2020

Comorbidities of Episodic Memory in the Pilocarpine Model of Temporal Lobe Epilepsy: Spontaneous Object Recognition for What, Where, and When

Cameron D'Orio

THE FLORIDA STATE UNIVERSITY

COLLEGE OF ARTS & SCIENCES

COMORBIDITIES OF EPISODIC MEMORY IN THE PILOCARPINE MODEL OF TEMPORAL LOBE EPILEPSY: SPONTANEOUS OBJECT RECOGNITION FOR *WHAT*, *WHERE*, AND *WHEN*

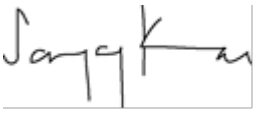
By

CAMERON D'ORIO

A Thesis submitted to the
Department of Biological Sciences
in partial fulfillment of the requirements for graduation with
Honors in the Major

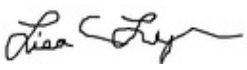
Degree Awarded:
Summer, 2020

The members of the Defense Committee approve the thesis of Cameron D'Orio defended On July 29, 2020.



Dr. Sanjay Kumar
Thesis Director

Dr. Devon Graham
Outside Committee Member



Dr. Lisa Lyons
Committee Member

SPECIFIC AIMS

The pathology of TLE is being continuously uncovered, with current findings suggesting significant hyper excitability in the temporal lobe and hippocampus (Santos et al. 2019), followed by neurodegeneration and neuroinflammation in the hippocampus and dentate gyrus (Kodam et al. 2019; Upadhya et al. 2019). Significant neuronal loss caused by TLE impairs learning and memory in both humans and rodents. Remote and long-term episodic memory deficits are common comorbidities expressed in human TLE models, in which patients exhibit functional and structural damage to hippocampal-parahippocampal networks that encode, consolidate, and retrieve memory for events with spatial and temporal associations. Epileptic rats are thought to display a decline in episodic memory comparable to human-related comorbidities of TLE, for damage to the hippocampus and parahippocampal regions impairs necessary neural networks to consolidate events with spatial and temporal contexts.

Aim 1: Highlight object, spatial, and temporal recognition comorbidities in pilocarpine-treated rats. Integrated memory for object perception, space, and time combine the unique *what*, *where*, and *when* experiences of human episodic memory. Studying episodic memory in rats is made possible through a combination of object recognition tests. The Novel Object Recognition (what), Novel Object Location (where), and Temporal Order (when) memory tasks utilize functional networks between the hippocampus, entorhinal cortex, perirhinal cortex and prefrontal cortex (Ennaceur and Delacour 1988; Ennaceur et al. 1997; Mitchell and Laiacona 1998). When integrated, impairments in neural networks responsible for consolidation of memory with spatial and temporal associations are apparent in pilocarpine-treated rats.

Aim 2: Emphasize TLE pathology in the hippocampus and surrounding cortical structures. Control and TLE-induced rats will be tested for the *what*, *where*, and *when* divisions of episodic memory separately, as well as for a combined episodic memory paradigm. Administering the NOR, NOL, and Temporal Order tasks individually will provide insight into the role of the hippocampus and cortical structures in each context of recognition memory, perception, space, time, and TLE pathology. We then aim to combine object recognition with spatial and temporal associations into one episodic memory task to analyze the damage associated with hippocampus-dependent learning networks. An integrated approach will highlight TLE-related damage to spatial and temporal changes simultaneously, revealing vital information about the role of hippocampal networks in episodic memory.

Aim 3: Combine behavioral paradigms with previous electrophysiology and cell biology studies in therapeutic treatments for TLE. Aim 1 will focus on the introduction of a behavioral paradigm to current and previous research into the pathology and treatment of TLE. In this phase of the study, we will discuss the impact of incorporating findings of episodic memory impairments in rats with research that highlights neurodegeneration, neuroinflammation, and electrophysiological changes found in pilocarpine-treated rats. Research analyzing modifications in learning and memory in rats after treatment with anti-epileptic drugs is limited, specifically research that highlights potential improvements in episodic memory in TLE-treated rats. Previous studies displaying mitigation of neurodegeneration in the hippocampus and medial entorhinal cortex, reduction in hyper excitability of entorhinal neurons, and anti-inflammatory properties of glial cells through D-serine intervention (Kumar et al. *unpublished*) would benefit significantly with the incorporation of modifications of episodic memory following treatment intervention. An integrated study of electrophysiology, cell biology, and behavior would create a sufficient chronic TLE model to expedite treatment methods for TLE-related comorbidities.

RESEARCH STRATEGY

Significance

Object recognition paradigms allow researchers to take advantage of a rat's innate tendency to explore novel environments. These experiments are not limited to specific areas of research and are consistently used in learning and behavior as a reliable measure of cognition and brain function. All four object recognition tests mentioned throughout this study are valuable as they require little habituation, they can be completed in short time frames, and they require no rewards or punishments (Silvers et al 2007). Further, object recognition tests place little to no stress on tested animals, a useful quality when considering increased anxiety-like behavior in epileptic rats.

While it is universally accepted that the perirhinal cortex plays a fundamental role in non-spatial object recognition memory (Brown et al. 2010), the hippocampus becomes essential when encoding object information with spatial or temporal experiences (Hammond et al. 2004; Barker and Warburton 2011a). Whether the hippocampus is fundamental in non-spatial memory remains a controversy, as many studies report little or no changes in novel object perception following hippocampal inactivation (Oliveira et al. 2010; Barker and Warburton 2011b; Cole et al. 2019); however, when animals are allowed longer habituation times, hippocampal lesions impair object recognition memory (Yi et al. 2016; Broadbent et al. 2010; Clark et al. 2000). These results relate to the increase in available experience associated with the novel object. NOR testing in the pilocarpine model of TLE is thus useful in highlighting differences in the pathology of hippocampal-dependent networks. As most damage is seen in hippocampal, entorhinal and subicular networks, the presence of spared OR memory in epileptic rats will support a lack of involvement of each structure in NOR memory. Impairment in the NOR task in epileptic rats would conversely suggest significant damage or lack of circuitry to the perirhinal cortex, or a greater involvement of the hippocampus and entorhinal cortices in OR memory than previously recorded.

Regarding object location memory, research suggests a double dissociation between non-spatial and spatial memory processing within the perirhinal cortex and hippocampus (Chao et al. 2016b; Winters et al. 2004). Damage to the structure and plasticity of the dorsal hippocampus is shown to disrupt object location recognition (De Landeta et al. 2020; Migues et al. 2019; Yu et al. 2018), while inactivation of the perirhinal cortex does not cause sufficient impairment in the NOL task (Barker et al. 2007). Similar disruption in the hippocampus did not have any effect in NOR performance in the same rats (De Landeta et al., 2020; Yu et al. 2018). Considering the studied networks between the perirhinal cortex and lateral entorhinal cortex, lesions to the lateral entorhinal cortex were also unable to impair object location memory (Wilson et al. 2013a, 2013b), while lesions to the medial entorhinal cortex resulted in impaired object location memory (Parron and Save 2004). Increasing significance of the role of the medial entorhinal cortex in object location memory is further emphasized in studies disrupting the lateral entorhinal cortex-medial prefrontal cortex circuits, which failed to impair OL memory (Chao et al., 2017). The visible differences in results of the NOR and NOL tasks are useful in emphasizing the role of the dorsal hippocampus in spatial recognition memory, specifically the likes of allocentric navigation. Both the NOR and NOL are vital memory tasks to sufficiently study rodent episodic memory, along with tasks of temporal order. Within the pilocarpine model of TLE, we hypothesize significant impairment in NOL memory, while sparing impairment in object recognition memory. While performance in the NOR task may decrease slightly when compared to controls due to disruption in hippocampal-dependent networks, the circuity of the perirhinal cortex, lateral entorhinal cortex, and medial prefrontal cortex should remain sufficient for NOR function.

Memory for temporal association in rodents is linked to many brain regions, including the hippocampus (Barker and Warburton, 2011b), entorhinal cortex (Marks et al. 2020), retrosplenial cortex (Powell et al. 2017), medial prefrontal cortex (Aggleton et al. 2020), medial dorsal thalamic nucleus (Cross et al. 2013), perirhinal cortex (Barker and Warburton 2011a), anterior thalamic tract (Dumont and Aggleton 2013), and mammillothalamic tract (Nelson and Vann 2017). A recent study claimed a fundamental role of the hippocampal-entorhinal network in temporal association memory in rats (Marks et al. 2020). Excitatory inputs from pyramidal cells in layer III in the medial entorhinal cortex onto the CA1 pyramidal cells guide temporal association memory, while inputs from calbindin-D28K cells from layer II of the medial entorhinal cortex onto the CA1 function in regulating layer III MEC inputs onto the CA1 through activation of stratum lacunosum interneurons, driving a feedforward inhibition response that regulates MEC-hippocampus activity (Marks et al. 2020). These findings are helpful for chronic TLE models, as they highlight the importance of the direct, temporoammonic pathway between LIII of the MEC to the CA1 of the hippocampus in temporal association memory. The temporoammonic pathway has previously been studied as a direct and fundamental pathway in epileptogenesis (Kumar et al. *unpublished*; Kumar et al. *unpublished*). Incorporation of temporal recency memory in a behavioral paradigm of TLE is thus useful is not only creating a unified model of episodic memory in rodents, but provides essential information highlighting involvement of the temporoammonic pathway in epileptogenesis. While the temporal order task utilizes multiple brain regions and networks for proper function, the greater role of the hippocampal-entorhinal network is useful in displaying impairments in temporal association memory in pilocarpine-treated rats. Epileptic rats will show impaired abilities to organize recency information of objects, exploring all objects equally, rather than proper differentiation of exposure times to each object, with control rats retaining the ability to recall more recent events, leading to greater exploration times for objects familiarized at earlier trials. Failure to report impaired temporal association memory in the temporal order task will entail multi-network sufficiency for rats to encode recency information about events.

Spatial and temporal experiences of episodic memory may be processed using different neural circuits throughout hippocampal sub-regions and parahippocampal structures, however evidence supports a convergence of information at the CA1 sub-region, where spatial and temporal aspects become integrated into complex representations of events (Eichenbaum 2017). The combined memory task for *what*, *where*, and *when* integrated memory (Kart-Teke et al. 2006) is sufficient to highlight complex processing of temporal and spatial information within the hippocampus, entorhinal cortex, perirhinal cortex, medial prefrontal cortex, and postrhinal cortex (Kesner and Rolls 2015). By incorporating objects with spatial and temporal change, exploration of events with multi-dimensional alterations becomes feasible in rats. The importance of testing each aspect of episodic memory, as well as integrated episodic memory is vital to understand communication in the temporal lobe and surrounding cortical structures. Singular testing will highlight fundamental brain regions responsible for object recognition memory, location memory, and recency memory, while combined testing will further examine the role of the hippocampal-entorhinal network in episodic memory, and whether hippocampus-dependent networks can reorganize contextual information about an event in the case of significant hippocampal damage.

Innovation

An integrated behavioral paradigm for episodic memory in rats will quantify data for object perception, space, and temporal consolidation. Previous research places most emphasis on impairments in spatial memory, rather than multiple aspects of memory impairment found in TLE patients (Pascante et al. 2016; Li et al. 2013;

Cavarsan et al. 2013). As new research explores the pathology of TLE and roles of hippocampus-dependent networks in epileptogenesis, reiteration of memory impairment is essential to provide a sufficient model of TLE. Previously, we discovered easement in hippocampal and entorhinal damage resulting from TLE through D-serine intervention in the medial entorhinal cortex (Kumar et al. *unpublished*; Kumar et al. *unpublished*). Further research on the role of hippocampal and entorhinal N-methyl-D-aspartate glutamatergic receptors in TLE pathology, and consequently learning and memory, combined with studies of episodic comorbidities associated with TLE, will create a unified model of TLE that encompasses circuitry, cell biology, and behavior simultaneously. Each area of study serves to emphasize the others, highlighting physiological and psychological pathology in epileptic rats.

Approach

The goals of this study are to conduct four object recognition memory tasks on rats that showcase TLE-related comorbidities in humans. To induce spontaneous recurrent seizures in rodents, rats will be injected intraperitoneally with scopolamine methyl nitrate, followed by a 20-minute waiting period before an intraperitoneal injection of pilocarpine. During the next two hours, seizure frequency and intensity will be recorded to monitor the onset of seizure activity and classification of status epilepticus (SE). After the two-hour period, we will administer Diazepam over the course of 20 minute periods as needed. Following classification of SE or non-SE dependent on the frequency and intensity of seizure activity during the pilocarpine model, rats will be video monitored over the next 29 days for 8 hours/day, 5 days/week. During the 29-day latent period, rats will be categorized into epileptic or non-epileptic experimental groups based on seizure frequency and intensity provided by the Racine scale of seizure severity (Racine 1972). After the 29-day period of video monitoring, rats will begin handling and later habituation to the apparatus.

The experimenter will wear a lab apron and gloves when conducting all experiments. All behavioral testing will be conducted between 8:00am and 5:00pm. Rats will be randomly separated into control (non-SE) and experimental (SE) groups prior to testing, distinguished by cage markings. Half of the animals will receive no treatment, while the other half will undergo epileptogenesis using the pilocarpine-induced spontaneous seizure model. To test disruption of episodic memory in rats, the experimental groups will be induced with spontaneous seizure activity prior to the habituation, familiarization, and retention phases of behavioral testing. If any animal is noticed to have spontaneous seizure activity during testing trials, the animal will be temporarily removed from the apparatus and only tested again after a period of 30 minutes with no seizure activity. Prior to testing, rats must be handled for five days at five minute intervals. This serves to reduce any stress the animal may experience during movement in and out of the apparatus. Throughout testing, video tracking and analysis will be completed through EthovisionXT by Noldus Information Technology, accompanied with a monochrome GigE camera. The software will also aid in defining exploration in rats. Multiple body point and head direction imaging will record behavior as exploratory if the rat is within 1 cm of the object with its head oriented towards the object. Climbing or rearing will not be recorded as exploratory behavior.

The arena is an acrylic chamber provided by Noldus Information Technology with the dimensions of 60 cm x 60 cm x 40 cm. The removable walls and base are painted matte black to eliminate a reflective floor and serve as a contrast to the white-pigmented rats upon video monitoring. Objects chosen for each memory task of the experiment are no larger than double the size of each rat, have a non-reflective surface, and weigh accordingly so the rats are unable to move the objects themselves. Objects may also be shaped accordingly to discourage any climbing, for this activity will not be scored throughout each trial. To provide an allocentric relationship

between rats and objects, a large white stripe will be used on one wall of the arena during the NOL and episodic memory tasks, providing contextual information about the location of each object. To eliminate object location bias, the position of the visual cue in relation to the objects may be altered between experimental groups, along with the use of multiple objects designated for each experimental group (Hale and Good 2005). To eliminate any confounding bias between each test, all memory tasks will be separated by 48-hour periods. The apparatus and objects will be thoroughly cleaned before habituation and in between all experimental trials with 70% isopropyl alcohol and DI water to remove any odors expelled by each animal.

The first task is the Novel Object Recognition task (Ennaceur and Delacour 1988) for non-spatial recognition memory. Following handling, rats will be placed in the empty apparatus with no external cues or objects for five minute intervals, once a day, for five days. Longer habituation periods are linked with greater recognition memory (Yi et al. 2016). Once habituated, rats will begin the familiarization phase of NOR. Two identical objects will be placed in opposite corners adjacent to the same wall, equidistant from the cornered walls. Allowing 10 cm between the objects and walls allows rats to move freely around all sides of each object, as well as allows the video tracking software to accurately map the apparatus. It is important to note that rats must be placed in opposite quadrants of the apparatus with their backs facing towards the objects to remove any initial bias before tracking starts. We will then allow 10 minutes of exploration time in the arena with the two identical objects. Once removed, rats will then be replaced in the arena following a 24-hour inter-trial interval (ITI). The 24-hour ITI is commonly used to test consolidation, rather than acquisition memory (Warburton et al. 2013). Rats will then be placed back into the apparatus; however, one familiar object will be replaced with a novel one. Control rats should differentiate the familiar object with the novel object, exploring the novel object a greater amount. Impaired rats explore the familiar and novel objects equally, as the rats fail to recognize the familiar object. Rats will be allowed 5 minutes in the test phase of the NOR trial.

The Novel Object Location task (Ennaceur et al. 1997) is identical in setup to the NOR task, however instead of replacing a familiar object with a novel object, one of the familiar objects now has a new location. During this task, a visual cue will be placed on one wall of the apparatus to incorporate an allocentric navigational cue to the task. During the familiarization phase, two identical objects will remain equidistant from corresponding corners, identical to the familiarization phase of the NOR task. Rats will have 10 minutes of exploration time during this phase. In the test phase of the NOL task, after a 24-hour ITI, one object will move to an opposite corner of the arena, still equidistant from the adjacent walls. Rats will then have 5 minutes in the test phase to explore the familiar object, and familiar object with a novel location, incorporating a spatial context in object recognition memory. Control rats will be able to consolidate the location of the unmoved familiar object, exploring the familiar object with a novel location for a greater amount of time, whereas impaired rats will explore both objects equally.

The Temporal Order task (Mitchell and Laiacona 1998) will begin like the NOR and NOL, with two identical objects placed equidistant from adjacent corners. This task will have three phases rather than two, as rats are exposed to two familiarization phases. During the first familiarization phase, rats will be allowed 10 minutes to explore two identical objects in the apparatus. Following a 1 hour ITI, rats will be replaced into the apparatus with a different set of two identical objects in the same locations as the previous set. Rats will be allowed 10 minutes to explore the new set of identical objects. After a second ITI of 24 hours, rats will be allowed to explore the same apparatus, however they are now exploring one old-familiar object, and one recent-familiar object. Control rats spend more time exploring old-familiar objects, as consolidation of recent-familiar objects is fresh. Impaired rats will spend an equal amount of time exploring both temporally-exposed objects, as they lose the ability to create temporal associations to object recognition memory.

The episodic memory task (Dere et al. 2005) will consist of two familiarization phases, and one test phase. During this task, a visual cue on one wall will also be necessary to incorporate an allocentric navigational cue. In the first familiarization phase, four identical objects are placed in the arena. Three objects will be placed equidistant from each other along one wall of the apparatus, while the fourth object is placed along the center of the opposite wall. This specific arrangement is essential in a square arena for the test phase of the memory task. Rats will be allowed 10 minutes to explore all four objects, followed by a 1 hour ITI. The second familiarization phase will consist of a different set of four identical objects, however arranged in a different arrangement. After a second ITI of 24 hours, rats will explore the arena for 10 minutes. Two objects from the old and recent familiarization trials will remain in the same location, while two objects from the old and recent familiarization trials will be moved to a new location. This results in four spatially- and temporally-different objects: old familiar stationary, old familiar displaced, recent familiar stationary, and recent familiar displaced. Control rats should be able to differentiate all four objects in a hierarchy of familiarity, exploring the old familiar displaced object the most, and recent familiar stationary the least. Exploration of the old familiar stationary versus the recent familiar displaced would indicate differential emphasis of spatial consolidation versus temporal consolidation. Intact episodic memory is apparent through discrimination of objects in precise locations and of their relative temporal order, demonstrating memory for *what*, *where*, and *when*.

Data analysis will consist of exploration times for each object and their relative novel appearance, location, or temporal order, calculated by (Exploration Ratio (ER) = object exploration time/total exploration time). Discrimination indexes will be calculated for the NOR, NOL, and Temporal Order tasks as [Discrimination Index (DI) = (novel object, novel location, or old familiar ER - familiar object, familiar location, or recent familiar ER)/(novel object, novel location, or old familiar ER + familiar object, familiar location, or recent familiar ER)] (Ennaceur and Delacour 1988; Dix and Aggleton 1999). During the episodic memory task, temporal factor and spatial factor ratios are calculated based on exploration ratios: [temporal factor (TF) = (old familiar stationary ER - recent familiar stationary ER)/(old familiar stationary ER + recent familiar stationary ER)]; [spatial factor (SF) = (recent familiar displaced ER - recent familiar stationary ER)/(recent familiar displaced ER + recent familiar stationary ER). The temporal factor will be calculated based on the comparison of exploration between an old stationary object versus a recent stationary object, while the spatial factor will be calculated based on the comparison of a recently displaced object versus a recent stationary object (DeVito and Eichenbaum 2010). Statistical analysis will be completed through EthovisionXT by Noldus Information Technology. The two-way ANOVA with Bonferroni posttest is useful in comparing ER's for each object in the NOR, NOL, and Temporal Order tasks, while a one-way ANOVA with posttest is useful in comparing the ER's of each object within each group during the episodic memory task (Jiang et al. 2017). Discrimination indexes for spatial and temporal factors between groups in the episodic memory task will be compared using a *T* test with a difference threshold of $P < 0.05$ for all statistical analysis testing.

While the collection of object recognition tasks mentioned above provides vital information relative to human episodic memory comorbidities in TLE patients, it is important to note that most previous studies were based on brain lesioning, knock-out, or optogenetic research. Few examined spatial and temporal comorbidities in pilocarpine-treated rats. All spontaneous object recognition tasks significantly depend on the exploratory behavior of subjects. Potential findings of decreased exploratory behavior in epileptic rats may provide insufficient data to reliably test object recognition. Habituation to the apparatus is thus fundamental to promote anxiety-free behavior and equal amounts of exploration between all control and experimental groups of rats. Another limitation of spontaneous object recognition lies in data interpretation and analysis. While each memory task mentioned above utilizes consistently-researched neural networks, it is easy to oversimplify results

as impairment in select brain regions. While the hippocampus and the entorhinal cortex are primary targets throughout this behavioral paradigm, many hippocampal-dependent and hippocampal-independent processes have been found to play roles in all types of object recognition. Thus, we cannot rule out the influences of extrahippocampal regions in the study of comorbidities of episodic memory in pilocarpine-treated rats. Our focus on the hippocampal-entorhinal networks also reveals alternative strategies that may be useful in the future for similar comorbidity studies. The benchmark being the Morris Water Maze (Morris 1984) task of allocentric navigation. Performance in the Morris Water Maze (MWM) is consistently linked to hippocampal-dependent spatial navigation, NMDA glutamatergic receptor function, and the roles of long-term potentiation and depression in spatial learning (Jeffery and Morris 1993; Bannerman et al. 1995; Moser et al. 1998; Morris et al. 1986). However, incorporation of the MWM task requires careful planning when the test is paired with other behavioral tasks, as the MWM may induce anxiety in tested animals, whereas object recognition tasks require no reward or punishment systems, resulting in stress-free paradigms. Incorporation of the MWM task will also lengthen experimental timelines, while the episodic memory tasks mentioned above can provide accurate and reliable behavioral measures in a relatively short time frame. Within the current proposed episodic memory tasks, only long-term remote memory is considered as we are most interested in consolidation of episodic memory. An efficient alternative strategy would be to incorporate short-term trials into the paradigm, as it will provide information regarding encoding and acquisition in addition to consolidation. One limitation of the spontaneous recognition task is that small changes in timing can produce variable results depending on the desired testing measures. Primarily in NOR and NOL studies, ITI's can be as short as 1 minute (Ennaceur 2010; Ennaceur and Delacour 1988), or as long as 48 hours (Ennaceur and Delacour 1988) depending on the stage of memory the researcher is interested in.

In conclusion, we have sufficient research and technology to include a behavioral paradigm in our current model of chronic TLE. Our goal ties back to understanding the pathology associated with TLE, the involved neural circuitry and networks, and improvements in therapeutic treatment for spontaneous recurrent seizures in rats. Performing an array of spontaneous recognition tasks will not only reveal information regarding the reliability of each memory task, but also provide essential data highlighting damage to hippocampal-dependent networks in the medial temporal lobe known to play fundamental roles in the process of epileptogenesis. Combined with previous and current research, behavioral paradigm additions to TLE studies will create a trifecta with pathophysiological and psychological data, filling longstanding gaps in data collection and opening the door to a visualization of anti-epileptic treatment plans following mitigation of hippocampal and cortical damage.

Timeline

The estimated timeline for the proposed paradigm depends heavily on the number of subjects used for the study. Following the initial 30-day period of epileptogenesis, handling and habituation to the apparatus will take approximately 10 days. Considering a random ordering of spontaneous object recognition tasks that take 2 days each to fully complete, followed by a 48-hour resting period in between each memory task, physical testing alone will take at least 2 months to complete. Fortunately, all control and experimental rats can participate in all memory tasks, as only long-term revisions have been made to each memory task. To incorporate short-term working memory into the paradigm, rats will not be tested in long-term recognition tasks simultaneously, as objects or the tasks themselves will not be considered novel once completed.

REFERENCES

Aggleton, J. P., & Nelson, A. J. D. (2020). Distributed interactive brain circuits for object-in-place memory: A place for time? *Brain and Neuroscience Advances* doi.org/10.1177/2398212820933471.

Bannerman DM, Good MA, Butcher SP, Ramsay M, Morris RGM. (1995) Distinct components of spatial learning revealed by prior training and NMDA receptor blockade. *Nature* 378:182–186.

Barker GRI, Bird F, Alexander V, Warburton EC (2007) Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *J Neurosci* 27:2948–2957.

Barker, GRI, Warburton, EC (2011a) Evaluating the neural basis of temporal order memory for visual stimuli in the rat. *European Journal of Neuroscience* 33(4): 705–716.

Barker, GRI, Warburton, EC (2011b) When is the hippocampus involved in recognition memory? *Journal of Neuroscience* 31(29): 10721–10731.

Broadbent NJ, Gaskin S, Squire LR, Clark RE (2010) Object recognition memory and the rodent hippocampus. *Learn Mem* 17:5–11.

Brown, MW, Warburton, EC, Aggleton, JP (2010) Recognition memory: Material processes and substrates. *Hippocampus* 20(11): 1228–1124.

Cavarsan CF, Queiroz CM, Dos Santos JG, et al. (2013) Reduced hippocampal dentate cell proliferation and impaired spatial memory performance in aged-epileptic rats. *Front Neurol.* 4:106.

Chao, OY, Huston, JP, Nikolaus, S, et al. (2016b) Concurrent assessment of memory for object and place: Evidence for different preferential importance of perirhinal cortex and hippocampus and for promnestic effect of a neurokinin-3 R agonist. *Neurobiology of Learning and Memory* 130: 149–158.

Chao, OY, Nikolaus, S, Brandao, ML, et al. (2017) Interaction between the medial prefrontal cortex and hippocampal CA1 area is essential for episodic-like memory in rats. *Neurobiology of Learning and Memory* 141: 72–77.

Clark RE, Zola SM, Squire LR (2000) Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci* 20: 8853–8860.

Cole, E., Simundic, A., Mossa, F.P. et al. (2019) Assessing object-recognition memory in rats: Pitfalls of the existent tasks and the advantages of a new test. *Learn Behav* 47, 141–155.

Cross, L, Brown, MW, Aggleton, JP, et al. (2013) The medial dorsal thalamic nucleus and the medial prefrontal cortex of the rat function together to support associative recognition and recency but not item recognition. *Learning & Memory* 20(1): 41–50.

De Landeta, AB, Pereyra, M, Medina, JH, et al. (2020) Anterior retrosplenial cortex is required for long-term object recognition memory. *Scientific Reports* 10(1): 1–3.

Denny, C. A., Kheirbek, M. A., Alba, E. L., Tanaka, K. F., Brachman, R. A., Laughman, K. B., Tomm, N. K., Turi, G. F., Losonczy, A., & Hen, R. (2014). Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis. *Neuron*, *83*(1), 189–201.

Dere E, Huston JP, De Souza Silva MA (2005) Integrated memory for objects, places, and temporal order: Evidence for episodic-like memory in mice. *Neurobiol Learn Mem* 84:214–221.

DeVito LM, Eichenbaum H. (2010) Distinct contributions of the hippocampus and medial prefrontal cortex to the "what-where-when" components of episodic-like memory in mice. *Behav Brain Res* 215(2):318-325.

Dix SL, Aggleton JP (1999) Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behav Brain Res* 99:191–200.

Dumont, JR, Aggleton, JP (2013) Dissociation of recognition and recency memory judgments after anterior thalamic nuclei lesions in rats. *Behavioral Neuroscience* 127(3): 415–431.

Eichenbaum, H (2017) Prefrontal–hippocampal interactions in episodic memory. *Nature Reviews Neuroscience* 18(9): 547–558.

Ennaceur A (2010) One-trial object recognition in rats and mice: methodological and theoretical issues. *Behav Brain Res* 215: 244–254.

Ennaceur A, Delacour J (1988) A new one-trial test for neurobiological studies of memory in rats. 1. Behavioral data. *Behav Brain Res* 31:47–59.

Ennaceur, A., N. Neave, J.P. Aggleton (1997) Spontaneous object recognition and object location memory in rats, the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix *Exp. Brain Res*., 113, pp. 509-519.

Hale G, Good M (2005) Impaired visuospatial recognition memory but normal object novelty detection and relative familiarity judgments in adult mice expressing the APPswe Alzheimer's disease mutation. *Behav Neurosci* 119:884–891.

Hammond RS, Tull LE, Stackman RW (2004) On the delay- dependent involvement of the hippocampus in object recognition memory. *Neurobiol Learn Mem* 82:26–34.

Jeffery KJ, Morris RGM. (1993) Cumulative long-term potentiation in the rat dentate gyrus correlates with, but does not modify, performance in the water maze. *Hippocampus* 3:133–140.

Kart-Teke E, De Souza Silva MA, Huston JP (2006) Wistar rats show episodic-like memory for unique experiences. *Neurobiol Learn Mem*. 85:173–82.

Kesner RP, Rolls ET. (2015) A computational theory of hippocampal function, and tests of the theory: new developments. *Neurosci Biobehav Rev*. 48:92–147.

Kodam A, Ourdev D, Maulik M, Hariharakrishnan J, Banerjee M, Wang Y, Kar S (2019) A role for astrocyte-derived amyloid beta peptides in the degeneration of neurons in an animal model of temporal lobe epilepsy. *Brain Pathol* 29:28-44.

Li L-Y, Li J-L, Zhang H-M, et al. (2013) TGFβ1 treatment reduces hippocampal damage, spontaneous recurrent seizures, and learning memory deficits in pilocarpine-treated rats. *J Mol Neurosci.* 50:109–23.

Marks, WD., Yamamoto. N., Kitamura, T. (2020) Complementary roles of differential medial entorhinal cortex inputs to the hippocampus for the formation and integration of temporal and contextual memory (Systems Neuroscience). *European Journal of Neuroscience* doi.org/10.1111/ejn.14737.

Migues, PV, Wong, J, Lyu, J, et al. (2019) NMDA receptor activity bidirectionally controls active decay of long-term spatial memory in the dorsal hippocampus. *Hippocampus* 29(9): 883–888.

Mitchell, JB, Laiacona, J (1998) The medial frontal cortex and temporal memory: Tests using spontaneous exploratory behaviour in the rat. *Behavioural Brain Research* 97(1–2): 107–113.

Montgomery K. C. (1955) The relation between fear induced by novel stimulation and exploratory behavior. *Journal of comparative and physiological psychology 48*(4), 254–260.

Morris RGM, Anderson E, Lynch GS, Baudry M. (1986) Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate antagonist, AP5. *Nature* 329:774–776.

Moser EI, Krobert KA, Moser MB, Morris RGM. (1998) Impaired spatial learning after saturation of long-term potentiation. *Science* 281:2038–2042.

Nelson, AJ, Vann, SD (2017) The importance of mammillary body efferents for recency memory: Towards a better understanding of diencephalic amnesia. *Brain Structure and Function* 222(5): 2143–2156.

Oliveira AMM, Hawk JD, Abel T, Havekes R (2010) Post-training reversible inactivation of the hippocampus enhances novel object recognition memory. *Learn Mem* 17:155–160.

Pascente R, Frigerio F, Rizzi M, et al. (2016) Cognitive deficits and brain myo-Inositol are early biomarkers of epileptogenesis in a rat model of epilepsy. *Neurobiol Dis.* 93:146–55.

Powell, AL, Vann, SD, Olarte-Sánchez, CM, et al. (2017) The retrosplenial cortex and object recency memory in the rat. *European Journal of Neuroscience* 45(11): 1451–1464.

Racine, R.J., Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin *Neurophysiol* 32 (3), 281-294 (1972).

Santos VR, Melo IS, Pacheco ALD, Castro OW (2019) Life and death in the hippocampus: What's bad? *Epilepsy Behav*:106595.

Silvers JM, Harrod SB, Mactutus CF, Booze RM (2007) Automation of the novel object recognition task for use in adolescent rats. *J Neurosci Met* 166:99–103

Upadhya D, Kodali M, Gitai D, Castro OW, Zanirati G, Upadhya R, Attaluri S, Mitra E, Shuai B, Hattiangady B, Shetty AK (2019) A Model of Chronic Temporal Lobe Epilepsy Presenting Constantly Rhythmic and Robust Spontaneous Seizures, Co-morbidities and Hippocampal Neuropathology. *Aging Dis* 10:915-936.

Warburton, E. C., Barker, G. R., and Brown, M. W. (2013). Investigations into the involvement of NMDA mechanisms in recognition memory. *Neuropharmacology* 74, 41–47. doi: 10.1016/j.neuropharm.2013.04.013.

Wilson, DI, Langston, RF, Schlesiger, MI, et al. (2013a) Lateral entorhinal cortex is critical for novel object-context recognition. *Hippocampus* 23(5): 352–366.

Wilson, DI, Watanabe, S, Milner, H, et al. (2013b) Lateral entorhinal cortex is necessary for associative but not nonassociative recognition memory. *Hippocampus* 23(12): 1280–1290.

Winters, BD, Forwood, SE, Cowell, RA, et al. (2004) Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: Heterogeneity of function within the temporal lobe. *Journal of Neuroscience* 24(26): 5901–5908.

Yi, J. H., Park, H. J., Kim, B. C., Kim, D. H., & Ryu, J. H. (2016). Evidences of the role of the rodent hippocampus in the non-spatial recognition memory. *Behavioural Brain Research* 297, 141–149. doi.org/10.1016/j.bbr.2015.10.018.

Yu, JY, Fang, P, Wang, C, et al. (2018) Dorsal CA1 interneurons contribute to acute stress-induced spatial memory deficits. *Neuropharmacology* 135: 474–486.

Committee Comments and Suggestions

Following the thesis defense of the above research, the committee suggested highly useful modifications and concerns when implementing an episodic memory paradigm.

One limitation mentioned above discusses sensitivity of exploration between control and epileptic groups, and the potential to find non-reliable information if one group naturally explores more than the other. This will be taken into consideration through monitoring of locomotor activity, recording of fluctuating weights in epileptic rats, modifying the method of calculating exploration in each rat, and tracking potential tendencies for rats to explore specific parameters of the arena upon habituation. With the help of EthovisionXT, distance traveled and velocity will be traced during habituation trials for both control and epileptic groups, providing data that compares pre-tested exploratory behavior. Predetermined parameters can categorize the arena into multiple quadrants, primarily separating the center of the arena from the area along the walls of the arena. This will show whether animals have the tendency to hug the arena walls (Montgomery 1955), indicating stressful behavior. When calculating exploratory behavior among each group, rather than use the above-mentioned exploration ratio which compared object exploration time to total exploration time, the use of comparing object exploration time to total time in the arena is less susceptible to unwanted variance in exploratory data.

Due to strain sensitivity in rat behavioral testing, it is imperative that rats are tested at the same time of day for all testing. Ideally, this is at the earliest point in their active cycle, such as 9:00 AM. Previous research discusses circadian rhythm dependence in learning and memory, therefore these studies need to showcase consistency in test timing.

When discussing possible incorporation of short-term working memory tasks, the proposition for remote memory studies was also introduced. In mice, remote memories can be stored in the hippocampus for up to 30 days before reorganization to the cortical sites (Denny et al. 2014), highlighting hippocampal involvement in remote consolidation. Remote memory studies would thus be useful in studying the ability of the hippocampus to store information in epileptic animals.

Due to the elaborate timeline involving four object recognition tasks, there is concern regarding animal motivation as tests proceed. All tests will be completed in the same apparatus, raising concerns as to whether rats will want to explore environments in general towards the end of the paradigm. To ease the concern, it is recommended that each test, or some of the tests, be completed in different apparatuses to provide newer environments, thus invoke higher levels of overall exploration. This can consist of a circular arena, rectangular, or square arena of a larger size. Regarding the integrated episodic memory task specifically, a larger size is recommended when using multiple objects, that way animals have sufficient room to explore all quadrants, including the center and walls, and objects comfortably. Concern was also placed on precise choices of objects for each study, specifically in tests with spatial associations. Textural differences in objects may skew exploration times, thus it is highly important that we choose objects that inhibit forms of climbing or rearing.

Upon the discussion of future studies involving the analysis of LTP and LTD influences in episodic memory, specifically in the hippocampus, there was a committee consensus that we need to keep past research, questioning limitations of LTP and LTD in learning, in mind. If research shows there is a limit regarding how much LTD can influence spatial learning, it'd be important to not push the said limit too far with too many tasks that trigger endogenous LTD.

The choice of using rats instead of mice throughout all behavioral tasks was universally agreed upon by the committee, as rats tend to show complex memory more comparable to human memory vs mice, especially in learned tasks.

Acknowledgements

This Honors in the Major Thesis could not have been completed without the immense assistance from Dr. Sanjay Kumar, Dr. Devon Graham, Dr. Lisa Lyons, Dr. Stephen Beesley, the fellow undergraduate and graduate students of the Kumar lab, the FSU Biology Department, and the FSU Honors Department.