IMPACT OF AGE ON THE HIPPOCAMPUS-PREFRONTAL CIRCUIT IN SPATIAL WORKING MEMORY

By

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Abstract

The hippocampus and prefrontal cortex are two brain regions that are critically important for executing cognitive processes involved in learning and memory. While these brain regions are known to be individually affected by age-related changes, the impact of aging on the hippocampal-prefrontal circuit remains unknown. For this study we used male Fischer 344 rats of two groups; young (8 months) and aged (22 months). These rats underwent a behavioral battery of cognitive tasks that tested hippocampus and prefrontal cortex function. They were trained on the spatial version of the Morris watermaze to test hippocampus function. Analysis of performance on this task shows that while both young and old rats show improvements in spatial learning across all four testing days, old rats show significantly worse performance on each day compared to young rats. Additionally, they were tested on the W-Maze spatial alternation task which looks at integrated hippocampus-prefrontal cortex function. This task consists of an inbound component testing spatial memory and an outbound component testing spatial working memory. For this task rats were yoked together across age groups and were then surgically implanted with dual-bundle hyperdrives targeting the ventral hippocampus and the medial prefrontal cortex. Analysis of behavioral results reveal that old rats learn the inbound component of the W-Maze task significantly slower than do young rats, and make significantly more errors on the outbound component of the W-Maze task. These results suggest that hippocampus-prefrontal cortex interactions degrade with age, and continued electrophysiological experiments on the spatial alternation task plan to investigate this. Understanding this mechanism may be important in understanding age-related cognitive decline.

Introduction

Increase in life expectancy across the world in developed and developing countries have led to a notable increase in an aged population (Christensen et al., 2009). These elderly populations are the most susceptible to detrimental factors of aging, and are correlated with a rise in chronic degenerative diseases as a cause of death (Stefánsson, 2005, Christensen et al., 2009). The risk and prevalence of neurodegenerative disorders such as Alzheimer's disease and dementia increase with longer lifespans. Even in healthy aged individuals we see notable cognitive decline, often seen as deficits in learning and memory (Glisky, 2007; Hou et al., 2019). Two of the most common cognitive declines are seen in long-term episodic memory and working memory. In order to address these problems, it is important to understand how these cognitive processes are affected by normative aging and their underlying mechanisms.

Memory can be defined as the process of encoding and recall of information about the world around us by neurons in the brain. Neurologically however, memory is a complex process involving dynamic alterations connections between neurons. Memory is thought to be made up of three distinct components: encoding, storage, and retrieval. Encoding involves the initial representation and processing of information (Melton, 1963). Storage is the mechanism of maintaining previously encoded memories, and retrieval involves the process of accessing and modification of past stored memories (Melton, 1963). Memory can also be thought of at different time scales: short-term memory and long-term memory. Short-term memory involves the ability to store a limited amount of readily-accessible information for a short period of time, while long-term memory refers to the storage of information for an indefinite period of time (Atkinson and Shiffrin, 1968). These memory processes require the involvement of multiple brain regions, with key involvement from the hippocampus and medial prefrontal cortex.

The hippocampus (HC) is a critical brain structure involved in learning and contributes to the initial formation, encoding, and storage of primarily episodic memories. Episodic memory reflects daily events and experiences, utilizing contextual information such as time, location, and emotion (Wheeler et al., 1997). The formation of an episodic memory is carried out by the hippocampus, and utilizes spatial and temporal representations in order to encode these memories (Tulving, 1983). Spatial experiences are relevant for eliciting retrieval of context for these episodic memories. An experiment performed on rats by Dostrovsky and O'Keefe found that a percentage of neurons in the hippocampus responded at an elevated rate when the rat was oriented in a particular spatial location and direction (Dostrovsky and O'Keefe, 1971). These neurons are called place cells. Place cells reflect specific locations of an environment - therefore forming a cognitive map of the space around an individual.

The prefrontal cortex plays an important role in guiding decision making and behavior through its involvement in working memory. Working memory is a memory system which allows for temporary storage of information important for completion of a cognitive task, such as reasoning or comprehension. Working memory is a form of short-term memory, and involves the utilization of memories to execute cognitive tasks and perform goal-directed behaviors (Baddeley, 2010). Baddeley suggests that the medial prefrontal cortex (mPFC) is specifically thought to have a major role in working memory by encoding and maintaining or updating information necessary to achieve a specific goal.

The integration of both spatial memory and working memory - known as spatial working memory - is particularly important for navigation and goal-directed action. Spatial working memory allows one to utilize and manipulate spatial memory representations for short periods of time (Spellman et al., 2015). In order to complete spatial working memory tasks, coordination

between the hippocampus and medial prefrontal cortex is thought to be necessary. Performing bilateral or contralateral lesions of the hippocampus and prefrontal cortex in rodents results in behavioral impairment on spatial working memory tasks, while unilateral lesions on the hippocampus and the prefrontal cortex leaves behavior intact, further supporting the necessity of interaction between these regions for proper spatial working memory function (Wang and Cai, 2006). Information transfer between these two brain regions is thought to take place via a circuit called the hippocampal-prefrontal circuit. In this circuit, neurons from the intermediate and ventral CA1 regions of the hippocampus project monosynaptically onto the infralimbic and prelimbic regions of the medial prefrontal cortex. Neurons from the medial prefrontal cortex then indirectly project back onto the dorsal and ventral CA1 hippocampus via the nucleus reuniens region of the thalamus (Jay and Witter, 1991; Vertes, 2006). Network oscillations play an important role in the transfer of information between different brain regions via temporal coordination. Network oscillations are seen in local field potential activity and are an important aspect of neural activity. There are multiple different network oscillation patterns in the brain, with some examples being alpha, beta, theta and gamma oscillations. Different network oscillations occur at distinct frequencies - allowing for neurons to synchronize activity at different timescales, assisting in both local information processing and communication and coordination between brain regions.

Network oscillations are thought to play an important role in numerous cognitive processes, including learning and memory. In the hippocampus, there are three distinct network oscillations present that are thought to contribute to cognitive processing: theta oscillations, gamma oscillations, and Sharp Wave Ripples (SWRs). Theta oscillations are relatively slow (6-12 Hz in rodents) rhythmic oscillations which are thought to play an important role in the

formation of new episodic memories. Theta oscillations occur during active locomotion in mammals (Rivas et al., 1996). During this process, neurons will align to different specific phases of theta oscillations (Skaggs et al., 1996). More recently, there has been research investigating the role of theta oscillations in the coordination of neural spiking in the hippocampus and mPFC during working memory tasks. During decision making, mPFC neurons will also phase-lock to hippocampal theta - allowing for synchronization of neurons from both regions (Jones and Wilson, 2005). From this, Jones and Wilson conclude that theta oscillations may allow the brain to more effectively assimilate relevant spatial information into decision-making processes during spatial working memory tasks. Numerous other studies further emphasize this, with Shin and Jadhav noting that improved performance on spatial working memory tasks is positively correlated with increased theta phase synchrony between the medial prefrontal cortex and dorsal hippocampus. (Shin and Jadhav, 2016). These findings imply that it is important for us to study theta oscillations in the hippocampal-medial prefrontal circuit while studying age-related deficits in working memory tasks.

Gamma oscillations are faster (30-100 Hz) oscillations that occur during similar timeframes as theta oscillations. Gamma oscillations are also believed to contribute to memory encoding processes, with research showing gamma oscillation activity during encoding events (Bragin et al., 1995; Nuñez and Buño, 2021). The coupling of theta oscillations with gamma oscillations is associated with further improved performance on spatial memory tasks, with theta-gamma coupling being observed during spatial memory processing and in the induction of long-term potentiation (Nuñez and Buño, 2021). Experiments performed by Tort et al. and Lisman and Jensen show that gamma synchrony with theta oscillations is increased during memory tasks, implying that theta-gamma coherence is important for spatial memory transfer and memory-

based behavior (Tort et al., 2007; Lisman and Jensen, 2013). HC-mPFC gamma synchrony also shows potential implications for improved cognitive performance - Sigurdsson and Duvarci note that increased gamma synchrony can be seen in a sample phase of a task prior to a working memory task, and other research notes potential unique roles of varying frequency bands in HC-mPFC interactions (Sigurdsson and Duvarci, 2016). Furthermore, Spellman et al. notes that in a rodent model of a spatial working memory task, subsets of mPFC neurons were phase-locked to ventral hippocampus gamma, and increased gamma phase synchrony is correlated with correct task performance - implying an important role for gamma oscillations for encoding of learning cues (2015).

Finally, SWRs in the hippocampus are fast (150-250 Hz) oscillations that occur in irregular, rapid bursts during both sleep and periods of immobility while awake. SWRs can reflectmemory consolidation through organizing the fast replay of place cell sequences specifically representing trajectory events, and are important for improved learning and task performance (Wilson and McNaughton, 1994). A study by Jadhav et al. finds that disruption of awake hippocampal SWRs leads to diminished performance in learning as well as memory retrieval and consolidation (Jadhav, 2012). Sharp wave ripples play a key role in HC-mPFC synchrony as well. Jadhav et al. reports that in rodents, awake SWRs have been shown to modulate a large number of mPFC neurons in a spatial working memory task (Jadhav et al., 2016). In addition, mPFC neurons that were modulated by SWRs had a significantly greater probability of also phase locking to HC theta - suggesting links between SWR-related activity and mPFC neuron reactivation (Jadhav et al., 2016). These results show distinct mPFC neuronal populations which are spatially correlated with hippocampal neurons, suggesting that these populations aided task preferences.

With the progression of aging, we see notable declines in performance on learning and memory tasks. The hippocampus and the prefrontal cortex are particularly negatively affected by the process of aging. Our aim is to understand how the mechanisms and role of hippocampal-mPFC interactions change with increased age. Prior research shows multiple differences in hippocampal and medial prefrontal cortex neural activity across different age groups. A study by Barnes et al. in 1997 showed that the stability of place fields deteriorates in aged rats compared to young rats (Barnes et al., 1997). While an initial study by Shen et al. in 1997 showed no significant differences in theta frequency between young and old rats, this study did not account for slower running speeds in older animals, as theta frequency is shown to increase with increases in running speed (Shen et al., 1997). A study by Crown et al. in 2022 that accounted for this difference in running speed showed that hippocampus theta frequency at any given running speed is higher in younger animals when compared with the theta frequency at the same running speed in older animals (Crown et al., 2022). However, the rate of increase of the theta frequency with running speed is the same for both old and young animals (Crown et al., 2022). When looking at the correlation between gamma frequency in the hippocampus and running speed, older rats had a decreased frequency as well as rate of increase when compared with young rats (Crown et al., 2022). Furthermore, a study performed by Insel et al. showed significant reductions in rhythmic gamma oscillatory activity from the mPFC in aged rats compared to young rats during testing on a three-arm, two-choice task (Insel, 2012). Finally, a study by Cowen et al. identified that aged and young rats had multiple differences in waking SWR activity in the hippocampus - potentially impacting the memory retrieval of aged rats (Cowen et al., 2018). These studies suggest the presence of age-related differences across the network activity in both the hippocampus and prefrontal cortex independently, suggesting impairments in

hippocampal-prefrontal synchrony. In order to address age-related cognitive deficits, it is crucial for us to understand the causal role that changes in hippocampus-medial prefrontal cortex interactions carry out. We plan to investigate this through the use of spatial working memory tasks.

Methods

Subjects

The data in this present study are collected from “young” (8 months) and “aged” (22 months) male Fischer 344 rats. Rats are single housed and maintained on a reverse 12h light/12h dark cycle. During Morris watermaze, food is provided *ad libitum* to rats as this is necessary for swimming. After behavioral battery, rats are placed on food deprivation, being steadily brought down to ~80-85% of their maximum weight.

Cognitive Behavioral Battery

Male F344 rats aged 8 months (young) and 22 months (aged) are tested on a cognitive battery across a span of 6 weeks in order to provide an initial assessment of hippocampus-dependent spatial memory as well as prefrontal cortex integrity between groups of rats. Furthermore, any rats that display indications of motor or visual impairments are removed from the study following this preliminary assessment. The first assessment in this battery utilizes the Morris watermaze, which tests hippocampus-dependent spatial memory. The Morris Water Maze consists of a removable platform and a large white circular pool divided into four equal quadrants by a virtual horizontal and vertical line. All quadrants have multiple platform inserts located at the bottom of the pool that allow for the platform to be moved and slightly submerged in water or slightly above the water line. The pool is additionally labeled with location markers – separated by 45-degree angles – denoting the starting locations where rats are placed into the water. White non-toxic chalk is mixed into the water in order to make it opaque and hide the platform, resulting in the rat requiring the usage of distal cues in order to orient itself.

The Barnes Lab utilizes the Morris watermaze through two different tasks: a spatial navigation task and a visual navigation task. Prior to the first trial, the rat is acclimated to the

environment by being placed onto the platform for a period of 60 seconds. The rat is then first tested on the spatial navigation component of the task. During the spatial navigation task, the platform is located in the same quadrant across all trials. The rat is first placed into the pool facing the wall at a location randomly selected at one of the eight starting markers. Once in the pool, the rat must locate the platform using only distal cues. The trial ends after 60 seconds or when the rat locates the platform, whichever comes first. In the case that the rat does not locate the platform within 60 seconds, the rat is led to the platform via a black rod and allowed to sit on the platform for a period of 30 seconds, and then repeats the process once more at a different marker. The rats are tested 6 times a day over a period of 4 days.

All rats are equipped with a stretch fabric backpack that contains a large black bar that allows for tracking of the trajectories of the rats through a video tracking software called ANY-maze. Path tracking of rats is then taken from ANY-maze to identify improved performance across trials as well as platform location learning. In order to account for variability between start locations across trials and swimming velocities between rats, analysis of spatial memory is calculated through the use of a Corrected Integrated Path Length (CIPL) score (Gallagher et al., 1993). CIPL score is calculated as the difference between the actual path taken by the rat and the most optimal path from the start location to the platform (a straight line). A lower CIPL score indicates better performance on the task.

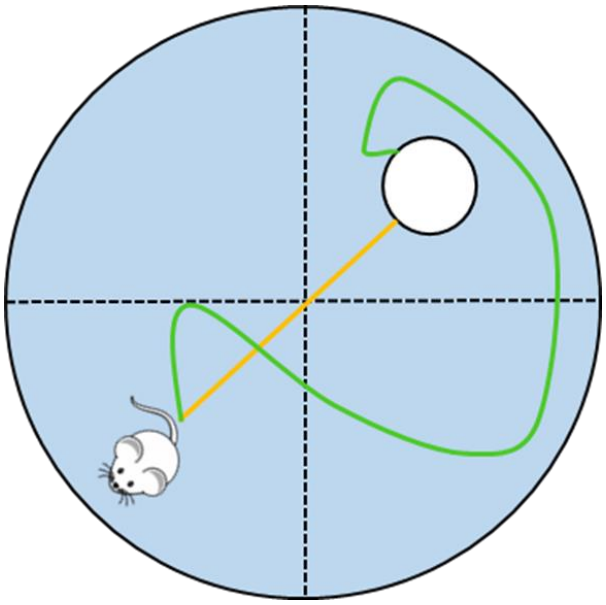

Figure 1. Schematic of CIPL score analysis in the Morris watermaze. CIPL is calculated as the difference between the actual pathway of the rat (green line) and the most efficient path to the platform (yellow line).

After completion of the spatial navigation task, rats are then tested on a probe trial in order to verify the rat's understanding of the location of the platform. The hidden platform is removed from the pool and rats are placed into the pool for a period of 60 seconds. The pathway that the rat takes during the swimming period is recorded to assess the rat's spatial memory of the platform location.

Following completion of the spatial navigation component, rats are then tested on the visual navigation task for a period of two days. The visual navigation task is performed similarly to the spatial navigation task, except that the platform is raised above the surface of the water and visible to the rat while actively swimming. The platform is also moved to a different location every trial. This task helps to identify rats who may have visual deficits prior to further training.

After completion of the Morris Water Maze, rats without evidence of motor, visual, or navigational deficits are food restricted to approximately 80-85% of their *ad libitum* weight. These rats are then trained on a Temporal Ordering task that tests working memory performance

and medial prefrontal cortex function. As this is a relatively new task in the cognitive battery which is still undergoing troubleshooting, we are not including the results in this thesis.

Linear Track

Rats are initially pre-trained on a linear track to shuttle from end to end to acquire Ensure, a nutrient-rich liquid food, on a linear track for a period of 7-10 days. The room containing the linear track apparatus is set up with white curtains, and 3 distal visual cues (posters) are hung up around the room. The room is additionally provided with moderate lighting, and at the end of each arm, a proximity IR sensor is used to detect the presence of the rat near the end of the track and triggers a solenoid to dispense 2mL of liquid nutrient (Ensure) when the rat reaches the end of each arm. The length of the sessions begins at 5 minutes, with session length being gradually increased across days until rats can average one lap per minute for 30 minutes consistently. Performance is calculated as the average number of laps each rat runs per 30-minute session. Based on performance during training, rats are yoked into pairs of one young rat and one old rat each, with the best performing old rat being paired with the best performing young rat, the second best performing old rat paired with the second-best young rat, etc.

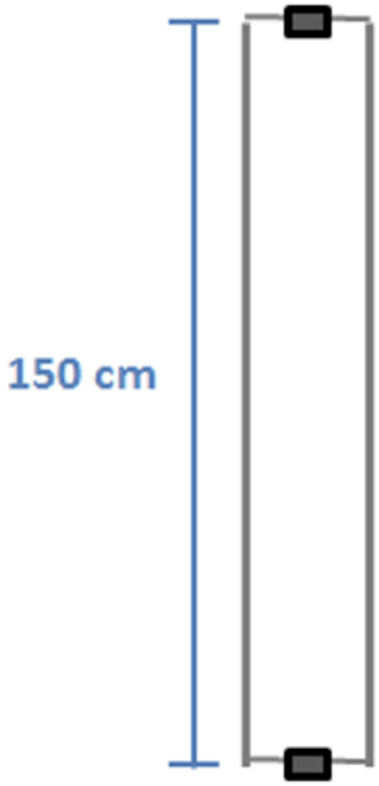

Figure 2. Schematic of the linear track apparatus used during pre-training.

Hyperdrive Implantation

All paired rats are then surgically implanted with dual-bundle hyperdrives. These drives target the ventral CA1 region of the hippocampus (vHC) [Coordinates: AP: -5.8mm, ML: 5.5mm, DV: 5.2-7.1mm] and the Infralimbic (IL) and Prelimbic (PL) regions of the medial prefrontal cortex [Coordinates: AP: 3.0mm, ML: 0.5mm, DV: 2.2-5.1mm] with nine movable tetrodes in each location. A cerebellar ground screw is used as a reference. Rats then undergo a period of post-surgery recovery for approximately 7 days. Following this recovery period, all implanted rats are then retrained on the liquid nutrient-rewarded linear track until they reach pre-surgery performance levels. Rats are then tested on spatial working memory performance through the use of a W-Maze spatial alternation task.

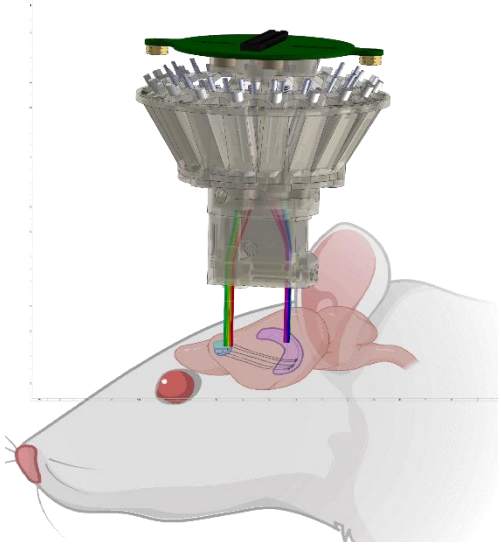

Figure 3. Schematic of a dual-bundle hyperdrive targeting the mPFC and HC, containing 9 tetrodes in each bundle.

W-Maze Spatial Alternation Task

The W-Maze spatial alternation task consists of two interleaved components – an "inbound" component testing spatial memory, and an "outbound" component testing working memory. The inbound component is primarily reliant on the hippocampus, while the outbound component utilizes both the hippocampus for spatial information and the prefrontal cortex to maintain a working memory of the most recently visited arm. Rats begin at the base of the center arm. After receiving a reward from the center arm, the rat then has two options – to go to the left or right outbound arm. After receiving a reward from the respective arm, the rat then must return back to the center arm and then visit the arm that they did not initially choose to visit. For example, if the rat initially chooses to visit the left outbound arm, the rat will have to return to

the center arm and then proceed to the right outbound arm. In order to differentiate the context from the linear track training, we create a different environment during the W-maze trials by covering the walls with black instead of white curtains, as well as introducing new visual cues.

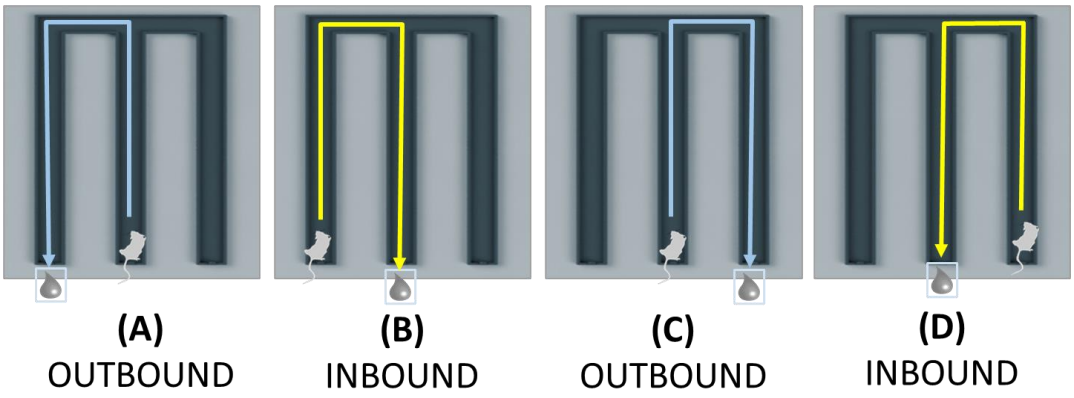

Figure 4. Schematic of the W-maze spatial alternation task. The rat begins at the bottom of the middle arm and travels down it to receive a liquid nutrient reward. **A)** The rat then makes a directional choice to travel down the left or right outbound arm (denoted by the blue trajectory). After visiting one of these arms, **B)** the rat will then travel back to the center inbound arm (denoted by the yellow trajectory) and **C)** then travel to the outbound arm which was not previously visited. **D)** The rat will then return to the center inbound arm and repeat this alternating pattern.

After an initial 30-minute sleep period, the rat is tested on the apparatus for a period of 1 hour or until reaching 30 correct outbound laps, whichever comes first, and then undergoes a second 30-minute sleep period. Rats are tested in the previously-determined yoked pairs, to control for the total number of learning trials per session. The old rat is typically tested first as they tend to complete fewer laps than do the younger rat and the young rat is allowed to run for the same total number of laps during the session. If the young rat is unable to match the same number of laps as the old rat, the rat order is swapped in the following session. This is done in order to increase consistency in number of laps between rat pairs. An overhead camera records position and speed of the rat, and is tracked by LEDs on the head stage of the rat.

Electrophysiological Data

The dual-bundle hyperdrives implanted in the vHC and mPFC record electrophysiological output simultaneously during both sleep phases and during the run phase itself, with 9 tetrodes in each location. Single-unit and LFP activity from both brain structures is collected through Neuralynx recording systems. During these sessions, an online clustering software known as SpikeSort3D is used in order to initially spike sort neurons from each tetrode into clusters in order to get an estimate of neuron cell count and type. Post-recording, these neurons are then spike sorted using MClust. Additionally, local field potential activity markers such as SWRs, theta oscillations, and spindles – indicators of hippocampal and prefrontal cortex activity – are recorded and observed in order to identify accurate tetrode placement in these regions. Tetrodes are moved at the end of every recording session in order to record new cells for following recording sessions.

Post-Experiment Procedures

Rats are given electrolytic marker lesions after completion of data collection in order to confirm the locations of tetrodes and verify the accuracy of placement in each brain region. A current of 20 microamps is passed through each tetrode for a period of ten seconds each. Lesioning forms a glial scar and releases iron from the tips of tetrodes. In histological analysis, we utilize two different staining methods. The first of these methods is Prussian Blue. Prussian Blue staining allows us to visualize the iron deposits from the tetrodes, allowing for us to see where tetrode tracks end and how deep the tetrodes were inserted. The second of these methods is Nissl staining. Nissl staining allows us to visualize the morphology of the tissue, and to identify where tetrode tracks are going in the brain.

Results

Behavioral Results of Morris Water Maze across Age Groups

For the spatial navigation trials in the Morris Water Maze task, the number of subjects was N=13 for old rats and N=12 for young rats. Age difference on spatial trials was calculated on each day using an unpaired T-test on the CIPL scores. In order to account for multiple analyses on the same dependent variable across different trials and different days, significance was calculated using Bonferroni adjusted p-values.

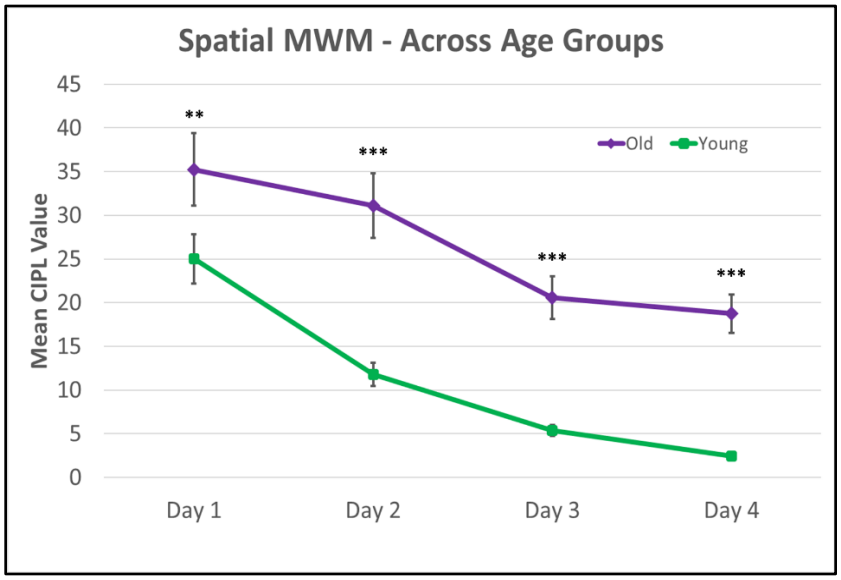

Figure 5. Comparison of the MWM spatial task performance across age groups. Bonferroni adjusted p-values: * < 0.005, ** < 0.001, *** < 0.0001. Young rats (8mo, N=12) perform significantly better than old rats (22mo, N=13) across all days of the MWM task.

Trial Day #	μ_{Young}	μ_{Old}	SEM_{Young}	SEM_{Old}	p-value
Day 1	25.02358974	35.24458333	2.833363598	4.153613979	0.0002
Day 2	11.77576923	31.10125	1.333343306	3.665317463	<0.0001
Day 3	5.379871795	20.58513889	0.609150528	2.425981883	<0.0001
Day 4	2.432692308	18.725	0.275448163	2.206762413	<0.0001

Table 1. CIPL score mean (μ), standard error of mean (SEM), and p-values for young and old F344 rats across spatial navigation trials on the Morris Water Maze apparatus.

The mean CIPL scores for old rats are significantly higher than for young rats across all four days. These results indicate that while both young and old rats show evidence of improved performance across task days, aged rats are spatially impaired on performance in the MWM task.

In order to confirm that differences seen across spatial trials were not due to visual deficits, rats were tested on a visual navigation task in the Morris Water Maze. Number of subjects was N=13 for old rats and N=12 for young rats. Age difference on visual trials was calculated on each day using an unpaired T-test on the CIPL scores.

	μ_{Young}	μ_{Old}	SEM_{Young}	SEM_{Old}	p-value
Average CIPL Score	2.513474026	3.244689619	0.209456169	0.290213862	0.2583

Table 2. Average CIPL score across day one and day two across visual navigation trials on the Morris Water Maze apparatus. CIPL score mean (μ), standard error of mean (SEM), and p-values are reported for young and old F344 rats.

Analysis of visual trials shows no significant difference in MWM performance when young and aged rats are able to see the platform (p = 0.2583). Furthermore, young and aged rats both perform notably better in comparison to a visually impaired rat (n=1), with young and aged

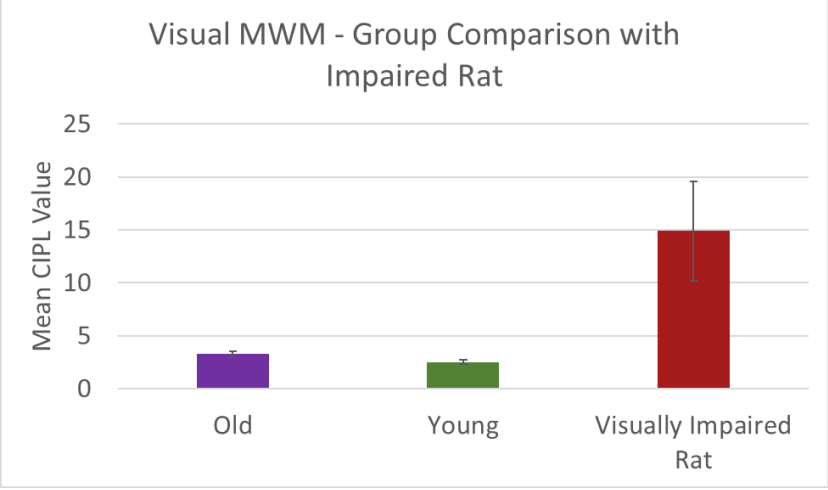

Figure 6. Comparison of MWM visual task performance across age groups. Bonferroni adjusted p-values: * < 0.0125, ** < 0.0025, *** < 0.00025. There is no significant difference in performance between young (N=12) and Old (N=13) rats (p = 0.2583), and both groups notably perform better compared to a visually-impaired rat (N=1).

rats both locating the platform much more efficiently.

Results of Spatial Alternation Task

12 young and 12 old rats were successfully trained on the spatial alternation task. Group performance on the inbound and outbound component of this task were measured using a two-way repeated measures ANOVA. On inbound trials, both young and old rats were able to reach close to 100% accuracy - however, old rats were significantly slower to reach this level of accuracy. On outbound trials, old rats make significantly more errors than young rats - old rats were able to reach only approximately 65% accuracy after 21 sessions, while young rats reached approximately 80% accuracy on trials. For the inbound component of the task $F(1,24) = 1.9$, p-value = 0.33. For the outbound component of the task $F(1,24) = 33.42$, p-value < 0.001.

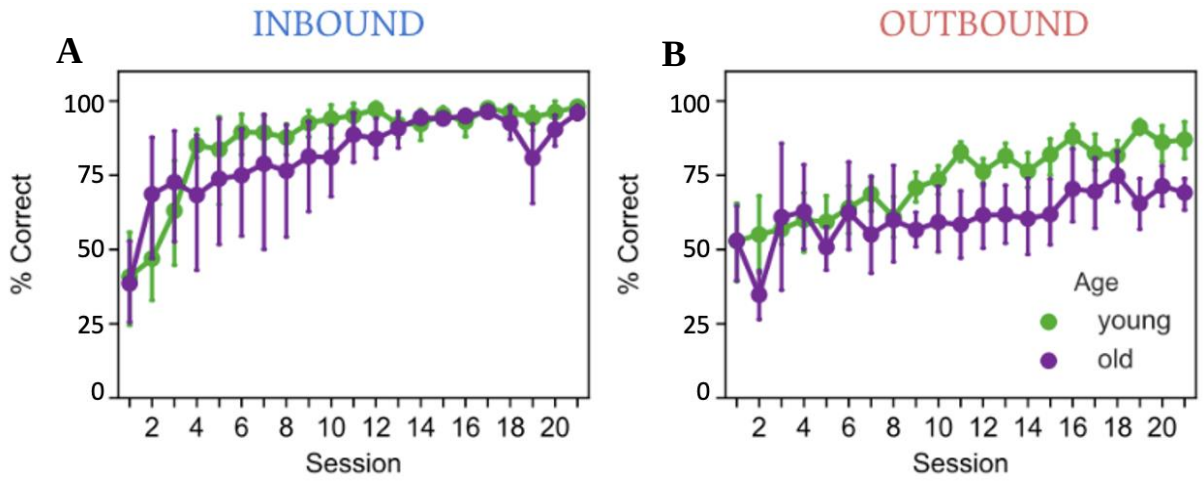

Figure 7. Percentage correct on the **A)** inbound component ($F(1,24) = 1.9$, p-value = 0.33) and the **B)** outbound component ($F(1,24) = 33.42$, p-value < 0.001) of the W-Maze spatial alternation task. Old rats (purple) learn the inbound component significantly slower than young rats (green), and make significantly more errors than young rats on the outbound component.

Inbound trials are trials in which the rat moves from an outer arm of the W-maze to the center arm of the W-maze. This assesses spatial memory performance by requiring the animal to remember to return to the center arm - a process dependent on hippocampal function. Outbound trials are trials in which the rat moves from the center arm of the W-maze to the correct outer

arm of the W-maze. This assesses working spatial working memory performance - utilizing both the mPFC and the hippocampus to maintain a working memory of the previously visited outbound arm as well as to locate the reward position in space (Kapellusch et al., 2018).

Verification of Tetrode Locations

Nissl stains of sections from implanted rats show tetrode tracks in the target locations of the infralimbic and prelimbic regions of the medial prefrontal cortex (Figure 8, left) and the ventral CA1 region of the hippocampus (Figure 8, right). Figure 9 represents schematics of modified Paxinos recording locations from one young and one old rat across the medial prefrontal cortex and hippocampus (Paxinos and Watson, 1998). The red dots represent recording locations while the blue dots represent references.

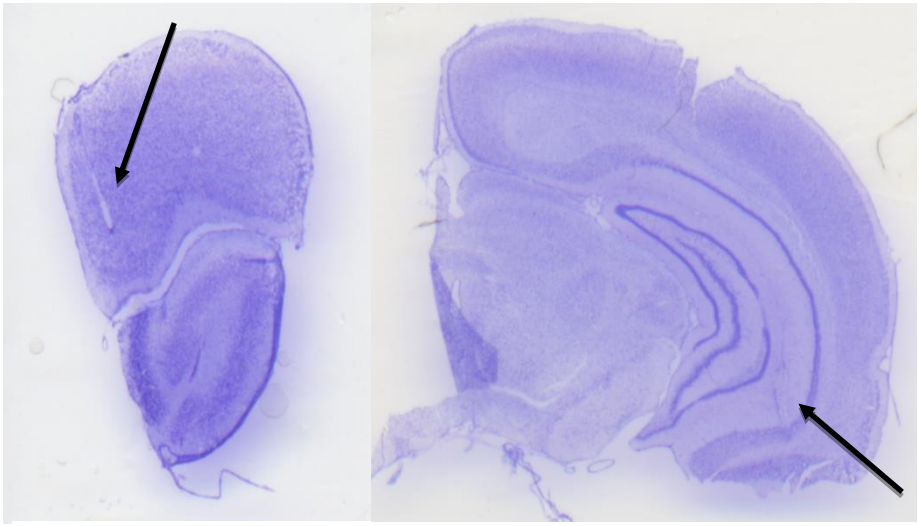

Figure 8. Coronal sections of the medial prefrontal cortex region (Left) and the ventral hippocampus region (Right) stained with Nissl stain. Tetrode tracks can be seen located in the desired regions.

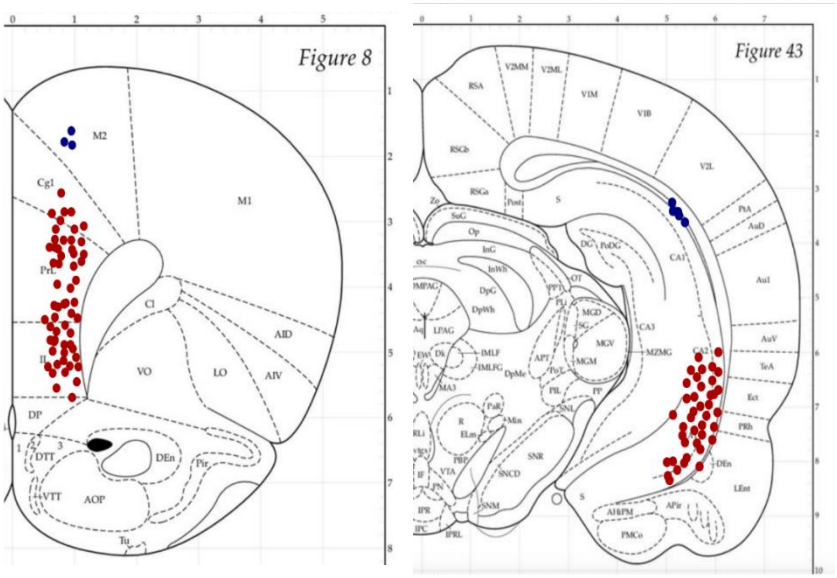

Figure 9. Representation of locations of tetrode tips targeting the mPFC (Left) and HC (Right). Blue dots represent references while red dots represent recording locations.

Electrophysiological activity during W-Maze sessions was recorded from a dual-bundle hyperdrive. We have been able to verify that neurons recorded from the hippocampus show spatial turning. Figure 10A is a representation of a spatially-tuned ventral hippocampus place cell recorded from an old rat. Figure 10B is a representation of a medial prefrontal cortex neuron which selectively fires at reward locations. Further analysis is needed in order to investigate how age-related differences from neurons in both of these regions correlate to task performance.

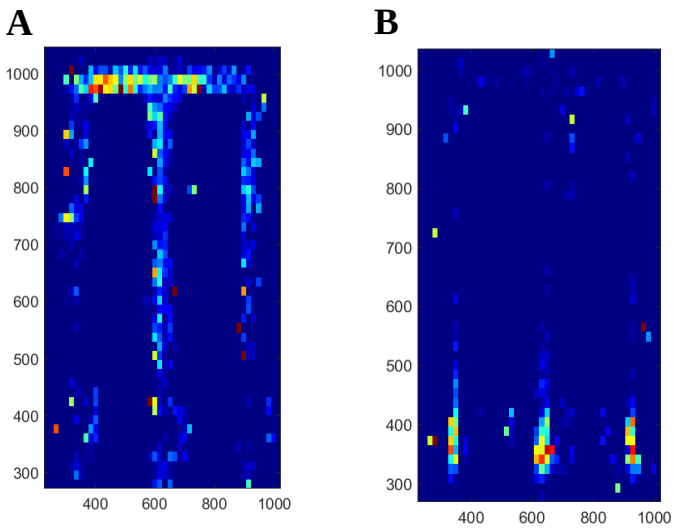

Figure 8. Heat map representations of firing from a spatially-turned ventral hippocampus place cell (Left) and a medial prefrontal cortex neuron (right). Colors represent the firing intensity of a single neuron.

Discussion

The hippocampus and medial prefrontal cortex play a key role in episodic memory and working memory. The hippocampus is integral for the formation of episodic memories and is necessary for spatial localization, while the prefrontal cortex is critically important in working memory function. Both of these regions independently show functional deficits with increased age, which implies impairments in the HC-mPFC circuit. Studying the interaction between these regions is important in order to identify the impacts of age-related declines in memory.

Hippocampal performance was first analyzed through examining spatial memory performance in the Morris watermaze apparatus. Results on this experiment revealed impairments in spatial memory performance in old rats compared to young rats. Furthermore, during analysis of performance on the W-Maze spatial alternation task, old rats learned the spatial memory component (inbound) significantly slower compared to young rats - however, old rats did eventually perform at the same levels as young rats on the spatial component. During analysis of spatial working memory performance on the W-Maze spatial alternation task, we saw that while old rats were performing above chance on this task, they were never able to match the performance of young rats. This implies that while old rats are capable of learning the outbound component of the W-Maze task, they are unable to meet the spatial working memory demands required to perform at the same level as young rats - emphasizing that there are impairments in the hippocampus-prefrontal cortex circuit that appear with age.

Currently, we have successfully implanted young and old rats with hyperdrives which have completed the spatial alternation task, and we are in the process of investigating the neural differences in the hippocampal and prefrontal cortex that correlate to task variables. We plan to

undergo further analysis in order to continue to investigate the underlying processes that drive these age-related changes.

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