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The role of reuniens and rhomboid thalamic nuclei in spatial memory

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The role of reuniens and rhomboid thalamic nuclei in spatial memory

Abstract
Spatial working memory is the ability to encode and temporarily store information for future retrieval to guide behavioral responses. Two areas of the brain that are important for this process are prefrontal cortex (PFC) and hippocampus. The hippocampus has strong connections to medial PFC, however there are no direct return projections from medial PFC to hippocampus. The reuniens (Re) and rhomboid (Rh) nuclei of ventral midline thalamus have anatomical connections with PFC and hippocampus. This dissertation sought to provide behavioral evidence for the role of the ventral midline thalamic nuclei in spatial working memory. Four experiments were conducted in rats using different methods to elucidate the role of Re and Rh nuclei in memory. Experiment 1 temporarily inactivated Re and Rh with pharmacological manipulations. Experiment 2 used permanent excitotoxic lesions to selectively damage Re or Rh nuclei. Experiment 3 used similar lesions on areas surrounding Re and Rh to rule out any potential contributions of these areas and Experiment 4 used event-related deep brain stimulation in Re and Rh to distinguish when during the memory process these nuclei are important. Results revealed impairments for the Re and Rh nuclei on different behavioral measures of spatial working memory that depend on the proper functioning of PFC and/or hippocampus. Temporal specificity was found for the storage and retrieval stages of the delayed nonmatching to position measure (DNMTP) of spatial working memory. These findings provide evidence that the ventral midline thalamic nuclei play an important role in spatial working memory, specifically for the communication of information across memory delays to guide memory responses.

Keywords
Psychology, Experimental, Psychology, Psychobiology
THE ROLE OF REUNIENS AND RHOMBOID THALAMIC NUCLEI IN SPATIAL MEMORY

BY

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DISSERTATION

Submitted to the University of New Hampshire in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy
In
Psychology

September, 2011
This dissertation has been examined and approved

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June 6, 2011

Date
DEDICATION

I would like to dedicate this work to my husband, Ian Short. I'm sure sometimes he has felt that he too was in graduate school. He was tirelessly by my side during my entire endeavor. He stayed up many nights while I typed into the wee hours of the morning, making sure my coffee cup was full. He was also always willing to read whatever I had written once I was done (usually as the sun was coming up). Thank you for being so understanding when I spent MANY MANY hours in the lab, I know you will get me back for it someday 😊

I would also like to thank my parents. They have always been very supportive in everything that I have done. They were also understanding of me bringing schoolwork home over most holidays, especially Christmas and knowing I could only stay for a few days. I appreciate all of your support and I know that this would not have been possible without it.
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Spatial working memory is the ability to encode and temporarily store information for future retrieval to guide behavioral responses. Two areas of the brain that are important for this process are prefrontal cortex (PFC) and hippocampus. The hippocampus has strong connections to medial PFC, however there are no direct return projections from medial PFC to hippocampus. The reuniens (Re) and rhomboid (Rh) nuclei of ventral midline thalamus have anatomical connections with PFC and hippocampus. This dissertation sought to provide behavioral evidence for the role of the ventral midline thalamic nuclei in spatial working memory.

Four experiments were conducted in rats using different methods to elucidate the role of Re and Rh nuclei in memory. Experiment 1 temporarily inactivated Re and Rh with pharmacological manipulations. Experiment 2 used permanent excitotoxic lesions to selectively damage Re or Rh nuclei. Experiment 3 used similar lesions on areas surrounding Re and Rh to rule out any potential contributions of these areas and Experiment 4 used event-related deep brain stimulation in Re and Rh to distinguish when during the memory process these nuclei are important. Results revealed impairments for the Re and Rh nuclei on
different behavioral measures of spatial working memory that depend on the proper functioning of PFC and/or hippocampus. Temporal specificity was found for the storage and retrieval stages of the delayed nonmatching to position measure (DNMTP) of spatial working memory. These findings provide evidence that the ventral midline thalamic nuclei play an important role in spatial working memory, specifically for the communication of information across memory delays to guide memory responses.
INTRODUCTION

Memory is the ability to encode, store and retrieve information. Encoding is the stage where information is processed and converted into a form that can then be stored in the brain for future use. The memory is then retained in the brain for a varying period of time. Finally, retrieval is when the information that was previously stored in the brain is recalled to be used. When any of these stages are disrupted, one is not able to accurately remember a particular piece of information (Sholl & Fraone, 2004).

One particular type of memory is working memory. Working memory is considered to be information that is encoded and temporarily stored for retrieval to guide a behavioral response (Baddeley, 1986). An example of this would be looking up a phone number and then being able to remember the number until you dial it on the phone. However working memory is not limited to only remembering numbers but can include various types of information that will be used for a direct action. This type of memory is thought to be important for a wide assortment of higher cognitive functions such as learning, comprehension and reasoning (Baddeley & Hitch, 1974; Baddeley, 2000). Tasks that involve working memory require one to keep trial-specific information on hand while also maintaining more long-term information about the task itself, such as the rules of the task and the surrounding environment. For the
successful completion of the task one must combine these types of information by encoding and then using that information at the correct time during the task (Goldman-Rakic, 1995; Zilli & Hasselmo, 2008).

Spatial memory involves remembering information about one's environment and its spatial orientation. An example of this is a person being able to navigate around a city. This can be done using allocentric or egocentric cues. The use of allocentric cues refers to the use of landmarks and other information from the environment to be able to accurately navigate. Egocentric cues on the other hand are using internal cues such as direction of turning (turning left or turning right) to be able to accurately navigate (Kesner, Farnsworth & DiMattia, 1989; Packard and McGaugh, 1996).

Different areas of the brain are important for the successful completion of spatial working memory tasks. Two areas of the brain that have been known to be important are prefrontal cortex and hippocampus (Yoon, Okada, Jung & Kim, 2008; Wang & Cai, 2006). The hippocampus has strong connections to medial prefrontal cortex; however, there are no direct return projections from medial prefrontal cortex to hippocampus (Thierry, Gioanni, Degenetais & Glowinski, 2000; Vertes, Hoover, Szigeti-Buck & Leranth, 2007). Therefore, there must be some other brain structure that is an intermediary between these two structures to facilitate this communication. Recent anatomical studies by Vertes and colleagues (Viana DiPrisco & Vertes, 2006, Vertes et al. 2007) suggest that two ventral midline thalamic nuclei, reuniens and rhomboid nuclei, may be the critical link between medial prefrontal cortex and hippocampus.
This dissertation provides behavioral evidence that the reuniens and rhomboid nuclei of thalamus are important for spatial working memory processing and that they thus may serve an intermediary role for communication between hippocampus and medial prefrontal cortex.
Animal Studies

Electrophysiological studies have provided evidence to support the role of prefrontal cortex in working memory (Passingham & Sakai, 2004). Funahashi, Bruce and Goldman-Rakic (1989, 1990) had primates remember a location of a visual stimulus before making a delayed response. They found elevations in prefrontal cortical activity during the period between the removal of the visual stimulus and that of the response cue. The neuronal activity increase accurately predicted whether the response the primate made would be correct. Interestingly, activity during the delay period was often increased (several hundred milliseconds) after the start of the visual cue, then continued during the delay period and ended just after the initial movement began for a response (Funahashi, 2006). This suggests that the prefrontal cortex is important for temporarily storing memory over a period of time until a response is made.

Numerous studies have also shown that delay-periods activity is shortened or prolonged dependent on the length of the delay given for the particular trial (Funahashi, Bruce & Goldman-Rakic, 1989; Fuster, 1973; Kojima & Goldman-Rakic, 1982). Interestingly comparable delay activity was not
observed when the monkey made an incorrect response (Fuster, 1973, Funahashi, Bruce & Goldman-Rakic, 1989).

Lesion studies in monkeys have provided evidence that prefrontal cortex is important for working memory (Mishkin & Manning, 1978; Paule et al., 1998) One task that tests working memory is the delayed matching to sample task (DMTS). There are different versions of the DMTS, however the concept is similar. A trial begins with a shape being illuminated in the center of a 3-button press-plate panel. An initial response would be completed by pressing the illuminated plate, causing it to turn off (sample stimulus). A delay could then be imposed to vary the difficulty of the task. Then all three buttons would illuminate, each with a different shape (choice stimulus). For a response to be correct, a press would need to be made to the button with the shape that matches the sample resulting in a food reward (Passingham, 1975; Rodriguez & Paule, 2009). Damage to prefrontal cortex produced deficits in accuracy performance on the DMTS in monkeys (Mishkin & Manning, 1978, Gaffan & Weiskrantz, 1980; Kolb, 1990; Paule et al. 1998).

Similar studies have been conducted in rats for assessing working memory (delayed match to position). In the DMTP, the subject is required to remember the particular side of a sample lever over the course of a delay and then make a response by choosing the same lever during the choice phase (Burk & Mair, 1998). Working memory is then able to be assessed over different retention intervals to determine the rate of forgetting.
There are two different outcomes for the rate of forgetting, delay-dependent and delay-independent deficits. Delay-dependent deficits are indicated when there are no impairments during short retention intervals but as the retention interval increases, there is an increase in performance deficits. Therefore, this shows the ability to complete a task using a specific rule, however as the demand on working memory is increased impairments become apparent. Delay-independent deficits are non-specific impairments that are present at all retention intervals and can be indicative of diminished factors. These include the failed ability to use a specific rule, diminished attentiveness or motivation to the task, or motor or sensory deficits (Dunnett, Wareham & Torres, 1990; vanHest & Steckler, 2001). This particular delay-effect distinction can be very useful when delineating differences between potentially related brain areas (Young, Stevens, Converse & Mair, 1996).

The prefrontal cortex is important in working memory (Passingham & Sakai, 2004). Specifically, the dorsal and ventral areas of medial prefrontal cortex are imperative for motor aspects of working memory and the response flexibility to be able to successfully complete the delayed matching to position task in rats (Kesner, 2000). Damage to these dorsal and ventral areas of medial prefrontal cortex in rats have been shown to produce delay-dependent deficits for delays ranging from 0 seconds to 24 seconds on the DMTP. Sloan and colleagues (2006) found deficits in overall accuracy of responding on the DMTP for longer delays and a spared performance for the 0, 2 and 4 second delays. Other lesion studies have found delay-independent deficits (Chudasama & Muir,
1997; Harrison & Mair 1996; Mair, Burk & Porter, 1998). It has been suggested that the delay-independent deficits are actually due to impairment in effectively utilizing mediation strategies to accurately solve the task rather than impairment in the memory itself (Chudasama & Muir, 1997).

One important difference between some of these other studies and Sloan Good and Dunnett (2006) was the inclusion of a lever on the opposite side of the operant chamber which serves as the start of each trial. Previous work in our lab has examined the differential effects of prefrontal cortical lesions on the delayed matching to position task, with the task including the lever on the opposite wall from the sample lever (Mair, Burk & Porter, 1998). This forces the animal to disengage from the sample lever during the delay and thus prevents any mediational responses such as allowing the animal to stay in the area of the choice during the memory delay period. Using this type of operant chamber, Mair and colleagues (1998) found delay independent deficits, in rats with lesions of the medial wall of prefrontal cortex.

An earlier study (Harrison & Mair, 1996) also examined the role of frontal cortex on a similar task of working memory, the delayed nonmatching to position task (DNMTP). They found delay-independent deficits for rats with lesions to either the medial wall or the rhinal sulcus of frontal cortex on the delayed nonmatching to position task with short imposed delays (0 seconds to 3 seconds). The findings of these studies provide overwhelming evidence that the prefrontal cortex plays a role in spatial working memory in rats and monkeys.
These studies help to provide a foundation to compare the results of these studies to then be able to apply them to humans with damage in similar areas.

**Human Studies**

Clinical studies have further supported the role of prefrontal cortex in short-term memory and executive functioning, such as planning a sequence of responses. Executive functions are thought to be critical for working memory and damage to prefrontal cortex creates deficits in these executive abilities (Shallace, 1982; Baddeley & Della Sala, 1996; Kimberg, D'Espisito & Farah, 1997). There is also evidence that prefrontal cortex is important for the temporal order for spatial locations, visual objects and linguistic information (Milner, Corsi & Leonard, 1991; Kesner, Hopkins & Fineman, 1994).

Humans with damage to the prefrontal cortex have shown similar impairments to studies with rats on delayed matching to position tasks (Fuster, 1997) as well as other spatial learning tasks and working memory tasks which involve remembering spatial response information and delayed spatial response tasks (Fuster, 1997; Leonard & Milner, 1991; Funahashi, Bruce & Goldman-Rakic, 1993).

With the advent of fMRI technology, researchers are now able to examine changes in activity in different areas of the brain during working memory tasks. Studies using this type of neuroimaging have consistently found activation in frontal cortical regions during tasks of working memory (Belger, Puce, Krystal,
Gore, Goldman-Rakic & McCarthy, 1998). For example, Kammer and colleagues (1997) scanned normal subjects while they were performing one of two working memory tasks involving letter detection. The task required subjects to respond by pressing a button whenever a letter was presented that was the same as the second to last letter in a given sequence. Control subjects were to respond to a single predefined letter so that both conditions were the same except for the task demand. Activation was higher in the dorsolateral prefrontal cortex in both hemispheres for subjects who underwent the working memory task compared to control subjects. There are two other recent examples of fMRI studies where bilateral activation of prefrontal cortex was found during a simple missing letter paradigm. Activation was specifically seen during the encoding and delay periods of the particular task (Cohen, Barch, Carter & Servan-Schreiber, 1999; Kerns, Cohen, Stenger & Carter, 2004) Cohen et al. 1999; Kerns et al., 2004). These studies provide evidence for the activation of prefrontal cortex not only during working memory processing but also for specific periods of this memory process.

There may be clinical applications for working memory tasks. For example, examining the activation of these areas of frontal cortex during working memory tasks may help to evaluate patients with frontal dysfunctions. However, prefrontal cortex is not the only brain region that is important for memory, more specifically working memory. The hippocampus is the other known structure involved in memory processes and therefore must also be examined for contributions in spatial working memory.
CHAPTER 2

HIPPOCAMPAL ASPECTS OF SPATIAL WORKING MEMORY

Animal Studies

Animal studies have shown hippocampus to be critical for spatial memory. A number of animal behavioral tasks have been used to test hippocampal-dependent aspects of spatial memory. These include, various radial arm maze tasks (RAM), DNMTP in the operant chamber and Morris water maze (MWM) tasks.

The MWM can be used to test two different types of memory, reference memory and working memory. In a typical reference memory water maze task, animals are trained to find a white platform in a fixed position that is submerged under a pool of white-colored water. After a set of training trials conducted over a set of days, the animals are given a delay period where there is no exposure to the water maze. This delay can be as little as one hour to twenty-four hours, or even as long as a few weeks. The animals are then given a memory probe trial where they are again exposed to the maze, however the platform is removed. This is where retention for spatial memory can be measured by examining the time animals spend in the quadrant that originally contained the platform training, the number of passes made where the platform was located and the proximity to
the platform area (Morris, Garrud, Rawlins & O'Keefe, 1982; Dolleman-van der Weel, Morris & Witter, 2009; Davoodi et al., 2009). The working memory version of the task consists of two trials per day over a series of days. In the first trial (acquisition), the animal needs to find the platform. The platform location varies each day of testing. The animal is then removed for a delay period. After the delay, the second trial is conducted (retrieval), where the platform is in the same location but the animal is released from a different place than in the acquisition trial (Davoodi et al., 2009).

In 1982, Richard Morris and colleagues first showed that hippocampal lesions impair spatial memory in rats on the MWM. Since then, more studies have shown that lesions in different parts of hippocampus and hippocampal lesions of different sizes impair performance in the MWM for spatial learning (Sloan, Good & Dunnett, 2006; Dolleman-van der Weel, Morris & Witter, 2009) and spatial memory (Broadbent, Squire & Clark, 2004; Clark, Broadbent & Squire, 2005; Dolleman-van der Weel, Morris & Witter, 2009).

Previous studies in our lab have examined the role of hippocampus in other spatial memory tasks. Mair, Burk and Porter (1998) examined the effects of permanent lesions in hippocampus on performance in rats using a varying choice delayed nonmatching to position task trained in the radial arm maze (VCDNM RAM) (see figure 1 for diagram of the maze). In this task, the animal starts in the central hub of the maze. One of any of the eight gates open and that particular arm serves as the sample arm. Once the animal runs down the arm and receives reinforcement, another randomly selected gate opens allowing
access to a new arm, which is then considered to be the delay arm. The animal is restricted to the delay arm for a variable amount of time and once the delay is over, the gate to the delay arm, the sample arm and another randomly selected arm are opened. For the animal to make a correct choice and receive water reinforcement, they must go down the arm they had not previously entered. Delay-dependent impairments were seen for the hippocampal lesion group compared to controls. The hippocampal lesion group performed normally at short delays but as the delay intervals increased, the impairments also increased. Delay-dependent deficits were also reported by Mumby and colleagues (1992, 1995) and Clark, West, Zola and Squire (2001) at the longest delays tested (600 seconds and 120 seconds respectively) in rats with hippocampal damage on versions of the DNMTS.

A similar delay-dependent impairment was found by Porter, Burk and Mair (2000) for the VC-DNM RAM when rats were given lesions of hippocampus. In the same study, Porter, Burk and Mair (2000), also used the DNMTP which was trained in operant chambers. They found delay-independent accuracy impairment for rats with hippocampal lesions on the DNMTP compared to controls. These deficits are corroborated by Hampson, Jarrard and Deadwyler (1999) and Aggleton, Keith, Rawlins, Hunt and Sahgal (1991) where they found accuracy impairments in rats with hippocampal damage. However, the impairments they found were delay-dependent. But, when damage to the area was more extensive, they saw delay independent deficits (Hampson, Jarrard & Deadwyler, 1999) similar to Porter, Burk & Mair (2000).
Primate studies have also examined performance on delayed non-matching to sample (DNMTS), specifically examining the brain areas that are important for the proper completion of the task. In primate studies, the delayed non-matching to sample (DNMTS) is similar to the delayed matching to sample (DMTS) except the animals are required to choose the item that does not match the sample object. It has been thought that monkeys are able to learn the DNMTS quicker than the DMTS because of the animal's natural tendency to attend to more novel stimuli (Rodriguez & Paule, 2009). This task has been instrumental in identifying brain areas involved in the recognition of previous stimuli. Studies have shown that the prefrontal cortex (Kowalska, Bachevalier & Mishkin, 1991; Meunier, Bachevalier & Mishkin, 1997) as well as the hippocampus (Beason-Held, Rosene, Killiany & Moss, 1999; Zola, Squire, Teng, Stefanacci, Buffalo & Clark, 2000) are necessary for this task.

Further evidence of the involvement of hippocampus was demonstrated by recording single unit activity in the hippocampus of monkeys. A number of single units had changes in discharge rate during a delayed response task (Watanabe & Niki, 1985). Another study by Wilson, Riches and Brown (1990) confirm similar hippocampal activity during the delayed nonmatching to position task. These results are consistent with the findings from lesion studies where damage to hippocampus produced deficits in spatial memory. Hippocampal activity is important during spatial memory tasks and when the hippocampus is damaged, spatial memory is impaired.
Human Studies

Clinical studies have provided convergent evidence of the role of hippocampus in memory. One famous patient with extensive hippocampal damage was the case of H.M. H.M. was a patient who had bilateral removal of his hippocampus. Following the surgery HM had many different memory issues. One particular memory issue that resulted was in relation to forming new memories. HM’s short-term memory was not as affected, however permanent long-term memory was impaired (Corkin, 1984).

Being able to navigate through the world is very important. Without this ability, we would never be able to find our house from work or our car in the parking lot. We use many different cues in the environment to complete these tasks such as landmarks. In analyzing the tasks that are available to assess spatial memory, it is difficult to make direct comparisons. This is due to the type of memory that is the focus of this research. Usually in humans, egocentric cues are examined in tasks such as learning a maze or learning where an object is located. However in nonhumans, allocentric memory is usually tested. Also, the term ‘spatial’ is varied wherein it can refer to vast array of different behavioral memory tests. One possible way to eliminate this variance is to use a virtual environment. A human version of the MWM was created to more accurately test aspects of spatial memory. This task is similar to the rat version except the participants navigate through a virtual pool using a video joystick and a speaker is used to be able to provide auditory feedback to the participant (Astur, Taylor, Mamelak, Philpott & Sutherland, 2002).
Typically, participants are told that they will be in a three-dimensional pool and that they should try to escape from the water as quick as possible. The computer gives feedback when the participant has escaped. After a series of learning trials, a probe trial will be conducted where no platform will be present in the pool and the participants will be given thirty seconds to swim around the pool in the location where they think the platform is. The participants were not told that the platform had been removed (Astur et al. 2002, Bartsch et al. 2010).

Astur and colleagues (2002) tested participants who had unilateral hippocampal damage on the virtual MWM. They found that when participants with hippocampal damage were required to use spatial cues to find the hidden platform, they were impaired compared to age-matched controls. Another study by Bartsch et al. (2010) used a similar version of the virtual MWM with patients who had transient focal hippocampal lesions. They also found impaired performance on memory probe trials when compared to control participants. Taken together, these findings show that the hippocampus is important for spatial memory processing.

**Integration of Prefrontal Cortex and Hippocampus in Spatial Working Memory**

Results of human and animal studies indicate that the hippocampus and prefrontal cortex are important for spatial working memory. Hippocampal function becomes more and more critical as the delay between when information is encoded and when it is retrieved increases. Subsequently, transient
disconnection of the hippocampal-prefrontal circuit with infusions of lidocaine or muscimol impair the retrieval process of spatial memory in radial arm maze tasks (Floresco, Seamans & Phillips, 1997) as well as delayed alternation tasks (Wang & Cai, 2006; Yoon et al., 2008). This suggests that not only are the prefrontal cortex and hippocampus important in spatial memory but the communication between these two brain areas is also imperative.

Further evidence for this is provided by Hyman et al. (2010) who compared activity during incorrect trials versus correct trials on the DNMTP. They found similar firing rates of medial prefrontal cortical cells regardless of the type of response, however, the theta-entrainment of medial prefrontal cortical neurons decreased during incorrect trials (17% versus 46%). There were also correlated theta-entrainment interactions of medial prefrontal cortex to hippocampus with successful performance of the DNMTP (Jones & Wilson, 2005a, 2005b; Hyman et al., 2010). These studies suggest that not only are prefrontal cortex and hippocampus important for spatial memory but the connections between these two structures are equally important.
CHAPTER 3

THALAMIC CONTRIBUTIONS

Midline Thalamic Nuclei

Midline and intralaminar nuclei are important sources of thalamocortical projections which have been implicated in the control of arousal, attention and awareness (Jones 1985, Steriade, Jones & McCormick, 1997, Van der Werf Van der Werf, Witter & Groenewegen, 2002). Clinical studies have linked damage of these nuclei with cognitive deficits affecting attention, memory and motor function and other aspects of executive functioning (Zola-Morgan & Squire, 1993; Braak & Braak, 1998; Gold & Squire, 2005) as well as deficits in awareness observed with coma, persistent vegetative state and akinetic mutism (Schiff, 2008).

The midline and intralaminar nuclei were once thought to be ‘nonspecific’ (Jones 1985, Groenewegen & Berendse 1994, Van der Werf, Witter & Groenewegen, 2002) because they did not seem to project to specific areas of cortex (Jones & Leavitt 1974). Also, when electrophysiological stimulation was applied to intralaminar nuclei, the result was widespread global changes in cortical activity (Dempsey & Morison, 1942; Moruzzi & Magoun, 1949; Jasper 1960).
More recently, studies using neuroanatomical tracing techniques have examined anatomical projections. From these studies, four different groups of midline thalamic nuclei have been identified, the lateral, the dorsal, the ventral medial, and the posterior group. These different groups potentially play different roles in brain functioning and patterns of connectivity. The lateral cluster consists of the anterior central medial, paracentral and central lateral nuclei (Van der Werf, Witter & Groenewegen, 2002). This cluster innervates medial prefrontal cortical areas along with the medial striatum. This area has been shown to play an important role in executive functions including different aspects of attention, working memory, memory for motor responses and decision making (Shallice, 1982; Baddeley & Della Sala, 1996; Kimberg, D'Espisito & Farrah, 1997). The posterior intralaminar cluster includes the centre median (in primates but not found in rats) and parafascicular nuclei and has robust connections with the basal ganglia. This cluster has limited cortical projections, primarily to the lateral agranular areas in the rat corresponding to primary and secondary motor cortex. These connections suggest a role related to response planning and selection and some aspects of motor control (Burk & Mair 2001, Mair, Koch, Newman, Howard & Burk, Hembrook & Mair, 2010). The ventral medial cluster consists of the rhomboid, reuniens and posterior central medial nuclei. This cluster has inputs to the hippocampus and parahippocampal cortex, two areas involved in spatial memory, therefore it is suggested that these nuclei might also play a role related to spatial memory (Van der Werf, Witter & Groenewegen 2002; Hembrook & Mair, 2010).
The hippocampus distributes strong projections to medial prefrontal cortex and has strong excitatory actions here, however are there are no direct return projections from medial prefrontal cortex to hippocampus (Hoover & Vertes, 2007). Recent anatomical work by Vertes and colleagues (2007) suggest that reuniens (Re) and rhomboid (Rh) nuclei may represent a critical link between medial prefrontal cortex and hippocampus. They used anterograde and retrograde tracers in order to visualize convergence. Anterograde injections of (PHA-L) were made into ventral portions of medial prefrontal cortex and retrograde injections (Flurogold) were made into the CA1/subiculum regions of hippocampus. Re was the only nucleus of the thalamus where fibers from medial prefrontal cortex and hippocampus showed strong convergence (Vertes et al. 2006; Vertes, Hoover, Szigeti-Buck & Leranth, 2007).

Both Re and Rh have projections to prefrontal cortex and the hippocampal system (CA1 and subiculum). However, Re is unique because medial prefrontal cortical fibers connect with the dendritic shafts of neurons in Re which directly project to hippocampus (Vertes et al., 2006). Based upon this and microstimulation work (Dolleman-Van der Weel, Lopes da Silva & Witter, 1997; Viana DiPrisco & Vertes, 2006), connections between the structures would form a loop through reuniens: CA1/subiculum to medial prefrontal cortex to Re and then back to CA1 (Vertes, Hoover, Szigeti-Buck & Leranth, 2007).

Re and Rh provide a critical link between hippocampus and prefrontal cortex thus ventral midline lesions should affect performance on behavioral tasks that depend on interactions between prefrontal cortex and hippocampus. The
following dissertation experiments examine effects of lesions, temporary drug inactivation and electrical deep brain stimulation (DBS) of ventral midline nuclei and surrounding areas to demonstrate the importance of these nuclei for spatial working memory. Experiment 1 examined the effects of inactivation produced by the drug muscimol to elucidate the role of the Re and Rh on spatial memory. Experiment 2 used excitotoxic lesions to selectively damage either Re or Rh and compared effects on a series of behavioral tasks, delayed nonmatching to position (DNMTP), serial reversal learning (SRL), radial arm maze tasks (standard 8 arm task (8-arm RAM) and a four-forced choice delay task (4F RAM)), and a reference memory water maze task. Experiment 3 compared effects of excitotoxic lesions that damaged Re and Rh to lesions of more dorsal and more lateral areas of thalamus on the same behavioral tasks as Experiment 2. Lastly, experiment 4 used event-related DBS to examine effects of activating Re and Rh during spatial working memory. As a control, the effects of DBS were compared for a spatial reference memory task (SRM). The combinations of these techniques provide convergent evidence elucidating the role of the reuniens and rhomboid nuclei in spatial working memory processing. The overarching hypothesis is that Re and Rh mediate interactions between hippocampus and prefrontal cortex that support capacity for spatial working memory.
GENERAL METHODS

Apparatus

Operant Chamber

*Delayed Non-matching to Position Task (DNMTP)*
*Serial Reversal Learning Task (SRL)*
*Spatial Reference Memory Task (SRM)*

The operant chamber consists of a computer-controlled chamber constructed of metal and Plexiglas. The chamber had Plexiglas on the ceiling, back wall and the door. The floor of the chamber consisted of metal bars and underneath was a pan with cedar shavings. One side of the chamber had a retractable lever in the middle of the wall with a house light above it. The other side consisted of two metal retractable levers on either side of a square opening. Inside the opening was a small hole where a dipper arm was raised up to give water reinforcement. The dipper was located outside of the chamber and was set in a small plastic tub that was freshly filled with water at the beginning of each testing day. The chamber itself was placed in a sound attenuating box that was fixed with a fan. Figure 2 shows the construction of the chamber and the outer box.

Radial Arm Maze

*Varying Choice Delayed Non-matching to Position Task (VC-DNM RAM)*
Eight-arm Task (8-arm RAM)
Four-Forced Choice Delay Task (4F RAM)

The radial arm maze consisted of a computer-controlled eight arm radial maze which was a modified version of one manufactured by Med Associates (Georgia, VT). Each of the arms was 60 cm long, 17.5 cm wide and 20.0 cm tall and was attached to an octagonal-shaped center hub, 30 cm in diameter and 33 cm in height. The floor of the maze was a white polycarbonate and the walls and ceilings of the arms were a clear polycarbonate. Motorized gates made of aluminum allowed access to each of the arms. Wells were milled into the floor of the apparatus at the end of each arm where water reinforcement was given by activating a miniature solenoid valve. Photocells were 4.0 cm off the ground and placed 4.5 cm from the end of the arm to register arm entries. Figure 1 depicts a diagram of the radial arm maze.

Morris Water Maze

Reference Memory Water Maze (RM-WM)

The water maze was a plastic circular pool, 140 cm in diameter and 55 cm high. The pool was filled with water to a temperature of +/- 25° C and the water level varied depending on the day of testing. The water was made white using non-toxic white powder paint. A ruler was used to ensure the proper water level under the platform as well as the water level relative to the platform. The pool was labeled; N, E, W, S, for ease of knowing which area to put the rat in. Room cues include different construction board papers at each major direction. This included a plus sign, a circle, a black rectangle and black/white horizontal stripes.
Other cues in the room included a cabinet, a cement platform and shelving. Once the rat was placed in the pool, the platform was the only way that the rat could escape from the water. The platform, 10 cm in diameter, was painted white and a sheet of white no-slip rug padding was secured on the top with string and rubber bands to provide grip for the rats. For some testing sessions; three white shower curtains were attached to the ceiling with Velcro around the pool to block out any external cues. Mounted on the ceiling in the middle of the pool was a video camera. The signal was fed to a computer tracking system in the next room to record the animal’s behavior in real-time.

**Animals**

All animals were obtained from Harlan Laboratories (Boston, MA) and were about 3 weeks of age upon arrival to the University of New Hampshire. Handling of the rats began at four weeks of age and rats began water deprivation after they reached a weight of 200 grams. Rats were individually housed in a temperature and humidity controlled room with a 12 hour light/dark cycle. All training and testing occurred during the light cycle. Water access was restricted for use as reinforcement for responding. Rats were given access to water during the behavioral training tasks and also for thirty minutes at the end of each training/testing day.
Surgical Procedure

All animals were food deprived 24 hours before the beginning of surgery. Prior to surgery, all surgical instruments were sterilized by an autoclave or by immersion into 70% alcohol. Rats were anesthetized with intramuscular injections of ketamine (85mg/kg) and xylazine (8.5mg/kg). The level of anesthesia was tested by checking reflexes to the foot. If any movement occurred, animals were given a 0.1mg supplement of ketamine. The rat’s head was then shaved, ears were punched for identification purposes and the head was placed into the stereotaxic instrument (David Kopf, Tujunga, CA). Ointment was applied to the eyes to prevent any dryness. After the application of betadine to the shaved area, a longitudinal incision was made along the midline and the skin retracted to expose the surgical field. The periosteum was then scraped away and measurements were taken of bregma and lambda relative to the interaural line to verify the stereotaxic plane. This is where the surgery differed depending on whether cannula/electrodes were implanted or lesions were created.

Cannula/Electrode Surgical Procedure

Four small holes were drilled into the cranium and screws were inserted without penetrating the dura. The skull was then opened with a trephine and either a 21 gauge guide cannula (Plastics One, Roanoke, VA) or a bipolar
electrode (twisted pairs of 0.125 mm SS wires with polyimide insulation, MS303-3, Plastics One, Roanoke, VA) was inserted.

For the cannula implantation, the guide cannula was angled to avoid the superior sagittal sinus. The guide cannula was aimed at the stereotaxic coordinates of, AP: 6.45, DV: 2.7 and ML: 0 relative to IA. These coordinates were determined using Paxinos and Watson (1998) and extended the guide cannula directly above Re, so when the cannula needle was inserted for testing sessions, it would extend 2mm past the guide cannula into both Re and Rh. For electrode implantation, the electrode was also angled to avoid the superior sagittal sinus and the tip of the electrode was directly aimed at ReRh with the targeted stereotaxic coordinates of, AP: 6.44, DV: 2.4 and ML: 0.0 relative to IA.

Cranioplastic cement was then applied around the guide cannula/electrode and the screws to secure it firmly to the skull. The skin was then sutured closed around the guide cannula/electrode and betadine applied to the stitches. Dust caps were put on to avoid infection into the area. A subcutaneous injection of butorphenal (0.2mg/kg s.c.) was administered to help with swelling and irritation. The rat was then returned to the home cage and monitored until consciousness was regained. Each animal was then given two weeks of recovery time, where they were monitored and given free access to water. If any rats during this time showed any signs of discomfort, they were given an injection of butorphenal (0.2mg/kg s.c.) to decrease the irritation.
Lesion Surgical Procedure

Excitotoxic lesions were induced by injecting a solution of 100 mM NMDA in normal buffered saline. This was done with a 26 gauge cannula, which was lowered down into the desired location. Tables 1 and 2, show the stereotaxic coordinates relative to IA for Experiment 2 and 3, respectively. A mini pump (Harvard Apparatus, Holliston, MA) was used to regulate the rate and volume of the NMDA (the volumes are also located on Tables 1 and 2). Each cannula was left in each site for at least three minutes after the injection to ensure that all of the substance had been properly injected. For control surgeries, the cannula was placed 2 mm into the brain and no substance was injected. After the last injection was completed the skin was sutured closed and betadine applied to the stitches. Animals were then returned to their home cage and monitored until they regained consciousness. They were then given two weeks of recovery with free access to food and water.
EXPERIMENT 1

INTRODUCTION

Previously our lab used excitotoxic lesions of NMDA to damage the reuniens and rhomboid (ReRh) thalamic nuclei and compared their performance to damage in rostral and caudal intralaminar thalamic nuclei (Hembrook & Mair, 2010). We found a double dissociation between ReRh lesions and caudal intralaminar lesions, where damage to caudal areas of the intralaminar nuclei produced deficits of reaction time for a visuo-spatial reaction time task but spared overall accuracy performance on radial arm maze measures of spatial memory. Conversely, ReRh lesions impaired radial arm maze measures of spatial memory while sparing the visuo-spatial reaction time task.

There are downsides to using lesions to examine deficits of behavioral function related to damage in the brain, one is that you need a large sample of animals. Lesion studies require between-group designs to compare performance of the experimental groups to that of a control group. The second downside is that once the lesion is produced, it creates permanent damage to the site and temporary impairment of adjacent tissue that recovers with the passage of time. Commonly, lesion animals exhibit a severe initial impairment in performance that improves over time as healing occurs or as other parts of the brain take over.
similar functions.

Experiment 1 used a technique called reversible inactivation to overcome these disadvantages. This technique can identify an acute impairment with inactivation of the injection area, in this case the cell bodies. This method required each animal to be implanted with a metal guide cannula directly above the area of interest, in this case, ReRh. Animals were trained on a behavioral task and then surgery was conducted to implant guide cannula into ReRh. After animals recovered from surgery, they were retrained on the behavioral task to a criterion. Then animals were injected with a GABA$_a$ receptor agonist muscimol into ReRh. This temporarily inactivated the area, while sparing fibers of passage, for a particular testing session. Overall performance was then able to be compared to other sessions where saline had been injected. The area of inactivation was also able to be varied by varying the concentration of the dose of muscimol. Another advantage to this method is criterion could be reestablished before the next injection session to ensure the animal was able to perform the behavioral task before each pharmacological manipulation.

Unfortunately, this technique also has a disadvantage that injected drugs may spread into surrounding areas of the brain and may lessen the specificity of the location of the effect. To test the localization of drug effects comparisons were made for results of accuracy performance for drug injections in anatomical control sites. Anatomical control injections were drug injections of muscimol into a more dorsally located area. Performance was then compared during the
anatomical control injection session to sessions with muscimol injections into ReRh and to saline injection sessions.

Two behavioral tasks were used to complete experiment 1; the DNMTP and the VC-DNM RAM. The DNMTP is affected by both hippocampal and prefrontal lesions (Porter, Burk & Mair, 2000). By contrast, VC-DNM RAM is affected by hippocampal but not prefrontal damage (Mair, Burk & Porter, 1998; Porter, Burk & Mair, 2000; Mair, Burk & Porter, 2003). If ReRh affects all hippocampal dependent memories, than both behavioral tasks should be affected by inactivation of ReRh. The hypothesis for Experiment 1 was that performance accuracy would be impaired for both behavioral tasks when ReRh was temporarily inactivated with muscimol, thus providing evidence for the role of ReRh in spatial working memory processes that rely on either prefrontal cortex or hippocampus.
EXPERIMENT 1

METHODS

Subjects

Twenty-four male Long Evans rats were used for this experiment. Animals were separated into two training groups of twelve and were trained on either the DNMTP or VC-DNM RAM.

Behavioral Tasks

DNMTP Task

At the start of each trial, the lever on the start end of the chamber extended. Once the lever was pressed, it retracted and one of the two levers on the other side of the chamber would extend out. After that lever was pressed, it would retract and the original lever on the start end would extend out again for a period of time. This was the stage where different delays were introduced (1 second, 5 second or 25 seconds). Shorter delays were used for training sessions. After the delay period, the lever retracted once the animal pressed it again and then both levers on the other side of the box extended. The animal was to choose the lever that had not been previously extended at the beginning.
of the trial (the sample lever). If the animal chose the correct response (the novel lever) then the animal would be reinforced with water in the port. If the animal was incorrect in responding, a correction trial was imposed wherein the trial was the same as the last trial conducted however there was no delay and the responses were not included in the final count. Performance on the DNMTP was measured by recording the number of correct responses and the amount of time required to make responses at each stage of the task. The sample stage was considered to be from the lever press of the initiate lever to the sample lever press. The choice stage was from the delay lever press (after the delay was over) to the choice lever press (Figure 3).

**VC-DNM RAM Task**

The animal was placed in the central hub at the beginning of the session. A randomly selected gate opened and the animal was required to break the photocell near the well in that particular arm to receive reinforcement (start arm). A second gate then opened and the animal had to break the photocell in that arm to again receive reinforcement (sample arm). Both gates were then closed and a delay period was imposed (1 second, 5 seconds or 25 seconds). After the end of the delay, the two gates that had previously been opened and a novel randomly selected gate opened. For a correct response to occur, the animal needed to break the photocell near the water port in the novel arm (choice arm). If the correct response was made, reinforcement was delivered and all the gates closed and a new trial commenced with the arm that was previously the choice...
arm then serving as the start arm. If an incorrect choice was made, the trial continued until the animal went down the correct arm and received water before all the gates would close. Figure 1 shows a diagram of the RAM. Performance on the VC-DNMTP was measured by the number of correct responses into the novel arms and the response time for each of the arms during the trials.

**Surgical Procedure**

The coordinates for the implantation of cannula were; AP: 6.45, DV: 2.7 and ML: 0. These coordinates were determined using Paxinos and Watson (1998) and would extend the guide cannula directly above Re, so when the cannula needle was inserted, it would extend 2mm past the guide cannula into both Re and Rh. See the general methods section for the surgical methods.

**Pre-surgical Training**

Animals were trained on a series of programs for either the DNMT or VC-DNM RAM. Once criterion was reached, surgery was performed. Criterion consisted of three consecutive days of 75% correct for an overall session with delays up to 25 seconds and all responses completed; 36 responses for the VC-DNM RAM and 60 responses for the DNMT.

**Microinjection Procedure**

Animals were wrapped tightly in a towel and hand-held by the researcher during the microinjection procedure. Treatments were administered by a 28
gauge internal cannula (Plastics One, Roanoke, VA) attached to a 250 μL syringe (Hamilton, Reno, NV) driven by a mini-pump (Harvard Apparatus, Holliston, MA). All equipment was sterilized with 70% ethyl alcohol before the drug was put into the tubing. The internal cannula extended approximately 2 mm below the tip of the implanted guide cannula. All injections consisted of 0.5 μL volume at a rate of 1.0 μL per minute. The internal cannula was left in for an additional minute following the injection to ensure all of the substance was injected. Animals were then returned to their home cage in the testing room for a period of ten minutes before being placed into the testing apparatus for behavioral testing.

An initial intracranial injection of saline was administered to familiarize the animal to the injection protocol. The results of this data were not included in the final analyses. Animals then received 10 counterbalanced injections of muscimol (0.4 nmol, 1.0 nmol, 2.5 nmol) and saline. All concentrations of muscimol were mixed ahead of time and stored in a freezer at -15° Celsius until they were thawed for use at the beginning of each week.

After the injection regimen was completed, 2 sessions of an anatomical control injection were conducted. Anatomical control injections were injections of the 2.5 nmol concentration of muscimol into a more dorsally located area. This was to show the specificity of the drug into the area in terms of the desired effects.
Post Surgical Training/Testing

Animals were trained back up to the original training criterion before beginning the microinjection procedure. Animals needed to complete all trials and have a performance of 75% the day before each injection session to ensure that there were no carry-over effects from the previous injection. The delays used for the testing sessions were 1 second, 5 seconds and 25 seconds.

No more than two injections were administered per week and there was a minimum of one testing session between injection sessions. Injections were repeated if any equipment malfunctions occurred during the microinjection procedure, testing apparatus or if the animal failed to perform a total of 30 (VC-DNM RAM) or 50 (DNMTP) responses.

Statistical Analyses

Overall performance and performance at each delay were analyzed using repeated measures ANOVAs for each behavioral task. Two-way ANOVAs were used to examine the effects of dose and delay for each task. Post hoc analyses of Bonferroni-Dunn ($\alpha = 0.05$) were conducted to examine any of the significant results from the omnibus ANOVAs to observe any significant effects.

Possible anatomical control effects were analyzed for each task separately with two-way with-in subject ANOVAs for delay and drug treatments. The drug treatments were the 2.5nmol muscimol dose in the control site versus
the 2.5nmol muscimol and saline in ReRh. Post hoc analyses (Bonferroni-Dunn, $\alpha = 0.05$) were used to compare any significant drug treatment effects.

Response time (RT) data was recorded for both behavioral tasks. This was to compare the time taken to make a sample response versus a choice response. For the DNMTP, measures for the sample RT were from when the initiate lever was pressed until the sample lever was pressed. Choice RT began when the delay lever was pressed and retracted until a choice response was made. For the VC-DNM RAM, hold RT and choice RT were measured. Hold RT was considered to be from when the gate to the sample arm was opened until they responded in the hold arm. Choice RT was from when the gates opened at the end of the delay period until they responded in the choice arm. Results for RT were based on the median RT for correct responses for each individual animal in each microinjection condition. RT was analyzed separately for each behavioral task.
EXPERIMENT 1

RESULTS

**Histological Findings**

A total of eighteen rats completed all the microinjection trials, nine animals for the DNMTP and nine for the VC-DNM RAM. For the DNMTP, seven out of the nine animals had injection sites within ReRh and for the VC-DNM RAM, eight out of nine had acceptable cannula placement. The three other animals had cannula placements which would have made the injection site more dorsal than ReRh, therefore they were excluded from the final analyses. For anatomical control injections, fourteen animals with acceptable injection sites were used for analyses (7 for DNMTP and 7 for VC-DNM RAM). See Figure 4 for the cannula placements for both the DNMTP and the VC-DNM RAM.

**Behavioral Findings**

Injections of muscimol produced dose-related impairments for both DNMTP (Figure 5) and VC-DNM RAM (Figure 6). Average performance for saline trials for the DNMTP and VC-DNM RAM were similar (84.4% and 81.5% respectively) (Figure 7). For the days in which animals were not injected, overall percent correct did not significantly change across the 6 weeks of testing for
either DNMTP ($F_{5,30}= 1.563, p= 0.2007$) or VC- DNM RAM ($F_{7,35}= 1.303, p= 0.2853$) (Figure 8). Performance decreased for both behavioral tasks, as the dose of muscimol increased from 0.4, 1.0 to 2.5 nmol; 67.5%, 60.0% and 56.5% for the DNMTP and 79.5%, 74.9% and 68.2% for the VC- DNM RAM. An omnibus ANOVA showed significant differences between the two behavioral tasks ($F_{1,13} = 4.957, p= 0.0443$), across delays ($F_{2,26}= 33.711, p<0.001$) as well as for drug treatment ($F_{3,36}= 5.321, p= 0.0036$) (Figure 7). There was a significant interaction between drug treatment and task ($F_{3,36}= 5.321, p= 0.0036$).

Two-way ANOVAs were conducted to examine the delay and dose effects for the individual behavioral tasks. For DNMTP, there were significant effects of delay ($F_{2,12}= 29.864, p<0.0001$) and drug treatment ($F_{3,18}= 16.749, p<0.0001$) with no significant interaction ($F_{6,26}= 1.887, p=0.110$). Post hoc analyses (Bonferroni- Dunn, $\alpha= .05$) showed significant effects of all doses of muscimol compared to saline (Figure 5). For the VC- DNM RAM, results also showed significant effects of delay ($F_{2,14} = 6.419, p= 0.0105$) and drug treatment ($F_{3,21}= 9.730, p=0.0003$) with no significant interaction ($F<1$) (Figure 6). Post hoc analyses using Bonferroni- Dunn ($\alpha = .05$) revealed a significant effect of muscimol at the 2.5 nmol (highest dose) compared to both saline and 0.4 nmol (lowest dose). While both tasks were affected at the highest dose (2.5nmol), only DNMTP was affected at low doses. This is consistent with a localized (low dose) effect on DNMTP but not VC-DNM RAM in ReRh (see Hembrook, Onos & Mair, 2011).
RT analyses for the DNMTP showed a significant difference between sample RT (mean = 3.004 seconds) and choice RT (mean = 1.943) ($F_{1,6}= 15.752$) (Figure 9). No significant effects were found for the dose of muscimol ($F_{3,18}= 2.667$, $p= 0.0787$) or for the interaction ($F_{3,18}= 1.428$, $p= 0.2679$). RT analyses for VC- DNM RAM also revealed significant differences between sample RT (mean= 3.125 seconds) and choice RT (mean = 7.054) ($F_{1,6}= 352.427$, $p<0.0001$) however in the opposite direction of DNMTP, meaning animals took longer to make a choice response (Figure 10). There was a significant effect of the dose of muscimol ($F_{3,18} = 5.226$, $p = .009$) with no interaction ($F<1$).

To examine the localization of the drug effects, separate analyses were conducted for anatomical control injections. For both behavioral tasks, injections of 2.5 nmol muscimol into the anatomical control site decreased the level of impairment compared to the same dose (2.5nmol) injected directly into ReRh. ANOVAs revealed a significant overall difference for both DNMTP ($F_{2,12}= 14.125$, $p = 0.0007$) and VC- DNM RAM ($F_{2,12} = 10.601$, $p= 0.0022$) when comparing the effects of 2.5 nmol muscimol in ReRh, the anatomical control injections and saline (Figure 7). Post hoc analyses (Bonferroni-Dunn, $\alpha = .05$) showed a significant difference between the 2.5 nmol dose of muscimol in ReRh compared to both the 2.5 nmol dose of muscimol in the anatomical control site for DNMTP but not for VC-DNM RAM. These findings support localized effects in
ReRh for the impairments seen on DNMTP, a task dependent on prefrontal cortex and hippocampus, but a lack of localized effects for the VC-DNM RAM, a task dependent on hippocampus.
Muscimol produced impairments in performance for both behavioral tasks, DNMTP and VC-DNM RAM. These impairments were dose-dependent, where higher concentrations of muscimol produced greater deficits in performance for both tasks (Figure 5 & 6). The effects on DNMTP were much greater than for VC-DNM RAM. These differences were confirmed by a significant dose by task interaction in the omnibus ANOVA and a significant effect of the drug on DNMTP at all muscimol levels (0.4nmol, 1.0nmol, and 2.5nmol). Impairments were only seen for VC-DNM RAM at the highest (2.5nmol) dose.

Response speed was unaffected for DNMTP for all treatments (Figure 9). Response time did increase significantly for VC-DNM RAM. The increase in response time was small, 0.91 seconds at the highest (2.5nmol) dose (Figure 10). Previous reports examining response speed for prefrontal lesions and intralaminar thalamic lesions have produced response latencies compared to controls on measures of spatial memory (Burk & Mair, 1998, 1999; Porter, Burk & Mair, 2000). But, because the impairment in accuracy was greater as the delays increased, it is unlikely that this small increase vitally contributed to the impairments of accuracy for the current study. These results are consistent with
a previous study by Porter, Koch and Mair (2001) where reversible inactivation of rostral intralaminar thalamic nuclei did not increase response time.

When applying drug injections directly into a particular area of the brain, the drug spreads into surrounding areas, potentially inactivating those areas as well. Drugs will spread over time and area, therefore there is a decreased concentration from the target area for that spread, and thus it is hard to know the area of actual inactivation at any point of time. Comparable to doses of muscimol used in this experiment, the spread and activity effects in thalamus can be up to 3 mm from the injection site. With lower doses being less concentrated farther away from the injection site and higher doses (3.5 nmol) having greater concentrations moving away from the site of injection (Edeline, Hars, Hennevin & Cotillon, 2002). It is difficult to translate these results into the exact inactivation area for the current study, however the highest concentration of 2.5nmol in this study was likely to inactivate areas well past the Re and Rh nuclei. To account for this, anatomical control injections where made at the highest concentration (2.5nmol muscimol) into a more dorsally located site. There was a greater significant impairment of performance accuracy on DNMTP for infusions of 2.5nmol into ReRh versus the anatomical site (Figure 7). Therefore, there was a localization of the effects to the area of ReRh for the DNMTP. The impairment seen for the lowest concentration dose (0.4nmol muscimol) in ReRh on DNMTP implies that the DNMTP is sensitive to inactivation in the area of ReRh. For the VC-DNM RAM, the anatomical control injections did not confirm a localized effect (Figure 7). Rather impairments were only seen at the highest dose (2.5nmol)
and were not significantly different than anatomical control performance. These findings show that for DNMTP, spread of inactivation into surrounding areas did not contribute to the impairments of spatial working memory, but this contribution from surrounding areas could not be ruled out for the VC-DNM RAM.

There are a couple of possible reasons why localization of the effects were found for DNMTP versus VC-DNM. The behavioral tasks; DNMTP and VC-DNM RAM require animals to respond based on spatial information which changes from trial-to-trial and must be remembered over short delay periods. However, there are differences, the type of spatial cues available for the rat to solve the tasks and the decisional response made by the animal depending on the task.

The type of spatial cue varies based on the behavioral task. The DNMTP can be solved with egocentric cues, which are cues related to direction of turning. The use of this type of spatial cue seems unlikely for the VC-DNM RAM, because the location of the correct choice arm and the direction of turning is not known until after the end of the delay period. The VC-DNM RAM was trained in an open-room, with light on and the arms of the maze have transparent sides and covers. Thus there are diverse external visual cues. The DNMTP on the other hand is completed in an operant chamber in sound insulated boxes blocking out external room cues. Therefore, it is possible that the lack of low-dose impairment seen for the VC-DNM RAM could be due to a spared ability to navigate the task based on allocentric information, whereby the animal used the visual spatial cues around them.
The other key difference between the two behavioral tasks is the actual decisional response the animal makes. The DNMTP requires the rat to choose between the same two levers on every trial, but the VC-DNM RAM allows 28 possible pairings for the arms selected for each trial. In the DNMTP each possible response is reinforced thirty times in each session but only 4.5 times in the VC-DNM RAM. This should make it more difficult to inhibit previous responses for the DNMTP. In the DNMTP, rats are faced with the same choice on every trial, whereas the VC-DNM RAM has 28 possible choices. This should increase demands on working memory as a result of proactive interference for DNMTP. Response alternatives stay the same and are repeated for every trial as either a sample or choice response, whereas they are exposed on average every fourth trial for the VC-DNM RAM. This should create a more difficult temporal discrimination for DNMTP. The DNMTP creates greater demands on inhibiting previous responses, as well as inhibiting proactive interference and also temporal discrimination.

It is also possible that the deficits seen in the VC-DNM RAM could reflect inactivation of more anterior nuclei. Lesions to the anterior thalamic nuclei have been shown to impair VC-DNM RAM performance (Mair, Burk & Porter, 2003) and to produce deficits in other allocentric spatial memory tasks (Aggleton, Hunt, Nagle & Neave, 1996; Byatt & Dalrymple-Aford, 1996). Lesions that spare these anterior nuclei but damage midline/intralaminar nuclei do not affect VC-DNM RAM performance (Bailey & Mair, 2005). According to the standard stereotaxic coordinates, the anterior thalamic nuclei are located within the potential spread
for the highest (2.5nmol) concentration of muscimol as well as the anatomical
control site (Paxinos & Watson, 1998; Edeline et al., 2002). Therefore, the
impairment seen for VC-DNM RAM could be due to inactivation to the anterior
nuclei and not specifically the disruption of ReRh.

The results from this current experiment are subject to some limitations.
First, the extent to which inactivation of tissue spread during the injections of
muscimol is uncertain. The distinct effects of low doses on both the DNMTP and
VC-DNM RAM supports a more localized effect of the injections on the DNMTP
but with this technique there is no way to tell which nuclei were inactivated by
particular doses. Anatomical controls help to provide supporting evidence for
localization on the DNMTP, but also cannot determine the precise area of
inactivation. Previously, Hembrook and Mair (2010) showed that discrete lesions
of Re and Rh affected spatial memory, but there is a gap in the published data
for the effects of ReRh lesions on the DNMTP and VC-DNM RAM.

The current finding of delay-independent impairments of accuracy on both
behavioral tasks question the actual functional specificity of the impairment seen
with inactivation of ventral midline thalamus. The delay-independent results
could stem from a specific memory process such as retrieval or encoding. This
could affect performance across all delay lengths. This type of impairment could
also be related to deficits in attention or perception. However, Hembrook and
Mair (2010) provides evidence against this, where a double dissociation was
found for ReRh lesions compared to caudal intralaminar thalamic lesions on
spatial memory tasks versus a visuospatial reaction time task. These results are
also comparable to both hippocampal and medial prefrontal cortical lesions which produced delay independent results on the DNMTP (Porter, Burk & Mair, 2000). This was also addressed further in Experiments 2, 3 and 4 by specifically comparing working memory and reference memory task performance. Therefore the results seen in Experiment 1 are consistent with both hippocampal and medial prefrontal disruption suggesting that ReRh is important in spatial working memory.

The Role of ReRh in Spatial Memory

It is impossible in this study to differentiate the effects muscimol had on Re and Rh because these nuclei are located directly on top of each other. Re is the largest of the nuclei in ventral midline thalamus. Rh is located directly dorsal to Re. The projections of Re are primarily to CA1 and subiculum, parahippocampal areas of cortex, and to infralimbic, prelimbic and orbital areas of prefrontal cortex. Rh, while having similar projections to Re, also projects to nucleus accumbens and the basolateral nucleus of the amygdala with more diffuse projections to cerebral cortex (Vertes et al., 2006). These nuclei are also driven by arousal and limbic inputs from brainstem and areas of forebrain as well as projections from medial prefrontal cortex to the reuniens nuclei (Krout, Belzer & Loewy 2002; McKenna & Vertes, 2004; Vertes et al. 2007).

Based on these anatomical connections, there are two possible hypotheses to explain the effects of inactivation of ReRh seen in this current study. The first is that inactivation of ReRh nuclei disrupts hippocampal function
by decreasing neuronal activation in CA1 and subiculum (Dolleman-van der Weel, Lopes da Silva & Witter, 1997; Bertram & Zhang, 1999). If this hypothesis is true, then disruption of any aspect of memory which has been seen to be impaired by hippocampal lesions should produce the same deficits. The second hypothesis is that the inactivation of ReRh could interfere with activity associated with interactions between hippocampus and medial prefrontal cortex (Schiff & Plum, 2000; Zhang & Bertram, 2002; Vertes et al., 2006, 2007; Dolleman-van der Weel, Morris & Witter, 2009). Therefore based on this hypothesis, inactivation of ReRh would produce differential impairments on tasks which are sensitive to both hippocampal and medial prefrontal lesions.

Findings from earlier studies in our lab as well as the results from Experiment 1 support the second hypothesis, that inactivation of ReRh interferes with activity associated with interactions between hippocampus and medial prefrontal cortex. Previous studies in our lab showed that the DNMTP and the VC-DNM RAM are affected by lesions to hippocampus, but the DNMTP is also affected by lesions to prefrontal cortex (Mair, Burk & Porter, 1998; Porter, Burk & Mair, 2000). The current study found localized effects for the low dose on the DNMTP but not for the VC-DNM RAM. This finding corroborates evidence that ReRh lesions affect radial maze win-shift tasks (8 arm task and 4F RAM), measures of spatial memory that have been shown to be sensitive to both hippocampal and medial prefrontal cortical lesions (Porter & Mair, 1997; Mair, Burk & Porter, 1998).
The lack of impairment seen for the low dose of muscimol on the VC-DNM RAM suggests that ReRh inactivation does not completely disrupt hippocampal functioning. The current findings are consistent with evidence from Dolleman-van der Weel and colleagues (2009) who found that larger Re lesions spared measures of a water-maze reference memory task which is comparable to other tasks that have been shown to be affected by hippocampal lesions but not medial prefrontal cortical lesions (Sloan, Good & Dunnett, 1996; Dolleman-van der Weel, Morris & Witter, 2009). This is in contrast to Davoodi et al. (2009) who found that reversible inactivation of ventral midline thalamus affects both working memory and reference memory tasks in the water maze. Since Davoodi et al. (2009) did not include an anatomical control procedure, it is impossible to determine the localization of these effects. Further, they inactivated with tetracaine, which also disrupts the neural transmission in the fibers of passage (Hilles, 1966, 1977; Ritchie, 1979) whereas muscimol spares these fiber tracts.

The results of the current study provide substantial evidence to confirm the hypothesis for ReRh involvement in spatial working memory and indicate that damage to these areas should affect measures which rely on the proper functioning of both prefrontal cortex and hippocampus.
EXPERIMENT 2

INTRODUCTION

Experiment 1 demonstrated the role of ReRh in two tasks of memory, one which required the proper functioning of hippocampus, the VC-DNM RAM, and the other which required the proper functioning of both hippocampus and medial prefrontal cortex, the DNMTP. The results from Experiment 1 revealed that the reuniens and rhomboid nuclei are important for spatial working memory and that overall accuracy impairments were greater on the DNMTP.

Experiment 1 does not indicate whether both Re and Rh are important for spatial working memory or if only one of these nuclei is the critical link. Experiment 2 sought to differentiate the importance of each of these nuclei by using permanent excitotoxic lesions to selectively damage either the Re or Rh.

Excitotoxic chemical lesions allow the experimenter to create a permanent lesion in a particular site that damages cell bodies but spares fiber tracts. Advantages to this method are that lesions can be localized to very small areas of the brain and histological analyses allow the experimenter to evaluate the amount of damage in a particular site and compare that to behavioral performances. However, disadvantages are that impairments must be inferred by comparison to a control group and permanent effects produced by damage to
an area can be confounded with transient disruption of surrounding tissue and by recovery of other related brain areas becoming involved.

In Experiment 2, animals were tested on a series of different behavioral tasks to compare working and reference memory to determine if deficits are related to memory demands. The first task was the DNMTP, a test of working memory used in Experiment 1. Then animals were tested on serial reversal learning (SRL), a reference memory task similar to the DNMTP but not dependent on hippocampus (Mair, Burk & Porter, 1998; Porter, Burk & Mair, 2000). The SRL is matched to the DNMTP for the choice response. The next tasks were two radial arm maze tasks, measures of spatial memory (Jarrard, 1993, Kesner, Bolland, & Dakis, 1993; MacDonald & White, 1993, 1995; Mair, Burk & Porter, 1998) as well as the same tasks used in Hembrook & Mair (2010) for comparison. Finally a water maze task (Eichenbaum, Stewart & Morris, 1990, Mair, Burk & Porter, 1998) a measure of reference memory was conducted. On the reference memory task, the responses made for a particular response are held constant across the trials (Prior, Schwegler & Ducker, 1997, Davoodi et al., 2009). The water maze measures allow for comparison with Dolleman-van der Weel et al. (2009) and Davoodi et al. (2009).

In this study, the expected results were that the lesions to Re and Rh would produce accuracy impairments compared to the controls on DNMTP based on the results from Experiment 1. On the SRL, there would be spared ability to perform at an errorless criterion and would also show positive transfer
between reversals (Porter, Burk & Mair, 2000; Mair, Burk & Porter, 1998). On the radial arm maze tasks, impairments would be seen for both Re and Rh lesion groups, also based on their anatomical connections with hippocampus and these deficits would increase as the delays for 4F RAM increased (Hembrook & Mair, 2010). Lastly, the RM-WM would not have deficits on either learning or memory impairments for finding the location of a hidden platform because the task is a test of reference memory.

Based on their projections to prefrontal cortex and hippocampus, both Re and Rh are hypothesized to be important for spatial working memory. However, Re has the unique attribute of receiving input from prefrontal cortex that terminate on neurons which project directly to hippocampus (Vertes et al. 2006), this has led some (Dolleman-van der Weel, Morris & Witter, 2009) to argue that Re is the critical structure for spatial memory. To test this idea we compared discrete Re and Rh lesions on different measures of spatial memory.
EXPERIMENT 2

METHODS

Subjects

Forty-one male Long Evans rats were obtained for use in this study. After animals were trained on the DNMTP, they were assigned by a randomized matching procedure, into one of four experimental groups; Re (n= 18), Rh (n= 10), or sham control (n= 13).

Behavioral Tasks

DNMTP Task

This task was similar to description in Experiment 1 (Figure 3), except trial delays were set as 1 second, 5 seconds, 10 seconds, 16 seconds and 25 seconds, intermixed randomly throughout each behavioral session.

SRL Task

SRL was conducted in the same chamber as the DNMTP. Figure 11 depicts the two different stages of the task. The trial began with the initiate lever extending (start end of the chamber). Once the lever was pressed, it retracted
and both levers on the other side of the chamber extended out. For the entire session, one lever was the reinforced lever (either the left lever or right lever), meaning when it was pressed, it retracted and water reinforcement was given. The session continued with one lever being reinforced until fifteen consecutive trials were completed correctly in a row or a total of 100 trials. The total number of errors was recorded for each session.

8-Arm RAM Task

Sessions began with the animal being placed in the center hub of the maze. After a ten second delay, all eight gates opened simultaneously. The gates remained open for the first eight entries that the animal made into the maze arms. A response was registered each time that the animal broke the photocell closest to the water well. If the entry into the particular arm was the first time that the animal entered the arm, the response was recorded as correct and two short pulses of water (0.2ml, 2 seconds apart) was given as reinforcement. If the arm had been previously entered during the specific trial, the response was recorded and added to the count of the number of arms entered and no reinforcement was given (Figure 1). A total of three trials were conducted per day. After the completion of the third trial, the animal was removed from the maze and placed back into their home cage.
4F RAM Task

The session began with the animal being placed in the central hub of the maze with all of the gates closed. The session began with one randomly selected gate opening. The animal then had to make a response into that particular arm, wherein reinforcement was given and another gate opened. This occurred for a total of four arms entries. This was to control the sequence in which the arms were entered. At the completion of the forth arm response, the gate closed for a delayed period (1 minute or 15 minutes). After the delay period, all eight gates opened and remained open for four arm responses, regardless of which arms were entered. If the arm entered was one not previously entered before the delay, the response was recorded as correct and reinforcement was given. If the arm entered was one that was previously entered before the delay, an error was recorded, the response was added to the total count and no reinforcement was given. All gates closed at the end of four entries and the session was ended (Figure 1). Animals were then removed from the maze and returned to their home cage. Each animal was tested for two sessions per day, one session with a 1 minute delay and the other session with a 15 minute delay. The delay was randomly selected for the first session for each day. A minimum of two hours elapsed between sessions.
The procedure for this task was adapted from Dolleman-van der Weel, Morris and Witter (2009). All animals were given free access to food and water for the entire duration of this testing protocol. All animals were handled by each of the experimenters who had contact with them during the testing sessions for the two days prior to the start of the testing. The animals were trained and tested for a total of 6 days.

Day 1: The curtain was hung around the pool for the entire session to exclude any external cues. The platform was placed into the northeast quadrant, 1 cm below the surface of the water. Trials began with the animal being placed with its front paws touching the side of the pool and lowered gently into the water. Four trials were conducted, one in each direction (N, S, E, W- randomly selected order) and allowed 120 seconds to find the platform. If the animal found the platform, the animal was allowed to stay on the platform for thirty seconds. If the animal was not able to find the platform in the 120 seconds, the experimenter picked up the rat and placed it onto the platform for a period of thirty seconds. After the thirty seconds regardless of the ability to originally find the platform, the next trial began. At the completion of four trials, the animal was towel dried and placed into a plastic tub under a heat lamp for a time period of ten minutes. At the end of ten minutes, the animal was returned to its home cage.

Day 2-4: These days were considered the learning sessions. There was no curtain for these sessions and external cues were present on the walls. The
platform was placed into the pool in the same northeast quadrant as before and was 1 cm below the surface of the water. Each session consisted of six total trials with the locations of each trial randomly selected. The animal was again given 120 seconds to find the platform and also again left on the platform for thirty seconds. If it did not find the platform in the allotted time, the animal was placed onto the platform for thirty seconds. After six trials were completed, the animal was towel dried, placed under the heat lamp for ten minutes and then returned to its home cage.

Day 5: This session was considered the memory probe trial and was completed twenty four hours after the completion of the trials on Day 4. The platform was removed from the pool for this session. The session consisted of one trial, wherein the animal was placed into the pool in a randomly selected location and given sixty seconds to swim in the pool. They were then removed from the pool, towel dried, placed under the heat lamp for ten minutes and returned to their home cage.

Day 6: The curtain was hung around the pool to remove any external cues. The platform was then placed in the same northeast quadrant except for these sets of trials; the platform was 1 cm above the surface of the water. The session consisted of four trials (one trial of each direction) randomly selected for the starting location. The animal was given 120 seconds to find the platform and was allowed to remain on the platform for thirty seconds. If the animal did not find the platform on any given trial, it was placed onto the platform for thirty seconds and then the next trial commenced. At the completion of the four trials
the animal was towel dried, placed under the heat lamp for ten minutes and then returned to its home cage.

**Surgical Procedure**

Surgical procedures were similar to Experiment 1 with the exception that excitotoxic lesions were induced with a solution of 100 mM NMDA in buffered saline. Targeted lesion sites are denoted on Table 1. See the General Methods section for the complete surgical procedure.

**Pre-surgical Training**

Animals were trained on a series of programs to learn the DNMTP. The final training program for the DNMTP included delays of 1 second, 5 seconds, 10 seconds, 16 seconds and 25 seconds. Animals continued on the training program until criterion was reached. Criterion for pre-surgery was three consecutive sessions, at least 50 total responses for each session and a performance of 75% correct.

**Post-surgical Testing**

After animals recovered from surgery, water deprivation was reestablished. Animals were then tested on the DNMTP with intermixed, randomized trial delays (1 second, 5 seconds, 10 seconds, 16 seconds, 25 seconds) for a total of fifteen sessions. Animals were then switched to the SRL. The direction of the original correct reinforced lever was determined by
examining which lever was preferred by the animal during their last two DNMTP sessions. The correct lever was then set to be the opposite of the preferred lever. In cases where there was no lever preference, the correct lever was randomly selected by a flip of a coin.

SRL testing continued for a total of seven reversals. A session continued until the animal completed 100 responses or it completed fifteen correct responses in a row. If the animal completed the fifteen correct responses in a row, the next session (on a new day) had the correct reinforced lever opposite of the last session. If the animal failed to complete the criterion of fifteen correct responses in a row, the animal continued on the same program (correct lever remained the same) until a session was completed where criterion was met.

Animals were then trained and tested on a series of radial arm maze tasks. Animals were first acclimated to the maze. This was done by putting an animal in one of the arms with all the gates closed. Animals then had to break the photocell nearest the gate and then the photocell nearest to the water well to receive water. Animals were allowed to make three to four responses in the arm and then was moved either clockwise or counterclockwise (randomly selected) to another arm. This was repeated with the direction staying constant until the animal had been exposed to each of the eight arms. The animals were then returned to their home cage. Acclimating occurred for three days.

Animals were then tested on the 8-arm RAM for three trials per session for a total of ten consecutive days. Animals were then tested on the 4F RAM. Animals were tested for two sessions per day for ten total days. Animals were
tested on each delay once a day. Delays were one minute or fifteen minutes and were randomly selected for which delay would be imposed for the first trial and then the other delay was implemented on the second trial.

There was concern that the animals might be solving the 4F RAM task by using odors to complete the task; meaning that they might have been using their own scent to determine which arms had previously been entered. One way we examined whether this strategy could be a factor was to remove as many odors as possible from the maze. This was done by testing the animals on the previously learned 4F RAM task with the delay of 15 minutes. Animals ran this task a total of four trials over the course of two days (2 trials per day, one of each condition). Each day, one session was considered the “clean” condition and the other session was considered the “no clean” condition.

Before the start of each session, the maze was entirely cleaned with a mixture of 1 gram of Alconox (a cleaning agent) and 1 Liter of water using a sponge and paper towels. During each session regardless of condition, animals made their first four responses and entered their delay periods. Once the last gate was closed for the delay, if the trial was a “clean” condition, the experimenter would take a different sponge and a new mixture of water and Alconox and clean out and dry the central hub and the other seven arms (except for the one that the animals were in for their delay) and dry them thoroughly with paper towels. Animals then completed their session. If the session was a “no clean” condition, the same protocol was followed except the sponge used was
dry and the experimenter would ‘pretend’ to clean and dry the central hub and arms during the delay period.

Animals were then given free access to water and handled for two days prior to the start of the RM-WM task protocol. This testing protocol was conducted for a total of six consecutive days.

**Statistical Analyses**

Since both Re and Rh are hypothesized to be important for spatial working memory, we used planned comparisons (Bonferroni-Dunn, $\alpha = 0.05$) to test for significant effects of Re and Rh lesion groups compared to control animals for each of the behavioral tasks.

Overall performance on the DNMTP was analyzed using repeated measures ANOVA to examine performance over the course of the 15 sessions by lesion group and also lesion group by delay length (1 second, 5 seconds, 10 seconds, 16 seconds, and 25 seconds). Response time (RT) analyses were conducted for the sample and choice responses similar to Experiment 1. RT was based on correct responses and the medians were found for each delay. Overall RT averages for sample and choice were then found based on the medians from each delay.

SRL was also analyzed using repeated measures ANOVA for the number of errors to criterion for each set of the seven reversals. Planned comparisons (Bonferroni-Dunn, $\alpha = 0.05$) were used to compare Re lesion and Rh lesion performance to control animal performance.
Radial arm maze performance was analyzed using repeated measures ANOVAs. The 8-arm RAM analyses examined overall performance over the course of the ten sessions by lesion group. The 4F RAM was analyzed with repeated measures ANOVAs for overall performance at each delay (1 minute, 15 minutes) by lesion group. A separate ANOVA was also conducted for the "clean/no clean" version of the 4F RAM at the 15 minute delay to compare performance between the lesion groups and condition. A paired t-test was also conducted to see if, regardless of lesion group, there were differences in performance between the clean and no clean conditions.

All RM-WM performance was videotaped and analyzed examining performance during the spatial learning days (Days 2-4) and the cue test (Day 6). Variables that were recorded were escape latencies and swim paths for all of the spatial learning days. The swim paths were analyzed to observe whether different lesion groups use a particular type of search strategy. Swim paths were categorized according to the same procedure used by Dolleman-van der Weel, Morris and Witter (2009). Swim path categories were, Edge (A), Random (B), Circle (C), Loop (D), Direct (E), Indirect (F) and Near miss (G). Examples are also pictured in Figure 12. The swim paths were analyzed by a blind observer (EB & DB). If more than one swim path was seen, the path that was more dominant during the trial was used at the category classification. For each animal, the number of swim paths per category was determined for each training trial. The total number of swim paths for each category was used for the analyses. An ANOVA was conducted for overall comparisons of type of swim
path by lesion group and planned comparisons were used to compare each lesion group to control animals. For the transfer memory probe trial, ANOVAs were conducted for swim speed, time spent in critical quadrant (the quadrant where the platform had been during the training sessions), total number of passes through the platform, swim path length and proximity.
EXPERIMENT 2

RESULTS

Histological Results

Of the 41 animals used for this study, 41 animals completed the DNMTP and 40 completed all of the other behavioral tasks. One animal was excluded from the Re group because he died during the course of the experiment, and was not used for any of the analyses because we were unable to determine the cause of death. Two additional animals were excluded from the SRL after being run on the wrong program; therefore the results could not be directly compared to all the other animals. One animal was excluded from the DNMTP because the animal stopped responding during a number of the sessions and his average performance therefore was two standard deviations below all the other averages in that particular group of animals. Therefore the analyses for the tasks included a total of 39 animals for the DNMTP, 38 for the SRL, and 39 animals for both the 8-arm RAM and 4F RAM and 40 for the RM-WM. Figure 13 shows a representative sample of each the Re lesion and the Rh lesion stained with cresyl violet.
Behavioral Findings

DNMTP Task

The Re lesion group tended to be impaired on performance accuracy compared to the control group and the Rh lesion group. No overall differences were found in performance accuracy between groups (Re, Rh and controls) ($F_{2, 36} = 1.179, p = 0.3192$). However there was a significant effect of delay ($F_{4, 8} = 65.238, p<0.0001$) where performance decreased at longer delays. There was no interaction between these factors ($F_{8, 144} = 1.385, p = 0.2073$). Planned comparisons of Bonferroni-Dunn ($\alpha = 0.05$) revealed a significant difference between the Re group and controls ($p= 0.0139$) but not for the Rh group compared to controls (Figure 14). The lack of an interaction effect for delay by group indicates that the effect was delay-independent.

Performance accuracy increased over the course of the fifteen sessions ($F_{14, 28} = 3.496, p < 0.0001$) but there was no interaction between the effects of session and lesion group ($F<1$). With data analyzed in this way, examining each day performance collapsed across delays, planned comparisons showed significant differences between Re group compared to controls ($p< 0.0001$) but not the Rh group and controls.

Examination of the response time for the overall sample and choice responses revealed no differences between the lesion groups ($F<1$) with a significant effect of the type of response ($F_{1, 2} = 30.437, p < 0.0001$) with no interaction effect ($F <1$). All rats were slower regardless of lesion group to make
a sample response (mean = 3.893) versus the choice response (mean = 1.79) (Figure 15).

**SRL Task**

As predicted all animals were able to perform at errorless criterion and showed positive transfer between problems. There were no significant differences between any of the lesion groups and the controls for errors to criterion on the seven reversals ($F_{2,34} = 2.952$, $p = 0.0657$) and no interaction between lesion group and errors to criterion across the seven reversals (Figure 16). When the total number of errors was collapsed across the reversals, there was a significant effect for the total number of errors by lesion group ($F_{7,14} = 40.150$, $p < 0.0001$). However, planned comparisons of Bonferroni-Dunn failed to show any differences between the Re group or the Rh group when compared to controls (Figure 17). This provides evidence of preserved rule-based responding sufficient to respond with out error on the two lever choice (SRL) and to learn about the task sufficient to produce positive transfer (Figure 16).

Also, the choice response for the SRL was identical to the DNMTP choice response, where for the SRL, animals pressed the start lever and then chose between two choice levers. This response was based on a fixed rule (SRL) rather than working memory (DNMTP).
Radial Arm Maze Tasks

The Re lesion group performed better than the controls for both the 8-arm and 4F RAM tasks. For the 8-arm RAM task, animals were tested over a series of ten days with two sessions per day. Performance was averaged for each day’s sessions. Performance accuracy increased throughout the days of training ($F_{2, 9} = 3.546, p= 0.0003$) with a difference between lesion groups ($F_{2, 36} = 4.027, p= 0.0264$) and a significant interaction ($F_{9, 18} = 1.702, p= 0.0376$). These results were confirmed by planned comparisons (Bonferroni-Dunn $\alpha = 0.05$) showing significant differences between the Rh lesion group and controls ($p< 0.0001$) but not the Re lesion group and controls (Figure 18).

For the 4F RAM task, repeated measures ANOVAs found no significant effects of lesion group ($F_{2, 35} = 1.898, p= 0.1650$), or delay ($F_{1, 2} = 3.117, p=0.0862$) with no interaction between the factors ($F<1$). There was an overall effect of day ($F_{9, 18} = 2.978, p= 0.0020$) that did interact with lesion group ($F<1$). Planned comparisons (Bonferroni-Dunn, $\alpha = 0.05$) showed no differences between either of the lesion groups compared to controls.

A separate analysis was conducted to determine whether the animals were using odors to determine the arms that they had previously entered to correctly perform the task. Analyses showed no differences in performances across lesions groups ($F<1$) as well as no differences between trials where the maze was completely cleaned out during the delay period versus the trials where no cleaning was conducted ($F<1$). Since there were no difference between
lesions groups for the two conditions, a paired t-test also confirmed no
differences between conditions, regardless of lesion group (t(38)= 0.626). The
average performance for the clean condition was 68.9 compared to the no clean
version where the average was 67.1 (Figure 20).

**RM-WM Task**

All groups performed similarly on training trials of escape latency at first,
however both lesion groups were slower to find the platform on training trials 3-7.
As the training trials continued, the lesion groups were similar to controls for
training trials 8-18. Learning trials were analyzed in two different ways. One was
to examine the trials as 18 individual trials, regardless of training day. This is a
commonly used method in water maze analyses, however this can sometimes be
misleading because animals are dropped in different locations and the distance
to the platform can vary from trial to trial. Another way to examine the data was
to use learning blocks, which were the averages of three trials put into artificial
block, and thus were 6 total training blocks. Dolleman-van der Weel and
colleagues (2009) used training blocks for their analyses and therefore any
results that we found could then be compared to their results.

For the learning trials, examining all 18 single trials, there were no overall
differences between lesion groups (F<1), but there was an effect of learning trial
(F_{17, 34} = 13.486, p < 0.0001) with no interaction (F_{34, 629} = 1.239, p = .1681)
(Figure 21). Planned comparisons of Bonferroni-Dunn found significant
differences between the Re group compared to the control group (p= 0.0234) but
not for Rh versus controls. This suggests an initial deficit in escape latency during the earlier learning trials to find the platform, trials 3-7, but this effect went away as the animals were all able to learn the task, trial 8 and on. When trials were grouped into the different learning blocks, similar results were found where there was only a significant effect of block ($F_{5, 10} = 25.215, p < 0.001$) where all animals improved over the course of the blocks (Figure 22) regardless of lesion group. Planned comparisons of Bonferroni-Dunn did not reveal any differences between the lesion groups compared to controls. Individual ANOVAs examining each block revealed no significant effect for lesion group for blocks 1 through 5 ($F_{2, 37} = 1.652, p = 0.2055$ for block 1, $F$'s $< 1$ for blocks 2-5) except for block 6 ($F_{2, 37} = 3.453, p = 0.0422$). Planned comparisons did not reveal any significant group differences for any of the learning blocks.

The type of swim paths was categorized into seven different types. For each learning trial, the path type that dominated the swim was recorded. The total number of each path type for all the trials was calculated for each animal. There was no significant effect of lesion ($F < 1$) but there was a significant effect of the type of path ($F_{6, 12} = 96.572, p < 0.0001$) with an interaction ($F_{12, 216} = 8.175, p = 0.0053$) (Figure 23). Planned comparisons did not reveal any differences between the swim paths for either lesion group compared to controls. Each swim path was analyzed by individual ANOVAs to examine whether any particular swim path varied between lesion groups and controls. There was only a significant effect of the "direct" swim path ($F_{2, 36} = 6.520, p < 0.0001$) and planned comparisons (Bonferroni-Dunn $\alpha = 0.05$) revealed differences between the
reuniens group and controls (p= 0.0011). All groups used the “direct” strategy for many of the learning trials. It is possible that controls learn this type of strategy to find the platform earlier in the training protocol than Re.

After the animals completed the trials where the platform was in the water, there was a 24-hour wait period where the animals remained in their home cages. Then one probe test trial was conducted wherein there was no platform in the pool and the animal had a 60 second free swim. Variables measured were swim path length, swim speed, number of passes through where the platform would have been located, the time spent in the quadrant where the platform was located and proximity. Individual ANOVAs did not reveal significant differences for any of the memory probe trial variables (F<1) (Figure 24). Planned comparisons showed no differences between Re or Rh compared to controls on any of the variables.

During last day of behavioral testing, the usually hidden platform was placed above the water level. This was done to examine whether there was any gross motor deficits that could contribute to any behavioral deficits seen during the learning stages of the tasks. There were no differences seen between lesion groups (F 2,37 = 1.54, p= 0.2278) but there was a significant effect of trial (F 3,6 = 5.832, p= 0.0010) with no interaction effect (F<1). Planned comparisons showed no differences between the Re or Rh lesion groups compared to controls, all the groups were able to learn where the platform was located (Figure 25). Therefore, any decreases in animals finding the platform or differences for the probe trials cannot be attributed to any motor deficits.
EXPERIMENT 2

DISCUSSION

Lesions of the Re and Rh nuclei produced differential effects on spatial working and reference memory behavioral tasks. Lesions to Re nuclei produced delay independent impairments on the DNMTP (Figure 14). Lesions to Rh nuclei produced accuracy impairments on the 8-arm RAM (Figure 18). No significant deficits were seen on the SRL, the 4F RAM or the RM-WM.

A double dissociation was revealed between the Re and Rh on the different behavioral tasks. On the DNTMP, Re lesioned rats had deficits in accuracy performance compared to Rh lesioned rats and controls. However, on the 8-arm RAM, performance was spared for the Re group but there were deficits in accuracy for the Rh group. There were no differences on response time between the lesion groups compared to controls on the DNMTP. Therefore, the deficits seen on DNMTP were not due to any motor deficits or the inability to complete the task in a reasonable time (Figure 15).

These findings behaviorally corroborate the anatomical connections of Re and Rh. Re projects to hippocampus, specifically CA1 and subiculum, parahippocampal areas of cortex and to infralimbic, prelimbic and orbital areas of prefrontal cortex. Rh has similar projections but also projects to nucleus
accumbens, amygdala and also has some diffuse projections to cerebral cortex (Vertes et al., 2006). These results suggest that the reuniens nuclei are critical for tasks which involve the integration of information of the prefrontal cortex and hippocampus. Rh, on the other hand, was only important for the 8-arm RAM of spatial memory, suggesting that it may play some sort of role in hippocampal-specific spatial memory.

For the reference memory tasks, there was spared performance on the SRL. Previous studies examining prefrontal cortex have shown that reversal learning was only impaired when stimuli were difficult to discriminate (Bussey, Muir, Everitt & Robbins, 1997) and it is possible that impairment was caused by the lack of being able to attend to the features of the stimulus. Birrell & Brown (2000) induced lesions in medial prefrontal cortex using ibotenic acid and found no impairments in the acquisition of a reversal learning task involving odor/texture discriminations. Conversely, Chudasama and Robbins (2003) found that lesions of the infralimbic cortex increased the number of sessions that were required to reach criterion during the learning of the reversals. In the current study, all groups had an increase in the number of errors to criterion for the first reversal condition, regardless of group. The lack of impairment shows that Re and Rh lesions do not impair positive transfer between problems based on a rule and also preserves reference memory.

This preserved reference memory was also seen for the RM-WM. There was an impairment of Re compared to controls on the amount of time to find the platform, however all groups were eventually able to learn the task (Figure 21).
There are two recent articles which have been published with inactivation of the reuniens nuclei on RM-WM. One study is in contrast to the current findings (Davoodi et al., 2009) where Re was reversibly inactivated with tetracaine during reference memory and working memory tasks. They found deficits on both the reference and working memory measures; however since they did not use any anatomical controls, there was the possibility that spread into surrounding areas could have accounted for the behavioral deficits. Also, tetracaine is a local anesthetic that acts on sodium channels that would potentially affect neural transmission in nearby white matter pathways. The current study used NMDA which spares these pathways and thus could have prevented the impairments that were seen by Davoodi and colleagues (2009).

This hypothesis is confirmed by another recent water maze study by Dolleman-van der Weel, Morris & Witter (2009). They used ibotenic acid, which has localized cell death with minimal effects on fibers of passage (Kohler & Schwarcz, 1983) to induce permanent lesions in Re. The method used for the water maze task was exactly the same method (RM-WM) we used for the current experiment to be able to directly compare the results. The RM-WM was also similar to the reference memory water maze task conducted in the experiment by Davoodi et al. (2009). Davoodi et al. (2009) found that animals with Re lesions were able to both learn the water maze task and also search in the area of where the platform would have been located during the memory probe. The results for Experiment 2 were similar to their findings as well as findings by Sloan, Good and Dunnett (2006) and Jo et al. (2007) where prefrontal lesions produced no
impairments for escape latencies, path length or memory retrieval of the location of the hidden platform. Therefore, Re and Rh are important for spatial working memory but not reference memory. Re is specifically important on tasks involving the integration of information between prefrontal cortex and hippocampus on working memory tasks that require a memory-guided response.
EXPERIMENT 3

INTRODUCTION

The results of experiment 2 revealed that the Re and Rh nuclei have differential effects on spatial and working memory, where Re lesions affected accuracy for DNMTP, a measure of spatial working memory, while Rh lesions affected RAM tasks. This suggests that lesions damaging both these nuclei affect spatial and working memory tasks that depend on prefrontal cortex and hippocampus.

Questions remain however about potential contributions of nearby nuclei. In Experiment 1 there was concern that inactivation spread into surrounding areas from the intended target in ventral midline thalamus. In Experiment 2, the role of individual ventral midline nuclei, Re and Rh, were confirmed to be critical for spatial working memory. However, the lesions induced in Re and Rh also could have damaged surrounding tissue. Experiment 3 addressed this issue by comparing discrete lesions of ReRh to lesions in more lateral and dorsal areas of thalamus (Figure 26). The areas targeted for the lateral lesions were the ventromedial thalamic nuclei (VM) and the targeted areas for the dorsal midline lesions were the paraventricular thalamic nuclei, the intermediodorsal thalamic nuclei and medial areas of the mediodorsal nucleus (DM). The effects of lesions
were compared for the same tasks used in Experiment 2. Based on the findings from Experiment 1 and 2 and Hembrook and Mair (2010), we expected lesions of ReRh nuclei to produce accuracy impairments on the DNMTP as well as both the RAM measures, while sparing learning and memory performance on the RM-WM. Performance should be spared on all behavioral measures for lesions in VM and DM compared to ReRh lesions.
EXPERIMENT 3

METHODS

Subjects

Thirty-two male Long Evans rats were obtained for use in this study. After animals were trained on the DNMTP, they were assigned by a randomized matching procedure into one of four experimental groups; ReRh, lateral thalamic nuclei (ventromedial thalamic nuclei- VM), dorsal midline thalamic nuclei (paraventricular, intermediodorsal and medial mediodorsal thalamic nuclei- DM) or sham control.

Behavioral Tasks

All behavior tasks were conducted in the same manner as Experiment 2. There was, however, one change for the 4F RAM. Results from Experiment 2 showed that the animals did not learn the task very well. It is possible that animals were not trained well enough on the longer delays. About half of the animals had a delay of 15 minutes for their first trial of the day. Perhaps 15 minutes was too long of a delay for a task that was not trained before surgery and rather animals needed to be trained more on shorter delays. For Experiment
3, instead of counterbalanced sessions using delays of 1 minute and 15 minutes; rats were tested for five days straight, two sessions per day on the same delay. There were three different delays, 1 minute, 5 minutes and 15 minutes. Therefore animals were tested for five days in a row on a 1 minute delay and then for five consecutive days on the 5 minute delay and finally five days on the 15 minute delay.

**Surgical Procedure**

All surgical procedures were completed the same as in Experiment 2. Table 2 depicts the sites for the different lesion grouped animals. Sham control surgeries were also conducted the same as in Experiment 2. See the General Methods section for complete surgery details.

**Pre-surgical Training**

Animals were trained on a series of programs to learn the DNMTP. The final program for the DNMTP included delays of 1 second, 5 seconds, 10 seconds, 16 seconds and 25 seconds. Animals continued on the training program until criterion was reached. Criterion for surgery was three consecutive sessions, at least 50 total responses for each session and a performance of 75% correct.
Post-surgical Testing

All post-surgical testing was conducted the same way as Experiment 2. Animals were tested on the DNMTP with delays (1 second, 5 seconds, 10 seconds, 16 seconds and 25 seconds) for a total of fifteen sessions. Animals were then switched to the SRL. SRL testing continued for a total of seven reversals. Animals were then trained and tested on the two RAM measures. Testing was conducted on the 8-arm RAM for three trials per session for a total of ten consecutive days. Animals were then tested on the 4F RAM. Testing occurred for two sessions per day for fifteen total days. Delays were 1 minute, then 5 minutes and then 15 minutes.

Animals then received free access to water for a period of at least two days and handled. Animals were then switched to the RM-WM for six consecutive days (Experimenters were EB, AC, BW and JH).

Statistical Analyses

All statistical analyses were conducted for all of the behavioral tasks in the same manner as Experiment 2. Since Re and Rh are hypothesized to be important in spatial memory and not the surrounding areas, we used planned comparisons (Bonferroni-Dunn, $\alpha = 0.05$) to test for significant effects of ReRh compared to VM and DM and controls. Planned comparisons (Bonferroni-Dunn, $\alpha = 0.05$) were also used to compare performance for VM and DM lesions.
compared to controls because we expected that both lesion groups would have spared performance on all behavioral measures.

For the RM-WM, the swim path analyses were completed by EB.
EXPERIMENT 3

RESULTS

Histological Analyses

A total of 32 animals completed all the behavioral tasks. One animal was excluded from the all the analyses because he failed to have damage in the target area (ReRh lesion group animal). The platform was left in the pool for one of the animal’s memory probe session and therefore the animal was excluded from the entire water maze analysis (Control group animal). Therefore the analyses for the tasks included a total of 31 animals for the DNMTP, SRL, 8-arm RAM, and the 4F RAM and 30 animals for the RM-WM. Figure 27 depicts a typical lesion for each of the lesion groups.

Behavioral Findings

DNMTP Task

For the DNMTP, there was no effect of lesion group (F < 1), a significant effect of delay (F_{4, 12} = 136.47, p < 0.001) and no interaction between these factors (F_{12, 108} = 1.029, p = 0.4279) (Figure 28). Overall performance for the DM lesions, VM lesions, control lesions and ReRh lesions were on similar average,
80.2%, 82.1%, 82.1% and 83.4% respectively. Planned comparisons revealed no differences between any of the lesion groups and controls and no differences between the ReRh group and VM or DM lesion groups.

There were no significant effects of lesion group on the RT of lever responses, whether the press was a sample response or a choice response ($F's < 1$) (Figure 29). There was a significant effect of the response type itself: the choice RT was quicker than the sample RT ($F_{1,3} = 133.612, p < 0.0001$) but this did not interact with lesion group ($F < 1$). Planned comparisons did not find any significant differences for RT for the comparisons of the different groups.

**SRL Task**

All animals had a similar number of errors to criterion for each group (Figure 30), were able to perform the task at errors of criterion and also showed positive transfer between problems. No significant differences were seen for the number of errors to criterion for lesion group ($F < 1$) on the SRL, but there was a significant effect of reversals ($F_{7,21} = 15.918, p < 0.0001$) with no interaction between these factors ($F < 1$) (Figure 30). All animals learned the task with an initial increase in the number of trials to criterion for the first reversal. There were no significant differences for any of the reversals between the lesion groups. Planned comparisons of Bonferroni-Dunn ($\alpha = 0.05$) showed no significant differences in performance between the different groups.
RAM Tasks

Spatial memory was measured overall accuracy performance on the 8-arm RAM, which was conducted over the course of ten days. ReRh lesion performance was compared to controls and the other lesion groups. There was a trend for the ReRh lesion group to be lower than all the other groups. The overall average of performance showed that the ReRh group had the lowest performance, 80.5% compared to 82.5%, 84.4% and 83.1% (DM lesions, VM lesions, controls) (Figure 31). Repeated measures ANOVA analysis of lesion and delay revealed no effect of lesion (F 3, 27= 2.676, p= 0.0671), a significant effect of day (F 9, 27= 3.736, p= 0.0002) with no interaction between factors (F 27, 243= 1.167, p= 0.2658). Planned comparisons of Bonferroni-Dunn revealed a significant difference between ReRh lesions and VM lesions (p= 0.0005), where ReRh was impaired on accuracy performance compared to VM lesions (Figure 31).

When animals were switched to the 4F RAM, where there was again a trend for the ReRh group to be lower than all the other groups. A repeated measures ANOVA analysis of lesion, delay and day revealed a significant effect for lesion (F 3, 27 = 1.157, p= 0.3443), a significant effect of delay (F 2, 8 = 3.359, p = 0.0422) and day (F 4, 12 = 3.543, p = 0.0093) with no interactions between the factors (Figure 32). Planned comparisons of Bonferroni-Dunn (α = 0.05) showed a significant difference for ReRh lesions compared to VM lesions (p= 0.0038) and
controls ($p=0.0020$), where the ReRh group was impaired on accuracy compared to both the VM group and controls when collapsed across delay.

**RM WM Task**

Learning trials were analyzed in the same manner as experiment 2 in both individual learning trials (18 trials) and learning blocks (6 blocks). For the learning trials, there was no significant difference of lesion ($F_{3,25}=3.015$, $p=0.0488$) but there was a significant effect of trial ($F_{17,51}=16.935$, $p<0.0001$) with an interaction between factors ($F_{51,425}=1.694$, $p=0.0030$) (Figure 33). Planned comparisons of Bonferroni-Dunn ($\alpha=0.05$) revealed significant escape latency differences for the ReRh lesions compared to VM lesions ($p=0.0001$) and DM lesions ($p<0.0001$) but not controls ($p=0.0484$). However, all animals were able to eventually learn the location of the fixed hidden platform. When examining the last training trial, the ReRh lesion group averaged 11.9 seconds compared to controls with an average of 15.2 seconds. An analysis of learning trials collapsed by learning blocks revealed effect of lesion group ($F_{3,26}=3.395$, $p=0.0328$), a significant effect of learning block ($F_{5,15}=20.703$, $p<0.0001$) with no interaction between factors ($F_{15,130}=2.099$, $p=0.0136$) (Figure 34). Planned comparison analyses (Bonferroni-Dunn $\alpha=0.05$) showed significant differences of ReRh lesions compared to DM lesions ($p=0.0019$) and VM lesions ($p=0.0046$) but not controls ($p=0.1889$). The overall average across the learning blocks for escape latency was 19.9 seconds for the ReRh group versus 13.0 seconds for the VM group, 12 seconds for the DM group and 15.7 seconds for the control group.
There was an initial increase in escape latency for the ReRh group, however all animals were able to learn the task regardless of group over the course of the training trials. Individual ANOVAs only showed significant differences for learning block 1 ($F_{3,26} = 4.451$, $p = 0.0119$). Planned comparisons of Bonferroni-Dunn ($\alpha = 0.05$) were conducted for each learning block and only revealed a significant differences for learning block 1 for ReRh lesions compared to VM lesions ($p=0.0057$) and DM lesions ($p=0.0027$).

The type of swim path was also examined for the set categories from Dolleman-van der Weel, Morris and Witter (2009) and from experiment 2 (Figure 12). There was no significant effect of lesion ($F<1$), a significant effect swim path type ($F_{6, 18} = 45.263$, $p < 0.0001$) with no interaction between factors ($F_{18, 156} = 1.548$, $p=0.0804$). Planned comparisons did not reveal any significant differences for any of the comparisons (ReRh compared to controls, ReRh compared to VM and dorsal medial, and VM and dorsal medial compared to controls) for any of the different swim path types (Figure 35). Individual ANOVAs were conducted to examine differences between each swim path type compared to the lesion groups. There were no differences between any of the swim path types and lesions ($A$-$F_{3,26} = 2.572$, $p = 0.0758$, $B$, $D$, $F$, $G$ - $F$'s <1, $C$-$F_{3,26} = 1.937$, $p = 0.1484$, $E$-$F_{3,26} = 2.093$, $p = 0.1256$). Planned comparisons revealed no differences for any comparisons for any of the swim path types.

The memory probe trial was conducted 24 hour after the last learning trial. The platform was removed from the pool for a one-trial memory probe test. Parameters analyzed were swim path length, swim speed, number of passes
through where the platform would have been located, the time spent in the quadrant where the platform was located and proximity. Individual ANOVAs were conducted for each variable. There was no significant effects of path length ($F_{3, 26} = 2.363, p = 0.0943$), speed ($F_{3, 27} = 2.383, p = 0.0923$), number of passes ($F_{3, 26} = 1.049, p = 0.3877$), time in quadrant ($F<1$), or proximity ($F<1$). Planned comparisons revealed no differences for any of the comparisons for any of the memory variables (Figure 36).

On the last day of testing, the platform was placed above the water level to measure any gross motor deficits. There was no differences between the lesion groups ($F_{3, 26} = 1.638, p = 0.2048$), a significant effect of trial ($F_{3, 9} = 3.936, p = 0.0114$) with no interaction between these factors ($F<1$). Similarly to Experiment 2, animals improved over the four trial sessions. Planned comparisons (Bonferroni-Dunn $\alpha = 0.05$) did not reveal any differences between lesion groups. The overall average across the four cue trials was 8.0 seconds for the ReRh group, 5.8 seconds for the lateral group, 6.1 seconds for the dorsal group and 7.5 seconds for the control group (Figure 37). Therefore, any deficits seen for learning or remembering the location of the platform were not because of motor deficits.
Lesions to ReRh impaired performance on the 8-arm RAM and 4F RAM compared to the VM lesions and only on the 4F RAM compared to control animals (Figure 26 & 27). There was a significant difference in the average learning trial performance for lesion to ReRh compared to VM and DM on the water maze, however all groups learned the platform location with no differences in memory performance. There were no impairments for ReRh lesions on the behavioral measures of DNMTP and SRL compared to control performance. Lesions to VM and DM did not produce deficits on any of the behavioral measures. The spared performance on the reference memory tasks of the SRL and the memory probe of the RM-WM for all lesion groups as well as the deficits in both the RAM tasks for the ReRh lesion group provide evidence that the deficits seen in experiment 1 and 2 and other studies (Davoodi et al. 2009, Hembrook & Mair, 2010, Onos, Hembrook & Mair, in prep) were not due to spread into surrounding areas.

The lesions for the dorsal midline (DM) were aimed at the paraventricular, intermediodorsal and medial mediodorsal thalamic nuclei (Figure 26 & 27c). There were no effects of DM lesions on the DNMTP. Research using lesions to
damage all of these nuclei is scarce. There have been a few studies examining the effects of mediodorsal thalamic nuclei. The mediodorsal nucleus is located lateral to the intermediodorsal nucleus and more ventral than the paraventricular nucleus, however histology for these studies show spread into these two nuclei (Burk & Mair, 1998; Zhang, Burk, Glode, & Mair, 1998; Neave, Sahgal & Aggleton, 1993; Bailey & Mair, 2005). Bailey and Mair (2005) found delay-dependent deficits for large mediodorsal lesions on the DMTP. Contrary to this, Burk and Mair (1998) examined the mediodorsal nuclei on the DMTP and found no deficits in accuracy or response speed compared to control animals.

Two other studies, one using 2-choice odor discriminations with go/no-go procedures (Zhang et al., 1998) and another using an automated DNMTP (Neave, Sahgal & Aggleton, 1993) found spared performance for the mediodorsal thalamic lesion animals. Also, on a spatial discrimination task and for a series of reversals, Neave, Sahgal and Aggleton (1993) did not see any deficits for mediodorsal lesions. These studies as well as the current study provide evidence that the dorsal medial nuclei do not contribute to impairments of spatial working memory.

To examine more lateral areas of thalamus, lesions were aimed at the ventromedial thalamic nuclei (VM) (Figure 26 & 27b) to avoid spread into ReRh. This area was also within the area of potential spread of pharmacological treatments. Mair and colleagues (1992) found no deficits on a DNMTP task when lesions were induced in lateral areas of thalamus. Their lesions were not specifically aimed at ventromedial thalamic nuclei, however based on their
histological analyses, there was spread into those areas for most of the animals. This is in contrast to evidence by Burk and Mair (1999) who examined lesions in VM on the DMTP. They found delay-dependent deficits of VM lesions on accuracy performance when compared to controls. In the same study, Burk and Mair (1999) found no impairments for the serial reversal learning task comparable to the results of the current study. However, the authors do caution that there was a limited amount of involvement of intralaminar nuclei when examining the damage radius area of the lesions.

Burk and Mair (1999) also found deficits in response speed for the DMTP task where the ventromedial lesion group was slower to make both sample and choice responses compared to controls. The possibility for this could be that in the rat, the VM nucleus is the area where convergence takes place for pathways involved in motor control (Herkenham, 1979). One possibility for the lack of an impairment seen in the current study could be due to the task being nonmatching to position, whereas the task used in Burk and Mair (1999) was matching to position. There is evidence that these two tasks have differential activation and thus are affected diversely by manipulation in different brain regions (Elliot & Dolan, 1999). Elliot and Dolan (1999) examined DNMTS and DMTS in humans and found activation in the bilateral head of caudate and medial orbitofrontal cortex on the DMTS whereas on the DNMS, there was activation in mediodorsal thalamus, bilateral lateral orbitofrontal cortex, and left premotor cortex. However, research on VM nuclei on the DNMTP has not been done as of the time of this dissertation.
Lesions in ReRh impaired performance on the 4F RAM (Figure 32). These impairments were similar to the impairments seen by Hembrook and Mair (2010). The lack of impairment for the DNMTP, suggests that damage in Re must be larger for deficits to occur. Lesions to ReRh were purposely made discrete to avoid overlap with areas damaged by VM or dorsal medial lesions (Figure 26 and 27a). These results provide evidence for the hypothesis that Re and Rh are important in spatial working memory functioning that are mediated by prefrontal cortex and hippocampus.
EXPERIMENT 4

INTRODUCTION

The results from experiment 1, 2 and 3, provide evidence that the reuniens and rhomboid nuclei play an important role in spatial working memory. However, these data do not indicate the stage of memory processing affected by these nuclei. Here we used deep brain stimulation (DBS) to address this issue. In event-related DBS, animals are trained on a behavioral task and then implanted with electrodes into the brain area of interest. Criterion is reestablished and sessions are conducted in which brief trains of DBS are applied at specific time during behavioral trials. This can allow for stimulation to occur during part of a task and not during others, as well as varying the amount of current being applied. Performance was then analyzed to compare when stimulation was applied versus when stimulation was not delivered. There are some disadvantages to using electrical stimulation, one is the potential for spread of current to other sites and another is that the exact alteration that occurs during the stimulation is not yet known.

Previously, our lab (Mair & Hembrook, 2008) used this technique to examine the effects of event-related electrical microstimulation in rostral intralaminar thalamic nuclei on the DMTP. Animals were tested on varying levels
of low and high levels of stimulation during different phases of the DMTP. The phases (with the different stages of memory in parentheses) were initiation (planning), sample (encoding), delay (storage), and choice (retrieval).

Animals were tested on three different delays of 1 second, 13 seconds and 23 seconds, in separate sessions. Electrical stimulation was applied during the different memory phases at brief (1 sec) trains of 0.2 millisecond constant current pulses at 120Hz. Results showed that stimulation affected performance when applied during the delay and retrieval stages. High levels of stimulation produced impairments and low levels enhanced the behavioral accuracy.

In the current study, electrodes were aimed at ReRh and stimulation applied while animals performed the DNMTP. Delays of 3 seconds and 15 seconds were intermixed randomly within sessions. Anatomical connections (Vertes et al. 2006, 2007) and findings from Experiments 1, 2 and 3 provide evidence that Re is the intermediary structure for communication between prefrontal cortex and hippocampus. Therefore, we expected that high levels of stimulation would produce impairments on the DNMTP measure of spatial working memory and low levels of stimulation would enhance behavioral accuracy similar to Mair and Hembrook (2008).

Previous research has shown that prefrontal cortical neurons have been found to fire during delay periods (Funahashi, Bruce and Goldman-Rakic, 1989; Batuev, Kursina & Shutov, 1990; Hyman et al., 2010) therefore stimulation on the
DNMTP would alter performance accuracy during the trials where current was delivered during the delay phase.

To test whether working memory demands were critical and not due to general disruption, the effects of event-related stimulation were compared for a spatial reference memory task (SRM). Previous studies and Experiment 2 have shown that ReRh, hippocampus and prefrontal cortex are important in spatial working memory (Mair, Burk & Porter, 1998; Porter, Burk & Mair, 2000; Bailey & Mair, 2005; Hembrook & Mair, 2010) and not important for spatial reference memory (Porter, Burk & Mair, 2000; Dolleman-van der Weel, Morris & Witter, 2009). The choice response for SRM was similar to the choice response for DNMTP. If working memory demands are critical than deficits would occur on the DNMTP for memory-guided responses, but not for the SRM for responses based on applying a rule. Therefore we expected that there would be no impairments seen when high current levels of stimulation were applied during any of the phases of the SRM, thus providing evidence for the specific nature of ReRh being critical for carrying decisional information across a memory delay to make a memory-guided response.
EXPERIMENT 4

METHODS

Subjects

Twelve male Long Evans rats were obtained for this study. All animals were trained on the lever DNMTP task before surgery was conducted to implant electrodes into ReRh.

Behavioral Tasks

DNMTP Task

This task was the same as Experiment 1, except for two differences. One, the imposed delays were 3 seconds and 15 seconds. The other difference was in the task itself, for each lever press, the animals had to press the lever twice for it to retract. This change was done to ensure that the animal was engaged in the direction of the lever to make certain to delineate the separate stages (Figure 3).
Spatial Reference Memory (SRM) Task

This task was similar to the SRL from Experiment 2 and 3 with some modifications. First, training consisted of 100 responses with a time limit of 40 minutes. The same lever side was reinforced for each session until a criterion of 80% accuracy performance was reached. The reinforced lever side was then switched (Figure 11). For the stimulation testing procedure, ten warm-up trials were presented at the beginning of the session to reinforce the particular lever side for the session. Stimulation then began on the 11th trial of the session.

Surgical Procedure

The surgical procedure was similar to that of Experiment 1 (see General Methods for details). Bipolar electrodes (twisted pairs of 0.125 mm SSD wires with polyimide insulation, MS303-3, Plastics One) were implanted into ReRh. The target coordinates for the implantation of the electrode were; AP: 6.44, DV: 2.4 and ML: 0.0. These coordinates were determined using Paxinos and Watson (1998) and the electrode was implanted at a 15° angle to avoid the midline.

Pre Surgical Training

Animals were trained on a series of DNMTP programs until they met a criterion of 50 responses with at least 75% correct for three consecutive days on
a delay set ranging from 1 second to 25 seconds. This training program did not have the double lever presses incorporated in the sessions.

**Electrical Stimulation Procedure**

Animals were put on a simple lever pressing task and stimulation applied. A staircase procedure was conducted to determine the threshold current for each animal. Threshold was considered to be the current level in which the animal paused (Table 4- shows each animal’s threshold stimulation level).

Electrical stimulation consisted of 1.0 second trains of 0.2 millisecond current pulses delivered at 120 Hertz from a constant current stimulus isolator (A365, WPI, Sarasota, FL) connected to an electrode through a commutator (SL2X2C, Plastics One, Roanoke, VA) which allowed the animals to freely move around the chamber.

For the DNMTP testing sessions; current levels were set below the animal’s threshold current level. Event-related stimulation was delivered at four different memory phases of the DNMTP. Each session consisted of randomly selected trials where stimulation occurred during one of the different memory stages, along with 20% trials where no current was applied. Figure 3 depicts the four different stages, trial initiation which is the planning stage; sample which is the encoding stage; delay which is the retention stage; and choice which is the retrieval stage. Animals were given one session between each stimulation session to assure that performance accuracy of 75% was reached. This was to
avoid any carry-over of stimulation from session to session. Animals were
stimulated no more than three sessions per week.

For the SRM, the previously set high current level of stimulation was used
for testing sessions. Current stimulation for each trial in the session was
randomly selected; control trial (no current applied), current applied before the
start of the initiation lever (inter-trial interval), current applied after the first press
of the initiate lever (start), or current applied as the initiate lever retracted
(choice).

**Post Surgical Testing**

Water deprivation was reestablished for all the animals. Animals began
DNMTP training sessions, with delays of 3 seconds and 15 seconds. Once the
animals were able to meet pre-surgical criterion, they were switched to the
DNMTP with double press responses. Animals were required to reach
performance of 75% on the double lever press sessions for the DNMTP for three
consecutive days. Testing sessions were conducted with varying current levels;
high, low and none; counterbalanced by block randomization. Animals
completed a minimum of 4 sessions at each current level with a total of 120 trials
(240 overall trials) for each delay.

Animals were then switched to the SRM. For training sessions, animals
were run on the same reinforced lever for every session until they completed a
session with at least 90% accuracy. After the animal performed at criterion for a
session, the reinforced lever side was switched. This reversal pattern continued until they had completed at least two reversals on each side.

Animals were then moved to the testing SRM. For this program, the animals had ten warm-up trials where no stimulation would occur and then 80 trials with the same lever being reinforced on every trial. Trials within the stimulation sessions were randomly intermixed for the phase that the stimulation would occur. Animals were trained on one side for a session (usually one or two sessions) until they performed with 80% accuracy and then the next session were plugged into the stimulator. For the stimulation testing session, the high current level was used and the reinforced lever stayed the same as the previous session to allow for maximum performance. The animals then were switched to the opposite lever side and the same procedure was followed. A total of two sessions where the animal was plugged in and given stimulation was completed for each reinforced lever side.

**Statistical Analyses**

An omnibus three-way repeated measures ANOVA was conducted to analyze DBS results for DNMTTP. The main factors were stimulation current level, delay and phase. Similarly, SRM results were analyzed with a repeated measures ANOVA with stimulation condition (no current versus high current) and phase (control, inter-trial interval, start and choice) as factors. Post hoc analyses
(Bonferroni-Dunn, $\alpha = 0.05$) were conducted to examine any of the significant effects.

To compare behavioral tasks, overall percent accuracy was examined for the DNMTP and SRL for no current and high current sessions. The delay and choice phases of the DNMTP were similar to the start and choice phases of the SRM. To test for the specificity of impairment on the DNMTP versus SRM, an ANOVA was conducted to compare the behavioral tasks, the current conditions and the two phases (delay/start and choice).
EXPERIMENT 4

RESULTS

Histological Analyses

Seven (of 12) animals completed both behavioral task protocols. Figure 38 depicts an example of a histological brain slice stained with thionin for the location of the electrode. Table 3 shows the location of the electrodes for each individual animal. A total of five animals were excluded from the study. One animal was excluded because he did not reach post-surgical criterion. Another animal was excluded because during one of the stimulation sessions, one of the prongs to the stimulator cable broke off into one of the animal's electrode sockets and could not be removed. One was excluded because of difficulty of finding a level of stimulation over 0.01mA (the minimum tested) that did not interfere with the ability to complete DNMTP trials. Another animal excluded when performance at its high current level of stimulation (0.06mA) drastically changed. Lastly, one animal was excluded because of incomplete data at the time of this writing.

The high level of stimulation current varied between animals, ranging from 0.03mA to 0.15mA. See table 5 for individual animal's high stimulation levels.
Based on previous results (Mair & Hembrook, 2008); all animals were tested at 0.01mA for their low current level of stimulation.

**DNMTP Task**

An omnibus repeated measures ANOVA showed significant effects for current level ($F_{2, 12} = 103.573, p < 0.0001$) (Figure 39), delay ($F_{1, 6} = 116.636, p < 0.0001$) (Figure 40) and phase ($F_{4, 24} = 5.048, p = 0.0043$) with a significant three-way interaction between these factors ($F_{8, 48} = 3.002, p = 0.0082$). The average accuracy performance at the 3 second delay was 86.5% compared to the 15 second delay, 68.7%. Performance was lower regardless of current level or phase for the longer delay. Performance also differed for sessions in which different stimulation currents were tested. The average accuracy for no current sessions was 85.3%; low current sessions was 82.5%; and 64.7% for high current sessions. Planned comparisons of Bonferroni-Dunn ($\alpha = 0.05$) revealed significant differences for high current sessions compared to low current sessions ($p < 0.0001$) and no current sessions ($p < 0.0001$) (Figure 39).

There was a significant interaction between delay and stimulation current level ($F_{2, 12} = 7.116, p = 0.0092$). This interaction was explored with two-way repeated measures ANOVAs for each delay. The 3 second delay revealed significant effects for current level ($F_{2, 12} = 130.593, p < 0.0001$) (Figure 41) and phase ($F_{4, 24} = 5.860, p = 0.0019$) and a significant interaction between these factors ($F_{8, 48} = 6.371, p < 0.0001$) (Figure 42). Exploring this interaction further for different current level sessions revealed no significant effects for the different
trial phases for no current sessions ($F_{4, 24} = 2.222, p = 0.0967$) and low current sessions ($F < 1$). There was a significant effect for the high current level sessions for the different trial phases ($F_{4, 24} = 6.955, p < 0.0001$). Bonferroni-Dunn ($\alpha = 0.05$) comparisons show significant differences between control trials (in which current was not applied) compared to trials where high current was applied during the delay ($p = 0.0028$) and choice ($p = 0.0005$) phases but not during the initiation ($p = 0.6528$) and sample ($p = 0.5902$) phases. Therefore within high current level sessions, current impaired performance specifically during the delay and choice trials but not during the initiate or sample trials (Figure 42).

The 15 second delay analyses revealed significant effects of current level ($F_{2, 12} = 36.435, p < 0.0001$) (Figure 43) with no effect of phase ($F < 1$) and no interaction between these factors ($F_{8, 48} = 1.964, p = 0.0717$) (Figure 44). Average performance on the high current sessions was 58.1\% compared to the low current sessions (71.7\%) and no current sessions (76.2\%).

The omnibus three-way repeated measures ANOVA also revealed significant interaction effect of current level by phase ($F_{8, 48} = 4.564, p = 0.0004$). Simple main effects revealed significant differences for current level for all the different memory phases. These analyses also showed that regardless of the phase the stimulation was delivered, performance was the worst for the high current sessions, even during the no current trials of the session.
Spatial Reference Memory Task

The choice response for the DNMTP and SRM are directly comparable. The effect of stimulation current was much more limited for the SRM than the DNMTP and was specifically striking for the choice response. On the SRM, the response accuracy differences were much smaller for the high current level versus sessions of no current than for the high current level for DNMTP. Response accuracy performance for the high current stimulation sessions dropped 8.5% from the no current level of stimulation sessions for the SRM compared to 20.6% for the DNMTP. An omnibus two-way ANOVA showed no significant effects for stimulation level (no current versus high current sessions) (F_{1,6} = 4.484, p = 0.0785) (Figure 45); phase (F_{3,18} = 3.023, p = 0.0566) or for the interaction of stimulation level and phase (F_{3,18} = 2.154, p = 0.1290). Therefore, during sessions of high current, where stimulation was applied during randomly selected phases (between trials, the initiate phase, the choice phase or no control trials) there was no difference in response accuracy performance (Figure 46).

Comparison of Behavioral Tasks

A two-way ANOVA was conducted to compare response accuracy performance for the sessions of no current and high current for the DNMTP and SRM. This revealed significant effects of task (F_{1,6} = 77.484, p< 0.0001) with significant effects of the stimulation current condition (F_{1,6} = 40.748, p = 0.0007)
and an interaction between task and stimulation current condition ($F_{1,6}=20.721$, $p=0.0039$). Average performance for the no current sessions was 85.3% for the DNMTP and 90.9% for the SRM. For the high current stimulation sessions, the average performance was 64.7% and 84.2% for the DNMTP (26.2% drop) and the SRM (6.7% drop), respectively (Figure 47).

The start and choice phases for the SRM and the delay and choice phases for the DNMTP were similar in nature. In both tasks, a single lever extends on one side of the chamber and then both levers on the other side of the chamber extend. The animal is required to press the same initial lever and one of the two choice levers, however the DNMTP is based on that particular trial information and the SRM is based on a consistent session rule, working memory versus reference memory. Response accuracy was examined for the start/delay and choice phase trials. A three-way ANOVA was conducted to examine the delay/start phase trials of each of the tasks for no current and high current sessions. There was an effect of the task ($F_{1,6}=101.941$, $p<0.0001$), stimulation current condition ($F_{1,6}=24.289$, $p=0.0026$) but no effect of phase ($F_{1,6}=2.089$, $p=0.1985$). There was a significant effect for the interaction of task by stimulation current (no current versus high current) ($F_{1,6}=47.651$, $p=0.0005$) but no interaction between task by phase ($F_{1,6}=2.202$, $p=0.1884$) or stimulation current by phase ($F<1$) (Figure 48). For SRM, accuracy performance was 90.3% (no current) and 86.0% (high current) for delay phase trials compared to 85.1% (no current) and 60.2% (high current) on the DNMTP. For the choice phase trials, SRM performance was 91.4% and 87.5% (no current versus high current).
and DNMTP was 88.0% on no current trials which dropped to 58.2% for high current trials. This suggests that performance was impaired selectively during the delay and choice phases of the DNMTP and not the SRM, providing evidence for the specificity of current affecting working memory demands across a memory delay.
EXPERIMENT 4

DISCUSSION

Experiment 4 examined the temporal specificity of inactivating Re and Rh nuclei with DBS. Brief trains of electrical pulses were applied at different times during the DNMTP. Constant current pulses were applied during four phases of the DNMTP, that correspond with different memory processes: initiation (planning), sample (encoding), delay (storage), and choice (retrieval) (Figure 3).

Accuracy performance was impaired with high current sessions of stimulation during trials where current was applied during the delay and choice phases for the imposed delay of three seconds (Figure 41). Sessions of low current levels did not show any improvement in performance for the 3 second delay. This could be due to the high accuracy of performing during sessions where no current was applied. The performance average for no current sessions was 94.406%, thus suggesting a ceiling effect where it was not possible for animals to perform better than their baseline accuracy.

On the other hand, there appeared to be a floor effect for the longer 15 second delay. For the no current sessions during the longer delay, average performance accuracy was 76.2%. This was much lower than the average for no current sessions during the shorter delay. There was a general decrease during the high current sessions for stimulation during the different phases compared to
control trials; however the decrease was not as substantial and not specific to the phases. To test carry-over effects, animals received control trials for 20% of the sessions for each of the low and high current stimulation level sessions. For the longer delay, there was also a decrease of accuracy on the no current trials during the high current sessions, suggesting a carry-over effect of the stimulation from the previous trials.

On the SRM task, no significant differences were seen between sessions where the high level of stimulation was applied versus when no current occurred (Figure 45). On average, the performance was 90.0% for no current sessions versus 84.2% for high current sessions. For the high current sessions, there were no differences between control trials and any of the phases for the SRM. When this performance was compared to the DNTMP task, there was an overall decrease in performance for no current sessions (85.3%) versus high current sessions (64.7%) (Figure 49). Current delivered for the high current stimulation sessions was examined for both delay/start and choice phases to compare performance between the tasks. Performance on the high current sessions for the SRM for the delay/start was 86% and choice 87.5% whereas performance on high current sessions for DNMTP was 60.2% for the delay phase and 58.3% for the choice phase (Figure 48). These results indicate that Re nuclei affect working memory processes involved in representing information during brief memory delays as well as in executing memory-guided responses. Therefore,
the lack of impairment seen of for the SRM indicates that the effects of Re nuclei stimulation depends on working memory requirements.

These findings are consistent with evidence from recording studies of both prefrontal cortical neurons (Hyman et al., 2010) and hippocampal neurons (Watanabe & Niki, 1985; Wilson, Riches & Brown, 1990). These neurons fire selectively during the delay period of a task and stop once the delay is over. Therefore, the information is able to be properly encoded but is disrupted during the delay process where communication to these structures is important for accurate responding.

Most of the research concerning microstimulation or DBS has been used for movement disorders such as Parkinson’s disease (Putzke, Wharen, Wszolek, Turk, Strongosky & Uitti, 2003) and controlling seizures (Velasco, Velasco, Velasco, Jimenez, Marquez & Rise, 1995). Recently, DBS has also been used in humans to treat depression (Velasco et al., 2006). And there is even one case study where stimulation in the rostral intralaminar thalamus helped improve behavior for a patient in a minimally conscious state (Schiff et al., 2007).

There is not much research using brief pulses of DBS stimulation. There have been a few monkey studies examining brief stimulation during a visual memory DMTP. Bisley, Zaksas and Pasternak (2001) stimulated the medial temporal lobe during the sample of some trials and the delay period during other trials. Stimulation that was applied during the sample period influenced performance by the monkey being more likely to choose the matching stimulus.
Other studies have begun examining the role of different areas of the brain in decision-making processes (Ditterich, Mazurek & Shadlen, 2003; Tehovnik, Slocum & Schiller, 1999, 2002, 2003).

Even though electrical stimulation has been around since at least the 1870’s (Fritsch & Hitzig, 1870) it has been argued that this type of stimulation is not precise enough to study the mechanisms underlying different processes. It is possible that this would be true, however evidence from the earlier studies from this dissertation (Experiments 1, 2 and 3) provide other types of manipulation techniques that have shown the Re and Rh nuclei to be important in spatial working memory.

Therefore, the findings of Experiment 4 provide evidence that stimulation can not only produce selective impairments during a spatial memory task versus a reference memory task, but also that these effects can be localized to a specific phase of the memory process. The impairments seen for the specific storage and retrieval phases are confirmatory to the overall behavioral results from Experiment 1 and other studies involving ventral midline thalamus (Hembrook & Mair, 2010, Hembrook, Onos & Mair, 2011).
GENERAL DISCUSSION

Re and Rh are two nuclei in midline thalamus which have robust anatomical connections with prefrontal cortex and hippocampus. Re nucleus is the largest of the ventral midline thalamic nuclei and the Rh nucleus is located above the caudal two thirds of Re. Re has projections to CA1 and subiculum of hippocampus as well as parahippocampal areas of cortex. Rh also has projections to all of these areas but has additional projections to nucleus accumbens and the amygdala, along with more diffuse widespread projections to the cerebral cortex (Vertes et al., 2006). Patients with amnesia have been shown to have damage in these areas (Gold & Squire, 2006; Van der Werf, Witter, Uylings & Jolles, 2000). But even with these findings and the strong connections, there has only been a limited amount of research on these nuclei.

What is the Critical Location?

Previous studies in rats have examined the role of prefrontal cortex and hippocampus in different types of behavioral memory tasks (Burk & Mair, 1998; Porter, Burk & Mair, 2000). Prefrontal damage has been associated with deficits on DMTP (Kesner, 2000; Sloan, Good & Dunnett, 2006). The hippocampus has been shown to be important for the proper performance of water maze memory.
tasks (Sloan, Good & Dunnett, 2006; Broadbent, Squire & Clark, 2004; Clark, Broadbent & Squire, 2005) and DNMTS (Mumby, Pinel & Datur, 1993; Mumby, Mana, Pinel, David & Banks, 1995; Clark, West, Zola and Squire, 2001).

However, behavioral research on Re and Rh has been more limited and have not been studied extensively on the above behavioral tasks. Recently, when Hembrook and Mair (2010) examined discrete lesions to Re and Rh nuclei, they found impairments of accuracy on measures of spatial memory in the RAM, but spared performance on a visuospatial reaction time task. There have been two recent studies specifically examining Re on different water maze tasks. Davoodi et al. (2009) found deficits on both reference memory and working memory tasks when reversibly inactivating Re nuclei with tetracaine. Dolleman-van der Weel, Morris and Witter (2009) did not find impairments on a reference memory task when lesions were produced in Re.

The experiments in this dissertation sought to continue examining these nuclei and pinpoint which of the nuclei are critical for spatial working memory. Experiment 1 used reversible inactivation of these nuclei during two different tasks of spatial working memory. Inactivation during working memory tasks decreased accuracy performance on hippocampal- and prefrontal- dependent tasks. There was evidence for a localized effect of inactivation on the DNMTP but not the VC-DNM RAM. This provided evidence for Re and Rh being important in working memory.
Experiment 2 was conducted to isolate damage to the individual nuclei to determine whether both nuclei are imperative for spatial and working memory. Results revealed delay independent effects on accuracy for Re but spared performance for Rh lesions on the DNMTP. The opposite was true for Rh where impairments were seen on an 8-arm RAM spatial memory measure but performance was spared for the Re lesions. Previous reports have found deficits on the DNMTP for both prefrontal and hippocampal lesions (Harrison & Mair, 1996; Young et al., 1996; Porter, Burk & Mair, 2000) and deficits on the standard 8-arm RAM for hippocampal lesions but not prefrontal cortex lesions (Young et al., 1996; Mair, Burk & Porter, 1998). These results from Experiment 2 are consistent with the hypothesis that Re was for behavioral tasks which rely on communication between hippocampus and prefrontal cortex and rhomboid nuclei were important for tasks which rely solely on hippocampus.

Dorsal Thalamic Areas

When inducing damage whether, permanent or temporary, to an area of the brain, there is always a possibility for damage to spread into other areas. This might contribute to overall impairments. Experiment 3 was conducted to rule out the surrounding more lateral and dorsal areas of thalamus on the same tasks which were used in Experiment 2.

In Experiment 3, we targeted the areas of the paraventricular, the intermediodorsal and the medial areas of the mediodorsal thalamic nuclei.
Previous studies have mostly examined damage in the mediodorsal thalamic nuclei (MD). The MD nuclei have reciprocal connections with prefrontal cortex (Kievit & Kuypers, 1977; Goldman-Rakic & Porrino, 1985; Giguere & Goldman-Rakic, 1988; Ray & Price, 1993) and there is evidence that the MD play some sort of role in working memory (Fuster & Alexander 1971, 1973; Kubota, Niki & Goto, 1972; Tanibuchi & Goldman-Rakic 2003).

In monkeys, lesions to the MD nuclei impaired performance accuracy on both DMTS (Aggleton and Mishkin 1983a, b; Parker, Eacott & Gaffan, 1997) and DNTMS tasks (Zola-Morgan & Squire, 1985). In rats, lesions to the MD nuclei, produced delay dependent impairments of accuracy on the DMTP (Bailey & Mair, 2005). In contrast, however, Burk and Mair (1998) found no deficits in accuracy on the DMTP and others found spared performance on a 2-choice odor discrimination task (Zhang et al., 1998), an automated DNMTP task (Neave, Sahgal & Aggleton, 1993), the VC-DNM RAM (Bailey & Mair, 2005) and a working memory task trained in the radial arm maze (Alexinsky, 2001).

When comparing the findings from Burk and Mair (1998) and Bailey and Mair (2005), the deficits seen in Bailey and Mair (2005) were small; the MD lesion group had an average overall performance of 85.1% compared to controls, 91.6%. In Burk and Mair (1998) the performance for the MD lesion group was 82.3% compared to 86.6% for controls, thus not reaching statistical significance. The impairment seen by Bailey & Mair (2005) was also delay dependent and it is possible that in the other studies where impairments were found, only a short
delay was used and multiple delay periods were not used (Kolb, Pittman, Sutherland & Whishaw, 1982; Chow, 1954).

On a reference memory task in the RAM, there were no deficits for animals with MD lesions compared to controls (Alexinsky, 2001). Animals were also able to correctly learn a series of reversal learning problems in the RAM (Alexinsky, 2001) and a spatial discrimination task and for a series of reversals (Neave, Sahgal & Aggleton, 1993), which suggests that the MD nuclei are not involved in the proper learning of a new set of rules. Therefore, even though the MD nuclei have connections to prefrontal cortex, lesions in this area do not seem to produce systematic memory impairments (Hunt & Aggleton, 1998).

In the current study (Experiment 3) no deficits were found for the dorsal thalamic nuclei lesions compared to controls on the spatial working memory task (DNMTP), however the overall accuracy average for the dorsal group was the lowest 80.21% compared to controls who were 83.24%. There were also no deficits seen for the SRL, the RAM tasks and the reference memory water maze task. The lack of impairment seen for any of these tasks provides evidence that any deficit produced by lesions or inactivation to Re and Rh nuclei was not due to damage of the MD nuclei.

The other area which is located directly above the rhomboid nuclei is the central median thalamic nuclei (CM). The CM nuclei are part of the rostral group of the intralaminar thalamic nuclei. Much of the literature has not examined this set of nuclei by itself. Most studies involve the surrounding dorsal areas or the
lateral areas. CM nuclei receive input from subcortical structures such as reticular formation, serotonergic cell groups, the supramammillary nuclei, the cholinergic pedunculopontine and laterodorsal tegmental nucleus, deep cerebellar nuclei such as the dentate and fastigial and posterior interpositus nuclei and superior intralaminar nuclei colliculus (Van der Werf, Witter & Groenewegen, 2002).

The CM nuclei have differential projections for the dorsal CM nuclei versus the rostral CM nuclei. The rostral area has projections to layers I, III and V of the anterior cingulate cortex and the caudal areas has projections to layers I, III and V of primary motor, gustatory, visceral and primary somatosensory cortices. All of CM nuclei project to subcortical structures of the caudate putamen and parts of the amygdala (Van der Werf, Witter & Groenewegen, 2002).

The CM nuclei have been implicated in supplying striatal neurons with information about sensory events related to behavior as well as in orienting attention (Matsumoto, Minamimoto, Graybiel, & Kimura, 2001; Minamimoto & Kimura, 2002). Behaviorally, Mumby and colleagues (2005) found deficits on object recognition tasks and a DNMTP in rats that had damage to the CM nuclei. However, this damage was not limited to the CM nuclei and was also produced using a method of thiamine deficiency.

Bailey and Mair, also in 2005, examined midline lesions which included the CM nuclei and found deficits on the DMTP task but spared performance on the VC-DNM RAM. Peinado-Manzano and Pozo-Garcia (1996) induced lesions
to the dorsomedial nuclei which included the CM nuclei and found moderate impairments on a delayed alternation task for delays up to forty seconds and a severe impairment for delays of eighty seconds. Therefore, it is possible that the CM nuclei are somehow important in memory and possibly in recalling specific response-related events. These results taken together, suggest that the CM could play a role in memory processing and it is not possible to completely rule out this area's importance.

**Lateral Thalamic Areas**

The Re and Rh nuclei are situated directly along the midline of thalamus. Studies have provided evidence that dorsal structures are not important for working or reference memory related to prefrontal or hippocampal systems (Neave, Sahgal & Aggleton, 1993; Burk & Mair, 1998; Zhang et al., 1998; Alexinsky, 2001; Bailey & Mair, 2005). There are two other areas could potentially explain impairments on these types of memory tasks because of their relative location to Re and Rh nuclei. These are the ventromedial thalamic nuclei (VM) and submedius thalamic nuclei (SubM).

The VM nuclei are often damaged when lesions are induced in the intralaminar thalamic nuclei and could possibly explain some of the impairments seen in those studies (Mair, 1994; Burk & Mair, 1998; Zhang et al., 1998). Specifically in the rat, the VM nuclei are where motor control pathways converge from the substantia nigra pars reticulata, entopeduncular nuclei, superior
colliculus and cerebellum. Projections are then diffused to layer 1 of cerebral cortex (Herkenham, 1979; Jones, 1985).

An extensive search of the literature from our lab and others, revealed an absence of studies examining the VM nuclei on a DNMTP and only one study by Bailey and Mair (2005) targeted VM in the DMTP and VC-DNM RAM. Bailey and Mair (2005) found no deficits on performance for the VC-DNM RAM, similar to the task in Experiment 1. However they did find delay independent deficits on the DMTP. Another study by Burk and Mair (1999) examined the effects of lesions to the VM nuclei on the DMTP and found similar delay independent impairments of both accuracy as well as a moderate impairment in response speed for sample and choice responses. In the same study, the VM lesion group was not impaired on the SRL, identical to the one used in Experiment 3. However, the cannula needle used to induce the lesions in the VM nuclei went through the locations of the paracentral and centrolateral nuclei and in some cases ReRh was affected, both of which could have contributed to this deficit (Burk & Mair, 1999). These results are similar to previous findings with damage to striatal and prefrontal cortical areas (Burk & Mair, 1999; Dunnet, 1990; Mair, Burk & Porter, 1998).

Experiment 3 found no deficits for large lateral thalamic lesions targeted at the VM nuclei on various behavioral tasks. This is corroborated based on the previous lack of impairment on the VC-DNM RAM (Bailey & Mair, 2005). Based upon the published articles and the connections of the ventromedial thalamic
nuclei with motor areas and cortex (Herkenham, 1979; Jones, 1985; Krout & Loewy, 2000; Hoover & Vertes, 2007) the likelihood of these nuclei contributing to the deficits seen in spatial working memory tasks is small.

No systematic studies have been conducted examining the role of the submedius thalamic nuclei (SubM) in working memory or reference memory. This is due to the lack of connections to either medial prefrontal cortex or hippocampus. Anatomical studies in both the cat and the rat have found that the SubM nuclei receives major projections from the trigeminal subnucleus caudalis and the spinal dorsal horn lamina I (Dado & Giesler, 1990; Yoshida, Dostrovsky, Sessle & Chiang, 1991; Yoshida, Dostrovsky & Chiang, 1992) and primarily projects to the ventrolateral orbital cortex (Coffield, Bowen & Miletic, 1992; Yoshida, Dostrovsky & Chiang, 1992). The ventrolateral orbital cortex projects to the midbrain periaqueductal gray (Hardy & Leichnetz, 1981; Craig, Wiegand & Price, 1982). The midbrain periaqueductal gray is an area involved in the modulation of nociception (Fields & Basbaum, 1999). Studies using electrophysiology have shown that neurons in the SubM nuclei are activated when exposed to noxious mechanical, thermal, chemical and electrical stimulation of the periphery (Kawakita, Dostrovsky, Tang & Chiang, 1993; Tang, Zhang & Jia, 1995). This suggests that the SubM nuclei are involved in processes concerning pain and not memory. Even though no studies have been done examining memory any spread into the SubM nuclei would probably not be the reason for any impairment seen in performance.
What is the Critical Function?

Evidence for the Re nucleus being important in prefrontal aspects of memory comes from the similar results seen for damage of Re versus damage in prefrontal cortex. Previous studies have shown that damage to prefrontal cortex impairs performance on DMTP tasks (Mair, Burk & Porter, 1998) as well as DNMTP (Harrison & Mair, 1996, Porter, Burk & Mair, 2000). Another prefrontal task is the recurring choice delayed nonmatching to position in the radial arm maze (RC-DNM RAM). Previous studies have found delay independent deficits on the RC-DNM RAM (Porter & Mair, 1997; Porter, Burk & Mair, 2000; Bailey & Mair, 2005). The RC-DNM RAM differs from both the DNMTP and the VC-DNM RAM. The RC-DNM RAM is trained and tested in a dark room with black covers on the arms of the maze. Only three arms are used on the maze (similar to a T configuration). The animal begins in one arm and is forced to go to one particular arm (sample) and then return to the original arm for a delay period (holding). Once the delay period ends, gates to three arms open (both are located 90 degrees from the holding arm, one to the left and one to the right) and the animal is supposed to make a response in the arm they had not previously entered. Thus if the animal went left for the sample, they should go right for the choice response to make a correct response and receive water reinforcement. The RC-DNM RAM forces the animal to use egocentric cues to solve the task, rather than allocentric cues of the environment around them (Whishaw, 1998).
A recent study by Onos, Hembrook & Mair (in prep) used muscimol to reversibly inactivate Re and Rh nuclei on the RC-DNM RAM. Deficits were seen at all delays compared to saline injection sessions. This suggests that the Re and Rh nuclei are important in mediating communication between prefrontal cortex and hippocampus. Disrupting that communication will also affect memory-related performance.

This is in agreement with lesions of Re and Rh nuclei on a visuospatial reaction time task and radial arm maze tasks of spatial memory. Lesions to Re and Rh nuclei spared performance on the visuospatial reaction time task, a motor-related task but impaired spatial memory accuracy in the RAM (Hembrook & Mair, 2010). Experiment 1, 2, and 3 of this dissertation are three studies which provide solid evidence for the role of Re and Rh nuclei in spatial working memory. However, the Re and Rh nuclei seem to be differentially involved in memory. Experiment 2 showed deficits for the Re nuclei for the DNMTP which relies on the proper functioning of prefrontal cortex and hippocampus versus the Rh nuclei which rely on hippocampal functioning.

The Re and Rh nuclei are important in the memory process for spatial working memory, however, it was not known if there is a particular stage of the memory process where the functioning of these nuclei is imperative. In Experiment 4, we used the technique of DBS. Electrodes were implanted into the area Re and Rh nuclei. Testing was then conducted during the same DNMTP, which had been used in the previous three studies of this dissertation.
The stages of the memory process are planning, encoding, storage and retrieval. Disruption during the DNMTP produced deficits at the delay and choice responding phases during high current stimulation sessions, which correspond with the storage and retrieval stages of memory. These results suggest that Re and Rh nuclei are important for the temporary storage of decisional information across memory delays.

**Clinical Applications**

Human patients with damage to thalamic nuclei have had deficits in cognitive functions such as attention, motor function, memory and aspects of executive functioning (Zola-Morgan & Squire, 1993; Braak & Braak, 1998; Gold & Squire, 2005) as well as deficits in awareness observed with persistent vegetative state (Schiff, 2008). One problem in human research of midline thalamic nuclei is that many of the studies do not actually state whether the midline nuclei were damaged and therefore do not differentiate impairments in patients with or without midline thalamic damage (von Cramon, Hebel & Schuri, 1985; Van der Werf et al., 2000).

Even with this limitation, there have been an increasing number of studies parceling out these midline thalamic structures. One particular study included a patient with bilateral infarction in medial thalamus had impaired anterograde/declarative memory, some retrograde amnesia but had spared performance on nondeclarative tests of memory (Gold & Squire, 2006). Nondeclarative memory
tasks were done in a set of different studies and included artificial grammar learning, cognitive skill learning, reading speed and priming of object naming (Knowlton, Ramus & Squire, 1992; Squire & Fransbach, 1990; Cave & Squire, 1992). This is corroborated with evidence from other clinical reports that have indicated that lesions in midline thalamic nuclei do not produce a global effect on cognition. Rather, there are impairments for executive cognitive functions related to the flexibility of using information but memory formation itself is spared (Van der Werf, Witter & Groenewegen, 2002).

**Deep Brain Stimulation**

DBS has been used for treatment of Parkinson’s disease (Putzke, Wharen, Wszolek, Turk, Strongosky & Uitti, 2003), seizure disorders (Velasco et al., 1995) and even depression (Velasco et al., 2006). More recently, the rostral intralaminar thalamic nuclei have been manipulated by DBS in a patient in a minimally conscious state. Stimulation in this area showed behavioral improvements in the frequency of cognitively mediated behaviors, functional limb control and oral feeding compared to periods where stimulation did not occur (Schiff et al., 2007).

All of these previous studies used longer pulses of electrical stimulation than in Hembrook and Mair (2008) where brief pulses of stimulation were applied during a memory task in rats. We found improvements in performance accuracy when low currents of stimulation were applied during the storage or the retrieval
of the memory. Experiment 4 from this dissertation did not find behavioral enhancement during low current sessions of stimulation during any of the phases of the behavioral task. Even so, the manipulation of these nuclei could lead to improvements in memory functioning to help facilitate communication through partially intact areas of reuniens and rhomboid nuclei to areas of prefrontal cortex and hippocampus.

**Limitations and Future Work**

The results from this dissertation are very promising for finding out the exact role of Re and Rh nuclei in spatial working memory. However, these results are not without limitations. First, it is not possible to rule out the potential contributions of CM thalamic nuclei in memory aspects. Second, even though the Re and Rh nuclei are important in memory-guided responding, lesions in these areas have produced ranges of impairments in behavioral tasks, from minimal to more moderate accuracy impairments (Experiments 2 and 3). Third, microstimulation in the area of Re and Rh nuclei produce impairments selectively during the DNMTP of working memory during delay and choice phases of the task with high currents of stimulation. However, the area of inactivation can not be known without future research. At the time of writing this dissertation, there have not been sufficient studies conducted recording neuronal activity of Re and Rh nuclei. Finding out when it is essential for these nuclei to be firing will help to
understand the type of activation needed to store the memory across the delay and guide the memory response.
List of References


127


Onos, K.D., Hembrook, J.R. & Mair, R.G. (in prep). Cholinergic modulation of the reuniens and rhomboid midline thalamic nuclei


Sloan, H.L., Good, M., & Dunnett, S.B. (2006). Double dissociation between hippocampal and prefrontal lesions on an operant delayed matching task


APPENDIX A
### Tables

<table>
<thead>
<tr>
<th>Lesion</th>
<th>AP</th>
<th>ML</th>
<th>DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhomboid</td>
<td>7.2</td>
<td>0</td>
<td>3.5</td>
</tr>
<tr>
<td>0.2ul/site</td>
<td>6.6</td>
<td>0</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>0</td>
<td>3.3</td>
</tr>
<tr>
<td>Reuniens (n= 9)</td>
<td>7.2</td>
<td>+/- 0.3</td>
<td>2.6</td>
</tr>
<tr>
<td>0.15ul/site</td>
<td>6.6</td>
<td>+/- 0.3</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>+/- 0.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Reuniens (n= 9)</td>
<td>7.2</td>
<td>+/- 0.4</td>
<td>2.2, 2.6</td>
</tr>
<tr>
<td>0.15ul/site</td>
<td>6.6</td>
<td>+/- 0.4</td>
<td>2.2, 2.4</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>+/- 0.4</td>
<td>1.8, 2.2</td>
</tr>
</tbody>
</table>

**Table 1:** Experiment 2: The stereotaxic coordinates for each of the different lesion groups, Rhomboids, Reuniens and controls. Coordinates were measured in millimeters with AP relative to interaural line. The amount of NMDA infused is listed for each site.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>AP</th>
<th>ML</th>
<th>DV</th>
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</thead>
<tbody>
<tr>
<td>ReRh</td>
<td>7.2</td>
<td>0</td>
<td>3.5, 2.6</td>
</tr>
<tr>
<td>0.2ul/site</td>
<td>6.6</td>
<td>0</td>
<td>3.3, 2.2</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>0</td>
<td>3.3, 2.2</td>
</tr>
<tr>
<td>Dorsal Midline</td>
<td>7.2</td>
<td>0</td>
<td>4.4, 5.0</td>
</tr>
<tr>
<td>0.2ul/site</td>
<td>6.6</td>
<td>0</td>
<td>4.0, 5.0</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>0</td>
<td>3.8, 4.8</td>
</tr>
<tr>
<td>Ventromedial</td>
<td>7.2</td>
<td>+/- 0.2</td>
<td>2.7</td>
</tr>
<tr>
<td>0.2ul/site</td>
<td>6.6</td>
<td>+/- 0.2</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>+/- 0.2</td>
<td>2.7</td>
</tr>
</tbody>
</table>

**Table 2:** Experiment 3: The stereotaxic coordinates for each of the different lesion groups, VM, DM, ReRh and controls. Coordinates were measured in millimeters with AP relative to interaural line. The amount of NMDA infused is listed for each site.
Table 3: Experiment 4: The histological stereotaxic coordinates for the electrode location for each individual animal. Location of the electrode site was based off of damage to the tissue and the location of the electrode tract.

<table>
<thead>
<tr>
<th></th>
<th>AP (IA)</th>
<th>ML</th>
<th>DV</th>
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<tbody>
<tr>
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<td>6.7</td>
<td>0</td>
<td>3.4</td>
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<tr>
<td>406</td>
<td>6.0</td>
<td>0.8</td>
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<td>420</td>
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<td>0</td>
<td>1.8</td>
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<td>421</td>
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<td>0</td>
<td>2.8</td>
</tr>
<tr>
<td>422</td>
<td>6.9</td>
<td>0.3</td>
<td>3.2</td>
</tr>
<tr>
<td>444</td>
<td>5.6</td>
<td>0</td>
<td>1.6</td>
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<tr>
<td>453</td>
<td>7.4</td>
<td>1.0</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Table 4: Experiment 4: The threshold stimulation levels for each animal. A staircase procedure was used, increasing the level of stimulation until the animal froze.

<table>
<thead>
<tr>
<th></th>
<th>Stimulation Level</th>
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<tbody>
<tr>
<td>404</td>
<td>0.6 mA</td>
</tr>
<tr>
<td>406</td>
<td>0.04 mA</td>
</tr>
<tr>
<td>420</td>
<td>0.5 mA</td>
</tr>
<tr>
<td>421</td>
<td>0.2 mA</td>
</tr>
<tr>
<td>422</td>
<td>0.4 mA</td>
</tr>
<tr>
<td>444</td>
<td>0.3 mA</td>
</tr>
<tr>
<td>453</td>
<td>0.1 mA</td>
</tr>
<tr>
<td></td>
<td>404</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Low Level</td>
<td>0.01mA</td>
</tr>
<tr>
<td>High Level</td>
<td>0.04mA</td>
</tr>
</tbody>
</table>

**Table 5:** Experiment 4: High and low stimulation levels for each individual animal. Each animal had the same low stimulation level of 0.01mA. The high level of stimulation varied depending on their threshold for the stimulation (see Table 4). The high level of stimulation that was used for behavioral testing was lower than their initial threshold level.
**Figure 1:** A diagram of the radial arm maze. There is a central hub that is in the shape of an octagon. Each side of the octagon has a motorized gate which can allow access into the arms. At the end of each arm is a set of photocells which record the animal's response into that particular arm. Also located at the end of each arm is a well where water reinforcement can be dispensed.

**Figure 2:** This is the operant chamber and the surrounding sound attenuating chamber. The operant box has motorized retractable levers and a cut-out on the center of the right side where water reinforcement can be given. For the electrical stimulation, there is a hole drilled through both the operant chamber and the sound attenuating chamber to allow a cable to fit through.
Figure 3: Diagram of the DNMTP. Each part of the diagram depicts which lever is out. During the DMTP, the animal is to choose the lever which has previously been the sample lever. During the DNMTP, for the animal to make a correct response, they need to choose the lever which has not previously been the sample lever.

![Diagram of the DNMTP](image)

Figure 4: Experiment 1: The cannula placements for both the DNMTP and the VC-DNM RAM. Each dot represents an individual animal as well as where the tip of the needle would have been placed for each injection. Anatomical control injections would have been 2 mm above each dot at an angle.

*picture copied from Hembrook, Onos & Mair, 2011
Figure 5: Experiment 1: The effects of dose on performance accuracy on the DNMTP. Performance accuracy was impaired for all doses of muscimol and all delays compared to saline performance.

Figure 6: Experiment 1: The effects of dose on performance accuracy on the VC-DNM RAM. Performance accuracy was only significantly affected at the highest dose (2.5 nmol).
* graph copied from Hembrook, Onos & Mair, 2011

**Figure 7:** Experiment 1: The overall performance accuracy of muscimol dose and saline for each of the behavioral tasks. For DNMTp, there were significant differences between the 2.5nmol in ReRh compared to saline in ReRh and 2.5nmol in the anatomical control site. This was not significant for the VC-DNM RAM.

* graph copied from Hembrook, Onos & Mair, 2011

**Figure 8:** Experiment 1: The performance accuracy for both DNMTp and VC-DNM RAM during the non-injection days. This graph shows there was no change in performance throughout the testing protocol.
Figure 9: Experiment 1: RT for the DNMTP. The sample response was recorded from the time the initiate lever was pressed until the time the sample lever was pressed. The choice lever was recorded from the time the delay lever retracted until the choice lever was pressed (regardless of correct or error choice). There were no significant differences in RT for the doses.

Figure 10: Experiment 1: RT for the VC-DNM RAM. The sample response was recorded from the time the gates were opened to allow the animal to exit the sample arm until they responded by breaking the photocell in the holding arm. The choice response time was recorded from the time the gates were opened to allow the animal to exit the holding arm to when the animal broke the photocell in the choice arm (regardless of a correct or error response). There was a significant effect of dose on RT, however there was no interaction between RT and the dose.
Figure 11: Diagram of the SRL and SRM. The two stages of the task are depicted, the first where an initial lever is extended and the second stage where two levers on each side of the water port extend out. One lever is the correct reinforcing lever for the entire training/testing session.

*copied from Dolleman-van der Weel et al. (2009)

Figure 12: Representative swim paths for each of the different swim path categories. Swim paths were analyzed for each animal for every learning trial. Swim path was considered the dominate swim path type for the particular learning trial.
Figure 13: Experiment 2: Representative lesions for Re (A) and Rh (B). Brain slices are stained with cresyl violet.
Figure 14: Experiment 2: Overall accuracy performance for the lesion groups compared to controls for different delays. There was a delay independent impairment for the Re lesion group compared to controls.

Figure 15: Experiment 2: RT for the DNMTCP. The sample response was recorded from the time the initiate lever was pressed until the time the sample lever was pressed. The choice lever was recorded from the time the delay lever retracted until the choice lever was pressed (regardless of correct or error choice). All animals were slower to make a sample response. There were no significant differences between lesion groups for RT.
**Figure 16:** Experiment 2: The number of errors to criterion for each of the lesion groups compared to controls. All animals were able to learn the SRL.

**Figure 17:** Experiment 2: The overall number of errors to criterion were compared for each lesion group compared to controls. There were no impairments on the number of errors to criterion.
Figure 18: Experiment 2: Percent correct for the 8-arm RAM collapsed across all testing session. There was no impairment for Re compared to controls. Rh lesion group was significantly impaired compared to controls.

4F RAM

Figure 19: Experiment 2: Overall performance for the 4F RAM for each delay (1 minute and 15 minutes). There were no differences in accuracy between the lesion groups and controls for either delays.
Figure 20: Experiment 2: Accuracy performance for the “clean” and “no clean” condition of the 4F RAM. Accuracy did not differ between the conditions.

Figure 21: Experiment 2: Escape latency for the 18 learning trials. The platform was placed in the same location for each trial and the location where the animal was placed into the water varied randomly across the trials. Planned comparisons revealed a significant effect for Re compared to controls.
Figure 22: Experiment 2: Escape latencies for the RM-WM learning blocks. There were no difference found on the time to find the platform for the lesion groups compared to controls for any of the learning blocks.

Figure 23: Experiment 2: Swim path strategies on the RM-WM for learning trials. Swim paths were categorized from methods used by Dolleman-van der Weel, Morris and Witter (2009). Planned comparisons revealed a difference between Re and controls on the "direct" swim path category. Control used the "direct" swim path significantly more often than Re.
A: Path Length

B: Swim Speed

C: Passes Through Platform
Figure 24: Experiment 2: Graphs for the RM-WM memory probe trial. Variables examined were path length (a), swim speed (b), number of passes through the platform area (c), time in the quadrant where the platform was located (d) and proximity (e). There were no significant effects for Re or Rh compared to controls on any of the measured variables.
Figure 25: Experiment 2: Escape latency average for the “visible” cue trials. The platform was placed in the same location but above the water level. There were no differences between the lesion groups and controls for the time to find the platform.

Figure 26: Experiment 3: Drawing of the different areas of interest for lesions. The DM lesion target is in red with some potential spread into more lateral areas. The VM lesion target is in green however there could be some potential spread into the submedial nucleus. The ReRh lesion group is labeled in blue.
Figure 27: Experiment 3: Representative lesions of ReRh (A), VM (B) and DM (C).
Figure 28: Experiment 3: Overall performance accuracy for each delay. There were no differences between performance accuracy for the lesion groups compared to controls.

Figure 29: Experiment 3: RT for the DNMTTP. The sample response was recorded from the time the initiate lever was pressed until the time the sample lever was pressed. The choice lever was recorded from the time the delay lever retracted until the choice lever was pressed (regardless of correct or error choice). All animals were slower to make a sample response. There were no significant differences between lesion groups for RT.
Figure 30: Experiment 3: The number of errors to criterion for each of the reversals on the SRL. There was an initial increase for the first reversal for all the groups. There were no differences between any of the groups and all animals were able to learn the task to criterion.

Figure 31: Experiment 3: Overall performance across all the sessions for the 8-arm RAM. Performance accuracy differed significantly for the ReRh lesions compared to the VM but not controls.
Figure 32: Experiment 3: Accuracy performance for 4F RAM at each delay interval. There was a significant difference of ReRh and controls and ReRh and VM for overall performance with no interaction of delay.

Figure 33: Experiment 3: Escape latencies for the RM-WM for the 18 learning trials. The platform was located in the same location for all trials and the location of the start point varied across the trials. Planned comparisons revealed a significant effect for ReRh compared to VM and DM but not controls.
Learning Blocks

**Figure 34:** Experiment 3: Escape latencies for the learning blocks. Planned comparisons revealed a significant difference for ReRh compared to VM and DM but not controls.

**Figure 35:** Experiment 3: Swim path strategies for the learning trials for the RM-WM. There were no differences between lesion groups and controls for the type of swim path strategy used on average.
A: Path Length

B: Swim Speed

C: Passes Through Platform
Figure 36: Experiment 3: Graphs for the RM-WM memory probe trial. Variables examined were path length (a), swim speed (b), number of passes through the platform area (c), time in the quadrant where the platform was located (d) and proximity (e). There were no significant effects for ReRh compared to controls or the other lesion groups on any of the variables measured.
Figure 37: Experiment 3: Escape latency average for the "visible" cue trials. The platform was placed in the same location but above the water level. There were no differences between the lesion groups and controls for the time to find the platform.

Figure 38: Experiment 4: An example of ReRh electrode placement. Brain slice is stained with thionin.
Figure 39: Experiment 4: Overall performance for the DNMTP for the different current levels of stimulation. There was a significant difference between high current sessions compared to no current and low current sessions.

Figure 40: Experiment 4: Overall performance on the DNMTP sessions for the different delay trials, 3 seconds and 15 seconds. There was a significant difference between the short delay trial performance and long delay trial performance.
Figure 41: Experiment 4: Overall accuracy performance for the DNMTP for 3 second delay trials for the different current level sessions. There was a significant effect of current session on percent correct for the high current compared to the no current and low current.

Figure 42: Experiment 4: Overall accuracy performance of the DNMTP for the 3 second trials for the different current level sessions for the different memory phase trials. There was a significant effect of the different memory phase trials, where there was a significant difference of performance for the high current stimulation sessions for the trials where current was applied during the delay and choice phases compared to control trials.
Figure 43: Experiment 4: Overall accuracy performance for the DNMTP for the 15 second delay trials for the different current level sessions. There was a significant effect of stimulation on accuracy performance.

Figure 44: Experiment 4: Accuracy performance for the DNMTP for the 15 second delay trials for the different current level sessions. Performance was examined at the difference phases of the task. There was no significant effect of memory phase on performance.
Figure 45: Experiment 4: Overall performance for the SRM for the different current sessions. There was no significant difference for accuracy performance for the no current sessions compared to the high current sessions.

Figure 46: Experiment 4: Accuracy performance for the SRM for no current and high current sessions for the different phases of the task. There were no significant effects for the different phases of the task for both no current and high current sessions.
**Task Comparison**

- **no stimulation**
- **high stimulation**

---

**Figure 47:** Experiment 4: Comparisons of the DNMT and SRM tasks. Session accuracy was compared for the no current sessions versus the high current sessions. There was a significant effect between the stimulation current and the task.

---

**Phase Comparison**

- **No Current**
  - Delay/start: 80%
  - Choice: 90%

- **High Current**
  - Delay/start: 60%
  - Choice: 65%

---

**Figure 48:** Experiment 4: Comparison of the DNMT and SRM tasks. The delay/start and choice phases were compared for control trials and high stimulation trials. There were no differences between the delay/start and choice phase trials for the no current sessions, but there was a significant effect of the delay/choice phase trials for the high current sessions. The high current of stimulation impaired performance on the DNMT while performance was not affected for those phases on high current sessions for the SRM.
03-Jul-2008

Mair, Robert G  
Psychology, Conant Hall  
Durham, NH 03824

IACUC #: 080605  
Project: Memory Function During Reversible Inactivation of Nucleus Reuniens  
Category: D  
Approval Date: 30-Jun-2008

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category D on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - the research involves chronic maintenance of animals with a disease/functional deficit and/or procedures potentially inducing moderate pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics. The IACUC made the following comment(s) on this protocol:

1. The Committee added the investigator to Section II, B (personnel information and occupational health program information).

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Jessica A. Bolker, Ph.D.  
Chair

cc: File
University of New Hampshire
Research Integrity Services, Office of Sponsored Research
Service Building, 51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

25-Mar-2009

Mair, Robert G
Psychology, Conant Hall
Durham, NH 03824

IACUC #: 090303
Project: The Role of Rhomboid and Reuniens Central Thalamic Nuclei on Memory System Function
Category: D
Approval Date: 25-Mar-2009

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category D on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - the research involves chronic maintenance of animals with a disease/functional deficit and/or procedures potentially inducing moderate pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics. The IACUC made the following comment(s) on this protocol:

1. In Section IV, A (experimental design), the IACUC inserted "Male" as the first word of the first paragraph, and replaced "will occur" with "consisting" in the third sentence of the third paragraph.

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
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2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Jessica A. Bolker, Ph.D.
Chair

cc: File
University of New Hampshire

Research Integrity Services, Office of Sponsored Research
Service Building, 51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

21-May-2010

Mair, Robert G
Psychology, Conant Hall
Durham, NH 03824

IACUC #: 100407
Project: Specificity of the ventral midline on spatial memory
Category: D
Approval Date: 23-Apr-2010

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol
submitted for this study under Category D on Page 5 of the Application for Review of Vertebrate
Animal Use in Research or Instruction - Animal use activities that involve accompanying pain or
distress to the animals for which appropriate anesthetic, analgesic, tranquilizing drugs or other
methods for relieving pain or distress are used.

Approval is granted for a period of three years from the approval date above. Continued
approval throughout the three year period is contingent upon completion of annual reports on
the use of animals. At the end of the three year approval period you may submit a new
application and request for extension to continue this project. Requests for extension must be
filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the
   UNH Occupational Health Program for persons handling animals. Participation is mandatory
   for all principal investigators and their affiliated personnel, employees of the University and
   students alike. A Medical History Questionnaire accompanies this approval; please copy and
distribute to all listed project staff who have not completed this form already. Completed
questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at
862-2003.

For the IACUC,

Jessica A. Bolker, Ph.D.
Chair

cc: File
21-May-2010

Mair, Robert G
Psychology, Conant Hall
Durham, NH 03824

IACUC #: 100408
Project: Event-Related Microstimulation of the Midline Thalamic Nuclei in the Rat
Category: D
Approval Date: 23-Apr-2010

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category D on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *Animal use activities that involve accompanying pain or distress to the animals for which appropriate anesthetic, analgesic, tranquilizing drugs or other methods for relieving pain or distress are used.*

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Jessica A. Bolker, Ph.D.
Chair

cc: File