ABSTRACT

Title of Dissertation:	ACUTE EXERCISE INDUCED MICROSTRUCTURAL AND FUNCTIONAL CHANGES IN THE HIPPOCAMPUS OF OLDER ADULTS
	Daniel D. Callow, Doctor of Philosophy, 2023
Dissertation directed by:	Professor, Jerome Carson Smith, Department of Kinesiology

Declining memory function is a common complaint of aging adults and a primary symptom of mild cognitive impairment (MCI) and Alzheimer's disease (AD). The hippocampus is often the first brain area to exhibit noticeable deficits in age and pathologically-related cognitive decline and is a necessary structure for proper memory function. More specifically, the dentate gyrus (DG) and the third cornu ammonis area (CA3) of the hippocampus directly support mnemonic discrimination (MD), which is the process of reducing interference among new representations and distinctly encoding them as independent memories. Poor MD is associated with age and is a presymptomatic biomarker of cognitive decline and is believed to result from reduced neurogenesis, angiogenesis, and synaptogenesis within the DG/CA3 subregion of the hippocampus. While causes and treatments for memory decline remain elusive, lifestyle interventions, especially physical activity, have received attention as cost-effective and safe means of ameliorating and potentially preventing cognitive decline in a growing aging population. Animal and human studies suggest exercise benefits the hippocampal structure, preserving neurogenesis and angiogenesis in aging rodents and macrostructure and memory in older adults. However, the mechanisms by which exercise affects the human hippocampus

remains a significant knowledge gap in the field and is a critical aspect in understanding the long-term impact exercise has on the aging hippocampus. To better address this gap, researchers have begun implementing acute exercise studies, which allow for greater control of non-exercise-related factors, are cheaper and more time efficient to conduct than training studies, and can predict and inform training-related adaptations. Unfortunately, limitations in the study designs, population tested, specificity of cognitive tasks, and spatial resolution of human imaging techniques have posed significant barriers to our understanding of how acute exercise relates to healthy brain aging at the functional and microstructural levels.

Therefore, the objective of this dissertation was to expand our understanding of how acute aerobic exercise alters the function and microstructure of the aging hippocampus. Three within-subject studies were conducted comparing the relationship between a 30-minute bout of moderate to vigorous intensity aerobic exercise vs seated rest on MD performance, hippocampal microstructure, and high-resolution hippocampal-subfield microstructure and functional activity in healthy older adults. In study one, acute exercise preserved MD performance compared to decrements exhibited after seated rest in a pre and post-condition study design. In study two, a post-condition-only study design, acute exercise elevated microstructural diffusion within the hippocampus, indicative of a hippocampal neuroinflammatory response and upregulation of neurotrophic factors. Finally, in study three, a post-condition-only study design, we found that acute exercise resulted in lower MD, suppressed MD-related DG/CA3 network hyperactivity (indicative of healthier network function), and led to higher DG/CA3 extracellular diffusion. However, these neuroimaging-based correlates of hippocampal neuroplasticity and network function were not associated with differences in MD performance. These findings suggest that higher-intensity acute exercise can alter memory performance and stimulate neuroplasticity and

neurotrophic cascades within the hippocampus and the DG/CA3 subfield, potentially via different mechanisms. Furthermore these results give insight into the immediate neurotrophic and behavioral effects of acute moderate to vigorous intensity aerobic exercise in older adults and provide new methods and tools for better understanding if and how exercise promotes healthy brain aging. Finally, these initial findings lay a foundation for optimizing exercise prescription and identifying future effective exercise treatments.

ACUTE EXERCISE INDUCED MICROSTRUCTURAL AND FUNCTIONAL CHANGES IN THE HIPPOCAMPUS OF OLDER ADULTS

by

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Chapter 1: Introduction

Importance and Overview

Dementia is a progressive decline in memory severe enough to interfere with independent function and affects one in ten Americans over the age of 65 [1]. A recent study found that dementia had the highest health care cost of any disease in the last five years of life; even before accounting for the emotional, mental, and physical toll of the projected 16 billion hours of unpaid care provided by families and loved ones to these individuals each year [2], [3]. Despite the widespread reach and growing cost of dementia care, there is currently no pharmacological means of treating this disease.

The following section will provide an introduction to various concepts and research methods that may support progress towards understanding issues of aging, cognitive decline, and how exercise may promote healthier brain aging. First, the unique relationship between the hippocampus, aging, cognitive decline, and mnemonic discrimination (MD) performance will be explored. Next we will discuss evidence for specific benefits of exercise and physical activity for hippocampal function in older adults, as well as gaps in our understanding of how acute exercise specifically may affect hippocampal function. We will then discuss the relationship between exercise and hippocampal structure and physiology, while also exploring how diffusion imaging techniques may provide novel insight into hippocampal microstructure and may prove to be more sensitive measures of hippocampal health and integrity than commonly used volumetric measures. Furthermore, we will overview the advantages and shortcomings of new multi-shell diffusion imaging techniques, including a discussion of the novel information the method can provide concerning hippocampal subfield glial activity and microstructural integrity in response to neuroinflammation. Finally, a discussion of these concepts and techniques will

then be incorporated into a proposed three-study dissertation project that will aim to advance our understanding of how acute aerobic exercise affects hippocampal function and microstructure of healthy older adults.

Hippocampal Function

The hippocampus has been at the center of memory research since the 1957 case study of patient HM, who famously lost the ability to form new declarative memories after surgical removal of the bilateral hippocampus for epilepsy treatment [4]. The importance of the hippocampus has grown as more research supports its distinct role in memory and its early susceptibility to aging and many neuropsychiatric diseases, such as AD [5]–[7]. The primary anatomical connectivity of the hippocampus is called the trisynaptic loop, which is implicated in hippocampal function, such as memory formation. In short, the entorhinal cortex (EC) provides the primary cortical input to the hippocampus, with most projections going via the performant path to the DG. DG then projects to the CA3, which projects to the CA1. However, many CA3 neurons also project onto other CA3 neurons, creating a recurrent collateral pathway that is the foundation of several theories on auto-associative networks and memory formation. Finally, CA1 projects back to the EC to complete the trisynaptic loop [8]. In particular, the trisynaptic loop is thought to play a critical role in processing episodic memory. Episodic memory is the ability to store and retrieve records of events and personal experiences [9], such as where you ate dinner last night, along with what you ate and who was with you. These event-related episodic memories are the foundation of how we understand and interact with the world [10]. However, general episodic memory is a complex cognitive process that incorporates numerous brain regions.

The hippocampus plays a central role in forming and storing these episodic memories through two distinct processes. Pattern separation is the process by which the DG subregion enhances the

resolution and reduces the interference of highly similar memories, so they are distinctly encoded in a non-overlapping manner [11], [12]. A unique property of DG is that it is one of only two brain regions to undergo neurogenesis across the lifespan in humans. These young adult-born DG granule cells are necessary for pattern separation; however, as these granule cells age (becoming old adult-born granule cells), they are thought to shift in function and contribute more towards pattern completion [13]. Meanwhile, pattern completion is the process of encoding or recalling old or degraded memories. The CA3's unique auto-associative network allows it to take incomplete information and "complete" or match a partial representation to recall a past memory [14]. Humans are continually undergoing experiences that share similar features (such as remembering where you put your phone or wallet). If pattern separation is impaired, newly encoded memories and information may overwrite previous memories. The healthy human brain can differentiate between similar experiences quite well, and thus, pattern separation is a crucial aspect of proper episodic memory storage and retrieval. Yet, extensive work indicates aging is associated with a shift away from pattern separation and toward pattern completion [15]–[19]. These pattern separation deficits prevent appropriate storage and retrieval of new information, which is believed to contribute to age-related cognitive impairment [16].

Mnemonic Discrimination - Measure of Hippocampal Subfield Function

The Mneumonic Similarity Task (MST) has been used widely in publications as a measure of hippocampal integrity by taxing behavioral pattern separation, which is often referred to as mnemonic discrimination (MD) in humans [20]. The task consists of showing participants a group of images that are either completely new (New), similar (Lure), or repeated (Old) (see **Figure 1**). Several studies using this task indicate that while nondemented older adults perform similarly to younger adults on object recall (identification of an old object), their ability to distinguish lures (similar but different from an old object), is significantly diminished [17], [19], [21]. This is thought to occur because older adults tend to

identify lure objects as old (pattern completion) at a much higher rate than younger adults. Meanwhile, standard object recognition rates (correct identification of a repeated object) are relatively consistent across young, unimpaired, and even impaired older adults [21]–[25]. This is particularly interesting given that standard object recognition is thought to be less reliant on the hippocampus, as animal and human studies have found that individuals with hippocampal damage maintain object recognition performance, but not MD [20], [26]. These results highlight the benefit of testing MD performance in older adults, as this cognitive process appears to deteriorate earlier and is a more nuanced and specific measure of hippocampal subfield integrity than general episodic memory or object recognition tasks

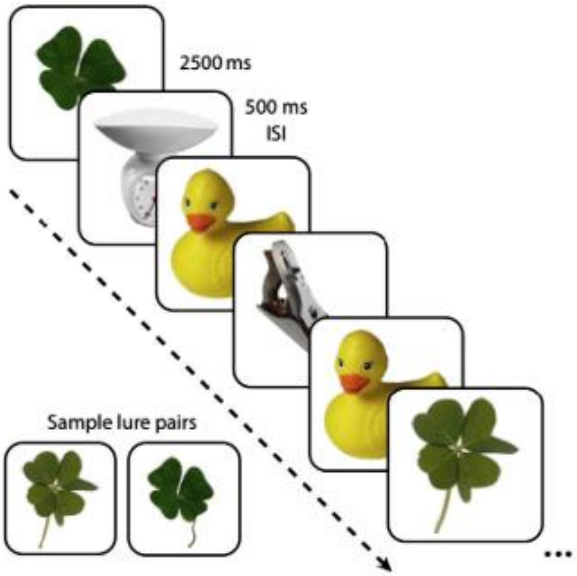

Figure 1: Continuous version of MST. Pictures of single items are presented for 2000 ms followed by a 500 ms ISI. New, old, and lure items are randomly shuffled in the task. Reproduced with permission from Dr. Craig Stark.

[10], [20].

The continuous fMRI variant of the MST leverages the repetition suppression effect, reduced blood oxygen level-dependent (BOLD) fMRI activity for repeated stimuli, to examine fMRI activity in hippocampal subfields during lure presentation. Human work corroborates animal and cellular findings, showing the BOLD response in the DG/CA3 for lures is much closer to that of new objects than repeat

objects, while other hippocampal and cortical regions exhibit the expected suppression effect for a highly similar item [12], [18], [24], [27]. This indicates the DG/CA3 region can indeed differentiate between highly similar and old representations in humans and suggests MD occurs in the DG/CA3 in humans (Note; due to limitations in the spatial resolution of fMRI, signals from the DG/CA3 are generally measured together).

Furthermore, hyperactivity and altered connectivity of the DG/CA3 hippocampal subfield during MD performance is associated with aging and pathological cognitive decline. Thus, hyperactivity or altered connectivity may represent deficiencies in proper DG/CA3 function and can be useful as a presymptomatic predictor of cognitive decline [21], [24], [28]–[30]. It is important to note however, that a recent study using high strength and resolution (7 Tesla) MRI found that the relationship between DG/CA3 activity, aging, and MD performance was nonlinear, finding that hyperactivity was positively associated with MD performance in middle-aged adults (26-45) but negatively related to performance in older adults (60-70 years) [31]. Therefore, it is important to keep in mind that changes in MD-related hippocampal subfield activity could be indicative of different underlying neurophysiological changes or processes in older adults compared to younger adults.

Effects of Fitness and Exercise Training on Hippocampal Function

A lack of successful treatments for dementia has led many researchers to focus on finding a cost-effective means of preventing or delaying its onset. Physical inactivity is considered to be one of the most significant modifiable risk factors for dementia, with substantial evidence for exercise and improved fitness preserving cognitive function in older adults [32]–[36]. By delaying cognitive decline, it may be possible to reduce the time spent with dementia or avoid it altogether. Given the wide range of

health benefits of aerobic exercise and its accessibility to those of any age, understanding how aerobic exercise preserves cognition during aging can have extensive societal and economic impacts.

Animal research indicates that freewheel running improves learning, increases neurogenesis, and alters the synaptic inputs to new DG adults born granule cells [37]–[39] and suggests these benefits counteract the adverse effects of aging on DG neurogenesis and synaptogenesis [38], [40], [41]. More specifically, several well developed and controlled animal studies have shown that freewheel running improves pattern separation performance via increased neurogenesis in the DG, rewiring of hippocampal circuitry, and upregulation of neurotrophic factors like BDNF [39], [42]–[45].

In humans, age is generally associated with episodic memory decline in older adults [7], [46], [47], while higher fitness levels and exercise training in older adults appears to improve if not preserve hippocampal-dependent cognitive tasks against these deleterious effects of age [34], [48]–[50]. Furthermore, higher cardiorespiratory fitness is associated with better MD performance in both younger [51] and older adults [52].

Meanwhile, exercise training studies have reported improved MD performance on high interference memory (disassociating between highly similar objects) in both young [53], [54] and older adults [55]. However, these benefits appear to be dependent on the type and intensity of the exercise training program and the extent of cardiorespiratory fitness improvements participants experienced during the intervention [53]–[55]. While a majority of this work has been limited to behavioral measures, a recent 12 week exercise intervention in younger adults found that improvements in cardiorespiratory fitness were associated with increased DG and CA3 subfield volumes and those fitness improvements mediated improvements in MD performance [56]. However, this and previous work has predominantly focused on younger adults, who are unlikely to be experiencing the more pronounced declines in hippocampal structure and function exhibited later in life. Therefore, more work is needed to

better understand the relationship between exercise, MD, and hippocampal function in older adults. Additionally, while this more recent intervention provides clinically relevant and location-specific evidence that exercise interventions can protect hippocampal subfield macrostructure and memory in younger adults, they fail to provide more nuanced and mechanistic evidence for how exercise might elicit these volumetric and behavioral changes.

Importance of Understanding the Effects of Acute Exercise on Hippocampus

While there is ample empirical evidence to support the cognitive benefits of exercise, the neurophysiological mechanisms are difficult to determine conclusively in humans. Over months or years, it may not be possible to disentangle the independent effects of exercise from those attributed to social interactions, sleep, or diet. However, suppose the exercise we do each day accumulates to produce brain benefits over time. In that case, it stands to reason that each session of exercise should produce effects from which these long-term adaptations occur. To ascertain the mechanistic effects of exercise on the aging hippocampus in older adults, we must first determine the immediate impact that an acute bout of exercise exerts on the hippocampus. These immediate effects can then be linked to longer-term changes in hippocampal function that might arise from exercise training interventions. This information would not only help elucidate the specific mechanistic effects of exercise for the aging hippocampus, but it may also allow for quicker development of optimized and targeted exercise programs and help us eventually identify individuals who are more likely to respond to exercise interventions.

Specific Effects of Acute Exercise on Memory

A wide range of study designs have been employed to ascertain the impact of a single session of exercise on memory [57], [58]. While acute exercise has consistently been shown to benefit performance

on executive function tasks, its effects on memory are less clear. A recent systematic review indicated that most studies looking at the impact of acute exercise on memory function in younger and middle-aged adults indicate a beneficial effect of exercise on some aspects of memory [59]. However, the length, intensity, and timing of the acute exercise intervention appear to moderate these effects. In fact, performing exercise before an episodic memory task (Rey Auditory Verbal Learning Task (RAVLT)) led to better performance than performing the task during, before, or without an exercise intervention [60], [61]. Furthermore, these benefits appeared to show an 'inverted-U' shaped association, with 15-20 minutes of moderate-intensity aerobic exercise eliciting optimal memory improvement over shorter or longer periods [62]. While these studies provide initial evidence for the beneficial effects of acute exercise for general hippocampal function, they have limitations.

Most recent acute exercise and episodic memory studies are limited to young college-aged adults [63], [64]. Considering some work suggests the benefits of acute exercise may be different for and possibly more pronounced in older adults [64], [65], paired with the fact that older adults generally experience a higher rate of memory decline, there is a need for research to determine the effects of acute exercise on memory specifically in older adults. Furthermore, most research focusing on acute exercise, hippocampal function, and memory has not incorporated imaging-based measures of the hippocampus, and many of these previous studies have not included a resting control condition. While we recently published a study from our lab showing an acute bout of moderate-intensity aerobic exercise is associated with greater hippocampal activation during a semantic memory task than after seated rest [66], there remains little evidence to date, for how acute exercise affects hippocampal specific structure and function in older adults. To better understand the immediate impact of aerobic exercise on proper hippocampal function, more accurate behavioral and neuroimaging measures of hippocampal integrity are needed. Episodic memory storage and retrieval is a complex process that incorporates numerous

cortical and subcortical structures; however, as previously discussed, a large body of animal and human research shows that the inability to perform MD accurately precedes general cognitive decline. Furthermore, MD performance on a task such as the MST is more specific to the hippocampal subfield integrity, particularly the DG/CA3 subregion, than other episodic memory or object recognition tasks [10], [11], [20]. Exploring the effects of acute exercise on MD performance in older adults can thus, provide strong evidence for the extent and location of acute exercise's effects on age susceptible memory processes and brain structures.

Acute Exercise Specific Effects on Mnemonic Discrimination

Two recent studies from Suwabe and colleagues assessed the effects of a single session of aerobic exercise on MD performance in younger college-aged adults. Using the MST, the first study established that younger adults could better discriminate highly similar objects after only 10 minutes of moderate-intensity exercise [67]. In the second study, participants performed the MST in the MRI scanner immediately after 10-minutes of light intensity aerobic exercise and after a seated rest condition [68]. They found elevated BOLD activity in the DG/CA3 hippocampal subregion in the post-exercise condition, and that enhanced DG/CA3 connectivity with cortical brain regions was related to improvements in discerning similar objects [68]. This work suggests that a single short bout of aerobic exercise can rapidly stimulate the DG/CA3 region, enhance its synchrony with cortical brain regions, and improve DG/CA3 dependent memory performance in younger adults.

Another recent study looked at the interaction effects that baseline physical activity levels and depression symptoms had on acute exercise-induced improvements (30 minutes of moderate-intensity aerobic exercise on a cycle ergometer) in MD performance in 18-49 year-olds [69]. They reported a relationship between baseline physical activity and MD performance that was moderated by age

(middle-aged participants saw a robust positive relationship between physical activity and MD). Interestingly, they did not report a main effect of acute exercise on changes in MD performance. However, unlike the two previously discussed experiments, participants performed a version of the MST before and after the exercise and rest condition. Yet, they only reported post-condition performance results. It is, therefore, possible that there could be some level of interference or potentially a main effect of time or interaction effect that went unreported. Nevertheless, Bernstein and Mcnally (2019) reported a significant 3-way interaction with baseline physical activity, depression symptoms, and post-condition MD performance differences. They found that individuals with the lowest depression symptoms and the highest baseline physical activity levels had better MD performance following the acute exercise session compared to following the rest condition. These findings suggest that acute exercise promotes immediate improvements in MD performance in younger adults, but that individual differences, as well as the intensity, duration, and timing of exercise and MST performance, may lead to differential effects.

Hippocampal Microstructure and Mechanisms

Aerobic Exercise, Hippocampal Microstructure, and Neuroinflammation

Animal work suggests that aerobic exercise training promotes hippocampal neurogenesis [38] and angiogenesis in the DG [70]. While human work indicates aerobic exercise training preserves hippocampal volume [71], vascularization [70], [72], and tissue density [73] in older adults. These neurogenic and angiogenic effects appear to occur over weeks, months, and even years of sustained exercise training. Yet, our understanding of the short-term neurophysiological responses occurring after a single exercise session and that are presumed to accumulate and promote observed long-term adaptations is severely lacking and predominantly limited to animal studies [74].

Research in animals suggests acute exercise increases hippocampal activity and upregulates neurotrophins in the hippocampus including hippocampal brain-derived neurotrophic factors, insulin-like growth factor, and vascular endothelial growth factor [75]–[78], which in turn are thought to be involved in eliciting the cellular changes resulting from sustained exercise training [74]. More recently, there has been growing interest in the anti-inflammatory properties of increased physical activity and exercise training. Yet, acute exercise appears to act as an acute stressor, with animal studies suggesting acute aerobic exercise elicits hippocampal neuroinflammation via glial activity and morphological changes [79]–[82]. Increased astrocyte and microglial activity are associated with neuroinflammation, brain repair, and clearance of pathological aggregates by redistributing these molecules to areas where they don't negatively impact the local environment. In response to inflammation, these glial cells, such as astrocytes and oligodendrocytes, experience proliferation, hypertrophy, and swelling [83]. While animal work suggests long-term exercise training reduces these biomarkers of neuroinflammation [84], acute exercise appears to increase glial activity and hypertrophy in the hippocampus [85]. Meanwhile, increased chronic hippocampal neuroinflammation is believed to impair neurogenesis and thus may contribute to the cognitive decline associated with aging and dementia pathology [86]. This suggests that while acute exercise may stress hippocampal homeostasis in the short-term, repeated bouts of exercise may lead to adaptations in the response and efficiency of the system to hippocampal neuroinflammation. Additionally, animal studies have shown that acute exercise-induced increases in hippocampal neuroinflammation are associated with better cognitive performance [87], [88]. While at first, this might seem counterintuitive due to the prevailing concept that chronic neuroinflammation inhibits neurogenesis [86], acute neuroinflammation has been shown to play a critical role in upregulating neurotrophic factors that promote neurogenesis [81], [82]. This further suggests that acute inflammation

induced by a single exercise session is beneficial in eliciting neurotrophic cascades that promote neurogenesis and could promote long-term and short-term cognitive improvements.

Novel Diffusion Imaging a Proxy for Glial Activity and Neuroinflammation

While gross hippocampal volume and atrophy measures are a hallmark of neurodegeneration, age-related memory decline, and dementia progression [89], they are not specific to the underlying neurophysiological changes occurring in hippocampal tissue. Most non-invasive human imaging techniques are not sensitive to physiological changes like neuroinflammation or glial-related hippocampal microstructure alterations. However, since astrocytes are permeable, their activity results in significant swelling and water movement, and thus changes in astrocyte structure and function from acute neuroinflammation can lead to significant changes in water diffusivity [83], [90]. Diffusion imaging is sensitive to the displacement of water molecules, which reflects the underlying microarchitecture of neural and glial morphology [90]–[93]. Although the majority of diffusion imaging work has focused on white matter, a growing body of research has focused on quantifying diffusion in cortical and subcortical gray matter regions [94]–[98]. Specifically, hippocampal diffusion provides information about tissue composition. For example, we recently reported that hippocampal diffusivity is more sensitive to development in children and is a better predictor of hippocampal-dependent source memory performance than hippocampal volume [99]. And for older adults, hippocampal diffusion is an earlier and more reliable predictor of memory decline, volumetric atrophy, and disease pathology and trajectory, particularly in cases of dementia conversion [100]–[107].

Furthermore, short-term changes in hippocampal diffusion are associated with learning and behavioral changes in humans, as well as glial activity in rats [108]–[110]. For example, improvements in 2 hours of a spatial learning task were found to be associated with hippocampal diffusion changes in both rats and humans, and in rats these diffusion changes were associated with glial and synaptic

changes [108], [110], [111]. Additionally, a recent study had 40 healthy younger adults learn to play a song on the piano for 45 minutes and found that this induced changes in diffusion within the motor cortex and cerebellum [112]. In summary, these findings not only indicate that hippocampal diffusion can provide novel information about tissue composition above and beyond tissue volume, but they also suggest that gray matter diffusion offers a novel measure sensitive to tissue plasticity over short timescales.

Technical Innovation in Diffusion Imaging

Previous work has predominantly applied the diffusion tensor model (DTI) [113] to obtain measures such as FA or mean diffusivity (MD; average diffusion within a voxel). These diffusion metrics are averaged across an entire brain voxel (for reference, a single standard 8mm^3 voxel could easily contain have over a million axons, neurons, and glia [90]) and thus, lack specificity for changes in different types of biological substrates and tissue that might contribute to changes in diffusion. New high angular resolution imaging (HARDI) protocols apply several non-zero b-gradients and can be analyzed with new multi-compartment tissue modeling techniques such as neurite orientation dispersion and density imaging (NODDI) [114]. NODDI addresses several limitations of the DTI technique through multi-shell acquisitions and multi-compartment diffusion modeling [115]. NODDI is arguably the most popular and widely used multi-compartment modeling technique. It attempts to parameterize diffusion signal into three microstructural compartments: intracellular diffusion (restricted diffusion within axons and dendrites), extracellular diffusion (hindered diffusion outside of axons and dendrites, such as within cell bodies, glial cells, extracellular matrices, and vascular structures)), and isotropic diffusion (i.e., free water). More specifically, the NODDI model provides three primary scalar values: the neurite density index (NDI; the proportion of intraneurite diffusion relative to extraneurite diffusion), the orientation dispersion index (ODI; 0 for perfectly aligned neurites and 1 for completely isotropic neurites), and the

volume fraction of isotropic diffusion (ISO; proportion of free water such as CSF). Importantly, NODDI has good test-retest reliability, accounts for partial volume effects, and has undergone histological validation in animals and humans [115]–[119]. Specifically, NODDI measures can account for CSF partial volume issues that plague DTI methods, particularly in gray matter, where the diffusion signal is generally isotropic. This partial volume issue arises because the strength of the diffusion signal within CSF is much greater, and therefore, even a small amount of CSF contamination can greatly influence diffusion summary metrics. HARDI and multi-compartment analysis methods such as NODDI provide higher resolution, signal to noise, and more biologically specific information of microarchitecture than other non-invasive human imaging methods [114], [120], [121].

Nazeri and colleagues [98], measured gray matter dendritic organization via NODDI in adults aged 21-84 and found that hippocampal and cortical neurite orientation dispersion index (ODI; dispersion coefficient of intracellular diffusion) was superior to volumetric measures for predicting differences in age, working memory, and processing speed [98]. Furthermore, two recent studies suggest that NODDI based microstructural differences in DG/CA3 microstructure are related to episodic memory performance and MD performance independent of age [106], [107]. Histological validation has shown that NODDI's ODI measure is sensitive to microglial density, activity, and morphological changes in the hippocampal DG region in response to neuroinflammation [122] and finally, a recent study comparing voxelwise associations between Positron Emission Topography imaging of neuroinflammation and NODDI (NDI and ODI) measures found significant associations between these two measures and neuroinflammation, with these associations being strongest within the medial temporal lobe [123]. In summary, HARDI-based multi-compartment diffusion imaging has gained increasing attention as a non-invasive means of measuring more biologically specific changes in human hippocampal microarchitecture [90], [94], [98], [119], [124]–[126].

Exercise and Hippocampal Diffusion

To date, only two studies have used DTI to show hippocampal MD is negatively associated with baseline aerobic fitness in older adults and improvements in fitness from a 6-month exercise training program [73], [127]. Additionally, we recently found increased cortical gray matter MD in healthy older adults and individuals with MCI following a 12-week exercise intervention, which was associated with improvements in verbal fluency and memory performance [128]. It is important to note that the two previous studies focusing on hippocampal diffusion specifically did not apply advanced processing methods to control for CSF contamination [73], [127], [129]. Additionally, these previously conducted studies have not looked at the effects of a single aerobic exercise session on hippocampal diffusion, have not applied more advanced diffusion acquisition or modeling methods such as NODDI, and have not looked at hippocampal subfields specifically [130]. The proposed studies will address these limitations and provide novel insight into the effects of acute aerobic exercise on hippocampal and hippocampal subfield-specific microstructure in older adults.

Study Aims

Aim Study 1

The results of previous acute exercise studies suggest that a single session of moderate-intensity aerobic exercise may have positive benefits for hippocampal-dependent memory. However, most of this work was conducted in younger college-aged adults. Considering the benefits of acute exercise may be different for and possibly more pronounced in older adults [63]–[65], paired with the fact that older adults generally experience a higher rate of hippocampal deterioration [6], [131], and exhibit lower MD performance [19], there is a need for research to determine the effects of acute exercise on MD

performance specifically in older adults. Yet, no research to date has explored this relationship in older adults. Given enhanced susceptibility to MD deficits and hippocampal deterioration in older adults and the pronounced protective effect of exercise training and fitness seen in this demographic, there is a need for research exploring the immediate effects of acute exercise on hippocampal function. Therefore, the aim of study 1 will be to determine whether an acute bout of moderate-intensity aerobic exercise alters MD performance on the MST task in healthy older adults.

Aim Study 2

A single session of wheel running has been shown to elicit hippocampal neuroinflammation, neurotrophic factors, and activity [75], [87], [88]. Furthermore, previous short-term spatial and motor learning studies suggest that diffusion tensor imaging is a novel and sensitive measure of short-term neural plasticity within the hippocampus [108], [110]–[112]. However, no work to date has established how a single session of aerobic exercise might immediately affect hippocampal neural plasticity. Therefore, the aim of study 2 is to determine whether an acute bout of moderate-intensity aerobic exercise alters whole hippocampal neural plasticity using diffusion tensor imaging in healthy older adults.

Aim Study 3

The aims of study 3 will advance our findings from studies 1 and 2 by determining how an acute bout of aerobic exercise alters hippocampal subfield function and microstructure in healthy older adults. This will be accomplished by conducting another within-subject study in which healthy older adults perform aerobic exercise or seated rest and then undergo high-resolution fMRI scans during the MST task and high-resolution HARDI scans.

Aim 3a: Previous work by Suwabe et al., 2018 found that acute aerobic exercise elicited higher hippocampal subfield BOLD fMRI activity during the MST task compared to after seated rest. However, this study was conducted on college-aged adults. This is a critical knowledge gap since age is a leading risk factor for cognitive decline, dementia, MD deficits, and is generally the demographic that exercise training clinical trials are conducted and most interested in [2]. Therefore, the purpose of Aim 3a will be to determine how a single 30-minute bout of moderate to vigorous intensity aerobic exercise affects hippocampal subfield BOLD fMRI activity during MD in older adults. We expect a higher BOLD fMRI response during lure discrimination in the DG/CA3 after the exercise condition than after seated rest.

Aim 3b: No studies to date have investigated if acute or exercise training affects hippocampal subfield microstructure using HARDI and multi-compartment diffusion imaging methods like NODDI, or how these effects might relate to behaviorally relevant changes in pattern separation performance. NODDI has better resolution, controls for partial volume effects and CSF contamination, and is more specific to underlying physiological changes in hippocampal microstructure than previously employed DTI methods. Therefore, NODDI measures such as ODI and NDI may better characterize the immediate effects of acute exercise on the hippocampal subfield microstructure. Furthermore, the HARDI scan quality and high resolution structural T2 scans of the hippocampus allow us to quantify hippocampal subfield diffusivity using an ROI approach. Thus, the purpose of Aim 3b will be to determine how a single 30-minute bout of moderate to vigorous intensity aerobic exercise affects hippocampal subfield microstructure. We expect higher ODI and lower NDI in the DG/CA3 after the exercise condition than after seated rest.

Aim 3c: Given the well-established relationship between MD performance and DG/CA3 integrity, the purpose of Aim 3c will be to determine if there is a relationship between exercise-induced differences in behavioral pattern separation performance and differences in hippocampal subfield BOLD

fMRI activity and ODI and NDI measures. We hypothesize that MD performance changes will be positively associated with differences in DG/CA3 BOLD fMRI activity and ODI values and negatively associated with differences in DG/CA3 NDI values. Characterizing these short-term microstructural alterations will help bridge the gap between animal and human acute exercise research and better understand the underlying mechanisms contributing to exercise's long-term benefits on the aging hippocampus.

Chapter 2: Effects of Acute Aerobic Exercise on Mnemonic Discrimination Performance in Older Adults

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Abstract

Ample evidence suggests exercise is beneficial for hippocampal function. Furthermore, a single session of aerobic exercise provides immediate benefits to mnemonic discrimination performance, a highly hippocampal specific memory process, in healthy younger adults. However, it is unknown if a single session of aerobic exercise alters mnemonic discrimination in older adults, who generally exhibit greater hippocampal deterioration and deficits in mnemonic discrimination performance. We conducted a within subject acute exercise study in 30 cognitively healthy and physically active older adults who underwent baseline testing and then completed two experimental visits in which they performed a mnemonic discrimination task before and after either 30 minutes of cycling exercise or 30 minutes of seated rest. Linear mixed-effects analyses were conducted in which condition order and age were controlled, time (pre vs. post) and condition (exercise vs. rest) were modeled as fixed effects, and subject as a random effect. No significant time by condition interaction effect was found for object recognition ($p=.254$, $\eta2=.01$), while a significant reduction in interference was found for mnemonic discrimination performance following the exercise condition ($p=.012$, $\eta2=.07$). A post-intervention only analysis indicated that there was no difference between condition for object recognition ($p=.186$, $\eta2=.06$), but that participants had better mnemonic discrimination performance ($p<.001$, $\eta2=.22$) following the exercise. Our results suggest a single session of moderate-intensity aerobic exercise may reduce interference and elicit better mnemonic discrimination performance in healthy older adults, suggesting benefits for hippocampal-specific memory function.

Introduction

Memory decline is a pervasive complaint of older adults and can exact an enormous toll on individuals, their loved ones, and society [132]. Physical exercise is a critical lifestyle intervention for promoting healthy brain aging, particularly preserving the hippocampus and memory function [133].

Meanwhile, growing evidence suggests a single session of aerobic exercise may provide immediate benefits for hippocampal integrity, function, and memory performance [58], [74], [134]. Specifically, a single session of low to moderate intensity aerobic exercise upregulates hippocampal BDNF expression in rats [75], [76], [135] and leads to small to moderate improvements in long term episodic memory performance in humans [58], [63], [136]. While a single exercise session may not elicit the same magnitude of benefit for memory performance as long-term exercise training protocols, acute exercise paradigms are ideally suited to understand the temporal interactions between exercise and phases of memory [58]. Furthermore, understanding these mechanisms and the timeframe by which a single acute session, or dose, of exercise may promote hippocampal function and integrity is critical to understanding and optimizing long-term brain health interventions in older adults. Nevertheless, previous acute exercise studies have predominantly been conducted on younger adults and utilized non-specific cognitive tasks that only partially tap into specific hippocampal function or integrity [137]. There is a need to incorporate cognitive tasks that more directly engage and challenge the integrity of important age- susceptible episodic memory circuits to better ascertain the relationship between acute aerobic exercise and hippocampal function in older adults.

One such cognitive task is the Mnemonic Similarity Task (MST), which behaviorally has been shown to engage hippocampal circuits and integrity by placing high demands on pattern separation. During the MST participants are asked to view colored images of everyday objects and then to determine whether the images they view are new, similar, or old compared to previously encoded images. The focus of the task is on quantifying how well participants can accurately distinguish between the previously viewed (old) stimuli and newly presented but visually similar stimuli. The new stimuli, which vary in their degree of similarity to the old items, are termed ‘lures’ [138]. The Lure

Discrimination Index (LDI) is a measure from the MST that represents the degree to which participants can successfully discriminate memories of highly similar old and new items. The LDI is the quantitative measure that operationally defines mnemonic discrimination capacity and has been strongly linked to hippocampal function [19]. Episodic memory encompasses the ability to encode, store, and retrieve unique facts or events in our life and is one of the most studied subdomains of memory as it is often the first process to deteriorate in both normal and pathological aging [139], [140]. Meanwhile, pattern separation, the process of reducing interference among memories, is thought to be a computation that supports episodic memory and is believed to be facilitated by the dentate gyrus (DG), a subfield of the hippocampus [11], [141]. Furthermore, the ability to accurately perform mnemonic discrimination and distinguish between similar experiences and event related memories is an important aspect of interacting with the world and functioning independently.

Mnemonic discrimination performance may be a useful marker of hippocampal function in older adults because the ability to behaviorally separate similar stimuli often declines earlier and more substantially during the aging process than other cognitive processes [19], [133], [142]. Furthermore, age-related deterioration in mnemonic discrimination performance has been strongly linked to deterioration of hippocampal subfield specific microstructure and function, particularly within the DG [11], [12], [138]. One hypothesis for this age-related deterioration is due to reduced neuroplasticity within the DG [13], as studies that directly stimulate neurogenesis within the DG show specific improvements in pattern separation performance [44].

Voluntary chronic exercise is known to reliably stimulate hippocampal neurogenesis. Animal studies have found regular exercise enhances neural plasticity, particularly within the DG of rats [37], and can counteract age-related reductions in neural plasticity [38], [41] and pattern separation performance [43], [45]. In humans, exercise training is associated with increased DG perfusion [70], DG

volume [56], [143], and better mnemonic discrimination performance [53], [54]. While most studies focusing on the relationship between exercise and mnemonic discrimination performance have been conducted in younger college-aged adults, Bullock et al. (2018) found a relationship between higher cardiorespiratory fitness and LDI across the lifespan and Kovacevic et al. (2019) found that a 3 month exercise training program improved LDI scores in older adults. While a growing body of research indicates that maintaining and improving aerobic fitness may benefit mnemonic discrimination and hippocampal function, less is known about the effects of a single session of exercise on hippocampal function in older adults.

Several recent studies suggest a single short bout of light to moderate-intensity aerobic exercise can benefit mnemonic discrimination and DG function in younger adults. Suwabe et al. (2017) found that 10 minutes of moderate-intensity aerobic exercise immediately improved the discrimination of high-interference memories during the MST in 21 younger adults, but there were no benefits for traditional object recognition. Suwabe et al. (2018) similarly found 10 minutes of low to moderate-intensity aerobic exercise led to improvements on moderate and high-interference memories, but not object recognition, and led to an increase in CA3/DG activity and connectivity with several cortical regions (left angular gyrus, left fusiform gyrus, and left perirhinal cortex) in 16 younger adults. Finally, Bernstein et al. (2019) found that 30 minutes of moderate-intensity aerobic exercise improved LDI in young to middle-aged adults with low depressive symptoms. Importantly, all three studies only tested mnemonic discrimination after the exercise intervention, and all three focused on young to middle-aged adults. Far fewer studies have tested the acute effects of exercise on memory in older adults [63], which is surprising given older adults are more likely to experience memory deterioration and, therefore, may experience greater or differential benefits from acute exercise than younger adults [64], [65], [137].

Two studies have explored the relationship between acute exercise and episodic memory in older adults. Segal et al. (2012) found that 6 minutes of moderate-intensity aerobic exercise increased the number of words recalled on the Rey Auditory Verbal Learning Task (RAVLT) in healthy older adults [144]. In addition, Etnier et al. (2021) recently showed that 20 minutes of moderate-intensity aerobic exercise led to better word recall on the RAVLT in healthy older adults. It is important to note however, that both studies incorporated post intervention only study designs, which permits comparisons between the exercise and rest conditions, but does not provide measures of change in memory performance from pre- to post-condition. Additionally, these two previous studies measured episodic memory with the RAVLT, which also involves language processing and verbal learning [145]. Meanwhile, the MST is a modified object recognition task and the LDI measure is a more sensitive and specific proxy of hippocampal function and aging than RAVLT scores due to its more robust demand on mnemonic discrimination [16], [20], [21]. Therefore, understanding the effects of acute exercise on mnemonic discrimination performance via the MST in older adults, while incorporating a pre- and post-exercise study design could better explain the relationship between an acute bout of exercise and hippocampal function and integrity.

The purpose of this study was to determine the effects of an acute bout of moderate-intensity aerobic exercise on mnemonic discrimination performance (measured via LDI on the MST) in healthy older adults. Based on previous studies reporting on the effects of acute aerobic exercise on LDI in younger adults [67], [69] and previous studies suggesting the benefits of acute exercise for memory may be even more pronounced in older adults [63]–[65], we hypothesized that acute aerobic exercise would lead to higher LDI scores compared to a seated rest control condition. However, given previous studies focusing on MST performance have only employed or reported post-intervention results, we predict that

there will be an interactive effect for mnemonic discrimination from pre- to post-intervention between the two conditions, but do not propose a specific directional hypothesis.

Methods

Participants

Forty-one physically active older adults (ages, 60-89 years) were recruited from the local community to participate in the study in accordance with the Helsinki Declaration. Participants were excluded if they reported a history of stroke, diabetes, untreated high blood pressure, neurological disease, major psychiatric disorder, had any contraindications to exercising on a bike, or were less physically active (less than 3 days/week of moderate intensity physical activity). Six participants dropped out before completing the study, leading to a final sample of 35. All participants completed a baseline session, a rest session, and an exercise session (order of Rest vs. Exercise counterbalanced across participants; see **Figure 2)**.

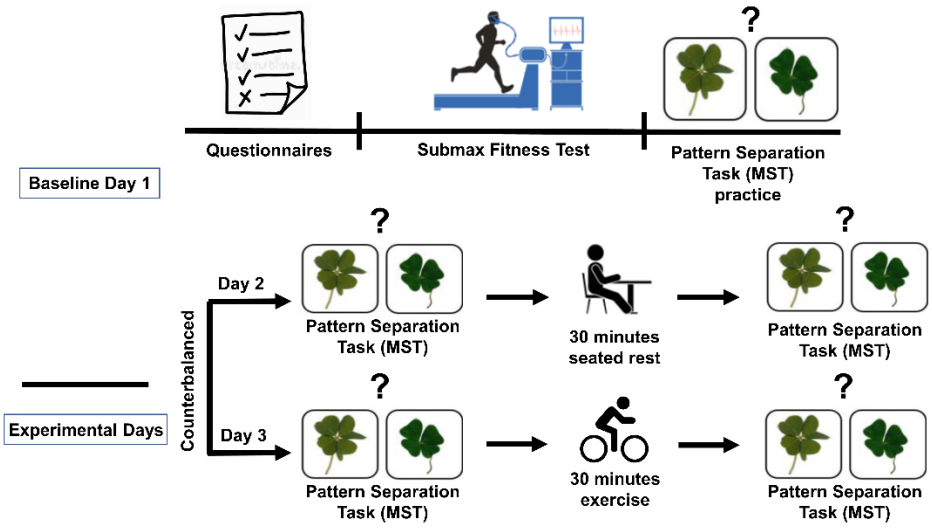

Figure 2: Graphical depiction of study design. Note, all participants completed all three days and all conditions with only the order of experimental conditions varying across participants. Comparisons between rest and exercise are therefore within-participant.

Submaximal Exercise Stress Test

Participants performed a submaximal stress test on a cycle ergometer (Corival, Lode, Netherlands) and respiratory gases were monitored via open-circuit spirometry (True One 2400 integrated metabolic system). Briefly, a staged ramp protocol [146] was employed where, following a two-minute warm up at 25W, an initial 30W resistance was set and increased by 10W/min until termination criteria was reached. Throughout the test heart rate (Polar H9, Polar) and measures of ventilation including rate of oxygen (O2) consumption, rate of carbon dioxide (CO2) production, and the respiratory exchange ratio (RER; CO2 production/O2 consumption) were collected, while the ratings of perceived exertion (RPE; 6-20 scale administered with instructions consistent with [147], [148]) scale was used to monitor subjective effort every minute. Tests were terminated upon attainment of 85% of participant's age predicted maximal heart rate response (220-Age), participant's request, or observations of exercise contraindications.

Mnemonic Similarity Task (MST)

The MST [21] was performed on a computer at five different time points and consists of an encoding and retrieval phase. During the encoding phase participants were shown 128 colored images of everyday objects, one at a time, for 2.5 s each (.5 s Interstimulus Interval) and then asked to indicate whether the object was an "indoor" or "outdoor" item. Immediately following the encoding phase, participants were shown a short 2-minute video that provided instructions for the retrieval phase. Participants then immediately performed the retrieval phase in which they were shown 192 colored images one at a time and were asked to identify whether the items were "old", "similar", or "new" with a button press. Of the 192 items, 64 were repeats from the encoding phase (targets), 64 were similar but not identical to an image in the encoding phase (lures), and 64 were new images (foils). Trial types were

presented randomly and separate stimulus sets were used for each test (Set 1 for the practice condition and Sets 2-5 counterbalanced across conditions). A total of 5 sets were used, with each set being equivalent in terms of the mnemonic similarity of their lures. Specifically, each lure image varied in its degree of similarity and was previously empirically ranked by assessing the false alarm rates (% old response) in a separate population [138]. These lures were then divided into 5 lure bins based on false alarm rates and each set was given an equal number of lures for each bin [21]. Given our sample consisted of older adults, we implemented the self-paced version of the MST in which participants were shown an image for 2 s, after which time the screen went blank, and then the program waited for a button response before continuing [19]. Two primary measures were obtained from the MST. The first was a traditional object recognition memory measure which was calculated as the rate of "Old" responses to repeats, minus "Old" responses to foils (Old | Target – Old | Foil) to account for response bias. The second measure was LDI, a measure of mnemonic discrimination, which was calculated as the rate of "Similar" responses to Lures minus "Similar" responses to new objects (Similar | Lure – Similar | foil) to again control for response bias.

Baseline Testing

Prior to the two experimental day visits, participants attended the baseline testing session. Upon arrival, participants provided written informed consent approved by Institutional Review Board. They then completed the Mini-Mental State Examination (MMSE), a 30-point questionnaire used to screen for global cognitive impairment, and demographic and baseline questionnaires to determine health history, physical activity (Stanford 7-day Physical Activity Recall questionnaire; [149]), anxiety symptoms (Geriatric Anxiety Scale; [150]), and depression symptoms (Geriatric Depression Scale; [151]). Finally, participants performed Set 1 of the MST to allow for familiarization with the task and to minimize practice effects during the following two experimental visits [19].

Exercise and Rest Conditions

At least 48 hours after the baseline testing day, all participants underwent counterbalanced control (seated rest) and exercise conditions on separate days, with each participant completing both experimental visits at the same time in the morning each day. Participants were asked to refrain from performing moderate to vigorous physical activities within 24 hours of testing, to eat a consistent breakfast, and to refrain from consuming coffee the morning of testing. All participants verbally confirmed that they followed the provided instructions at the beginning of each visit. Upon arrival participants completed the MST (Sets (2-5) and condition order (Exercise or Rest first) were counterbalanced across participants). Then participants completed either 20-minutes of moderate intensity aerobic exercise or 30 minutes of seated rest. During both the exercise and rest condition participants' heart rate (HR), RPE, and subjective valence (pleasantness) and arousal ratings (Self Assessment Manikin (SAM) [152]) were taken every 5 minutes. For the moderate intensity aerobic exercise condition, participants warmed up for the first 5 minutes and cooled down for the last 5 minutes of the 30 minute session. During the middle 20 minutes of the moderate intensity exercise session participants were instructed to exercise at a subjective rating of perceived exertion of 13 to 15 on the Borg 6-20 RPE scale (associated with a verbal anchor of "somewhat hard" to "hard") and were permitted to adjust the resistance on the cycle ergometer in order to maintain a consistent relative moderate intensity. After a 5-minute seated cooldown period, participants completed a different set of the MST.

Statistical Analysis

As a manipulation check, paired t-tests were conducted to determine differences in HR, RPE, valence, and arousal between the exercise and rest conditions. First, we looked at the relationship between age and pre-intervention LDI and object recognition performance using a linear mixed-effects

model while controlling for gender. To determine the effects of acute exercise on memory performance, we next compared the change in object recognition and LDI performance between the two intervention conditions with a linear mixed-effects model in which participant ID was a random effect and Condition (exercise vs rest) by Time (pre vs post) was modeled as a fixed interaction effect. Main effects of Condition and Time were further reported for both object recognition and LDI scores. To control for any variance related to the age of the participant and order of conditions, Order and Age were included in the model as fixed effects. To compare our findings to previous studies using only post-intervention designs and to further determine the effects on object recognition and LDI performance, we performed a post-intervention only analysis with Order, Age, and Condition modeled as fixed effects and participant ID as a random effect. Finally, to compare our results to previous studies in younger [68] and older adults[144], we conducted a post-hoc analysis to determine whether exercise-induced differences (Exercise minus Rest) in arousal levels were associated with exercise-induced differences (Exercise minus Rest) in post-intervention LDI scores using a Pearson correlation. To accomplish this, we ran a Pearson correlation on differences in arousal scores between the exercise and rest condition with differences in post condition LDI scores between exercise and rest. Statistical significance was set to an a priori threshold of $p<0.05$. All statistical analyses were performed using the R 4.0.1 statistical package.

Results

Participants

Of the 35 participants who completed all study protocols, four participants (2 males and 2 females) were excluded from further analysis due to exceptionally poor performance (< 50%) on MST's traditional object recognition memory component and one additional (male) participant was excluded due to a failure to use the "similar" response button at least ten times. These criteria have been similarly

employed to remove participants that were not following task instructions (Kolarik,Stark,and Stark 2020). A final sample size of 30 participants were included in the analyses (see **Table 1**).

Table 1: Participant Demographic Information (n = 31).

		Total sample (n=31) Mean (SD)
Demographics		
	Age (years)	70.2 (6.1)
	Sex	23 Female, 8 Male
	Education (n,(%), ≥ Graduate School)	20 (67%)
Health		
	Height (cm)	166.6 (8.9)
	Weight (kg)	71.3 (14.1)
	BMI (kg/m^2)	25.7 (4.3)
	$HR_{resting}$ (bpm)	68.7 (10.9)
Cardiorespiratory Fitness and Leisure-Time Physical Activity		
	VO_{2peak} (kg/ml/min)	21.2 (6.1) [a]
	7-day Physical Activity Energy Expenditure (MET-hours/week)	56.5 (27.7) [b]
Cognitive Status, Depression, and Anxiety		
	MMSE	29.8 (.4) [c]
	Geriatric Depression Score	3.1 (4.6) [d]
	Geriatric Anxiety Score	4.6 (3.7) [e]

Notes: bpm = beats per minute; RHR = Resting Heart Rate; HR_{max} = Maximum Age predicted heart rate; MMSE = Mini Mental State Examination. kg/ml/min = kilogram per milliliter per minute. MET = ratio of working metabolic rate relative to energy at rest. 7-day Energy Expenditure = the total MET- hours completed in the last 7 day period. MET is a unit of energy expenditure relative to the resting metabolic rate. VO_{2peak} = Peak oxygen consumption estimated from submaximal exercise stress test. [a] American College of Sports Medicine 50th percentile for peak oxygen consumption of older adults aged 60+ is approximately 30 (male) & 27 (female). [b] American Heart Association physical activity guidelines suggest 8.3-16.66 MET-hours/week for significant health benefits. [c] MMSE scores below 27 indicate potential mild cognitive impairment. [d] Geriatric Depression Scores between 9-15 indicate moderate to severe depression symptoms. [e] Geriatric Anxiety Scores between 16-63 indicate moderate to severe anxiety symptoms.

Participants were physically active, with an average 7 day metabolic equivalent (MET) of 56.5 hours and with all participants getting at least 8.3 MET hours (consistent with physical activity

guidelines of 150 minutes of moderate intensity physical activity per week (Kaminsky & Montoye 2014)). Additionally, participants were cognitively healthy (MMSE>26) and did not have major depressive (GDS<12) or anxiety (GAS<22) symptoms.

Age and Baseline Behavioral Performance

While controlling for gender, we found that pre-intervention LDI scores ($F(1,30)=5.08$, $p=.032$), but not object recognition scores ($F(1,30)=0.684$, $p=.415$), were negatively associated with age.

Experimental Manipulation Check

As expected, HR ($t(59)=22.21$, $p<.001$), RPE ($t(59)=39.20$, $p<.001$), and arousal ($t(59)=-5.82$, $p<.001$) were significantly higher during the exercise condition compared to the seated rest condition, see **Table 2**. There were no significant differences in valence ($t(59)=-0.37$, $p=.709$) values between conditions.

Table 2: Experimental condition outcomes and manipulation check.

Measure	**Mean (SD)**		
	Rest	**Exercise**	**p**
HR (BPM)	65.6 (9.4)	117.4 (16.9)	**<.001**
RPE (Borg 6-20 scale)	6.5 (1.0)	13.53 (0.9)	**<.001**
Valence	2.5 (0.9)	2.4 (0.8)	.709
Arousal	3.0 (0.9)	3.7 (1.2)	**<.001**
Reaction Time (milliseconds)	1700 (449)	1687 (467)	.557

Notes: SD = Standard Deviation. Measures of HR = heart rate; BPM = beats per minute; RPE = rating of perceived exertion. Valence = subjective measure of valence; Arousal = subjective measure of arousal; All measures were averaged and compared over the final 10 minutes of the moderate intensity exercise session (minutes 15-25 of the experimental conditions). Reaction Time = the average response time (in milliseconds) that participants took for each response during the test phase of the MST. Average participant heart rate in the final 10 minutes of the exercise condition was approximately 78% (SD 11%) of age predicted maximal heart rate. This is consistent with a moderate to hard intensity rating based on ACSM guidelines [153].

Time by Condition Analysis

While controlling for the Order and participant Age, the interaction effect of Time by Condition on object recognition performance was not significant ($F(1,90)=1.32$, $p=.254$, $\eta^2=.01$). Furthermore, there was no significant main effect of condition ($F(90,1)=537.$,$p=.770$), but there was a significant main effect of Time with participants on average performing better post-intervention ($F(1,90)=1.32$, $p=.004$, $\eta^2=.09$). Additionally, while there was no difference in pre-exercise vs rest ($t(93)=0.23$, $p=.992$) or for post-exercise vs rest object recognition performance ($t(93)=-.13$, $p=.561$), there was a significant increase in object recognition performance from pre- to post-exercise ($t(93)=2.88$, $p=.025$) (see **Figure 3**). With respect to LDI performance, there was a significant main effect of Time ($F(1,90)=4.20$, $p=.04$, $\eta^2=.04$), but not Condition ($F(1,90)=2.17$, $p=.144$), with participants on average performing worse post-intervention compared to pre-intervention. Furthermore, there was a significant interaction effect of Time by Condition on LDI performance ($F(1,90)=6.65$, $p=.012$ $\eta^2=.07$). Specifically, while pre-rest LDI was not significantly different from pre-exercise LDI ($t(93)=0.77$, $p=.868$), there was a significant decline in LDI from pre-rest to post-rest ($t(93)=-3.22$, $p=.010$). Additionally, there was no significant decline in LDI from pre-exercise to post-exercise ($t(93)=0.37.$, $p=.983$).

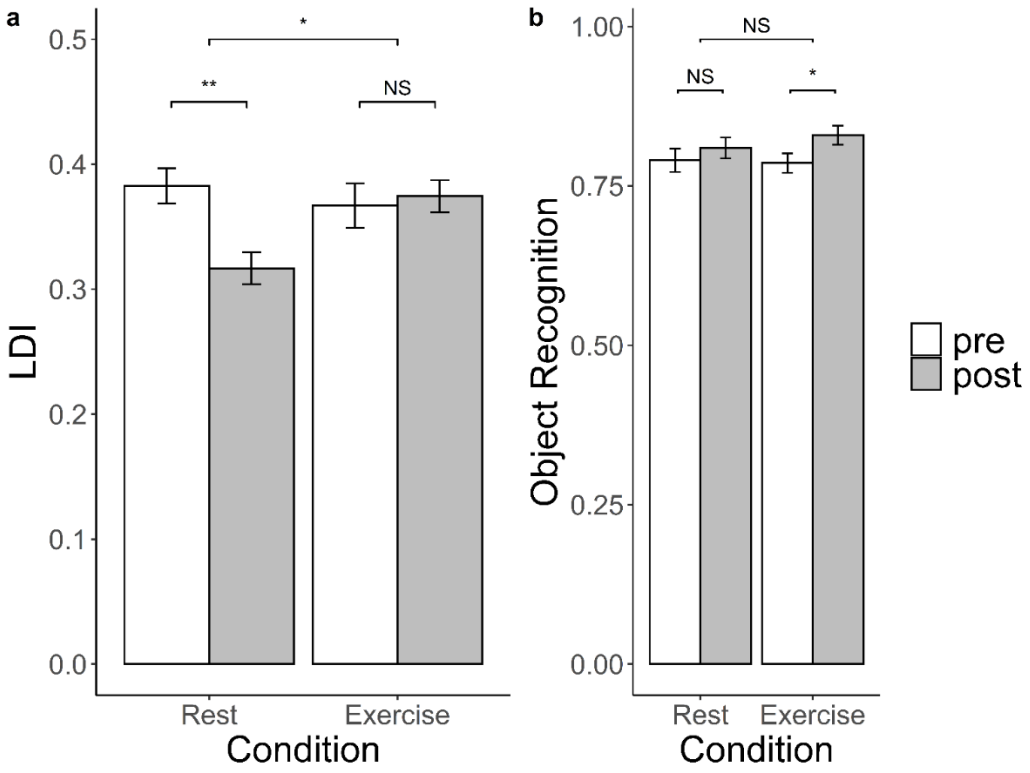

Figure 3: Panel a) depicts raw LDI (Lure Discrimination Index) score before and following both the exercise and rest condition (error bars = 1 SEM). * Indicates a significant interactive effect of Time (pre vs. post) by Cond (exercise vs rest) on LDI performance while controlling for condition Order and participant Age. ** Indicates a significant decrease in LDI from pre to post rest while NS indicates a non significant difference in LDI scores from pre to post exercise. Panel b) depicts raw Object Recognition performance before and following both the exercise and rest condition (error bars = 1 SEM). * Indicates a significant increase in object recognition from pre to post exercise while NS indicates a non significant difference in object recognition scores from pre to post rest and a non significant interactive effect. *p<.05; **p<.01; NS (p>.05).

Post Intervention Analysis

While controlling for condition Order and participant Age, the post-intervention analysis showed no significant effect of Condition on post-condition object recognition performance ($F(1,30)=1.83$, $p=.186$, $\eta^2=.06$). Meanwhile, LDI was significantly higher following exercise compared to rest ($F(1,30)=8.29$, $p<.001$, $\eta^2=.22$), see **Figure 4**.

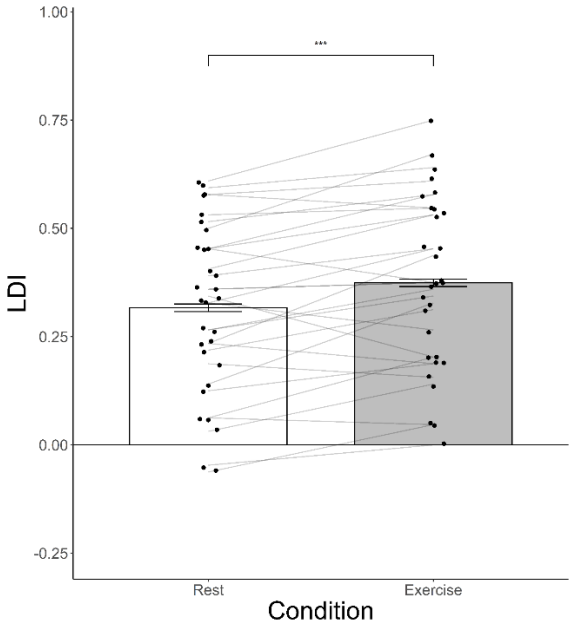

Figure 4: Bar graph of raw LDI (Lure Discrimination Index) performance following both the exercise and rest condition (error bars depict standard errors). *** Indicates a main effect of Condition (Exercise vs Rest) on LDI performance while controlling for condition Order and participant Age. *** p<.001.

Arousal and Post Condition Behavioral Performance

We found a significant increase in self-rated arousal in the last 10 minutes of the exercise condition compared to the rest condition (t(59) = -5.88, p-value <.001), see **Table 2**. However, exercise-induced differences in post-intervention LDI scores were not associated with exercise-induced differences in self-rated arousal levels (r=.044, p=.817).

Discussion

The primary purpose of this study was to determine if moderate-intensity aerobic exercise alters mnemonic discrimination performance in healthy older adults. In a group of 30 healthy older adults, we found that a 30-minute session of moderate-intensity aerobic exercise did not have a significant interactive effect or main effect of Condition on object recognition. However, acute exercise led to better maintenance of LDI scores from pre- to post-condition performance compared to the seated rest condition. Exercise also resulted in a significantly higher post-exercise LDI score compared to after

seated rest. Additionally, we found that pre-intervention LDI scores were negatively related to age. These results suggest moderate-intensity aerobic exercise may promote better hippocampal specific function via reduced interference and better mnemonic discrimination of similar objects in healthy older adults.

LDI scores on the two pre-intervention tasks were negatively related to age which is consistent with numerous previous studies [19], [21], [142], showing that aging is associated with poorer discrimination of similar objects on the MST task, but not with object recognition. We further explored this relationship and found that age was negatively associated with correctly identifying lure images and was positively associated with incorrectly identifying a lure image as a repeated image, suggesting a worsening ability to discriminate images with increased age and indicating a shift in older age from behavioral pattern separation to pattern completion [154]. Critically, there was no significant relationship between age and object recognition performance (i.e., the ability to identify a repeated image correctly). This is consistent with widely reported age-specific effects on behavioral pattern separation that cannot be attributed to age-related differences in object recognition memory [21], [22].

These behavioral findings corroborate previous computational and animal findings. Specifically, computational and animal models of pattern separation propose that newborn neurons within the dentate gyrus of the hippocampus are critical for distinguishing between highly similar information and storing the information as separate and distinct representations [155]–[157]. Furthermore, aging rodents [158] and potentially humans [159], [160] appear to exhibit fewer newborn neurons within the dentate gyrus. Meanwhile, reductions in newborn DG neurons are directly associated with poorer pattern separation performance in rodents [43], [44]. While intriguing to consider, it is not possible to directly measure DG neurogenesis in humans, and thus, we can not definitively determine the underlying mechanisms for this relationship. Additionally, a recent study suggests that LDI deficits are significantly associated with

deficits in visual perception [161] and thus, age related deficits in visual perception could be partially contributing to our finding of an age and behavior relationship.

Interestingly, while we found an improvement in object recognition from pre to post-intervention, we found no interactive effect or main effect of condition for object recognition performance. This suggests that an acute bout of exercise does not appear to immediately affect simple object recognition performance for healthy older adults when compared to seated rest. Notably, the MST's measure of object recognition performance provides a valuable comparison to pattern separation performance, as object recognition is considered to be less heavily implicated in hippocampal function [20], given it is relatively robust to aging and in those who have sustained hippocampal damage [21], [26]. This finding aligns with previous acute exercise studies that have examined pattern separation performance in younger adults. For example, Suwabe et al. (2017, 2018) report improvements in pattern separation performance but not object recognition [67], [68]. Furthermore, Bernstein et al. (2019) did not report a significant effect of acute moderate-intensity aerobic exercise on object recognition performance compared to a stretching condition [69]. Taken together, this suggests that the effects of acute aerobic exercise on memory and hippocampal function, at least for visual objects, may be somewhat specific to behavioral pattern separation performance instead of object recognition.

Meanwhile, we found that LDI scores were better maintained following the exercise condition while decreasing after rest. Furthermore, we found a main effect of time in which participants had worse LDI scores during the post-intervention test than the pre-intervention test, likely predominantly driven by the rest condition. While decreased or maintained performance may seem counterintuitive to the expected increase in performance after exercise (or due to practice), this finding suggests that participants were further challenged when performing the task for a second time in the post-intervention task, which required disentangling similar images in the context of extra interference induced by the

previously viewed images from the pre-intervention task. Notably, participants performed different versions of the task at each time point with entirely different images that did not overlap with each other [20]. Previous work by Stark and colleagues (2015) has shown that the MST can be used repeatedly in the same person to determine changes in performance over time [19]. However, these previous studies performed repeat tests using different temporal spacings (either immediately or days between tests) and often compared standard vs. overt instructions during the encoding phase, to try to and determine the effects of repeated testing on performance [19], [162]. However, to the best of our knowledge, previous work has not compared repeated task performance within the moderate time window we employed (approximately 35 minutes). Interestingly, however, we had numerous cases in which participants provided unsolicited feedback after the experimental session had ended that they found the post-condition version of the task more challenging than the pre-condition version due to interfering memories of the pre-condition task images. While the main effect of reduced LDI scores over time might suggest that reductions in post-condition task performance could be due to some level of interference, future studies will need to specifically test whether repeated testing over short time intervals might negatively affect LDI performance.

Nevertheless, we did find an interaction effect in which participants' LDI scores were maintained from pre to post-test for the exercise compared to the rest condition (as supported by the t-tests conducted within each condition). The exercise group's LDI performance remained relatively stable. Previous acute exercise studies focusing on the MST and pattern separation have thus far only performed post-intervention study designs [67], [68] or have failed to report pre to post-intervention results [69]. Additionally, while several previous studies have found that the effects of acute exercise were dependent on the degree of lure similarity in healthy younger adults [67], [68], we did not find that the degree of lure similarity interacted significantly with treatment condition in our older adult sample

(see Table 3 and Figure 5). Interestingly, Segal et al. (2012) found that 6 minutes of walking after viewing a set of emotional images led to elevated endogenous norepinephrine and better recall of emotional image details in older adults [144]. Suggesting that a short bout of moderate-intensity aerobic exercise may retrogradely enhance memory consolidation of images. Concerning our current findings, this could mean that participants who performed exercise between the two sets of MST tasks may remember more details of the previously depicted images, including the temporal sequence of exposure to the images, and thus, be more resistant to interference from the previously viewed images during the post-intervention test phase.

Table 3: Interaction Effects of Condition and Time by Lure Similarity

Lure Similarity Bins	F (df)	p-value
Lure Bin 1	F (1,90) = 0.762	.385
Lure Bin 2	**F (1,90) = 4.04**	**.047**
Lure Bin 3	**F (1,90) = 4.30**	**.041**
Lure Bin 4	F (1,90) = 3.24	.075
Lure Bin 5	F (1,90) = 0.683	.411

Notes: Results from a linear mixed effects analysis of Condition (Exercise vs Rest) and Time (Pre vs Post) on LDI Bin scores while controlling for Age and Order of condition. Lure Bins ranked from 1 (most similar) to 5 (least similar). F(df) = F-value (degrees of freedom).

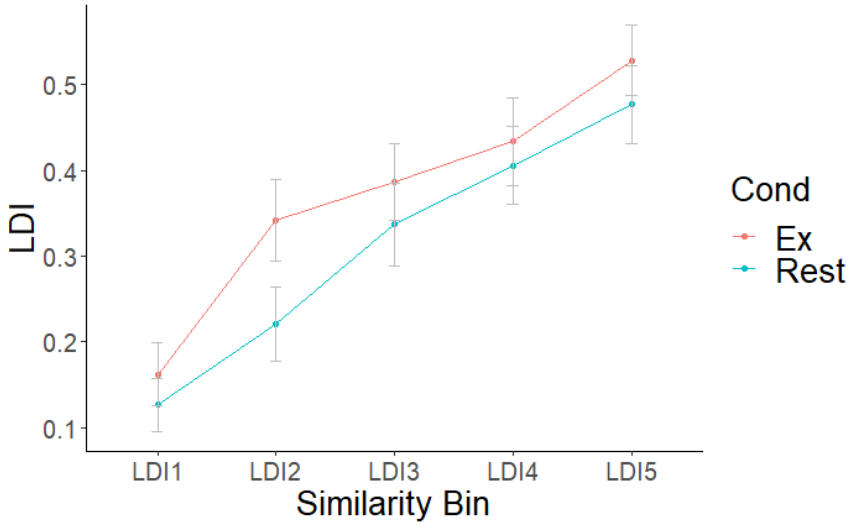

Figure 5: Line graph showing LDI scores following the Exercise and Rest condition and varying across lure similarity bins. With similarity bins scaling from 1 (most similar) to 5 (least similar).

In support of the hypothesis that exercise may reduce interference effects on later memory performance, Etnier et al. (2021) recently tested the effects of acute exercise timing on RAVLT performance in healthy older adults. They found that 20 minutes of moderate-intensity aerobic exercise improved the number of words recalled in healthy middle-aged and older adults. Specifically, they found that an exercise prior condition led to the greatest improvement in words recalled and that this improvement was specific to the short-term (Trial 1), learning portion (Trials 1-5), and the post interference recall portion (Trial 7) of the RAVLT, but not for the interference trial (Trial 6) [64]. This suggests that while acute aerobic exercise may not affect verbal recall on an interference list, it did lead to significantly better post interference recall, and suggests that aerobic exercise might help overcome interference issues when performing memory tasks. However, it is also important to note that there was not a significant improvement in LDI from pre to post exercise condition. Given our control condition consisted of 30 minutes of quite seated rest, which has the potential to elicit negative emotional and cognitive effects, could be leading to a reduction in LDI. Yet, there were no differences in subjective

measures of pleasantness (valence) between the exercise and rest condition and differences in arousal between the two conditions were not related to differences in LDI scores. Thus, our data do not support the view that unpleasant emotion or boredom of the rest condition explains the memory performance differences between the conditions. Nevertheless, future studies should consider implementing more active or engaging control conditions to better determine if benefits in MST performance are specific to a single session of exercise.

Previous studies looking at the immediate effects of acute aerobic exercise on behavioral pattern separation performance in younger adults have only analyzed post-exercise effects. We took the same approach and found that following the 30 minutes of moderate-intensity aerobic exercise, older adults had significantly higher LDI scores compared to following the seated rest condition. This finding is in line with three previous studies conducted in healthy younger adults, all of which found that 10-30 minutes of light to moderate-intensity aerobic exercise on a cycle ergometer led to better LDI scores via improvements in the discrimination of mnemonically similar objects [67]–[69]. Furthermore, previous work has shown an inverted-U-shaped dose-response relationship, where moderate-intensity aerobic exercise between 15-30 minutes appears to be optimal for eliciting benefits in complex cognitive processes, including memory [62], [65]. It is important to note that acute exercise paradigms are ideally suited to understand the temporal interactions between exercise and phases of memory. In particular, looking at the effects of exercise before the encoding phase and the incorporation of retrieval shortly after suggests these effects may be more specific to encoding mechanisms [58]. However, to truly disentangle these effects from storage/consolidation, a study that specifically compares pre-encoding vs post encoding interventions (between the encoding and test phase) would be needed. Nevertheless, these potential benefits are relevant given that numerous previous studies conducted in college-aged adults have shown that exercising shortly before, but not during or after a memory task leads to improvements

in both short and long-term memory [60], [71], [136]. Meanwhile, Etnier et al. (2021) similarly found improvements in short and long-term memory performance on the RAVLT in healthy older adults when employing a similar length (20 minutes) and intensity (moderate) of acute aerobic exercise in middle and older-aged adults [64]. Interestingly, we also found a relatively large positive effect of exercise on post-condition LDI scores (η^2=.22), which is consistent with the effects reported in older adults by Etnier et al. (2021) [64]. Unfortunately, previous acute exercise studies on pattern separation and younger adults did not report effect sizes. Thus, it is hard to make direct comparisons [67]–[69]. However, our finding of a relatively large effect size supports our previous hypothesis, which may relate the premise that older adults have more room to benefit from behavioral interventions given they are theorized to have less cognitive reserve, and younger adults have greater potential for performance ceiling effects [58], [64], [65], [163]. However, since our study only includes older adults from a relatively narrow age range, future studies are needed to test for a moderating effect of age specifically.

Our study is the first to show, in healthy older adults, positive effects of moderate-intensity aerobic exercise on a highly hippocampal specific and age susceptible mnemonic discrimination task. Furthermore, this is the first study to measure and compare MST performance before and after an exercise and control intervention, providing more rigorous insight into the immediate effects of aerobic exercise on mnemonic discrimination. By accounting for baseline LDI scores, we were able to determine how participants' performance changed over time. However, our study also suffers from several limitations. Specifically, our sample was predominantly white, well-educated, and female, making it challenging to generalize these findings at the population level. Furthermore, our volunteers were all physically active individuals, who may respond differently to a single exercise session compared to more sedentary individuals.

In conclusion, this is the first study to show that a single 30-minute session of moderate-intensity aerobic exercise can improve mnemonic discrimination in healthy older adults relative to rest. We found that exercise led to better discrimination of similar objects and better maintenance of pattern separation capacity than following the seated rest control condition. Furthermore, and consistent with the literature, we found that baseline mnemonic discrimination was negatively related to age in our sample. Our results suggest that a single session of moderate-intensity aerobic exercise may provide immediate benefits for hippocampal-specific memory function and thus, provide evidence for exercise as a safe and easy-to-implement intervention to maintain healthy cognitive function.

Chapter 3: Microstructural Plasticity in the Hippocampus of Healthy Older Adults After Acute Exercise

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Abstract

The hippocampus experiences structural and functional decline with age and is a critical region for memory and many cognitive processes. Exercise is beneficial for the aging brain and shows preferential benefits for hippocampal volume, activation, and memory-related cognitive processes. However, research thus far has primarily focused on the effects of exercise on long-term volumetric changes in the hippocampus using structural MRI. Critically, microstructural alterations within the hippocampus over short time intervals are associated with neuroplasticity and cognitive changes that do not alter its volume but are still functionally relevant. However, it is not yet known if microstructural neuroplasticity occurs in the hippocampus in response to a single session of exercise. We used a within subject-design to determine if a 30-minute bout of moderate-intensity aerobic exercise altered bilateral hippocampal diffusion tensor imaging (DTI) measures in healthy older adults (n=30) compared to a seated rest control condition. Significantly lower fractional anisotropy (FA) and higher mean diffusivity (MD) were found after exercise relative to seated rest within the bilateral hippocampus, and this effect was driven by higher radial diffusivity (D_r). No significant differences in axial diffusivity (D_a) were observed. These findings suggest that a single exercise session can lead to microstructural alterations in the hippocampus of healthy older adults. These differences may be associated with changes in the extracellular space and glial, synaptic, and dendritic processes within the hippocampus. Repeated microstructural alterations resulting from acute bouts of exercise may accumulate and precede larger volumetric and functional improvements in the hippocampus.

Introduction

The hippocampus is considered a fundamental structure for human cognition. In addition to its well-documented participation in memory and learning systems, the hippocampus is implicated in several aspects of cognition including executive function, processing speed, fluid intelligence, path integration, and spatial processing [164]. Hippocampal structure is also specifically susceptible to neurodegenerative diseases such as dementia, with numerous neuroimaging studies showing smaller hippocampal volume in those with mild cognitive impairment (MCI) and Alzheimer's disease (AD), compared to age-matched controls [165]. Healthy aging is similarly associated with consistent declines in hippocampal volume and a range of memory and hippocampal-related cognitive abilities [131]. The importance of the hippocampus in many cognitive domains, its high degree of endogenous plasticity throughout the lifespan, and its vulnerability to aging and various disease states has sparked interest in measures that are more specific and sensitive to cognition and the underlying tissue structure [166].

Advancements in diffusion-weighted imaging and anatomical segmentation techniques have allowed researchers to ask questions related to the underlying tissue microstructure of the hippocampus and its role in aging and cognition. While analyses of diffusion tensor imaging (DTI) data have traditionally been used to examine white matter tract structure and integrity, recent studies have focused on quantifying diffusivity within the gray matter itself [94]. By quantifying the diffusion of water molecules in cortical and sub-cortical tissue, one can infer the underlying microstructure's functional and structural properties [92]. The two most common DTI measures of local tissue diffusivity are fractional anisotropy (FA), a measure of the degree of diffusion anisotropy within a brain voxel, and mean diffusivity (MD), a measure of the average diffusion properties of the underlying tissue within each voxel [113].

Diffusivity measures of the hippocampus have been particularly crucial for examining cognitive decline in aging and neurodegenerative diseases. For instance, compared to conventional volumetric measurements, studies have shown that diffusion-based measures of microstructural alterations in the hippocampus may be earlier indicators of MCI, more sensitive to episodic memory impairments, and more predictive of transition to MCI and AD [100], [103]. In the context of cognitive aging, hippocampal FA is negatively and MD positively associated with age [167], [168]. Both higher and lower hippocampal FA and MD have been related to improvements in cognitive processes, such as pattern separation, working memory, fluid memory, processing speed, and navigation skills [18], [101], [167]. Decreases in synaptic, dendritic, and neuronal density are thought to drive these long-term changes in hippocampal diffusivity with age and pathology. Furthermore, animal and human work has shown that changes in gray matter diffusivity, and more specifically hippocampal diffusivity are associated with improved short-term learning over a period of days and even hours [108], [109]. However, these short-term gray matter diffusion changes are thought to result from different physiological mechanisms, such as altered neural activity and structural remodeling of synapses and glial processes, tissue swelling, and differences in the ratio between intracellular and extracellular volumes after neural excitation [109].

The World Health Organization recently reported that lifestyle changes, such as physical activity, offer the best evidence to date in the prevention of cognitive decline and dementia [169]. A growing body of evidence suggests exercise is beneficial for cognition and functional and structural measures of brain health in older adults [170]. Specifically, converging lines of both animal and human work have found that some of the most potent benefits of exercise for age-related cognitive impairment are seen in the hippocampus [133], with exercise training, physical activity, and improvements in cardiorespiratory fitness associated with preservation of hippocampal volume and hippocampal-dependent neurocognitive

measures in older adults [48], [72], [171]. Furthermore, a single session of aerobic exercise has also been shown to have an immediate impact on hippocampal dependent memory performance and hippocampal activity, connectivity and perfusion [66], [68], [172]. Meanwhile, animal work suggests a single bout of wheel running upregulates hippocampal inflammatory markers, neurotrophins, neurotransmitters, and glial activity [80], [85], [173]. A better understanding of the immediate neurophysiological effects that an acute bout of aerobic exercise has on the hippocampus is critical for formulating a comprehensive understanding of the relationship between exercise and hippocampal function as the immediate responses to an acute bout of exercise are believed to accumulate and elicit long term structural and function changes that are more established in the literature [174].

Despite consistent findings that exercise training preserves hippocampal volume [71] and increases hippocampal perfusion in cognitively healthy older adults [72], only two studies have looked at the effects of exercise or cardiorespiratory fitness on microstructural changes of the hippocampus using DTI. Tian and colleagues conducted a cross-sectional study in sedentary older adults and found that hippocampal mean diffusivity (MD) was negatively associated with cardiorespiratory fitness [127]. Furthermore, Kleemeyer and colleagues conducted a 6-month exercise intervention and found that changes in cardiorespiratory fitness were negatively associated with changes in MD of the hippocampus [73]. While these studies provide evidence that cardiorespiratory fitness and exercise training may preserve diffusion-related indices of hippocampal integrity, there is little evidence for how a single exercise session might affect hippocampal microstructure in older adults.

Given this evidence for hippocampal-dependent functional and cognitive changes after acute exercise, we hypothesized that a single aerobic exercise session would also affect the microstructural properties of the hippocampus in healthy older adults. While exercise training and cross-sectional exercise studies have shown associations between higher cardiorespiratory fitness and higher FA and

lower MD in the hippocampus [73], [127], short-term learning studies focusing on diffusion within gray matter have found conflicting results with respect to the directionality of diffusion changes [108], [109] and no research has previously explored how acute exercise affects hippocampal diffusion. Thus, we did not have a directional hypothesis but rather predicted that there would be differences in hippocampal FA and MD measures between the acute exercise and seated rest conditions. To test this hypothesis, we assessed the effect of a single session of moderate-intensity aerobic exercise on hippocampal diffusivity metrics in cognitively healthy and physically active older adults.

Methods

Subjects

Thirty-two physically active, cognitively healthy, non-smoking, right-handed older adults (ages 55-85 years) were recruited for this study. Participants were pre-screened with a questionnaire battery that included MRI safety, the 7-day physical activity recall (33), the Beck Depression Inventory-II (34), and the Mini-Mental State Exam (MMSE) (35). Participants without contraindications to MRI scanning, who indicated participation in at least three days of moderate-intensity exercise per week and scored at least 27 on the MMSE, were eligible to participate (see (24) for details on recruitment and final sample determination). Participants were instructed to refrain from exercising for 24 hours before testing. Participants who completed all experimental sessions were paid for their participation. Written informed consent was obtained, and this study was conducted according to the Helsinki Declaration of 1975, and was approved by the Institutional Review Board at The University of Maryland.

Exercise and Rest Conditions

Using a within-subject design, participants performed two experimental conditions (exercise and rest) on separate days in counterbalanced order. Due to scheduling restrictions, some of the participants performed the experimental conditions at different times of day for each condition; all scans were obtained within a few hours of the same time of day. Before both conditions, participants were fitted with a heart rate (HR) monitor and were provided standardized instructions for the Borg 6-20 Ratings of Perceived Exertion (RPE) scale [147]. For the exercise condition, participants completed a continuous bout of cycling on a Monark cycle ergometer (Varberg, Sweden) located outside the MRI scanner. They were free to adjust the resistance of the bike while maintaining a cadence between (60-80 rpm) to achieve the target RPE. Participants performed a 5-minute warm-up at a self-selected pace, followed by a 20-minute bout of cycling at a target RPE of 15 on the Borg 6-20 RPE scale (associated with the verbal anchor of "hard"), and finished with a 5-minute cooldown. Heart Rate and RPE were collected every five minutes. They received water *ad libitum* during both conditions, and after the exercise condition, they were provided with a towel and clean and dry clothing for the scan. During the rest condition, participants were seated quietly for 30 minutes, while HR and RPE were measured every five minutes. Participants did not have access to cell phones, and excessive talking was discouraged. Approximately 20 minutes elapsed from the end of each condition to the initiation of MRI scanning. This was part of a larger acute exercise study that included multiple MRI sequences [66] including a semantic memory and flanker task based scan. The diffusion-weighted scan was acquired at the end of the scanning session and thus, was collected approximately 80 minutes after the end of each experimental condition.

MRI Acquisition

Immediately following both the rest and exercise condition, participants were prepared for MRI scanning on a Siemens 3.0 Tesla MR scanner (Magnetom Trio Tim Syngo, Munich, Germany). A 32-channel head coil was used for radiofrequency transmission and reception, and foam padding was positioned within the head coil to minimize head movement. Furthermore, for each individual scan, the MRI operator was trained to specify the imaging prescriptions (brain coverage, slice orientation, etc.) as uniformly as possible across all participants and particularly within subjects to further minimize the variability. High-resolution T1-weighted anatomical images were acquired with the following sequence parameters: Magnetization Prepared Rapid Acquisition of Gradient Echo (MPRAGE), matrix = 256 x 256, field-of-view (FOV) = 230 mm x 230 mm^2, pixel size = 0.9 × 0.9 mm^2, slices = 192 (sagittal plane, acquired right to left), slice thickness = 0.9 mm, repetition time (TR) = 1900 ms, echo time (TE) = 2.32 ms, inversion time (TI) = 900 ms, flip angle = 9°, sequence duration = 4:26 min. Diffusion images were acquired with a twice-refocused spin-echo single-shot Echo Planar Imaging sequence. The protocol included a set of 64 non-collinear diffusion-weighted acquisitions with b =1000 s/mm^2 and a single T2-weighted b = 0 s/mm^2 acquisition (TR/TE = 6300/106 ms, 128x128 matrix, 1.64 x 1.64 mm^2 in-plane resolution, flip angle = 90°, and a bandwidth of 1221 Hz/Px comprising 96 3-mm-thick slices). A pair of magnitude and a single-phase image were acquired (TE1 = 4.92 ms and TE2 = 7.38 ms, TR = 560 ms, 53 slices, slice gap = 0.625 mm, 2 mm isotropic voxels, acquisition = 96 x 96 mm, and FOV = 192 x 192 mm^2) for field map-based unwarping of diffusion-weighted images (DWI) to correct for distortion artifacts and improve the registration of the DWI to anatomical image [175].

Anatomical Image Processing

T1-weighted anatomical images were processed with the FreeSurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/, version 6.0). The cross-sectional "recon-all" processing stream was

implemented to perform initial motion correction, intensity normalization, and computation of the transformation to Talairach standard space, followed by non-brain tissue removal, cortical reconstruction, and volumetric segmentation of cortical and subcortical structures. To reduce variability and to improve skull stripping and segmentation performance across time points, images were then processed with the FreeSurfer longitudinal stream [176]. Specifically, an unbiased within-subject template space and image were created and then further steps, such as skull stripping, computation of transformation to Talairach standard space, and atlas registration and parcellations [176] were initialized with information from the within-subject template, increasing reliability and statistical power. Hippocampal volume was obtained from the longitudinal stream results and was extracted in native anatomical space. All reconstructed data were visually checked for skull removal and segmentation accuracy. No manual intervention with the MRI data was needed.

Diffusion-Weighted Image Processing

Diffusion-weighted images were processed using MRtrix3 commands or MRtrix3 scripts [177] that link the FMRIB Software Library (FSL v6.0.1) (Image Analysis Group, FMRIB, Oxford, UK; [178]). First, physiological noise due to water molecules' thermal motion was removed, followed by eliminating Gibbs ringing artifacts, bias field correction, and then brain extraction using the dwi2mask command. Finally, we up-sampled the processed data to 1.5 x 1.5 x 1.5 mm^3 isotropic voxels to improve the spatial intensity contrast in later modeling steps [179]. The FSL dtifit program was then used to fit a diffusion tensor model of three eigenvectors and three eigenvalues to each brain voxel, as well as FA, MD, axial diffusivity (D_a, amount of diffusion in the primary diffusion direction), and radial diffusivity (D_r, average diffusion perpendicular to the primary diffusion direction) values [113]. It is important to note that FA and MD are summative measures that are dependent on axial and radial diffusivity.

Hippocampal, Amygdala, and Middle Temporal Cortex Segmentation and Registration to Diffusion Space

Spatial normalization of diffusion data is susceptible to the accumulation of interpolation, tensor reorientation, and misregistration errors [175]. When trying to determine the effects of a single session of exercise, smaller physiological effects are expected and an ROI analysis approach in each participant's native diffusion space may be more appropriate to reduce noise and increase sensitivity to differences between conditions [175]. Our within-subject design allowed us to use each participant as their own control and thus segment and extract hippocampal diffusion values from native space, thus reducing registration artifacts and partial volume effects that may occur with smoothing and normalization. **Figure 6** displays an example of a single participant's hippocampal segmentation, registration to diffusion space, and extraction of hippocampal FA measures.

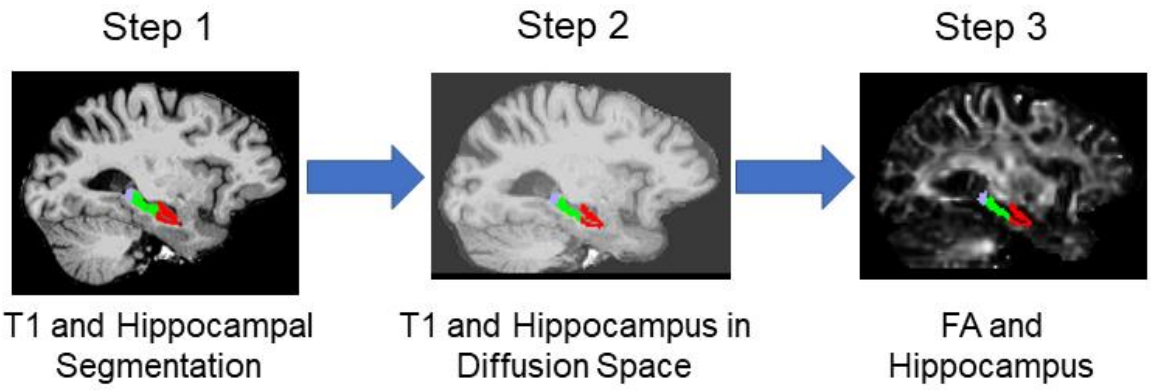

Figure 6: Example of a single participant's hippocampal segmentation and extraction process, including the hippocampal tail (blue), body (green), and head (red). First, the T1-weighted images were segmented (Step 1); then, the T1-weighted segmentation was aligned with the diffusion-weighted image (Step 2), and finally, the diffusivity metrics were aligned with the hippocampal mask in the participant's native space (Step 3). Full hippocampal ROIs were created by combining tail, body, and head segmentations. Posterior hippocampal ROIs were created by combining the tail and body segmentations while the head segmentation was used to create the anterior hippocampal ROI. T1 = T1-weighted anatomical scan. FA = fractional anisotropy image.

Automated hippocampal subregion segmentation was performed for each participant's T1 weighted image using the Freesurfer longitudinal hippocampal subregion segmentation program [180]. This method uses a computational atlas of the hippocampus based on ultra-high resolution, ex vivo MRI and incorporates a Bayesian longitudinal segmentation approach using subject-specific atlases that significantly improve test-retest reliability and sensitivity over cross-sectional methods [180]. Segmentation of the bilateral amygdala and bilateral middle temporal cortex was performed using Freesurfer's longitudinally processed subcortical segmentation and cortical parcellation using the Deskian-Killany atlas [176]. The amygdala is associated with emotional regulation and is a nearby subcortical gray matter region to the hippocampus, while the middle temporal cortex is a part of the medial temporal lobe, in which the hippocampus resides. Finally, a whole brain gray matter mask was created using Freesurfer's tissue segmentation. These additional segmentations were created and analyzed to determine if there was a more global exercise effect on gray matter diffusivity. The Advanced Normalization Tool (ANTs;[181]) was used to correct potential b0 inhomogeneities in the diffusion data and improve diffusion to anatomical co-registration. Specifically, the b0 image was registered to the bias-corrected and skull stripped anatomical image using the antsIntermodalityIntrasubject.sh script, which uses ANTs robust SyN nonlinear registration algorithm with a Mutual Information criterion that is optimized for within-subject registration across image modalities. This method has been shown to have equal, if not superior, performance to standard field map methods for correcting b0 inhomogeneities and aligning b0 and T1 images [182]. The inverse transformations of the b0 to T1 registration was then applied to the T1-weighted image and hippocampal, amygdala, and cortical segmentations to get these ROI's in native diffusion space. Registration of T1-weighted images and ROIs to diffusion space was visually inspected by overlaying the T1 and ROI segmentations over the FA and MD images in native diffusion space.

Control for CSF Contamination and Partial Volume Effects

When examining gray matter diffusivity, particularly in older adults or patient populations, it is essential to consider partial volume effects that might arise from CSF contamination. To control for CSF contamination in the estimated ROI's, we applied a free-water contamination mask using MRtrix3Tissue (https://3Tissue.github.io), a fork of MRtrix3 [177]. MRtrix3Tissue allows for the estimation of 3-tissue constrained spherical deconvolution results from single shell diffusion data. The three tissue compartments can determine the contribution of free water CSF-like, WM-like, and GM-like signal within each voxel and has been shown to exhibit high reliability (intraclass correlation above 0.95), particularly for estimating the contribution of free water CSF-like diffusion [179]. We used a slightly more stringent threshold than previously reported [179], with each subjects ROI's thresholded to remove voxels that were considered to have more than 20 percent free water CSF-like signal.

Statistical Analyses

Statistical analyses were performed with R [183]. A Shapiro Wilkes test was performed for diffusion measures to test for normalcy. The Shapiro Wilkes test indicated a non-normal distribution for hippocampal FA ($p = .004$), Da ($p = .008$), and age ($p = .016$), and a normal distribution for MD ($p = .702$), and D_r ($p = .331$). However, given our smaller sample size, we chose to perform a Wilcoxon paired t-test to compare all diffusion measures. To check for bias in our diffusion measures that could be attributed to inconsistencies in hippocampal segmentation across conditions, a student paired t-test was used to test for significant differences in hippocampal volume between exercise and rest days. Additionally, to test for bias that might be caused by order of conditions, a repeated measures analysis of variance was used to compare diffusion differences between participants who performed exercise and then rest and those who performed rest and then exercise. Although MD and FA have been shown to be associated with age, the focus of this analysis was on within-subject differences in hippocampal

diffusivity. A Pearson correlation test was performed for bivariate data with normal distributions, and a Spearman's rank-order correlation test was performed for bivariate data with non-normal distributions. There were no associations between age and the difference in exercise and rest FA ($r = 0.05$, $p = 0.79$), D_a ($r = .09$, $p = .63$), D_r ($r = -.03$, $p=.86$), or MD ($r = -.01$, $p = 0.98$) values, and thus age was not included as a covariate in the within-subject analysis; see **Supplemental Figure 1**. Finally, all FA and MD values fell within a reasonable range of previously reported hippocampal diffusion measures in older adults [167], [168] and use of a Wilcoxon rank order t-test to compare these values reduces the influence of any potential outliers.

Results

Participants

Demographic, physical, and cognitive data for all participants are provided in **Table 4**. Of the 32 participants who completed the entire study protocol two participants were excluded from the analysis because of failure to obtain a DWI scan at one of the experimental time points.

Table 4: Participant Characteristics (n = 30).

Variables	***Mean (SD)***
Demographics	
Sex	7 M, 23 F
Age (years)	66.4 (±7.5)
≤ College	11 (36.7%)
≥ Graduate	19 (63.3%)
Health	
Height (cm)	166.6 (±8.9)
Weight (kg)	71.3 (±14.1)
BMI (kg/m^2)	25.7 (±4.3)
RHR	65.3 (±6.4)
7-Day Physical Activity Recall Score	
kJ/kg/day	133.2 (±17.2)
Cognition and Depression	
MMSE	29.2 (±1.1)

Beck Depression Inventory II	1.9 (±1.9)

Notes: M = male; F = female; bpm = beats per minute; RHR = Resting Heart Rate; HR_{max} = Maximum Age Predicted Heart Rate; MMSE = Mini Mental State Examination. kg/ml/min = kilogram per milliliter per minute. BMI = Body Mass Index.

Exercise Manipulation, Hippocampal Volume, and Order of Condition

HR and RPE data during the exercise and rest condition, hippocampal volume measured after each condition, and differences in hippocampal diffusion for both condition orders are reported in **Table 5**. As expected, HR was significantly greater during the exercise condition (133.56 ± 19.0) relative to rest (66.48 ± 8.65) [t = 18.134, df = 29, p<.001]. RPE was similarly greater during the exercise condition (13.95 ± 1.12) compared to the rest (6.11 ± 0.27) [t = -32.569, df = 29, p <0.001] and no significant hippocampal volume differences were found between the exercise (5355.3 ± 733.3) and rest conditions (5478.0 ± 808.6) [Z =1.34, p = 0.181]. Finally, there was no significant interaction effect between conditions based on order for hippocampal FA [F(1,28) = 2.37, p = 0.135] and MD measures [F(1,28) = 0.56, p = 0.460].

Table 5: Experimental condition outcomes and manipulation check.

Measure	**Mean (SD)**		
	Rest	**Exercise**	**p**
HR (BPM)	66.5 (±8.65)	133.6 (±19.0)	**<.001**
RPE (Borg 6-20 scale)	6.1 (±0.27)	14.0 (±1.12)	**<.001**
Hippocampal Volume (mm^3)	5478 (± 808.6)	5355 (± 733.3)	.181
	Order 1	**Order 2**	
Difference in Hippocampal FA	-0.006 (± .007)	-0.005 (± .020)	.135
Difference in Hippocampal MD *(10^{-3} mm/s^2)*	0.011 (± .019)	0.008 (± .043)	.460

Notes: HR = heart rate; BPM = beats per minute; RPE = rating of perceived exertion. Hippocampal volume measured in cubic millimeters. Order 1 = exercise and then rest; Order 2 = rest and then exercise. FA = fractional anisotropy. MD = mean diffusivity.

Diffusivity Differences Between Exercise and Rest

Analyses of diffusion values within the bilateral hippocampus showed significantly lower FA (Z = 2.85, p = 0.004) and higher MD (Z = 2.01, p = 0.045) and D_r (Z = 2.55, p = 0.011) values following the exercise condition compared to rest, but no significant difference in values for D_a (Z = .916, p = 0.360) between conditions; see **Figure 7**. The effects for both FA and D_r survived FDR correction while the effect for MD did not. Analyses of diffusion values within the bilateral amygdala and middle temporal cortex showed no significant differences in FA (Z = 1.73, p-value = .084; Z =.548, p=.584) or MD (Z = .040, p-value = .968; Z =.223, p = .824) between conditions, respectively. Additionally, there were no significant differences in whole brain gray matter mask for FA (Z = -1.71, p = .088) or MD (Z = -1.31, p = .191) values.

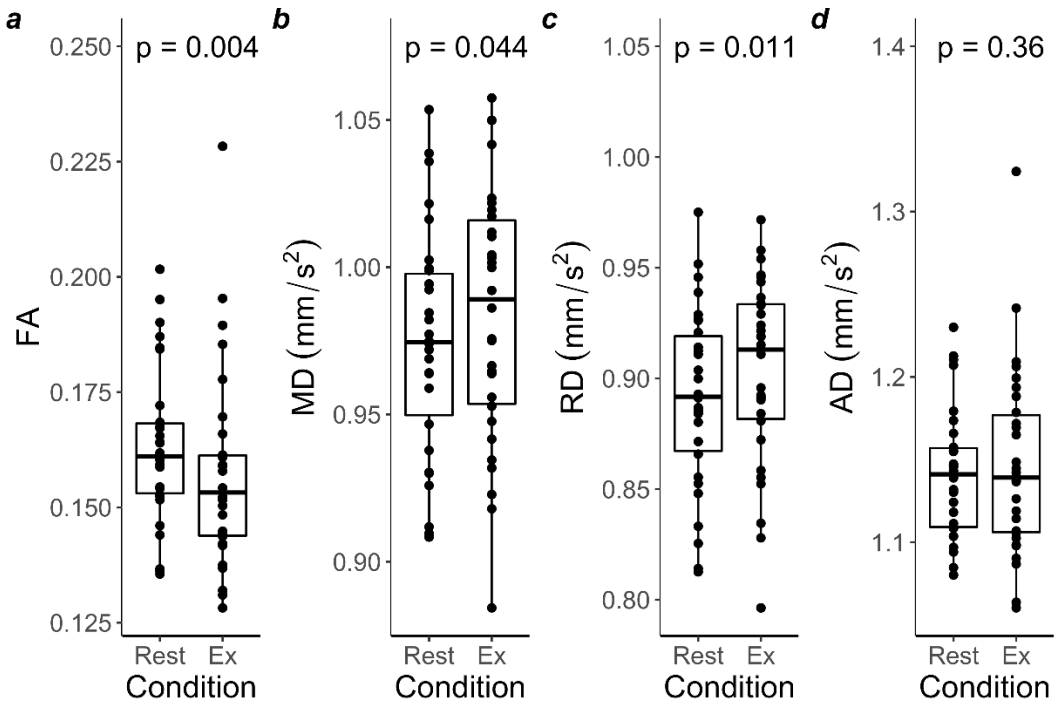

Figure 7: Results of a Wilcoxon signed-rank test comparing bilateral hippocampal diffusivity following 30 minutes of aerobic exercise (Ex) versus after a seated rest condition. a) Fractional anisotropy (FA) was significantly lower following exercise compared to seated rest; b) Mean diffusivity (MD) was

significantly higher following exercise compared to rest. c) Radial diffusivity (D_r) was significantly higher following exercise than after seated rest. d) Axial diffusivity (D_a) was not significantly higher following the exercise condition.

Location and Direction of Diffusion Differences

Given the differences between exercise and rest reported for FA, we performed a post hoc analysis of the anterior and posterior bilateral hippocampus to test if hippocampal diffusivity differences were regionally specific. An initial analysis indicated that there were no differences in laterality for the right and left hippocampus and therefore, these diffusion differences were reported as bilateral effects. FA of the anterior ($Z = 2.33$, $p = .020$) and posterior ($Z = 2.31$, $p = .021$) hippocampus were both significantly lower following exercise compared to seated rest. Meanwhile, anterior D_r ($Z = 2.18$, $p = .029$) was significantly different between conditions and anterior MD ($Z = 1.88$, $p = .061$) was trending toward significance. A comprehensive comparison of diffusivity measures for the full bilateral hippocampus and anterior and posterior hippocampus subregions following exercise and rest are shown in **Table 6**.

Table 6: A comprehensive comparison of diffusivity measures for whole brain gray matter and the full, anterior, and posterior hippocampal subregions between exercise and rest conditions.

Measure	**Region**	**Mean (SD)**		**p**	**(95% CI)**	**Effect Size (*r*)**
		Rest	**Exercise**			
FA	Whole Brain Gray Matter	0.153 (±.014)	0.152 (±.010)	.088	(-.001, .005)	.302
	Bilateral Hippocampus *Subregion*	0.163 (±.017)	0.157 (±.021)	**.004**	**(.002, .008)**	**.422**
	Posterior (Tail/Body)	0.171 (±.017)	0.168 (±.023)	**.021**	**(.001, .008)**	**.248**
	Anterior (Head)	0.154 (±.021)	0.146(±.024)	**.020**	**(.001, .012)**	**.406**
MD (10^{-3} mm/s^2)	Whole Brain Gray Matter	0.985 (±.027)	0.988 (±.026)	.191	(-.002, .007)	.231
	Bilateral Hippocampus *Subregion*	0.974 (±.038)	0.984 (±.044)	**.045**	**(-.017, -.001)**	**.322**
	Posterior (Tail/Body)	0.950 (±.042)	0.957 (±.049)	.177	(-.017, .003)	.205

	Anterior (Head)	1.002 (±.046)	1.014 (±.045)	.061	(-.022, .001)	.352
D_r *(10^{-3} mm/s^2)*	Whole Brain Gray Matter	0.914 (±.029)	0.915 (±.025)	.655	(-.003, .006)	.079
	Full Hippocampus	0.977 (±.054)	0.993 (±.054)	**.011**	**(-.018, -.002)**	**.389**
	Subregion					
	Posterior (Tail/Body)	0.866 (±.042)	0.871 (±.045)	.105	(-.018, .001)	.260
	Anterior (Head)	0.920 (±.050)	0.936 (±.047)	**.029**	**(-.026, -.001)**	**.377**
D_a *(10^{-3} mm/s^2)*	Whole Brain Gray Matter	1.129 (±.028)	1.134 (±.029)	.074	(-.001, .011)	.317
	Full Hippocampus	1.222 (±.049)	1.225 (±.053)	.360	(-.015, .001)	.153
	Subregion					
	Posterior (Tail/Body)	1.120 (±.048)	1.126 (±.066)	.529	(-.016, .007)	.122
	Anterior (Head)	1.165 (±.046)	1.170 (±.053)	.299	(-.016, .006)	.172

Notes: FA = fractional anisotropy, MD = mean diffusivity, D_r = radial diffusivity, Da = axial diffusivity; SD = standard deviation; CI = confidence interval; Posterior = posterior hippocampus, consisting of tail and body subregion; Anterior = anterior hippocampus, consisting of the head subregion section.

Discussion

These DTI results suggest that a single session of moderate-intensity exercise may elicit short-term microstructural alterations in the bilateral hippocampus of healthy and physically active older adults. Specifically, we found that healthy older adults exhibited lower hippocampal FA and higher MD after the exercise session compared to after the seated rest condition. A post hoc analysis of diffusivity within the anterior and posterior hippocampus indicates that differences in FA occurred in both anterior and posterior portions, suggesting the effects of exercise were not specific to only a subregion of the hippocampus. Furthermore, D_r but not D_a of the hippocampus was significantly different between conditions, suggesting the higher hippocampal diffusion following exercise was primarily driven by diffusion occurring perpendicular to the primary tensor value. Lastly, no effects of exercise on diffusivity were observed in the amygdala, middle temporal cortex, or whole brain gray matter,

however, this does not preclude the possibility that these effects are actually diffuse and present in trace amounts in other regions.

Higher hippocampal FA and lower MD is associated with better performance on various neurocognitive measures and is thought to be indicative of higher vascular, synaptic, and neuronal density of the hippocampus [100], [104], [167]. However, we found *lower* FA and *higher MD* following a single session of exercise, primarily driven by *increased* D_r. It is important to note that the current findings are of short-term within-participant differences in response to a physical exercise stimulus. This may not be entirely surprising as acute exercise is a physiological stressor that is believed to elicit numerous immediate neurophysiological effects. In contrast, most of the current literature reporting decreased FA and increased MD with increasing age, and cognitive decline have been cross-sectional or longitudinal in nature, taking place over several months or years.

Similarly, cross-sectional differences in diffusivity are associated with cardiorespiratory fitness [127], and changes in diffusion over a six-month exercise training intervention are associated with changes in cardiorespiratory fitness [73]. Kleemeyer and colleagues argue that these associations may be due to a combination of cellular changes such as angiogenesis, neurogenesis, and synaptogenesis [73]. The underlying differences in hippocampal microstructure over a lifetime or with exercise training are the result of numerous bouts of exercise [74]. However, the timescale of our study and single sessions of exercise are not consistent with some of these proposed mechanistic changes, such as neurogenesis, which occurs over weeks. There is currently no evidence demonstrating changes in neurogenesis, angiogenesis, synaptogenesis, or axonal density within hours of a single session of exercise; however, it is plausible that changes in hippocampal diffusivity may co-occur with cellular processes that promote eventual neurogenic and/or angiogenic effects [74].

Few studies have focused on short-term neuroplastic changes in diffusion within the hippocampus. The results of this line of work have been inconsistent, with both increases and decreases in the microstructure of gray matter associated with improved cognitive performance, glial activity, morphological changes [108], [109]. As in the current study, some effects are in the opposite direction of what one would expect based on long-term aging and disease progression studies — suggesting that differences between clinical groups or over the lifespan arise from different mechanisms than the differences observed in short-term studies [108], [109]. Previous research indicates that gray matter diffusivity changes in response to acute manipulations or learning may be the result of synaptogenesis and both morphological and functional changes in glial, synaptic, and dendritic processes [108], [109]. Specifically, Blumenfeld et al. (2011) found that decreases in gray matter MD and FA were associated with glial cell activity and increases in glial volume after learning a spatial navigation task [108]. Meanwhile, Sagi et al. (2012) found that decreases in hippocampal MD after learning a spatial navigation task was associated with glial activity in rats [109]. Our results indicated that healthy older adults exhibited lower hippocampal FA and higher MD after the exercise session compared to after the rest condition, which contrasts with results of short-term learning studies. This could be the result of the 80 min delay between exercise and the diffusion scan, as previous short-term hippocampal diffusion studies have performed diffusion scans immediately or shortly after completion of the learning task [109]. However, it is also important to note that exercise is an acute physiological stressor, and 30 minutes of moderate-intensity aerobic exercise may elicit differential effects on the microstructure of the hippocampus compared to those experienced when learning a new task at a resting metabolic rate.

Interpretation of changes in gray matter diffusivity is particularly challenging due to the more isotropic nature of the underlying microstructure [94]. Previous studies have reported changes in hippocampal MD and FA; in our study we also found differences in hippocampal FA and MD. FA is a

highly sensitive, but also non-specific biomarker of the neural architecture and is often more susceptible to changes than MD, which may constitute the large effect size of differences in FA that we report [113]. FA reflects the shape of the ellipsoid based on the three tensor eigenvectors and thus, we further analyzed the tensor components to shed light on which aspects of the tensor model were driving differences in FA and MD. Increased D_r and not D_a was observed, suggesting higher diffusivity in the two non-primary eigenvectors following exercise, but no difference in the primary eigenvector. It is important to note however, that within a highly isotropic gray matter structure like the hippocampus the distinction between radial and axial diffusivity is controversial [113]. Therefore, caution should be taken when interpreting physiological changes solely based on the differences seen in radial or axial diffusivity.

Previous short-term learning studies have employed human and animal study designs and have found associations between hippocampal diffusivity changes and glial processes and biomarkers of glial expression [108], [109]. Gliosis is a response by glial cells, such as astrocytes and oligodendrocytes, to damage or inflammation that leads to proliferation, hypertrophy, and swelling of the cell [83]. Meanwhile, exercise is an acute stressor that can increase hippocampal neuroinflammation [88], and while long-term training can reduce age-related glial hypertrophy [84], acute exercise has been shown to elicit higher glial activity and hypertrophy [85]. A primary function of astrocytes is the redistribution of ions and osmotically active molecules to areas where they will not negatively impact the local environment. Since astrocytes are permeable, their activity can result in significant swelling and water movement, and thus changes in astrocyte structure and function can lead to significant changes in diffusivity [83]. Furthermore, astrocytes in the hippocampus have been shown to increase activity and undergo morphological change following both acute and chronic exercise [85], [184]. Our results indicate a change in anisotropy, which might indicate changes in the morphology of glia, synapses, and

dendrites after acute exercise, as these cellular processes tend to be less uniformly oriented than axons. While there is no evidence for significant increases in neurogenesis or myelination at this time scale, changes at the molecular level and may precede the detectable changes in neurogenesis, gliogenesis, and myelination that are reported in exercise training studies [74]. Another possible mechanism may be an increase in hippocampal cerebral blood flow. However, studies to date have found conflicting results within 1 hour of exercise and no study to date has determined whether perfusion differences past this 1 hour time point persist [172]. Additionally, the fast diffusing free-water compartment absorbs a large portion of perfusion effects and thus, helps limit contamination in the diffusion signal from intravoxel incoherent motion of blood due to differences in capillary perfusion [185]. Suggesting these effects are likely independent of differences in hippocampal perfusion.

A recent meta-analysis of randomized controlled exercise trials provides evidence that exercise training preserves hippocampal volume in older adults [71]. However, an important question is whether acute exercise impacts cognition and hippocampal function which may then relate to adaptations to exercise training that impact both neurophysiological and cognitive changes. For instance, it is hypothesized that long-term changes in hippocampal plasticity following exercise training result from the accumulation of physiological adaptations that accompany numerous short sessions of exercise [66], [74]. Acute exercise-related alterations to the hippocampus are supported by animal work showing upregulation of hippocampal BDNF protein levels in rats two hours after an acute bout of exercise [135] and higher functional activation, connectivity, and blood flow [66], [68] in humans following an acute session of exercise. Furthermore, some of these neurophysiological findings were further linked to acute aerobic exercise induced improvements in a highly hippocampal dependent pattern separation task [68]. Unfortunately, there remains little evidence for how an acute aerobic exercise effects the neurophysiology of the aging hippocampus in humans due to, methodological limitations that prevent

the measurements of central levels of neurotrophic factors or cellular processes in the human brain. Thus, imaging techniques such as DWI that are susceptible to the hippocampal microstructure should be explored to further our understanding of the physiological and cellular adaptations that occur in response to exercise.

Our study provides novel findings that a single session of aerobic exercise can elicit microstructural alterations in the hippocampus of healthy older adults; however, the study does suffer from several limitations. Our participants were well educated, predominantly white, healthy, and physically active older adults, and therefore, our findings may not generalize to a more diverse population. Due to the spatial resolution of DWI in humans and lack of direct histological measurements, it is impractical to infer individual biological processes from differences in FA and MD, and these changes are likely due to a host of microstructural alterations that cannot be determined explicitly with current human imaging techniques. Furthermore, diffusivity measures were only taken post-exercise and rest, and therefore it is impossible to imply causality or that these effects are specifically induced by the aerobic exercise bout, although the use of a randomly counterbalanced within subject study design and the lack of any order effect strengthens the argument for exercise induced changes. Additionally, this study did not include a hippocampal-dependent cognitive measure that could be tested or associated with differences in diffusivity between conditions and therefore, we are unable to determine how these differences may related to behavioral changes. We were also not able to measure hydration levels throughout the experiment, however, participants were provided water ad-libitum, and our free-water control method helps control for differences in CSF and intravoxel incoherent motion that would be most likely to be affected by hydration levels. Finally, we measured hippocampal diffusion approximately 80-minutes after each exercise and rest condition, which may raise concerns regarding head motion related to participant fatigue. Nevertheless, there were no significant

differences in head motion parameters between the two conditions and head motion parameters throughout the scan session were well below previously suggested cutoffs [186]. Therefore, it is unlikely the observed effects were due to participant head motion during the DWI scan. Moreover, because these effects were detected more than one hour after the exercise had ended, it is quite possible the differences in diffusivity may be greater if measured more proximally to the end of the exercise session.

In this within-subject study, healthy and physically active older adults exhibited short-term microstructural alterations in the hippocampus after an acute bout of moderate-intensity aerobic exercise compared to seated rest. These findings suggest that for older adults, a single session of moderate-intensity exercise can elicit small but significant alterations in the microstructure of the hippocampus. Over time, with exercise training or a more active lifestyle, these alterations may lead to adaptations that accumulate and elicit long-term changes in hippocampal diffusivity, volume, and hippocampal-dependent cognitive function. Future work should explore the relationship between short-term changes and long-term adaptations in hippocampal diffusivity and should incorporate hippocampal-dependent neurocognitive measures to determine whether short-term microstructural changes in the hippocampus are related to changes in cognition.

Chapter 4: Acute Cycling Exercise on Hippocampal Subfield Function and Microstructure

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Abstract

Aging is associated with deterioration in Dentate Gyrus (DG) and CA3, both crucial hippocampal subfields for age susceptible memory processes such as mnemonic discrimination (MD). Meanwhile, a single aerobic exercise session alters DG/CA3 function and neural activity in both rats and younger adults and can elicit short-term microstructural alterations in the hippocampus of older adults. However, our understanding of the effects of acute exercise on hippocampal subfield integrity via function and microstructure is limited to younger adults and gross hippocampal measures. Thus, a within subject-design was employed to determine if 20-minutes of moderate to vigorous aerobic exercise alters bilateral hippocampal subfield function and microstructure using high-resolution functional magnetic resonance imaging (fMRI) during an MD task (n=35) and high angular resolution multi-shell diffusion imaging (n=31), in healthy older adults, compared to seated rest. Following the exercise condition, participants exhibited poorer MD performance, particularly on low-similarity images. Exercise was also related to lower MD-related activity within the DG/CA3 but not CA1 subfield. Finally, after controlling for whole brain gray matter diffusion, exercise was associated with lower neurite density index (NDI) within the DG/CA3. However, exercise-related differences in DG/CA3 activity and NDI were not associated with differences in MD performance. Our results suggest moderate to vigorous aerobic exercise may temporarily inhibit MD performance, and suppress DG/CA3 MD-related activity and NDI, potentially through neuroinflammatory/glial processes. However, additional studies are needed to confirm whether these short-term changes in behavior and hippocampal subfield neurophysiology are beneficial and how they might relate to long-term adaptations.

Introduction

Across the world, the number of older adults is expanding rapidly as advances in medicine, public health, and education prolong the human lifespan [187], [188]. This extended lifespan is unfortunately accompanied by a growing number of individuals developing dementia and memory impairments [169]. However, growing evidence suggests that modifiable lifestyle factors, such as exercise, may help delay or prevent the onset of cognitive impairment in older adults [132]. In particular, exercise training preserves memory and can preferentially protect age-susceptible and memory-critical structures such as the hippocampus [133]. However, although aerobic exercise training protects various memory networks and reduces age-related cognitive decline and dementia risk, the benefits appear to vary greatly across individuals, and several randomized controlled trials (RCTs) have failed to show positive effects [133], [189], [190]. While RCTs are a gold standard for determining the effects of exercise interventions on the aging memory system, they have high costs concerning time and money, limiting their use [191]. Additionally, inconsistencies and variability in previous RCTs may result from a lack of consistency in cognitive domains tested, a failure to tailor exercise protocols and track physiological adaptions and responses, and an inability to control for other lifestyle factors (sleep, diet, environmental enrichment, and socialization) [133].

Notwithstanding, identification of the short-term behavioral and neurophysiological responses of a single (acute) aerobic exercise session on specific age-susceptible memory processes and structures can provide more nuanced insight into the relationship between exercise and memory. Within-subject acute exercise study designs generally occur over a shorter period of time and allow subjects to act as their own control, providing an opportunity to better attribute behavioral and neurophysiological changes specifically to exercise. Acute exercise adaptations may also predict training-related outcomes [133], [174]. Therefore, characterizing acute exercise effects may help inform more individualized and

optimized exercise interventions. Thus, acute exercise interventions that specifically probe age-susceptible memory constructs and structures are crucial for better addressing how exercise affects the aging memory system.

Growing evidence indicates acute aerobic exercise can alter hippocampal integrity, function, and memory performance [58], [174], [192], [193]. However, these effects may depend on the duration, timing, and intensity of the exercise, the age of the participants, and the specific memory construct tested [58]. For example, a short bout of light to moderate-intensity aerobic exercise can lead to better mnemonic discrimination (MD) on highly similar stimuli in college-aged younger adults [67], [68]. Meanwhile 20 minutes of moderate-intensity exercise can preserve pre to post MD performance in healthy older adults compared to seated rest [193]. MD is a memory construct that engages the Dentate Gyrus (DG) and CA3 subfields of the hippocampus by placing a high demand on pattern separation [11], [20] and represents a process by which individuals accurately discern between previously viewed (old) stimuli and newly presented but visually similar (lure) stimuli. Prior studies investigating acute exercise effects on MD have been predominantly limited to younger adults [67]–[69], but MD performance is of particular interest for understanding aging memory systems. This is because MD and the ability to behaviorally separate similar stimuli often declines earlier and more rapidly during aging compared to other cognitive processes, potentially due to declines in neurogenesis within the DG [13], [19], [44], [142].

Various mechanisms may underlie age-related memory decline, such as increased oxidative stress and neuroinflammation, as well as alterations in plasticity, connectivity, excitability, and neurogenesis within hippocampal subfield circuitry [164], [194]. Research supports that wheel running-induced upregulation of neurogenesis within the DG directly improves MD related performance in mice

and rats [39], [43]. Meanwhile, human studies in older adults suggest improving fitness through exercise training may preserve hippocampal volume and memory performance [71], [143], [195]. Understanding of short-term effects of acute exercise that may compound over time to elicit long-term adaptations in hippocampal subfield-dependent memory and structure in healthy aging remains limited. Acute exercise effects on memory may be due to changes in arousal, hippocampal subfield neural activity and synchrony, neurotransmitter and neurotrophic release, and hippocampal neurogenic inflammatory mediators [68], [75], [80], [81], [173]. It is thus essential to employ sensitive and specific neurophysiological biomarkers to characterize acute exercise-related alterations to the integrity of the aging memory system.

MD-related DG/CA3 functional magnetic resonance imaging (fMRI) hyperactivity is associated with age- and neurodegeneration-related memory impairment in older adults and those with mild cognitive impairment [18], [24]. Indeed, a drug-induced reduction of DG/CA3 hyperactivity has been associated with better MD performance, suggesting that hyperactivity reflects neural distress rather than adaptive compensation [28]. Animal studies have also shown that DG/CA3 hyperactivity may be due to a loss of inhibitory neurons within the CA3 [164]. However, the relationship between MD performance and MD-related DG/CA3 fMRI activity appears to be age-dependent [31]. DG/CA3 hyperactivity represents enhanced network integrity in younger but network dysfunction in older adults.

In addition to MD-related hippocampal subfield activation, indices of hippocampal microstructure may also be valuable biomarkers for understanding acute exercise-related changes in age-susceptible memory circuitry. While diffusion-weighted imaging has predominantly been employed to ascertain the microstructural integrity of white matter tracts [90], improvements in multi-shell diffusion imaging sequences and modeling approaches now allow researchers to better probe gray matter integrity

with more biophysically relevant measures, particularly within the hippocampus [94], [107], [114], [196]. For example, it has been reported that hippocampal microstructure may better predict mild cognitive impairment and dementia than hippocampal volume [100], [103] and is more closely related to age and hippocampal-dependent memory processes [18], [99], [106], [107], [197]. Furthermore, short-term changes in hippocampal diffusion have been linked to learning [108], [111], as well as the expression of hippocampal brain derived neurotrophic factor (BDNF), synaptophysin, and glial related activity and morphological changes [108], [111], [122]. Thus, coupling hippocampal subfield fMRI activity and hippocampal microstructural diffusion in healthy older adults could provide independent and complimentary insight into the impacts of short-term perturbations and alterations to the aging memory system.

Unfortunately, most of the evidence for the effects of acute exercise on memory-related circuits is limited to animal studies, behavioral measures, or samples of younger adults in humans. Animal studies have found that a single session of low to moderate-intensity wheel running increases hippocampal activity and promotes neurogenesis [75], [198], while higher-intensity wheel running may upregulate hippocampal neuroinflammatory mediators that are neurogenic [79]–[81], [87]. Meanwhile, younger adults exhibited higher MD-related DG/CA3 activity following 10 minutes of light-intensity exercise [68]. Furthermore, we recently reported that 20 minutes of moderate to vigorous aerobic exercise elevated whole hippocampal diffusion compared to seated rest in healthy older adults [192]. However, the effects of acute aerobic exercise on highly age-susceptible hippocampal function and microstructure in healthy older adults are currently unknown. Thus, this study aimed to determine how 20 minutes of moderate to vigorous aerobic exercise affects MD performance, MD-related DG/CA3 activity, and DG/CA3 microstructural integrity compared to seated rest, using a within-subject counterbalanced crossover design and high-resolution hippocampal subfield specific imaging

approaches. We hypothesized that following the acute exercise session participants would perform better on the MD task, would exhibit reduced MD-related DG/CA3 activity (consistent better network function in older adults), and have elevated DG/CA3 extracellular diffusion (consistent with lower NDI and higher ODI and indicative of an upregulation of neurotrophic factors and neuroinflammatory mediators).

Methods

Subjects

Forty-one cognitively healthy and physically active older adults (ages, 60-89 years) were recruited from the local community to participate in the study in accordance with the Helsinki Declaration. Participants were excluded if they reported a history of stroke, diabetes, untreated high blood pressure, neurological disease, major psychiatric disorder, contraindications to undergoing an MRI scan (claustrophobia/ferromagnetic metal in body), and any contraindications to exercising on a bike. All participants completed a phone screening, baseline session, rest session, and an exercise session (order of Rest and Exercise sessions were counterbalanced across participants).

Baseline Visit

Prior to the two experimental day visits participants attended a baseline visit in which they first provided written informed consent approved by the Institutional Review Board. Participants then completed the Montreal Cognitive Assessment (MoCA) [199], a 30 point questionnaire used to screen for cognitive impairment (participants had to score ≥ 26 to participate in the study). Next, participants completed a battery of questionnaires to determine health history, as well information about sleep habits (PSQI), physical activity (Stanford 7-day Physical Activity Recall questionnaire; [149]), anxiety symptoms (Geriatric Anxiety Scale; [150]), and depression symptoms (Geriatric Depression Scale;

[151]). Then, participants performed a submaximal stress test to determine baseline cardiorespiratory fitness levels. Finally, participants watched a video with standardized instructions and then performed Set 1 of the continuous MST to allow for familiarization with the task and to minimize practice effects during the following two experimental visits [19].

Submaximal Exercise Stress Test

Participants performed a submaximal stress test on a cycle ergometer (Corival, Lode, Netherlands) and respiratory gases were monitored via open-circuit spirometry (True One 2400 integrated metabolic system). Briefly, a staged ramp protocol [146] was employed where, following a two-minute warm up at 25W, an initial 30W resistance was set and increased by 10W/min until termination criteria was reached. Throughout the test heart rate (Polar H9, Polar) and measures of ventilation including rate of oxygen (O2) consumption, rate of carbon dioxide (CO2) production, and the respiratory exchange ratio (RER; CO2 production/O2 consumption) were collected, while the ratings of perceived exertion (RPE; 6-20 scale administered with instructions consistent with (Borg 1982; Cook et al., 1997)) scale was used to monitor subjective effort every minute. Tests were terminated upon attainment of 85% of participant's age predicted maximal heart rate response (220-Age), participant's request, or observations of exercise contraindications

Mnemonic Similarity Task

Participants completed the continuous version of the Mnemonic Similarity Task (MST) at 3 different time points. Participants completed the continuous version of the MST on the computer at the baseline visit and in the mock scanner and scanner during the two experimental visits. During each task, participants were shown 140 colored images of everyday objects, one at a time, for 2.4 seconds (.3s Interstimulus Interval) and then asked to indicate whether the item was an "old", "similar", or "new"

image. The 140 items consisted of 80 new (foils), 30 similar (lures), and 30 old (repeat) images. Trial types were presented pseudo-randomly and separate images were used for each visit. Each participant completed Set 1 for practice at the baseline visit and an abbreviated version of Set 6 (30 total images) for practice in the mock scanner at the beginning of each experimental day to familiarize participants with the task. Then, each participant completed Set 2 and 3 in the scanner during their first experimental visit and Set 4 and 5 in the scanner during their second experimental visit. A total of 6 Sets were used, with each set being equivalent in terms of the mnemonic similarity of their lures. Specifically, each lure image varied in its degree of similarity and was previously empirically ranked by assessing the false alarm rates (% old response) in a separate population (Lacy et al., 2011). These lures were then divided into 5 lure bins based on false alarm rates and each set was given an equal number of lures for each bin (Stark et al. 2013). Furthermore, as previously conducted by Suwabe et al. 2018, the lag order (how far apart lures or repeated images were displayed after they were initially displayed) was consistent across Sets and all lures and repeats occurred between 10-30 images following initial presentation to limit use of working memory and to keep a consistent difficulty of the task (Suwabe et al., 2018). The MST provides two primary behavioral measures. First, a traditional object recognition memory measure was calculated as rate of "Old" responses minus "Old" responses that were foils (Old | Target – Old | Foil) to account for response bias to the "Old button". Second, the lure discrimination index (LDI) was calculated, a measure of MD calculated as the rate of "Similar" responses to lures minus Similar responses to new objects (Similar | Lure – Similar | Foil) to control for response bias of the choice "Similar". LDI is a quantitative measure that operationally defines MD and is closely linked to DG/CA3 function (Stark et al., 2019; Yassa & Stark, 2011).

Exercise and Rest Conditions

Using a within-subject design, participants performed two experimental conditions (exercise and rest) on separate days (spaced 1 to 7 days apart) in counterbalanced order. Participants completed both experimental sessions at the Maryland Neuroimaging Center within a week of each other and completed the exercise and rest sessions in the room adjacent to the scanner to minimize time between completing conditions and initiating scanning. Due to scheduling restrictions, some of the participants performed the experimental conditions at slightly different times of day for each condition; however each participants two scans were obtained at most two hours apart of the same time of day. Before both conditions, participants were provided standardized instructions for the Borg 6-20 Ratings of Perceived Exertion (RPE) and Self Assessment Manikin (SAM) scale (Borg, 1982; Bradley & Lang, 1994). Additionally, on both days participants would again watch the standardized video of MST directions and would then practice another abbreviated version (30 images) of MST Set 6 using identical button boxes to those used in the scanner to provide familiarity and limit practice effects.

For the exercise condition, participants completed a continuous bout of cycling on a Monark cycle ergometer (Varberg, Sweden) located outside the MRI scanner. They were free to adjust the resistance of the bike while maintaining a cadence between (60-80 rpm) to achieve the target RPE. Participants performed a 5-minute warm-up at a self-selected pace, followed by a 20-minute bout of cycling at a target RPE of 13-15 on the Borg 6-20 RPE scale (corresponding to moderate-vigorous intensity and associated with the verbal anchor of "somewhat hard" to "hard"), and finished with a 5-minute cooldown. Heart Rate, RPE, and a subjective valence (pleasantness) and arousal measure via the SAM scale were collected every five minutes. They received water *ad libitum* during both conditions, and after the exercise condition, they were provided with a towel and clean and dry clothing for the scan. During the rest condition, participants were seated on the same cycle ergometer and asked to sit quietly

for 30 minutes, while HR and RPE were measured every five minutes. Participants did not have access to cell phones, and excessive talking was discouraged during both conditions. Following the cooldown, participants changed, had an opportunity to go to the bathroom, and then immediately entered the scanner room to prepare for scanning. Approximately 10-15 minutes elapsed from the end of each condition to the initiation of the first MRI scan.

MRI Acquisition

Immediately following the rest and exercise condition, participants were prepared for MRI scanning on a Siemens Prisma 3.0 Tesla MR scanner. A 32-channel head coil was used for radiofrequency transmission and reception, and foam padding was positioned within the head coil to minimize head movement. Furthermore, for each individual scan, the MRI operator was trained to specify the imaging prescriptions (brain coverage, slice orientation, etc.) as uniformly as possible across all participants, particularly within subjects, to minimize the variability. High-resolution T1-weighted anatomical images were acquired with the following sequence parameters: Magnetization Prepared Rapid Acquisition of Gradient Echo (MPRAGE), field-of-view (FOV) = 256 mm x 256 mm^2, voxel size = 0.8x0.8x0.8 mm, repetition time (TR) = 2400 ms, echo time (TE) = 2.32 ms, inversion time (TI) = 1060 ms, flip angle = 8°, and parallel acceleration factor=2 were used to achieve a scan duration = 6:36 min. Following the functional and diffusion scans, a high-resolution medial temporal lobe (MTL) T2-weighted fast spin echo scan, and then a whole brain T2-weighted scan with the same FOV and resolution settings were also collected for each session to improve hippocampal subfield segmentation and registrations for later analysis.

Following the anatomical T1 scan, the Mnemonic Similarity Task (MST) event-related data were acquired using the following sequence parameters; A Simultaneous Multi-Slice (SMS) echo planar

imaging sequence with a multi-slice acceleration factor of 4 combined with a parallel imaging factor of 2, FOV = 210 mm x 210 mm^2, voxel size = 1.5x1.5x1.5 mm, TR/TE = 2000/32 ms, Bandwidth = 2298 Hz/Px, sequence duration = 6 min 40 sec per run (2 runs per scan).

Then, high angular resolution diffusion-weighted images were acquired with a twice-refocused spin-echo single-shot sequence using Multi-Band Echo Planar Imaging at an acceleration factor of 4. The protocol included two sets of 64 non-collinear diffusion-weighted acquisitions collected, using phase-coding in the AP and PA directions, respectively. Each set included 2 diffusion weightings (b = 1500, and 3000 s/mm^2) and 4 single T2-weighted b = 0 s/mm^2 acquisition (TR/TE = 3500/102 ms, 1.7x1.7x1.7 voxel size, flip angle = 90°, and a bandwidth of 1698 Hz/Px).

Functional Image Processing

Functional analysis results included in this manuscript come from preprocessing performed using fMRIPrep 21.0.2 (Esteban et al., 2019), which is based on Nipype 1.6.1 (Gorgolewski et al., 2011). In short, functional images were corrected for susceptibility induced distortions using FSL's topup function. Anatomical images were then skull stripped and a subject specific anatomical template was created with freesrufer's mri_robust_template function using each subject's 2 T1-weighted and 2 T2-weighted scans. Next a one step volume based registration was calculated between T1 weighted images, subject template space, and our target template in MNI space (ICBM 152 Nonlinear Asymmetrical template version 2009c 1mm^3 resolution) and was then executed with ANTS (Avants et al., 2008). For all distortion corrected functional images the following preprocessing steps were then performed: skull stripping, head motion parameter estimation with FSL's mcflirt, slice time correction with AFNI's 3dTshift, coregistration to subject T1 with bbregister, and frame wise displacement, global signal, and physiological regressor estimation based on freesurfer segmentation masks. Within each BOLD time

series, volumes before, during, and after a frame wise displacement > 0.5mm were identified and flagged for statistical removal via dummy coding. BOLD time-series were resampled into standard space, generating preprocessed BOLD runs in MNI152NLin2009cAsym space. A reference volume and its skull-stripped version were generated using a custom methodology of fMRIPrep. All resamplings were performed with a single interpolation step by composing all pertinent transformations (i.e., head-motion transform matrices, susceptibility distortion correction when available, and co-registrations to anatomical and output spaces) with ANTS.

Functional Image Analysis

MD-related activity was first assessed within the hippocampal region of interest as described below and similarly to previous papers (Klippenstein et al., 2020; Suwabe et al., 2018). Specifically, analyses were limited to an anatomical mask of the bilateral hippocampus (>40% probability in FSL's Harvard-Oxford subcortical atlas). First level analysis was conducted for each subject and time point with behavioral vectors (i.e., correct or incorrect identification of targets, foils, or lures) using a deconvolution approach based on multiple linear regression (3dDeconvolve). Deconvolution of the hemodynamic response was achieved with tent functions covering stimulus onset to 16 s after onset with 9 estimator functions across the time window. Motion parameters and global signal regressors for CSF and WM were further entered into the model to suppress their effect on task-related parameters. Statistical fit coefficients were estimated from the regression analysis and represented differences in activity between trial types and baseline (response of new for novel foil) at a given time point in a voxel. The sum of the fit coefficient taken over the expected hemodynamic response (3-11 s after trial onset) was used for the model's estimate of the relative response to each trial type.

Next, group-level analyses were performed on all scans to determine voxels within the hippocampus that were sensitive to MD by using a linear mixed effects model (3dLME). Participant's MD-related activity (i.e., activity modulated by our MD-related contrast of interest (correct lure identification vs incorrect "old" response to a lure) irrespective of exercise or rest condition) was thresholded at $P<.05$, with a cluster corrected threshold of 20 voxels to create a mask of MD active voxels within the hippocampus. Note, our goal here is not to determine whether reliable activity, that survives a full correction for multiple comparisons exists for this contrast within the hippocampus as other papers have done as much. Our goal is to first identify task-relevant voxels (or remove task-irrelevant voxels) at the group level and determine whether these are altered by exercise. Thus, we intentionally use a more liberal threshold in this initial pass to reduce voxel selection biases and set our final alpha at the end, when examining the effects of exercise. With these task-selective voxels, identified, we next collapsed any sub-clusters that fell within the same hippocampal subfield ROIs (bilateral DG/CA3, CA1, Subiculum), based on a previously used hippocampal subfield template (Stark et al., 2021). This resulted in a bilateral DG/CA3 ROI (derived from 5 clusters) and a bilateral CA1 ROI (derived from 7 clusters), see **Table 7.** The average BOLD response within these two ROIs was then extracted for all scans for second-level analysis to determine the difference between exercise and rest conditions on MD-related hippocampal subfield activity. This approach and keeping the hippocampal subfield ROIs as bilateral helped improve SNR and reduce the number of multiple comparisons as we had no a priori reason to separate left from right for this task. fMRI processing scripts for this analysis can be found in the following GitHub repository (https://github.com/CallowBrainProject/Acute-Cycling-Hippocampal-Subfields).

Table 7: Task Active Hippocampal Subfield Cluster Coordinates.

Region	Cluster	Voxels	x	y	z

DG/CA3	1	39	-30.8	34.8	-3.1
	2	37	-30.9	20.2	-16.8
	3	30	19.3	18.4	-18.2
	4	27	27.1	20.9	-16.5
	5	22	-30.3	12.5	-23.5
CA1	1	42	*27.3*	7.8	-22.7
	2	28	*18.0*	13.8	-18.9
	3	22	*-30.8*	8.0	-30.0
	4	22	*11.5*	-18.1	-14.0
	5	22	*-16.8*	6.7	-21.5
	6	21	*-25.8*	15.8	-16.3
	7	21	*34.0*	27.1	-10.9

Notes: Table of task active clusters for contrast Similar | Lure – Old | Lure. Task active clusters identified as DG/CA3 or CA1 hippocampal subfield.

Diffusion-Weighted Image Processing

Group level statistical analyses were performed with R (R Core Team, 2018). First, we ran paired t-tests to determine whether there were statistically significant differences in measures of effort between the final 10 minutes of the exercise condition compared to the final 10 minutes of the rest condition. Additionally, to determine the independent effect of exercise on hippocampal subfield function and microstructure we employed a linear mixed effects model with condition (exercise vs rest), order (which condition was performed first), days apart (number of days between two experimental visits), and age as fixed effects and subject as a random effect for all additional behavioral and neuroimaging analysis. Specifically, first we tested whether there was a significant fixed effect of condition on overall MD performance, followed by testing whether an effect of condition persisted within each of the 5 similarity bins (1 most similar – 5 least similar). Next, we identified MD-related voxels within the hippocampal subfields and then tested for the independent effect of condition on DG/CA3 and CA1 activity. We then tested for a main effect of condition on extracted ODI and NDI values from the hippocampus and DG/CA3 and CA1, while again controlling for the order of the conditions, the number of days between

conditions, and the age of participant. Furthermore, to determine specificity to the subfield regions, we then added whole brain gray matter ODI and NDI values as an additional covariate in the diffusion analysis. Finally, partial correlations controlling for age, order and number of days apart were performed to determine relationships between differences in HR, RPE, valence, and arousal with differences in MD performance and neuroimaging measures, as well as to determine if there were significant relationships between MD performance and neuroimaging measures. Statistical significance was set based on a two-tailed alpha <.05 and Bonferroni family wise error rate (FWER) correction within each behavioral and neuroimaging analysis.

Hippocampal and Subfield ROI analysis

Automated hippocampal subregion segmentation was performed for each participant's T1 and high resolution hippocampal T2 scan using the Automatic Segmentation of Hippocampal Subfields (ASHS) software [204]. Using ASHS, hippocampal Subfield ROIs for the Dentate Gyrus and CA3 (DG/CA3), CA1, and Subiculum were created for each subject in native T1 space. To further threshold and make sure that diffusion values were extracted from hippocampal gray matter, a whole brain gray matter mask was created using FSL's FAST tissue segmentation algorithm and hippocampal and hippocampal subfields ROI were further thresholded based on only keeping voxels that were present within the gray matter mask [205]. These subfield ROIs and gray matter mask segmentations were then warped into subject template space using previously calculated nonlinear warps with ANTS. At which point, both ODI and NDI values were extracted from the whole brain gray matter mask, bilateral DG/CA3, CA1, and whole hippocampal ROIs.

Statistical Analyses

Group level statistical analyses were performed with R (R Core Team, 2018). First, we ran paired t-tests to determine whether there were statistically significant differences in measures of effort between the final 10 minutes of the exercise condition compared to the final 10 minutes of the rest condition. Additionally, to determine the independent effect of exercise on hippocampal subfield function and microstructure we employed a linear mixed effects model with condition (exercise vs rest), order (which condition was performed first), days apart (number of days between two experimental visits), and age as fixed effects and subject as a random effect for all additional behavioral and neuroimaging analysis. Specifically, first we tested whether there was a significant fixed effect of condition on overall MD performance, followed by testing whether an effect of condition persisted within each of the 5 similarity bins (1 most similar – 5 least similar). Next, we identified MD-related voxels within the hippocampal subfields and then tested for the independent effect of condition on DG/CA3 and CA1 activity. We then tested for a main effect of condition on extracted ODI and NDI values from the hippocampus and DG/CA3 and CA1, while again controlling for the order of the conditions, the number of days between conditions, and the age of participant. Furthermore, to determine specificity to the subfield regions, we then added whole brain gray matter ODI and NDI values as an additional covariate in the diffusion analysis. Finally, partial correlations controlling for age, order and number of days apart were performed to determine relationships between differences in HR, RPE, valence, and arousal with differences in MD performance and neuroimaging measures, as well as to determine if there were significant relationships between MD performance and neuroimaging measures. Statistical significance was set based on a two-tailed alpha <.05 and Bonferroni family wise error rate (FWER) correction within each behavioral and neuroimaging analysis.

Results

Participants

Of the 41 participants who completed all study protocols, one participant (female) was excluded from the behavioral analysis due to exceptionally poor performance (below 0.5 for object recognition) and one more (female) was excluded due to failure to use the "similar" response button at least ten times. This criteria has been similarly employed to remove participants who failed to follow task instructions (Callow, Pena, et al., 2022). An additional three subjects (females) were excluded due to MRI goggles that fogged and prevented completion of the fMRI task at one of the two time points. An additional one subject (male) was removed from the fMRI and diffusion analysis due to claustrophobia and failure to complete the scanning sessions. Furthermore, 9 subjects' diffusion scans were excluded from the analysis due to protocol sequence errors. The final samples were 36 participants for the behavioral analysis, 35 for fMRI analysis, and 31 for the diffusion analysis. Participants were cognitively healthy (MoCA ≥ 26), had an average age of 67.1 years and were predominantly female (30 Female, 6 Male) (see **Table 8**).

Table 8: Participant Demographic Information (n = 36).

		Total sample (n=36)
		Mean (SD)
Demographics		
	Age (years)	67.1 (4.3)
	Sex	30 Female, 6 Male
	Education (n,(%), ≥ Graduate School)	24 (67%)
Health		
	BMI (kg/m^2)	25.7 (4.3)
	$HR_{resting}$ (bpm)	68.7 (10.9)
Cardiorespiratory Fitness and Leisure-Time Physical Activity		

	VO_{2peak} (kg/ml/min)	22.7 (7.3) [a]
	7-day Physical Activity Energy Expenditure (MET/week)	94.7 (39.4) [b]
Cognitive Status, Depression, and Anxiety		
	MoCA	28.0 (1.3) [c]
	Geriatric Depression Score	2.8 (2.7) [d]
	Geriatric Anxiety Score	6.8 (7.5) [e]

Notes: bpm = beats per minute; RHR = Resting Heart Rate; HR_{max} = Maximum Age predicted heart rate; MoCA = Montreal Cognitive Assessment. kg/ml/min = kilogram per milliliter per minute. MET = ratio of working metabolic rate relative to energy at rest. 7-day Energy Expenditure = the total MET- hours completed in the last 7 day period. MET is a unit of energy expenditure relative to the resting metabolic rate with 1 MET = 1kcal/kg/hour. VO_{2peak} = Peak oxygen consumption estimated from submaximal exercise stress test. [a] American College of Sports Medicine 50th percentile for peak oxygen consumption of older adults aged 60+ is approximately 30 (male) & 27 (female). [b] American Heart Association physical activity guidelines suggest at least 10 MET/week for significant health benefits. [c] MMSE scores below 27 indicate potential mild cognitive impairment. [d] Geriatric Depression Scores between 9-15 indicate moderate to severe depression symptoms. [e] Geriatric Anxiety Scores between 16-63 indicate moderate to severe anxiety symptoms.

Experimental Check

As expected, HR ($t(35)=15.9$, $p<.001$), RPE ($t(35)=35.9$, $p<.001$), and arousal ($t(35)=3.86$, $p<.001$) were significantly higher during the exercise condition compared to the seated rest condition. Valence (pleasantness) was not found to be significantly different between the two conditions ($t(35) = 1.3$, $p = .195$) (see **Table 9**).

Table 9: Experimental condition outcomes and manipulation check.

Measure	**Mean (SD)**		
	Rest	**Exercise**	**p**
HR (BPM)	68.7 (15.2)	125.6 (36.4)	**<.001**
RPE (Borg 6-20 scale)	6.2 (0.5)	13.1 (1.6)	**<.001**
Valence	7.1 (2.5)	6.7 (2.4)	0.195
Arousal	4.6 (2.4)	5.8 (2.5)	**<.001**

Notes: SD = Standard Deviation. p = p-value from paired t-tests performed between Exercise and Rest conditions. Measures of HR = heart rate; BPM = beats per minute; RPE = rating of perceived exertion. Valence = subjective measure of valence; Arousal = subjective measure of arousal; All measures were averaged and compared over the final 10 minutes of the moderate to vigorous intensity exercise session (minutes 15-25 of the

experimental conditions). Average participant heart rate in the final 10 minutes of the exercise condition was approximately 82% (SD 17%) of age predicted maximal heart rate. This is consistent with a moderate to hard intensity rating based on ACSM guidelines (American College of Sports Medicine, 2013).

Behavioral Performance

While controlling for order of condition, age, and days between the two visits, participants had significantly worse LDI scores after exercise compared to after seated rest ($F(1,35) = 4.73$, $\eta^2=.12$, $p = .036$). More specifically, following the exercise condition participants responded old when the object was a lure (Old | Lure) at a significantly higher rate than following the rest condition ($F (1, 35) = 4.39$, $\eta^2=.09$, $= .043$). Additionally, only Lure Bin 5 (least similar bin of lures) LDI scores were significantly lower following the exercise condition ($F(1,35) = 10.17$, $\eta^2=.26$, $p = .003$). There was no significant difference between conditions for traditional object recognition performance ($F(1,35) = 3.53$, $\eta^2=.09$, $p = .068$), see **Figure 8.** Controlling for age, order, and number of days apart, differences in valence ($r(33) = .02$, $p=.892$), arousal ($r(33) = .11$, $p=.559$), and HR ($r(33) = -.05$, $p=.754$) were not associated with differences in LDI. However, differences in RPE between conditions were negatively associated with differences in LDI ($r(33) = -.40$, $p = .022$; uncorrected). Furthermore, a post hoc analysis indicated that greater differences in RPE were related to a higher likelihood of a false alarm when viewing a lure after exercise compared to after rest ($r = .52$, $p = .002$).

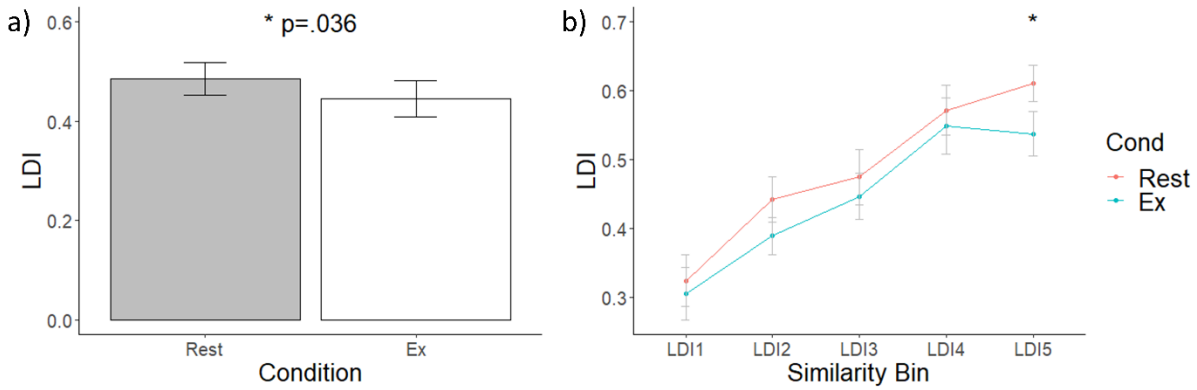

Figure 8: Acute exercise effects on Lure Discrimination performance. LDI = Lure Discrimination Index. a) Comparison of post Rest vs post Ex (Exercise) LDI performance while controlling for order of conditions, age, and number of days apart. b) Comparison of post Rest vs post Ex LDI performance across the similarity bins (1 = most similar; 5 = least similar). * indicates significant at p-value < .05.

MD-related fMRI Activity

While controlling for order of the conditions, age, and days between the visits, MD related DG/CA3 activation was significantly lower in the exercise condition compared to the rest condition ($F(1,33) = 9.02$, $\eta^2=.21$, $p = .005$). There was no significant difference in MD related CA1 activity between the exercise and rest conditions ($F(1,33) = 0.06$, $\eta^2<.01$, $p = .806$), see **Figure 9**.

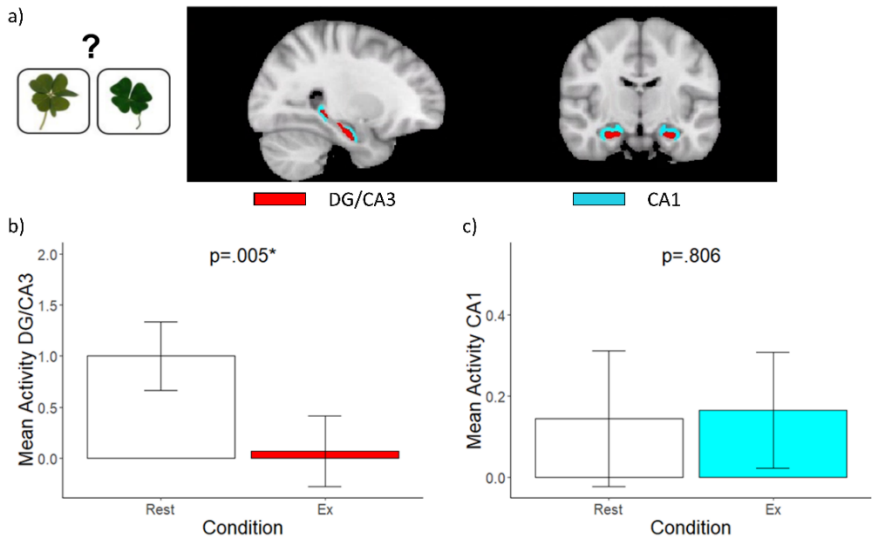

Figure 9: a) Hippocampal Subfield segmentation of the DG/CA3 (red) and CA1 (blue) and an example of a lure pair image condition under which mean activity was extracted. b) Significant difference of mnemonic discrimination related DG/CA3 activity following exercise (Ex) and rest (Rest) while controlling for order of conditions, age, and number of days apart. c) Nonsignificant difference of mnemonic discrimination related CA1 activity following exercise and rest. p = p-value. * $p < .05$.

Subfield NODDI Analysis

Controlling for condition order, age, and days between visits, whole hippocampal ODI was significantly higher ($F(1,31) = 6.44$, $p = .016$), while NDI was lower following exercise compared to rest ($F(1,31) = 8.93$, $p = .005$). Additionally, DG/CA3 ODI was significantly higher ($F(1,31) = 6.67$, $p = .015$), and NDI was significantly lower following exercise compared to rest ($F(1,31) = 8.52$, $p = .006$). Finally, CA1 ODI ($F(1,31) = 3.60$, $p = .067$) and NDI ($F(1,31) = 4.59$, $p = .040$) and Subiculum ODI ($F(1,31) = 4.48$, $p = .041$) and NDI ($F(1,31) = 4.56$, $p = .042$) were not significantly different between exercise and rest after accounting for multiple comparisons.

However, we also found that exercise was related to higher global gray matter ODI ($F(1,31) = 38.70$, $\eta^2 = .56$, $p < .001$) but was not associated with significant differences in global gray matter NDI ($F(1,31) = 2.19$, $\eta^2 = .07$, $p = .149$). Thus, to determine whether exercise related differences in hippocampal NDI and ODI were specific to the DG/CA3 or were driven by global differences in these

metrics, we re-ran the analysis while controlling for whole brain NDI and ODI values. Following the addition of global diffusion to the model, we found that DG/CA3 ODI was no longer significantly different between the two conditions ($F(1, 31) = 0.55$, $\eta^2=.01$, $p = .464$), but exercise-related suppression of DG/CA3 NDI remained robust ($F(1,31) = 7.10$, $\eta^2=.18$, $p = .012$). Meanwhile, neither CA1 ODI ($F(1,31) = 5.32$, $\eta^2=.11$, $p = .030$) and NDI ($F(1,31) = 2.62$, $\eta^2=.08$, $p = .116$), or Subiculum ODI ($F(1,31) = 0.15$, $\eta^2<.01$, $p = .700$) and NDI ($F(1,31) = 2.63$, $\eta^2=.08$, $p = .115$) differences between conditions were significant following correction for global diffusion and multiple comparisons. This suggests that the effects of acute exercise on DG/CA3 ODI may be attributed to a more global effect of exercise on gray matter ODI, while the exercise-related effects on NDI may be more specific to the DG/CA3, see **Table 10**.

Table 10: Linear mixed effects analysis looking at the effect of acute aerobic exercise on ODI and NDI measures for whole brain gray matter, whole hippocampus and DG/CA3 and CA1 hippocampal subregions.

Region	Diffusion Measure	Exercise Effect (F-value)	p-value	Effect Controlling for Whole Brain GM Diffusion (F-value)	Effect Size (η^2)	Corrected p-value
Whole Brain GM	ODI	38.70			**+ .56**	<.001*
	NDI	2.19			- .07	.149
Whole Hippocampus	ODI	6.44	**.016***	2.09	+ .05	.156
	NDI	8.93	**.005***	6.28	**- .17**	**.018***
DG/CA3	ODI	6.67	**.015***	0.55	+ .01	.464
	NDI	8.52	**.006***	7.10	**- .18**	**.012***
CA1	ODI	3.60	.067	5.07	+ .11	*.030*
	NDI	4.59	*.040*	2.62	- .08	.116
SUB	ODI	4.48	*.041*	0.15	+ <.01	.700
	NDI	4.56	*.042*	2.63	- .08	.115

Notes: For effect sizes + = higher following exercise. – = lower following exercise. GM= Gray Matter. ODI= Orientation Dispersion Index, NDI=Neurite Density Index. Bold and ***** indicate significant effect of exercise in linear mixed effect model, while controlling for age, order of condition (exercise or rest first), and number of days apart between two visits based on a FWER corrected p-value <.05. Corrected F and p-values

indicate the effect of exercise when additionally controlling for whole brain gray matter diffusion values in the model. Italicized p-values indicate not significant after multiple comparison correction.

Exercise Related Behavioral and Hippocampal Subfield Neuroimaging Associations

Differences in both DG/CA3 ($r(33) = -.19$, $p = .354$) and CA1 ($r(33) = -.24$, $p = .232$) MD-related activation were not associated with differences in LDI performance. Additionally, differences in DG/CA3 ODI ($r(24) = -.09$, $p = .653$) and NDI ($r(24) = -.17$, $p = .418$), CA1 ODI ($r(24) = -.12$, $p = .57$) and NDI ($r(24) = -.05$, $p = .801$), and Subiculum ODI ($r(24) = -.17$, $p = .409$) and NDI ($r(24) = -.36$, $p = .071$), were not associated with differences in LDI performance. Finally, differences in valence, HR, RPE, and arousal were not related to differences in DG/CA3 MD related activity, or with differences in DG/CA3 ODI or NDI measures (all $p > .05$).

Discussion

Our results are the first to show that an acute 20-minute bout of moderate to vigorous intensity aerobic exercise can alter DG/CA3 specific function, activity, and microstructural diffusion in healthy older adults. More specifically, we found that moderate to vigorous acute exercise led to lower MD performance, particularly in low-similarity images. Additionally, MD-related DG/CA3 activation, but not CA1 activation, was lower following exercise than seated rest. Finally, exercise was associated with greater extracellular diffusion within the DG/CA3, but not CA1 or Subiculum. These findings suggest that an acute bout of moderate to vigorous intensity aerobic exercise can lead to short-term alterations in DG/CA3 function and microstructure in healthy older adults, indicating that higher-intensity acute exercise may elicit neurophysiological perturbations to age-susceptible hippocampal networks.

We report that moderate to vigorous aerobic exercise may suppress MD performance in healthy older adults. Furthermore, exercise-related decrements in MD performance were most likely from a higher response rate of false alarms to lures (responding 'old' when presented with a dissimilar lure image) and greater decrements were related to a higher subjective perception of effort during the exercise versus rest condition. Meanwhile, several smaller studies in younger adults show that acute exercise improves MD performance. For example, Suwabe et al. (2017 & 2018) report that 10 minutes of light and moderate-intensity aerobic exercise in college-aged adults was associated with better MD on highly similar lures. Additionally, we have recently reported that moderate-intensity exercise in active older adults was associated with reduced MD interference seen from pre to post-seated rest; however, MD did not specifically improve from pre to post-exercise (Callow, Pena, et al., 2022). While our current finding of reduced MD performance was not in the direction we hypothesized or reported in Suwabe et al. (2017 & 2018), there are several reasons why a longer bout of acute moderate to vigorous intensity exercise in healthy older adults may have led to lower MD performance.

Notably, the effect of acute exercise on hippocampal memory depends on the timing, length, and intensity of the exercise bout and the cognitive task tested (Chang et al., 2012; Loprinzi et al., 2019, 2021; Marchant et al., 2020; Sng et al., 2018). Although moderate to vigorous intensity acute aerobic exercise has generally been shown to provide benefits for cognition and executive function in older adults (Chang et al., 2012; Moreau & Chou, 2019), the effects of acute exercise for hippocampal-specific MD and general memory function are far less established (Callow, Pena, et al., 2022; Etnier et al., 2021; Griebler et al., 2022). In fact, several studies suggest that higher-intensity exercise may be stressful, elevating cortisol and lactate levels and specifically interfering with hippocampal-dependent memory (Basso et al., 2015; Marchant et al., 2020; Soya et al., 2007). Consistent with this argument, our results suggest that individuals whose effort was perceived as a greater proportion of their maximal

capacity saw a greater decline in MD following exercise compared to rest (although this relation did not survive correction for multiple comparisons).

There are few reports of the effects of acute exercise on episodic memory in older adults. Etnier et al. (2021) found acute exercise improved Rey Auditory Verbal Learning task (RAVLT) performance compared to a rest control condition in middle-aged and older adults. Importantly, episodic memory was tested immediately following a 20-minute bout of moderate-intensity exercise, while our participants completed a memory task approximately 20-25 minutes after exercise termination. Additionally, while the RPE of the participants was similar to those we report, the percentage of age-predicted maximal heart rate (75% vs 82%) was lower, suggesting our participants' effort may have been slightly more vigorous. Finally, our participants performed the MST, which measures MD of objects, and is a heavily hippocampal subfield-dependent task. Meanwhile, the RAVLT is an auditory verbal learning task that also involves executive control processes and does not heavily tax MD and may measure the function of different hippocampal memory systems and circuitry.

Importantly, in the current study, participants performed a time-restricted continuous version of the MST in the MRI scanner, exercised at a slightly higher percentage of age-predicted maximum heart rate, and only completed the task post-intervention. While the continuous version of the MST shows good consistency with the study test version (Stark et al., 2015), the time-restricted and forced choice nature of the continuous MST performed in the scanner may relate to poorer performance, particularly for older adults (Stark et al., 2015). Specifically, we found that acute aerobic exercise shifted performance from greater pattern separation-related behavior to greater pattern completion. This shift in MD to pattern completion is generally seen with aging (Stark et al., 2015) and is associated with compensation or dysfunction within the hippocampal circuitry (Leal & Yassa, 2015; Yassa et al., 2011).

However, acute exercise could also facilitate this compensatory mechanism in older adults when performing such a time-restricted task by promoting more efficient encoding and recall of the "gist" of viewed objects, as opposed to the greater detail needed to accurately distinguish between old and new yet similar objects. Notably, the acute exercise-related MD suppression we observed does not indicate that higher-intensity exercise will lead to adverse long-term outcomes for hippocampal memory function. As a recent study found that 12 weeks of higher-intensity exercise training in older adults led to greater benefits for MD performance than lighter-intensity exercise and a control condition (Kovacevic et al., 2019). This suggests that short-term perturbations to MD following a single moderate to vigorous aerobic exercise session may stimulate network adaptations that improve MD and do not indicate longer-term network dysfunction. However, additional studies that pair acute exercise and exercise training interventions are needed to determine how short-term perturbations to MD may relate to longer-term behavioral trajectories (Voss, Weng, et al., 2019).

Our findings suggest that 20 minutes of moderate to vigorous exercise in healthy older adults is associated with lower MD-related activity within the DG/CA3, but not CA1. Meanwhile, Suwabe et al. (2018), report that 10 minutes of very light-intensity aerobic exercise in 16 college-aged adults was associated with elevated MD-related hippocampal subfield activity, including in the DG/CA3 (Suwabe et al., 2018). However, similar to our analysis, they did not find that differences in hippocampal subfield activity were associated with differences in behavioral MD performance. This suggests that exercise-related DG/CA3 activity differences may not be sensitive or specific to short-term differences in MD performance. Instead, Suwabe and colleagues argue that light-intensity exercise modulated MD-related activity via increased arousal and cholinergic neurotransmission, which may help facilitate hippocampal networks in proper memory storage and recall (Hasselmo et al., 1995). However, while we did find exercise was associated with a higher level of subjective arousal via the SAM, differences in arousal

were not associated with differences in behavioral performance or MD-related DG/CA3 activation, indicating these exercise-related differences are likely due to different underlying factors.

Lower-intensity acute exercise has been shown to elicit higher DG/CA3 activity in rats compared to more stressful moderate and vigorous intensity exercise (Soya et al., 2007) and Suwabe et al. (2018), showed that acute low-intensity exercise elevated MD-related hippocampal activity in younger adults (Suwabe et al., 2018). However, we report moderate to vigorous exercise suppressed MD-related DG/CA3 activity in healthy older adults. Interestingly, in younger adults, MD-related DG/CA3 hyperactivity is associated with better hippocampal function and integrity (Riphagen et al., 2020), while in older adults, DG/CA3 hyperactivity is associated with poor hippocampal function and integrity (Bakker et al., 2012; Riphagen et al., 2020; Yassa, Stark, et al., 2010). In fact, hippocampal hyperactivity is observed in conditions of elevated risk for Alzheimer's disease (Putcha et al., 2011; Yassa & Stark, 2011), and a previous clinical trial has shown that lowering MD-related DG/CA3 activity with levetiracetam in older patients with mild cognitive impairment can improve cognitive performance compared to those receiving a placebo (Bakker et al., 2012). This MD-related DG/CA3 hyperactivity is also associated with poorer hippocampal function in non-demented healthy older adults (Yassa, Lacy, et al., 2010) and is linked to a loss of inhibitory neurons and an inability of these CA3 neurons to encode new information in aging animals (Leal & Yassa, 2015). Therefore, given that DG/CA3 hyperactivity indicates hippocampal network distress, our finding of acute exercise-related reduction of MD-related DG/CA3 activity in healthy older adults suggests short-term therapeutic and beneficial effects for hippocampal subfield network function. Unfortunately, no previous research has determined exercise training-related effects on MD-related DG/CA3 activity in healthy older adults. Thus, additional studies will be needed to confirm and determine the long-term nature of these acute exercise-related changes in DG/CA3 activity.

In addition to acute exercise-related functional and behavioral changes, our study provides new evidence for acute exercise-related changes to hippocampal subfield microstructure using an advanced high angular resolution diffusion imaging technique. Specifically, we found moderate to vigorous aerobic exercise was related to higher ODI (greater neurite dispersion) and lower NDI (higher extracellular diffusion) in the hippocampus. Importantly, we found a global effect of acute exercise on whole brain gray matter ODI, but not NDI. And after controlling for global diffusion values, only the effect of acute exercise on hippocampal NDI remained significant. Furthermore, the effect of acute exercise on hippocampal NDI was specific to the DG/CA3, but not the CA1 or Subiculum. This suggests acute exercise may lead to elevated extracellular water diffusion, particularly within the DG/CA3. Indeed, we have previously reported a similar effect of acute exercise leading to high diffusion and dispersion within the whole hippocampus in healthy older adults (Callow, Purcell, et al., 2022). However, the current study uses high-resolution multi-shell diffusion scans that allow for the use of the NODDI model, which better accounts for partial volume effects and is critical for accurately ascertaining microstructural differences in the aging hippocampal structure (Henf et al., 2018). Our analysis approach also allowed us to control for whole brain global gray matter diffusion metrics and better attribute these microstructural effects of acute exercise to the DG/CA3 structure. The current results largely replicate those we have previously reported and further support the interpretation that acute exercise leads to higher diffusion of water within the hippocampal gray matter. Our use of high-resolution multi-shell diffusion scans and the NODDI model provide additional confidence that this increased extracellular diffusion is not associated with cerebrospinal fluid or free water and is independent of more global differences in gray matter diffusion. Furthermore, the higher resolution of our diffusion scan (1.7mm isotropic) and our hippocampal-specific structural scans allowed us to further delineate these microstructural effects to the DG/CA3 subfield specifically. This is particularly

noteworthy given that previous animal research indicates that exercise-related benefits for the hippocampus may be specific to the DG/CA3 structure due to its importance for neurogenesis (Bekinschtein et al., 2011; Creer et al., 2010; Pereira et al., 2007; Voss, Soto, et al., 2019).

We have previously shown that cardiorespiratory fitness and exercise training are related to mean diffusion and NDI in the hippocampus of healthy younger adults and cortical regions in individuals diagnosed with MCI, respectively (Callow, Purcell, et al., 2022; Callow, Won, Pena, et al., 2021). Furthermore, Kleemeyer et al. (2016) found that exercise training-related fitness changes were associated with decreased hippocampal mean diffusion via increased neural density (Kleemeyer et al., 2016). Meanwhile, lower DG/CA3 NDI mediates age-related decrements in episodic memory performance (Radhakrishnan et al., 2020). DG/CA3 extracellular diffusion can also distinguish between healthy individuals and those with MCI and AD and is associated with neurofilament light chain, a plasma biomarker for neuroaxonal damage (Shahid et al., 2022). Greater extracellular diffusion and dispersion are also associated with elevated glial cell count, glial activity, and neuroinflammation within the hippocampus (Radhakrishnan et al., 2022; Sone et al., 2020; Yi et al., 2019). While this novel diffusion imaging approach allows for greater spatial resolution and provides more biophysically relevant information about hippocampal subfield microstructure than our previous study (Callow, Won, Alfini, et al., 2021), it is still not possible in humans to determine the effects of acute exercise on neurophysiological changes at the cellular level. However, given the nature of the intervention and the time scale of these effects, they are unlikely to result from previously reported neurogenesis-mediated benefits of exercise (Creer et al., 2010; Sahay et al., 2011).

Rather, our finding of acute exercise-related elevation of extracellular diffusion in the DG/CA3 is more likely to reflect several underlying neurophysiological changes that may prove to be neurogenic

through repeated bouts. For example, several studies have found that short-term learning, on a similar time scale as a single session of acute exercise, is associated with diffusion changes in the hippocampus that relate to glial activity, remodeling, and promotion of neurotrophic and inflammatory factors (Blumenfeld-Katzir et al., 2011; Sagi, Tavor, & Assaf, 2012; Tavor et al., 2020). Meanwhile, higher-intensity wheel running is also associated with acutely increased hippocampal neuroinflammatory and neurogenic mediators (Basso & Suzuki, 2016; Ferris et al., 2007; Nogueira et al., 2019; Packer & Hoffman-Goetz, 2015; Pervaiz & Hoffman-Goetz, 2012). While chronic levels of neuroinflammation are generally maladaptive and associated with reductions in hippocampal integrity and neurogenesis, the short-term elevation of hippocampal neuroinflammatory mediators promotes neurotrophic factor expression (Belarbi & Rosi, 2013; Fan & Pang, 2017; Pervaiz & Hoffman-Goetz, 2012; Whitney et al., 2009). Therefore, acute exercise-related DG/CA3 elevation of extracellular diffusion could indicate increased glial activity associated with the expression of neuroinflammatory mediators and neurotrophic factors which may in turn upregulate neurogenesis and promote long-term adaptations that benefit older adults' brain health (El-Sayes et al., 2019). However, additional studies that link short-term diffusion changes to long-term exercise training adaptations are still needed to determine if short-term differences in NDI are beneficial and can promote a healthy aging memory system.

This study provides novel evidence of acute exercise-related functional and structural neuroplasticity in the hippocampal subfields of healthy older adults. However, when interpreting these results, it is important to note several limitations. Our convenience sample consisted of predominantly female, well-educated Caucasian participants, limiting generalizability to the broader population. Neuroimaging and behavioral measures were only collected post-exercise and rest, limiting our ability to infer changes in performance between the two conditions. However, participants confirmed similar pre-test day routines, the order of conditions was counterbalanced across participants, and condition order

was controlled for in all analyses, providing greater confidence in attributing differences to the acute exercise intervention. Additionally, given safety limitations in performing maximal effort stress tests in older adults, it was impossible to tailor acute exercise intensity based on individual maximal heart rates or percentage of maximal oxygen uptake. Instead, we asked participants to exercise based on subjective ratings of perceived exertion, which has been shown to control the relative exercise intensity among individuals who may vary in their absolute maximal capacity to perform cycle ergometer work (Dishman, 1994; Dunbar et al., 1994). Finally, while our novel imaging techniques provide new insight into differences in hippocampal subfield function and microstructure, they are not specific to any underlying neurophysiological mechanism. Additionally, neither MRI measure was associated with differences in MD performance between the exercise and rest conditions. Thus, it is impossible from this study to link these structural and functional network effects to behavioral changes.

We provide new evidence that 20 minutes of moderate to vigorous intensity acute cycling exercise, compared to a seated rest control condition, is associated with differences in DG/CA3 function and microstructure in healthy older adults. Specifically, we found acute aerobic exercise reduced MD performance, MD-related DG/CA3 fMRI activation, and DG/CA3 NDI, but the differences in these measures were not associated. These findings suggest that a single session of higher-intensity aerobic exercise may rapidly alter the DG/CA3 hippocampal subfield through several different mechanisms and pathways. Future studies are needed to determine whether different durations, intensities, and/or types of acute exercise might modify the effects of acute exercise on the aging memory system and link these shorter-term alterations in the hippocampal subfields to longer-term training-induced adaptations.

Chapter 5: General Discussion

Memory shapes who we are by influencing how we interact with the world and helping us predict what is coming. This makes neurodegenerative diseases such as Alzheimer's all the more devastating, as it can rob individuals of their personalities and the ability to interact and function as a part of society. Unfortunately, due to a growing aging population and barring any considerable advances in treatment, the number of Americans living with Alzheimer's related dementia is expected to double in the next few decades. Meanwhile, Alzheimer's is the 5th leading cause of death for Americans aged 65 or older and has the highest healthcare cost of any disease within the last five years of life [3]. Sadly, these costs don't account for the enormous financial, physical, and emotional toll loved ones and family experience when providing approximately 16 billion hours of unpaid care each year [219]. Nevertheless, despite extraordinary efforts and funding for Alzheimer's research over the last decade, there remains no cure or effective treatment, which has led to a significant push in the field to identify ways to prevent or delay the onset of dementia. A recent report concluded that modifiable lifestyle factors may account for approximately 40% of all dementia cases [132]. Thus, proper lifestyle interventions and preventative measures could drastically improve the lives of millions of individuals and families. However, many questions remain concerning how and which lifestyle interventions may most effectively prevent or delay dementia and memory impairment in an aging population.

In particular, obtaining adequate levels of physical activity and exercise shows great promise for promoting healthy cognitive aging and dementia prevention [132]. However, large exercise training randomized controlled trials (RCTs) have produced mixed results [133], [189], [190]. Indeed, while exercise training randomized controlled trials (RCTs) are the gold standard for determining the cumulative effects of exercise for brain health, they are costly, take a long time to conduct, and due to the difficulty of controlling for confounding factors, often make it challenging to attribute

neurophysiological changes specifically to exercise [189]. Thus, a burgeoning field of research has attempted to identify the effects of a single bout of exercise on the brain [65], [206]. The use of within-subject designs where the individual serves as their own control, shorter study length, the ability to control for outside factors, and control over the timing of testing in relation to a subject's last exercise session make acute exercise interventions particularly practical for isolating the neurophysiological effects of exercise in humans [74], [173]. Importantly, if exercise is responsible for changes seen in long-term exercise interventions, we would expect that these changes result from the culmination of small adaptation afforded over numerous acute exercise bouts. Thus, the short term effects of acute exercise may also predict longer-term training based adaptations and allow for efficient identification of optimal interventions in diverse populations [74], [209].

In addition to limitations of observational and long-term exercise training study designs, previous research focusing on the effects of exercise for healthy brain aging have mostly utilized broad and nonspecific behavioral or neuroimaging-based measures [133]. Meanwhile, the hippocampus and its subfield structures play a critical role in proper memory formation and retrieval and displays particular susceptibility in the earliest stages of neurodegeneration and memory impairment [11], [194], [220], [221]. Specifically, the dentate gyrus (DG) and the third cornu ammonis area (CA3) of the hippocampus support mnemonic discrimination (MD), the process of reducing interference among new but similar items and encoding and retrieving them as distinct memories (e.g., remembering where you parked the car today vs. yesterday) [11], [222]. The inability to appropriately perform MD is associated with age, a presymptomatic biomarker of cognitive decline, and hypothesized to result from reduced neurogenesis, angiogenesis, and synaptogenesis within the DG/CA3 subfields of the hippocampus [13], [16], [21], [23], [223]. Interestingly, exercise appears to preferentially protect these age susceptible hippocampal subfields and hippocampal-dependent memory constructs [56], [57], [71], [133], potentially due to an

evolutionary need or affinity for spatial navigation and resource acquisition [224], [225]. In fact, animal studies show wheel running upregulates neurogenesis and alters the wiring of new adult born granule cells in the DG, which leads to improvements in MD related behavior [39], [43]–[45]. While 3-4 months of higher intensity exercise training is associated with preservation of DG/CA3 volume [143] and improvements in MD performance in healthy older adults [55].

Unfortunately, despite growing evidence of the benefits of exercise for hippocampal dependent memory and preservation of gross hippocampal structure, the specific mechanisms by which a single session of exercise provides these benefits in humans remain unclear. Acute exercise studies in animals suggest a single session of wheel running may increase DG/CA3 activity [75], [198], neuroinflammatory mediators [79], [80], [87], and vascular and neurotrophic growth factors [75], [135], [226]. Yet, despite numerous studies having explored the behavioral effects of acute exercise for multiple domains of memory in young adults [58], [65], [136], very few studies have been conducted in older adults [64], [137] and no research had explored the effects of acute aerobic exercise on hippocampal specific MD in older adults. Furthermore, given methodological limitations, our understanding of the neurophysiological effects of acute exercise on the human hippocampus remained lacking and were predominantly limited to gross volumetric changes that occur over months, years or even decades and were non specific to underlying tissue integrity [71], [133]. However, recent technological advancements in imaging protocols now allow for high resolution functional and microstructural imaging of the hippocampus and its specific subfields [31], [68], [106], [227]. Providing an opportunity to probe network activity/disfunction and biophysically relevant microstructural properties within highly age susceptible hippocampal subfields.

Therefore, this dissertation aimed to advance the current literature by providing translational evidence for the effects of moderate to vigorous intensity exercise on age susceptible hippocampal

memory network function and microstructure in healthy older adults. To accomplish this, three within subject research projects were conducted in which the effects of acute exercise on hippocampal dependent memory, hippocampal microstructure, and hippocampal subfield neural activity and microstructure were explored.

Summary of Results

The first objective of this dissertation sought to understand the behavioral effects of acute moderate to vigorous intensity exercise on the hippocampal-dependent MD in older adults. While a growing number of studies had identified the beneficial effects of a single session of exercise on episodic memory of college-aged adults, far fewer had been conducted in older adults [58], [64]. Additionally, acute exercise's effects on MD were completely limited to younger adults [67], [68]. To address this gap in the literature, we used a pre and post-intervention study design to determine the effects of acute exercise on MD performance compared to seated rest in healthy physically active older adults. In a post condition only analysis it was found that participants performed better following the exercise condition compared to seated rest. However, when analyzing the results of the pre to post study design for MD performance, we found a significant interactive effect in which participants performed significantly worse from pre to post rest, while MD performance was maintained, but not improved from pre to post exercise. These results were interpreted to suggest acute exercise may have reduced some of the interference participants appeared to experience performing multiple iterations of the MST in short succession.

In addition to this significant interaction, we received subjective feedback from numerous participants indicating that they found the post condition task more challenging than the precondition task due to interference and uncertainty as to whether images in the post condition test phase were from the pre or post condition study phase of that day. It is crucial to note that all images were different

between test Sets and thus, there was no crossover between task performances. This interactive effect could have occurred due to reduced MD performance from pre to post rest, potentially due to interference and issues with false memory. Interestingly, acute moderate to vigorous exercise in younger adults has been shown to reduce the rate of false memories, potentially through enhanced executive function [57], [228]. Therefore, our results could indicate that while an acute bout of exercise did not lead to improvements in MD performance, it may have helped these older participants limit their rate of false memories.

Furthermore, we used a self selected pace protocol for the task in which there was no time limit for recall and performance, which is a common approach when having older adults perform the MST [21]. Nevertheless, in a post hoc analysis we did not find that acute exercise was associated with differences in reaction time on the task, suggesting the protective effect of exercise for MD and memory interference were not due to exercise related differences in reaction time or vision [161]. Finally, differences in performance were not associated with differences in subjective measures of arousal. Previously a short 10 minute bout of light intensity exercise in younger adults was shown to be associated with enhanced arousal and concurrent benefits in MD performance [68]. While we did find the acute exercise session did elevate subjective levels of arousal, they were not associated with differences in MD performance. Thus, we infer that the effect of higher intensity acute exercise on MD performance in older adults is not due to differences in arousal and may be due to different underlying neurophysiological mechanisms.

The second dissertation objective was to determine if acute exercise in healthy older adults was associated with differences in hippocampal microstructure compared to seated rest. Previous acute exercise studies focusing on brain health are predominantly limited to behavioral performance, and those studies that have employed imaging approaches have focused on executive function and global effects of

hemodynamic-related functional activity [58], [65], [206], [229], [230]. However, several animal studies indicate that a single session of exercise can specifically stimulate the hippocampal memory network via neuroinflammatory mediators [79], [80], [85], [87], and vascular and neurotrophic growth factors [75], [135], [226]. However, given technological imaging constraints, it remains impossible to measure any of these factors within the human brain directly. In particular, changes in hippocampal volume are not a strong proxy for changes in any of these factors, especially within the context of acute exercise. Nevertheless, diffusion-weighted imaging, which measures the movement of water molecules within brain tissue and is a proxy for tissue integrity, is sensitive to short-term learning and the upregulation of related hippocampal neuroplastic factors, such as synaptosin, brain-derived neurotrophic factor, and glial morphological changes and activity [108], [111], [112].

Thus, to accomplish the second objective of identifying acute exercise-related changes in hippocampal microstructure, a within-subject post-intervention-only study design was conducted in which participants completed 20 minutes of moderate-intensity aerobic exercise or seated rest followed by a diffusion imaging scan. We found 20 minutes of moderate to vigorous intensity aerobic exercise was associated with greater dispersion and elevated hippocampal diffusion compared to following seated rest. Elevated diffusion signal within the hippocampus is consistent with changes in glial morphology and activity, which often accompany and help facilitate neuroinflammatory responses [90], [231]–[233]. Interestingly, acute wheel running at higher intensities is also associated with upregulation of neuroinflammatory mediators through changes in glial morphology and activity [80], [85], [87]. Unfortunately, no hippocampal-specific behavioral data were collected, and the diffusion imaging was obtained approximately 60-80 minutes following exercise termination. However, this does suggest that these microstructural diffusion effects persisted for an hour to an hour and a half post exercise, which is

consistent with studies in animals suggesting neurotrophins are upregulated for up to two hours following a single wheel running session [173].

Furthermore, younger adults exhibit elevated hippocampal blood perfusion for up to an hour following an acute bout of exercise [172]. Notably, our analysis approach used a novel single-shell processing approach to limit the impact of free water, cerebrospinal fluid, blood flow, and partial volume effects on our hippocampal diffusion metrics. However, it remains impossible to rule out these potential confounding factors given the resolution and diffusion sequence specifically used. Additionally, a lack of hippocampal-specific behavioral measures makes it challenging to determine whether these acute exercise-related microstructural effects are associated with hippocampal function and whether they are beneficial. Nevertheless, these results are particularly exciting as they provide the first evidence that a single session of aerobic exercise can elicit neuroplastic microstructural changes within the hippocampus of humans.

The third dissertation objective was to determine the effects of acute moderate to vigorous-intensity aerobic exercise on hippocampal subfield-specific function and microstructure. This third study aimed to build on the two previous studies and to extend some of the research performed by Suwabe et al. (2018), to older adults [68]. Specifically, we showed in our previous two studies that 20 minutes of acute moderate to vigorous aerobic exercise could alter hippocampal-dependent memory performance and hippocampal microstructure in older adults. Meanwhile, Suwabe et al. (2018) found that 10 minutes of light aerobic exercise improved MD and increased MD-related hippocampal subfield activity, including within the DG/CA3 of younger adults [68]. Notably, MD is closely tied to DG/CA3 integrity [18], [29], [234]. At the same time, age and pathological memory impairment are linked to DG/CA3 dysfunction [28], [29], [163]. Meanwhile, animal studies suggest that acute and chronic wheel running stimulates MD-related behavior and neurogenic and angiogenic processes within the DG/CA3 [43]–[45],

[70], [75], [198], [226]. Thus, we employed high-resolution and hippocampal subfield-specific and sensitive functional, anatomical, and diffusion imaging in healthy older adults following a 20-minute bout of moderate to vigorous intensity aerobic exercise versus a seated rest condition. Specifically, we found that the higher-intensity acute exercise session led to lower MD performance, suppressed MD-related DG/CA3 activity, and elevated DG/CA3 extracellular diffusion.

Our behavioral findings were in direct contrast to those studies reporting 10 minutes of light and moderate-intensity aerobic exercise led to better MD performance on highly similar lures in college-aged adults [67], [68]. However, as previously discussed, our higher intensity and longer exercise session may have been more stressful and elevated cortisol and lactate levels, which may specifically interfere with hippocampal-dependent memory [75], [78], [207]. Additionally, while we did not show pre to post-improvements in MD in study one, exercise preserved MD performance compared to a seated rest control condition potentially through more executive function-related pathways by reducing interference and false memory rates [57], [193], [228]. Importantly, in study three, participants performed a time-restricted continuous version of the MST in the MRI scanner and only completed the task post-intervention. Thus, the time-restricted and forced choice nature of the continuous MST performed in the scanner could have led to poorer MD performance, particularly for older adults [19]. Specifically, this observed shift from better MD to more pattern completion-related behavior is generally associated with aging, compensation, or dysfunction within the hippocampal circuitry [18], [164]. However, it could also be the case that higher-intensity acute exercise increases this compensatory mechanism in older adults, thus, when performing a time-restricted task, they favor a more efficient encoding and recall approach by focusing on the "gist" of viewed objects. Notably, our findings of MD suppression do not suggest that repeated bouts of higher-intensity exercise will lead to adverse long-term

outcomes concerning hippocampal memory function as higher-intensity exercise training in older adults can provide greater benefits for MD performance than lighter-intensity exercise [55].

Again, our finding of reduced MD-related DG/CA3 fMRI activity is in contrasts with the results of Suwabe et al. (2018), in which they found 10 minutes of light aerobic exercise led to elevated MD-related DG/CA3 activity [68]. As discussed in the difference in behavioral findings, a longer and more intense bout of exercise may be the reason for suppressed rather than elevated DG/CA3 activity. However, previous research linking MD-related DG/CA3 activity to hippocampal dysfunction and behavior may suggest that participant age led to these differential findings and that both studies suggest acute exercise benefits DG/CA3 function and integrity [31]. Specifically, MD-related DG/CA3 hyperactivity is related to dysfunction in older adults but better health and performance in younger adults [24], [29], [31]. Meanwhile, reducing DG/CA3 activity in older adults via interventions is linked to better hippocampal function and behavioral performance. One limitation to our finding is that we did not find acute exercise-related reductions in DG/CA3 activity were associated with acute exercise-related differences in MD performance. Yet, Suwabe et al. (2018) did not report this association either, thus, acute exercise-related differences in DG/CA3 activity may be associated with hippocampal subfield network alterations that are not specific or sensitive to short-term differences in MD performance, and these behavioral differences could be due to different underlying mechanisms.

Finally, we found that acute exercise was associated with elevated extracellular diffusion in the hippocampus and specifically DG/CA3 subfield. This replicates and extends our findings in study two. Specifically, in study two, we reported that exercise elevated hippocampal diffusion and dispersion within the hippocampus [235]. In study 3, we utilized a higher resolution multi-shell diffusion imaging approach that allowed us to break the diffusion signal into biophysically relevant components (i.e. free water, intracellular, and extracellular), better control for partial volume effects of surrounding

cerebrospinal fluid and white matter, and specifically isolate the effects to the hippocampal subfields, such as DG/CA3. Additionally, our analysis and statistical approach allowed us to control for additional confounding factors, particularly the effect of exercise on global gray matter diffusion. Thus, our findings are exciting and novel in that they confirm acute exercise leads to microstructural alterations in the aging hippocampus independent of more global effects and that these microstructural differences are specific to the DG/CA3 subfield. These translational findings are also particularly exciting as differences in hippocampal extracellular diffusion and microstructure are associated with hippocampal integrity and neuroplastic factors that may be neurogenic [81], [108], [111], [122], [123]. In fact, these findings corroborate acute wheel running studies that suggest exercise specifically increases DG/CA3 neurotrophic factors, inflammatory mediators, and glial activity and morphological changes [75], [79], [80], [87], [226], but that repeated bouts lead to long term reductions in neuroinflammation, increase neurogenesis and the viability of new neurons to integrate into networks, as well as better cognitive performance and brain health [236]–[239].

These three studies suggest that a single moderate to vigorous aerobic exercise session can alter hippocampal subfield-specific behavior, function, and microstructure. This provides critical insight into our understanding of the effects of exercise on the aging brain given hippocampal subfields are critical to proper memory function and are susceptible to aging and neurodegenerative disease. Furthermore, the current optimal health guidelines for older American adults are 150 minutes of moderate to vigorous aerobic physical activity. Our studies support this statement and suggest that 20-30 minutes of moderate to vigorous aerobic exercise performed several days a week can potentially promote a healthy hippocampus and memory system and may be protective against age-related memory decline and hippocampal deterioration. However, while these findings are promising, they provide the field with

many more questions and opportunities for future research that focuses on optimizing interventions and understanding the neurophysiological effects of exercise on the aging memory system.

Remaining Questions and Future Directions

One of the remaining questions of these studies was that differences in MD performance were not related to differences in our neuroimaging measures. This was a methodological limitation in study 2 that we hoped to address in study 3, given that MD performance via the MST has consistently been shown to relate to hippocampal subfield-specific integrity and function [20]. Meanwhile, we found that acute exercise led to differences in MD-related DG/CA3 activity and DG/CA3 microstructure. However, this makes interpreting the benefits or risks of these exercise-related differences in hippocampal subfield function and microstructure challenging, as we could not link these changes to material differences on a behavioral task. An important limitation of study 3 was that we limited our behavioral measures to the continuous MST being performed in the scanner. We took this approach because there is ample evidence that acute exercise could alter MD performance in younger individuals and that MD is closely related to hippocampal integrity, which was the dissertation's focus.

Meanwhile, acute exercise consistently benefits other cognitive domains, such as executive function in older adults [65], [240]. Thus, these acute neurophysiological changes we probed via neuroimaging might more closely relate to other cognitive tasks, such as executive function or working memory [65], [240]. Additionally, these underlying neurophysiological changes elicited in the DG/CA3 by acute exercise may not be specifically associated with short-term changes in behavior. They may be more closely related to longer-term adaptive physiological processes such as neurogenesis [74], [241]. Thus, future studies must determine if these acute exercise-related neuroimaging differences are associated with other cognitive processes over short and longer-term time frames.

Furthermore, there is currently an enormous gap in the field concerning the link between acute exercise-related neurophysiological and behavioral effects and long-term exercise training-related adaptations [74], [209]. Acute exercise interventions provide an optimal study design to identify and link neurophysiological processes and changes specifically to exercise [74]. However, from a public health perspective, what is arguably more important is to determine how these neurophysiological processes accumulate through repeated bouts of physical activity to provide beneficial adaptations to the aging memory system [133], [209]. Thus, acute exercise interventions also provide a cost-effective and time-efficient opportunity to identify optimal and more personalized exercise interventions for brain health. In fact, these types of acute exercise interventions could one day help determine if an individual will or will not respond well to specific types of exercise interventions based solely on how they respond to a single session of exercise. Therefore, future acute exercise studies are needed to characterize the effects of different types, intensities, and modalities of exercise on individuals of different races, ages, fitness backgrounds, chronic conditions, and genetics. Additionally, studies linking acute exercise-related neurophysiological changes to long-term exercise training outcomes are critical for advancing the field forward and identifying the most efficient and effective exercise interventions for brain health.

While this dissertation employed novel high-resolution neuroimaging approaches to characterize the effects of acute exercise on the underlying neurophysiology of older adults, these approaches and techniques are still limited. In particular, while multi-shell diffusion imaging approaches are sensitive and specific to broad biophysical properties such as diffusion within intracellular, extracellular, and free water tissue compartments, it is by no means specific or at the resolution of resolving cell types and or processes [115], [214]. Unfortunately, imaging at this scale and to this level of specificity within live humans remains a major technological limitation. However, scanner technology and new scan sequences continue to be developed and push the field forward. For example, while imaging techniques such as

positron emission topography (PET) are more specific to underlying neural substrates, they generally have poor resolution and have limited use in humans due to the use of radioactive isotope tracers.

An imaging technique that is more specific to a single neural substrates and can be performed multiple times and across a wide range of individuals is magnetic resonance spectroscopy (MRS). MRS measures the concentration of specific brain metabolites (including N-acetyl aspartate (NAA), glutamate, creatine, lactate, and GABA), by capturing the magnetic resonance of molecules other than bulk water [242]. Yet, as water is a more abundant molecule throughout the human body, spatial resolution or scan time must be sacrificed to obtain a strong enough signal. Therefore, MRS is generally acquired using "single voxel spectroscopy," in which the concentration of a specific metabolite is extracted from a large voxel (i.e., size of the entire hippocampus). Despite this limitation, MRS measures specific metabolic levels and, thus, has higher specificity than MRI while being safer than PET [242]. Given this niche, the use of MRS to study the effects of acute exercise on the brain is limited. Of two studies conducted to date, one animal study showed acute exhaustive exercise in rats was associated with increased glutamate and glutamine concentrations (a marker of astrocytic activity) in the cerebellum, striatum, and hippocampus [243] and the other small human study found a vigorous bout of aerobic exercise resulted in higher lactate, glutamate, and glutamine concentrations in the occipital cortex of healthy younger adults [244]. While these two studies support our diffusion-weighted imaging findings and our interpretation of elevated neuroinflammation, no work has looked at acute exercise's effect on hippocampal metabolite concentrations in humans or older adults. Therefore, future studies should determine how acute exercise alters hippocampal metabolites such as N-acetyl aspartate (neural integrity and density), Myo-inositol (measure of glial cell volume changes due to activity), and lactate (measure of neural and astrocytic energy consumption which can be modified due to

neuroinflammation) in older adults and how these changes relate to other measures of hippocampal function and microstructure [245].

Finally, while exercise is a leading modifiable lifestyle factor for preserving the aging memory system and dementia prevention, other lifestyle factors could provide similar or complementary benefits to those reported in the literature and this dissertation [132]. Ample evidence suggests that obtaining adequate sleep and eating a healthy diet may play an essential role in healthy aging, particularly concerning brain health and memory [246], [247]. While we asked participants to maintain their sleep and diet the two nights before and the days of each experimental visit for all three studies, we did not subjectively or objectively measure either and differences in sleep or diet quality could interact with the acute exercise effects [248]. Thus, future acute exercise intervention studies should objectively measure and account for sleep and diet quality in the days leading up to and on the days of the intervention.

Additionally, several animal studies suggest that environmental enrichment provides complimentary benefits to exercise for the hippocampal memory system. For example, wheel running upregulates neurogenesis by stimulating more progenitor cells in the dentate gyrus. At the same time, environmental enrichment helps these new adult-born granule cells survive and interface with existing neural networks [249], [250]. Indicating that exercise and adequate environmental enrichment may be necessary to produce both behavioral and neurogenic benefits in aging animals and potentially humans [249], [251]. In fact, a recent non-randomized study reported the additive benefits of a simultaneous video game and exercise intervention for MST performance and hippocampal volume and connectivity [252]. Of note, our acute exercise and rest sessions had participants performing these interventions while facing a blank wall and in a very low-stimulus environment (no screens, music, or socialization with research assistants). Furthermore, the exercise session was performed on a stationary cycle ergometer, so participants did not have to navigate or engage with the world as they might if they were

to go on a walk or bike ride through the neighborhood or along a trail. Therefore, future studies should determine whether exercising in an open vs closed environment may differentially alter the effects of exercise on hippocampal subfield function and microstructure.

In conclusion, the results of this dissertation indicate that acute moderate to vigorous aerobic exercise directly impacts hippocampal subfield-specific behavior, function, and microstructure in healthy older adults. However, these findings also suggest that the impact of acute aerobic exercise on the aging brain is nuanced and dependent on the participant, intervention, and cognitive task performed. For example, acute exercise can both impair and improve short-term performance on hippocampal-dependent behavior and memory. However these effects depend on individual factors, such as the age of participants, and the intervention factors, such as the length, intensity, and timing of the acute exercise session, and the memory task. Furthermore, acute aerobic exercise also suppresses DG/CA3 hyperactivity during MD in healthy older adults, which is consistent with better regulation of hippocampal subfield network function. This supports previous findings and theories that the DG/CA3 differentially processes information during MD with age and that the effects of acute exercise on the hippocampus are, thus, similarly age dependent. Finally, higher-intensity acute exercise in older adults elicits neuroplastic changes specific to the hippocampus and DG/CA3. These acute exercise-induced microstructural diffusion changes are consistent with animal studies showing that brief challenges to allostasis (such as higher intensity exercise) can stimulate cascades of neurotrophic factors and neuroinflammatory mediators within the aging hippocampus that in turn promote longer-term neurogenic adaptations over time and through repeated bouts of exercise.

Thus, this dissertation helps advance our understanding of the relationship between exercise and brain health, particularly within the hippocampal structure, which is critical to proper memory performance and is highly susceptible to aging and neurodegenerative diseases such as Alzheimer's.

Furthermore, these findings help establish new high-resolution imaging techniques for identifying exercise-related hippocampal neuroplasticity in humans. These methods can be incorporated into future research and link acute exercise-induced neurophysiological changes to exercise training intervention-based adaptations in order to efficiently identify optimal intervention approaches. Finally, while these findings are novel and impactful to the field, numerous questions remain concerning the identification of optimal and individualized lifestyle interventions to promote brain health. Yet, with continued incremental progress, there is hope that one day soon, it may be possible to delay or even prevent the onset of the majority of dementia cases, drastically improving the lives of both the potentially afflicted and their loved ones.

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