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Mapping Out Learning: How Aerobic Exercise, Sex and Alzheimer's Disease Impact Learning

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And to close, I'd like to thank God for the gifts of cognitive function and exercise. It is a privilege and honor to learn about the systems you've created and how to best care for them.

Abstract

Exercise has the power to ameliorate the outward symptoms of Alzheimer's Disease (AD) by rescuing hippocampal-dependent learning and memory. The goal of this research was to map out how exercise and sex impact behavioral learning in AD mouse models by looking outside of the traditional measurements in the Morris water maze (MWM), as they fail to describe the process of learning itself. I calculated heading error at maximum velocity, manually categorized search strategy in the MWM across trials, and determined flexibility in the probe trial for transgenic and wild-type mice who were exposed to exercise or control housing. Mice significantly switched from nonspatial to spatial learning strategies over time across all groups, however, only half were using a spatial strategy by the end of the fourth day of testing. There were no group effects on preference for spatial strategy, flexibility in the probe trial, or in decreasing heading error at maximum velocity, though targeted M/TG/CON and F/WT/EX groups displayed a preference for spatial strategy over time. Spatial strategies were superior to all nonspatial strategies in efficiency to the platform but one: thigmotaxis. This particular nonspatial strategy can save cognitive resources by allowing for efficient maze performance without the primary use of the hippocampus, challenging the common thought that the MWM is a hippocampal-dependent task. Moving forward, it is imperative to think more carefully about how the MWM is used to parse out learning, and to better understand advanced aspects of AD and how to overcome challenges to learning and memory.

Introduction

Exercise and Alzheimer's Disease (AD) differentially impact the hippocampus. Because the hippocampus is involved with cellular mechanisms crucial for memory storage, the relationship between exercise, AD, and learning and memory is of great interest. An established method to assess hippocampal learning and memory in rodents is the Morris Water Maze (MWM). Traditionally, subjects with the shortest distance and latency to the platform as well as the highest percentage of time spent in the target quadrant during the probe trial were thought to use spatial strategies to solve the maze, while worse performance indicated a nonspatial strategy. However, without watching each mouse navigate the maze, it is nearly impossible to assert that those with the shortest distance and latencies used spatial strategies. Below, I build the context for my theory that there are different ways to learn, that the MWM can be solved in a variety of ways, and that the MWM is only a hippocampal-dependent task if a subject chooses to solve it using a strategy that requires the hippocampus (spatial).

Alzheimer's Disease

Prevalence & Symptoms

Alzheimer's Disease (AD) is of interest to this study because of its relationship with the hippocampus as well as its relevance in the world today. AD is an irreversible neurodegenerative disease and the sixth leading cause of death in the United States ("Alzheimer's Disease Fact Sheet"). Because AD currently affects 5.6 million people in the United States alone, and even more worldwide ("Alzheimer's Disease Fact Sheet"), it is important to understand its pathology and how to improve the lives of those who have AD. Hallmarked by loss of memory and personality change, this disease is known to take an emotional toll on families by slowly eroding memories and skills that were fundamental to the affected person's personality.

The most common early symptom of AD is difficulty remembering newly acquired information. In the mild stages, typical challenges can include spatial issues, impaired reasoning and judgement, repeated questions, and personality and behavior changes. As the disease progresses into the moderate stages, memory loss grows worse, along with the ability to carry out multi-step tasks and cope with new situations. At this point, hallucinations and paranoia are not uncommon. The severe stages are characterized by the inability to communicate, becoming entirely dependent on others for care, and remaining in bed for most of the time as the body slowly shuts down. On average, a person lives with AD for four to eight years after diagnosis, meanwhile the disease transforms loved ones into characters unable to process the future or remember the past, save for distant memories. The strongest risk factor for this disease is age, with the majority of onset cases taking place after 65 years of age ("2019 Alzheimer's Disease Facts and Figures"), and with the risk increasing each decade.

Cellular Mechanisms & Prevention

The disease symptoms manifest themselves in outward behavior but originate within the structures of the brain-- specifically the medial temporal lobe as the primary site of impact. The two hallmarks of the disease come in the form of neurofibrillary tangles and amyloid plaques. In the brain, key nutrients, organelles, proteins, and enzymes are transported throughout the cells via microtubules. Tau is a protein predominantly found in neurons and plays an important role in internally stabilizing the microtubules (Spillantini & Goedert, 1998). Importantly, phosphate molecules regularly bind to microtubules, which helps stabilize them and regulate the transport of signals along the pre-synaptic axon, through the cell body, to the dendrites and along the post-synaptic axon. Propagating signals across cells is crucial because it allows for all movement, perception, cognitive and neuronal function. However, one indication of AD is the

hyperphosphorylation and thus the dysfunction of the tau protein, leading to the dysregulation of the microtubules (Alonso, Zaidi, Grundke-Iqbal & Iqbal, 1994). This microtubule instability in neurons can lead to the formation of tau threads and paired helical filaments. These threads and helices can become tangled in the cell, collapsing the internal transport network of neurons, thus inhibiting the ability of neurons to work together (Kocahan & Doğan, 2017). This breakdown damages properties that signal propagation controls, such as movement, perception, and cognitive function. This is one of the driving factors for symptoms of AD: intracellular neurofibrillary tangles.

The other hallmark of AD is the extracellular deposition of plaques. The protein involved with these plaques is called the Amyloid-beta precursor protein (APP), and this large membrane protein plays a vital role in promoting synaptic activity, synapse formation, dendritic spine formation, and neuronal plasticity (Hoe, Lee & Pak, 2010). This protein is activated once it is cut by enzymes, but under some circumstances, it can be improperly cut by the B-secretase enzyme rather than the typical a-secretase. After APP has been cut by the B-secretase, it can transform into a sticky version of itself and form small clusters called oligomers. Then, the oligomers form chains (fibrils), which form beta-sheets. The final stage of plaque formation occurs when the beta sheets transform into clumps and other substances that aggregate and disrupt cell communication (Parihar & Brewer, 2010). The clumps, or plaques, also activate immune cells, triggering inflammation and eventually destroying neurons (Morgan et. al, 2004). Neuronal death and the loss of synapses contribute to progressive cognitive decline.

Figure 1.

Healthy Vs. AD Brain

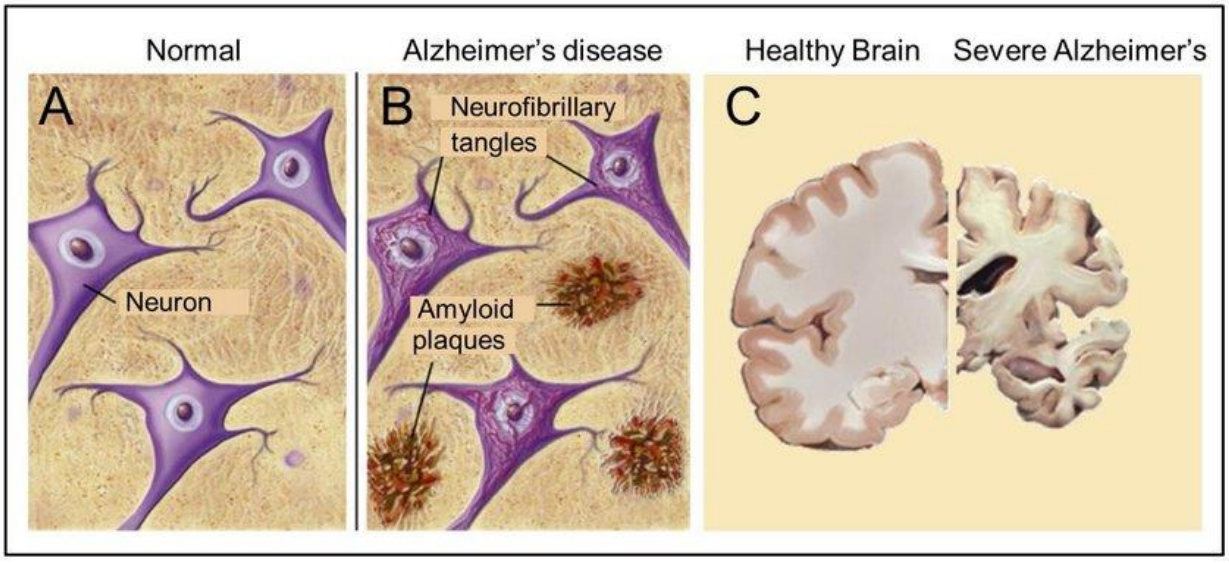


Figure 1. Hallmarks of Alzheimer's Disease include, but are not limited to, neurofibrillary tangles, amyloid plaques, and brain atrophy. (Loof & Schoofs, 2019).

Though neurofibrillary tangles and amyloid plaques are defining characteristics of AD, they are not the only factors that play into the steady decline of an AD-riddled brain. Researchers have determined that along with plaques and tangles, additional patterns in AD exist. For one, there is a marked decrease in the neurotransmitter Acetylcholine, the main neurotransmitter in the neuromuscular junction that is plays a role in mechanisms of memory (Giovannini, Lana & Pepeu, 2015). Moreover, another pattern includes rapid neurodegeneration and brain atrophy beginning in the medial temporal lobe, but spreading outwards from the hippocampus to the temporal, frontal and parietal lobes over time ("2019 Alzheimer's Disease Facts and Figures"). There are several hypotheses for what else contributes to the cognitive decline, but today, few medications exist to target different players in the disease. Although treatments for symptoms are available and research continues, there is no current cure for AD.

The next best thing we can do as a scientific community is step up the research for understanding how to prevent the disease from taking hold in the first place. Previous research suggests that consistent enhancements to daily life may work to prevent AD, such as consistent sleep (Barnard et. al, 2014), regular mental activity (Curlik & Shors, 2013), environmental enrichment (Arendash et. al, 2004; Jankowsky et. al, 2005), aerobic exercise (Hötting & Röder, 2013), and an antioxidant-rich diet (Morris et. al, 2006). The relationship between AD and exercise as a medium of prevention of AD-related cognitive decline is of interest in this study.

Learning and Memory

NMDA Receptor & LTP

The cellular mechanisms of AD take root in the hippocampus and expand outward over time. Therefore, symptoms of AD such as cognitive decline and memory loss are thought to occur due to their close relationship with the hippocampus for optimal processing. Specifically, learning and memory are linked to the proper function of the N-Methyl-D-aspartate (NMDA) receptor, but AD is linked to an overactivation of this receptor, which leads to cell death. The cellular mechanism that drives long-term memory formation is called long-term potentiation (LTP), and it requires the NMDA and the α--amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor to function properly (Lynch, 2004). The process of LTP begins with NMDA and AMPA receptors. When glutamate interacts with the AMPA receptor, frequent action potentials will cause the postsynaptic neuron to depolarize. This depolarization inside of the cell will eventually remove the magnesium ion blockade from the NMDA receptor and allow calcium to flow through the NMDA channel, as well. The influx of calcium initiates a feedback loop in which more AMPA receptors are inserted into the cell membrane, thus allowing more glutamate to bind, more depolarization to occur, and more calcium to flow through the recently

opened NMDA receptors. These chain reactions strengthen the synapse and make it more likely to be activated in the future; this is the basic definition of LTP. Research has demonstrated that these strengthened synapses maintain stronger memories. LTP, which requires the NMDA receptor, plays a key role in learning (Bashir et. al, 1991), so impaired NMDA receptors, in the case of AD patients, disrupt learning and memory.

To prove that LTP was necessary for learning and memory, some researchers inhibited the NMDA receptor. In a study conducted by Richard Morris, the NMDA receptor was inhibited using the enzyme d,L-AP5 (s o,r.-2-amino-5phosphonopentanoic acid), and rodents' learning and memory was assessed using the Morris water maze (1989). One main finding from the experiment was that the inhibition of the NMDA receptor caused impaired performance and a slower escape from the maze. AP5 did not cause impairment to vision or motivation in the maze. The main takeaway was that the NMDA receptor, and thus LTP, is crucial to processing, storage, and retrieval of information during place navigation, as the AP5 caused significant impairment to learning.

One medication intended to treat symptoms of AD focuses on controlling the overactivation of NMDA receptors. The improper function of the NMDA receptor is one of the cellular consequences of plaques and tangles. In an AD brain, the magnesium blockade that is usually in place until the process of LTP begins is displaced, and too much calcium enters through the NMDA receptor. This heightened influx of calcium leads to excitotoxicity and cell death (Tariot et. al, 2004) in the hippocampus, compromising LTP and the ability to process and store new memories. Therefore, controlling the overactivation of the NMDA receptors by blocking the receptor through medication can ameliorate the memory decline associated with AD.

Hippocampus

Furthermore, because NMDA receptors are most highly concentrated in the hippocampus (Olverman, Jones & Watkins, 1984), LTP occurs most prominently in the hippocampus. As such, the hippocampus is generally considered to be linked to specific types of memory, especially spatial memory (Kim & Diamond, 2002). Some of the earliest research documents the discovery of 'place cells' in the hippocampus, or cells that fire preferentially based upon the rodent's specific spatial location (O'Keefe & Dostrovsky, 1971). Since then, an abundance of research has indicated that the hippocampus is the main region involved in spatial memory (Bird & Burgess, 2008; Deng, Aimone & Gage, 2010; Erickson et. al, 2011). Spatial memory, therefore, is one of the first skills lost during the onset of AD because plaques and tangles riddle the hippocampus and impair the proper function of the NMDA receptors.

Additional studies have looked at the plasticity of the hippocampus, or its ability to adapt and grow in response to internal or external environments. Larger posterior and smaller anterior hippocampi were found in London taxi drivers who needed to memorize their way around the city as a profession; gray matter increases were observed in the posterior hippocampus for medical students preparing for a major examination; and transient increases of gray matter in the hippocampus occurred in adults learning to juggle. Studies have also found that the hippocampus is generally more plastic in younger adults, and that the volume of the hippocampus overall decreases significantly as we age. In response to understanding the plasticity of the hippocampus and the significance of maintaining its function, researchers trained participants in spatial navigation and determined that hippocampal volume can be protected from age-related loss through consistent spatial navigation training (Lövdén et. al, 2012). That is to say, there is hope

that certain practices can enhance the function of the hippocampus and protect it from the destructive nature of the plaques and tangles of AD.

Exercise

Aerobic exercise is one such lifestyle enhancement that can protect those with AD from some cognitive symptoms (Hötting et. al, 2013). Specifically, aerobic exercise can induce changes in the hippocampus to protect an AD brain from losing even more memory processing. Some of the myriad of ways that aerobic exercise alters the brain include increases in brain-derived neurotrophic factor (BDNF), glial cell proliferation, neurogenesis and plasticity.

BDNF is a protein found in the brain and spinal cord that promotes the survival, growth repair, and differentiation of neurons; it also supports synaptic plasticity and cellular homeostasis. BDNF is most highly concentrated in the hippocampus, cortex, and hypothalamus (Voss et. al, 2019). Increases in BDNF are thought to be related to improved synaptic plasticity, neurogenesis, and cell survival (Voss et. al, 2019). On the contrary, decreases in BDNF are linked to impairment in memory function. BDNF is known to be inversely correlated with age. Recent research suggests that mechanisms involved in increasing BDNF levels may be activated through aerobic exercise in both humans and rodents (Choi et. al, 2018; Mandolesi et. al, 2018; Voss et. al, 2019; Voss et. al, 2013). Indeed, research indicates that increased BDNF gene and mRNA expression was found in the cerebellum, caudal cortex and hippocampus after short and long periods of exercise with either daily or alternating running days (Voss et. al, 2013). In addition, these changes were observed for at least two weeks after the exercise had ended (Voss et. al, 2013). Other studies have shown that exercise raises BDNF gene expression in the lumbar spinal cord, cerebellum, amygdala, caudal neocortex, and perirhinal cortex in addition to the dentate gyrus in the hippocampus. (Voss et. al, 2013). Notably, the positive effect

of increased BDNF gene expression in response to exercise is only present following aerobic exercise, and not in anaerobic exercise (Voss et. al, 2019).

Neurogenesis, the process by which new neurons are born which is regulated by BDNF (Mandolesi et. al, 2018), can also be induced by exercise. The benefit of neurogenesis is that it can improve brain cognition and plasticity. In mice lacking hippocampal neurogenesis, there was a marked decrease in performance on spatial memory tasks and in separation/discrimination tasks (Mandolesi et. al, 2018). On the other hand, increases in neurogenesis are thought to underlie positive effects on Y-maze performance, or increases in learning and memory (Van der Borght et. al, 2007). There is growing evidence suggesting that exercise is an activity that can induce neuronal stem cell proliferation and differentiation (Quan et. al, 2020; Voss et. al, 2013; Voss et. al, 2019), particularly in the dentate gyrus in the hippocampus (Mandolesi et. al, 2018).

Exercise can elevate levels of BDNF throughout the body, and this makes a considerable impact in those with AD, because a physiological consequence of AD is a decrease in overall BDNF (Quan et. al, 2020). Recent research has proposed that irisin, a hormone, and its precursor, fibronectin type III domain-containing protein 5, mediate the beneficial effects of exercise on the brain, which may include the elevation of levels of BDNF; when increased, this hormone and its precursor can rescue LTP and synaptic plasticity in AD model mice (Lourenco et. al, 2019). Moreover, a recent study took to genetically and pharmacologically mimicking the effect of exercise through increasing BDNF levels and inducing neurogenesis in the hippocampus (Choi et. al, 2018). This mimic of exercise protected neuronal cell death and improved cognition in mouse models of AD. These studies suggest that exercise, and even mimics of exercise, can positively alter mechanisms that prove to make a difference in the outward symptoms of AD.

Additionally, increases in glial cell proliferation in the hippocampus and in the neocortex were observed post-exercise (Mandolesi et. al, 2018). Recent waves of information suggest that glial cells are intimately involved in the regulation of neuronal activity and synaptic transmission (Chung, Allen & Eroglu, 2015). Therefore, glia proliferation in the hippocampus is especially important for hippocampal synapse efficiency. Specifically, synapse elimination is controlled by astrocytes, a type of glia, and synapse elimination is most likely a feature of prioritization and reorganization of information in our nervous system during learning (Chung et. al, 2015). Therefore, exercise-induced glia proliferation in the hippocampus can increase synapse efficiency, which has a positive effect on learning and memory.

Furthermore, exercise-induced neurogenesis is particularly important in regard to DNA repair and synaptic plasticity (Vilela et.al, 2020). Not only can exercise induce neurogenesis, but it can increase dendritic spine density in the entorhinal cortex and CA1 pyramidal cells (of the hippocampus), lengthen dendritic spines, and increase the volume of the granule cell layer (Voss et. al, 2013). These changes in the neuron morphology serve to increase connections within the brain and improve overall cognition. Importantly, exercise was also found to significantly increase slope and amplitude of the excitatory postsynaptic potential (EPSP) (Dastgerdi, Radahmadi & Reisi, 2020), leading to the increased functionality of LTP. This is important for understanding how exercise can improve learning and memory.

While researchers can study neurogenesis and synapses in rodents, there is no way to replicate these studies with humans in a non-invasive manner. The next best alternative that researchers have at the moment is looking at gray matter volume and blood flow to the brain. In these studies, researchers have found that exercise can increase the volume of gray matter in the frontal lobes and hippocampus in humans and rodents (Mandolesi et. al, 2018).

Behaviorally, the effects of increased BDNF, EPSP, LTP, neurogenesis, glial cell proliferation and gray matter volume of the neocortex and hippocampus all result in greater executive function, visuospatial memory, pattern separation, academic achievement, attention and processing speed, learning and memory, and decreased anxiety (Voss et. al, 2013). Applied to a human population, exercise has the potential to improve quality of life of those who have AD by ameliorating the impact of the loss of LTP and synaptic plasticity.

Sex Differences

For decades, female rodents and human participants were overlooked in research studies due to their estrous and menstrual cycles, which result in constantly fluctuating levels of estrogen and progesterone (Marcondes, Bianchi & Tanno, 2002). Now that both sexes are being included in studies, researchers have found that performance on these spatial tasks may be linked to hormone levels (Kimura & Hampson, 1994; Galea et. al, 1995). In studies where females are treated with testosterone, they tend to perform better on spatial tasks (Roof & Havens, 1992); similarly, males tend to perform worse on spatial tasks after they have been gonadectomized (Gibbs & Johnson, 2008) and better when treated with testosterone (Bimonte-Nelson et. al, 2003). Since testosterone can be aromatized to estrogen (Bimonte-Nelson et. al, 2003), researchers are unsure whether the testosterone or the aromatized estrogen is responsible for these spatial benefits.

Morris Water Maze

With regard to AD, plaques and tangles cannot be analyzed without performing post-mortem analysis. Therefore, using tasks that assess spatial learning, such as the MWM, can serve a non-invasive and significant role to monitor the progress of AD because the hippocampus is the starting point for AD and crucial for processing and storing memories. The MWM is a maze

that was developed by Richard Morris that has been used to test learning and memory in rodents for the last 35 years.

The idea of the maze was to introduce a task that challenges hippocampal learning. Because rodents are averse to water and will be motivated to swim to find an escape, the test takes place in a pool of water (Vorhees & Williams, 2006). Ensuing trials contain a platform as respite for the rodents. However, the platform is occluded by submersion in opaque water (Morris, 1984). The maze itself is divided into four equal quadrants, and the platform is positioned in the middle of one quadrant (Vorhees et. al, 2006). It is the rodents' goal to find the hidden platform. In each trial, the platform is left in the same spot, while the rodent is placed into the maze from different locations. Cues to the platform within the maze given from senses of sight, hearing and smell are minimal (Morris, 1984). This means that the rodent must rely on spatial memory, or extra-maze cues, to find the platform (Morris, 1984).

Figure 2.

Visual Representation of MWM

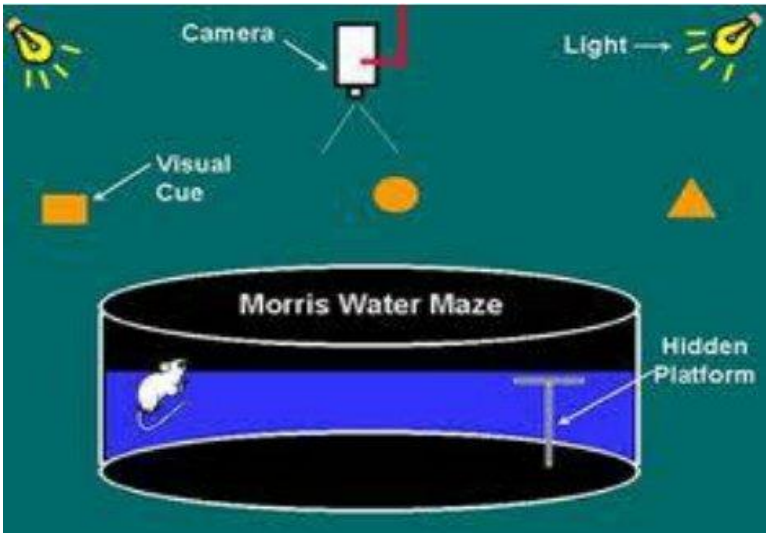


Figure 2. Illustration of MWM created by Shojaeepour et. al (2013).

The MWM forces rodents to continually monitor their position in space, using their own proprioception, distal cues such as the testing room itself, and memory to find the platform and solve the task (Morris, 1984). In the final trial, the probe trial, researchers remove the platform and have the rodents attempt to find the missing platform. The most common purpose of the probe trial is to see whether the rodents will go to where the platform used to be, and how long they will spend in the quadrant where it should have been, which is considered to be a measurement of their spatial memory. A key feature of the MWM is that it is versatile; it is widely used in research today to analyze the relationship between memory and disease, pharmacological agents, lesions and more in rodent populations and even human populations using a virtual maze (Vorhees et. al, 2006). Moreover, the maze requires little training ahead of time and exhibits high validity as a measure of spatial memory driven by the hippocampus (Vorhees et. al, 2006).

Evidence of Hippocampal Dependence of MWM

Many researchers have taken their experiments to a physiological level, looking at the specific regions in the brain associated with learning and memory. One of the earliest studies lesioned rats' hippocampi; those with lesions suffered profound navigational impairment in the maze and never reached the same level of learning as the control group (Morris et. al, 1982), indicating that the hippocampus is a primary region of the brain involved with consolidating information required for performance improvement in the MWM. In a similar experiment, hippocampal-lesioned rats showed high spatial learning impairment, but did learn to perform consistently above chance (Morris et. al, 1986). Additional research has confirmed that as long as the platform is not visible, the MWM is a hippocampus-dependent task (Cornwell et. al, 2008; Logue, Paylor & Wehner, 1997). In lesion studies, researchers have discovered that rats with

fimbria-fornix lesions are unable to encode and store spatial information (Nilsson et. al, 1987). These rats instead adopted a random, nonspatial search strategy in the MWM (Sutherland & Rodriguez, 1989). This tracks with the necessity of the hippocampus a high degree of spatial based on performance in the MWM because the role of the fimbria-fornix is to transmit information from the hippocampus to the cortex (Cassel et. al, 1997).

Previous research has linked MWM performance to LTP and NMDA receptor function, key participants in the circuitry of the hippocampus (Vorhees et. al, 2006). Similarly, Morris et. al reported research using the MWM that confirms that the infusion of d,L-AP5, a competitive NMDA receptor antagonist, caused impairment of spatial learning without affecting overall learning (1986). This specific finding suggests that the NMDA receptor, required for LTP, is similarly required for spatial learning and memory; when the NMDA receptor is inhibited, it consequently inhibits spatial learning and memory. In the case of those with AD, the NMDA receptor is compromised, which reduces spatial learning and memory in the MWM.

Measurements in the MWM

Most researchers analyze performance on the MWM by measuring the latency and distance traveled for the rodent to reach the platform over a series of trials. Measurements are normally taken with a stopwatch and video tracking software. The ideology behind these measurements is that a rodent that has greater capacity for using spatial strategies and forming memory should learn where the platform is and decrease its latency and distance to the platform over trials. In the probe trial, a rodent with greater capacity for spatial memory should spend more time in the quadrant where the platform used to be. In addition to measuring latency and distance to the platform to quantify learning and memory in the MWM, some researchers look at other measurements. These include swim speed, search error, Whishaw's error (% path outside a

corridor that is the most direct path to the platform), path efficiency (% path during which speed toward platform is 75% or more), proximity index (average distance from target) (Fritz, Amrein & Wolfer, 2017), and heading error.

Heading error is a measure of direction estimation, or accuracy to the platform (Astur, Oritza, & Sutherland, 1998). This measurement is the difference between direction of movement in the maze and the direction of the target platform (Sneider et. al, 2015), and is thought to be linked to parahippocampal areas (Morris et. al, 1986). Different researchers assess this differently; some measure the angle difference after 25% of the total diameter of the pool has been swum (Sneider et. al, 2015). Others measure the angle difference after 12 cm have been swum (Whishaw, 1985), and others measure the angle difference when the rodent is swimming at its peak speed throughout the trial (Blankenship et. al, 2019). Regardless of nuances between studies in measuring heading error, it serves as a key measurement of hippocampal dependent spatial information processing (Schneider et. al, 2017).

Performance in the MWM

Virtual reality studies analyzing performance on a human analog of the MWM have validated previous studies using rodent performance in the MWM as a measure of spatial memory (Newman & Kaszniak, 2000). In the human analog of the MWM, participants navigate to a submerged platform with a joystick. Participants navigate from different start locations and build a cognitive map using distal visual cues, just like rodents in the MWM. Learning is quantified using distance and/or latency to the platform across trials (Cornwell et. al, 2008). Similar to the results of many rodent studies, human analog studies have reported that hippocampal volume was related to performance as measured by latency and distance to the platform in an aged population, suggesting again that the virtual MWM is hippocampal

dependent (Cornwell et. al, 2008). This is consistent with another human fMRI finding, reporting that there is a positive relationship between hippocampal activation and virtual navigation performance (Maguire et. al, 1998).

Similarly, statistically significant differences in virtual MWM performance have been documented in both humans and rodents between participants with AD and control participants (Schneider et. al, 2017). A host of abnormalities in the brain, including the build-up of amyloid plaques, neurofibrillary tangles and the overactivation of the NMDA receptor are thought to be the cause of neurodegeneration and brain atrophy beginning in the medial temporal lobe and extending outwards. However, the AD mouse strain only exhibits amyloid plaques, and not neurofibrillary tangles. Nonetheless, the decline in cognitive function and memory overall has been documented in both humans and rodents. In MWM studies, mice genetically manipulated to have increased amyloid plaque in their brain, mimicking AD symptoms, have demonstrated worse performance (higher latencies and distances to the platform) compared to their control counterparts (Edwards et. al, 2014).

Recent research has also reported an inverse relationship between age and spatial memory, with younger rats performing significantly better than aged rats as quantified by latency, distance (Frick et. al, 1994; Guidi et. al, 2014), and heading error (Guan et. al, 2015), and with humans in aged populations making more errors in visual rotation questions (Segen et. al, 2020). Human studies have found that older adults showed impaired performance compared to their younger counterparts, as well (Newman et. al, 2000).

On the other hand, sex differences have also been found in both rodents and human participants using latency, distance (Astur et. al, 2004; Bucci et. al, 1995; Einon, 1980; Roof et. al, 1992; Roof et. al, 1993), and heading error (Astur et. al, 1998; Sneider et. al, 2015) with

males performing significantly better than females in the MWM. Researchers are not positive what causes these divergences in performance, but some have drawn a connection to spatial memory and hormone levels (Bimonte-Nelson et. al, 2003; Galea et. al, 1995; Gibbs et. al, 2008; Roof et. al, 1992). These studies extend a significant body of research from rodents to humans.

In addition to age, sex, and disease, exercise is a variable that impacts learning and memory. Aerobic exercise impacts the hippocampus by increasing neurogenesis (Voss et. al, 2013), glial cell proliferation (Mandolesi et. al, 2018), plasticity (Vilela et. al, 2020), and levels of BDNF (Voss et. al, 2019). These alterations may be the cause of greater performance in the MWM. Specifically, changes in the hippocampus protect an AD brain from losing even more learning and memory processing. This is only possible through the myriad of ways that aerobic exercise alters the brain.

Indeed, studies have reported that exercise groups of rodents perform significantly better on the MWM in terms of latency and distance to platform compared to their control counterparts (Alaei, Moloudi & Sarkaki, 2008; Uysal et. al, 2005). Another study examined the effects of exercise, hormones, and the combined treatment on hippocampal BDNF levels in transient congenital hypothyroid rats (Rashidy-Pour et. al, 2020). Importantly, they found that exercise, hormones, and the combined treatment increased hippocampal BDNF, but that only exercise and the combined treatment produced significant effects on learning and memory. These results suggest that while increased amounts of BDNF may influence learning and memory, this mechanism does not work alone to produce a significant effect.

Search Strategy

Memory in the MWM has traditionally been assessed using latency and distance to platform, but there are some limitations to these measurements. While these indexes are

correlated with some aspects of performance in the maze, they do not describe learning itself. That is to say, even when looking at multiple indexes at once (including swim speed, path directionality, path tortuosity, turning preferences, etc.) in an attempt to create a more holistic approach to performance, aspects of behavior are merely "summed up" (Graziano, Petrosini & Bartoletti, 2003). However, another approach exists to curb this limitation: analyzing search strategy using manual categorizations. This methodology provides a qualitative description of complex explorative behaviors, furthering our understanding of performance and learning in the MWM (Graziano et. al, 2003). The lack of extensive research in search strategy is likely a result of the time-intensive nature of this data collection, but it can open up more involved discourse regarding more accurate description of multifaceted explorative behaviors.

Garthe et. al has characterized search strategies by efficiency (2013). The rodents that employed the most efficient strategies directly approached the platform or its vicinity. These efficient strategies signified the presence of an allocentric map, requiring the hippocampus (Garthe et. al, 2013). The study shows that increasing allocentric knowledge resulted in an increase in the accuracy with which the rodent approached the platform. Allocentric strategies, or spatial strategies that included the presence of an allocentric map, included having an idea of where the platform is and swimming in its direction (directed search), knowing generally where the platform is but missing it by a hair (focal search), and knowing exactly where the platform is and swimming to it (direct swimming) (Fig. 3). Egocentric strategies, or nonspatial strategies that indicated the absence of an allocentric map, included circling the pool on the outside (thigmotaxis), random searching, methodically going back and forth through the maze (scanning), and swimming in circles toward the middle of the pool (chaining) (Fig. 3).

Figure 3.

How Lesions Impact Search Strategy

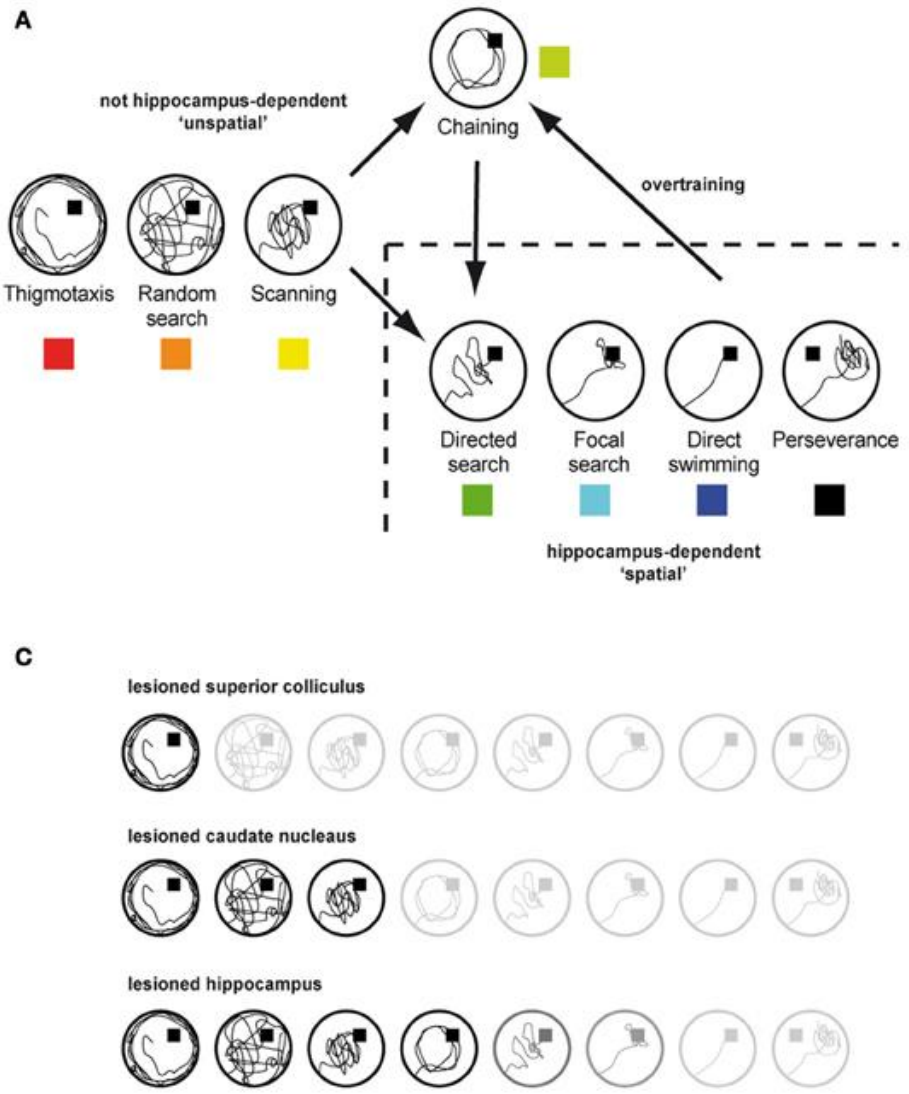


Figure 3. Visual representation of search strategies as affected by lesions of the superior colliculus, caudate nucleus, and hippocampus conducted by Garthe et. al, 2013.

The results of this study indicate that certain strategies are dependent upon particular regions of the brain. For example, in rodents with a lesioned superior colliculus, only thigmotaxis was employed. In those with a lesioned caudate nucleus, thigmotaxis, random search, and scanning were the strategies in use. Finally, in those with a lesioned hippocampus,

thigmotaxis, random searching, scanning and chaining were all strategies employed by the rodents. The spatial strategies were only utilized in those with an intact hippocampus. Rodents lacking a hippocampus were unable to form an allocentric cognitive map of the maze and had to rely solely on egocentric strategies, which were less efficient in solving the maze.

In addition to the search strategies and measures of learning and memory that are used in the MWM, similar measures are in place for the Barnes maze. The Barnes maze was invented in 1979 as a spatial learning task. The task relies on rodents' innate preference for dark, enclosed spaces opposed to bright, open spaces. In the maze, rodents are exposed in a brightly lit room, and their goal is to find shelter via an escape hole in the maze. Rodents learn where this escape hole is, despite the presence of many holes that provide no escape, by paying attention to intra and extra-maze cues. Thanks to certain advantages of the Barnes maze, such as that it has a clear spatial component but does not induce stress by the requirement of swimming, it proves to be a suitable complement to the MWM (Harrison et. al, 2006). Van Den Herrewegen et. al conducted a study assessing search strategies in epileptic rodents using the Barnes Maze (2018). Similar to chaining in the study conducted by Garthe et. al, the study at hand identified a serial search strategy, where the rodent would travel serially from one hole to the adjacent hole and so forth looking for the escape route (2013). The random search strategy and spatial strategy in the Barnes Maze are analogous to the random search strategy and spatial strategies in the MWM. The study showed that learning and memory impairment was specific to the spatial strategy, because the epileptic mouse model was able to use random and serial strategies but failed to use a spatial strategy to find the escape hole (Van Den Herrewegen et. al, 2018).

Search Strategy Results

In addition to differences in overall latency and distance to platform, previous studies have found differences in search strategy. Specifically, AD mice models (APP mice) were found to display non-search behaviors such as floating and circling, and random, non-goal directed strategies, compared to control mice who used goal-directed strategies (Baeta-Corral & Gimenez-Llort, 2015). In other studies, APP mice were also found to use chaining (Janus, 2003) and random (Wiesmann et. al, 2013) search strategies, but diet influenced APP mice to exhibit a chaining strategy, which is preferable to a random search strategy (Wiesmann et. al, 2013). Finally, some APP mice used repetitive looping (also known as thigmotaxis as classified by Garthe et. al, 2013) as a nonspatial strategy, but were able to adopt more spatial oriented strategies and less repetitive looping with training (Brody & Holtzman, 2006).

Recent literature suggests that male dominance in the MWM, while attributed to a male-favored strength for spatial skills, may also be attributed to their preference for spatial strategies to solve the maze (Astur et. al, 2004). Invariably linked with performance, hormone levels and sex may also influence search strategy in the MWM. Spatial strategies most referenced in papers that assess sex differences are geometric vs. cue-based, allocentric vs. egocentric, and place vs. response. Geometric, allocentric and place strategies are all spatial strategies that require subjects to create a cognitive map and know where they are within that space, relating objects to other objects; cue-based, egocentric, and response strategies are all non-spatial strategies that require subjects to know where they are in relation to other objects (Ferguson, Livingstone-Lee, & Skelton, 2019). Previous literature suggests that males typically employ spatial strategies more often than females (Astur et. al, 2016; Jonasson, 2005; Sandstrom, Kaurman & Huettel, 1998; van Gerven et. al, 2012).

Females are well-known to heavily prefer cue-based search strategies (looking only at one cue in the maze), and thus non-spatial search strategies (Brake, 2018; Jonasson, 2005; Picucci, 2011; Sandstrom et. al, 1998). Subjects using spatial strategies are more likely to search directly for the platform in the MWM, whereas subjects using non-spatial strategies are more likely to use thigmotaxis or serial searching, chaining, and scanning (Astur et. al, 2004; Schoenfeld et. al, 2010). The male advantage in the MWM may stem from strategies used, because allocentric strategies allow for a more direct search for the platform, and thus lower time and distance to the platform. However, females learn these tasks equally well, but typically use different strategies, or even a combination of strategies. The main difference in performance in the MWM is that males tend to develop more efficient strategies earlier than females, even though their performance is comparable by the end of training.

Presently, there are no published studies that have looked at the impact of exercise on search strategy. Given the fact that spatial strategies are dependent upon the hippocampus, rodents with less intact hippocampi tend to solve the maze using nonspatial strategies. But in light of the myriad of benefits exercise imparts on the hippocampus, search strategy may shift following robust practice in aerobic exercise. Furthermore, there are few studies in general that assess search strategy and no published studies to date that combine exercise with sex or AD status while assessing search strategy or heading error in the MWM. By only looking at distance and latency in the MWM, data is missing from the literature that can improve our understanding of how rodents learn, and how learning is intimately impacted by certain interventions and treatments.

The Current Study

Although many studies have argued in favor of the hippocampal dependence of the MWM, previous literature also indicates that spatial tasks can be completed without the hippocampus. Even though spatial tasks are not solved as efficiently by rodents with hippocampal lesions, these rodents still perform above chance (Morris et. al, 1986). The idea behind this is that typically deemed spatial tasks can be solved with both spatial and non-spatial strategies, though non-spatial strategies are initially less efficient. Defining search strategy is imperative to determine how each rodent is learning and whether the hippocampus plays a role in each rodent's learning, because the task is only hippocampal-dependent if a spatial strategy is used to solve the maze.

In sum, the ramifications of exercise, AD, and sex are broad in the context of learning and memory in the hippocampus. Typical measures of the MWM (latency, distance, amount of time spent in the target quadrant) are descriptive but fail to tell the entire story about spatial learning and memory in the hippocampus. The aim of this study is to look at measures such as heading error and search strategy to draw more comprehensive conclusions about the role of the hippocampus in spatial learning. My main research interests are 1) If exercise lowers heading error such that exercise mice will have a smaller heading error than control mice, 2) If transgenic APP/PS1 mice intended to mimic AD symptoms (referred to as TG mice) will have bigger heading error than control, wild-type mice (referred to as WT), and 3) If male mice will have a smaller heading error than females, 4) Whether exercise impacts preference towards a spatial strategy, 5) Whether TG mice show a preference for nonspatial strategies compared to WT mice, 6) Whether male mice will show a preference for spatial strategies compared to female mice, and 7) Whether groups shift strategies significantly across days.

Method

Subjects

Seventy-four mice (TG = 37, WT = 37; F = 37, M = 37; EX = 35, CON = 39) from two separate, previous studies were included in this analysis. The mice used for this experiment were transgenic APP/PS1 mice (5xFAD; Jackson Laboratory Strain Tg6799), intended to mimic AD symptoms in a rodent model. 5xFAD mice carry five genetic mutations that lead to increased plaque formation. These genes include three mutated copies of the APP gene and two of the PS1 gene. These transgenic mice exhibit neurodegeneration and amyloid plaque formation by about three months of age, making them ideal models of AD ("Jax Mice and Services"). A hemizygous female 5xFAD mouse was bred with a male WT F2 mouse to produce offspring for this study. Approximately half of each litter carried the set of transgenes and half did not. Mice carrying the set of transgenes are referred to as transgenic (TG) mice; mice lacking this set of transgenes are referred to as wild-type (WT) mice. All animal procedures were carried out in accordance with the Institutional Animal Care and Use Committee.

Materials

Following birth and weaning, half of the mice were housed in same-sex environmental enrichment groups at one month of age. Environmental enrichment housing was larger (76x40x19 cm) than control housing (40x20x22 cm) and included running wheels, tubes, wooden toys, and nesting materials. The presence of these novel stimuli and a larger cage size were in place to encourage these mice to be more active and engage in more aerobic exercise than the control group.

Researchers filled a circular pool (5 ft. diameter, 2 ft. depth) with opaque, non-toxic, water (19-21ºC). The pool was divided into four quadrants: NE, NW, SE, SW. The water maze

was in a room that contained novel objects on the walls, and these objects served as distal cues for the mice. The cues remained unchanged throughout the experiment. A submerged platform remained in the SW quadrant, 2 cm below the surface, throughout the experiment. The water was opaque to ensure that mice would use exploration and memory to find the platform rather than vision. Researchers positioned a camera directly above the center of the maze.

Procedure

Behavioral Testing Procedure as Conducted by 2019 and 2020 Studies

Data used for this project came from two existing studies conducted in 2019 and 2020. At three months of age in the 2019 study, and six months of age in the 2020 study, the mice were tested in the MWM. The mice were tested in the MWM for six trials per day over five consecutive days. In each trial, the start positions randomly varied (North, South, East, West), but over the course of six trials, each cardinal direction was used as a start position at least once. The platform remained in the same position across all testing trials. The mouse was placed into the water and, once it found the platform, was removed from the water, dried off, and returned to its cage. If after 60 seconds the mouse had not found the platform, the researcher guided the mouse to the platform before removing it from the maze. Each trial per mouse was spaced out by three to six minutes. This continued for five consecutive days. Extra-maze cues remained in place and the escape platform remained in the SW quadrant for the duration of the experiment. Twenty-four hours after the fifth day of testing, the mice underwent their last trial: the probe trial. In this trial, the escape platform was removed from the maze and each mouse swum for 60 seconds. Each trial was recorded using the video tracking program EthoVision XT12.

Experimental procedure for the current experiment

Independent variables included genotype, exercise, and sex. Animals were divided into the following experimental groups: F/M, WT/TG and EX/CON, as well as more specialized experimental groups, F/WT/EX, F/WT/CON, F/TG/EX, F/TG/CON, M/WT/EX, M/WT/CON, M/TG/EX, M/WT/CON. Dependent variables for the present experiment were heading error on day 4 and search strategy on day 2, day 4 and the probe trial.

Videos of each trial recorded using the video tracking program EthoVision XT12 were housed on a computer for later use. The first step for gathering data for the present experiment was determining which videos from the previous experiments to analyze. I did this first by selecting only the control subjects and the exercise subjects (in the enrichment group) from the previous experiments. All mice manipulated in other ways were not included in this analysis. Once I determined which subjects to use, it was important to decide which days and trials of testing to analyze. Days 2 and 4 of testing and the probe trial were selected for an evenly spaced assessment of learning over time. Because I wanted to compare strategy across days and trials, the mice needed to start off in the same start position (N, E, S, W) for all of the videos per mouse, to ensure they were required to travel the same distance to reach the platform. Therefore, trials for days 2 and 4 were determined by the start position of each mouse in the probe trial. For example, if a mouse's probe trial started out in the N quadrant, I selected the latest trial possible (3, 4, 5 or 6) on each respective day (2 and 4) that also started from the N quadrant.

Heading error was determined using EthoVision Software. The program calculated both maximum velocity and heading to point, or the angle difference between the mouse's heading direction and the location of the platform. Maximum velocity was chosen for the point in time to assess heading to point because it may imply a level of confidence for which the mouse believes it knows where the platform should be. Once I knew the time at which the mouse had reached

maximum velocity, I accessed this time point and determined what the mouse's heading to point was according to the EthoVision Software, and I refer to this as heading error at the point of maximum velocity.

Search strategy was classified in a series of steps. The first step was to watch the videos of the mice solving the maze and take notes on how each mouse solved each trial. After this was completed, patterns between trials were identified. Some of these patterns included mice circling the pool at a certain distance, going straight to the platform from their start position, or scanning back and forth in the maze to search for the platform. Each strategy was manually categorized based on observation, with the help of terms that previous researchers have coined. I decided on six different search strategies (Table 1): direct (Fig. 4A), landmark (Fig. 4B), thigmotaxis (Fig. 4C), scanning (Fig. 4D), random (Fig. 4E) and looping (Fig. 4F). This process was used to classify strategies on days 2 and 4, differently from how strategies were classified for the probe trial. These strategies were ranked with direct as best and looping as worst, on a scale from 1-6. Later, some strategies were combined in a system of ranks for the purpose of running statistical tests.

Table 1.

Verbal Classifications of Search Strategy

Direct	Mouse travels directly to the platform, normally achieved in a straight path but sometimes spins in a circle before approaching the platform.
Landmark	Mouse heads directly to an area of the maze (landmark) and uses that landmark to find the platform.
Thigmotaxis	Mouse circles the pool at a correct distance from the wall to find the platform.
Scanning	Mouse swims back and forth in a linear fashion, zig zagging methodically until it finds the platform.
Random	Mouse has no identifiable pattern but swims randomly around the maze until it finds the platform.
Looping	Mouse hugs the wall, often circling around the maze without leaving the wall.

Table 1 includes the operational definitions for how I classified each search strategy, including direct, landmark, thigmotaxis, scanning, random and looping.

Figure 4.

Visual Classification of Search Strategy

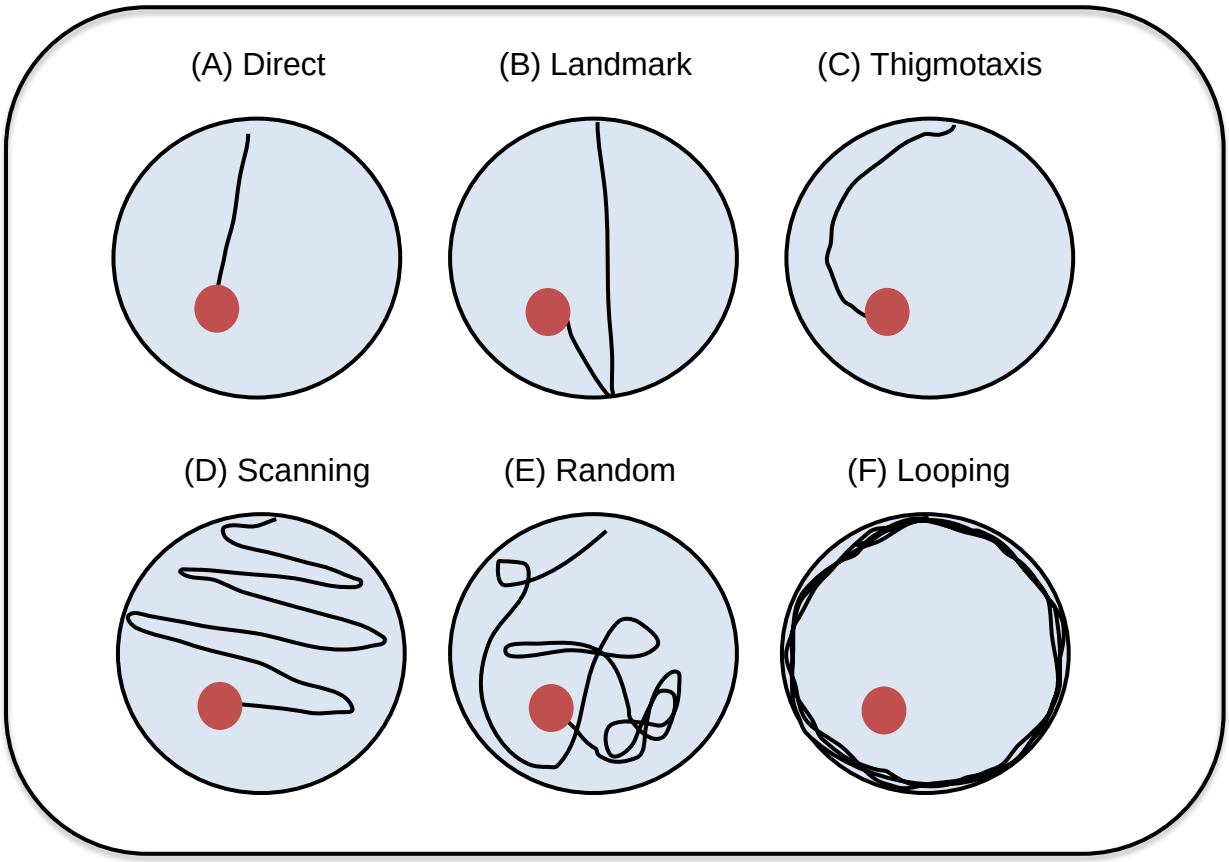


Figure 4 includes visual examples of each search strategy as classified for the purposes of this project. Strategies included are direct, landmark, thigmotaxis, scanning, random and looping.

For the probe trial, a modified process was used to determine strategy. Since the platform had been removed, there was more time for each mouse to swim around and attempt to find it. Therefore, there was the opportunity for multiple strategies to be used in an attempt to find the platform. If the mouse started off by heading directly to the platform or a landmark, it was classified as such. If the mouse completed one or more full loop the correct distance away from the wall, it was classified as thigmotaxis. If it engaged in scanning, random, or looping behavior for 15 seconds or more, it was classified as such. Given the nature of the probe trial

lacking the platform, mice could have an initial, second, and/or third search strategy. For example, a mouse may start off using a direct strategy, which would be recorded as its initial strategy. Once that strategy failed to help the mouse arrive to the platform, it may try another strategy, such as random. This would be recorded as its second strategy. However, for group-wise Mann-Whitney Wilcoxon U-tests, only the initial strategy used by each animal was included in the analysis. In sum, probe trial data collected included strategies used as well as whether mice remained in the target quadrant or left to explore when they realized that the platform was not where they expected it to be.

Results

One of my main research interests was whether sex, genotype or exercise lowers heading error at maximum velocity (hypotheses 1, 2, and 3). To test this, I ran independent samples t-tests for each of the three independent variables (F/M, TG/WT, EX/CON). I hypothesized that males would have smaller heading error than females, WT mice would have smaller heading error than TG mice, and exercise mice would have smaller heading error than control mice. Results yielded no statistically significant difference in heading error to platform at maximum velocity on day 4 between male and female, $t(72) = 0.685$, $p = 0.728$; transgenic and wild-type, $t(72) = 0.757$, $p = 0.452$; or exercise and control $t(72) = 1.275$, $p = 0.207$ groups.

Another research interest was whether sex, genotype, or exercise impacted preference for spatial strategy (hypotheses 4, 5, and 6). Because these spatial strategies were categorical in nature, all statistics were nonparametric. The Mann-Whitney Wilcoxon U-test (MWW-U test) is normally used to assess whether two categorical samples are statistically similar or different; in this case, I used the MWW-U test to determine whether belonging to a certain group influenced preference for spatial strategy within each day. I hypothesized that for each day 2, day 4, and the

initial probe strategy, males would have a higher use of spatial strategies compared to females, WT mice would have higher use of spatial strategy compared to TG mice, and exercise mice would have higher use of spatial strategy compared to control mice. For the purposes of this analysis, search strategies were ranked. Direct was ranked as 1, thigmotaxis ranked as 2, landmark, scanning and random ranked as 3, and looping ranked as 4. Landmark strategies were categorized under "random/scanning" because there were so few mice employing that specific nonspatial strategy. The MWW-U test determined whether groups demonstrated preferences for search strategies when compared to their complement group, and the probe strategy used was the initial strategy presented. The MWW-U test yielded no significant results for day 2, day 4, or initial probe trial strategy (statistical results presented in Table 2).

Table 2.

Search Strategy per Day vs. Group of Interest

	Exercise vs. Control	Female vs. Male	Wild-type vs. Transgenic
Day 2	0.544	0.533	0.941
Day 4	0.848	0.684	0.723
Probe	0.326	0.931	0.202

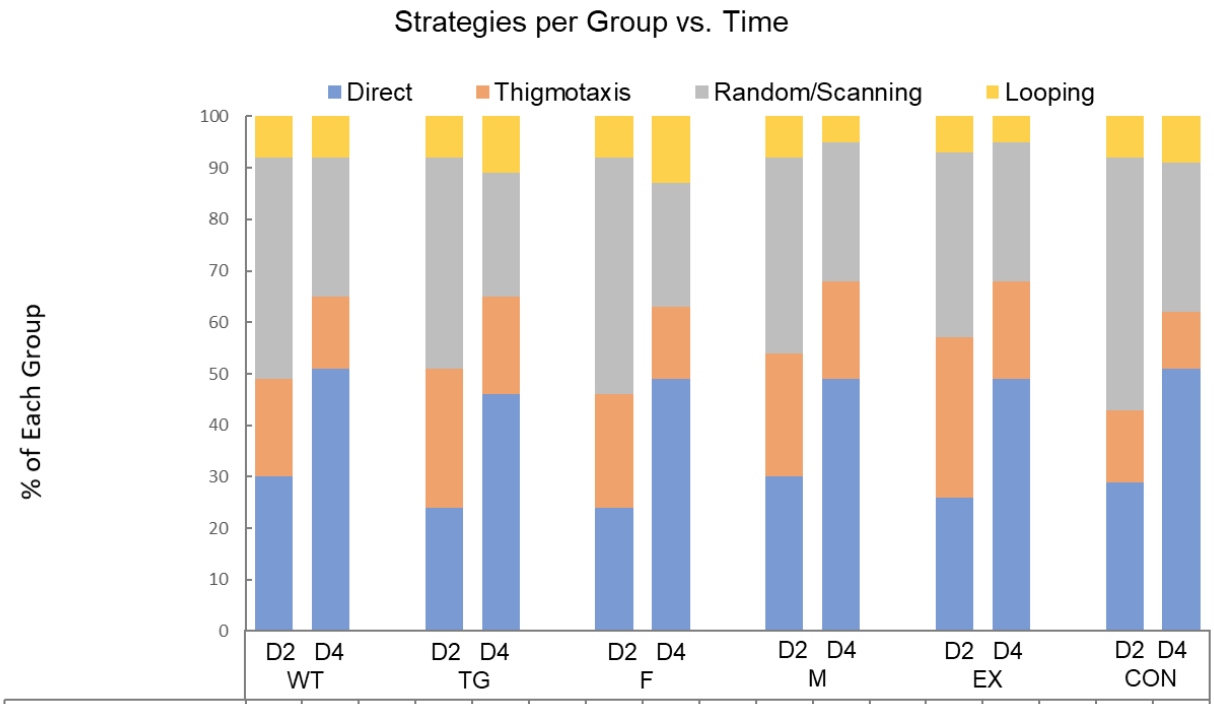
Table 2. P values from MWW-U test analyzing whether groups of interest displayed differences in search strategies employed on day 2, day 4, and probe; no comparison yielded statistical significance.

Our next research interest was whether sex, genotype or exercise would influence a shift in strategy significantly between days 2 and 4 (hypothesis 7). I was interested in comparing

within groups for this analysis, given that the MWW-U test did not indicate group-wise differences. The probe trial was excluded from this analysis because most mice employed multiple strategies in the absence of the platform. I used the Friedman test, the nonparametric alternative to the parametric repeated measures one-way ANOVA, which uses a system of values of ranks to detect differences in groups when the variable is ordinal. All groups combined did experience a shift such that direct strategies were used more in day 4 than day 2, X^2 (1) = 6.721, p = 0.010, which can be seen in the increased size of the blue bars and percentage of subjects using direct strategies in Figure 5. Concomitantly, there was a shift from more random and thigmotaxis strategies in day 2 to fewer on day 4 (Fig. 5).

Figure 5.

Strategies per Group vs. Time



	D2 WT	D4 WT		D2 TG	D4 TG		D2 F	D4 F		D2 M	D4 M		D2 EX	D4 EX		D2 CON	D4 CON
Looping	8	8		8	11		8	13		8	5		7	5		8	9
Random/Scanning	43	27		41	24		46	24		38	27		36	27		49	29
Thigmotaxis	19	14		27	19		22	14		24	19		31	19		14	11
Direct	30	51		24	46		24	49		30	49		26	49		29	51

Figure 5. Identified search strategy for each group on day 2 and day 4 are displayed for each group of interest. Numbers presented in the chart below the figure represent the percentage of each group using each respective strategy. Each independent variable, genotype (WT/TG), sex (F/M) and exercise (EX/CON) has one column representing day 2 of testing and one column representing day 4 of testing. Shifts in strategy are visually represented with color. When combined, all groups showed a significant shift in strategy over time.

We expanded upon the previous analysis by assessing whether each specific treatment condition (i.e. F/TG/EX) shifted their strategy as learning improved using the Friedman test. This analysis was used to identify a specific and individual piece of data that is not represented in the

larger test previously run. Results suggest a significant difference in strategy between days 2 and 4 for the M/TG/CON group, X^2 (1) = 4.00, p = 0.046 (Fig. 6) and the F/WT/EX group, X^2 (1) = 8.00, p = 0.018 (Fig. 7), both exhibiting an increase in the use of direct strategies. Though no other group demonstrated statistical significance, the F/TG/CON group was trending towards significance.

Figure 6.

Strategy Shift Over Time for M/TG/CON Group of Interest

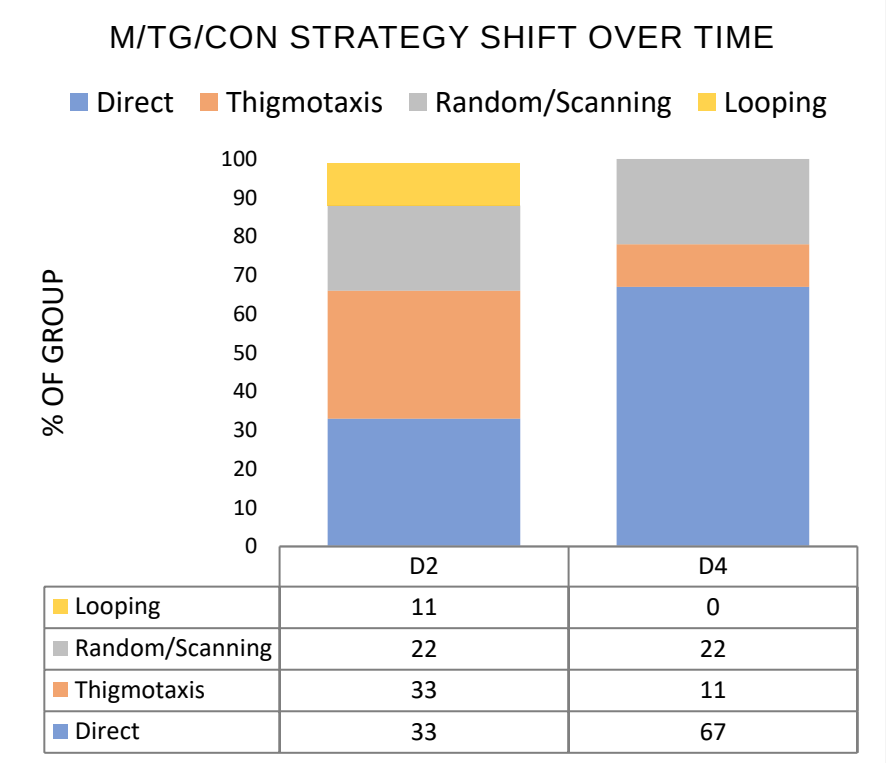


Figure 6. The male, transgenic, non-exercise group demonstrated a significant shift in strategy from day 2 to day 4. Exact percentages of this group are displayed above.

Figure 7.

Strategy Shift Over Time for F/WT/EX Group of Interest

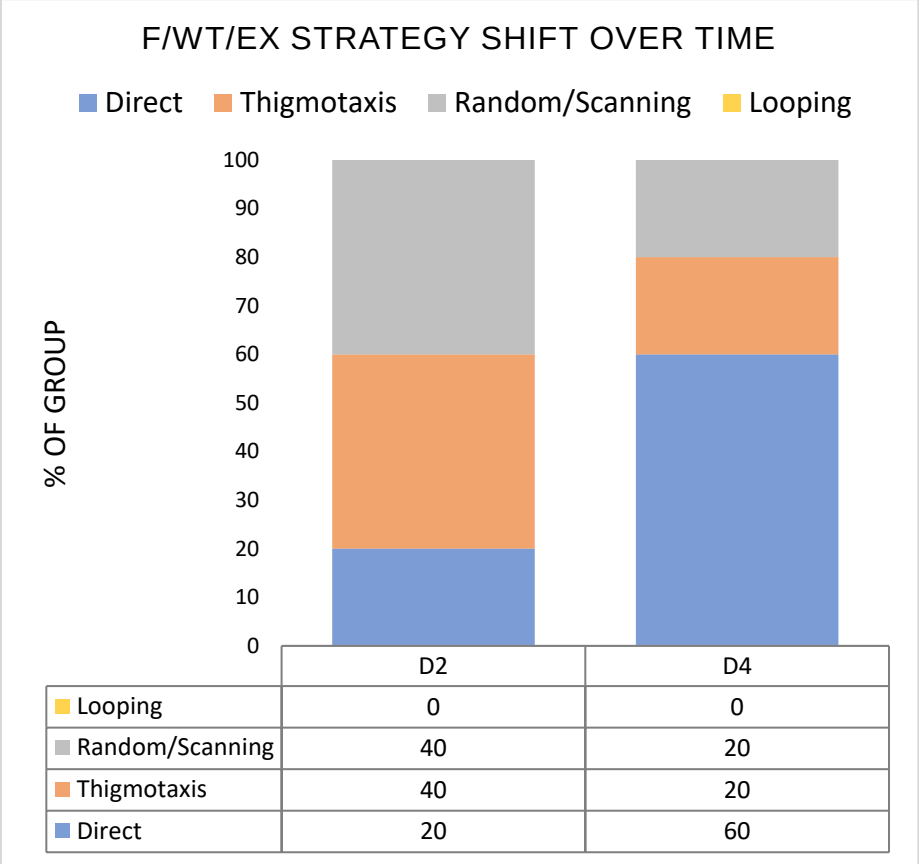


Figure 7. The female, wild-type, exercise group demonstrated a significant shift in strategy from day 2 to day 4. Exact percentages of this group are displayed above.

Though flexibility of strategy during the probe trial was not initially a variable of interest, in the process of watching and scoring these trials, it became clear that some animals perseverated on a strategy and location within the maze during the probe trial while others shifted strategies. To explore this shift in strategy, which may actually be considered more flexible problem solving, I conducted a chi-square analysis. This test determined if the number of strategies used and exploration outside of the target quadrant, and thus flexibility or learning, within the probe trial is similar or different across each group (F/M, TG/WT,

EX/CON). For chi-square this analysis, mice were categorized into one of two groups: 1) explored outside of the target quadrant and employed multiple strategies throughout the probe trial or 2) remained in the target quadrant for the duration of the probe trial and used only one strategy to search for the platform. Results yielded no statistically significant difference in number of strategies in the probe between male and female, X^2 (1) = 1.689, p = 0.297; transgenic and wild-type, X^2 (1) = 0.901, p = 0.342; and exercise and control, X^2 (1) = 0.001, p = 0.980.

Discussion

I hypothesized that exercise, genotype, and sex would influence preference for search strategy, such that female, transgenic, and control (non-exercise) groups would prefer non-spatial strategies (thigmotaxis, landmark, scanning, random, looping) and male, wild-type and exercise groups would prefer spatial strategies (direct). The data did not support these hypotheses. I was also interested in which of these groups would switch search strategies significantly between days 2 and 4, suggesting a learning pattern that favored spatial strategies. None of the umbrella group variables (M/F, TG/WT, EX/CON) showed statistically significant differences between days 2 and 4, but the targeted groups M/TG/CON and F/WT/EX groups did display significant differences in search strategy between days 2 and 4. Additionally, I hypothesized that heading error at maximum velocity would be less accurately targeted to the platform in female, transgenic, and control (non-exercise) mice, and that heading error at maximum velocity would be more accurate in male, wild-type and exercise mice. Again, the data did not support these hypotheses. Although not included in my original hypotheses, I became interested in knowing which groups would switch strategies more than others in the probe trial as a measure of flexibility, but none of the groups showed significant differences.

While meaningful findings regarding group variables were scarce in this study, there remain relevant implications for the MWM and learning as overarching themes. There was an overall trend from nonspatial strategies on day 2 to a spatial strategy on day 4. While the three independent variable groups (M/F, WT/TG, EX/CON) did not show differences in preference for spatial strategy within or between days, the existence of a significant shift overall indicates that spatial learning is evident across the board, and for some groups, takes more time to develop. Moreover, I did find a significant shift between days for the individual F/WT/EX and M/TG/CON groups. Since these two groups do not share any variables, it is difficult to claim that any one of these variables was more important for learning than another, but demonstrates that some animals were using more efficient strategies by the end of testing. Finally, we found that by the end of testing, only about half of the mice used a spatial strategy to arrive to the platform.

Fitting in with Previous Research

I expected to find that wild-type mice would display a preference for spatial strategies would shift to spatial strategies over time in greater quantities, and would have smaller heading errors than transgenic mice. All of these hypotheses were supported by the literature affirming the myriad of abnormalities that occur in the AD brain to make memory processing and storage less efficient. Some of these include neurofibrillary tangles, which destabilize the transmission of signals along the axon (Spillantini et. al, 1998), thus damaging properties such as cognitive function (Kocahan et. al, 2017); amyloid plaques, which disrupt cell communication and trigger neuronal death (Parihar et. al, 2010), contributing to cognitive decline (Morgan et. al, 2004); and overactivation of the NMDA receptor, which plays a critical role in LTP and thus learning and memory (Bashir et. al, 1991). Additional studies support my original hypotheses by finding that mice transgenically manipulated to display AD symptoms were more likely to use nonspatial,

random strategies as well as looping to solve the maze compared to the control group (Baeta-Corral et. al, 2015 & Wiesmann et. al, 2013). Spatial strategies indicate, unlike nonspatial strategies, that the mouse knows exactly where the platform is. With cognitive impairment, I would expect TG mice to employ nonspatial strategies because I would expect them to not know exactly where the platform was. However, I did not find a statistically significant difference between the WT and TG mice.

Furthermore, I expected mice in the aerobic exercise group compared to the control group to reflect my predictions of the WT group compared to the TG group: demonstrating a higher preference for spatial strategies, greater shift from nonspatial to spatial strategies over time, and smaller heading error. These predictions were similar because exercise-induced changes in the brain such as increased BDNF (Choi et. al, 2018; Mandolesi et. al, 2018; Voss et. al, 2013; Voss et. al, 2019), glial cell proliferation (Mandolesi et. al, 2018; Quan et. al, 2020; Voss et. al, 2013; Voss et. al, 2019), neurogenesis (Mandolesi et. al, 2018; Van der Borght et. al, 2007, and plasticity (Vilela et. al, 2020) all alter brain morphology to increase the functionality of LTP and have a positive impact on learning and memory. There were few studies combining exercise and search strategy as independent and dependent variables, so this study aimed to fill an important gap. I predicted benefits of exercise on spatial learning, but found no significant difference between the exercise and control groups.

Finally, my hypotheses that males would prefer spatial strategies, shift from nonspatial to spatial strategies more than females over time, and that males would have smaller heading error to the platform than females were also supported by a wealth of previous literature suggesting that males are wired to use spatial strategies more than females (Astur et. al, 2004; Brake, 2018; Jonasson, 2005; Picucci, Caffo & Bosco, 2011; Roof et. al, 1992; Sandstrom et. al, 1998;

Schoenfeld et. al, 2010). This wiring may be a result of hormones (Galea et. al, 1995; Roof et. al, 1992) and greater usage of certain regions of the brain for the respective sexes. Again, however, the data did not support these predictions.

Study Limitations

Though the data did not support my hypotheses, other studies' data have. Limitations such as small sample size, short period of time given the nature of a senior thesis project, and errors made in the previous studies from which I pulled data may contribute to the misalignment of my results to other studies' results. Because of the unpredictability of the COVID-19 pandemic, instead of collecting my own data, I combined two previously collected sets of data that came with their own shortcomings. For example, in 2019 subjects, the transgenically manipulated mice used for the project began testing at only three months of age, the age where plaques are just beginning to build. Therefore, these mice were too young to display AD symptoms. Accordingly, I would expect that a number of the TG mice used in this study did not behave as I would have expected typical TG mice to behave. Additionally, the two studies from which I drew data did not directly require exercise of the mice in the exercise group. The mice in the exercise group were housed in enriched housing, which is larger than the control cage and contains toys, tubes, and a running wheel. Though the running wheel was always accessible to them, encouraging exercise, there was no way to determine that all mice assigned to the exercise group actively and equally engaged in aerobic exercise as I had hoped for this study.

Importantly, the study of the processes of learning by means of search strategy is time and labor intensive. Each mouse completed 31 trials of testing throughout the duration of the experiment, and while I watched each mouse for at least one trial on day 2, day 4, and the probe trial, this is an incomplete representation of each mouse's learning process. I did not analyze

search strategy for the vast majority of trials per mouse. The results of this study and the mapping out of spatial learning over time would surely be more complete if each of these trials were analyzed. This is a limitation of the MWM as a general protocol. But while watching recordings of rodents in the MWM can be labor and time-intensive, it is important to keep in mind the aim of the research: to better understand learning processes with the goal of helping populations overcome roadblocks to their learning. Future studies may find more conclusive results about the processes of learning if search strategy is assessed over more trials and days, and the categorization of strategy and flexibility of learning may be best achieved by watching the mice as they learn. However, the sheer number of trials that need to be watched in order to categorize search strategy and draw conclusions about the process of learning is a limitation of the MWM protocol itself.

Meaningful Implications

After categorizing the search strategy of hundreds of trials in the MWM, it is clear that the spatial strategy (direct) is superior in efficiency to all nonspatial strategies but one: thigmotaxis. Anecdotally, mice employing thigmotaxis, circling the maze at a specific distance from the perimeter, as their search strategy used a commensurate amount of time and distance to find the platform compared to mice employing a direct search strategy. Therefore, the use of this particular nonspatial strategy suggests that even when a mouse does not know exactly where the platform is, thigmotaxis can save cognitive resources and make it equal in efficiency to a mouse that does (Garthe et. al, 2013). Notably, the shift in strategy from random on day 2 to direct on day 4 was more apparent than a shift away from thigmotaxis to direct. Essentially, there is less shift away from thigmotaxis than random over time. Mice employing thigmotaxis as a strategy

on day 2 may have kept their strategy throughout trials and days because thigmotaxis was an efficient strategy in the first place.

In MWM research, rodents that have low latency and distance to the platform are typically deemed “spatial learners.” However, my results demonstrate that there are nonspatial learners that achieve the same level of performance as quantified by distance or latency to the platform as spatial learners. Moreover, only half of the mice in this study, and likely in other studies, even use a spatial strategy by the end of the maze. Therefore, an assessment of strategy is necessary to claim whether rodents are spatial learners or nonspatial learners. This finding also challenges the claim that the MWM is strictly a hippocampal-dependent task. Previous research states that rodents lacking a hippocampus are unable to form an allocentric cognitive map of the maze and relied only on inefficient egocentric, or nonspatial, strategies (Garthe et. al, 2013). However, these results suggest that a percentage both begin and end the maze using an efficient nonspatial strategy; these rodents, therefore, are solving the maze as efficiently, yet without the primary use of the hippocampus.

Moving Forward

The goal of this research was to help map out the behavioral process of learning in a mouse model and extrapolate it to a human population. Understanding the process of learning in WT and TG mice can help us understand how to improve learning in populations where there are challenges in learning and memory as a result of AD. The study of sex helps illustrate how inherent differences in males and females can impact learning. Meanwhile, the study of aerobic exercise helps illustrate how lifestyle choices can impact the processes and outcomes of learning. Uniquely, aerobic exercise is a free treatment that can ameliorate some effects of AD on learning and memory, so understanding its impact is invaluable. In studying the processes of learning on a

behavioral scale, we can identify potential routes to help AD populations curb disadvantages to learning and memory, and potentially lead to a more fulfilling life.

The goals of this research and ideas behind it can still be explored. There are few studies that assess learning and memory with the goal of understanding how the process of learning occurs. In a rodent model, search strategy in the MWM is vastly understudied in comparison to latency and distance. Furthermore, there is a dearth of research combining search strategy with exercise: a free treatment that yields a prolific number of health and brain benefits. One important future avenue of study would be to assess the impact of exercise on search strategy, without the complication of voluntary exercise. This might be achieved using a treadmill. Another avenue of future study would be to use properly aged TG mice and determine how transgenic status impacts search strategy.

In addition to search strategy, another measure of MWM performance that would suggest patterns in learning is flexibility in the probe trial, or the number of strategies used and persistence with which a rodent commits to the quadrant where the platform used to be. Many current studies deem evidence of a positive measure of spatial learning in the probe trial as the animal spending significantly more than 25% of the time in the platform quadrant. Yet, this may be another traditional MWM measurement that is limited. Instead, determining the strategy of the rodent in the probe trial and taking note of the point where the rodent has learned that the platform is not there and explores other options may be a better indication of learning, and in fact flexibility of learning.

Several published studies have looked at flexibility, the inverse of perseverance, in the probe trial by way of moving the platform, rather than removing it altogether. This may be referred to as platform reversal or reverse probe trial. Studies have deemed perseverated

swimming to the old location of the platform to be indicative of a lack of cognitive flexibility. Moreover, findings have pointed to a lack of newborn hippocampal neurons to be linked to strong perseverating preference for the old location of the platform, and thus the inability to master spatial task flexibility and efficiency (Garthe, Behr & Kempermann, 2009). Another study analyzed the relationship between the expression of astrocytes and flexibility in the reverse probe trial. Indeed, glial cells have been recognized as key players in synaptic transmission and thus neuronal activity; Wolfer et. al found that L1 astrocytes appear to be linked to increases in flexibility while learning by way of positively affecting LTP (2001). From these papers, it is clear that the traditional measure of percentage of time spent in the probe trial suggests not better learning, but perseverated learning that is indicative of a lack of cognitive flexibility.

The MWW U-test results for the current study as a measure of flexibility of learning are preliminary, as well as basic and secondary to my primary interests of the processes of learning over time. Further analyses and additional observations in each trial would be necessary to move forward in this area. Notwithstanding, this area of research is largely untouched however vital to consider as we think more carefully about how to use the MWM to parse out if and how rodents learn.

Conclusion

In this study, group differences between transgenic and wild-type, female and male, and exercise and control in search strategy were not identified, but these variables may have relevant implications for learning and memory and should continue to be studied. By identifying how learning occurs through search strategy over time and through other measurements, such as flexibility of learning, we can intentionally seek interventions that aid individuals and populations in surmounting roadblocks to learning and memory.

In sum, the MWM as a measure of spatial learning is limited in how researchers use it today. Latency and distance to platform as well as amount of time spent in the target quadrant during the probe trial are all typical measures that are meant to determine whether learning occurs, but are severely limited in that they do not represent the process of learning nor cognitive flexibility. Likewise, many studies refer to mice with lower latencies and distances to the platform as spatial learners, but as we see in this study, only about half of the mice by the end of testing even use a spatial strategy. Instead, thigmotaxis as a nonspatial search strategy is highly employed by mice in the maze and functions as a shortcut to preserve cognitive resources while solving the maze as efficiently as mice using a spatial strategy. Because this efficient nonspatial strategy does not require the use of the hippocampus, this finding challenges the claim that the MWM is strictly a hippocampal-dependent task. Overall, as a scientific community, we need to think more carefully about how we use the MWM to parse out if and how mice learn to better understand advanced aspects of AD and how to overcome challenges to learning and memory.

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