OPTOGENETIC SUPPRESSION OF THALAMIC NUCLEUS REUNIENS DURING SPATIAL WORKING MEMORY

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Neuroscience

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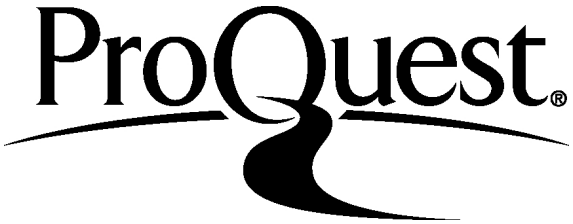

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ABSTRACT

Spatial working memory (SWM), the temporary storage and manipulation of spatially relevant information in order to guide goal-directed behavior, has been demonstrated to depend on functional interactions between the medial prefrontal cortex (mPFC) and hippocampus (HPC). Recent demonstrations have shown that the nucleus reuniens (RE), part of the ventral midline thalamus reciprocally connected to both the HPC and mPFC, plays an essential role in coordinating interactions within the HPC-mPFC circuit during SWM. However, the precise circuitry that facilitates this coordination and the role of RE in distinct aspects of SWM has yet to be determined. Therefore, we investigated the functional involvement of RE in SWM by optogenetically silencing RE in rats performing the SWM-dependent delayed non-match to position (DNMP) task. Silencing RE during each experimental condition: sample phase, choice phase, delay period, and entire trial, showed impaired choice accuracy on all four conditions. These findings implicate RE in all aspects of SWM and set the stage for future experiments to investigate the possibility of pathway-specific roles of RE afferents during distinct stages of the SWM-decision making process.

Chapter 1

INTRODUCTION

1.1 Hippocampal-Prefrontal Interactions and Spatial Working Memory

Spatial working memory (SWM) refers to an essential cognitive operation involving the temporary storage and manipulation of task-relevant spatial information in order to guide goal-directed behavior. SWM can be further fractionalized into distinct components: encoding, maintenance, and retrieval (Dudchenko 2004). Oscillatory synchrony within the hippocampal-prefrontal circuit has been suggested to be critical for SWM (Griffin, 2015; Colgin 2011; Gordon, 2015). Although hippocampal-prefrontal interactions have been correlated with SWM-task performance in rodents (Jones and Wilson 2005; Hyman et al., 2010; Sigurdsson et al., 2010; O'Neill et al., 2010; Spellman et al., 2015), the neurophysiological mechanisms that underlie this synchrony remain poorly understood. Lesions of the rodent dorsal (Dudchenko et al., 2000; Ainge et al., 2007; Czerniawski et al., 2009; Hallock et al., 2013a), but not ventral (Czerniawski et al., 2009), hippocampus impair SWM, and the rodent medial prefrontal cortex functionally synchronizes with the dorsal hippocampus during successful SWM-task performance (Jones and Wilson 2005; Hyman et al., 2010; Sigurdsson et al., 2010; O'Neill et al., 2013), supporting the hypothesis that maintaining behaviorally-relevant information in SWM requires the rodent dorsal hippocampus. Despite numerous studies demonstrating that dorsal hippocampal-prefrontal interactions are both correlated with (Jones and Wilson 2005; Hyman et al., 2010; Sigurdsson et al., 2010), and necessary for (Lee and Kesner, 2003), SWM in

rodents, the rodent mPFC receives direct input only from the ventral hippocampus (Swanson, 1981; Jay et al., 1989; Thierry et al., 2000). Taken together, this supports an indirect pathway synchronizing the dorsal hippocampus and mPFC during SWM.

The ventral midline thalamic nucleus reuniens (RE) is ideally situated for meditating hippocampal-mPFC synchrony due to its bidirectional connectivity with both the mPFC and hippocampus (Vertes et al., 2006; Vertes et al., 2007). RE fibers exclusively synapse on distal CA1 dendrites in the stratum lacunosum-moleculare cell layer of the hippocampus (Herkenham, 1979), while mPFC projecting RE fibers synapse most heavily in the infralimbic (IL) and prelimbic (PL) subregions of the mPFC with fewer projections to the anterior cingulate cortex (ACC) (Vertes et al., 2006). In turn, the PL, IL, and ACC send direct return projections back to RE, forming asymmetric peri-dendritic synapses on a number of HPC-projecting neurons, possibly allowing for cortical modulation of RE-HPC interactions (Vertes et al., 2007). Additionally, a small subset of RE neurons sends collateral projections to both the HPC and mPFC, possibly allowing RE to orchestrate cooperative activity between the HPC and mPFC. Thus, RE is likely to contribute to SWM by dynamically modulating hippocampal-prefrontal interactions. In support of this hypothesis, pharmacological inactivation of RE has been shown to impair performance during SWM-tasks (Hembrook and Mair, 2011; Hembrook et al., 2012; Hallock et al., 2013b; Layfield et al., 2015).

1.2 Dynamic Role of Nucleus Reuniens in Thalamo-Hippocampal-Prefrontal Circuit

A recent set of experiments in our lab have shown that pharmacological inactivation of RE concomitantly disrupts SWM-task performance as well as decreasing several measures of HPC-PFC synchrony. Specifically, RE inactivation impaired performance on a SWM-dependent delayed alternation (DA) task which requires both the dorsal hippocampus and RE (Ainge et al., 2007; Hallock et al., 2013a), while also decreasing mPFC single unit entrainment to dHPC theta oscillations, phase-amplitude coupling between dHPC theta and mPFC slow gamma oscillations, as well as dHPC-mPFC theta phase coherence (Hallock et al., In Review). Previous studies have shown that entrainment is strongest to past phases of HPC theta during SWM-guided behavior in rodents (Siapas et al., 2005; Sigurdsson et al., 2010), in other words, mPFC neurons preferentially fire to past phases of HPC theta cycles. Our findings revealed mPFC neurons entrain to past dHPC theta cycles specifically during the delay period of the DA task, as shown by a negative shift in the distribution of lag values at which mPFC neurons maximally phase-lock to dHPC theta. Following RE inactivation, this delay-specific entrainment was significantly reduced, suggesting that HPC theta oscillations organize spike timing in the mPFC during SWM-guided behavior and that RE critically regulates this interaction. Furthermore, the directionality of communication between the dHPC and mPFC during DA task performance was found to occur in a delay period- and frequency-specific manner. Using granger causality analysis, lead index values indicated that changes in dHPC local field potential (LFP) activity led changes in the mPFC LFP in the theta frequency band specifically during the delay-period, suggesting dHPC theta may

transmit previously encoded spatial information to the mPFC in order to successfully guide upcoming performance.

Following the delay-period of this SWM-dependent DA task, animals must use their memory of the previously visited location in order to choose the correct arm at the choice point of the T-maze. As animals approached the choice point, changes in the mPFC LFP led changes in dHPC LFP in the slow gamma band, indicating mPFC to dHPC directionality of communication during choice point traversals. Possibly reflecting the retrieval of trial-specific information by the dHPC from the mPFC in order to guide successful SWM-decision making. Following RE inactivation, decreased theta-gamma coupling and theta phase coherence specific to choice point traversals was observed. These results demonstrate that RE modulates hippocampal-prefrontal synchrony during SWM and further show that RE is necessary for bi-directional communication between the dHPC and mPFC during SWM-task performance (Hallock et al., 2016). One likely interpretation of these results is that projections from the dHPC (through the dorsal subiculum) to RE (Varela et al., 2014) support information maintenance during the delay-period of the DA task, and afferents from the mPFC to RE may support the retrieval of trial-specific information during successful SWM-guided decisions.

Recent evidence has supported the hypothesis that distinct pathways within this circuit transmit SWM-specific information at different stages of the encoding, maintenance, and retrieval process. Optogenetic silencing of RE neurons has been shown to decrease trajectory coding in dHPC CA1, but not CA3, neurons during continuous alternation task performance (Ito et al., 2015), suggesting RE may transmit route-specific information during goal-directed navigation. Another study has further

highlighted the complexity of this circuit by demonstrating the direct monosynaptic projection from ventral hippocampus to mPFC supports the encoding, but not maintenance or retrieval, of spatial information during the sample phase of a delayed non match to position (DNMP) T-maze task (Spellman et al., 2015). Importantly, the representation of sample goal location in the mPFC didn't require direct vHPC input during choice phases (retrieval), possibly reflecting a transition in the structures needed for processing previously encoded information. RE is a candidate structure in mediating this switch in information processing due to its bidirectional connectivity with both the dorsal and ventral hippocampus and mPFC (Vertes et al., 2006; Vertes et al., 2007).

A likely interpretation of these results are that during the encoding of SWM information the HPC needs to interact with the mPFC, as demonstrated by HPC driven synchrony during presumable encoding portions of SWM tasks, thus it is likely that synchrony is facilitated by RE circuit involvement. We also believe that RE is involved in supporting the maintenance of the spatial information needed for successful SWM, either through conjunctive involvement with the mPFC or by playing a role in wider circuit level coordination. During retrieval, interregional interactions between the mPFC and HPC allude to a need for an indirect pathway, possibly through RE, synchronizing these structures in order to support the retrieval of necessary spatial information. Due to the possible mechanisms underlying encoding, maintenance, and retrieval, all of which involve RE, we believe that inactivating RE activity during any portion of the task will induce performance deficits.

Chapter 2

MATERIALS AND METHODS

2.1 Experimental Overview

This project was conducted as three parallel experiments, each aimed at characterizing the differential involvement of RE and its afferent projections during SWM. The specific experiment discussed in this thesis is the optogenetic silencing of RE neurons during distinct phases of the DNMP task. The advantage of using this task is that it features temporally distinct task-phases effectively separating the encoding (sample) and retrieval (choice) aspects of SWM. Thus, utilizing this approach allows us to directly compare across each SWM process.

In this experiment we transfected a group of opsin-positive rats with a viral construct encoding the transmembrane protein archaerhodopsin-T (ArchT), a light responsive outward proton pump, targeted at RE neurons. All rats within this group were implanted with an optical fiber in RE in order to control opsin kinetics during task performance. Two additional control groups will be added consisting of an opsin-negative group and a fiber-only group. The opsin-negative group differs only from the opsin-positive group in that the viral construct delivered does not encode for the opsin protein, thus controlling for any effects induced by viral transduction of the opsin as well as any effects seen during illumination conditions. The fiber only group will consists of rats not exposed to either virus and are implanted only with optic fibers in RE to control for the possibility of prolonged illumination resulting in heat-induced effects. Currently, only opsin-positive rats have been tested.

All rats included in the present experiment were trained to asymptotic performance levels (>80%) on the DNMP T-maze task, after which they were tested on four distinct experimental conditions: sample-only light, delay-light, choice-only light, and entire-trial light. Each testing session occurred pseudorandomly on separate days with blocks of no-light trials interleaved with light trials, allowing for within session comparisons. The dependent variable measured was the percentage of correct trials per session block. Currently, statistical analysis has not been performed due to a small sample size.

2.2 Subjects

Nine (4-9 months old) male Long-Evans Hooded rats (Harlan, IN) served as subjects for the experiment. All subjects were housed individually in a temperature- and humidity-controlled colony room on a 12 hour light-dark cycle with ad libitum access to food and water. During periods of behavioral training and testing, rats were held at 90% of their free-feeding weight. All training and testing occurred during the light phase on a T-maze modified with return arms.

2.3 Viral Construct

The proviral plasmid used for packaging the rAAV is flanked by serotype-5 inverted terminal repeats (ITRs). The rAAV vector contains the CAG (chicken beta-actin) promoter enhanced with CMV, the outward proton pump from archaerhodopsin T009 fused to GFP, a woodchuck hepatitis posttranscriptional regulatory element (WPRE), and a SV4 PA terminator sequence. The concentrated viral titer of the construct, rAAV5-CAG-ArchT-GFP, at the time of injection was determined by the UNC Core Facility using the dot blot method to be of titer exceeding $4x10^{12}$ vg/ml.

2.4 Surgical Preparation

Rats were anesthetized with isoflurane and the skull surface was exposed. An appropriately sized trephine was used to drill a single hole for the viral injections and fiber optic cannula, and burrs were used to drill 4 holes for anchor screws. Dura was gently punctured using a 25-gauge needle and sterile saline-soaked gel foam was used to control bleeding as well as keeping the brain surface from drying out. The virus was pressure injected using a Harvard Apparatus PHD2200 programmable syringe pump interfaced to a 1µm Hamilton Syringe. The viral suspension was then injected into RE (injection 1: -2.0 AP; +/- 2.0 ML; -7.2 DV; injection 2: -2.5AP; +/- 2.0 ML; -7.4 DV) at a flow rate of 0.1µl/min for 5 min, with a 10 minute rest period between injections. A fiber optic cannula (Ø2.3mm ceramic ferrule, Ø200µm core, 0.66 NA, L=10mm) was then implanted slowly (-2.3 AP; +/-2.0 ML; -7.0 DV). A sealant (Quik-Sil) was then applied around the cannula to act as a barrier between the acrylic and brain surface. Dental acrylic was then applied around all of the anchor screws and the base of the implant. Antibiotic ointment was then applied to the incision site and an injection of banamine was administered subcutaneously for pain relief. The rat was then placed in a clean cage atop a heating pad and observed until anesthesia wore off.

2.5 Behavioral Training

All pre-training procedures began a minimum of 4 weeks after surgery in order to allow for ~6 weeks between surgery and LED stimulation. This time frame allowed for the sufficient expression of the opsin in RE cell bodies. LED stimulation occurred after successful completion of all pre-training and training procedures as defined below.

2.5.1 Goal Box Training

During goal-box training, subjects were placed in the reward zones of the T-maze, located at the ends of each reward arm, and barricaded from the rest of the maze. In the reward zone, a rimmed plastic bottle cap containing chocolate sprinkles was fastened to the floor of the maze. During each goal box trial the rat was given three minutes to consume the sprinkles, and at the end of each trial the rat was picked up and placed in the other goal-zone to perform the subsequent trial. Each goal-box session contained six trials, and one session was performed per day until the rat consumed the reward on every trial in less than 90 seconds for two consecutive sessions. Achieving this level of performance advanced the rat to the next stage of pre-training.

2.5.2 Forced-Run Training

Each forced run trial consisted of the rat entering the central stem from the delay pedestal, traversing the stem, entering a goal-zone, eating the reward, and returning to the delay pedestal via the return arm. Rats were unable to make a choice at the intersection of the maze due to a wooden barrier occluding one goal arm. Rats were prevented from turning around or entering the return arms by the experimenter. The sequence of blocked goal arms was pseudorandomly chosen (Fellows, 1967), and each session consisted of 12 trials performed once a day. Successful completion of at least 10 trials per session for two consecutive sessions advanced the rat to behavioral task training. A successful trail entailed the rat traversing the maze smoothly and consuming the reward in the goal-zone.

2.6 DNMP Protocol

Rats were trained on a DNMP T-maze task. Sessions consisted of 12 trials during training and increased to 18 trials for testing. This was done for practical reasons, as rats initially learning the task take a longer time to complete a session, which is about the same time as 18 trials when they have learned it. Sessions were run once a day until the rats reached asymptotic performance levels (>80% trials correct for two consecutive sessions). Each trial consisted of two maze traversals, a sample phase and a choice phase, in this order. At the start of each phase, the barrier confining the rat to the pedestal was removed and the rat ran down the central stem until reaching the choice point where the goal arms intersect with the stem. At this point, on sample phase traversals, one of the two goal arms was blocked by a wooden barrier, forcing the rat to enter the available goal arm and subsequent goal zone containing the reward. After consuming the reward, the rat returned to the pedestal via the return arm and a barrier was placed between the maze and the pedestal preventing the rat from reentering the maze. After the sample phase concluded, rats were confined to the pedestal for a delay period of 20 seconds. Following the delay, the barrier was then removed signifying the start of the choice phase. The rat again traveled down the central stem, however when reaching the choice point both goal arms were open whereby the rat had to choose between them. If the rat chooses the arm that was not visited during the previous sample phase, then he finds a sprinkle reward and returns via the return arm to the pedestal. If the rat chooses incorrectly, then he is not rewarded and returns to the pedestal. After a choice phase traversal, regardless of its outcome, rats were confined to the pedestal by the barrier for an inter-trial-delay (ITI) of 40 seconds. The long ITI is implanted to help the rat distinguish between phases. After waiting for 40 seconds the rat begins a new sample phase run and repeats the

trial process. The goal arm blocked on each sample phase was determined pseudorandomly (Fellows, 1967). After a rat achieved a performance score of 15 correct trials or better for two consecutive sessions, the following day's session was conducted with the fiber-optic cable attached. If the rat traversed the maze smoothly and successfully completing at least 15 trials during this session then behavioral testing began.

2.7 LED Stimulation Protocol

Testing was performed under the same parameters as described above, however, during testing with LED stimulation, the fiber-optic patch cable was attached for each session. Performance was recorded from four distinct experimental conditions: sample-only light, delay-only light, choice-only light, and entire-trial light sessions. All light sessions began with six no-light trials followed by six light trials, where light was delivered during condition-specific times, followed by another six no-light trials, followed by a final six light-on trials. This no-light/light-on blocking method allows for within session comparison of performance. For light-on trials, LED stimulation was delivered via a compact LED module (Plexon, INC) emitting 550nm light through a patch cable attached to the ferrule of the implanted optic fiber. In all conditions, light was delivered while rats were on return arms or during the ITI, reducing the possibility of light-induced heat effecting behavioral performance. The order in which the experimental conditions were performed was pseudorandomly determined (Fellows, 1967). All experimental conditions took place on separate days to prevent carry over effects from previous trials. An additional experimenter in the testing room (behind a curtain) triggered the control of LED output. This experimenter

watched a live feed of the rat being run on the maze, using the real time position of the rat to trigger the LED when necessary.

2.8 Histological Analysis

Rats were euthanized with an overdose administration of sodium pentobarbital and perfused transcardially with saline and 4% paraformaldehyde. The brain was then removed and placed in a 4% paraformaldehyde solution. After soaking in paraformaldehyde for 1-2 days, the brains were then transferred to a sucrose solution. After sinking, indicative of tissue saturation, the brains were frozen and sectioned using a cryostat. The sections were then mounted on slides and prepared for immunohistochemistry. For immunohistochemical analysis, slides went through three five-minute washes in 1X PBS (pH 7.2-7.4). Slides were then incubated at room temperature for one hour in blocking buffer solution containing 5% goat-serum, 0.3% Triton-X, and 1X PBS. After a second washing procedure, slides were then transferred to a cold room for 24 hours to incubate in the primary antibody solution. After this 24 hour period, slides were removed from the cold room and washed in 1X PBS. The secondary antibody solution (goat anti-rabbit IgG, ThermoFisher Scientific, and blocking solution) was then applied to the slides and allowed to incubate in the dark at room temperature for one hour. After this secondary incubation period, slides were rinsed with 1X PBS and allowed to dry for 10 minutes. Once dry, Prolong Gold anti-fade reagent was applied to the tissue and a cover slip was placed over it. Slides were imaged using a confocal microscope to identify the extent of viral expression.

Chapter 3

RESULTS

3.1 Task Acquisition

Figures 1 and 2 represent the learning data collected in opsin-positive RE animals (N=4). The average number of training sessions needed for animals to reach performance criterion, at least 80% correct for two consecutive sessions, were recorded (Figure 1; mean=7.5, SEM=1.7).

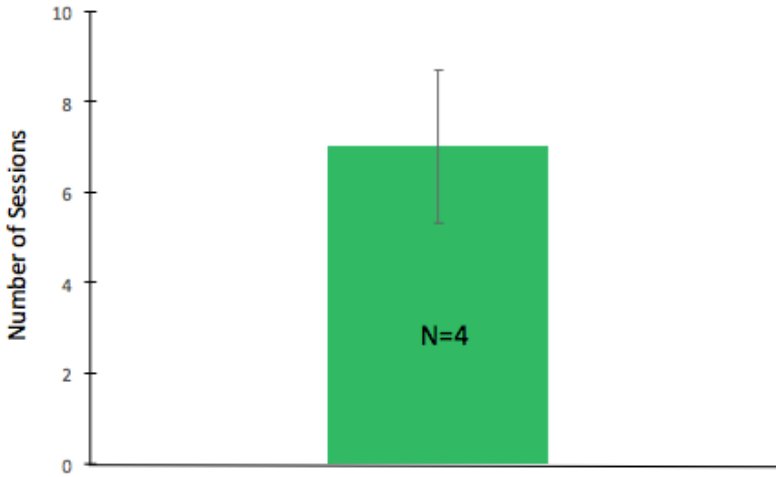


Figure 1. Average number of sessions needed to hit criterion in ArchT RE rats (N=4). Mean and SEM

Figure 2 represents the number of sessions needed for each animal to reach criterion. Of these four animals, only ArchT Rat 3 and ArchT Rat 4 advanced to behavioral testing.

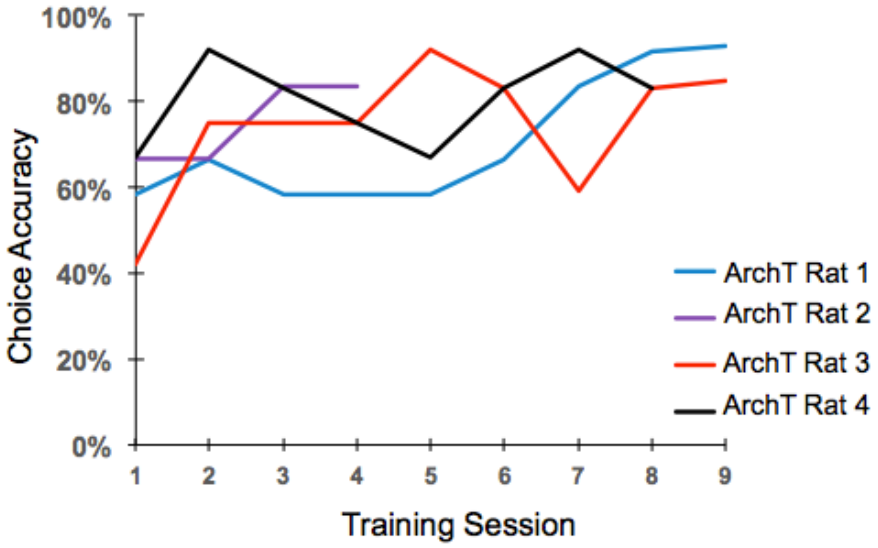


Figure 2. Number of sessions needed to reach criterion to advance to LED stimulation in ArchT RE rats (N=4). Each individual color represents data from 4 separate rats.

3.2 Behavioral Testing

The results from two individual animals that have undergone behavioral testing are represented in figures 3 and 4. Each color represents the four different testing conditions conducted on separate days. Choice accuracy was recorded in blocks of no-light and light trials. Although insufficient sample size prevented statistical analysis, there appears to be a decrease in choice accuracy in both animals specific to light trials

for all four testing conditions. In both figures, overlapping lines are the result of choice accuracy being equal among data points.

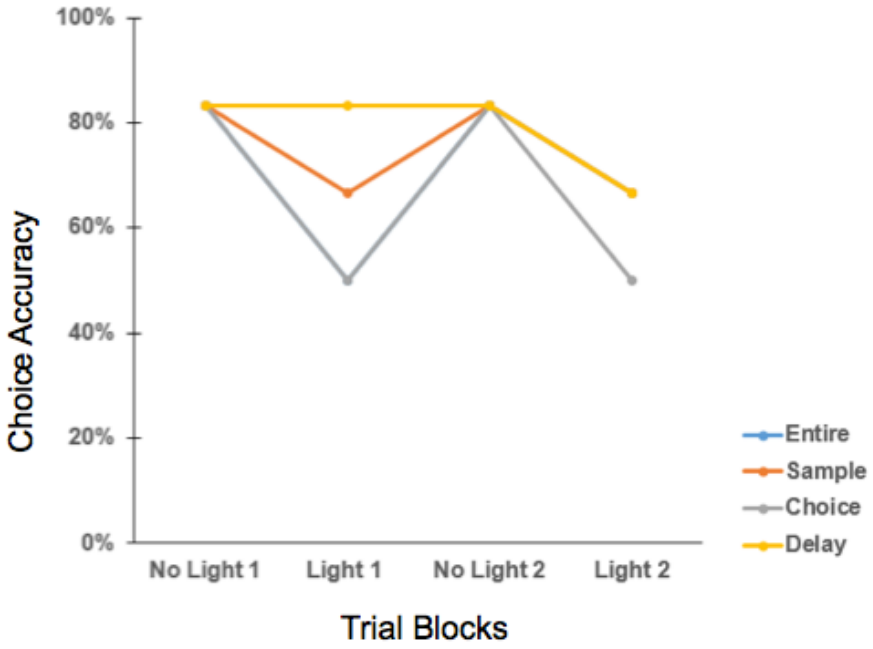


Figure 3. Task performance during LED stimulation in the first ArchT expressing animal. Each colored line represents the different testing conditions.

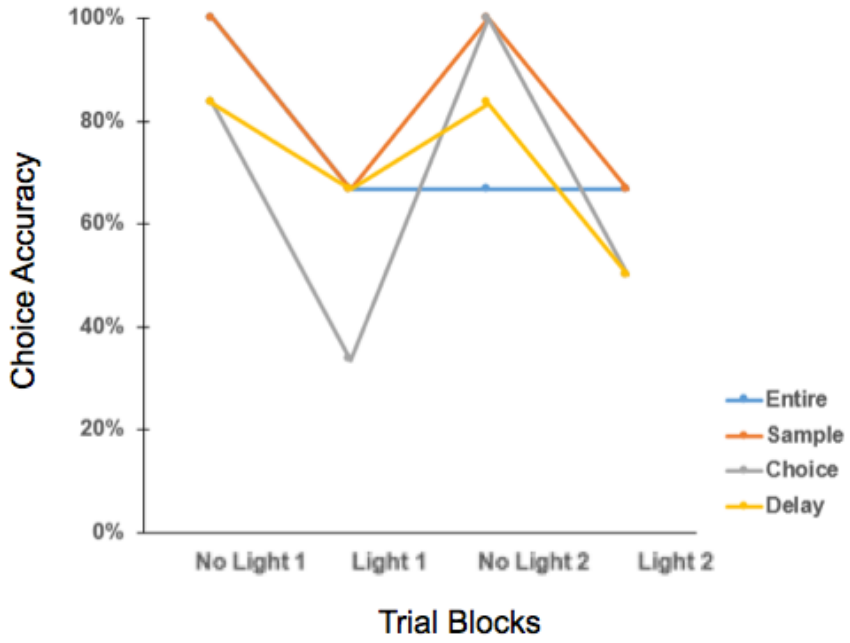


Figure 4. Task performance during LED stimulation in the second ArchT expressing animal. Each colored line represents the different testing conditions.

3.3 Imaging

Currently only one opsin-positive ArchT animal has undergone imaging. As shown in figure 4a, opsin expression was relatively confined to RE. Additionally, figure 4b demonstrates that fiber-optic cannula placement terminated just dorsal of RE neurons expressing ArchT, indicated by the presence of green fluorescence.

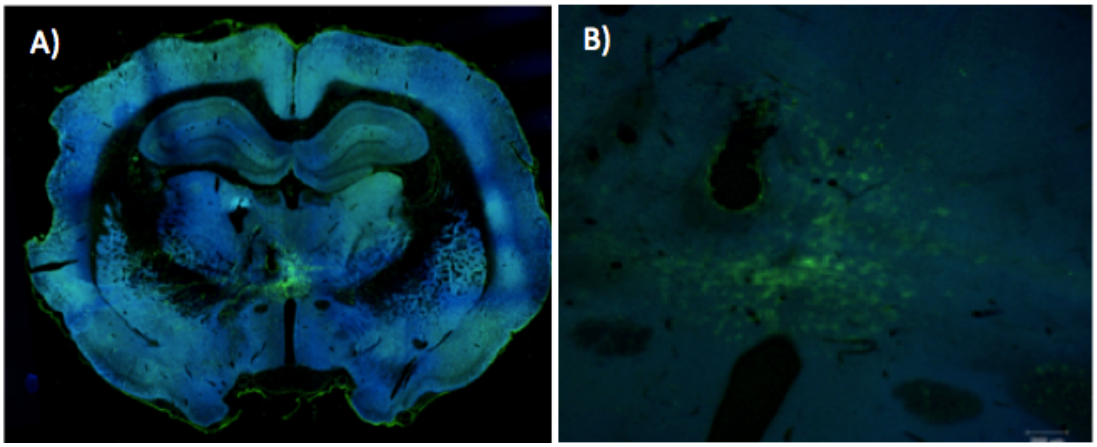


Figure 5. Example confocal images of RE neurons expressing ArchT. Part a represents a coronal section at 5X magnification. 4b represents the same image at 20X magnification.

Chapter 4

DISCUSSION

4.1 Continuing Data Collection and Future Directions

Although these findings implicate RE in all aspects of SWM, more animals are needed in order to run statistical analysis. Currently, only 4 animals have successfully reached criterion and 2 animals have been tested. Additionally, by including opsin-negative and fiber only controls, we can rule out any confounding variables due to viral transduction and heat associated behavioral impairments. Furthermore, more imaging is needed to verify the degree of viral expression and accuracy of fiber optic placement within RE. Incorporating electrophysiological recordings into this project, which our lab has recently begun, will allow us to record from RE while silencing neural activity during performance of the DNMP task. These recordings would allow us to first determine the degree of suppression achieved under the current protocol, which is a major limitation of the current study. Additionally, these recordings could further elucidate the electrophysiological signatures of RE unit firing and ensemble activity, data that is critically lacking. Other possibilities could include the incorporation of different techniques, such as DREADDs combined with optogenetics and triple site recordings of the HPC-RE-mPFC circuit, which would allow precise manipulation of numerous pathways and their effects on synchrony and behavior.

While these preliminary results support our hypothesis that RE is involved in the encoding, maintenance, and retrieval aspects of SWM, additional preliminary data from the experiments conducted in parallel to this one have shown that terminal suppression of dorsal subicular input to RE caused a selective deficit on the sample-

only light condition, suggesting that dSub-RE projections are critical for the encoding of task relevant spatial information. Conversely, terminal suppression of mPFC input into RE caused a selective deficit on the delay-only light condition, suggesting that mPFC-RE projections support the encoding and maintenance of spatial information necessary for successful SWM-behavior. Importantly, in support of the main hypothesis of this thesis, these results demonstrate that RE is involved in the encoding, maintenance, and retrieval aspects of SWM. Furthermore, pathway-specific roles for RE afferents during the encoding and maintenance of spatial information appear to be critical for successful SWM.

REFERENCES

Cassel, J. C., de Vasconcelos, A. P., Loureiro, M., Cholvin, T., Dalrymple-Alford, J. C., & Vertes, R. P. (2013). The reuniens and rhomboid nuclei: neuroanatomy, electrophysiological characteristics and behavioral implications. Progress in neurobiology, 111, 34-52.

Churchwell, J. C., Morris, A. M., Musso, N. D., & Kesner, R. P. (2010). Prefrontal and hippocampal contributions to encoding and retrieval of spatial memory. Neurobiology of learning and memory, 93(3), 415-421.

Colgin LL. (2011). Oscillations and hippocampal-prefrontal synchrony. Current Opinion in Neurobiology, 21: 467-474.

Czerniawski, J., Yoon, T., & Otto, T. (2009). Dissociating space and trace in dorsal and ventral hippocampus. *Hippocampus*, *19*(1), 20-32.

Dudchenko, P. A. (2004). An overview of the tasks used to test working memory in rodents. *Neuroscience & Biobehavioral Reviews*, *28*(7), 699-709.

Ferino, F., Thierry, A. M., & Glowinski, J. (1987). Anatomical and electrophysiological evidence for a direct projection from Ammon's horn to the medial prefrontal cortex in the rat. Experimental Brain Research, 65(2), 421-426.

Ferino, F., Thierry, A. M., Saffroy, M., & Glowinski, J. (1987). Interhemispheric and subcortical collaterals of medial prefrontal cortical neurons in the rat. Brain research, 417(2), 257-266.

Fellows, B.J. (1967). Change stimulus sequences for discrimination tasks. Psychological Bulletin, 67 (1967), pp. 87-92

Griffin AL, Eichenbaum H, Hasselmo ME. 2007. Spatial representations of hippocampal CA1 neurons are modulated by behavioral context in a hippocampus-dependent memory task. J Neurosci 27:2416–2423.

Griffin, A. L. (2015). Role of the thalamic nucleus reuniens in mediating interactions between the hippocampus and medial prefrontal cortex during spatial working memory. Frontiers in systems neuroscience, 9.

Han, Xue et al. “A High-Light Sensitivity Optical Neural Silencer: Development and Application to Optogenetic Control of Non-Human Primate Cortex.” Frontiers in Systems Neuroscience 5 (2011): 1-8.

Harris, A. Z., & Gordon, J. A. (2015). Long-Range Neural Synchrony in Behavior. *Annual review of neuroscience*, *38*, 171-194.

Hallock, H. L., & Griffin, A. L. (2013a). Dynamic coding of dorsal hippocampal neurons between tasks that differ in structure and memory demand.*Hippocampus*, *23*(2), 169-186.

Hallock, H. L., Wang, A., Shaw, C. L., & Griffin, A. L. (2013b). Transient inactivation of the thalamic nucleus reuniens and rhomboid nucleus produces deficits of a working-memory dependent tactile-visual conditional discrimination task. Behavioral neuroscience, 127(6), 860.

Hallock, H.L., Wang, A., & Griffin, A.L. (2016). The ventral midline thalamus is critical for hippocampal-prefrontal synchrony and spatial working memory. Manuscript submitted for publication.

Herkenham, M. (1979). The afferent and efferent connections of the ventromedial thalamic nucleus in the rat. Journal of Comparative Neurology,183(3), 487-517.

Hembrook, J. R., & Mair, R. G. (2011). Lesions of reuniens and rhomboid thalamic nuclei impair radial maze win-shift performance. Hippocampus,21(8), 815-826.

Hembrook, J. R., Onos, K. D., & Mair, R. G. (2012). Inactivation of ventral midline thalamus produces selective spatial delayed conditional discrimination impairment in the rat. Hippocampus, 22(4), 853-860.

Hoover, W. B., & Vertes, R. P. (2007). Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Structure and Function, 212(2), 149-179.

Ito, H. T., Zhang, S. J., Witter, M. P., Moser, E. I., & Moser, M. B. (2015). A prefrontal-thalamo-hippocampal circuit for goal-directed spatial navigation.Nature.

Jay, T. M., Burette, F., & Laroche, S. (1996). Plasticity of the hippocampal-prefrontal cortex synapses. Journal of Physiology-Paris, 90(5), 361-366.

Jones, M. W., & Wilson, M. A. (2005). Theta rhythms coordinate hippocampal–prefrontal interactions in a spatial memory task. PLoS Biol,3(12), e402.

Kesner, R. P., Hunt, M. E., Williams, J. M., & Long, J. M. (1996). Prefrontal cortex and working memory for spatial response, spatial location, and visual object information in the rat. Cerebral Cortex, 6(2), 311-318.

Lee, I., & Kesner, R. P. (2003). Time-dependent relationship between the dorsal hippocampus and the prefrontal cortex in spatial memory. The Journal of Neuroscience, 23: 1517-1523.

Layfield, D. M., Patel, M., Hallock, H., & Griffin, A. L. (2015). Inactivation of the nucleus reuniens/rhomboid causes a delay-dependent impairment of spatial working memory. Neurobiology of learning and memory, 125, 163-167.

Pastalkova, E., Itskov, V., Amarasingham, A., & Buzsáki, G. (2008). Internally generated cell assembly sequences in the rat hippocampus.Science, 321(5894), 1322-1327.

Pettersson-Yeo, W., Allen, P., Benetti, S., McGuire, P., & Mechelli, A. (2011). Dysconnectivity in schizophrenia: where are we now?. *Neuroscience & Biobehavioral Reviews*, *35*(5), 1110-1124.

Siapas, A. G., Lubenov, E. V., & Wilson, M. A. (2005). Prefrontal phase locking to hippocampal theta oscillations. Neuron, 46(1), 141-151.

Sigurdsson, T., Stark, K. L., Karayiorgou, M., Gogos, J. A., & Gordon, J. A. (2010). Impaired hippocampal–prefrontal synchrony in a genetic mouse model of schizophrenia. Nature, 464(7289), 763-767.

Sigurdsson, T., & Duvarci, S. (2015). Hippocampal-prefrontal interactions in cognition, behavior and psychiatric disease. *Frontiers in Systems Neuroscience*, *9*, 190.

Shapiro, M. L., Kennedy, P. J., & Ferbinteanu, J. (2006). Representing episodes in the mammalian brain. *Current opinion in neurobiology*, *16*(6), 701-709.

Shevtsova, Z., Malik, J. M. I., Michel, U., Bähr, M., & Kügler, S. (2005). Promoters and serotypes: targeting of adeno-associated virus vectors for gene transfer in the rat central nervous system in vitro and in vivo.Experimental physiology, 90(1), 53-59.

Swanson, L. W., Sawchenko, P. E., & Cowan, W. M. (1981). Evidence for collateral projections by neurons in Ammon's horn, the dentate gyrus, and the subiculum: a multiple retrograde labeling study in the rat. The Journal of Neuroscience, 1(5), 548-559.

Tierney, P. L., Dégenètais, E., Thierry, A. M., Glowinski, J., & Gioanni, Y. (2004). Influence of the hippocampus on interneurons of the rat prefrontal cortex. European Journal of Neuroscience, 20(2), 514-524.

Uhlhaas, P. J., & Singer, W. (2012). Neuronal dynamics and neuropsychiatric disorders: toward a translational paradigm for dysfunctional large-scale networks. *Neuron*, *75*(6), 963-980.

Varela, C., Kumar, S., Yang, J. Y., & Wilson, M. A. (2014). Anatomical substrates for direct interactions between hippocampus, medial prefrontal cortex, and the thalamic nucleus reuniens. Brain Structure and Function,219(3), 911-929.

Vertes, R. P. (2006). Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. Neuroscience, 142(1), 1-20.

Vertes, R. P., Hoover, W. B., Do Valle, A. C., Sherman, A., & Rodriguez, J. J. (2006). Efferent projections of reuniens and rhomboid nuclei of the thalamus in the rat. Journal of Comparative Neurology, 499(5), 768-796.

Vertes, R. P., Hoover, W. B., Szigeti-Buck, K., & Leranth, C. (2007). Nucleus reuniens of the midline thalamus: link between the medial prefrontal cortex and the hippocampus. Brain research bulletin, 71(6), 601-609.

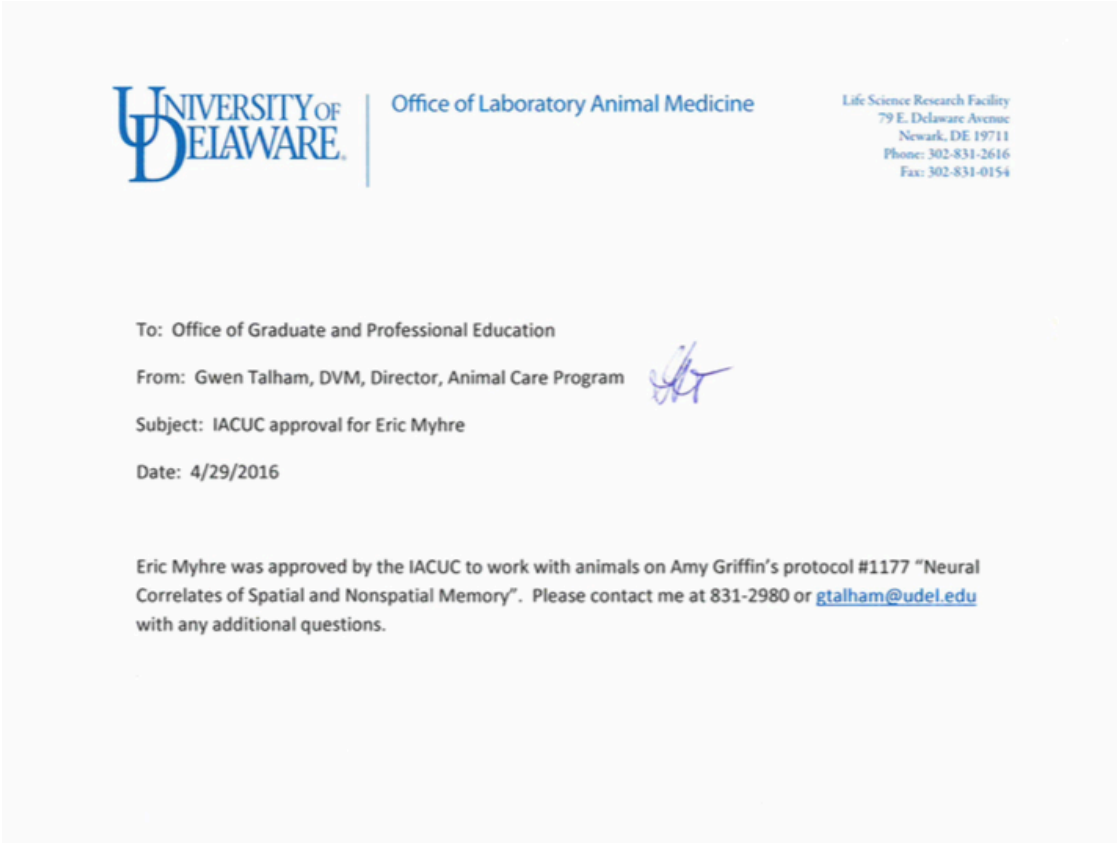
Wang, G. W., & Cai, J. X. (2006). Disconnection of the hippocampal–prefrontal cortical circuits impairs spatial working memory performance in rats. Behavioural brain research, 175(2), 329-336.

Wu, Z., Asokan, A., & Samulski, R. J. (2006). Adeno-associated virus serotypes: vector toolkit for human gene therapy. Molecular therapy, 14(3), 316-327.

Yizhar, O., Fenno, L. E., Davidson, T. J., Mogri, M., & Deisseroth, K. (2011). Optogenetics in neural systems. *Neuron*, *71*(1), 9-34.

Appendix

APPROVAL FOR THE USE OF ANIMAL SUBJECTS



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To: Office of Graduate and Professional Education

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

Subject: IACUC approval for Eric Myhre

Date: 4/29/2016

Eric Myhre 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.