DELAY-SPECIFIC EFFECTS OF MEDIAL SEPTUM SUPPRESSION ON A SPATIAL WORKING MEMORY TASK

by

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ABSTRACT

Spatial working memory (SWM) is defined as the ability to process and maintain spatially-relevant, goal-directed information over a temporal gap. Several brain regions are known to be essential for successful SWM performance, including the hippocampus (HPC). One way to characterize the activity of the HPC is by measuring the local field potential (LFP) within the structure. LFP is the summated electrical activity of a population of cells, made up of the graded potentials of the neurons. One of the most prominent oscillations in the HPC is theta, which is known to support SWM processes. The medial septum (MS) is one of the septal nuclei that projects to all cell fields of the HPC and provides the rhythmic drive for theta oscillations. We investigated whether disrupting the normal temporal organization of the HPC via suppression of MS activity could cause task-specific deficits on a delayed non-match to position (DNMP) task by suppression MS activity during the sample, delay, or choice phases of the task. We found deficits specific to the delay period of the task. This indicates that the MS supports the maintenance of goal-relevant information over a temporal delay during SWM tasks. This deficit may be due to an interruption of planning abilities, where the disruption of normal theta causes deficits in the rats' ability to plan a future decision using previously acquired information.

Chapter 1

INTRODUCTION

Spatial working memory (SWM) is defined as the ability to process and maintain spatially-relevant, goal-directed information over a temporal gap. Several brain regions are known to be essential for successful SWM performance, including the hippocampus (HPC). HPC lesions causes deficits in acquisition and performance of SWM tasks in rats (Aggleton et al. 1986, Hallock et al. 2013, Jarrard 1993). Other, simpler tasks that may not have as high of SWM demand can be performed even after HPC lesioning. One task that is spared after HPC lesions is the continuous alternation T-maze task, in which rats are trained to alternate between two arms of a T-maze in order to receive a reward with no delay between trials. When there is no delay between trials, the rat is still utilizing spatial information, but they are still able to perform with complete hippocampal lesions after learning (Roberts, Dember, & Brodwick, 1962). When a delay is introduced between trials, rats with lesioned hippocampi are no longer able to perform the task (Ainge et al. 2007, Racine & Kimble 1965). Therefore, the higher the demand on SWM, the more necessary the HPC is for successful completion of the task.

One way to characterize the activity of the HPC is by measuring the local field potential (LFP) within the structure. LFP is the summated electrical activity of a population of cells, made up of the graded potentials of the neurons. LFP is composed

of several different oscillations. One of the most prominent oscillations in the HPC is theta, commonly defined as 4-12 Hz (Buzsáki 2002). Theta activity is correlated with movement (Vanderwolf 1969, Terrasas et al. 2005) as the rat explores an environment as well as sensory actions such as whisking (Berg & Kleinfeld 2003) and sniffing (Macrides, Eichenbaum, & Forbes 1982). Because of theta's connection to the intake of sensory information, it has been hypothesized that theta oscillations allow multiple types of sensory information to be combined into one distinct representation (Buzsáki & Moser 2013; Gupta et al. 2012). This process is referred to as "chunking," where simplified chunks of information from multiple senses are used to represent a larger global image; i.e., the environment as a whole (Gupta et al. 2012; Uchida, Kepecs, & Mainen 2006). Multiple cell assemblies can be linked within the time frame of a theta cycle, connecting them initially so that they may be more readily recalled later (Buzsáki & Moser 2013). In this way, theta represents the "on-line" state of the HPC (Buzsáki 2002), where the HPC is able to incorporate and organize information when the rat is actively exploring an environment or performing a task. Interestingly, using a valued-guided decision-making task with multiple reward zones, Wikenheiser & Redish (2015) found that spatial representations within theta sequences were biased toward rats' preferred reward zone in an environment. In this way, theta sequences appeared to reflect future trajectories in a goal-dependent manner. This suggests that theta sequences may be important for planning future decisions.

Theta rhythms arise from two important mechanisms: a rhythm generator, which is responsible for creating and controlling the frequency and pattern of the

oscillation, and a current generator, which influences the power and amplitude of the theta wave via differences in transmembrane currents (Buszáki 2002). The medial septum (MS) is one of the septal nuclei that projects to all cell fields of the HPC and provides the rhythmic drive for theta oscillations (Mitchell et al. 1981). Septal cholinergic neurons innervate both excitatory and inhibitory neurons while septal GABAergic neurons selectively innervate HPC GABAergic neurons. Some of these inhibitory hippocampal neurons project back to the MS, forming the septo-hippocampal-septal loop (Jakab & Leranth 1995).

Stimulating the MS at a 6 Hz frequency (within the theta range) using optogenetic techniques changed the frequency of HPC theta to closer match the stimulation frequency. The efficacy of this stimulation depended on the velocity of the animal, reflecting the influence of the animal's running speed on theta frequency (Blumberg et al. 2016). The MS is believed to drive the theta rhythm in the HPC via GABAergic projections. Theta is eliminated when these projections are inhibited (Tóth, Freund, & Miles 1997). Hyperpolarization-activated, cyclic nuceleotide-gated non-selective cation (HCN) channels are expressed in the GABAergic cells of the MS and generate a hyperpolarization-activated current. Activation of this current via hyperpolarization leads to depolarization of the membrane, which in turn leads to after hypolarization, repeating the cycle. It is this hyperpolarization/depolarization balance that causes the pacemaker effect of these cells (Wahl-Schott & Biel 2009). GABAergic neurons that express HCN channels have been shown fire rhythmically at theta rhythms. When the temporal relationship between the firing of these HCN

neurons was compared to HPC interneurons and HPC network activity, the MS pacemaker cells consistently lead putative HPC interneuron spiking, which in turn lead hippocampal theta (Hangya et al. 2009). Together, these results have led investigators to hypothesize that MS GABAergic cells pace the HPC theta rhythm.

MS cholinergic cells, however, do not fire at theta frequencies, so they are unlikely to aid in rhythm generation (Simon et al. 2006). Eliminating the septohippocampal cholinergic projection with an immunotoxic lesion caused a decrease in HPC theta power, but the frequency remained unchanged. This power decrease showed a dose-dependent relationship to the amount of toxin used (Lee et al. 1994), suggesting that cholinergic projections from the MS help control the amplitude and power of the theta oscillation. Therefore, the cholinergic cells in the MS act as a current generator. The cholinergic projection may also help promote theta activity in the HPC by quieting other oscillations. During behavior, stimulating the septohippocampal cholinergic projections in mice resulted in not only an increase in theta power, but a relative decrease in sharp-wave ripple activity and the power of oscillations outside of the theta frequency band within the HPC (Vandecasteele et al. 2014).

Disruption of MS activity not only affects HPC theta, but also causes SWM deficits. When the MS was lesioned in rats following learning of a SWM task, they were no longer able to perform the task (Rashidy-Pour, Motamedi, & Motahed-Larijani 1996; Walsh & Stackman 1998; Winson 1978). Performance deficits have been found both when a GABAergic antagonist (Chobak et al. 1992) or a cholinergic

antagonist (Chang & Gold 2004; Lee et al. 1994) was administered before performance on previously learned SWM tasks. Together, these findings suggest that although the GABAergic and cholinergic septohippocampal projections may influence different aspects of the theta oscillation, both are necessary for successful SWM performance.

In order to determine what aspects of SWM are supported by theta oscillations, Mizumori et al. (1990) used a delayed non-match to position (DNMP) task, which employed a 30-minute delay between the sample and choice phases. Tetracaine, a short-acting local anesthetic, was injected into the MS before the sample, at the beginning of the delay period, or at the end of the delay period before the choice phase. Injections before the sample or choice phased caused deficits in performance, while injection before the delay period had no effect. The authors speculated that MS input to the HPC is necessary for the initial encoding of information during a sample phase and the retrieval of relevant information during the choice phase, but was not important for the maintenance of information over the delay period. However, they also showed that tetracaine produces decreased theta in the HPC for a period of about 15 minutes.

Another study aimed at determining the contribution of MS to SWM tasks involved permanently lesioning the MS after rats had learned a delay alternation task. Following lesioning, rats dropped to chance levels of alternation. Over time, their behavior improved, but never to levels equal to that of control rats (Thoman, Brito, & Stein 1980). The ability to inhibit the MS on a precise timescale during the task, rather

than permanently lesioning the region or using long-lasting pharmacological inhibition methods, would allow for a better understanding of how MS-generated theta helps animals perform SWM tasks.

Optogenetic techniques allow neural activity to be manipulated with a resolution of milliseconds (Yizhar et al. 2011). For example, this technique has been used to inhibit the pathway from ventral HPC (vHPC) to the medial prefrontal cortex (mPFC) in mice during either the sample, delay, or choice phases of a DNMP task. This study showed a choice accuracy deficit only when the optogenetic suppression was delivered during the sample traversal and not during the choice traversal or delay period, suggesting that the vHPC to mPFC pathway is selectively critical for encoding during SWM (Spellman et al. 2015). Therefore, the current study used optogenetic techniques that allowed us to restrict MS inhibition to the sample, delay, or choice phases of a DNMP task to determine if there is a task phase-specific deficit.

In summary, theta activity is necessary for a functioning HPC (Rashidy-Pour, Motamedi, & Motahed-Larijani 1996; Walsh & Stackman 1998; Winson 1978). In this study, we used optogenetics to suppress MS activity during distinct phases of a spatial working memory task in order to perturb normal HPC theta. We found deficits specific to the delay period of the task. This indicates that the MS supports the maintenance of goal-relevant information over a temporal delay during SWM tasks. This deficit may be due to an interruption of planning abilities, where the disruption of normal theta causes deficits in the rats' ability to plan a future decision using previously acquired information.

Chapter 2

METHODS

Subjects

Subjects were 13 adult (>90 day) male Long Evans hooded rats. Rats were housed in a temperature and humidity-controlled room with a 12-hour light-dark cycle. Rats were housed in pairs up until the time of surgery and then housed individually throughout the rest of the experiment. Rats were given *ad libitum* access to food until pre-training begins and given *ad libitum* access to water throughout the experiment. Food-deprived rats were kept at 90% of their free-feeding body weight and were weighed weekly. Rats averaged 431 grams at the time of surgery. Rats were divided into three groups: the experimental group and two control groups. Six rats were assigned to the experimental ArchT group, where an opsin-positive virus was injected and ferrule was placed into the MS. Four rats were assigned to an opsin-negative control group, where an opsin-negative virus was injected and ferrule was placed into the MS. This allowed us to control for stress effects from surgery as well as light and heating effects in the MS. Three rats were used as site controls, where an opsin-positive virus was injected and ferrule was placed in the lateral septum. This allowed us to control for stress effects of surgery as well as showing specificity of effect to our target brain region.

Viral Construct

Two recombinant adeno associated viral (rAAV) vectors were constructed (UNC Vector Core). The first was an opsin-positive viral vector constructed of titer

3.1 x 10^{12} virus molecules/mL containing a CAG promotor (cytomegalovirus early enhancer element, first exon and intron chicken beta-actin gene, and the splice acceptor of the rabbit beta-globin gene), and a archaerhodopsin-tdtomato red fluorescent protein (ArchT-tdtomato) fusion. An opsin-negative viral vector was constructed of titer 4.3 x 10^{12} virus molecules/mL containing the same CAG promotor and tdtomato red fluorescent protein (CAG-tdtomato).

ArchT is a derivative of archaerhodopsin-3, a gene derived from the organism *Halorubrum sodomense* that encodes for a light-activated proton pump. When exposed to light of wavelength 566 nm, the pump will remove protons from the cell, hyperpolarizing it. This decreases the likelihood that the cell will depolarize and therefore fire an action potential, suppressing the activity of the cell (Chow et al. 2010, Yizer et al. 2011).

Surgical Preparation

Prior to surgery, rats were given a subcutaneous injection of atropine (Atroject, 0.05mg/kg). All instruments were sterilized using a bead sterilizer at 300°C and cleaned in Chlorhex solution. Rats were anesthetized using 3.5% isoflurane in oxygen in a Plexiglass induction chamber. Once anesthetized, Paralube opthalmic lubricant was applied to eyes and the rats' heads were shaved at the incision site. For the duration of the surgery, rats were head-fixed in a stereotax to ensure continuous flow of isoflurane in oxygen. The stereotax was placed on a heating pad set to medium to help regulate the rats' body temperature. The rats' breathing, heart rate, and oxygen

levels were monitored throughout surgery using a pulse oximeter via foot clamp. The incision site was cleaned using Chlorhex solution and a subcutaneous injection of Lidocaine was given prior to the incision. The skull was cleaned and leveled by comparing the dorsal-ventral (DV) coordinates of bregma and lambda. Once leveled, a bregma measurement was taken to determine its anterior-posterior (AP) and medial-lateral (ML) coordinates. This measurement was used to mark the skull where the virus were injection and ferrule were implanted (see sections below for coordinates). Four holes were drilled into the skull using a stereotaxically mounted drill (Fine Science Tools) and bone screws were inserted. The screws later helped stabilize the dental acrylic later used to hold the ferrule in place.

A trephine was then used to remove the skull over the medial septum. Vetspon absorbable gelatin sponge soaked in saline was applied to the exposed tissue to prevent it from drying out. The virus was injected and the ferrule was implanted into the MS (see following sections). Approximately 30-45 minutes before the end of the surgery rats were given a subcutaneous injection of Banamine to help manage pain. Dental acrylic was used to anchor the ferrule to the skull and bone screws. Neosporin was applied to the incision site and rats were placed in a clean cage with access to food and *ad libitum* water containing children's ibuprofen for five days. The cage was placed on a heating pad set to low and monitored for several hours, until they are awake and alert. They were continually monitored as they healed over the next five days.

Virus Injections

All injections were performed using a Harvard Apparatus PHD 700 programmable syringe pump. Virus was injected into the MS at a 10° angle at two coordinates in order to ensure viral spread across the entirety of the region. AP and ML were measured from bregma. DV were measured from the surface of the brain at the injection site. All measurements are in millimeters. For the experimental group and opsin negative control group, injection one coordinates were AP +0.7/ML +1.1/DV -6.0 and injection two coordinates AP +0.6/ML +1.1/DV -5.5. For the opsin positive site control group, injection one coordinates were AP +0.7/ML +1.1/DV-5.0 and injection two coordinates were AP +0.7/ML +1.1/DV -4.5. The needle was lowered to the more ventral site first. Before beginning the injection, there were a two-minute wait to allow tissue to settle around the needle. The virus were injected at a rate of 0.1 µl/min for 5 minutes for a total of 0.5 µl per injection site. The needle will then be raised to the more dorsal injection site. Following the second injection, there were five-minute wait before removing the needle from the brain in order to be sure the virus was able to diffuse into the tissue. The exposed tissue were covered in Vetspon soaked in saline.

Fiber Optic Ferrule Implantation

A scored fiber optic ferrule (2.5 mm ceramic ferrule length, 200 mm fiber core) was positioned using a stereotaxic cannula arm at AP +0.7/ML +1.1/DV -5.3 at a 10° angle for the experimental group and opsin negative control groups. The ferrule was implanted at AP +0.7/ML +1.1/DV -4.3 for the site control group. Gluture was

used to secure the fiber. Once the Gluture was dry, dental acrylic (Lang Dental) was used to anchor it to the skull and bone screws.

Behavioral Training

After five days of post-surgery recovery, rats began pre-training. First, the rats were handled in order to grow accustomed to the researcher for 10-15 minutes per day for five days. Following handling, rats were returned to home cages with a small weigh boat of chocolate sprinkles. This was when food-deprivation began. Rats were weighed once per week so ensure they remained at 90% of their free-feeding body weight.

Following handling, rats began a goal box exercise where they are introduced to a T-maze. The goal zones of the maze were isolated using wooden blockers and the rats were placed in these areas, alternating left and right sides, and tasked with eating from a small weigh boat of chocolate sprinkles. Rats performed six trials per day until they ate the chocolate sprinkles within 90 seconds for each trial on two consecutive days.

Next, the rats began forced runs. A rat was placed in the delay pedestal of the maze. One arm of the maze was blocked off using a wooden blocker. The rat must run up the stem, turn down the open arm, and eat the chocolate sprinkles in the goal arm. He must then return to the delay pedestal. Forced runs consisted of twelve trials, six left and six right, presented in a pseudorandom order. The rat was able to move on to

DNMP task training when he successfully ate during ten of the twelve trials for two consecutive days.

DNMP Training

After the rat has completed pre-training successfully, he began training. The DNMP task consists of a sample phase, a delay phase, and choice phase (Figure 1). Initially, the rat will run up the stem for the sample phase and be forced to turn in one direction due to a wood blocker placed at the choice point. He was able to eat the chocolate sprinkles located in that arm's goal zone and then return to the delay pedestal, where he must wait for 20 seconds. Following this delay, he was allowed to reenter the maze, where he had a free choice to turn in either direction. In order to receive a reward, he must turn down the opposite arm. Once he has made his choice and returned to the delay pedestal, there was a 40 second inter-trial interval delay while the maze was set-up for the next trial. Each daily session consisted of 24 trials. On average, it took rats 5 days of training to learn the task to criteria (80% correct on 2 consecutive days).

The DNMP task is used in order to break down a complex working memory task down into smaller components. The sample or cued-based phase is thought of as the encoding phase, where the rat must take in information from the environment in order to guide his later decision. The delay phase is thought to require maintenance of that goal-relevant information. The choice phase is used as a proxy for retrieval, where

the animal must retrieve the information from working memory in order to make a goal-directed decision.

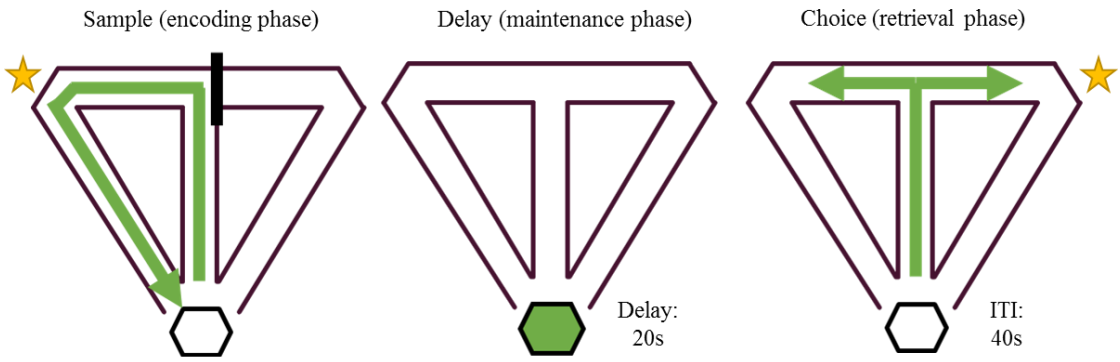

Figure 1 Task schematic. Rats initially enter the stem from the delay pedestal and are forced in one direction by a wooden blocker at the choice point. They receive a reward (indicated by yellow star) and return to the delay pedestal for a 20 second delay. Following the delay, they are allowed to re-enter the maze and have a free choice of direction at the choice point. In order to receive a reward, they must pick the opposite arm from the sample phase. The inter-trial interval (ITI) is 40 second. Green indicates when light was on for each testing condition. Entire trial condition encompassed all three light timings.

Behavioral Testing

Once rats were able to consistently perform the task (2 consecutive days at 80% choice accuracy or higher), they moved on to the behavioral testing portion of the experiment. Prior to testing, the rat had one day of training while the ferrule is tethered to the light source to ensure that is did not affect performance. The testing phase was comprised of four light conditions: sample light, delay light, choice light and entire trial light (Figure 1). For the sample light condition, the light was on for the rat's first traversal of the maze, but remained off for the delay and choice parts of the trial. For

the delay condition, the light will only be on for the 20 second delay between sample and choice traversals. For the choice condition, the light was on for the second traversal of the stem until the rat had made a decision and turned down one of the goal arms. For the entire trial condition, the light was on for the entirety of the trial and only off during the inter-trial interval. Each day of testing consisted of 24 trials, 12 light and 12 no light, presented in a pseudorandom order.

Data Analysis

In order to determine if there is a deficit in choice accuracy when the light is turned on, a 2 (light: on, off) x 3 (group: experimental, opsin-negative, and site control) ANOVA was performed for each stimulation condition (sample, delay, choice, and entire trial), followed by Bonferroni-correct posthoc tests.

Histological Analysis

Following testing, rats were given a lethal dose of sodium pentobarbital and perfused transcardially with tris-buffered saline (TBS) and 4% paraformaldehype (PFA) in TBS. Brains were immediately extracted post-perfusion and stored in 4% PFA for 24 hours and then switched to TBS. Within a week, the brains were frozen and sliced using a Cryostat into 20 µm slices and mounted on slides. The slides were stained by applying Prolong Diamond containing 4′,6-diamidino-2-phenylindole (DAPI) (ThermoFisher), which is used to help visualize cell bodies by staining nuclear DNA (Tarnowski, Spinale, & Nicholson 1991). The slides were then coverslipped and

generic clear nail polish was used to seal the coverslips. The slices were imaged using a confocal microscope (Delaware Biotech Institute).

Experimental Overview

This experiment aimed to determine how inactivating the MS during different task phases will impair spatial working memory performance in rats. Rats underwent surgical injection of an adeno-associated virus that allowed for light-controlled inactivation of cell activity in the MS. Another group of rats were injected with a control virus in MS, and another received an activated injection into a nearby region. Both viral vectors contain a fluorescent protein to allow for post-hoc imaging. A fiber optic ferrule was also implanted into the MS or the control region in order to project light into the structure. Rats were trained on a delayed non-match to position (DNMP) task until they reach a criterion of at least 80% correct for two consecutive days before testing began. Rats were then tested under four experimental light conditions: sample phase light, delay phase light, choice phase light, and entire trial light. There were an equal number of light and no light trials per session, presented in a pseudorandom order. Each rat underwent one day of testing at each condition for a total of four testing days. Following testing, rats were perfused and their brains extracted and sliced. The slices were then imaged using a confocal microscope to confirm virus spread and ferrule location.

Chapter 3

RESULTS

Histological Analysis

All rats were injected with virus that was tagged with tdtomato. This allowed for imaging of the virus spread. After perfusion, brains were extracted and sliced at 20µm thickness. DAPI was applied to visualize cell bodies. Slices were then imaged using a confocal microscope and compared to the Paxinos Watson Brain Atlas. Figure 2 shows a representative photograph from one rat. MS is outlined in white. The white arrow indicates the terminal end of the fiber optic cannula.

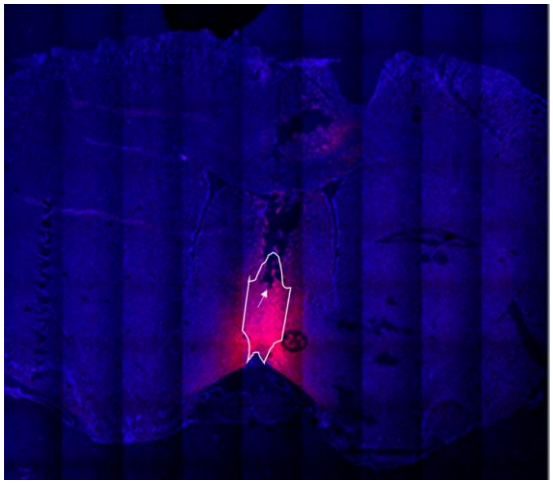

Figure 2 Representative image of virus spread and fiber placement. The MS, as defined by the Paxinos Watson Brain Atlas, is outlined in white. The white arrow indicates the terminal end of the fiber optic cannula.

Behavioral Results

Each animal was tested on four conditions to identify how MS suppression during different phases of a DNMP task affected performance. Three groups of

animals were used: an opsin-positive MS experimental group, an opsin-positive site control group, and an opsin-negative MS control group. Independent samples Mann-Whitney U Tests were performed for each condition to compare the difference scores (percent correct with light on minus percent correct for light off) for the site controls and opsin negatives controls; there was no significant differences between the two on either condition, so the groups were combined into one control group (entire: p=0.700; sample: p=0.200; delay: p=1.000; choice: p=0.800).

For each animal at each condition, a percent change score was calculated using (light on percent correct)/(light off percent correct) *100% to determine the extent to which each animal's behavior changed on light on condition relative to his behavior on the light off condition. An outlier analysis was performed to exclude any animals that performed above or below the 95% confidence interval for each group on the basis of this change score.

The behavioral results are displayed in Figure 3. The mean difference score (percent correct light on minus percent correct light off) for each group is shown. Error bars represent standard error of the mean. A 2 (group: experimental, control) x 2 (light: off, on) mixed design ANOVA was performed for each condition to determine if there was an interaction between group and light. No significant interaction was found for experimental conditions where the light was on for the entire trial (*F(1,8)=1.231, p=0.299*), for only the sample traversal (*F(1,6)=3.429, P=0.114*), or for only the choice traversal (*F(1,6)=0.273, P=0.620*). However, there was a significant interaction between group and light for the delay condition *(F(1,7)=7.099, p=0.032).* A paired samples t-test showed that animals in the opsin positive experimental group performed poorer on light on trials (M=71.0%, SD=0.133) than

light off trials (M=83.3%, SD=0.155; t(5)=4.922, p=0.019). Conversely, animals in the control groups performed no differently for light on trials (M=87.5%, SD=0.10) than light off trials (M=85.4%, SD=0.07; t(4)=-0.397, p=0.595).

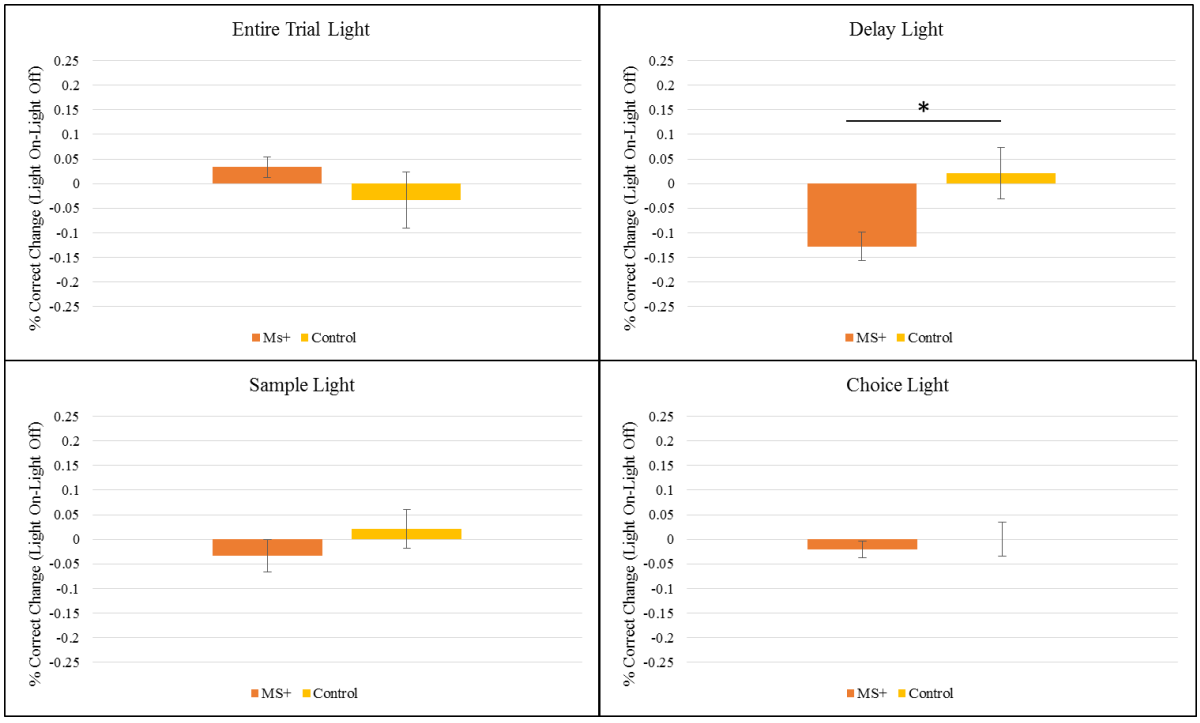

Figure 3 The behavioral results are shown above. Bars indicate the mean difference score (percent correct light on – percent correct light off), orange for the experimental group and yellow for the combined control group. Error bars represent standard error of the mean.

The order of light conditions was counterbalanced across animals. To ensure that there were no order effects, repeated measures analysis of variance was conducted for overall choice accuracy per session with testing day used as the within-subjects factor. Testing day had no effect on animal performance (*F(3)=0.249, p=0.860).*

Chapter 4

DISCUSSION

This study showed a selective SWM deficit associated with optogenetic MS suppression during the delay phase of a DNMP task. This suggests that normal hippocampal theta, which is paced by an intact medial septum, is important for the performance of a DNMP task during this period. However, no impairments were found on trials that suppressed MS activity during the sample and choice phases, indicating that SWM tasks can still be performed without an intact MS during the sample and choice phases of the task. There are multiple ways in which the theta rhythm may influence circuit level function and behavioral performance during this period, with two of them being helping to maintain task-relevant information during this temporal gap, or facilitating changes in hippocampal states necessary for optimally encoding and retrieving trial-specific information.

One function of theta may be temporally-organize task-relevant, goal-directed information available to the animal that is relevant to achieving a goal. Cell assemblies may be linked within a theta cycle to connect previously-learned information that is relevant to the task, such as task rules and knowledge of goals, with multiple types of on-going sensory information (Buzsáki & Moser 2013). This may allow for encoding of the meaning of task-relevant environmental landmarks to effective SWM. The timing of cell firing within a theta cycle has been shown to help encode certain aspects of an environment. For example, the shifting of a place cell firing from the end of a theta phase to the beginning as the animal runs through the place field has been

proposed as a mechanism for encoding space (O'Keefe & Reece, 1993). Place cell firing during theta cycles not only represent a rat's current position, but also extend slightly behind and in front of the animal, reflecting recent past and prospective future locations as well (Buzsáki & Moser 2013). Firing of cell related to future locations extend farther in front of the animal when rewards are further away from closer, indicating that these sequences may important for goal-directed planning (Wikenheiser & Redish 2015). While it is well-established that theta allows for the compression of spatial information into ensemble representation, current research aims to understand what other types of information may be encoded using theta-dependent mechanisms. For a DNMP task, information concerning the recency with which a rat visited the right versus left reward zone would be necessary for the rat to appropriately choose a reward zone during the choice phase of the trial (Hasselmo 2009). Therefore, both spatial and temporal information are necessary to make a correct decision, although it is not clear how these two type of information are integrated.

It has previously been shown that cells in the hippocampus fire at distinct times during the delay period of a previously-learned SWM task, and collectivity this activity extends throughout the entirety of the delay. Furthermore, these cells fire differently depending on the type of trial when rats perform multiple tasks on the same apparatus (MacDonald et al. 2001) or even preceding correct or incorrect choices (Pastelkova 2008). These "time cells" may help encode the delay between task-relevant events, such as a sample period and a choice period, and their differential firing patterns between trial types is believed to help aid in successful choice accuracy

(MacDonald et al. 2011). Theta may help organize a combination of temporal information from time cells and spatial information from place cells (Hasselmo 2009). The theta cycle may provide the optimal temporal window necessary for linking these types of information, which are both represented within the hippocampal circuit (Mizuseki et al. 2009). By disrupting hippocampal theta during the delay period of a DNMP task, we are disrupting the ability of the hippocampal network to effectively integrate multiple types of task-relevant information in order to guide a correct decision.

However, this hypothesis is unable to answer the question of whether it is the maintenance of information that is being disrupted, or a shift between the encoding of one type of information to the retrieval of another type of information. Further studies are needed to probe the whether it is only one or both of these processes that is being disrupted. Studies in humans have attempted to answer this question by using two separate tasks: one that simply requires the maintenance of information during a delay, and another that requires the manipulation of information (D'Esposito et al. 1999). This could be investigated by using a radial arm maze with two different types of tasks: a maintenance task, where the animal simply has to return to arms he has previously been exposed to following a delay, and a manipulation task, where he has to return to previously exposed arms in a specific order; for example, in the opposite order they were originally presented.

Another interesting finding from this study is that the entire trial MS suppression did not cause a performance deficit, although it encompassed the delay

period. It is possible that suppression of theta activity throughout the entire trial engages compensatory mechanisms that allow the animal to perform normally. In a previous study, rats trained to asymptotic performance on a delayed alternation task initially dropped to chance levels in performance following MS lesions, but were able to recover to approximately 75% correct after six post-operative testing sessions. This level of choice accuracy was still significantly poorer than control rats, who performed the task with 90-95% accuracy post-operation, but provides evidence that a potential compensatory mechanism could allow for this recovery (Thomas, Brito, & Stein 1980). It has been hypothesized that projections from the medial frontal cortex to entorhinal cortex could be behind this recovery of function (Beckstead 1979), and that continued testing post-operation allows for the strengthening of these connections and resulting recovery of performance (Thomas & Brito 1980). However, this hypothesis has never been explicitly tested. Attempting to examine a brain region as allowing for a potential compensatory mechanism is difficult, as many of these regions may also make contributions to task performance during normal behavior.

In order to fully interpret and understand these results, follow-up analyses will be performed. To determine any differences in behavior beyond choice accuracy, the behavior of rats on the maze will be examined by recording the time spent at the choice point of the maze for all trials and the running behavior on the stem. This data will be analyzed using a 2 (group: experimental, control) x 2 (light: on, off) x 2 (traversal: sample, choice) ANOVA, followed by Bonferroni-correct posthoc tests, for

each testing condition to determine any differences. This will allow us to see if the light is causing any behavioral effects beyond differences in choice accuracy.

Furthermore, electrophysiological confirmation is necessary to fully understand these results. As previously mentioned, there are multiple cells types in the MS which serve different functions. The two main projections from MS to HPC are the cholinergic and GABAergic projections, and disruption of these pathways causes different effects on the theta rhythm (Lee et al. 1994; Mitchell et al. 1981; Simon et al. 2006; Tóth, Freund, & Miles, 1997). However, disrupting either projection leads to deficits in SWM performance (Chang & Gold 2004; Chobak et al. 1992; Givens & Olton 1990; Lee et al. 1994). The promotor used for the AAV in this experiment, CAG, is not cell-type specific and causes high expression of opsin within all cells (Yizhar et al. 2011). The opsin used, ArchT, causes expression of a proton pump which, when exposed to light, removes protons from the cell, inducing a neural silencing current of approximately 900 pA (Chow et al. 2010). Because of this, optogenetic suppression in this experiment should function similarly to a reversible lesion of the area, with a substantial reduction in firing of the affected cells. However, measuring the local field potential in HPC to determine the exact effects on the theta rhythm is a necessary proof-of-concept experiment to ensure the desired effect is taking place during the desired timeframe.

In conclusion, we have shown that MS inactivation impairs DNMP performance in a condition-specific manner, where suppression during the delay period causes choice accuracy deficits while suppression during sample and choice

phases does not. This deficit may reflect a perturbation of the HPC theta rhythm's ability to integrate goal-relevant spatial and temporal information. Further studies are needed to appropriately characterize the effects of MS optogenetic suppression on HPC theta in order to better understand its specific contributions to successful performance of the DNMP task.

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Appendix A

ANIMAL PROTOCOL PERMISSION

UNIVERSITY OF DELAWARE | Institutional Animal Care and Use Committee (IACUC)

Newark, DE 19716-1561
Phone: 302-831-2616
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To: Office of Graduate and Professional Education

From: Gwen Talham, DVM, Director, Animal Care Program

Subject: IACUC approval for Margaret Donahue

Date: 4/18/2018

Margaret Donahue was approved by the IACUC to work with animals on Amy Griffin's protocol #1177 "Neural Correlates of Spatial and Nonspatial Memory". Please contact me at 831-2980 or gtalham@udel.edu with any additional questions.