of the cresyl-stained and DAPI-stained brain slices using Adobe Illustrator.

2.9 Data Analysis

A repeated-measures, one-way ANOVA was used to compare behavioral performance on saline and muscimol sessions between the rats on the DA30 task and CA task, separately. We expected to find a main effect of session as the DA30 task places significantly higher demand on working memory than does the CA task. We then combined the raw data from the DA30 task with that from the CA task and ran a 2 (delay) x (4 session), mixed-factor ANOVA. Finally, we normalized the scores

between the two tasks to account for the difference in saline session performance seen between the subjects in the two tasks. In order to normalize the scores, we took the difference of the percent performance on saline sessions and the percent performance on each of the muscimol sessions, and we compared those scores using a 2 (delay) x 4 (session) mixed-factor ANOVA, as well as two separate repeated-measures ANOVAs for each group, similar to the raw data analysis. We predicted that there would be a significant interaction and that post hoc tests would reveal a significant drop in performance on the muscimol day for the DA30 group, but not for the CA group. From this, we hypothesized that RE is not strongly involved in modulating the execution of the CA task, but that it would become increasingly necessary as the temporal demand on working memory increased to 30-seconds.

Chapter 3

RESULTS

3.1 Raw-score results

A 2 (delay) x 4 (session) mixed-factor ANOVA revealed no significant interaction, $F(1.726, 25.892) = .835$, $p = .430$. The same mixed-factor ANOVA revealed a significant within-subjects main effect of drug, $F(1.726, 25.892) = 10.795$, $p = .001$, and a significant between-subjects main effect of delay, $F(1, 15) = 11.854$, $p = .004$. Two, separate repeated-measures ANOVAs revealed a within-subjects main effect of session for the DA30 group, $F(1.543, 13.885) = 11.824$, $p = .002$, and revealed no significant within-subjects main effect of session for the CA group, $F(3, 18) = 2.571$, $p = .086$ (see Figure 1).

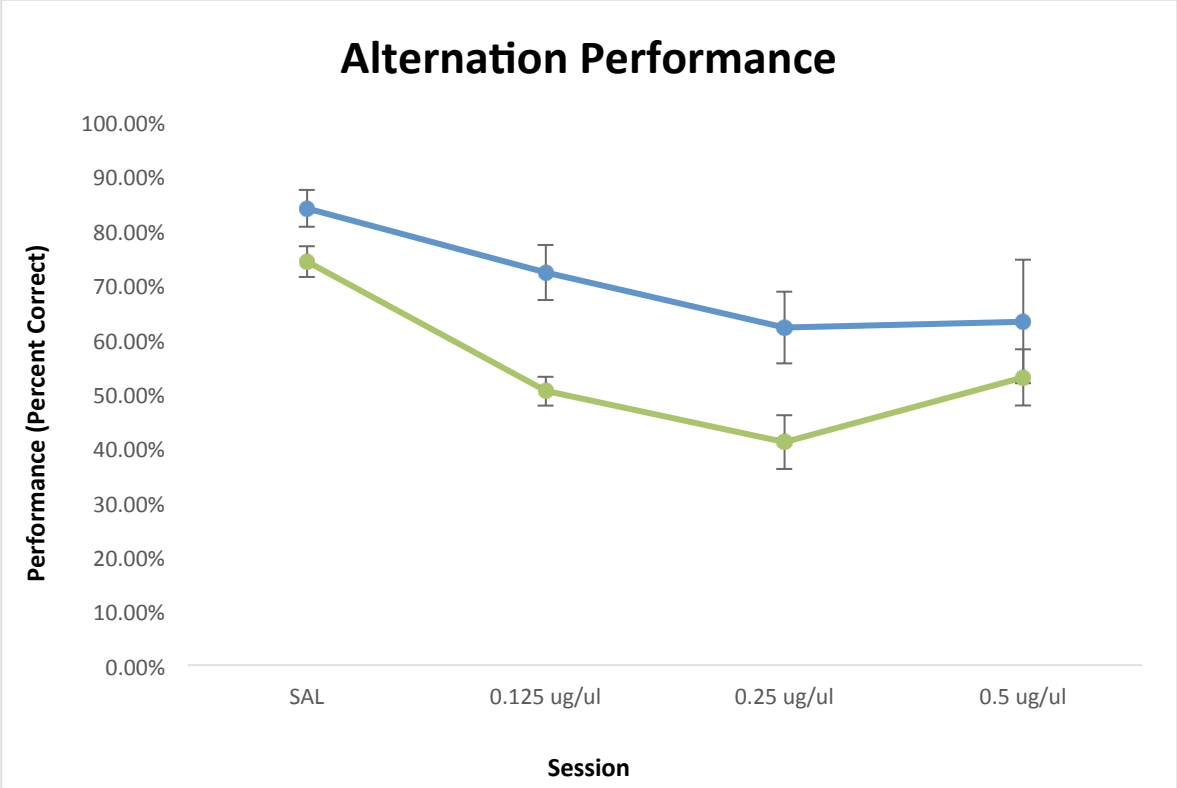

Figure 1. Raw results for performance (percent correct) of subjects on both the CA (blue) and DA30 (green) tasks after receiving saline and muscimol infusions.

3.2 Normalized-score results

A 2 (delay) x 4 (session) mixed-factor ANOVA revealed no significant interaction, $F(1.724, 25.853) = .840$, $p = .427$, a significant within-subjects main effect of drug, $F(1.724, 25.853) = 10.752$, $p = .001$, and no significant between-subjects main effect of delay, $F(1, 15) = 1.749$, $p = .206$. Two, separate repeated-measures ANOVAs revealed a within-subjects main effect of session for the DA30 group, $F(1.543, 13.885) = 11.824$, $p = .002$, and revealed no significant within-subjects main effect of session for the CA group, $F(3, 18) = 2.551$, $p = .088$. Post-hoc pairwise comparisons revealed that normalized performance on DA30 during muscimol

sessions did not significantly differ from one another, but normalized performance during each muscimol session was significantly higher than normalized performance during the saline session, ($p < 0.05$) (see Figure 2).

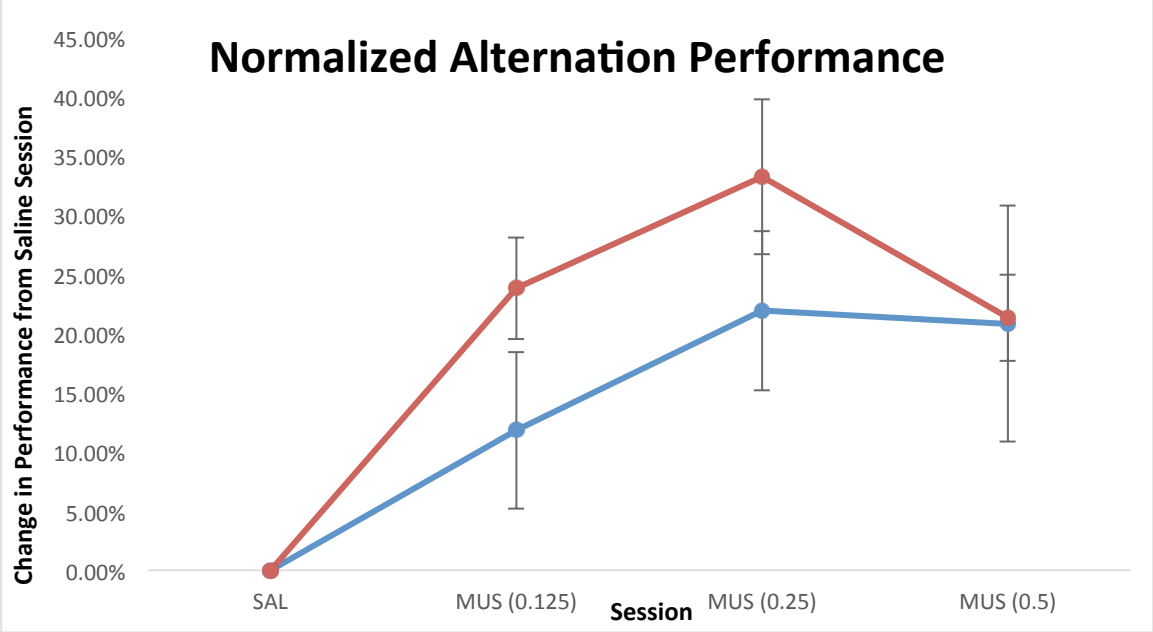

Figure 2. Normalized results for performance (percent correct) of subjects on both the CA (blue) and DA30 (red) tasks after receiving saline and muscimol infusions.

3.3 Histology

Out of the implanted rats, 6 rats in the CA group had cannula placements localized to RE/Rh (see Figure 3a), and 10 rats in the DA30 group had cannula placements localized to RE/Rh (see Figure 3b). Muscimol location was confirmed via

confocal microscopy and revealed that fluorescence was mainly restricted to RE and Rh (see Figure 4).

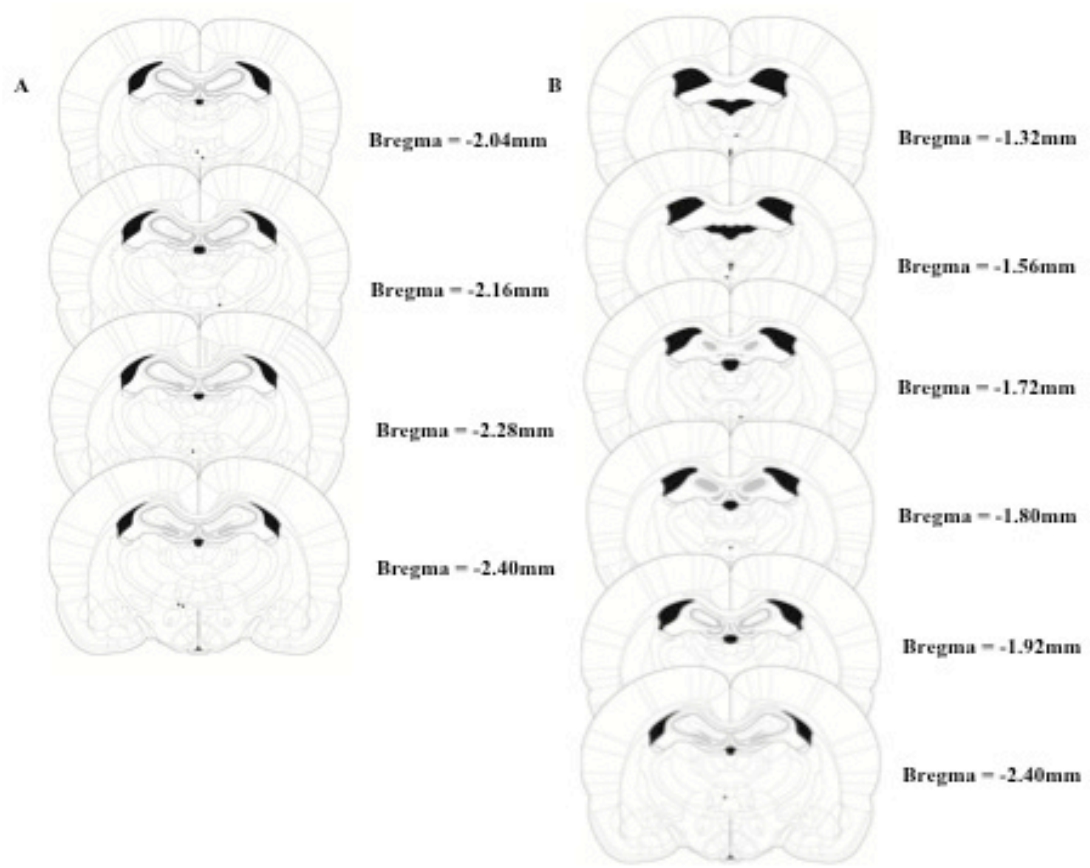

Figure 3. Cannula placements in RE for rats in the CA group (A) and for rats in the DA30 group (B).

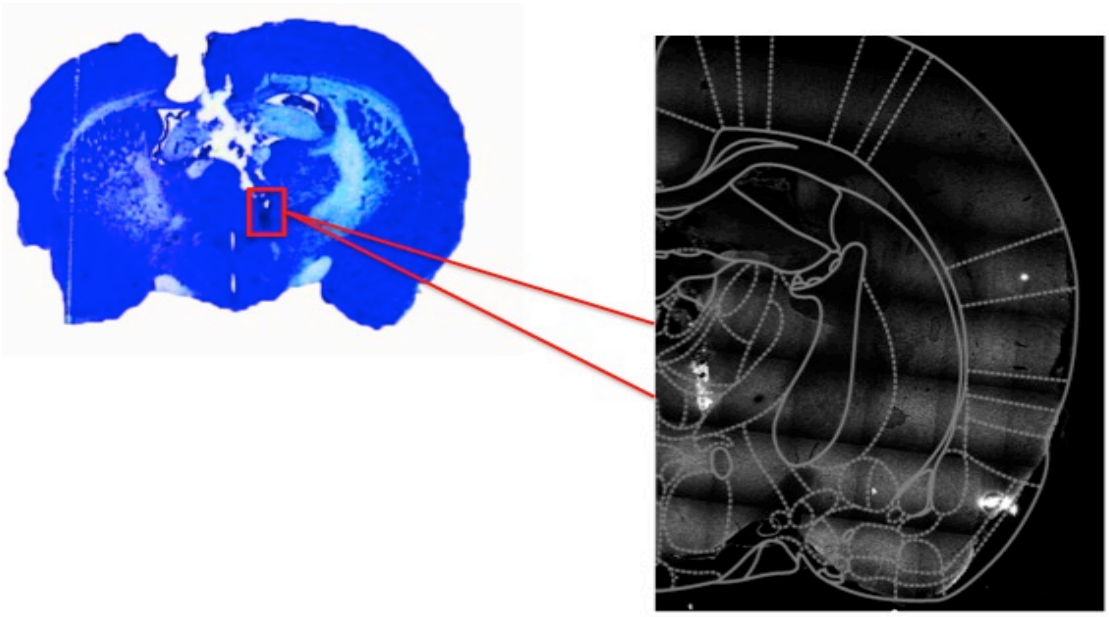

Figure 4. Nissl-stained coronal slice with cannula track and fluorescent image taken from the same rat with a plate from the Paxinos and Watson (2005) rat brain atlas overlaying it. Fluorescence at the tip of the injector cannula track was restricted to RE.

Chapter 4

DISCUSSION

The results of the present study indicate that RE inactivation by muscimol significantly impairs performance on DA30, while the performance on CA was not as strongly affected. This suggests that RE may have a delay-dependent function in working memory tasks.

4.1 Effects on CA and DA30

Electrophysiological evidence has shown that the hippocampus and prefrontal cortex functionally synchronize during working memory tasks in rodents, indicating that the two brain structures form a neural circuit that is important for working memory performance (Hallock et al., *in preparation*; Gordon, 2011). Current hypotheses propose a possible time-dependent functional relationship between HPC and mPFC, with results of functional inactivation studies suggesting that HPC and mPFC act together during performance of tasks that require working memory over long delays, and operate in parallel or have dissociable functions over short delays (Lee & Kesner, 2003; Churchwell & Kesner, 2011).

With reciprocal connections to both DH and mPFC, RE is a strong candidate to gate the flow of information between the two areas (Vertes et al., 2006). Previous studies in our lab have shown that inactivation of RE impairs the performance of a working memory-dependent conditional discrimination task, while leaving the

performance of a non-working memory-dependent version of the same task intact (Hallock et al., 2013). Furthermore, Hembrook et al. (2012) showed that RE inactivation compromised performance only on a task that required the use of both the HPC and mPFC, compared to a task that was only disrupted by HPC lesions. Taken together, these results suggest a role for RE in gating the flow of information between the two areas when communication between the two areas is needed, potentially during working memory tasks.

The hippocampus has been implicated in spatial alternation tasks in previous studies. CA1 place cells are known to fire differentially on the stem of a T-maze during continuous spatial alternation tasks (Wood et al., 2000). Ainge et al. (2007) showed that, while this firing may occur on the stem of the T-maze when there is no delay between trials, once a delay is imposed, such context-dependent hippocampal activity occurs during the delay period, and not on the stem of the T-maze. Furthermore, these same authors went on to show that this context-dependent hippocampal activity does not regulate alternation behavior in the continuous alternation task, as rats with hippocampal lesions were able to perform the task as well as the control rats. Hippocampal-lesioned rats, however, were significantly impaired when delays (2s or 10s) were imposed. Taken together, these findings suggests a role for context-dependent hippocampal activity in spatial alternation tasks, but that the mechanism underlying the behavior may change as the task demand changes. Our results provide further support for this idea. Because a significant within-subjects main effect was observed only on DA30 and not on CA during RE inactivation, it is

possible that RE may become important during spatial working memory as the temporal demand on working memory is increased, such as when a delay is imposed between trials.

While no interaction between performance on the DA30 and CA tasks was observed, this is because DA30 subjects began at a lower performance baseline than did the CA subjects, presumably due to the increase in difficulty of the DA30 task. It is possible, however, that this increase in difficulty is not entirely due to a heightened working memory demand in DA30. While DA30 does place a greater requirement on working memory, it also requires rats to exit the maze during each delay period to sit on an adjacent pedestal. Conversely, CA requires the rats to continuously run through the maze until the testing session is complete. This difference in strategies may introduce differences in attention or response-selection mechanisms, which may, in turn, affect the performance baselines on the two tasks.

4.2 Conclusion and future directions

While this study showed an effect of RE inactivation on working memory performance, similar studies provide conflicting evidence. In a lesion study on impulsive and compulsive behaviors, Prasad et al. (2012) found that RE lesions on a 5-choice reaction time task did not affect overall response accuracy in rats; however, the task used was a test of visuospatial attention and inhibition control, and may not have required the use of spatial working memory. In a separate study similar to the current study, Hembrook et al. (2012) inactivated RE on two different tests of spatial

working memory, a delayed nonmatching to position task (DNMTP) and a varying choice radial maze delayed nonmatching task (VC-DNM), and the results did not show a delay-specific difference in task performance. These contrasting results may be due to the demands of the DNMTP task itself; while the task had been characterized as being hippocampal-prefrontal dependent, it had not been characterized as having a delay-dependent component. The use of tasks that we know are dependent on hippocampal function, as well as the comparison across tasks for which the main difference is in the intertrial delay period duration, allowed us to better interpret the data in this study to suggest that RE may only be crucial when there is a delay present between trials.

Another advantage of our approach over previous investigations was the use of fluorescent muscimol, which allowed for better visualization of the spread of the infusate than is possible simply by examining the cannula tracks. For our study, muscimol localization to RE was confirmed in all of the subjects, confirming that inactivation was restricted to the RE and that muscimol did not spread to adjacent brain regions. We predicted that behavioral deficits would only occur on the task with a higher working memory requirement, DA30, and while we ended up seeing some deficits in a few subjects in the CA group, it is possible that RE still becomes crucial as the duration of the delay period over which information must be held is increased. In future studies, one way to control for the variance in performance seen in the CA group could be to investigate the effects of inactivating RE on a more intermediate delay, such as a five second delay, with a DA5 task.

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