ROLE OF PREFRONTAL-HIPPOCAMPAL INTERACTIONS IN AGE-RELATED DEFICITS IN SPATIAL WORKING MEMORY

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Elizabeth Reed Church

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As members of the Master's Committee, we certify that we have read the thesis prepared by Elizabeth Church, titled Role of Prefrontal-Hippocampal Interactions in Age-Related Deficits in Spatial Working Memory, and recommend that it be accepted as fulfilling the dissertation requirement for the Master's Degree.

C. L. Barnes — Date: 5/9/23
Carol A. Barnes, Ph.D.

Stephen L. Cowen — Date: 5/9/23
Stephen L. Cowen, Ph.D.

Torsten Falk — Date: 5/9/23
Torsten Falk, Ph.D.

Final approval and acceptance of this thesis is contingent upon the candidate's submission of the final copies of the thesis to the Graduate College.

I hereby certify that I have read this thesis prepared under my direction and recommend that it be accepted as fulfilling the Master's requirement.

C. L. Barnes — Date: 5/9/23
Carol A. Barnes, Ph.D.
Master's Thesis Committee Chair
Physiology

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Abstract

The aging population is particularly susceptible to cognitive deficits, and as aging progresses, deficits in learning and memory can be observed. The hippocampus (HC) and prefrontal cortex (PFC) are vital regions in decision-making and spatial memory tasks. However, changes in aging are not uniform across these brain structures. Deteriorations in HC-PFC synchrony can lead to alterations in memory encoding, storage, and retrieval, ultimately resulting in age-related deficits in cognitive performance. The medial prefrontal cortex (mPFC) supports working memory, while the HC supports the acquisition of new episodic memories, including spatial memory. Studying age-related changes in these regions, and interactions between these regions, could give us valuable insight into the underlying mechanism of these age-related deficits. To investigate the functional differences of the mPFC and HC with age, healthy young (8 months) and aged (22 months) F344 rats were tested on a multi-stage cognitive behavioral battery of tasks that assess integrated and independent HC and mPFC function. On the Morris watermaze, subjects underwent spatial navigation trials (spatial memory), visual trials, and working memory trials. They were then tested on the temporal order memory task (working memory). The final task is a spatial alternation task rendered with an inbound component (spatial memory) and an outbound component (spatial memory and working memory). An implanted dual-bundle hyperdrive simultaneously records electrophysiological signals from the CA1 regions of the ventral HC and infralimbic and prelimbic regions of mPFC during the spatial alternation task. When comparing the performance of aged and young rats, aged rats were more impaired in the spatial navigation and working memory tasks on the Morris Watermaze, the Temporal Order Memory task, and the outbound component of the spatial alternation task. These results indicate impairments in the PFC and hippocampal regions, as well as interactions between them.

Introduction

The World Health Organization predicts that by 2050, the world's population aged 60 years and older is estimated to nearly double from 12% to 22% which comes to approximately 2.1 billion people. As life expectancies continue to rise, it is critical to understand the normative process of aging to optimize cognitive health throughout the lifespan and generate a baseline of cognitive and neural performance across age groups to compare with those who succumb to neurodegenerative disease.

The aging population is particularly susceptible to cognitive deficits related to executive functions, particularly working memory (Belleville et al., 1996; Glisky, 2007). Working memory is a limited capacity system, supported by the prefrontal cortex (PFC), that involves the active manipulation of information currently being maintained in focal attention (Petrides & Milner, 1982; Goldman-Rakic, 1987; Cowan, 2005; Baddeley, 2003). A decline in working memory can result in deficits in decision-making, problem-solving, and planning of goal-directed behavior (Glisky, 2007). Aging populations are also susceptible to cognitive deficits in long-term memory, more specifically episodic and semantic memory. Episodic memory is responsible for linking personally experienced events set in a spatiotemporal context (Tulving, 1987; Burgess, 2003). Semantic memory, on the other hand, is one's acquired knowledge about the world. Episodic memory usually evokes an "I remember" response, while semantic memory evokes an "I know" response (Kausler, 1994). The acquisition of new episodic memories, including spatial memory, is associated with the hippocampus (Smith & Milner, 1981; Morris et al., 1982). The cognitive map theory proposes that the hippocampus represents environments, locations within those environments, and their contents, thus providing the basis for spatial memory (O'Keefe & Nadel, 1978).

The storage and consolidation of memory is a complex process that involves multiple brain regions and neural computations that are currently not well understood. Two brain regions that are important in the formation and consolidation of memory are the hippocampus and the PFC. The hippocampus is critical in the process of memory integration, particularly for spatial episodic memory. Memory formation is broadly organized into three stages: encoding, ongoing storage, and retrieval (Cohen & Eichenbaum, 1993). The hippocampus is thought to encode and store information related to spatial context, and binds episodic experience in multiple domains to that context. This binding is thought to occur through the coordination of hippocampal place cells to theta oscillations forming distinct sequences (Eichenbaum et al., 1999). O'Keefe and Dostrovsky (1971) referred to hippocampal neurons in freely behaving rats' spatial receptive fields as place cells. Place cells form a map of the space around them with each neuron encoding a specific location in the given environment and thus plays an essential role in spatial navigation and memory. These hippocampal neurons form an ensemble of neurons that collectively fire in phase with a theta rhythm during locomotion (Moffat & Resnick, 2002; Skaggs et al., 1996). When at rest, in both awake and sleep states, this ensemble activity is reactivated at faster timescales. This reactivation preserves the order that the neurons originally fired in and can be detected in local field potentials as high-frequency oscillations termed sharp-wave ripple (SWR) (Axmacher et al., 2006; Diekelmann & Born, 2010). Ongoing storage and retrieval of memory occur through synaptic consolidation facilitated by the reactivation of these ensembles through hippocampal oscillations (McGaugh, 2000).

However, the hippocampus is not solely responsible for memory functions but instead integrates this information with other brain regions. A part of the neocortex called the PFC is specifically associated with executive functions, including working memory, and functions

related to making decisions that guide behavior. This type of memory is much more short-term but does have the potential to be consolidated long-term (Miller, 2001). The PFC, however, does not influence working memory independently and relies on projections to and from the hippocampus. Previous studies have demonstrated unidirectional, ipsilateral, and monosynaptic projections from the hippocampus to the PFC (Ferino et al., 1987; Goldman-Rakic & Friedman, 1991; Jay et al., 1989; Jay & Witter, 1991; Rosene & Van Hoesen, 1977; Swanson, 1981). The PFC does not directly project to the hippocampus but is routed via sub-regions of the thalamus and via direct projections to the entorhinal cortex. These neural projections to and from the hippocampus and PFC are often referred to as the hippocampal-prefrontal circuit. Previous hippocampal lesioning studies have demonstrated that during a task that tests for both spatial and spatial working memory, HC-mPFC projections are required for spatial working memory performance. Hippocampal lesions compromise the circuit's ability to function, and the ability to consolidate new spatial memories (Morris et al., 1982). Conversely, PFC lesions damage working memory performance but not the formation of episodic spatial memories **(**Wang & Cai, 2006).

Two prevailing theories of memory consolidation are the dual-system theory and the multiple trace theory. The dual-system theory proposes that memory is initially stored in the hippocampus, but over time these memories are transferred to the neocortex, and eventually become independent of the hippocampus. When new memories are created, they initially depend on both the hippocampus and neocortex, but as time passes memory consolidation occurs. Memory consolidation is a process by which the hippocampus guides information to be stored in the neocortex so that it eventually becomes independent of the hippocampus. This idea can be applied to both episodic and semantic memories (Müller and Pilzecker 1900; Lechner et al.

1999). This theory is supported by studies of patients with retrograde amnesia (Squire et al., 1995). Nadel and Moscovitch (1997) proposed multiple memory trace theory (MTT), which challenged this standard model of memory consolidation. MTT emphasized that the role of the hippocampus is in fact not temporary and is always involved in the retrieval of spatial episodic information. MTT proposes that every time novel information is presented it is neurally encoded, thus the hippocampus is constantly storing and retrieving information. However, semantic memory is represented in the neocortex. Electrophysiological studies in rodents and fMRI studies in humans show both hippocampal and neocortical activity during memory consolidation, thus providing evidence of continued PFC-hippocampal interactions and supporting the multiple-trace theory (Euston et al., 2021; Morici et al., 2015; Preston & Eichenbaum, 2013; Ryan et al., 2001).

Electrophysiology is the study of the electrical properties of biological cells and tissues and is key to the study of brain circuits. When studying the electrophysiological activity produced during behavioral tasks, we look for specific signatures in the patterns of neural activity that correlate with specific aspects of the behavior. Neural oscillations, specifically theta oscillations, are prevalent during locomotion and are important in spatial navigation, as place cells fire during specific phases of theta (O'Keefe & Recce, 1993). Neurons in the hippocampus and PFC phase lock to hippocampal theta (Skaggs et al., 1996, Jones & Wilson, 2005) with high-phase coherence of theta oscillations occurring during memory-guided decision-making or correct trials. (Benchenane et al., 2011; Spellman et al., 2015). However, as aging occurs hippocampal place cell representations become less stable (Barnes et al., 1997; Schimanski et al., 2013). Mixed results have been observed in studying hippocampal theta with some reporting no age differences (Abe et al., 1999) and others reporting a reduction in theta frequency in old rats

during locomotion (Shen et al., 1997). Recently, Crown et al (2022) found that young rats hippocampal theta frequency is higher across all speeds when compared to aged rats. However, the rate at which theta frequency increased was the same across running speeds in both aged and young rats.

Additionally, high-amplitude oscillations known as SWRs (sharp wave ripples) can be utilized as indicators of memory consolidation and/or retrieval events (Kudrimoti et al., 1999; Buzsáki et al., 2015). SWRs occur both during awake states and slow-wave sleep. During sleep, temporal synchrony of hippocampal SWRs and prefrontal cortical spindles improves hippocampus-dependent memory (Maingret et al., 2016). mPFC neurons temporally correlate with awake SWRs to influence decision-making on spatial tasks (Sin et al., 2019; Tang et al., 2021). Both awake and sleep-state SWRs carry information about past and future trajectories which are consolidated for spatial memory. These high amplitude oscillations are the result of synchronous depolarization of CA1/CA3 neurons that occur during rest in either awake or sleep states (Buzsáki et al., 1983; Buzsáki et al., 1992). However, a reduction in oscillation frequencies corresponds to behavior slowing, and the frequency of SWRs and the content of replayed trajectories vary with age (Wiegand et al., 2016). One study demonstrated that when SWRs in the hippocampus are disrupted, spatial working memory processes are affected (Jadhav et al., 2012). The frequency of SWRs in sleep is significantly less in aged animals (Wiegand et al., 2016). Additionally, gamma frequency in the PFC is slower in aged animals (Insel et al., 2012), and PFC pyramidal cells undergo increased inhibition in aging (Carpenter et al., 2016). These studies suggest variability in age-related changes in neural activity across both the hippocampus and medial prefrontal cortex, independently. This is further reason to believe that tasks that are

dependent on hippocampal-mPFC synchrony are likely to be impaired with aging due to a lack of synchrony of neural activity at multiple timescales between these regions.

While hippocampus-dependent activity is affected by aging, the number of neurons in the hippocampus does not decrease, suggesting alterations in hippocampal functioning rather than cell number(Calhoun, 1998). Hippocampal plasticity defects also occur during the aging process, resulting in changes in synaptic communications which can be detrimental to memory consolidation (Nicholson et al., 2004). Since there is no general loss of neurons, we expect to see a lack of synchrony in neural activity between the two regions (Rapp & Gallagher, 1996). Deteriorations in hippocampal-PFC synchrony can lead to alterations in memory storage and retrieval. As aging progresses, defects in learning and memory can be observed, especially in navigation tasks. Self-motion and environmental cues are integral parts of spatial navigation and require hippocampal place and grid cells to operate. Place cell firing characteristics can become altered during the process of aging. Older memory-impaired rats often generate new spatial maps upon re-exposure to familiar spatial environments, which is referred to as global remapping (Barnes et al., 1997). Additionally, memory retrieval becomes more challenging with age as sleep-dependent memory deteriorates in older individuals. Given that these regions are particularly susceptible to the effects of aging it is critical for us to investigate the changes in the circuit, as it could give us valuable insight into age-related deficits and disease. We plan to accomplish this via behavioral and electrophysiological studies.

There are two distinct age groups in these studies, young (9-month-old rats) and old (23-month-old rats). The behavioral component of the study assesses the subject's baseline hippocampal and PFC integrity by having them undergo a multi-stage cognitive behavioral battery. The Morris watermaze evaluates hippocampal function by conducting trials meant to test

spatial memory, visual capabilities, and working memory. The temporal order memory task tests working memory, thus testing the mPFC. Afterward, subjects are trained on the linear track, where they learn to transverse a track and collect food rewards. After linear track training, subjects undergo hyperdrive implantation surgery so electrophysiological data can be collected as the rats complete the spatial alternation task. The W-Maze spatial alternation task (Frank et al., 2000), is the final stage in the behavioral battery and is designed to test spatial memory and spatial working memory capabilities. A functional HC-mPFC circuit is essential, as both brain regions must cooperate to successfully perform the task. Together we aim to assess the performance of aged rats on tasks that independently require hippocampal and prefrontal cortex integrity as well as an intact HC-mPFC circuit to understand how these regions are affected with age.

Methods

Behavioral Battery

The subjects for this study are male Fischer 344 rats that are categorized at the start of the behavioral battery as either “young” (8 months) or “old” (22 months). Rats are single-housed and maintained on a reverse 12hr light/12hr dark cycle. These rats are tested on a multi-stage cognitive behavioral battery to assess baseline hippocampal and PFC integrity and observe the differences in memory function that can be observed between age groups.

Morris Watermaze

The Morris watermaze is the first apparatus utilized in the cognitive behavioral battery. The Barnes’ Lab adaptation of the Morris watermaze involves the use of a white circular pool that is 120cm in diameter and 36cm deep. The pool is divided into 4 equal quadrants by a virtual vertical and horizontal line generated by the program ANY-maze. Each quadrant has a designated platform insert at the bottom of the pool that allows a removable platform to either be slightly submerged or raised above the water line. The circular platform is 11.5cm in diameter and has velcro tape on the surface to help the rats to grip the platform and climb on top of it. To prevent the subjects from being able to see the platform while it is submerged below the water, the water is made opaque with non-toxic chalk. The pool is further divided by 8 location markers along the circumference of the pool, that are equidistant from each other. These are the locations where the rat will be released into the water. Animal handling and data recording occur in the room adjacent to the apparatus. While the trials are being conducted, the experimenter views the rats' position via a video camera that is recording above the pool.

Spatial Navigation

The spatial navigation trials are designed to evaluate the rats' spatial localization capabilities utilizing the distal cues surrounding the Morris Watermaze apparatus (Figure 1). The opaque water prevents the rats from seeing the platform when it is submerged under the water, thus forcing the rats to rely on the distal cues (posters) in the rooms to create a representation of space in order to navigate to the escape platform.

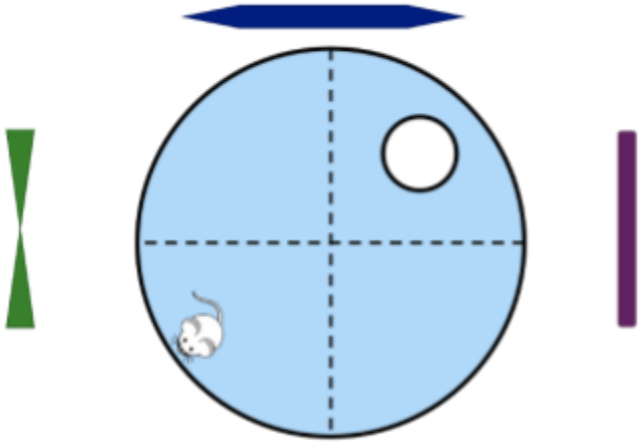

Figure 1. Schematic diagram of Morris Watermaze apparatus with platform, distal cues, and quadrant divisions.

The first trial conducted is the acclimation trial. The subject is placed on top of the platform for 30 seconds so that they can acclimate to the new spatial environment. Afterward, the subjects start the spatial navigation task, which lasts for 4 consecutive days with 6 trials per day. Throughout the spatial navigation task, the platform remains in the same quadrant. Rats are released into the pool at one of eight randomized start locations, with their head facing the pool's edge. The trial ends either when the rat climbs on top of the platform, or when the rat has swum for 60 seconds. In cases where the rat does not find the platform within the allotted 60 seconds, the rat is led to the platform with a rod covered in black and yellow electrical tape and sits atop the platform for 30 additional seconds before being dropped at the next location. The video camera above the pool records the rat's swim path.

Visual Trials

After the completion of the spatial navigation trials, the rats participate in the visual navigation trials for 2 consecutive days, 6 trials per day. The visual navigation task is conducted with a similar methodology as the spatial navigation task, except in this task the platform is elevated above the water line so that the black electrical tape around the platform is visible. Additionally, the platform is moved to a different location at the start of every trial. The aim of this task is to identify any rats that have visual defects. Any rats that fail the visual trials are excluded from the rest of the cognitive battery.

CIPL Score

Different release locations are varying distances from the escape platform. This and the fact that there are differences in the rats' swimming velocities means there is variability in the latency it takes for an animal reach the escape platform. To ensure comparability of the rats' performance across different release locations a corrected integrated path length (CIPL) score is calculated. The CIPL score measures the difference between the rat's cumulative distance from the drop location to the escape platform (Figure 2, purple line) against the

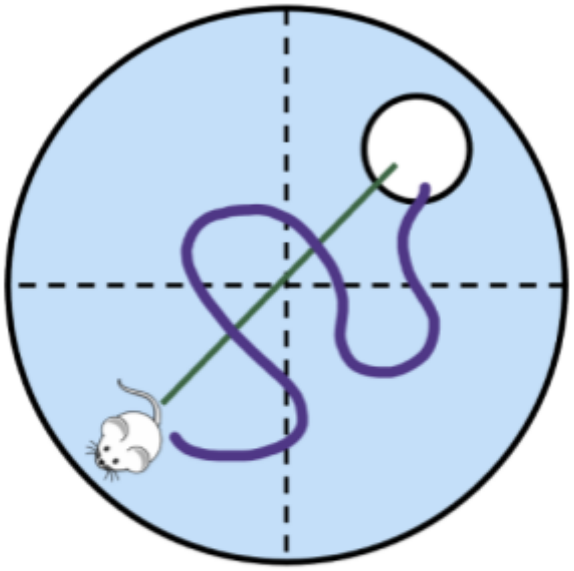

Figure 2. Example CIPL score calculation. CIPL score is the difference between the subjects' actual swim path from the release location and the platform (purple line) and the most efficient path from the release location to the platform (green line).

most efficient path to the escape platform (Figure 2, green line). The most efficient path from the starting point to the platform is typically a straight line. Therefore, as the rats learn the task and start spending more time swimming toward the escape platform, we would expect their CIPL scores to decrease over the course of the trials. (Gallagher et al., 1993).

Working Memory Trials

The final set of trials performed with the Morris watermaze apparatus is the working memory trials. Using the same apparatus, a different spatial environment is created by changing the distal cues and switching the color of the curtains on the walls from white to black. Working memory trials last for 9 days, 2 trials per day, with the escape platform's location changing daily. This task operates in 2 phases: the informational trial and the retention trial. The information trial acts as an opportunity for the animal to learn the location of the platform. The retention trial then tests the animal's working memory capabilities, to see if the subject can find the platform at the same location they were exposed to in the information trial. Retention trials are conducted after a 30-second, 30-minute, or 2-hour delay. All animals are tested within the same delay time for that day; eventually, all animals will complete the task with each delay 3 times. As the delay time increases, so does the working memory demand.

Latency

For the working memory trials, the latency difference between the information and retention trials is utilized as the measure of performance, not the CIPL score. During the working memory trials, path length does not vary as much as it does during the spatial navigation trials since the starting location is consistent between animals. Additionally, velocity is not affected

because the difference between the information and the retention trail times is performed by the same rat. Hence, latency is the more appropriate form of measure. A smaller latency value is indicative of worse performance as it shows a lack of improvement in the time it took the rat to reach the platform during the retention trial. A larger latency value is indicative of improved performance.

Temporal Order Memory Task

The apparatus for the temporal order memory task is a 50 x 50 x 30cm (L x W x H) wooden box, painted black. Objects for exploration are made from nonporous materials and are secured to the floor using reclosable fasteners. The room is lit by several overhead red lights. Prior to the start of the task, the subjects are food restricted and steadily brought down to ~80-85% of their starting weight. This is done to encourage foraging behaviors throughout the trials. On days 1 and 2, the animals are habituated to the empty apparatus for 10 minutes. On days 3 and 4 the subjects undergo a trial consisting of 3 phases: 2 exploration phases and 1 test phase (Figure 3). During the 1st exploration phase, the subject is given 4 minutes to explore a pair of novel identical objects (Figure 3, object A) followed by a 1-hour delay period. The same procedure is followed for the 2nd exploration phase, with a different pair of novel identical objects (Figure 3, object B) presented. After a 2-hour retention period, the test phase is

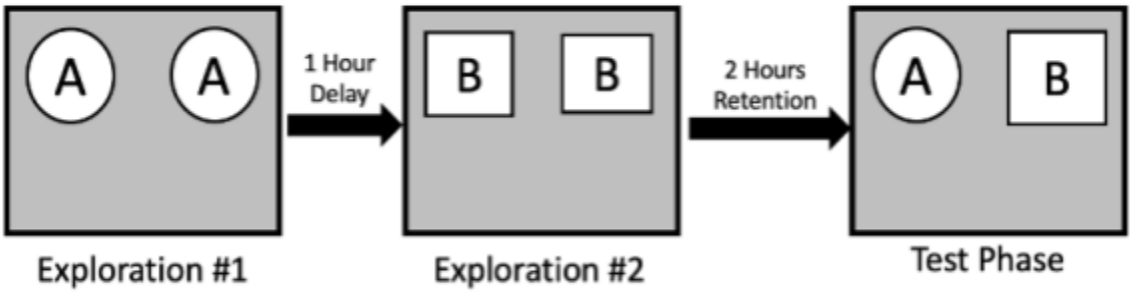

Figure 3. Schematic diagram of the progression of the temporal order memory task across trial periods.

administered using one object from both exploration phases. During the test phases, the object positions were counterbalanced so that each subject experienced both potential configurations. Different objects were used on each day of the task.

Discrimination Score

To evaluate the rat's performance on the temporal order memory task a discrimination ratio is calculated from the test phase scores. To calculate the discrimination ratio for context and recency variables the difference between the exploration time of the last object (object B) and the first object (object A) is calculated and divided by the total exploration time. A positive discrimination ratio indicates a novelty preference, a negative discrimination ratio indicates a familiarity preference, and a discrimination ratio of 0 indicates no preference for either object. Any subject that showed insufficient exploration was excluded from this data set. This included if subjects did not explore both objects during either exploration phase or if their total exploration time during the test phase was less than 10 seconds.

$$\frac{t_{last\ object} - t_{first\ object}}{t_{total}} = \textbf{Discrimination Ratio}$$

Linear Track

If the rats do not have any visual or motor impairments (as determined in the cued version of the Morris Watermaze) they continue onto linear track training for 7-10 days. The linear track apparatus consists of a 150cm long track that dispenses liquid food rewards into feeder dishes at both ends of the track (Figure 4). These animals continue food restriction during linear track training to encourage the rats to collect the liquid food rewards. The rat must run the entire length of the track to receive the reward, and no reward is given for consecutive repeat visits to the same food dish. The food rewards are dispensed into feeder dishes via solenoids,

which are triggered by an automated system controlled by the Arduino Uno microcontroller. Infrared motion sensors planted next to the feeder dishes are hooked up to the microcontroller. When the rat breaks the plane of the sensor, it triggers the opening of the solenoid to dispense a 50:50 mixture of water and vanilla Ensure™ into the feeder dish. A computer application tells the experimenter how many laps were completed and how many errors the animal made. Additionally, a camera mounted on the ceiling allows the experimenter to watch the rat while in the adjacent recording room.

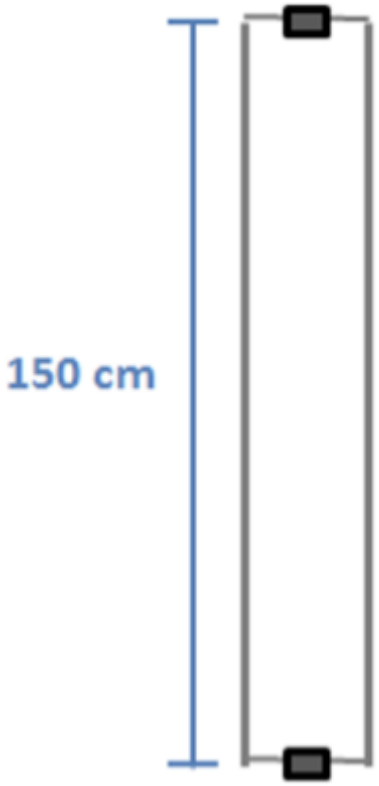

Figure 4. Schematic of the linear track apparatus.

The room with the apparatus is set up with white curtains facing the track and 4 distal visual cues (posters) are hung up at the 4 directional points of the maze. The visual cues and the color of the curtains are important in the creation of the spatial environment. During the task, the main room lights are turned off and low illumination is instead kept on.

The purpose of the linear track is to train the rats to traverse the track to collect liquid food rewards, while also assessing the speed and motivation of the rats to shuttle back and forth for food rewards on the track. Rats are trained once a day and the length of the sessions increases gradually over the days until they are capable of reaching the criteria of collecting 30 food rewards in 30 minutes for 2 consecutive days. If an animal is not able to reach the criteria after 3 weeks of training, it will be excluded from the cohort.

Yoking

The rats are yoked to maintain consistency in the number of trials, to account for the difference in physical ability between the two groups, and to ensure that the better-performing rat is exposed to the same number of training laps as the worse-performing rat.

Based on performances on the linear track, the two rats from each age group that performed the best are yoked together and the two second-best-performing rats are yoked together. In these yoked rat pairs, the rat that performed worse on the linear track will run the spatial alternation task first, typically this is the older rat as it is assumed they might be physically unable to run the same number of laps as the younger rat. The first rat will run until they reach the criterion for the spatial alternation task. The rat that performed better on the linear track will then run until they reach the same number of total laps as the first rat. In cases where the rat that typically runs first outperforms the rat that runs second on the task, the experimenter will switch the order in which the rats are run the following day in order to maintain consistency in the total number of laps run.

Spatial Alternation Task: W-Maze

Rats are tested on the spatial alternation task (W-Maze) for 21 sessions. No prior exposure is given on the W-Maze, so all learning of the task is done during these 21 sessions. The recording room that contains the W-Maze apparatus is the same room that is used during linear track training. A different spatial environment is created by switching the colors of the curtains on the walls from white to black and changing the distal cues (posters) on the walls. The automated food-dispensing system is also controlled by the Arduino Uno microcontroller. At the

end of each arm of the W-Maze, there are three solenoids, three feeder dishes, tubing, and three infrared motion sensors hooked up to the microcontroller. Similar to linear track training, when the rat breaks the plane of the sensor, it triggers the opening of the solenoid to dispense the liquid food reward into the feeder dish. Again, the computer application will tell the experimenter how many laps the animal completed, how many errors the animal made, and which arm is the next correct destination for the animal. Additionally, a camera mounted on the ceiling records video data for future behavioral analysis.

The W-Maze is a continuous spatial alternation task that consists of an inbound and outbound component. The inbound component is designed to test spatial memory and the outbound component tests for spatial working memory. The rat starts the W-Maze task at the base of the center arm. Once the subject runs down the length of the center arm, they receive their first food reward. They then must return from the end of the center arm and are then tasked with choosing whether to go down the right or left outbound arm (Figure 5a). They must then run down the length of the outbound arm to receive their next food reward

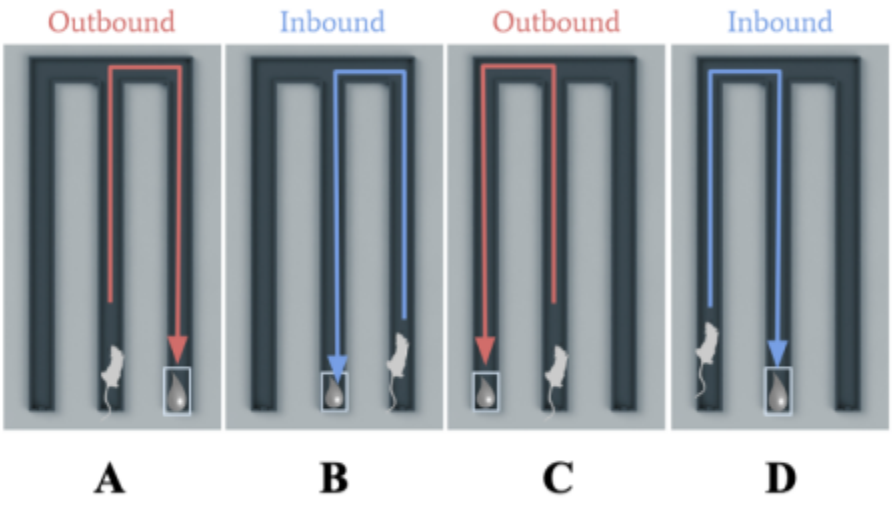

Figure 5. Progression of the spatial alternation (W-Maze) task with W-Maze apparatus. The rat is placed at the bottom of the center arm at the start of the trial **A)** After running to the end of the center arm a liquid nutrient reward is dispensed. The rat must them visit one of the outbound arms **B)** return to the center arm **C)** and visit the outbound arm not previously visited. **D)** The rat will then return to the center arm and repeat this alternating pattern.

and return to the end of the center arm (inbound trial) (Figure 5b). To receive a food reward, the subject must run down whichever outbound arm they did not initially visit (outbound trial) (Figure 5c) and return to the end of the center arm (Figure 5d). This results in a continuous alternation pattern. For example, if the rat went down the right outbound arm the first time it must return to the center arm and then proceed down the left outbound arm to receive food reward. If the rat reaches the end of the correct arm they are rewarded with liquid nutrients. They do not receive a food reward if they visit the incorrect arm of the track. The criteria for this task are that the rat either runs for 1 hour or until they reach 30 correct outbound laps.

Hyperdrive Implantation

After two yoked pairs are identified, they are surgically implanted with a custom-built dual-bundle micro-manipulator, known as a hyperdrive. The hyperdrive allows the experimenter to manipulate the placement of tetrodes along the dorsal-ventral axis post-surgery. The first 8 tetrodes are designed to target the medial prefrontal cortex (mPFC), while the second 8 tetrodes are designed to target the ventral CA1 region of the hippocampus (vHC). The implant coordinates are mPFC (prelimbic and infralimbic regions) (AP 3.0, ML:0.5, DV:2.0-5.5) and vHC (CA1 regions) (AP -5.8, ML 5.5, DV 5.8-7.2).

Figure 6. Dual-Bundle hyperdrive placement

Hyperdrives are implanted in the right hemisphere, as previous inactivation studies have demonstrated that rats can lateralize place learning to the right hippocampus (Fenton & Bures, 1993). This trend in lateralization can also be seen in humans. Maguire et al. (2000) conducted a study of the brain anatomy of London taxi drivers compared to age-matched nontaxi drivers, and found significant differences in gray matter volumes in the right posterior hippocampus. Abrahams et al. (1997) also demonstrated spatial memory deficits in patients with right hippocampal damage. Due to this trend in lateralization, the hyperdrive is always implanted in the right hemisphere of the brain (Burgess 2002). During the W-Maze sessions the dual-bundle hyperdrives record neural activity at a single unit and population level from the mPFC and vHC. To help eliminate excess noise from the environment, cerebellar references are utilized, along with 2 reference tetrodes, one in each bundle. The overhead camera records the rats' position and speed and is tracked by LEDs on the head stage.

When electrophysiological recordings take place, the hyperdrive is connected to a tether attachment. This tether consists of a quick-clip head-stage and cable that connects the hyperdrive to the Neuralynx electrophysiology data acquisition system. The rats sleep 30 minutes prior to the start of the W-Maze run and 30 minutes after the run to compare neural activity before and after training on the task. These sleep sessions are conducted while the animal is in a rubber pot with a towel, and while connected to a tether.

Electrolytic Lesioning

Once the W-Maze task is concluded, the location of the tetrodes from the dual-bundle hyperdrive needs to be verified. The first step in the histological verification process is electrolytic lesioning. An electric current of 20μA is passed through each individual tetrode for

10 seconds. This is done to promote the formation of a glial scar and to release iron deposits from the tips of the tetrodes. A few days after lesioning, the animals are perfused with a 4% Paraformaldehyde solution. The brains were then extracted and stored in a 30% sucrose solution, so the brains are cryoprotected during the sectioning process. After the brains have reached a similar density as the sucrose solution, the brains are hemisected, and coronally sectioned at 40 microns on a cryostat.

Histology

Two stains are applied to the collected tissue sections: Nissl stain and Prussian Blue stain. The Prussian Blue stain (Figure 7) allows us to visualize the last recording position of the tetrode bundles by staining for iron deposits released during electrolytic lesioning.

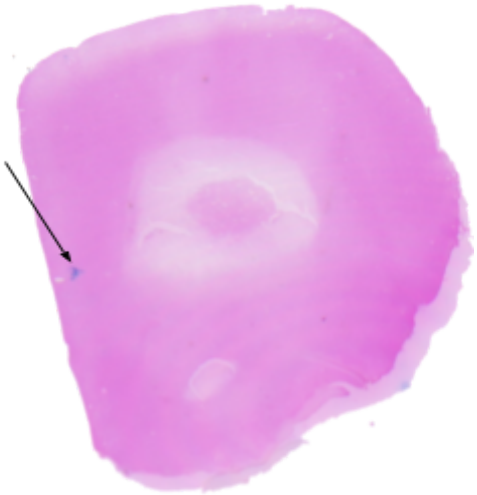

Figure 7: Coronal section of mPFC stained with Prussian Blue stain. Blue spot indicates iron deposits released from the end of tetrode track.

The Prussian Blue staining procedure starts by dehydrating the tissue in washes of 95% and 100% ethanol, followed by a clearing wash using Xylene. The tissue is then rehydrated and placed into a solution of 10% potassium ferrocyanide and 20% HCl solution. This solution is referred to as the Prussian Blue stain and will cause the iron deposits in the tissue to appear blue. The slides are then rinsed in three separate washes of Milli-Q H2O before being counterstained with 0.15% Basic Fuchsin, which gives the stain a pink color making the blue dots easier to identify. The slides are again rinsed with Milli-Q H2O, then submerged in 95% and 100% ethanol for

final dehydration, and submerged in xylene for the final clearing. Slides are coverslipped immediately after, using toluene.

The Nissl stain (Figure 8), stains chromophilic free ribosomes on the rough endoplasmic reticulum, revealing the morphology of the tissue and the trajectory of the tetrodes. For Cresyl Violet staining, we dehydrate the mounted tissue by submerging the slides in ethanol washes of increasing concentration, from 50% to 70% to 95% to 100%. This process removes residual salts and water from the tissue. The slides are then placed in Histoclear, a clearing agent used to make the tissue transparent, for 5 minutes. The slides are then rehydrated by putting the tissue in the same concentrations of ethanol, in decreasing order from 100% to 50%, followed by deionized H2O. This process effectively rehydrates the tissue and removes excess Histoclear. The slides are then submerged in a 0.5% Cresyl Violet stain until the desired levels of color are achieved. Finally, the slides go through final differentiation, dehydration and clearing washes. Slides are immediately cover-slipped using toluene.

Figure 8: Coronal section of mPFC stained with Nissl stain.

Results

Morris Watermaze

Spatial Navigation Trials

For the spatial navigation trials in the Morris watermaze subjects were $n_{old} = 36$ and $n_{young} = 32$. To ensure comparability of the rats' performance across different release locations with varied swimming distances to the platform, and to account for differences in the subject's swimming velocities, a corrected integrated path length (CIPL) score was used as the performance metric on this task. CIPL score measures the difference in the rat's cumulative distance from the drop location to the escape platform against the most effective path to the escape platform. As the rats learn the task and start spending more time swimming toward the escape platform, we would expect their CIPL scores to decrease over the course of the trials. (Gallagher et al., 1993). All rats displayed a trend of improvement on the task as CIPL scores decreased across the 4 days of learning (Figure 9).

An unpaired t-test was conducted to investigate age-related differences on each day of the task. When comparing the two age groups, young animals achieved significantly lower CIPL scores than the old animals on trial days 2, 3, and 4 (Table 1). Bonferroni corrected p-values were calculated to reduce the possibility of type 1 error occurrence. Over the course of the trials, the difference in performance between young and old animals increased in magnitude, with young animals performing better on every day of the task (Figure 9).

Trial Day #	μ_{old}	μ_{young}	σ_{old}	σ_{young}	SEM_{old}	SEM_{young}	p-value	df
Day 1	34.5268	29.7519	16.7525	16.8331	2.7921	2.9757	0.246	66
Day 2	29.1891	16.8551	16.2931	16.0361	2.7155	2.8348	0.0025	66
Day 3	17.8010	6.5347	15.8863	8.9392	2.6477	1.5802	0.0007	66
Day 4	15.3056	3.2618	15.3805	4.9149	2.5634	0.8688	0.0001	66

Table 1. CIPL Score mean (μ), standard deviation (σ), standard error of mean (SEM), degrees of freedom (df), and p-values for young and old F344 rats during spatial navigation trials on Morris Watermaze apparatus.

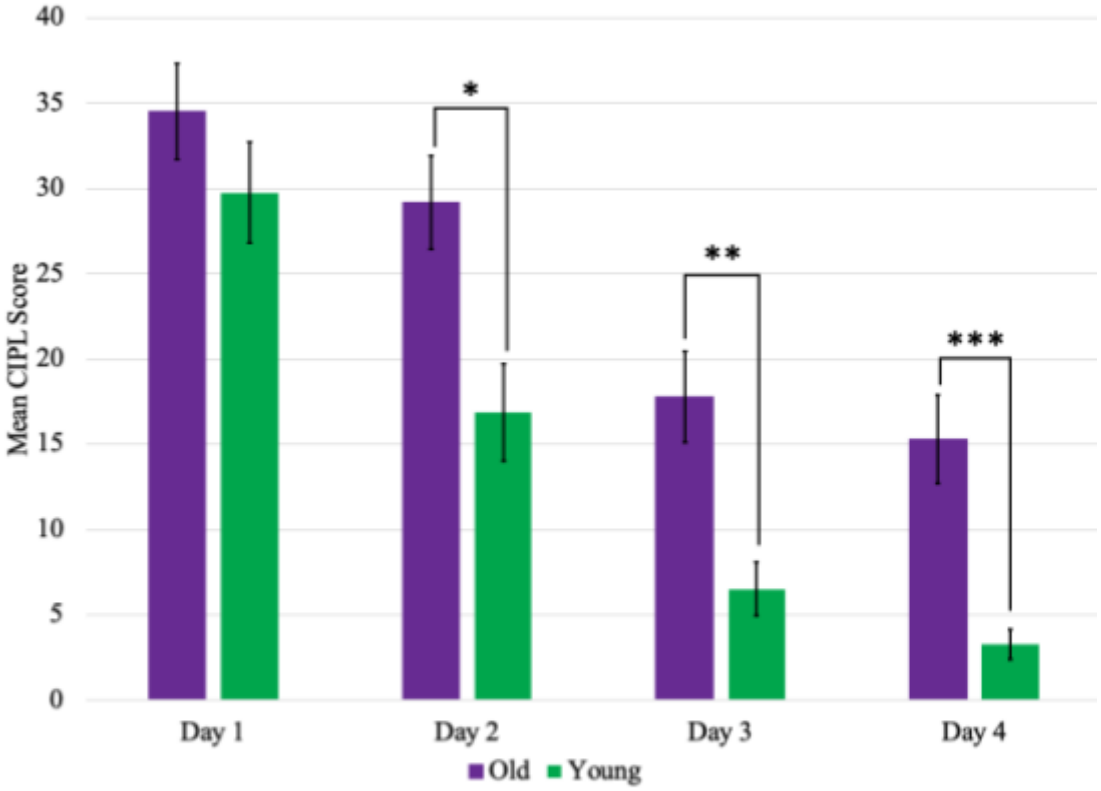

Figure 9. Performance of young and old F344 rats on the Morris Watermaze task during spatial navigation trials. Shown are the mean CIPL scores (±SEM) and Bonferroni-Corrected p-values: *p <0.005, **p<0.001, ***p<0.0001.

Visual Trials

To confirm the spatial memory impairments in older animals were not due to visual deficits, and to ensure that all rats had adequate vision to participate in the remainder of the behavioral battery, the rats performed a visually cued version of the Morris watermaze. For the visual navigation trials, the subjects were $n_{old} = 36$ and $n_{young} = 32$ with one old rat being excluded from the dataset due to visual impairments (Table 2). While the performance of all rats improved over the course of the 2 days, no significant difference was found between the age groups on either day of the task, or in the overall group performance between days 1 and 2 (Figure 10).

Trial Day #	μ_{old}	μ_{young}	σ_{old}	σ_{young}	SEM_{old}	SEM_{young}	p-value	df
Day 1	3.3305	3.2356	5.6189	6.6727	0.9365	1.1796	0.9494	66
Day 2	2.2781	1.6021	4.0585	2.2374	0.6764	0.3955	0.4063	66

Table 2. CIPL Score mean (μ), standard deviation (σ), standard error of mean (SEM), degrees of freedom (df), and p-values for young and old F344 rats during visual trials on Morris Watermaze apparatus.

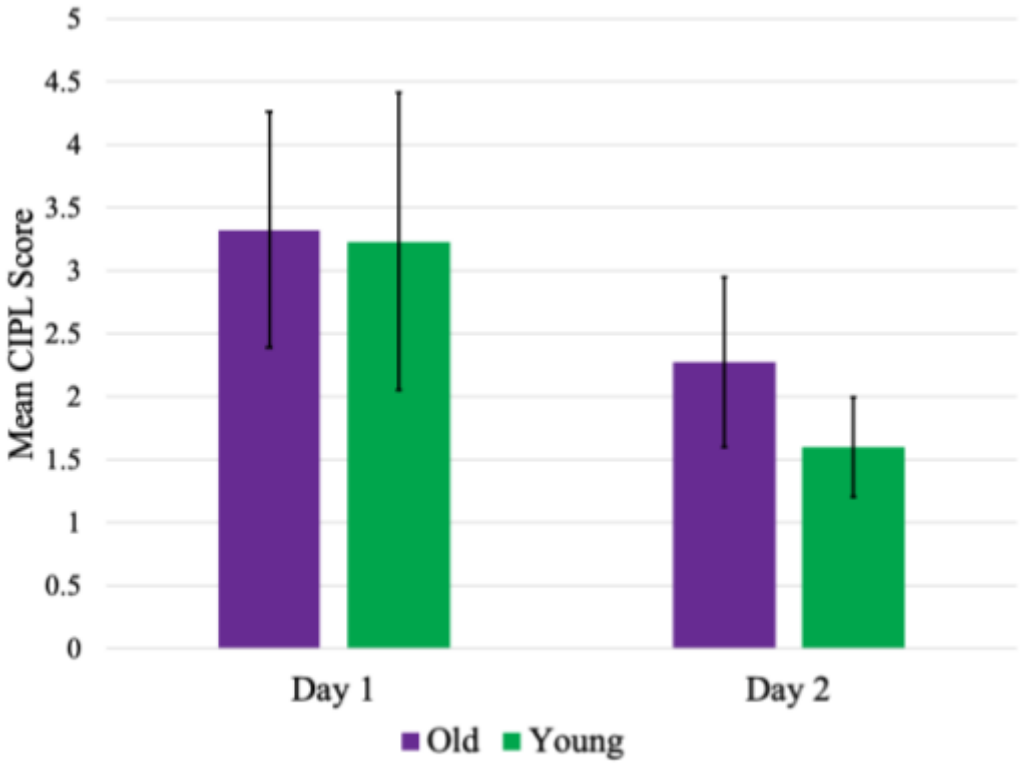

Figure 10. Performance of young and old F344 rats on the Morris Watermaze task during visual trials. Shown are the mean CIPL scores (±SEM).

Working Memory Trials

For the working memory trials in the Morris watermaze subjects were $n_{old} = 15$ and $n_{young} = 13$. The time taken to reach the platform in the information and retention trials is calculated. The performance metric utilized for working memory trials on the Morris watermaze apparatus is the difference in temporal latency between the information trial and the retention trial. This is due to the fact that optimal path length does not vary as it does during the spatial navigation trials, because the starting location is consistent between animals. Additionally, velocity is not affected because the difference between the information and the retention trail times is performed by the same animal. Latency directly correlates with the temporal component of working memory. A smaller latency value is indicative of worse performance as it demonstrates a lack of improvement in the time it took the rat to reach the platform during the retention trial. A larger latency value is indicative of improved performance (Table 3). When evaluating the performance of all animals across the three conditions, there is a trend of animals performing worse as the time from retention and information trials increases. The mean

Condition	μ	σ	SEM
0.5min	2.9029	2.6639	0.5034
30min	2.2439	3.8731	0.7319
120min	0.5018	2.0744	0.3920

Table 3. Latency difference between the informational and retention trials. Mean (μ), standard deviation (σ), standard error of mean (SEM), degrees of freedom (df), and p-values for all F344 rats during working memory trials on Morris Watermaze apparatus.

Condition Comparison	p-value	df
0.5min v. 30min	0.4615	54
30min v. 120min.	0.0406	54
120min v. 0.5min	0.0004	54

Table 4. Unpaired t-test p-values, and degrees of freedom (df) for comparisons of the time conditions of working memory trials on Morris Watermaze apparatus. All subjects included in dataset, not separated by age.

latency time difference progressively decreases as the time between the information and retention trials increase (Figure 11a).

When comparing overall animal performance across the 3 conditions, there is a significant difference between the performance on the 0.5min condition and the 120min condition, with subjects performing considerably worse in the 120min condition (Table 4).

Condition	μ_{old}	μ_{young}	σ_{old}	σ_{young}	SEM_{old}	SEM_{young}	p-value	df
0.5min	2.0633	3.8718	2.4936	2.6105	0.6438	0.7240	0.0724	26
30min	1.6906	2.8825	3.4766	4.3389	0.8976	1.2034	1.4172	26
120min	0.3327	0.6969	2.3479	1.7813	0.6062	0.4941	0.6519	26

Table 5. Latency difference between the informational and retention trials. Mean (μ), standard deviation (σ), standard error of mean (SEM), degrees of freedom (df), and p-values for young and old F344 rats during working memory trials on Morris Watermaze apparatus.

Breaking this performance down by age group (Figure 11b), reveals a trend of both young and old rats performing worse as the time from initial exposure increases. The younger rats outperform the older rats in all 3 conditions, but there is no statistically significant difference when comparing the age groups in each of the conditions (Table 5). The only statistically significant difference occurred when comparing the performance of the young rats in the 0.5min and 120min conditions of the task (Table 6). All other comparisons were considered not statistically significant by Bonferroni corrected p-values.

Age Group	Condition Comparison	p-value	df
Young	0.5min v. 30min	0.488	28
	30min v. 120min	0.1059	28
	120min v. 0.5min	0.0014	28
Old	0.5min v. 30min	0.7383	24
	30min v. 120min	0.2204	24
	120min v. 0.5min	0.0604	24

Table 6. Unpaired t-test p-values, and degrees of freedom (df) for comparisons of the time conditions of working memory trials on Morris Watermaze apparatus. All subjects included in dataset, separated by age group.

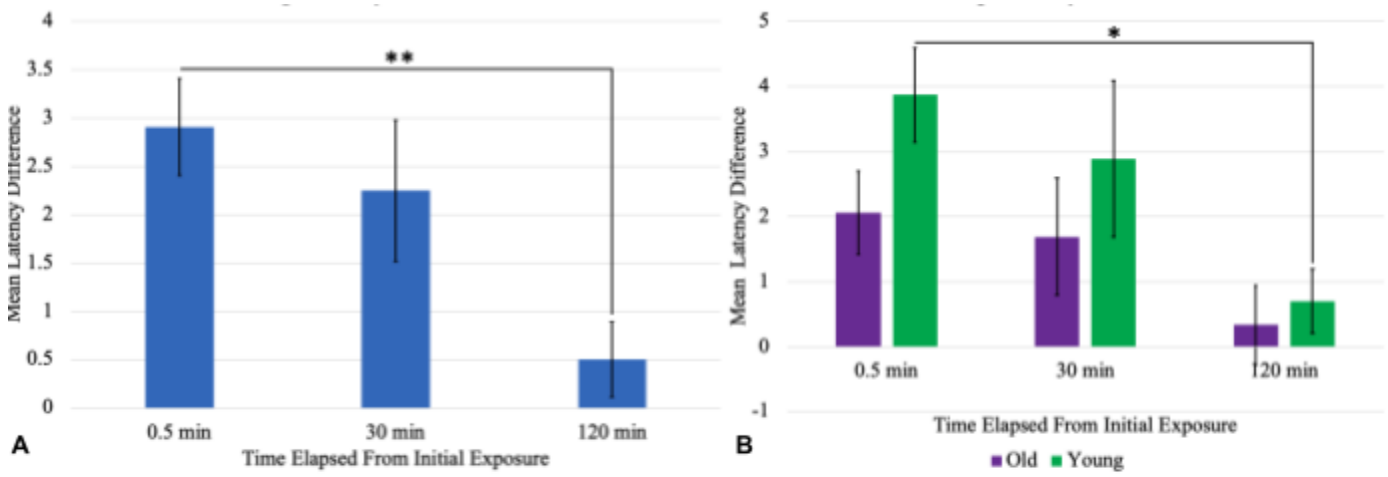

Figure 11. **A)** Overall performance of F344 rats on working memory trials Morris Watermaze apparatus. Shown are the mean latency difference (±SEM) for each condition. **B)** Performance of young and old F344 rats on the Morris Watermaze task during the working memory trials. Shown are the mean latency difference (±SEM) for each condition *p<0.005556, **p<0.001111, ***p<0.0001111.

Temporal Order Memory Task

To evaluate the rat's performance on the temporal order memory task a discrimination ratio is calculated from the test phase scores. To calculate the discrimination ratio for context and recency the difference between the exploration time of the last object (object B) and the first object (object A) is calculated and divided by the total exploration time. A positive discrimination ratio indicates a novelty preference, a negative discrimination ratio indicates a familiarity preference, and a discrimination ratio of 0 indicates no preference for either object. Several rats from both age groups were excluded either for not meeting the threshold for exploration (4 seconds of overall exploration for the test phase), or for not exploring both objects during either exploration phase. Video recordings were scored for time spent exploring each object by a scorer who was blind to the conditions. Discrimination ratios and object exploration times from the test phase were analyzed using a mixed-effects model and post hoc analysis was completed using Šídák's multiple comparisons test (Day 3: Old v. Young p-value = 0.6228, Day

4: Old v. Young p-value = 0.0305*). There is a trend of discrimination ratios, for both age groups, decreasing on day 4, which is indicative of worse performance, and then rising on day 5 (Figure 12). When compared to the young rats, older rats have lower discrimination ratios on all days of testing. However, none of the comparisons are statistically significant (Table 7).

Test Day #	n_{old}	n_{young}	μ_{old}	μ_{young}	σ_{old}	σ_{young}	SEM_{old}	SEM_{young}	p-value	df
Day 3	17	16	0.07548	0.2175	0.3001	0.2610	0.0905	0.0754	0.158	31
Day 4	12	12	0.00719	0.1007	0.3430	0.3229	0.1296	0.1142	0.4991	22
Day 5	5	4	0.1636	0.4633	0.1406	0.2518	0.0531	0.0890	0.0568	7

Table 7. Number of subjects (n), Discrimination Ration mean (μ), standard deviation (σ), standard error of mean (SEM), degrees of freedom (df), and p-values for young and old F344 rats during Temporal Order Memory task.

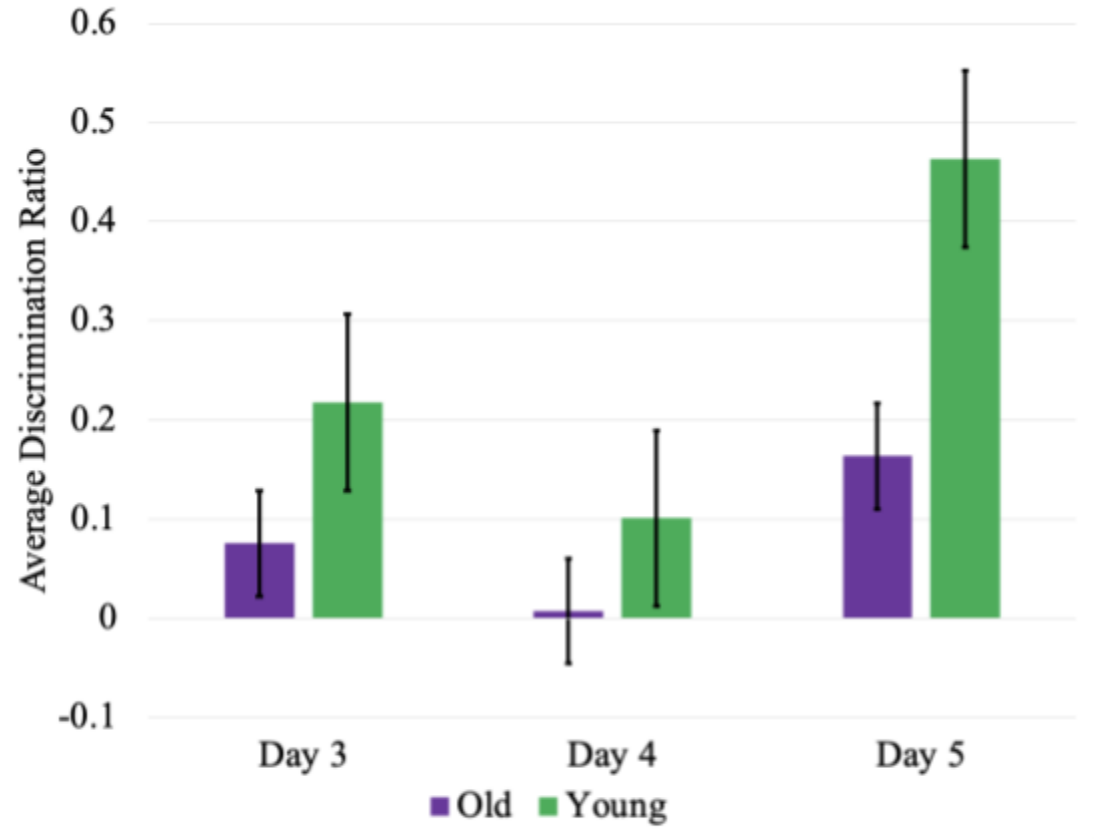

Figure 12. Performance of young and old F344 rats on the Temporal Order Memory task. Shown are the discrimination ratio mean (±SEM).

Spatial Alternation Task: W-Maze

12 young and 12 old rats successfully completed the spatial alternation task. Group performance was measured using a two-way repeated measures ANOVA and individual and group learning was evaluated using a hierarchical state space model (Kapellusch et al., 2018).

Two-Way Repeated Measures ANOVA

When comparing the percentage of correct inbound vs. outbound trials over the course of the 21 sessions, both age groups reach near 100 ±5% accuracy for the inbound component of the task with the older animals taking longer to reach near 100 ±5% correct (Figure 13a). Meanwhile, on the outbound portion of the task, the older rats make significantly more errors than the younger rats. Young animals typically achieve an average of 80% correct by the end of the 21 sessions, while older animals end at an average of 65% accuracy (Figure 13b). A two-way repeated measures analysis of variance (ANOVA) is utilized to compare the group performance of young and aged animals. No significant difference between age groups was found when performance across all days was compared. However, there was a significant difference in performance

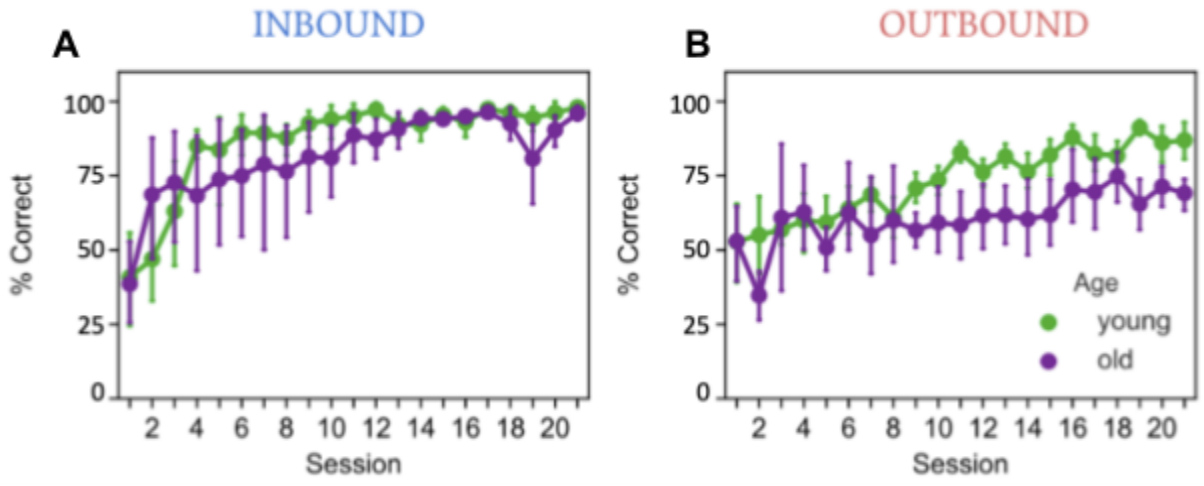

Figure 13. Percentage correct for old (purple) and young (green) F344 rats on **A)** inbound ($F_{(1,24)}$ = 1.9, p-value = 0.33 repeated-measures ANOVA) and **B)** outbound ($F_{(1,23)}$ = 33.42, p-value < 0.001 repeated-measures ANOVA) components of spatial alternation task over the course of 21 sessions.

between the two groups when comparing the inbound ($F_{(1,24)} = 1.9$, p-value = 0.33) and outbound ($F_{(1,23)} = 33.42$, p-value < 0.001) components of the task.

Learning Sessions

Young and old animals learn the two components of the W-Maze task at different rates. An animal is considered to have "learned" the task when the lower 90% confidence bound of its performance is above chance and stays above chance for the remaining trials in the testing period. The session number where the animal successfully achieves learning criteria is the "learning trial." For example, if an animal reaches the learning criteria for the outbound component of the task during session 17, and continues to remain above the learning criteria for sessions 18, 19, 20, and 21, session 17 would be considered their "learning trial" for the outbound component.

Learning sessions for the inbound (Figure 14a) and outbound (Figure 14b) trials for each age group were plotted and compared using the nonparametric Mann-U Whitney test. Both age groups of animals learn the inbound component of the task at similar rates. However, older animals

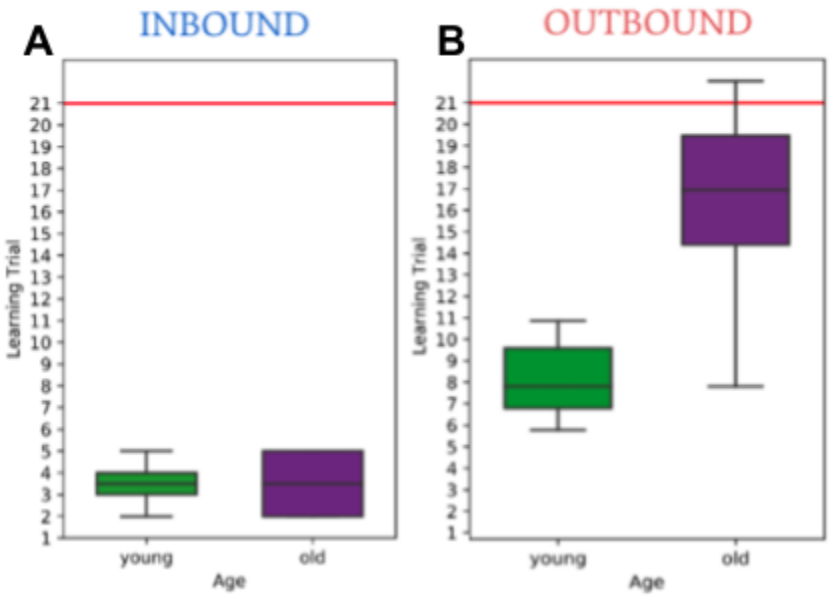

Figure 14. Median learning session for the **A)** inbound [young = 3.32 ± 1.14, old = 4.41 ± 3.09] p-value = 0.6 and **B)** outbound [young = 7.6 ± 3.45, old = 17.4 ± 5.78] p-value = 0.008 components of the task for both the young (green) and old (purple) F344 rats. The red line signifies the end of the 21 sessions.

learn the outbound portion of the task significantly later than younger animals (Table 8).

Trial	U	μ_{old}	μ_{young}	σ_{old}	σ_{young}	p-value
Inbound	2.9	4.41	3.32	3.09	1.14	0.6
Outbound	18.5	17.4	7.6	5.78	3.45	0.008

Table 8. Test statistic for the Mann-Whitney U Test (U), mean (μ), standard deviation (σ), and p-values for young and old F344 rats during Spatial Alternation (W-Maze) task.

Hierarchical State-Space Model

A hierarchical state-space random effects model is used to fit the data for each rat to get an estimate of learning across the two groups (Smith et al., 2004). Figure 15 shows the learning curves from the state-space analysis of each individual animal for inbound (Figure 15a) and outbound (Figure 15b) decisions across the 21 sessions All animals' performance tends to improve across time, with both age groups learning the inbound component of the task quicker than the outbound component. However, younger animals learn the inbound component of the task significantly faster than older rats. Additionally, the results support the evidence that old rats overall cannot match the younger rats' performance on the outbound component.

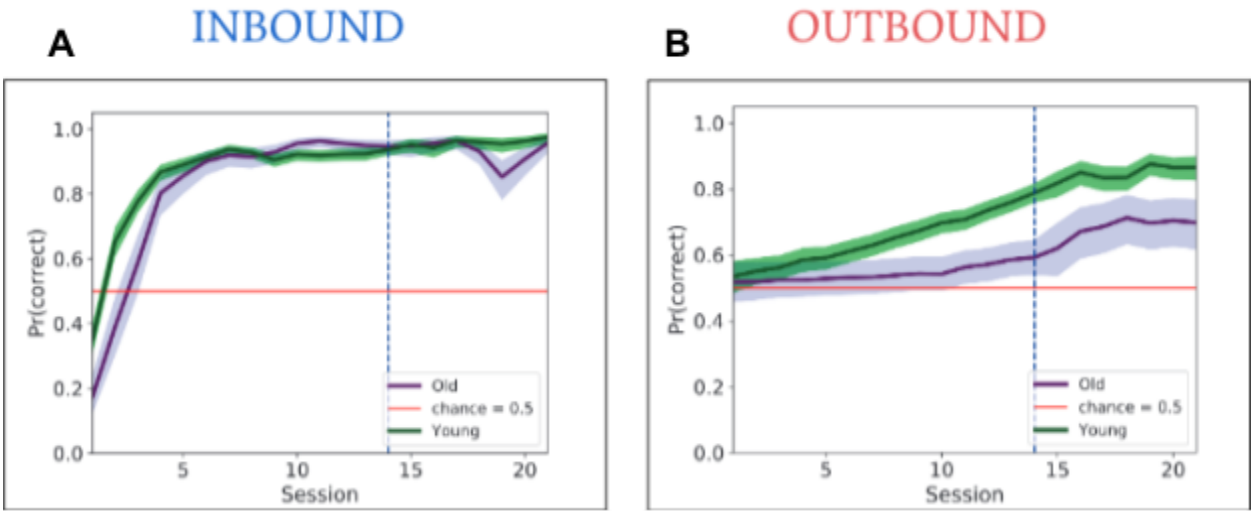

Figure 15. Hierarchical state space model, and the resulting learning curves for **A)** inbound and **B)** outbound components of the task for both the young (green) and old (purple) F344 rats.

Histology

Previous histology revealed that the tetrodes were not in the target location in both the hippocampus and the mPFC. The anterior tetrode bundle was angling laterally towards the motor cortex (Figure 16a), and the posterior tetrode bundle was also angling laterally, past the ventral hippocampus (Figure 16b).

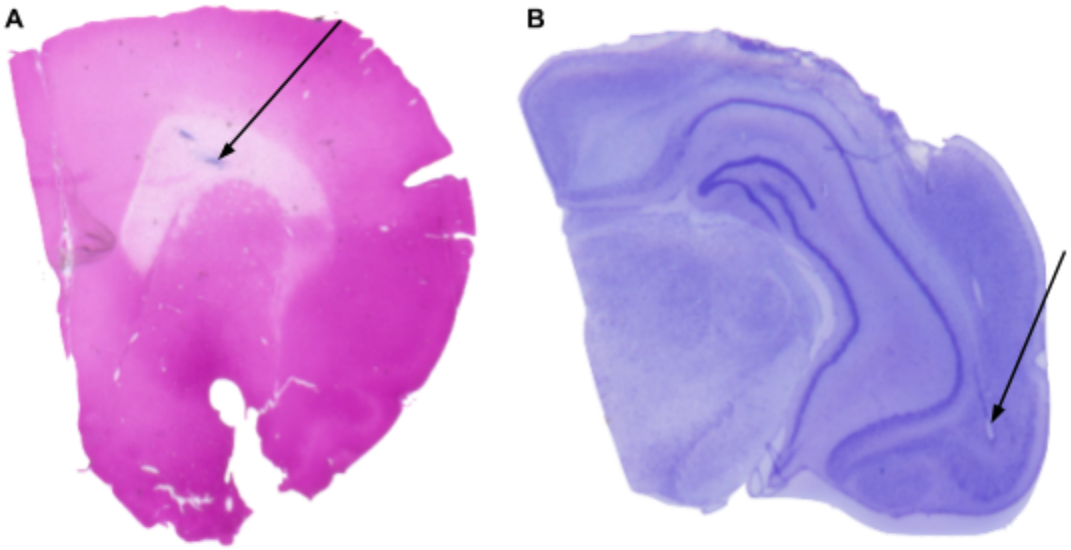

Figure 16. **A)** Coronal sections of mPFC region. Tetrode tip is angled too laterally, and ends in the motor cortex, not the desired mPFC region. **B)** Coronal sections of the ventral hippocampus, tetrodes are too lateral and are not in target location.

After modifications were made to the hyperdrive design, more recent histology has revealed that we are in the target location of the mPFC (Figures 17a and 18a) and ventral hippocampus (Figures 17b and 18b).

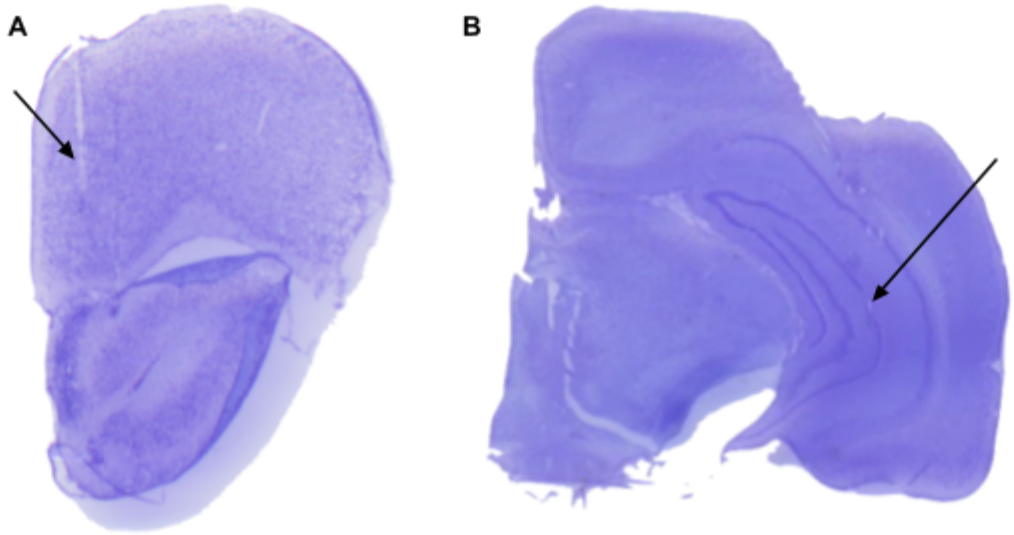

Figure 17. **A)** Coronal sections of the mPFC region stained with Nissl stain. **B)** Coronal sections of the ventral hippocampus region stained with Nissl stain. Tetrodes are in the desired regions.

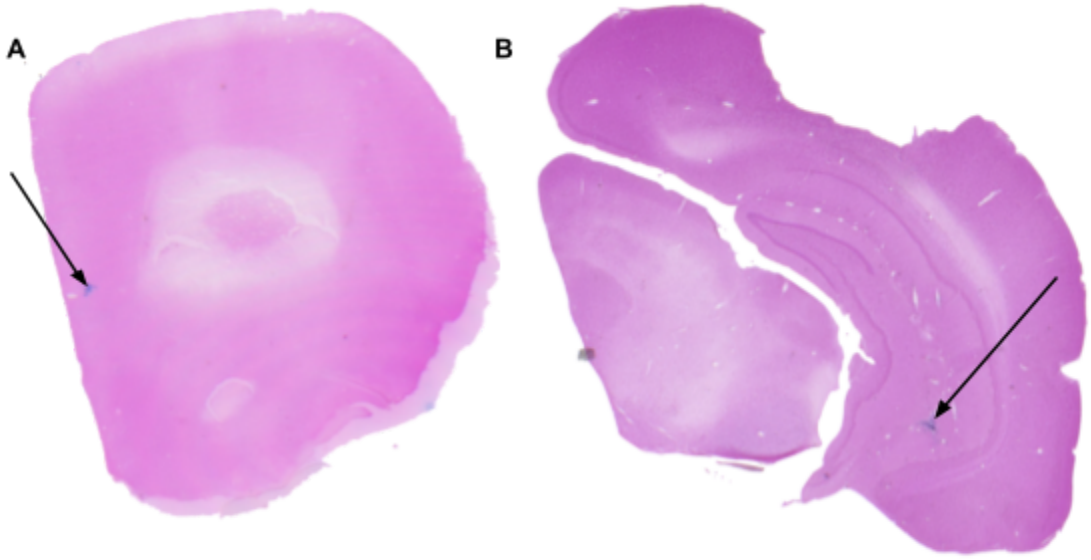

Figure 18. **A)** Coronal sections of the mPFC region stained with Prussian Blue stain. **B)** Coronal sections of the ventral hippocampus region stained with Prussian Blue stain. Tetrodes are in the desired regions.

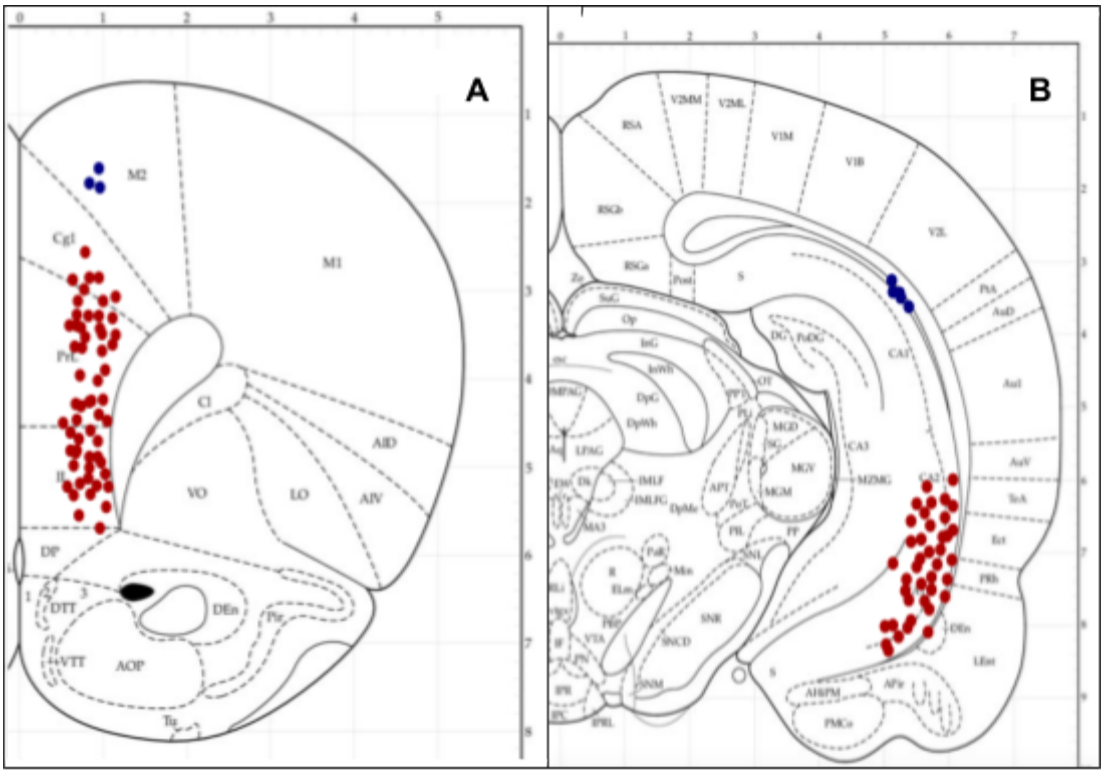

Figure 19. Locations of the last recording position of the tips of the tetrodes. Locations of the tips of the tetrodes in the **A)** medial prefrontal-cortex (mPFC), and the **B)** ventral hippocampus (vHC). Maps of brain coronal sections are modified from those contained in the atlas of Paxinos and Watson (1986).

Discussion

As normative aging occurs, it is known that cognitive performance declines. This decline can be attributed to several regions of the brain; however, it is not well understood how these changes occur over time and how these changes work in concert with each other. Some mechanisms that are particularly vulnerable to age-related declines are mechanisms involved in functional plasticity, executive functions, learning, and memory (Burke & Barnes, 2006). However, neuroimaging studies have revealed that an aging brain may undergo both neural decline and compensatory neural recruitment. These findings support the scaffolding theory of cognitive aging, which proposes that there are compensatory cognitive processes that attempt to alleviate age-related declines in cognitive function by preserving certain cognitive abilities (Park, & Reuter-Lorenz, 2009). Recent reports have stated that in normally aged animals, some age-related changes in neural plasticity enhance network function (Gray & Barnes, 2015).

The hippocampus and PFC are two vital structures for learning and memory (Goldman-Rakic, 1987; Morris, Garrud, Rawlins, & O'Keefe, 1982; O'Keefe and Speakman, 1987). The mPFC is critical for working memory, which contributes to behaviors involved in decision-making, problem-solving, and planning goal-directed behavior. Meanwhile, the hippocampus is critical for memory integration, particularly for spatial episodic memory. Declines in these regions lead to deficits in spatial memory, working memory, and spatial working memory. Each stage of the behavioral battery is designed to target these regions both individually and collectively.

The spatial navigation trials of the Morris watermaze target spatial memory, and thus tests the integrity of the hippocampus. While aged animals did improve over the course of the trials, their CIPL scores were significantly higher than the younger animals. This implicates

significant impairments to the older rat's spatial memory capabilities across all days of learning when compared to the young rat. Since the visual trials demonstrated that all rats had adequate vision, this suggests that the aged rats' performance is the result of impaired hippocampal function.

The working memory trials of the Morris watermaze target working memory capabilities, and thus tests the integrity of the mPFC. There is a trend of older animals not recalling the location of the platform on the retention trial as much as their younger counterparts during any of the delay conditions. Older animals experiencing impairments in working memory performance is indicative of impairments in prefrontal cortex function, however, the results concluded that the difference in performance was not significantly affected by age. There are caveats with completing the working memory trials on the Morris watermaze apparatus. Older rats fatigue quickly after one round of swimming, which affects their latency scores on the task. Additionally, the Morris watermaze working memory trials take close to two weeks to complete and would take place immediately after the spatial navigation trials. The increased physical stress on the animals, and the amount of time it took to complete these tasks, can increase the rate of deterioration in older animals by the time they get to the spatial alternation task. The working memory portion of the Morris Watermaze has since been retired and replaced with the temporal order memory task that can also reflect working memory but is not as strenuous.

The temporal order memory task is an efficient way of testing the integrity of a subject's working memory capabilities. The results indicate that the aged rats are impaired compared to the younger animals in their ability to recall which object was most recently experienced. Animals with mPFC lesions show impairments in discriminating between the temporal order of presented objects (Mitchell and Laiacona, 1998; Barker et al., 2007). Older animals show a

similar trend. However, it is worth noting that in early test phases, there was a variance in aged rats' performance between days 3 and 4. This difference was previously thought to be due to an object preference anomaly. However, a baseline object preference test for all objects has been conducted to eliminate objects that rats tend to gravitate towards for non-task-related reasons. None of the results from the object preference test proved to be statisically significant. Other explanations for this difference include the fact that earlier cohorts that participated in the task were not food restricted as much as later cohorts were, and may have resulted in the animals not displaying as much foraging behavior as other subjects. Another potential explaination for this difference is that the exploration time criteria needs to be longer. Barker et al. (2007) requires their subjects to meet a criteria of 15 seconds of exploration time for the exploration phase and 10 seconds of exploration time for the test phase.

The final behavioral task was the W-Maze spatial alternation task. This task consisted of two components: an “outbound” or alternation component that tests for spatial working memory capabilities, and an “inbound” component requiring spatial memory capabilities. Cooperation between the mPFC and hippocampus is required to successfully complete the task, particularly the outbound trial. The results revealed that older rats made more errors overall and took longer to reach the learning criterion on the W-Maze spatial alternation task than did younger rats. When evaluating the individual parts of the task, aged rats made more errors in the outbound trials. However, overall performance on the inbound component was not significantly affected by age. This performance deficit on the outbound component in aged rats suggests not only impairments in individual brain regions but impairments in hippocampal–prefrontal interactions.

In summary, older rats made more errors overall on all stages of the behavioral battery than did the younger rats. Older animals were most impaired in the spatial memory and spatial

working memory aspects of the tasks. The deficit in spatial working memory indicates disruptions in the interactions between the mPFC and the hippocampal systems. The aging hippocampus does not undergo a loss of cells or synapses (Calhoun, 1998), but does show evidence of changes in population activity and place cell dynamics (Barnes 1997, Schimanski 2013). Conversely, reduced gamma frequency was found in the PFC of aged rats during both behavior and rest (Insel et al., 2012). Together this suggests that the dynamics of the neural synchrony between these regions change with age. We are continuing to investigate these changes. To that end, future directions of this work should include an integration of the electrophysiology recordings from the mPFC and hippocampus in correlation with behavioral performance. By implanting rats with modified hyperdrives, and through histological verification, we have ensured that we can accurately and stably record from both regions. Integration of these data sets for both age groups will provide a more thorough insight into the electrophysiological changes that occur in these regions during aging, and how these changes reflect in behavior. Additionally, a female cohort of rats should be planned for the future in order to investigate sex differences in aging.

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