EXERCISE, SPATIAL MEMORY AND HIPPOCAMPAL FUNCTION

EXERCISE ENHANCES ALLOCENTRIC PROCESSING AND HIPPOCAMPAL FUNCTION IN THE ADULT BRAIN

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TITLE: EXERCISE ENHANCES ALLOCENTRIC PROCESSING AND HIPPOCAMPAL FUNCTION IN THE ADULT BRAIN

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ABSTRACT

This experiment explored whether a long-term aerobic exercise program may induce significant structural and functional changes in the hippocampus, an area of the brain that is important for spatial navigation and memory formation. Based on existing rodent studies, we hypothesize that exercise will cause a shift to allocentric processing, away from a less robust egocentric learning strategy. It is possible that exercise-induced relief of chronic stress, which contributes to improved hippocampal function, will increase reliance on allocentric spatial navigation. Neurogenesis, which occurs in the dentate gyrus region of the hippocampus, is another indicator of hippocampal function that may influence this shift to allocentric learning.

The current study examines whether six weeks of aerobic exercise enhances allocentric processing in healthy young adults. Forty-nine young adults (35 female; age range 18-29 years) were randomly assigned to one of three groups: 1) High intensity interval training group, 2) Moderate intensity training group, or 3) Non-exercising control group. Hippocampus-dependent memory was assessed before and after the intervention on a *Virtual Reality Water Maze* task, and a high interference memory task, the *Mnemonic Similarity Task* (MST) which may be dependent on hippocampal neurogenesis. Levels of chronic stress and depression were measured using the Beck Depression Inventory II. It was expected that exercise would improve spatial memory performance on the water maze task, and that performance would improve in proportion to enhanced fitness levels. This improvement in spatial memory performance was expected to correlate with the two indicators of hippocampal function that were assessed in the current study—chronic stress and performance on the high interference memory task.

Six weeks of regular aerobic exercise resulted in a 21.5% improvement in spatial memory performance on the water maze task, indicating improved hippocampusmediated spatial memory function. Improvements displayed by high intensity exercisers were greater than those observed in the moderate intensity exercisers, suggesting that higher intensity exercise may be more effective in enhancing hippocampal function. Importantly, low responders to exercise exhibited a 30% improvement in water maze performance, suggesting that even minor fitness improvements can lead to significant cognitive gains. Chronic stress and depression, and performance on the MST were not significantly associated with changes in spatial memory performance; however trends observed may offer some explanation to the aforementioned changes in spatial memory. Findings from the current study have important implications for treatment options in populations that are currently, or at risk of suffering from impaired hippocampal function.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
BDI-II	Beck Depression Inventory II
BrdU	Bromodeoxyuridine
CA1	Cornus ammonis 1
CA3	Cornus ammonis 3
DG	Dentate gyrus
EC	Entorhinal cortex
fMRI	Functional magnetic resonance imaging
HIIT	High Intensity Interval Training
HR	Heart rate
MIT	Moderate Intensity Training
MRI	Magnetic resonance imaging
MST	Mnemonic Similarity Task
RPE	Ratings of perceived exertion
VO2 peak	Peak oxygen consumption

DECLARATION OF ACADEMIC ACHIEVEMENT

S. McLaughlin's role:

- Contributed to study concept, design, and measurement selection
- Contributed to participant recruitment
- Trained and supervised volunteers who assisted with data collection
- Responsible for data collection, analysis, and interpretation
- Responsible for preparation of manuscript

Role of SB:

- Lead contributor to study concept, design, and measurement selection
- Contributed to obtained study funding

INTRODUCTION

Aerobic exercise benefits overall brain health, causing significant improvements in cognitive function (Hillman, Erickson & Kramer, 2008). The benefits of exercise include protection from age-related neurodegenerative diseases (Um et al., 2008; Yaffe, Barnes, Nevitt, Lui & Covinsky, 2001), the alleviation of chronic stress and depression (Binder, Droste, Ohl & Reul, 2004; Singh et al., 2005), and learning and memory enhancements (Creer, Romberg, Saksida, van Praag & Bussey, 2010; Dery et al., 2013). Much of our understanding derives from animal studies, which have helped scientists identify many of the cellular and molecular modifications underlying the neurological benefits of exercise. Findings can then be translated from animal models to human physiology so that appropriate healthrelated recommendations can be made. The exercise-induced structural and functional changes that occur in the brain are largely due to synaptic plasticity and the processes that support plasticity, such as neurogenesis, the birth of new brain cells (Farmer et al., 2004; van Praag et al., 1999a). The effects of exercise on the hippocampus are particularly interesting, as the hippocampus is a structure in the brain that is important for certain kinds of associative memory, including memory for sequences, contexts and episodes (Chun & Phelps, 1999; Fortin, Agster & Eichenbaum, 2002; Scoville & Milner, 1957; Tulving & Markowitsch, 1998) and is also critical for spatial memory and navigation (Bohbot, Iara & Petrides, 2004; King, Burgess, Hartley, Vargha-Khadem & O'Keefe, 2002; Morris, Garrud, Rawlins & O'Keefe, 1982; Smith & Mizumori, 2006)-the focus of this study. Extensive rodent literature supports the contention that exercise enhances hippocampus-dependent spatial memory performance (Mustroph et al., 2012; van Praag, Christie, Sejnowski & Gage, 1999a; Praag, Shubert, Zhao & Gage, 2005). It is possible that exercise can also influence spatial memory and spatial navigation strategy choice in humans. Consequently, the main research question addressed by this study is whether exercise will improve hippocampus-dependent spatial memory performance on a virtual reality water maze task in healthy young adults. Understanding how exercise can be used to induce such neurobiological changes in humans is vital, since it may allow scientists to provide advice on preventative and therapeutic measures for various brain disorders, such as Alzheimer's disease.

Spatial Navigation strategies: Allocentric and Egocentric Processing

Imagine that you just moved to a new city to take up a job offer. The day before your first day of work, with your briefcase in one hand and a map in the other, you start walking from your apartment to the address of your new company. There are two main strategies you may use to read the map and ensure you remember the route tomorrow. If you are an allocentric spatial navigator, you will navigate using topographic relations between distant landmarks as points of reference. You will mentally rotate your surroundings until your imagined heading matches the orientation of the map. As you make your way to the office building, you may notice, for example, that your workplace is east towards the waterfront, then north in the direction of the mountain range.

On the other hand, if you are an egocentric navigator, you will navigate using your previous position as a point of reference. You will rotate the map until it is in line with your physical motion so that you can use internal cues or self-movement to guide yourself, in combination with proximal cues. For instance, you may remember that upon leaving your apartment, you should walk towards the church, and then turn right at the first cross-street.

Allocentric Navigation. Allocentric spatial navigation of an environment involves encoding spatial relationships between multiple landmarks-salient cues located at a distance from the observer. This type of mental representation of the environment is known as a *cognitive map*—a term first introduced by Edward Tolman (1948). As observers move through the environment, their focus shifts to different parts of the map. As their focus shifts, the map is continuously updated with detailed and relevant information, consequently leading to a more robust spatial memory (O'Keefe & Nadel, 1978). Cognitive mapping is considered to be a cognitively complex process compared to simpler navigation strategies such as cue learning. This is due to the flexibility of the cognitive map. Unlike cue learning, the map is not limited to helping the navigator learn one specific route; it can be used to navigate an unlimited number of routes and locations within the map. Additionally, the map allows for adaptation to environmental change, so that if one or a set of distal cues is removed or modified, the relationship between the remaining cues can still assist in navigation to a particular place (O'Keefe & Nadel, 1978).

Cognitive mapping is heavily reliant upon the hippocampus, and is supported also by the entorhinal cortex (EC) and other surrounding structures in the medial temporal lobes (Okeefe & Nadel 1978; Remondes & Schuman, 2004). One important line of research that supports the importance of the hippocampus in allocentric spatial navigation is lesion studies in rodents. This research shows that hippocampal damage leads to severe deficits in performing various types of allocentric spatial navigation tasks (Morris et al., 1982; M. Moser, E. Moser, Forrest, Andersen & Morris, 1995). Human research comparing patients with varying levels of hippocampal damage has shown that spatial recall is strongly associated with bilateral hippocampal volumes (Guderian et al., 2015). Pyramidal cells known as *place cells* make the hippocampus structurally and functionally suitable for cognitive mapping. Place cells, reported in both rodents (Wilson et al., 2003) and humans (Ekstrom et al., 2003), become active when an animal occupies a specific location within its environment, known as a 'place field'(O'Keefe & Nadel, 1978;Wilson et al., 2003). Subsets of place cells collectively form a cognitive map for each environment the animal experiences (O'Keefe & Nadel, 1978). In processing the spatial information, these place cells communicate with other specialized cells in the hippocampus, EC and surrounding areas (Remondes & Schuman, 2004). While place cell firing depends on an animal's location in the environment, it does not depend on the animal's orientation or rotational movements made when navigating the environment (O'Keefe & Nadel, 1978). For this reason, long-term spatial memory relies on allocentric representations, because the body will not likely be in the same location or orientation during memory retrieval (Byrne, Becker & Burgess, 2007).

Functional differentiation for spatial learning has been found within the hippocampus. For example, electrophysiological recordings of rats indicate that the dorsal hippocampus has a higher proportion of place cells than the ventral hippocampus (Jung, Wiener & McNaughton, 1994). Moreover, lesioned rats in a Morris Water Maze paradigm required the dorsal, but not the ventral, hippocampus for spatial learning (M. Moser et al., 1995). Neuroimaging methods have allowed researchers to non-invasively confirm these findings in humans. For instance, structural magnetic resonance imaging (MRI) studies revealed that more experienced taxi drivers had greater dorsal hippocampi and smaller ventral hippocampi than less experienced taxi drivers, and that all taxi drivers had greater dorsal hippocampi than subjects who did not drive taxis (Maguire et al., 2000).

Loss of function within the hippocampus causes animals to shift away from allocentric processing, instead relying on extra-hippocampal systems that support a different type of spatial learning known as egocentric processing (Kim, Lee, Han & Packard, 2001; Schwabe, Dalm, Schächinger, & Oitzl, 2008).

Egocentric Navigation. Egocentric navigation involves executing particular orientations and movements in the presence of specific cues in order to reach a target location (O'Keefe & Nadel, 1978). In other words, it is finding your way using proximal or internal cues. *Proximal cues* are objects that are close to the observer (versus landmarks, which are farther away) and act as markers of what direction to move in. *Internal cues* include information on rate of movement and on direction. Information on rate of movement comes from proprioceptors, which are sensory nerve endings commonly found in muscles, tendons, joints and the inner ear (Vorhees & Williams, 2014). Information on heading and direction of movement comes from specialized cells found within an area of the brain called the dorsal striatum.

The dorsal striatum- in particular, the caudate nucleus- is strongly associated with egocentric processing (Potegal, 1969). Rats with striatal lesions perform more poorly than controls on navigation tasks requiring egocentric processing (Potegal, 1969). Specialized cells called head direction cells make the dorsal striatum critical to egocentric processing. Head direction cells are neurons that are

activated when an animal's head points in a specific direction along the horizontal plane of an environment. Weiner (1993) conducted the first neurophysiological recordings of the dorsal striatum during a navigation task, successfully providing evidence of the existence of head direction cells in rats. Recordings were taken from five dehydrated rats as they searched for water in a square-walled open environment. The recordings revealed location-selective changes in firing rates—that is, the firing rate of cells selectively increased when the rat's head was oriented towards a certain direction, regardless of where the rat was located within the environment. Structural and functional imaging in humans further supports the role of the caudate in egocentric spatial cognition. For example, MRI analyses of older adults who completed a virtual navigation task revealed that use of an egocentric strategy is negatively associated with hippocampal grey matter (Konishi & Bohbot, 2013).

Allocentric versus Egocentric Navigation. Literature supports that allocentric and egocentric memory systems work cooperatively to allow animals to navigate their environments (Rice, Wallace & Hamilton, 2015). Each system has distinguishing features, which are ideal under different circumstances. Allocentric representations are beneficial for long-term spatial memory formation, because they allow one to retrieve a memory even when encountering a scene from a novel viewpoint. Egocentric representations, on the other hand, may be more important for short-term spatial memory, when the local layout of objects around the observer must be maintained as they change their head and body position (Byrne et al., 2007). Additionally, repeated exposure or over-learning of egocentric representations results in procedural, or habitual, memory. This means, for example, that the more often you take a particular route to a target destination, the more you will be able to follow the route without thinking about it (Vorhees & Williams, 2014).

Perhaps the largest difference between allocentric and egocentric processing is flexibility. While, as previously described, cognitive maps allow the observer to readily adjust to changes in the environment, egocentric navigation must be used in the correct sequence, based on specific cues. Thus, disorientation, momentary distraction, or a cue that no longer exists, can prevent egocentric navigators from reaching their target locations (O'Keefe & Nadel, 1978). For instance, if a person is used to turning left at the red mailbox on their way from the bus stop to the dentist's office, the person may miss their turn if the owners of the mailbox decide to paint it purple instead.

Additionally, while cognitive maps allow navigators to remain oriented no matter where they are within the environment, subjective rotations within an egocentric space may cause the navigators to lose their bearings and become confused about which direction to head (O'Keefe & Nadel, 1978).

In sum, two main memory systems work together to allow animals to navigate their environments: a cognitively complex allocentric system, and a simpler

egocentric system. Since the two systems primarily rely on different areas of the brain, the egocentric processing system can compensate for loss of function, due to factors such as aging or neurodegenerative disease, within the allocentric processing system.

Measuring spatial strategy choice: the Morris Water Maze

The Morris Water Maze (also referred to as 'water maze' in the current paper) is one of the most widely used tasks to assess allocentric spatial memory. The paradigm was originally developed by Morris to assess spatial or place learning in rodents (Morris, 1984). Essentially, the task involved placing a rat into a circular pool of opaque water and observing as it escaped to a platform hidden beneath the water. On each trial, the rat started from a different location around the perimeter of the tank and could use distal cues surrounding the pool to allocentrically navigate its way back to the same target location. In other words, the rats could form a cognitive map that represented the geometric relationship between the platform and a set of distal cues. Stronger water maze performance may be indicated by decreased time to reach the hidden platform (latency), decreased path length from starting point to the hidden platform, and more time spent in the quadrant where the hidden platform is located.

Since the development of the original Morris Water Maze, several additions to the procedure and different versions have been developed. In addition to distal cues, some mazes have proximal cues within the pool, which allow for the use of an egocentric strategy. In the water maze, this strategy entails defining the platform position according to a heading vector by learning the direction and distance of the platform from a particular cue located within the pool. Pearce, Roberts and Good (1998) confirmed the use of this model for determining allocentric versus egocentric spatial encoding by testing rats in a Morris Water Maze. Results showed that rats with hippocampal damage tend to rely on intramaze cues to find a hidden platform location (egocentric strategy), while control rats relied more heavily upon distal cues surrounding the pool. Recently, a virtual adaptation has been used to investigate spatial navigation strategies in humans (e.g. de Castell, Larios, Jenson & Smith, 2015; Hamilton, Driscoll & Sutherland, 2002). The concept behind the Virtual Morris Water Maze is the same as the original maze, except the user is controlling an avatar (the virtual "rat").

Sex has been shown to affect water maze performance. Males have demonstrated better spatial memory performance than females in the hidden platform trials of a virtual water maze task (de Castell et al., 2015). Analyses also revealed a statistically significant main effect of trial in males, who had decreasing path lengths across trials. Females, on the other hand, did not show a statistically significant main effect of trial. In other words, males acquired the water maze task

quicker and to a greater degree than females. Although the current study did not have equal numbers of male and female participants, the sexes were evenly distributed across the intervention groups in order to prevent biases in betweengroup comparisons.

Exercise enhances hippocampal functioning

Animal research has shown that the brain can respond to external stimuli through structural and functional changes. In fact, it has been shown that exercise alone can induce such plasticity, particularly in the hippocampus (Farmer et al., 2004). Hippocampal atrophy and loss of function reduce an animal's use of the hippocampus-mediated allocentric processing system, in favor of striatal egocentric processing. A long-term aerobic exercise program, on the other hand, may cause a shift back from egocentric processing to a more robust allocentric learning strategy by enhancing hippocampal function. The effects of exercise on allocentric learning have been widely demonstrated with rodents in the water maze paradigm. Studies show that compared to non-exercising control groups, mice assigned to running interventions showed steeper learning curves on water maze learning trials, as demonstrated by decreased latency and decreased path length from the starting point to the hidden platform (Mustroph et al., 2012; van Praag et al., 1999a). A study by van Praag et al. (2005) divided young mice, three months of age, into two groups: a running group, which had unlimited access to a running wheel, and a non-exercising control group. Similar groups were created for a set of old mice (19 months of age). Test results revealed that in comparison to non-exercising controls, both young and old runners displayed significantly improved acquisition of the water maze, exhibiting a significant bias for the area of the pool in which the hidden platform was located. Little research exists supporting the benefits of exercise on allocentric processing in humans.

The neurobiological mechanisms underlying the exercise-induced enhancements in hippocampal function remain unclear. Two indicators of improved hippocampal function that are hypothesized to affect the exercise-induced shifts between egocentric and allocentric learning are increased *neurogenesis* and reduced *chronic stress*.

Underlying Mechanisms: Neurogenesis. A part of the hippocampus known as the dentate gyrus (DG) is one of two areas in the mammalian brain that has the ability to generate new neurons into adulthood. The newly born cells are generated in the granule cell layer of the DG (Eriksson et al., 1998). Exercise promotes neurogenesis by increasing cell proliferation and survival in the DG. This has been confirmed in animal studies using immunohistological techniques to label and count dividing cells (van Praag et al., 1999a,b). For example, mice assigned to a running intervention had significantly more granule cells labelled

with bromodeoxyuridine (BrdU) than non-exercising control mice (van Praag et al., 1999a).

Neurogenesis and Pattern Separation. The contribution of the newborn granule cells to hippocampal memory formation remains unclear. One of the most supported hypotheses is that the neurons facilitate performance on high interference memory tasks by increasing *pattern separation* in the DG. Pattern separation is the process of creating distinct memory traces for highly similar patterns, rendering these overlapping representations less similar. The DG is anatomically suited to conduct pattern separation. Information primarily flows to DG neurons from the entorhinal cortex (EC). The DG has 5 to 10 times more neurons than does the EC, and yet the mature dentate granule cells fire very sparsely, so information for highly similar events can be projected to the DG in more distinct detail. After the input information is separated within the DG, it is projected to a region of the hippocampus called the CA3 for further processing (for a review, see Deng et al., 2010). Without this DG-mediated discrimination, highly similar events may be encoded too similarly and consequently may converge into a single memory. Putative neurogenesis-dependent computer tasks are thought to be dependent upon pattern separation in humans. Of particular interest to the current study is the Mnemonic Similarity Task (MST; formerly called the Behavioral Pattern Separation task), which is a recognition task that was first developed by Kirwan and Stark (2007). The study used functional magnetic resonance imaging (fMRI) to demonstrate that hippocampal DG activity correlated with performance on high interference trials requiring participants to distinguish between two similar items. Another study demonstrated that young adults who participated in a long-term aerobic exercise intervention had enhanced post-intervention performance on an adapted version of the MST. The study also found that those who experienced greater fitness improvements exhibited stronger performance (Dery et al., 2013). Moreover, those with high stress and depression scores performed more poorly on this task (Dery et al., 2013). The opposing effects of stress and exercise on neurogenesis in rodents and on performance on the MST in humans are consistent with the hypothesis that performance on the MST may be dependent upon neurogenesis levels.

Neurogenesis and hippocampus-dependent spatial memory. Whether the newborn granule cells are required for hippocampus-dependent spatial memory is under debate. Several studies have shown a positive correlation between neurogenesis and allocentric learning. For example, in aged rats (10 to 11 months old), the top 8 water maze performers (shorter distance to platform or lowest latency) had the highest numbers of newly born granule cells and the highest numbers of surviving neurons, while the worst 8 performers had the lowest numbers (Drapeau et al., 2003). Furthermore, conditions known to improve hippocampal neurogenesis, such as an enriched environment, or a long-term aerobic exercise intervention, also improve allocentric memory in a water maze

task (Kemperman, Kuhn & Gage, 1997; van Praag et al., 1999a; van Praag et al., 2005). This positive association between neurogenesis and allocentric learning has led to the hypothesis that the newly born neurons may partially contribute to exercise-induced improvements in spatial memory.

On the other hand, emerging evidence attributes the effects of exercise on hippocampus-dependent spatial memory to factors other than neurogenesis. For example, a study by Siette et al. (2013) found that 12 weeks of voluntary running improved spatial memory performance in aged but not younger mice and upregulated hippocampal neurogenesis in both groups. However, it was found that hippocampal neurogenesis did not correlate with place memory performance.

Further research is necessary to clarify the relationship between neurogenesis and allocentric memory.

Chronic Stress. Many studies have found that exercise relieves chronic stress and other symptoms of depression. For example, one study found that exercise caused a 50% reduction in depressive symptoms in participants, and that these effects were observed more frequently with high intensity exercise than with low intensity exercise (Singh et al., 2005). The exercise-induced relief of depression has implications for hippocampal functioning.

The hippocampus contains a high concentration of receptors for adrenal steroids, which alter hippocampal morphology and function (for a review, see McEwen, 2004). Prolonged release of stress hormones can have deleterious effects. For example, human patients who have experienced multiple episodes of depression exhibit reduced hippocampal volume (MacQueen et al., 2003). Moreover, studies in rodents have shown that chronic stress inhibits neurogenesis and neuronal survival in the dentate gyrus (Gould, McEwen, Tanapat, Galea & Fuchs, 1997; Gould, Tanapat, McEwen, Flügge & Fuchs, 1998). The ensuing loss of function within the allocentric processing system forces animals to rely on extrahippocampal systems. For example, studies have shown that rodents and humans exposed to both acute stress (Kim et al., 2001; Schwabe et al., 2007) and chronic stress (Schwabe et al., 2008) tended to use a non-hippocampal-based strategy to acquire certain spatial navigation tasks, while controls preferred allocentric learning to a greater degree. These studies support that stress causes a shift from hippocampal-based learning and memory, to a simpler, more rigid stimulusresponse strategy called egocentric navigation.

Conversely, animal models have demonstrated that stress-induced trauma in the hippocampus can be reversed to some degree. For instance, interventions that have antidepressant action, such as aerobic exercise and antidepressant medication, also stimulate neurogenesis (Malberg, Eisch, Nestler & Duman, 2000; van Praag et al., 1999a). It is therefore possible that exercise-induced relief of stress and depressive symptoms contributes to improved hippocampus-dependent

spatial memory and that these changes occur partially through the upregulation of neurogenesis.

Purpose and Hypothesis

The present study examined the effects of six weeks of cycling on performance on a virtual reality water maze task and on several control tasks in healthy young adults. The aim of the study was to determine whether aerobic exercise improves hippocampus-dependent spatial memory, or, allocentric memory. In addition, the study aimed to provide support for the role of neurogenesis in allocentric learning. 49 young adults were randomly assigned to one of three groups: 1) High intensity interval training (HIIT) group, 2) Moderate intensity training (MIT) group, or 3) Non-exercising control group. A batter of hippocampus-dependent and other memory tasks and fitness levels were assessed pre- and post-intervention. Based on the literature, it was hypothesized that exercise would improve spatial memory on the water maze task, and that performance would improve in proportion to enhanced fitness levels (Figure 1: H1). Additionally, it was expected that the improvement in spatial memory performance would be associated with two indicators of improved hippocampal function: a) Improved performance on a putative neurogenesis-dependent task (Figure 1: H2), and b) Improved ratings of depression (Figure 1: H3). These two indicators are expected to be negatively correlated with each other.

Figure 1. Hypotheses.



METHOD

The research described in this paper is part of a larger study that investigates the effects of physical exercise and cognitive training on neurocognitive function in young adults. This paper specifically focuses on data relating to the effects of physical exercise on spatial navigation strategy. All parts of this study were approved by the Hamilton Integrated Research Ethics Board (HIREB).

Participants

Data come from a larger research study with sixty-two healthy young adult participants. Of these, 49 participants (14 male and 35 female; mean age: 19.98 years; age range: 18-29 years) provided complete data on all the variables of interest in this study. Participants were recruited using ethics board-approved advertisements posted at the McMaster University campus and through a database of Introductory Psychology students who consented to future contact. All participants provided written informed consent and met the inclusion criteria described as follows. Recruitment targeted participants between 18 and 30 years of age in order to minimize age-related variations in cognitive and physical fitness performance. Verbal confirmation was used to ensure that in their regular daily lives, participants previously engaged in less than one hour of vigorous physical exercise per week, with the aim of participants achieving greater fitness improvements, and thus greater cognitive benefits, from the exercise interventions. The Physical Activity Readiness Questionnaire (Thomas, Reading & Shepard, 1992) was employed to ensure participants were generally healthy and had no pre-existing condition such as cardiovascular disease that would increase their risk of developing an exercise-related injury. Finally, we ensured through verbal confirmation that participants had not previously been diagnosed with any psychiatric disorders, in order to minimize any illness-related cognitive deficits that may have affected our results. Of particular concern to our study were severe cases of stress-related psychiatric disorders such as major depression, which may cause significant damage or volume loss to the hippocampus (MacQueen et al., 2003). Such damage would overshadow the cognitive gains attained through our exercise intervention.

Study Design & Measures

All participants completed pre- and post- physical, mood and cognitive tests, separated by a 6-week intervention. The study pseudo-randomly assigned each participant to one of three groups: a non-exercising control group, or one of two exercise groups—either a high intensity interval training (HIIT) group or a moderate intensity training (MIT) group. These three groups were chosen with the goal of generating a range of fitness improvements, so that we could observe

whether varying degrees of cognitive improvement would be induced. Physical, mood and cognitive tests were administered one week prior to the intervention in order to establish baseline functioning, and then one week after the intervention in order to measure any exercise-induced change. No fitness or cognitive performance changes were expected in the non-exercising control group.

Dery et al. (2013) demonstrated that 6 weeks of high intensity aerobic exercise can yield significant changes in fitness and cognition in a sample size of 12 healthy young adults. To increase the power of our study, we increased the sample size (HIIT: 16 (5 male, 11 female); MIT: 16 (5 Male, 11 female); Control: 17 (4 Male; 13 female)).

Fitness Measure

Manipulation Check – VO₂ peak Test. Aerobic fitness was determined by measuring maximum oxygen consumption (VO₂ peak), which is the maximum amount of oxygen one's body can use while performing submaximal aerobic exercise. VO₂ peak is widely accepted as the best measure of cardiovascular fitness (Fletcher, Froelicher, Hartley, Haskell & Pollock, 1990). When aerobic exercise is initiated, oxygen uptake by the lungs quickly rises. As exercise intensity increases, oxygen consumption increases linearly until a maximum level $(VO_2 \text{ peak})$ that depends on the individual's aerobic fitness. We measured VO_2 peak levels using the submaximal bicycle test, which is considered to be the most objective, reliable and valid indicator of aerobic capacity (Vanhees et al., 2005). Participants completed the incremental exercise test to exhaustion on a cycle ergometer while breathing through a specialized mask. Participants warmed up for one minute at 50 watts (W). Workload was then increased by 1W every 30 seconds until the participants reached volitional fatigue, or when their pedaling rate fell below 80rpm. Expired air was continuously analyzed for volume of oxygen consumed by an online gas collection system (MOXU modular VO₂ System; AEI Technologies, Pittsburgh, PA). Oxygen consumption (mL/kg/min) and power output (W) were recorded every 15 seconds. VO₂ peak was considered to be the highest oxygen consumption value and max wattage obtained from the peak power output value. Maximum heart rate was measured using Polar RS300X heart rate monitors. For analysis purposes, absolute VO₂ peak scores were converted to VO_2 peak relative values, which integrate the effect of a participant's weight into the calculation.

Exercise Intervention

Independent Variables – *High Intensity Interval Training (HIIT) and Moderate Intensity Training (MIT).* Participants completed 6 weeks of exercise, 3 times per week with at least one day of rest between each session and the next, in order to allow for muscle recovery. All participants, including the nonexercising control group, were instructed to refrain from additional exercise outside of the study requirements. Training was completed on stationary cycle ergometers (*Lifecycle 95Ci*).

All training sessions began with a 3 minute warm up and ended with a 2 minute cool down at a resistance of 50W. The HIIT protocol consisted of ten one-minute high intensity intervals at 80% VO₂ peak and approximately 90-95% maximal HR, interleaved with ten one-minute active recovery intervals, for a total of 20 minutes of exercise. After each interval, heart rate and ratings of perceived exertion (RPE, scale 6-20) were recorded (Borg & Linderholm, 1970). The MIT protocol consisted of 27.5 minutes of cycling at 40% VO₂ peak and 70-75% maximal HR. HR and RPE were recorded every second minute (Borg & Linderholm, 1970). As fitness adaptations occurred throughout the 6 weeks, wattage was increased to ensure individuals continued to achieve their target heart rate.

The VO₂ peak levels were chosen based on the recommended aerobic exercise intensity values for cardiovascular fitness benefit in healthy individuals (moderate intensity: 40-50% VO₂ peak; vigorous intensity: 60-85% VO₂ peak) (Lippincott, Williams & Wilkins, 2013). Based on methods used by Bartlett et al. (2011), to equate average caloric expenditure across conditions, there was no difference in average VO₂ peak between our HIIT and MIT exercise protocols (i.e. isocaloric conditions).

Fitness gains achieved from a six-week high intensity exercise intervention are sufficient to demonstrate significant cognitive benefits (Dery et al., 2013). Furthermore, studies have shown that a MIT protocol with the same total workload or energy expenditure as a HIIT protocol induces similar physiological and health-related improvements (Hwang, Wu & Chu, 2011). Using isocaloric protocols therefore allowed us to determine whether a lower intensity protocol (MIT), which may be safer and more tolerable for certain demographics such as aging populations, could yield similar cognitive gains.

Cognitive Measures

Spatial navigation strategy choice was assessed using a virtual reality version of the *Morris Water Maze*, which was run on a computer. A putative neurogenesis-dependent task known as the *Mnemonic Similarity Task* (MST) was used, as performance on this task is known to co-vary with known correlates of neurogenesis: stress levels and exercise-induced changes in fitness (Dery et al., 2013). Participants also completed other cognitive tasks that assessed executive function and reaction; however these results are outside of the scope of this paper and are not reported.

Dependent Variable – Spatial Memory Task. Hippocampus-dependent spatial memory performance was assessed with a Virtual Morris Water Maze, which was created in Second Life by David Harris Smith based on a version by de Castell et al. (2014). The participant controls a human avatar using the four arrow keys on a computer keyboard. Each trial begins by teleporting the avatar from a starting location to one of four randomly selected cardinal positions (North, South, East, West) within a circular pool. In our water maze, the distal cues include mountains and rocks placed asymmetrically around the outside of the pool. The proximal cues include pictures of five different marine creatures that are placed asymmetrically along the inside of the pool wall. X-Y coordinates of the avatar are recorded during navigation. These coordinates may be used to determine the efficiency of navigation (e.g. navigation time or path length) from the starting point to the hidden platform; however, these data were not analyzed for the current study. The participant completes a total of 7 trials for one target location. On the first trial, the participant searches the pool until the hidden platform is found, or for a maximum of three minutes. After three minutes, the hidden platform location is revealed and the participant must navigate the avatar to the platform. The participant is then given a chance to observe the surroundings and is instructed to use any cues they wish in order to find the same target location as efficiently as possible in subsequent trials. This is repeated four more times for a total of five "learning trials". In the final two "probe trials", the participant is instructed to navigate the avatar to the same hidden platform location and indicate when they think they have arrived. The actual platform location is not revealed in probe trials. On the first probe trial, the participant can use any of the proximal and/or distal cues they used during the learning trials. On the last probe trial, the proximal cues are removed. Using the X-Y coordinates, we can determine whether or not the participant's performance was hindered by removing the proximal cues, thus allowing us to determine what strategy was predominantly used during spatial encoding. For the current study, water maze performance was assessed by the last probe trial. A smaller distance between the participant's guess and the actual target location indicates stronger allocentric encoding.

Covariate – *High Interference Memory Task.* An adapted version of Kirwan and Stark's (2007) Mnemonic Similarity Task (MST) was used to assess hippocampus-dependent memory function. The computer task begins with a learning phase, during which a series of 60 colored objects are presented on the screen for two seconds each, and participants must classify the objects as indoor or outdoor. The purpose of this phase, which is not revealed to the participants, is to encourage object learning. The second phase of the task is a 3-alternative forced-choice visual recognition task, in which 90 colored objects are presented sequentially: 30 of the objects are 'repetitions', which were previously presented during learning phase; 30 are 'lures', which are new but highly similar to previously presented objects; and 30 are 'foils', which are new and completely unrelated to the previously presented objects. Objects appear on the screen in a

randomized order for 2500ms each and participants are asked to classify each object as 1) old (repetitions), 2) similar (lures), or 3) new (foils), in comparison to the 60 objects seen during the learning phase. Pattern separation performance is measured by the ability to distinguish between previously presented objects and highly similar objects in order to correctly identify the lures as 'similar'. Performance for each participant was quantitatively measured by subtracting the proportion of incorrect 'similar' responses given the presentation of an unrelated foil, from the proportion of correctly identified 'similar' responses given the presentation of a lure [proportion of correctly identified lures = p("Similar"|Lure) - p("Similar"|Foil)]. The change in MST performance due to the intervention was then measured by subtracting the proportion. It is hypothesized that enhanced neurogenesis improves task performance, which is reflected by a higher proportion of correctly identified lures.

Mood Measures

Covariate – *Beck Depression Inventory-II (BDI-II)*. The severity of chronic stress and depressive symptoms exhibited by participants were measured using the BDI-II, a standardized 21-item self-report questionnaire based on a clinically-determined scale (Beck, Steer & Brown, 1996). A higher BDI-II score indicates more severe symptoms of depression. While participant data was made anonymous to experimenters, a code linking the data to their personal information was given to a third party for assessment. If a participant was deemed at risk for depression or suicide based on their score, their contact information was forwarded to a psychological counselor at the McMaster Student Wellness Centre, who would contact the participant to discuss and offer possible treatments. Participants were made aware of this process on the consent form.

Statistical Analysis

Manipulation Checks. To ensure intervention groups did not differ on key measures of fitness and memory performance at baseline, a one-way analysis of variance (ANOVA) was conducted on pre-intervention data with a between-subject factor of intervention group.

To verify that the exercise interventions yielded the expected changes in fitness levels, paired samples t-tests were used to compare pre- and post-intervention VO_2 peak scores.

To determine whether HIIT and MIT interventions yielded significantly different changes in VO_2 peak, the means of the post- minus pre-intervention changes in VO_2 peak scores for the two groups were compared using independent

samples t-tests. Non-significant results would suggest that the two groups could be combined into an 'Exercisers' group for analysis purposes.

To verify that the exercise interventions yielded the expected relief of depressive symptoms, paired samples t-tests were used to compare pre- and post-intervention BDI-II scores.

To verify that the exercise interventions yielded the expected performance improvements on a putative neurogenesis-dependent task, paired samples t-tests were used to compare pre- and post-intervention MST scores.

To check whether sex played a significant role in allocentric spatial learning, male and female water maze test scores were compared at baseline, and on post- minus pre-intervention difference scores, in case exercise affected males and females differently. Since the two groups had unequal sample sizes, Welch's t-test was used to determine if the two group means significantly differ.

Group-Based Analyses. Participant data were analyzed based on three sets of comparison groups: Intervention groups (HIIT versus MIT versus Control); Combined intervention groups (Exercisers: HIIT, MIT versus Non-exercisers: Control); and Responders to exercise (High versus Low). Responders to exercise were grouped based on post- minus pre-intervention changes in VO₂ peak. Participants in each exercise group were separately ranked from smallest to largest VO₂ peak improvements. Participants with a change below the medians were considered low responders to exercise, while those above the medians were considered high responders. The low responders from the HIIT and MIT groups were then combined into one "Low Responders" group, while the high responders were combined into one "High Responders" group.

Within-Group Analyses. To evaluate the effect of the interventions on water maze performance within each group, paired samples t-tests were performed using pre- and post-intervention water maze scores.

To evaluate the effect of the interventions on ratings of depression within each group, paired samples t-tests were used to compare pre- and postintervention BDI-II scores.

To verify the relationship between performance on a putative neurogenesis-dependent task (MST) and ratings of depression (BDI-II), Pearson's correlation was calculated for each analysis group.

Between-Group Analyses. To compare the effects of the interventions on changes in water maze performance between groups, one-way analyses of covariance (ANCOVAs) were conducted on post- minus pre-intervention water maze scores. Analyses included a between-subject factor of group, while controlling for water maze pre-test scores and age, as age is known to affect hippocampal functioning.

To draw inferences about the roles of neurogenesis and depressive symptoms in spatial memory, the ANCOVA was re-calculated multiple times. Calculations included post- minus pre-intervention MST scores and/or post-minus pre-intervention BDI-II scores as a covariates, in addition to the other two covariates. These analyses gave insight into whether exercise-induced changes in water maze performance are related to changes in these indicators of hippocampal function (i.e. the ability to distinguish between highly similar items in the MST, and ratings of depression on the BDI-II).

It is noted that in order to eliminate outliers so that statistical analyses could be performed, some post- minus pre-intervention difference scores were recoded. Outliers were replaced with the highest or lowest non-outlier value in the individual's group, depending on whether the outlier was high or low. The recoded values were included in calculations of the mean difference scores for each group.

For Pearson's correlation and all t-tests, a *p*-value =<0.05 (one-tailed) was considered significant. One-tailed t-tests were used for two reasons: 1) The t-tests are testing for the possibility of relationships in particular, hypothesized directions, and 2) Due to small sample sizes, one-tailed tests are beneficial because they have more power. Results are presented as mean (M) +/- standard deviation (SD).

RESULTS

Manipulation Checks

At baseline, intervention groups did not differ on key measures of fitness, mood scores or memory performance (all p > 0.062) (Table 1).

No significant differences in spatial memory were observed between males and females on baseline scores (all p > 0.079), nor on post- minus pre-intervention difference scores (all p > 0.224). Gender was thus not included in further analyses.

The interventions resulted in the expected fitness improvements (Figure 2). High intensity interval training resulted in the greatest improvement, a 15.47% increase in VO₂ peak [t(15) = 4.303, p < 0.001], followed by moderate intensity training, which induced a 10.59% increase in VO₂ peak [t(15) = 2.318, p < 0.05]. Change in fitness level in the non-exercising control group was non-significant.

Comparisons of fitness improvements exhibited by the HIIT versus MIT groups revealed that the effects of the two interventions on VO₂ peak change did not differ significantly [t(30) = 0.997, p = 0.163]. Therefore, HIIT and MIT groups were combined into an 'Exercisers' group for some group-based analyses, in order to compare to the non-exercising controls.

Tables 1, 2 and 3 present mean scores on the MST task. On average, the exercise interventions slightly improved the ability to correctly identify a lure as similar during the MST; however, results were non-significant (all p > 0.225). A

non-significant decline in MST performance was observed in the control group. It is noted that high responders to exercise showed a significant improvement in their ability to identify a lure as similar on the MST [t(15) = 2.076, p <0.05].

Tables 1, 2 and 3 present mean BDI-II scores, with higher scores indicating more severe depressive symptoms. Tables 1 and 2 show that after the 6week intervention, the non-exercising control group experienced a 29.45% increase depressive symptoms [t(16) = 2.922, p < 0.01]. Overall, exercisers displayed a 0.28% decrease in depressive symptoms (Table 2) but interestingly, when split into intervention groups (Table 1), it is seen that the HIIT group displayed a 16.35% increase in depressive symptoms, while the MIT group displayed a 17.66% decrease in depressive symptoms (all non-significant). Similarly, Table 3 shows that high responders to exercise displayed a 10.89% increase in depressive symptoms, while low responders displayed a 9.31% decrease in depressive symptoms (both non-significant).

Within-Group Analyses

Within-group effects of exercise on water maze performance. Water maze performance on the second probe trial, with proximal cues absent, was assessed by analyzing the mean distance between the estimated and actual target locations. A smaller mean distance indicates better water maze performance. Table 1 presents mean water maze performance for the three intervention groups. Figure 3 displays the data graphically. The HIIT group displayed a 25.00% postintervention improvement in water maze performance. This improvement was marginally significant [t(15) = 1.371, p = 0.095]. The MIT group displayed an 18.52% improvement, however results were non-significant. Table 2 and Figure 4 show that overall, exercisers significantly improved water maze performance by 21.49% [t(31) = 1.92, p < 0.05]. In comparison, the non-exercising control group displayed a 0.82% decline in performance (non-significant). Table 3 compares mean water maze performance in high versus low responders to exercise. Figure 5 depicts these results graphically. Interestingly, it is seen that high responders, who improved by 12.46% (non-significant), did not improve as much as low responders, who improved by 30.09% [t(15) = 1.743, p = 0.05]. Curiously, the high intensity exercise group showed greater water maze improvements than the moderate intensity group, while low responders to exercise showed greater improvements than high responders. An additional graph (Figure 6) was created in order to help clarify these counterintuitive results. The most apparent contributing factor according to the graph is that the high responders to exercise in the MIT group experienced markedly smaller improvements in water maze performance in comparison to the rest of the exercising participants.

Relationship between exercised-induced change in performance on the MST and depression. Results revealed that within the HIIT group, change in MST performance is marginally related to change in BDI-II scores (r = -0.365, p =0.082). This means that for HIIT participants, less severe depressive symptoms relate to improved performance on the MST, a task that we hypothesized to be neurogenesis-dependent. The correlation was also negative for the Control group, while it was positive for the MIT group; however, neither correlation was statistically significant. Change in MST performance was negatively correlated to change in BDI-II scores for both exercisers and non-exercisers (non-significant). Change in MST performance was also negatively correlated to change in BDI-II scores for low responders to exercise, while the relationship was positive for high responders (non-significant).

Between-Group Analyses

Between-group effects of exercise on water maze performance. ANCOVA results (see Appendix) reveal no statistically significant effects of group on change in water maze performance when controlling for various combinations of age, pre-intervention water maze scores, change in ratings of depression on the BDI-II and change in performance on the MST. However, a marginally significant difference between exercisers and non-exercisers was found when controlling for age, pre-intervention water maze scores and change in performance on the MST [F(1, 44) = 3.056, p = 0.087], with exercisers exhibiting greater water maze improvements than non-exercisers.

A potential role for neurogenesis and depression in mediating exerciseinduced spatial memory changes. Although ANCOVA results were nonsignificant, a review of the amount of variance accounted for by each model (SS_M) created reveals interesting trends (see Appendix). For comparisons between intervention groups, it was found that the variation in spatial memory performance, SS_M, is greater when controlling for change in MST performance (SS_M = 519.06 units) than when controlling for change in ratings of depression (SS_M = 507.12 units). When simultaneously controlling for the two variables, SS_M was even greater (SS_M = 529.69 units). Comparisons of exercisers versus nonexercisers revealed the same trend (MST change: SS_M = 511.7 units; BDI change: SS_M = 495.10 units; Both: SS_M = 518.43 units). The amount of variance accounted for was approximately the same across all models when comparing high versus low responders to exercise.

		Mean VO2 peak [mL/(kg*m)]	MWM: mean distance to target	Mean BDI- II score	MST: proportion of lures correctly identified as similar
	Pre	31.74 (1.86)	6.12 (1.64)	11.50 (1.97)	0.52 (0.04)
HIIT (n = 16)	Post	36.66 (2.30)	4.18 (0.84)	13.25 (1.78)	0.53 (0.05)
	\triangle	4.91*** (1.14)	-1.53# (0.99)	1.88 (1.98)	0.04 (0.03)
	Pre	29.74 (1.28)	7.02 (1.20)	10.25 (2.17)	0.49 (0.03)
MIT (n = 16)	Post	32.88 (1.53)	5.28 (0.40)	8.44 (2.24)	0.53 (0.05)
	\triangle	3.15* (1.36)	-1.30 (1.04)	-1.81 (1.55)	0.04 (0.05)
	Pre	29.43 (1.42)	6.09 (1.11)	17.18 (2.33)	0.50 (0.03)
CTRL (n = 17)	Post	29.57 (1.44)	6.14 (1.20)	23.00 (2.76)	0.46 (0.04)
	Δ	0.52 (0.64)	0.05 (1.26)	5.06** (1.66)	-0.04 (0.05)

Table 1. Mean and standard error of fitness, memory performance and mood across intervention groups.

Paired samples t-test (pre-intervention data, post-intervention data): # 0.05 (marginally significant); * <math>p < 0.05; **p < 0.01; ***p < 0.001



Figure 2. Mean post-intervention fitness improvement across intervention groups, with standard error bars.

The HIIT intervention induced a 15.47% improvement in VO₂ peak (p=0.001); MIT induced a 10.59% improvement (p<0.05); the control group displayed a statistically non-significant change in fitness level.

Figure 3. Mean pre- and post-intervention water maze scores across intervention groups, with standard error bars.



Post-intervention performance: HIIT: 25.00% closer to target (p = 0.095); MIT: 18.52% closer to target (non-significant); Control: 0.82% further away from target (non-significant).

		Mean VO2 peak [mL/(kg*m)]	MWM: mean distance to target	Mean BDI-II score	MST: proportion of lures correctly identified as similar
		30.74	6.56	10.88	0.51
	Pre	(1.12)	(1.00)	(1.45)	(0.02)
Exercisers		34.77	4.73	10.84	0.53
(n = 32)	Post	(1.40)	(0.47)	(1.47)	(0.04)
		4.03***	-1.41*	0.03	0.04
	\triangle	(0.89)	(0.71)	(1.28)	(0.03)
		29.43	6.09	17.18	0.50
Nor	Pre	(1.42)	(1.11)	(2.33)	(0.03)
INON- Exercisers		29.57	6.14	23.00	0.46
(n-17)	Post	(1.44)	(1.20)	(2.76)	(0.04)
(n - 17)		0.52	0.05	5.06**	-0.04
	\triangle	(0.64)	(1.26)	(1.66)	(0.05)

Table 2. Mean and standard error of memory performance and mood in exercisers and non-exercisers.

Figure 4. Mean pre- and post-intervention water maze scores in exercisers and non-exercisers, with standard error bars.



Post-intervention performance: Exercisers: 21.49% closer to target (p < 0.05); Non-Exercisers: 0.82% further away from target (non-significant).

Paired samples t-test (pre-intervention data, post-intervention data): * p < 0.05; ** p < 0.01; ***p < 0.0001.

		Mean VO2 peak [mL/(kg*m)]	MWM: mean distance to target	Mean BDI-II score	MST: proportion of lures correctly identified as similar
		30.80	6.34	10.38	0.54
High	Pre	(1.90)	(1.43)	(1.65)	(0.03)
Responders		38.74	5.11	11.50	0.61
(n-16)	Post	(2.14)	(0.69)	(2.34)	(0.03)
(n - 10)		7.94***	-0.79	1.13	0.07*
	\triangle	(0.85)	(1.01)	(1.80)	(0.04)
		30.68	6.78	11.38	0.48
T	Pre	(1.27)	(1.46)	(2.43)	(0.04)
LOW Responders		30.80	4.35	10.19	0.45
(n-16)	Post	(1.18)	(0.64)	(1.85)	(0.06)
(n - 10)		0.12	-2.04#	-1.06	0.01
	\triangle	(0.70)	(0.99)	(1.84)	(0.04)

Table 3. Mean and standard error of fitness, memory performance and mood scores for high and low responders to exercise.

Paired samples t-test (pre-intervention data, post-intervention data): # p = 0.05; * p < 0.05; **p < 0.001.

Figure 5. Mean pre- and post-intervention water maze scores in high and low responders to exercise, with standard error bars.



Post-intervention performance: High Responders: 12.46% closer to target (non-significant); Low Responders: 30.09% closer to target (p = 0.05).

Figure 6. Average improvement in water maze scores in HIIT and MIT groups, broken down into high and low responders to exercise, with standard error bars.



DISCUSSION

The present study examined the effects of exercise on hippocampus-mediated spatial performance in sedentary but otherwise healthy young adults. The study also explored the potential roles of neurogenesis and the relief of depressive symptoms in mediating the observed changes. Participants were randomly assigned to a high intensity interval training group, a moderate intensity training group, or a non-exercising control group. As expected, within each exercise group, individuals displayed a wide range of responses to the exercise regimes, allowing for additional comparisons of high and low responders to exercise. It is noted that these diverse responses to exercise could be explained by characteristics of the HIIT and MIT programs, genetics, or lifestyle factors such as sleep, stress and dietary intake (Mann, Lamberts & Lambert, 2014). Significant relationships between exercise and performance on a virtual reality water maze task were revealed. Insight was gained into the potential influence of neurogenesis and depressive symptoms on exercise-induced spatial memory improvements.

Effects of exercise on spatial learning (H1)

Exercisers and Non-Exercisers. An abundance of research supports that loss of function within the allocentric processing system due to hippocampal atrophy and dysfunction causes animals to rely more heavily upon extrahippocampal systems. Conversely, literature supports that aerobic exercise

improves allocentric processing in rodents (Mustroph et al., 2012; van Praag et al., 1999a; van Praag et al., 2005). Less evidence exists to support this relationship in humans. The current study demonstrated that after six weeks of regular aerobic exercise, human subjects show a statistically significant improvement in performance on a virtual reality water maze task. It is likely that exercise-induced enhancements in the allocentric processing system allowed participants to form more robust spatial memory by relying more heavily on place cells and cognitive mapping in order to acquire the task. A heavier reliance on cognitive mapping likely improved water maze performance for two main reasons. First, forming a cognitive map of the environment during learning trials limits the amount of confusion that is experienced due to each trial beginning at random locations within the pool. This is because place cell firing depends on location within the environment, rather than on orientation or rotational movements, so the navigator will remain oriented no matter where in the environment the learning trial begins. Second, cognitive maps for the water maze task are formed based on relationships between the platform and sets of distal cues. Thus the removal of the proximal pool cues during the second probe trial would not affect an allocentric navigator's ability to find the hidden target.

The improvements in allocentric processing displayed by exercisers in the current study have important implications for clinical therapies aimed at counteracting disease-related loss of hippocampal function. Results suggest that physical exercise should be used in conjunction with traditional therapies in order to better improve hippocampal functioning in populations such as adults at risk for, or suffering from Alzheimer's disease.

In comparison to exercising participants, non-exercising controls displayed slightly worse post-intervention water maze performance. Results indicate that during the six weeks between pre-testing and post-testing, non-exercisers shifted away from allocentric, and towards egocentric processing. This may have occurred due to factors external to the experiment that caused hippocampal damage, such as stress. Consistent with this interpretation, non-exercising controls exhibited a greater increase in depressive symptoms at post-test than did either exercising group. This could be due to the time in the semester during which posttesting occurred. A shift towards egocentric learning resulted in poorer performance because egocentric learning is less flexible than allocentric learning. For egocentric navigators, beginning each trial in a new, random position would have disrupted their ability to follow a defined sequence of movements based on specific cues, thus increasing the difficulty of learning the hidden target location. In addition, removing the pool cues during the second probe trial meant removing the proximal cues that egocentric learners use to navigate to the target. This would have caused disorientation, as the navigator was forced to rely on an unfamiliar distal environment.

Exercise intensity. Assessing exercisers based on their intervention groups (HIIT and MIT) gave further insight into how an exercise regime may be constructed in order to ensure cognitive enhancements are realized. In the current study, a high intensity exercise regime caused large, statistically significant improvements in water maze performance. The lower intensity exercise regime also resulted in distinct performance improvements, however the results were non-significant. These findings suggest that while both high and low intensity exercise regimes improve allocentric processing in humans, a higher intensity program may have greater effects.

Response to exercise. Low responders to exercise displayed statistically significant improvements in water maze performance. High responders also showed distinct improvements, however the results were non-significant. These findings suggest that even minor fitness improvements may lead to significant cognitive gains.

Results, however, do not support the hypothesis that water maze improvements increase in proportion to improved fitness levels. Also, the results are contrary to findings from Dery et al. (2013), which showed that in comparison to low responders to exercise, high responders experience greater enhancements in performance on a high interference task. The smaller improvements displayed by high responders in the current study may be due to the subsection of high responders from the MIT group, who displayed much less improvement in the water maze task than the rest of the exercising study participants. The reasons for this are unclear and may be partially explained by low sample sizes (n = 8 for MIT high responders). Another explanation relates to the potential mediating effects of depression and neurogenesis on shifts in between egocentric and allocentric processing. This will be discussed later on.

Between-group comparisons. Between-group comparisons of water maze improvement, calculated multiple times with various combinations of covariates, revealed no statistically significant results. A marginally significant difference between exercisers (HIIT + MIT) and non-exercisers was observed, indicating that larger sample sizes for each intervention group may have yielded significant results.

Summary. Think back to our example in which you had just moved to a new city and were attempting to navigate from your new apartment to the location of your new job. Assuming you were previously an egocentric navigator and have just completed one of the exercise regimes from the current study, you may now have improved allocentric navigation skills due to enhanced hippocampal functioning. Thus upon leaving your apartment, you may still note that you should walk towards the church then turn right at the first cross-street; however you may also begin to allocentrically understand your target location in relation to the distal mountain range to the north and the waterfront to the east. Therefore, if one day

you are forced to find a detour because the cross-street before the church is closed due to construction, you will be able to use the distal cues of your cognitive map to ensure you head in the right direction. According to the current study, whether you had completed the high or the low intensity exercise regime, and whether you are a low or high responder to the intervention, you may have experienced this shift towards allocentric processing.

The next step in understanding how exercise may be used to confer cognitive gains in spatial learning is to investigate the underlying mechanisms. The current study analyzed two indicators of hippocampal function, hypothesizing that these factors may mediate the effects of exercise on the observed cognitive gains.

Underlying mechanisms: Neurogenesis and depression as mediators of shifts in spatial strategy

Neurogenesis (H2). All exercising groups had improved post-intervention performance on the putative neurogenesis-dependent task, MST. However, improvements were generally smaller than what was expected based on past studies (Dery et al., 2013), with only high responders displaying a statistically significant improvement. According to rodent literature, it is likely that exercisers experienced increased cell proliferation and survival in the dentate gyrus (van Praag et al., 1999a, b), and that these changes were more pronounced for high responders to exercise. The non-exercising control group displayed slightly worse (non-significant) MST performance, indicating decreased rates of neurogenesis. Again, it is noted that non-exercisers had reduced resilience against stress due to lack of exercise, which may explain the decreased rates of neurogenesis (Gould et al., 1997).

Analyses suggest that neurogenesis, as indexed by MST performance, may be involved in allocentric processing enhancements. Models created for betweengroup comparisons of spatial memory change in the intervention groups found that controlling for post- minus pre-intervention MST scores reduced the amount of unexplained variance. In other words, changes in neurogenesis may partially explain why certain groups displayed greater water maze improvements than others. In the current study, both MST and water maze performance improved in all exercising groups, and worsened in non-exercisers. This is consistent with the aforementioned studies that have shown a positive correlation between neurogenesis and allocentric learning (Drapeau et al, 2003; Kempermann et al, 1997; Schwabe et al, 2012; van Praag et al, 1999a; van Praag et al, 2005). The positive correlation between exercise-induced changes in MST and water maze performance suggests that the mechanisms underlying MST performance improvement (presumably neurogenesis) may partially explain the exerciseinduced spatial memory improvements. Alternatively, it is possible that exercise affects memory via two routes: one via stress reduction, which increases use of a hippocampus-dependent spatial strategy, and two, via exercise-induced enhancements in neurogenesis, which could selectively affect performance on high interference memory tasks. Future research should further investigate whether exercise-induced neurogenesis plays a role in allocentric processing, or if the effects are specific to high interference memory.

Depression (H3). Unexpectedly, exercise did not result in significantly improved ratings of depression (as reflected by BDI-II scores). On the other hand, non-exercising controls experienced a statistically significant increase in severity of depressive symptoms. Taken together, these observations suggest that environmental conditions external to the experiment, but shared by all participants, became increasingly stressful towards the end of the experiment. Indeed, pre-testing took place early in the semester, while post-testing took place towards the end of the semester, around exam time. Exams likely increased rates of chronic stress, which masked the benefits of exercise for relief of depressive symptoms.

Moreover, results revealed that HIIT participants and high responders to exercise experienced increased severity of depressive symptoms, while MIT participants and low responders had reduced severity. This is contrary to findings of Singh et al. (2005), that high intensity exercise is more likely to reduce depression than low intensity exercise. It is possible that the formerly sedentary participants in the current study experienced greater amounts of psychological stress from undergoing a high intensity exercise program versus a lower intensity program, and/or from being more physically affected by the exercise regimes versus experiencing little to no physical changes. The accumulation of both exam-related stress and exercise-related stress in HIIT participants and high responders may have outweighed the therapeutic benefits of exercise, thus leading to more severe ratings of depression.

The unexpected persistence of depressive symptoms may have offset the potentially beneficial effects of exercise on spatial memory. Models created for between-group comparisons of spatial memory improvement found that controlling for post- minus pre-intervention BDI-II scores reduced the amount of unexplained variance. Again, this trend suggests that changes in the severity of depressive symptoms experienced by in individual may also partially predict changes in water maze performance. This is in line with rodent research, which supports that stress and depression can damage hippocampal functioning, causing a shift from hippocampus-mediated spatial learning, to a striatal egocentric strategy (Kim et al., 2001; Schwabe et al., 2007; Schwabe et al., 2008). Following this, the increase in depressive symptoms exhibited by high responders to exercise likely hindered exercise-induced improvements in hippocampal functioning, thus

explaining why high responders showed weaker improvements than low responders, who had decreased ratings of depression.

Combined effects of Neurogenesis and Depression. Between-group variances in water maze performance were best explained by the model that controlled for both MST performance and BDI-II. It was hypothesized that MST performance would reflect levels of neurogenesis, while BDI-II scores would reflect chronic stress levels and in turn would be negatively correlated with neurogenesis levels. It is possible that both neurogenesis and depression partially predict shifts between egocentric and allocentric processing. Therefore, in understanding the relationship between these two variables and spatial memory, it is also important to consider how the variables interact with each other. It is wellknown that a negative relationship between neurogenesis and severity of depression exists. Based on the indicators used in the current study, a negative relationship between MST performance and BDI-II scores was expected. This relationship was observed for all analysis groups besides the MIT group and high responders to exercise (all non-significant). The non-significance of the relationship, and the results for the two non-conforming groups, may have been due to other confounding variables outside the scope of this study, or may have simply been due to small sample sizes. Nonetheless, the persistence of depressive symptoms in study participants likely hindered increases in neurogenesis, as reflected by the minor, non-significant improvements in MST scores. It is possible that greater relief of depressive symptoms would have allowed for more significant increases in neurogenesis, which in turn, may have led to greater water maze improvements in the exercising analysis groups.

Summary. Experimental results suggested that the spatial memory changes exhibited by participants may have been partially mediated by changes in neurogenesis (which was assumed based on performance on a putative neurogenesis-dependent task) and changes in depressive symptoms. However, the relationships in the current study were non-significant. It is possible that the exercise-induced increases in neurogenesis improved spatial performance by promoting the survival and proliferation of hippocampal cells. However, stress (exam- and/or exercise-induced) likely hindered increases in neurogenesis, which in turn limited improvements in hippocampal functioning, and the degree to which exercisers shifted towards allocentric learning. Without the persistence of stress, exercise-induced water maze improvements may have been even greater. Future research should further investigate the underlying mechanisms of allocentric processing improvements in human subjects. Uncovering these underlying mechanisms will allow researchers to better understand how exercise leads to cognitive enhancements so that more specialized and targeted therapies may be developed.

Limitations & Future Directions

While the present study has made important contributions to understanding the benefits of aerobic exercise for hippocampus-dependent spatial processing, experimental limitations do exist.

It is possible that statistically significant results could have been obtained with larger sample sizes. Future studies should increase the sample size in order to help clarify and support results found in the current study.

Post-testing occurred around exam time, which is a period of high stress in comparison to early in the university semester when pre-testing took place. The stress experienced by study participants may have minimized the potential for exercise-induced increases in neurogenesis and enhancements in allocentric processing. In designing future studies, researchers should account for any predictable periods of high stress that may affect participant performance on hippocampus-dependent cognitive tasks.

Neurogenesis cannot be directly measured non-invasively at the cellular and molecular level in human subjects. Instead, the current study used cognitive tasks to make inferences about hippocampal function. However, perhaps the most reliable method of measuring hippocampal function in humans is through imaging techniques, in particular, MRI. For example, the current study could have used MRI in conjunction with cognitive testing in order to relate dorsal hippocampi growth to water maze performance (Maguire et al., 2000). By the same token, MRI measures of cerebral blood volume in the dentate gyrus could have been used in conjunction with the MST as an index of neurogenesis (Pereira et al., 2007). Future studies should employ imaging techniques in addition to cognitive testing, in order to provide more precise inferences about hippocampal function.

Future studies using the virtual reality water maze task may collect more data that will further assist in understanding the changes in spatial performance. For instance, data may be collected that reveals how quickly participants learned the location of the hidden target by measuring path length and latency.

Despite the limitations of the current study, results are consistent with the hypothesis that regular exercise benefits the brain and cognition. Such findings may be particularly relevant to clinical populations, such as older adults who are at risk for, or who are suffering from dementia. Furthermore, it has been shown that these changes can be induced by lower intensity exercise regimes that are more accessible to older adults and clinical populations. Therefore, a logical next step would be to conduct the current study on older adults and clinical populations. Such research would provide further support for the benefits of incorporating exercise into clinical therapies aimed at enhancing hippocampal

function. Another important next step would be to compare the effectiveness of treating clinical populations using traditional cognitive therapies, versus using a combination of exercise and cognitive therapy.

Conclusion

In summary, exercise improved hippocampus-dependent spatial memory performance on a virtual water maze task. Neurogenesis and depression may have partially influenced the observed changes in spatial memory, however the results are unclear. Findings suggest that physical exercise should be used in conjunction with traditional cognitive therapies in order to help improve hippocampal functioning. Findings from this study are especially important because improvements in spatial memory were achieved from relatively short (6-week) exercise regimes of both high and low intensities. Thus, even greater cognitive gains may be observed from a more prolonged intervention and interventions may be adjusted to suit the physical abilities of each patient.

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Appendix

Legend

- "diff" indicates mean post- minus pre-intervention difference score calculation was used
- BDI: Score on Beck Depression Inventory II
- MST: Score on Mnemonic Similarity Task
- MWM-pre: Pre-intervention score on water maze task

Covariates	Effect of group on change in water maze performance after controlling covariates	Unexplained variance (SS _R)	Amount of variance accounted for by the model (SS_M)
Age, MWM-pre	F(2, 44) = 1.509, p = 0.232	452.870	499.071
Age, MWM-pre, diffBDI	F(2, 43) = 1.251, p = 0.296	444.818	507.123
Age, MWM-pre, diffMST	F(2, 43) = 1.885, p = 0.164	432.880	519.062
Age, MWM-pre, diffMST, diffBDI	F(2, 42) = 1.515, p = 0.232	422.249	529.693

ANCOVA: Intervention Groups

ANCOVA: Exercisers vs Non-Exercisers

Covariates	Effect of group on	Unexplained	Amount of
	change in water	variance (SS_R)	variance
	maze performance		accounted for by
	after controlling		the model (SS_M)
	covariates		
Age, MWM-pre	F(1, 45) = 2.202, p =	461.365	490.577
	0.145		
Age, MWM-pre,	F(1, 44) = 13.863, p	456.843	495.099
diffBDI	= 0.254		
Age, MWM-pre,	F(1, 44) = 3.056, p =	440.243	511.698
diffMST	0.087		
Age, MWM-pre,	F(1, 43) = 1.905, p =	433.514	518.427
diffMST, diffBDI	0.175		

ANCOVA:	High vs	Low re	esponders
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Covariates	Effect of group on change in water maze performance after controlling	Unexplained variance (SS _R)	Amount of variance accounted for by the model (SS _M)
Age, MWM-pre	<i>covariates</i> F(1, 28) = 1.318, p = 0.261	136.36	358.604
Age, MWM-pre, diffBDI	F(1, 27) = 1.259, p = 0.272	136.346	358.618
Age, MWM-pre, diffMST	F(1, 27) = 1.218, p = 0.279	136.359	358.604
Age, MWM-pre, diffMST, diffBDI	F(1, 26) = 1.161, p = 0.291	136.346	358.618