INVESTIGATING THE EFFECTS OF A 6-MONTH AEROBIC EXERCISE INTERVENTION ON BRAIN FUNCTION AND MEMORY IN OLDER ADULTS

BY

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THESIS

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ABSTRACT

Episodic memory is particularly susceptible to age-related decline. Research in animal models and cross-sectional studies in humans provide evidence that engaging in aerobic exercise may mitigate the effects of aging on memory. Yet, despite abundant evidence from animal literature, many aerobic exercise interventions in humans have failed to demonstrate improvements in learning and memory (Erickson et al., 2011a; Tamura et al., 2014; Ngandu et al., 2015). Further, we know little about how aerobic exercise affects brain function and whether functional changes are behaviorally relevant. In the present study, we investigated the effect of a 6-month aerobic exercise intervention on episodic memory retrieval using fMRI in healthy older adults. Specifically, 137 older adults (60 - 80 years of age) were randomized across four treatment groups: walking, walking plus nutrition, dance, and stretching and toning (active control). Although we found that the walking groups increased cardiorespiratory fitness (CRF) relative to the dance and control groups, there were no group differences in episodic memory. Consistent with previous studies, we found no association between change in CRF and change in memory performance. However, we did find that change in CRF, across intervention groups, was positively associated with change in hippocampal activation during recognition 'hits'. Furthermore, increased hippocampal activation was associated with improvements in memory performance. Thus, these results demonstrate a mechanism by which increases in CRF may indirectly relate to memory performance improvements in the absence of a significant behavioral effect.

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CHAPTER 1

INTRODUCTION

1.1 Aging brain and memory

One of the most pervasive signs of cognitive aging is decline in episodic memory, which begins in the third decade of life and declines steadily thereafter (Salthouse, 2003). Episodic memory is a type of long-term memory about events and the relation between events situated in time and place (Tulving, 1972). Research unambiguously shows decline in episodic memory across the lifespan. A meta-analysis of 91 studies found that episodic memory is significantly worse in older adults compared to young adults (Verhaeghen et al., 1993). While they found that material that is more easily categorized reduces age-related differences, learning type (intentional versus incidental), method of presentation (self-paced versus experimenter pacing), and recall delay did not attenuate age-related differences. A cross-sectional study found that for memory of sequences of events older adults were not only impaired relative to younger adults, but also memory performance declined linearly with age within the older adult group (Allen et al., 2015).

While most studies investigating age effects compare groups of young and older adults, one large (N= 1400), cross-sectional study investigated incidental and intentional learning using everyday stimuli across the lifespan (ages 18 – 70 (Crook III et al., 1993)).While they found associations between age and memory for both types of learning, they found a stronger association between age and intentional learning, the type of episodic memory we employed in the current study. Salthouse and colleagues investigated whether cognitive aging is a normal or pathological process. They reasoned that if cognitive aging is a normal process, between-subject variability should be consistent across age; however, if variability increases with age, pathology could be underlying age-related cognitive decline (Salthouse, 2003). Indeed, they found that

between-subject variability is homogenous across age, indicating that cognitive aging is a normal, ubiquitous process.

1.2 Aging brain and functional (fMRI) changes in memory

There are detectable age-related changes in brain structure and function in regions primarily implicated in episodic memory, such as the hippocampus and prefrontal cortex. The frontal lobe theory of aging posits that age-related cognitive decline is primarily due to functional and neuroanatomical changes in the frontal lobe, a region especially vulnerable to age-related plasticity (Grady et al., 2006). Indeed, many studies implicate the prefrontal cortex in episodic memory retrieval, suggesting that it is involved in guiding knowledge recovery (Bunge et al., 2004; Hayes et al., 2004). Specifically, studies have found activation in the dorsolateral prefrontal cortex (DLPFC) during recognition memory and some suggest that the right DLPFC may monitor stimuli familiarity (Bunge et al., 2004; Hayes et al., 2004; Achim and Lepage, 2005). Another study investigated the involvement of the DLPFC in post-retrieval monitoring and observed greater bilateral DLPFC activation for high-monitoring trials compared to low-monitoring trials (Achim and Lepage, 2005). Using PET imaging, one study found that younger adults exhibit greater right PFC activations during retrieval than older adults and older adults also showed compensatory left PFC activation (Cabeza et al., 2000). Another study testing recognition for words and pictures found a linear decrease with age for task-associated activation, including the bilateral PFC, and a linear increase with age for default mode areas (Grady et al., 2006). Both of the above described studies found no difference in behavioral performance between groups. Although most studies have shown that PFC activation during retrieval decreases with age, some studies have demonstrated an increase in activation. Madden and colleagues used PET imaging during encoding and retrieval for word list learning and found

that while memory performance decreased with age, older adults had greater activation in the many bilateral PFC regions compared to young adults (Madden et al., 1999). Still, other studies report no age-related difference in DLPFC during retrieval (Giovanello and Schacter, 2012).

Many studies across rodents and humans have demonstrated the role of the hippocampus in episodic memory. Studies have shown that activation is greater for retrieval of relational versus non-relational material and for successfully retrieved trials ('hits') versus unsuccessfully retrieved trials ('misses') (Bunge et al., 2004). Although it is difficult to interpret the behavioral relevance of changes in functional activation in the absence of a behavioral effect, most studies report less hippocampal activation in older adults compared to younger adults during relational memory tasks. Despite no group differences in memory performance, one study found greater activation in the left hippocampus in young adults compared to older adults (Giovanello and Schacter, 2012). Another study found that for successful source memory recognition, young adults exhibited greater activation in several frontal regions and the hippocampus relative to older adults (Cansino et al., 2015). In this study, the authors did cite a significant difference in source memory hits, with the young adults outperforming the older adults. However, some studies have found less activation in young adults, attributing it to neural efficiency. For instance, Backman and colleagues used PET imaging during word list retrieval and found increased MTL activation in older adults relative to young adults (Bäckman et al., 1997). Yet, other studies have failed to find age-related differences in hippocampal activation during retrieval (Daselaar, 2003). Thus, the behavioral relevance of changes in activation in the DLPFC and hippocampus across the lifespan are still not fully understood.

1.3 Cardiorespiratory fitness (CRF) and memory

Research in rodent models consistently demonstrates aerobic exercise-induced enhancements in learning and memory (Uysal et al., 2005; van Praag et al., 2005; Cotman and Berchtold, 2007). Although mechanisms underlying these cognitive gains are not fully understood, research has shown that exercise upregulates synaptogenesis and increases dendritic length, complexity, and spine density (Cotman and Berchtold, 2007; Ambrogini et al., 2013). Aerobic exercise stimulates the production of growth factors, such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF), which support cell proliferation and survival (Voss et al., 2011). In fact, several days of wheel running increases the translation of the BDNF protein across all hippocampal subfields. Furthermore, blocking BDNF receptors during exercise obliterates exercise-induced cognitive benefits (Cotman et al., 2007; Voss et al., 2011). Exercise upregulates IGF-1 in both the CNS and periphery and helps regulate hippocampal neurogenesis and angiogenesis (Cotman et al., 2007). VEGF plays a critical role in angiogenesis and neurogenesis. In fact, preventing VEGF from crossing the blood brain barrier obliterates exercise-induced hippocampal neurogenesis and administering VEGF peripherally in the absence of exercise increases neurogenesis (Fabel et al., 2003). Although the behavioral relevance of these new neurons is still debated, it is known that new neurons do have a lower threshold for excitability, which suggests one mechanism by which they may enhance learning and plasticity (Cotman et al., 2007).

Brain structures that exhibit the greatest plasticity during development and aging (e.g., frontal and temporal lobes) appear to benefit the most from aerobic exercise. Thus, aerobic exercise exerts particular benefits for cognitive abilities such as executive function and hippocampal-dependent memory. Yet, despite abundant evidence from animal literature (van Praag et al., 2005), the association between aerobic exercise and learning and memory has been

far less robust in humans. Most research has focused on the benefits of aerobic exercise on executive functioning. In fact, a meta-analysis investigated the effect of 18 aerobic training interventions on cognition in sedentary older adults and found that aerobic exercise had the largest effect on executive functioning (Colcombe and Kramer, 2003). In contrast, a meta-analysis from 19 memory studies (collapsed across memory type) found that long-term aerobic exercise had a small but significant effect on memory; however, for the 11 studies that specifically investigated the effect of chronic aerobic exercise on long-term memory, there was a non-significant effect (Roig et al., 2013).

Yet, many cross-sectional studies do find a positive association between CRF and episodic memory performance. In fact, a study using both an equation to calculate CRF and the gold standard GTX, found that both measures of fitness were significantly associated with spatial working memory and hippocampal volume in older adults (McAuley et al., 2011). Another cross-sectional study investigated the relationship between fitness and cognition in healthy young and older adults (Hayes et al., 2014a). They found that CRF is positively related to executive function and episodic memory in older adults but this relationship does not hold for young adults. In middle-aged adults, greater estimated CRF is associated with greater gray matter volumes in Alzheimer's disease (AD)-relevant areas such as the hippocampus, amygdala, precuneus, supra marginal gyrus, and middle frontal gyrus (Boots et al., 2014). Chaddock and colleagues found that higher fit children had larger bilateral hippocampi and better performance on a relational memory task than their lower fit peers (Chaddock et al., 2010). Thus, these cross-sectional findings suggest that children and older adults are particularly sensitive to aerobic exercise induced brain plasticity.

Longitudinal studies have largely failed to find exercise intervention induced improvements in memory performance. A very large (N=1260) 2-year longitudinal study investigated the effects of an aerobic exercise and nutrition intervention on cognition in healthy older adults and found significant improvements in executive functioning and processing speed compared to the control group (Ngandu et al., 2015). However, they found no differences for episodic memory. A smaller scale (N=110) 2-year mild aerobic exercise intervention improved attentional shift and prevented prefrontal cortical decline in healthy older adults (Tamura et al., 2014). Another study found that a one-year aerobic intervention in older adults led to a nearly 2% increase in hippocampal volume, whereas the active control group had a nearly 1.4% decrease (Erickson et al., 2011a). Yet, despite the hippocampal gain, there were no group differences in memory performance after the intervention. At baseline, they did find that aerobic fitness was positively correlated with spatial learning performance. Thus, it remains unknown whether episodic memory can be enhanced through an aerobic exercise intervention. Perhaps the cognitive tasks used are not sensitive enough to capture change in memory performance due to aerobic exercise and were instead diluted by within subject variability and test-retest effects.

1.4 Physical activity and memory

Physical activity is defined as "any bodily movement produced by the skeletal muscles that results in energy expenditure" (Caspersen et al. 1985). Physical activity encompasses activities of daily living (e.g., laundry, gardening) and intentional exercise (e.g., tennis, walking). Cross-sectional studies generally indicate that higher physical activity is positively associated with memory performance. Beilak and colleagues found a positive correlation between baseline physical activity and a fluid cognitive composite score (perceptual speed, working memory, short-term memory, and episodic memory) across the lifespan, but the strongest association was

in the young adults cohort (20-24), followed by the oldest (60-64), and last, the middle-aged (40-44) (Bielak et al., 2014). A cross-sectional study in middle-aged and older adults showed that higher physically active adults had fewer errors in a paired associates learning task and better performance on a spatial recognition memory task (Czajkowska et al., 2014). A study in a large sample of older women (N=9,344) found that self-reported measures of physical activity across the lifespan, but particularly during teenage years, were negatively correlated with cognitive impairment as measured by MMSE (Middleton et al., 2010). There is evidence that physical activity affects brain structure as well. Varma and colleagues (2015) found that low intensity daily walking is associated with larger hippocampi in women but not men (Varma et al., 2015). Although the authors speculate that this finding could be due to the fact that the women in the study were more obese.

Evidence from longitudinal data suggests that increasing physical activity participation may reduce risk for cognitive decline and increase volume in brain regions sensitive to age-related atrophy. A longitudinal study found that physical activity at 36 years old significantly predicted change in memory score from 43 to 53 years of age, with greater physical activity engagement during middle age resulting in slower decline (Richards et al., 2003). A large 24-month intervention study investigated the effects of a moderate-intensity physical activity program, including walking, strengthening, and stretching, on cognition in sedentary older adults (Sink et al., 2015). They found no differences in cognitive change between the intervention and active control group. Declining episodic memory is a hallmark feature of cognitive impairment. Although Erickson and colleagues (2010) did not directly measure episodic memory, they found that greater self-reported walking distance was associated nine years later with reduced cognitive impairment. Furthermore, greater walking distance is associated with greater volume in key areas

that support episodic memory such as the lateral and medial prefrontal cortex, lateral temporal cortex, lateral parietal cortex, and medial temporal lobe (Erickson et al., 2011b). A six-month intervention investigated whether exercise-intensity mediated change for episodic memory in older adults (Ruscheweyh et al., 2011). They found that mild and moderate intensity exercise led to equivalent improvements in episodic memory. Furthermore, since they found that even control participants increased their daily physical activity during the study, they collapsed groups and found a positive correlation between change in physical activity and change in episodic memory recall score. Thus, regardless of improvements in CRF, this study suggests that older adults who engage in more physical activity can improve episodic memory.

1.5 Fitness, physical activity, and brain function

Although there is much less research investigating the effect of fitness and physical activity on brain function, evidence suggests that higher fit older adults show greater activation in task-related brain regions. Colcombe and colleagues were the first to investigate the association between CRF and brain activation during a modified Ericksen flanker task. Interestingly, they found that both the high fit (cross-sectional) and aerobically trained (longitudinal) older adults showed greater activation in the middle frontal gyrus and superior frontal gyrus, and less activation in the anterior cingulate compared to their low fit and stretching and toning counterparts (Colcombe et al., 2004). Importantly, these differences in activation are behaviorally relevant. In fact, both the high fit and aerobically trained groups showed better performance, with greater efficiency in dealing with conflict trials and a reduction in behavioral conflict from baseline to post-test, respectively. Another study investigated whether a 6-month combined fitness and cognitive training intervention would improve spatial memory in middle-aged adults. Although they found no relationship between spatial learning performance and CRF,

they did find that changes in CRF positively correlated with changes in brain activation in the middle frontal gyrus and the cuneus (Holzschneider et al., 2012). At post-test (cross-sectional) they found positive associations between CRF and activation in the hippocampus and frontal areas. However, one caveat to note is that for both the cross-sectional and longitudinal results described, associations were only found in the spatial training groups. Similarly, Prakash and colleagues found increases in activation for task-relevant brain regions for higher fit older adults. Specifically, they found that higher fitness was positively associated with better performance and greater recruitment of frontal and parietal regions during a Stroop task (Prakash et al., 2011). In an aerobic exercise intervention in healthy older adults, Voss and colleagues (2012) found that increased temporal lobe connectivity between the parahippocampal gyrus and middle frontal gyrus was positively associated with growth factors that mediate aerobic exercise induced plasticity (e.g., BDNF, IGF-1, and VEGF), but only for the aerobic exercise group.

While most studies have investigated the effects of CRF on brain activation, one study investigated the relationship between self-reported physical activity frequency and intensity and brain activation. Interestingly, they found increases in brain activation in areas involved in semantic memory for the high physical activity and high Alzheimer's risk group (Smith et al., 2011). Although the majority of studies find positive associations between brain activation in task-relevant regions and CRF, one study found that the aerobic exercise and coordination training groups decreased prefrontal activation during an executive control task relative to a control group (Voelcker-Rehage et al., 2011). They interpreted this decrease as neural efficiency, despite no relationship between change in activation and behavioral performance. Thus, the relationship between activation and CRF/physical activity remains uncertain and the relationship becomes difficult to interpret in the absence of a behavioral effect.

1.6 Study aims and hypotheses

In the present study, we investigated the effect of a 6-month aerobic exercise intervention on episodic memory retrieval using fMRI in healthy older adults. This intervention included four groups: walking, walking plus nutrition, dance, and stretching and toning (active control). Because the face-scene task we used requires participants to discriminate whether familiar stimuli were previously displayed together, it specifically requires relational memory processing, a type of hippocampal-dependent memory sensitive to aerobic exercise. We used d' as our outcome measure of memory performance because it takes into account both hit rate and false alarm rate. We first analyzed whether groups showed differential gains in memory performance due to the intervention. Based on previous literature, we hypothesized that the walking groups would show larger gains in CRF and memory performance compared to the control. However, we did not have specific hypotheses for whether the dance group, a form of cognitive and aerobic training, would perform better or worse than the two aerobic groups because this is the first study to include both within a single intervention. Second, we wanted to test baseline associations among CRF, physical activity, hippocampal and DLPFC activation, and memory performance. Based on previous findings (Czajkowska et al., 2014; Hayes et al., 2014b), we hypothesized that both CRF and physical activity would be positively related to memory performance and hippocampal and DLPFC activation at baseline. Less is known about the relationship between baseline CRF/physical activity and patterns of brain activation, but some research suggests a positive relationship between fitness and task-relevant brain activation (Colcombe et al., 2004). Thus, we hypothesize that greater baseline CRF and physical activity will be positively correlated with hippocampal and DLPFC activation. Furthermore, we hypothesize that greater activation would correlate with better memory performance. Third, we

also tested the longitudinal relationships among change in CRF, change in physical activity, change in hippocampal and DLPFC activation, and change in memory performance. Although the relationship between change in CRF/physical activity and change in episodic memory has been much less reliable in humans, we hypothesized that a greater increase in CRF and physical activity would be positively correlated with an increase in memory performance. Based on two previous studies (Colcombe et al., 2004; Holzschneider et al., 2012), we hypothesized that change in CRF and physical activity would be positively correlated with change in activation in the hippocampus and DLPFC and that this change in brain activation would mediate the relationship between change in CRF/physical activity and change in memory performance.

CHAPTER 2

MATERIALS AND METHODS

2.1 Participants

The University of Illinois Institutional Review Board approved the study and written informed consent was obtained from all participants. The data presented in this paper were collected as part of a randomized controlled exercise trial "Influence of Fitness on Brain and Cognition II" (registered at ClinicalTrials.gov and clinical study identifier NCT01472744), which involved a series of neuroimaging, cognitive and cardiorespiratory testing, before and after a six-month lifestyle intervention program. Healthy, sedentary older adults were recruited from the Champaign county area via fliers and were financially reimbursed for study participation. Eligible participants met the following criteria: (1) were between the ages of 60 and 80 years old, (2) were free from psychiatric and neurological illness and had no history of stroke or transient ischemic attack, (3) scored ≥ 24 on the Mini-Mental State Exam (MMSE); (4) scored >21 on a Telephone Interview of Cognitive Status (TICS-M) questionnaire, (5) scored < 10 on the geriatric depression scale (GDS-15), (6) scored ≥ 75% right-handedness on the Edinburgh Handedness Questionnaire, (7) demonstrated normal or corrected-to-normal vision of at least 20/40 and no color blindness, (8) were cleared for suitability in the MRI environment, that is, no metallic implants that could interfere with the magnetic field or cause injury, no claustrophobia, and no history of head trauma, (9) were not taking medication for cardiovascular disease (e.g., beta blocker, diuretics), neurological, or psychiatric conditions (e.g., antidepressant, neuroleptic, anxiolytic), (10) participated in no more than two moderate bouts of exercise per week within the past six months.

Of the 1119 participants who contacted the study directors, 247 (n=169 female) met inclusion criteria, agreed to enroll in the study, and were randomized into one of the four intervention groups (walking n=54, walking+nutrition n=54, dance n=69, stretching-toning n=70). Of the 247 participants who completed the intervention, only 137 had (1) no missing data at baseline and post-intervention, (2) positive *d'* scores at baseline and post-intervention (negative scores indicate that a participant does not understand the episodic memory task), and (3) good quality fMRI data (less than 10 volumes with motion per run) data for the face-scene memory task. Randomization was successful as groups (walking n=35; walking+nutrition n=29; dance n=40; and stretching control n=33) did not differ with respect to age ($F=0.49$, $p=0.69$), sex ($F=0.13$, $p=0.95$), education ($F=1.35$, $p=0.26$), MMSE ($F=0.03$, $p=0.99$), health ($F=0.24$, $p=0.87$), or baseline CRF ($F=0.004$, $p=1.0$).

All intervention groups met three times per week for one-hour per session for six months. Both the walking and walking+nutrition groups were designed to increase cardiorespiratory fitness (CRF). The only difference between these groups was that the participants in the walking+nutrition group were instructed drink a beta-alanine supplement known to increase the growth of lean muscle mass. The dance group was designed to enhance CRF and cognition, as these participant's learned complex partner dance sequences that became progressively more difficult through the program. The stretching and toning group served as an active control, accounting for the psychosocial benefits experienced in the experimental groups. The sample contained more females because fewer older males met the above inclusion criteria or showed willingness to participate in the study.

2.2 Cardiorespiratory fitness

All participants obtained physician's approval to engage in cardiorespiratory fitness (CRF) testing. CRF was defined as peak oxygen consumption [ml/kg/min], measured with indirect calorimetry during a modified Balke graded maximal exercise test on a motor-driven treadmill test. Oxygen consumption (VO_2) was calculated from expired air sampled at 30-s intervals until peak VO_2 was reached or the test was terminated due to volitional exhaustion and/or symptom limitation. CRF was defined as the highest recorded VO_2 value (VO_2max) after two of three criteria were met: (1) a plateau in VO_2 after increase in workload; (2) a respiratory exchange ratio >1.10; and (3) a maximal heart rate within 10bpm of their age-predicted maximum. Our subjects represented a broad range of CRF values that were normally distributed and fell within the 90% peak VO_2max percentile according to gender- and age-specific norms (ACSM's Guidelines for Exercise Testing and Prescription, www.acsm.org).

2.3 Accelerometer

Actigraph accelerometers (Pensacola, FL: models GT3X and GT1M) were used to objectively measure physical activity. Accelerometers were initialized to sample movement in 60-second epochs and in the vertical axis only. Participants were instructed to wear the accelerometer on their left hip during their waking hours for seven consecutive days prior to the intervention and during the final week of the intervention. A day was considered valid if participants had at least 10 hours of wear time using a non-interruption period of 60 minutes. Participants with at least three valid days of data were retained for analyses (Troiano et al., 2008; Evenson et al., 2012). Modified Freedson cutpoints (1998) were used to classify activity as sedentary, light, moderate, and vigorous. Moderate and vigorous activity levels were condensed into one classification of moderate-to-vigorous physical activity (MVPA). All processing and scoring was conducted using Actilife software (Version 6.0 Actilife, Pensacola, FL).

2.4 MRI parameters

Participants were scanned at the University of Illinois in Beckman Institute on a Siemens Trio 3-Tesla full body magnet using 12-channel head coil. Functional BOLD images were collected with a T2*-weighted echo-planar imaging sequence of 35 contiguous axial slices collected in ascending order [time to repetition (TR) = 2000 ms; echo time (TE)= 25 ms; BOLD volumes = 296; flip angle = 80°; field of view (FOV) = 220mm; voxel size = 3.4 × 3.4 × 4.0 mm]. Structural images were acquired using a T1-weighted 3D magnetization prepared rapid gradient echo imaging (MPRAGE) protocol of 192 contiguous sagittal slices collected in ascending order parallel to the anterior commissure-posterior commissure line [TR = 1900 ms; TE = 2.32 ms; FOV = 220mm; flip angle = 9°; voxel size = 0.9 × 0.9 × 0.9 mm].

2.5 Episodic memory paradigm

Although the baseline and post-intervention assessments included a 2-hour MRI session of several scans (structural scans, resting state, DTI, attention task), this paper will only focus on the face-scene episodic memory fMRI task (described in Monti et al., 2013, 2015). In brief, this task is divided into three runs and each run contains 24 encoding trials followed by a 20 second break and then 24 recognition trials. During both encoding and recognition, a single trial consists of (1) a fixation cross displayed during the intertrial interval (ITI) for 2000 to 12000 msec, followed by (2) an outdoor scene image displayed for 2000 msec, and then, (3) the same outdoor scene image with a face overlaid on the scene for an additional 2000 msec. Importantly, the 72 face images were equally divided by sex and age (e.g., 36 male, 36 female; 36 young, 36 older). For each encoding trial participants were asked to make a 'yes' or 'no' judgment via button press indicating whether the face "fit" with the scene. This was done to elicit deep encoding. At recognition, two trial types (12 trials of each type) were presented, (1) "intact" trials, in which

face-scene pairs were identical combinations presented during encoding, and (2) "re-pair" trials, in which face-scene pairs were recombined to create a previously-seen face and a previously-seen scene that were not shown together during encoding. Thus, all stimuli were equally familiar at recognition, making the task rely specifically on relational memory processes. During recognition, participants were asked to make a 'yes' or 'no' judgment via button press as to whether the face-scene pair was an exact match of the pair shown during encoding. For intact pair trials, a correct response was classified as a 'hit' and an incorrect response was classified as a 'miss'. For re-pair trials, a correct response was categorized as a 'correct rejection', whereas an incorrect response was categorized as a 'false alarm.' The hit rate for each participant was calculated by dividing their hits by the sum of their hits and misses. The false alarm rate for each participant was calculated by dividing their false alarms by the sum of their false alarms and correct rejections. *D'* is a standard measure of memory performance that takes into account both hit rate and false alarm. We calculated each participant's *d'* score by subtracting the z-scored false alarm rate from the z-scored hit rate. In the event that hit rate was equal to one, we recalculated it using 1 – (1/2N) where N is the number of hits + misses. In the case that false alarm rate was equal to zero, we recalculated it using 1/2N where N is the number of false alarms + correct rejections. The analyses in this paper will focus on 'hit' events during the recognition phase. Before the MRI session, participants practiced this task outside of the scanner to ensure that they understood the instructions.

2.6 Image pre-processing

Preprocessing and data analysis were carried out using FSL 5.0.4 (FMRIB's Software Library, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). We applied the following preprocessing steps to the functional data: rigid body motion correction using MCFLIRT (Jenkinson et al., 2002), B0

unwarping (effective EPI echo spacing: 0.275 ms; EPI TE: 25 ms; unwarp direction: -y; signal loss threshold: 10%), removal of non-brain structures using BET (Smith, 2002), spatial smoothing using a Gaussian kernel of FWHM 6.0 mm, temporal filtering with a high pass frequency cut-off of 100 s. Images were co-registered to the subject's mean functional scan and placed into Montreal Neurological Institute (MNI) template space.

Regression-based analysis of fMRI data were carried out using FEAT (fMRI Expert Analysis Tool, http://www.fmrib. ox.ac.uk/analysis/research/feat/) version 6.00. First-level analyses were conducted on each participant's three runs individually at baseline and post-intervention. We used FILM prewhitening and included motion as a covariate in this model. The hemodynamic response was convolved with the double-gamma HRF. The statistical model for the task contained three regressors from encoding (hits, misses, other) and five regressors from recognition (hits, misses, correct rejection, false alarm, and no response). All regressors corresponded to the onset of the scene image. This model was then regressed onto the observed fMRI data. We then ran three types of second-level analyses to average the three runs: (1) we concatenated the three runs at baseline using fixed-effect FEAT higher-level analysis, (2) we concatenated the three runs at post-intervention using fixed-effect FEAT higher-level analysis, and (3) we used a model that contrasted an individual's three baseline and three post-intervention runs using fixed-effects FEAT higher-level analysis. This paper will focus on the contrast 'recognition hits' > baseline, where baseline represented anything not included in the EVs. Runs that included too much motion (greater than 10 volumes) were excluded from analysis and subjects were included in analysis if they had at least 2 quality runs.

2.7 ROI analysis

We selected two *a priori* regions of interest (ROI), the hippocampus and dorsolateral prefrontal cortex (DLPFC), as they are both associated with episodic memory (Daselaar, 2003; Kim et al., 2011; Manenti et al., 2013; Rugg and Vilberg, 2013) and sensitive to age-related decline and fitness-related improvement (Raz et al., 1997; Erickson et al., 2011b; Hayes et al., 2013). We created the hippocampal ROIs (right, left, and bilateral) using a Harvard-Oxford Cortical Structural probabilistic atlas with a 50% threshold and binarized. We created the DLPFC ROI (right, left, and bilateral) using an automated meta-analysis synthesized by Neurosynth (neurosynth.org), which compiled the peak voxel from over 300 studies with the term "DLPFC". After creating each mask, we extracted the mean activation for the ROI using the FEATQUERY command in FSL. We used the mean activation for further analyses described below.

2.8 Statistical Analyses

All data were analyzed using IBM SPSS Statistics version 22. Before conducting general linear model (GLM) and Pearson bivariate correlation analyses, all variables were checked for normality and skew. We tested for outliers using Tukey's outlier labeling rule with g=2.2 (Hoaglin and Iglewicz, 1987) and found that one variable (post-intervention left hippocampal activation) had an outlier. Because we found no differences in the results when we ran the analyses with and without this outlier, we decided to keep this participant in all further analyses to increase power. All predictor variables for GLM analysis were mean-centered. We calculated intervention change scores by subtracting the baseline score from the post-intervention score and dividing the difference by the standard deviation of the sample at baseline. We calculated change scores for CRF, light physical activity, moderate-vigorous physical activity, and memory performance (*d'*).

CHAPTER 3

RESULTS

3.1 Intervention Group

3.1.1 Group differences in CRF

Randomization was successful as groups (walking n=35; walking+nutrition n=29; dance n=40; and stretching control n=33) did not differ at baseline with respect to age (F=0.49, p=0.69), sex (F=0.13, p=0.95), education (F=1.35, p=0.26), MMSE (F=0.03, p=0.99), health (F=0.24, p=0.87), or baseline CRF (F=0.004, p=1.0). Using repeated-measures ANOVA with CRF as the dependent variable, time as the within subject variable (baseline and post-intervention), and treatment group (walking n=35; walking+nutrition n=29; dance n=40; and stretching control n=33) as the between subject variable, we found a main effect of time (F=11.00, p=0.001), indicating that all groups increased fitness. There was a trending significant time × group interaction (F=2.660, p=0.051), demonstrating that both walking groups showed larger gains in CRF from the intervention than the dance or stretching control.

3.1.2 No hemispheric ROI differences across time

To determine whether the right and left hemispheres differed in main effects we conducted a 2 (time) × 2 (hemisphere) repeated measures general linear modeling (GLM) analysis separately for the hippocampus and dorsolateral prefrontal cortex (DLPFC). In the model, we entered time (baseline and post-intervention) and hemisphere (right and left) as within subject factors, activation during recognition 'hit' trials as the dependent variable, and treatment group, change in fitness (continuous independent variable), age, and sex as covariates. For both the hippocampus and DLPFC, there was no main effect of time, meaning that activation in these ROIs did not change with time (HPC: F=0.76, p=0.39; DLPFC: F=0.04, p=0.84). There was a

main effect of hemisphere indicating that the left DLPFC had greater activation than the right, whereas the right hippocampus had greater activation than the left (HPC: $F=23.25$, $p=0.000$; DLPFC: $F=9.24$, $p=0.003$). The hippocampal finding fits with previous literature (Bunge et al., 2004). There were no significant first or second order interactions (for both HPC and DLPFC, all $F<3.5$, $p>0.06$). Although we had significant differences in hemisphere, we decided to use bilateral hippocampus and bilateral DLPFC for all further analyses.

3.1.3 No regional differences across time

To determine whether bilateral hippocampus and bilateral DLPFC responded similarly to the fitness intervention, we conducted a 2 (time) × 2 (ROI) repeated measures general linear modeling (GLM) analysis. In the model, we entered time (baseline and post-intervention) and ROI (HPC and DLPFC) as within subject factors, activation during recognition 'hit' trials as the dependent variable, and treatment group, change in fitness (continuous independent variable), age, and sex as covariates. Although there was no change in activation for either ROI after the intervention ($F=0.26$, $p=0.61$), there was a significant difference in activation between the DLPFC and hippocampus ($F=33.12$, $p=0.000$), such that the DLPFC had greater activation than the hippocampus for all pair-wise combinations (pair-wise t-test, all $p<0.000$). There were no significant first-or second-order interactions (all $F<3.3$, $p>0.07$). Although the hippocampus and DLPFC responded similarly to the intervention, since activation in the hippocampus and DLPFC were highly correlated (all $R>0.49$, $p=0.000$), we decided to test region-specific hypotheses of group in relation to function and task performance in models separate by region to avoid bias from collinearity.

3.1.4 No group differences in memory at baseline

To ensure that participants were properly randomized at baseline, we ran univariate GLM regressions separately for the DLPFC and hippocampus. In the model, we entered memory performance at baseline as the dependent variable and treatment group, ROI activation during recognition 'hits' at baseline, age, and sex as covariates. We included all bivariate interactions and removed any that were not above $p<0.05$. As expected, there was no main effect of group, indicating that memory performance was similar across groups at baseline (HPC: $F=0.30$, $p=0.83$; DLPFC: $F=0.26$, $p=0.85$). A significant age × hippocampus interaction indicated that the relationship between hippocampal activation and memory performance is positive for older adults less than 70 years old, but negative for older adults 70 years and older ($F=6.92$, $p=0.01$). One caveat to note about the robustness of this particular analysis is that there were many more participants in the young-old group (N=110) than the old-old group (N=27). The group × hippocampus interaction indicated that the control and dance groups showed a positive relationship between hippocampal activation and memory performance at baseline, whereas the walking groups showed a negative relationship ($F=2.77$, $p=0.045$).

3.1.5 No group differences in change in memory performance

To determine the effect of treatment group on change in memory performance, we conducted a repeated measures general linear modeling (GLM) analysis with d-prime score as the dependent variable, time (baseline and post-intervention) as the within subject factor, and change in ROI activation, age, and sex as covariates. We ran this model separately for the hippocampus and DLPFC. There was no main effect of time indicating that memory performance did not increase from baseline to post-test (HPC: $F=0.26$, $p=0.61$; DLPFC: $F=0.12$, $p=0.73$). Based on the CRF results, we hypothesized that the walking groups would show the greatest improvement in memory performance; however, we found no significant time × group

interaction (HPC: F=0.81, p=0.49; DLPFC: F=0.38, p=0.77). Because there were no groups effects of memory, we decided to collapse all groups and use CRF as a continuous variable to investigate its effect on ROI activation and memory performance at baseline and longitudinally.

3.2 Fitness as a continuous variable

3.2.1 CRF and hippocampal activation not associated with memory at baseline

We ran a univariate GLM regression to test the hypotheses that (1) baseline CRF predicts memory performance (*d'*) and (2) activation in the hippocampus mediates the relationship between CRF and memory performance. In the model, we entered memory performance at baseline as the dependent variable and CRF at baseline, whether the participant peaked during CRF testing at baseline, hippocampal activation during recognition 'hits' at baseline, age, and sex as covariates. Contrary to our hypothesis, there was no relationship between CRF and memory performance (F=0.01, p=0.92) or hippocampal activation during recognition 'hits' and memory performance (F=2.08, p=0.15). Furthermore, although we hypothesized that activation in the hippocampus would mediate the relationship between CRF and memory performance, the CRF × hippocampal activation interaction term indicated no evidence of mediation (F=0.94, p=0.33). We ran bivariate partial correlations post-hoc controlling for age, sex, and whether participant peaked during CRF testing to further investigate the relationships among CRF, hippocampal activation, and memory performance. We found no relationships between CRF and memory performance (R=0.009, p=0.92), memory performance and hippocampal activation during recognition 'hits' (R=0.13, p=0.15), or CRF and hippocampal activation (R=-0.004, p=0.97).

3.2.2 CRF and DLPFC activation not associated with memory at baseline

Similar to the above described analysis, we ran a univariate GLM regression to test the hypotheses that (1) baseline CRF predicts memory performance (*d'*) and (2) activation in the DLPFC mediates the relationship between CRF and memory performance. In the model, we entered memory performance at baseline as the dependent variable and CRF at baseline, whether the participant peaked during CRF testing at baseline, DLPFC activation during recognition 'hits' at baseline, age, and sex as covariates. Although we hypothesized that CRF and memory performance would be positively related, we did not find a significant relationship (F=0.007, p=0.93). Greater DLPFC activation during recognition 'hit' trials is (trending) positively associated with memory performance (F=2.58, p=0.11). Furthermore, there was no evidence that the DLPFC mediates the relationship between CRF and memory performance (F=1.56, p=0.214). We ran bivariate partial correlations post-hoc controlling for age and sex to further investigate the relationships among CRF, DLPFC activation, and memory performance. We found no relationship between CRF and memory performance (R=0.009, p=0.92), memory performance and DLPFC activation during recognition 'hits' (R=0.14, p=0.11), or CRF and DLPFC activation (R=0.01, p=0.91).

3.2.3 Change in hippocampal activation positively related to change in CRF and memory

To determine the effect of change in CRF and change in hippocampal activation on memory performance due to the fitness intervention, we conducted a repeated measures general linear modeling (GLM) analysis with d-prime score as the dependent variable, time (baseline and post-intervention) as the within subject factor, and change in CRF, change in hippocampal activation, whether participant peaked during CRF testing, age, and sex as covariates. There was a nearly significant main effect of time (F=3.52, p=0.06). Those who peaked during CRF testing at both baseline and post-test showed improvements in memory performance, while those that

did not peak at least once showed no improvements (F=3.36, p=0.069). Females improved memory performance over time, while males declined (F=7.02, p=0.009). We found no evidence of mediation by the hippocampus on the relationship between change in fitness and change in memory performance as evidenced by the non-significant Δhippocampal activation × ΔCRF interaction term (F=1.82, p=0.18). We ran bivariate partial correlations controlling for whether the participant peaked during CRF testing, age, and sex to further dissociate the relationships among change in CRF, change in hippocampal activation, and change in memory performance. Although we hypothesized that change in CRF would be positively correlated with change in memory performance, we found no relationship (R=-0.010, p=0.91). We did find that change in CRF was positively associated with change in hippocampal activation during recognition 'hits' (R=0.18, p=0.04) such that greater change in fitness is associated with a larger change in activation. Furthermore, change in hippocampal activation is associated with greater improvement in memory performance (R=0.25, p=0.004).

3.2.4 Change in DLPFC activation positively correlated with change in memory

We ran the same analyses described above to determine the effect of change in CRF and change in DLPFC activation on memory performance. Thus, we conducted a repeated measures general linear modeling (GLM) analysis with d-prime score as the dependent variable, time (baseline and post-intervention) as the within subject factor, and change in CRF, change in DLPFC activation, age, and sex as covariates. For this analysis, however, there was no main effect of time (F=1.47, p=0.23). Again, there was a significant time × sex interaction (F=4.55, p=0.04), indicating that across time females increased memory performance whereas males decreased. Furthermore, we found no evidence of mediation by the DLPFC on CRF and memory performance (F=0.23, p=0.63). To better interpret these data, we ran bivariate partial correlations

among change in CRF, change in DLPFC, and change in memory performance while controlling for whether the participant peaked during CRF testing, age and sex. There was no association between change in CRF and change in DLPFC activation during recognition 'hits' (R=0.07, p=0.44). However, similar to the hippocampus, we found that change in DLPFC activation positively correlated with change in memory performance (R=0.37, p=0.000).

3.3 Light Physical Activity as a Continuous Variable

3.3.1 Light physical activity decreases memory performance at baseline

Because several participants had missing accelerometer data at baseline or post-intervention, this sample size for the following physical activity analyses is 130. We ran a univariate GLM regression to test the hypotheses that (1) baseline light physical activity (PA) predict memory performance (*d'*) and (2) activation in the hippocampus and DLPFC mediates the relationship between light PA and memory performance. In the model, we entered memory performance at baseline as the dependent variable and light PA at baseline, ROI activation during recognition 'hits' at baseline, age, and sex as covariates. We ran analyses separately for hippocampus and DLPFC. We found a significant relationship between light PA and memory performance (HPC: F=3.68, p=0.036; DLPFC: F=4.87, p=0.029). However, it was in the opposite direction we predicted, with higher amounts of light PA predicting worse memory performance. More activation in the hippocampus was related to better memory performance (F=4.28, p=0.041), but there was no evidence of mediation by the hippocampus (F=0.00, p=0.98). There was no relationship between activation in the DLPFC and memory performance (F=0.08, p=0.77) and no evidence of mediation by the DLPFC (F=0.12, p=0.74). We further investigated the relationships among ROI activation, light PA, and memory performance using partial bivariate correlation while controlling for age and sex. Light PA was negatively correlated

with memory performance (R=-0.20, p=0.023). There was no relationship between light PA and hippocampal activation (R=-0.13, p=0.14), but a negative relationship between light PA and DLPFC activation (R=-0.19, p=0.028). Both hippocampal and DLPFC activation were positively correlated with memory performance (HPC: R=0.20, p=0.026; DLPFC: R=0.20, p=0.021). In other words, less light PA and more hippocampal activation separately contributed to better memory performance. While in the DLPFC, less PA was correlated with more activation in the DLPFC and more activation was related to better memory performance.

3.3.2 No relationship between change in light PA and change in hippocampal activation

To determine the effect of change in light PA and change in hippocampal activation on memory performance due to the fitness intervention, we conducted a repeated measures general linear model (GLM) analysis with d-prime score as the dependent variable, time (baseline and post-intervention) as the within subject factor, and change in light PA, change in hippocampal activation, age, and sex as covariates. There was no main effect of time (F=0.21, p=0.65). There was no evidence of mediation by the hippocampus on the relationship between light PA and memory performance (F=0.49, p=0.48). Bivariate partial correlation analysis revealed no relationship between change in light PA and change in hippocampal activation (R=0.09, p=0.34) or change in light PA and change in memory performance (R=0.05, p=0.55). There was a positive relationship between change in hippocampal activation and change in memory performance (R=0.25, p=0.005).

3.3.3 No relationship between change in light PA and change in DLPFC activation

We investigated the effect of change in light PA and change in DLPFC activation on memory performance using a repeated measures general linear model (GLM) analysis with d-prime score as the dependent variable, time (baseline and post-intervention) as the within subject

factor, and change in light PA, change in DLPFC activation, age, and sex as covariates. Once again, there was no main effect of time (F=0.09, p=0.76). There was no evidence of mediation by the DLPFC on the relationship between change in light PA and change in memory performance (F=1.08, p=0.30). Using bivariate partial correlation controlling for age and sex, there was a positive relationship between change in DLPFC and change in memory performance (R=0.33, p=0.00), yet no relationship between change in light PA and change in DLPFC (R=-0.02, p=0.87).

3.4 Moderate-Vigorous Physical Activity as a Continuous Variable

3.4.1 Hippocampus mediates relationship between MVPA and memory at baseline

We ran a univariate GLM regression to test the hypotheses that (1) baseline MVPA predicts memory performance (*d'*) and (2) activation in the hippocampus mediates the relationship between MVPA and memory performance. In the model, we entered memory performance at baseline as the dependent variable and MVPA at baseline, hippocampal activation during recognition 'hits' at baseline, age, and sex as covariates. Greater hippocampal activation predicted better memory performance (F=5.03, p=0.027) and activation in the hippocampus mediated the relationship between MVPA and memory (F=5.21, p=0.024). To better disentangle the mediation, we ran bivariate partial correlations controlling for age and sex. Both of the relationships between MVPA and memory performance (R=-0.076, p=0.39) and MVPA and hippocampal activation (R=-0.12, p=0.18) were non-significant but trending in a negative direction. Furthermore, there was a positive correlation between hippocampal activation and memory performance (R=0.20, p=0.026). Against our hypotheses, less MVPA may facilitate more hippocampal activation and more hippocampal activation is associated with better memory performance.

3.4.2 DLPFC mediates relationship between MVPA and memory during baseline

Similar to the analysis in the hippocampus, we used a univariate GLM regression with memory performance at baseline as the dependent variable and MVPA at baseline, DLPFC activation during recognition 'hits' at baseline, age, and sex as covariates. There was no association between MVPA and memory performance ($F=0.86$, $p=0.34$) or between activation in the DLPFC and memory performance ($F=2.55$, $p=0.11$). Furthermore, the activation in the DLPFC mediated the relationship between MVPA and memory performance ($F=6.97$, $p=0.009$). Using bivariate partial correlation while controlling for age and sex, we found a positive relationship between DLPFC and memory ($R=0.20$, $p=0.021$) but no relationship between MVPA and DLPFC ($R=-0.03$, $p=0.70$). Thus, similar to the hippocampus, less MVPA is associated with more DLPFC activation, which supports better memory performance.

3.4.3 Change in MVPA and change in hippocampal activation increase memory

To determine the effect of change in MVPA and change in hippocampal activation on memory performance due to the fitness intervention, we conducted a repeated measures general linear model (GLM) analysis with d-prime score as the dependent variable, time (baseline and post-intervention) as the within subject factor, and change in MVPA, change in hippocampal activation, age, and sex as covariates. There was a significant time × ΔMVPA interaction ($F=4.25$, $p=0.041$), indicating that d-prime score at post-intervention was positively associated with change in MVPA. There was no evidence of mediation by the hippocampus ($F=0.28$, $p=0.60$). Using bivariate partial correlation analysis, we more deeply explored the relationship among change in MVPA, change in hippocampal activation, and change in memory performance. There was no association between change in MVPA and change in hippocampal activation ($R=-0.01$, $p=0.091$). However, there were positive associations between change in

MVPA and change in memory performance (R=0.17, p=0.057) and between change in hippocampal activation and change in memory performance (R=0.27, p=0.003). Thus, both change in MVPA and change in hippocampal activation increase memory performance.

3.4.4 Change in MVPA and change in DLPFC activation increase memory

Similarly to the above described analysis, we used a repeated measures general linear model (GLM) with d-prime score as the dependent variable, time (baseline and post-intervention) as the within subject factor, and change in MVPA, change in DLPFC activation, age, and sex as covariates. There was a nearly significant mediation by the DLPFC (F=3.00, p=0.086). Using bivariate partial correlation, there was no significant relationship between change in MVPA and change in DLPFC (R=0.11, p=0.22). However, there were positive relationships between change in DLPFC and change in memory performance (R=0.33, p=0.000) and change in MVPA and change in memory performance (R=0.17, p=0.057). Thus, similar to the analysis in the hippocampus, both change in MVPA and change in DLPFC activation support better memory performance.

CHAPTER 4

DISCUSSION

4.1 Intervention Groups

Although we found that our intervention successfully improves CRF for the walking groups, we failed to find greater improvement in memory performance for these groups. Despite the positive relationship between CRF and memory in animal models and cross-sectional studies in humans, our results are in line with other longitudinal studies, which have largely failed to find group differences in change in memory performance from exercise interventions (Erickson et al., 2011b; Tamura et al., 2014; Ngandu et al., 2015). Our results mirror the findings of Ruscheweyh and colleagues (2011). They found that even though self-reported physical activity significantly increased in the two exercise conditions relative to control in a 6-month intervention, there were no group differences in memory performance. They only found an association between memory and physical activity when they collapsed across groups. Thus, we decided to collapse across groups and investigate the effect of CRF as a continuous variable. One potential reason we see no difference in memory performance between walking and control groups is that the control group increased their daily level of activity outside of the intervention, while the walking groups decreased their physical activity because they saw the exercise they were doing in the intervention as sufficient.

Most studies show that older adults have worse memory performance and diminished hippocampal activation during episodic memory retrieval compared to younger adults. By comparing extreme groups (e.g., young adults versus older adults) to understand how the brain changes with age, we fail to detect heterogeneous effects of age on cognition and brain function within older adults. In this study we found that young-older adults (less than 70 years old)

demonstrated a positive relationship between hippocampal activation and memory performance whereas old-older adults (70 – 80 years old) showed a negative relationship. Perhaps, in the young-older adult group individuals that show greater hippocampal activation resemble the young adults and this leads to better memory performance; whereas, in the old-older adult group greater hippocampal activation may signal the need for widespread compensatory activation and result in worse memory performance. More work investigating age-related trajectory of memory performance and brain activation across last few decades of life are needed to better disentangle these relationships.

4.2 CRF as a Continuous Variable

We collapsed across groups to investigate the relationship between CRF, hippocampal and DLPFC activation, and memory performance at baseline. We found no relationship between CRF and memory performance at baseline. This is surprising given previous cross-sectional studies that cite a positive relationship between CRF and memory performance (McAuley et al., 2011; Hayes et al., 2014a). In fact, McAuley and colleagues found positive correlations between CRF and subjective memory complaints and a frequency of forgetting questionnaire. Hayes and colleagues found a positive relationship between CRF and a face-name memory task, similar to our face-scene task. We hypothesized that CRF would be positively correlated with activation in the DLPFC and hippocampus based on evidence that (1) activation in DLPFC and hippocampus generally declines with age during retrieval tasks and (2) higher fit older adults have greater activation in task-relevant brain regions (Colcombe et al., 2004; Prakash et al., 2011; Holzschneider et al., 2012). Yet, we found no relationship between CRF and activation in either the hippocampus and DLPFC. We also found no relationship between memory performance and

activation in the hippocampus and DLPFC, although it was trending in a positive direction. It is unlikely that we were simply underpowered given that we had 137 participants in this study.

Although most studies do not mention the potential problem of participants not attaining "peak" during the GXT testing for CRF, many older participants voluntarily end the test before the criteria for peak is reach. This means that the VO2max that we use in analyses is quite noisy. We accounted for this by using a nuisance regressor during longitudinal analysis. Although the interaction term did not quite reach significant ($p=0.069$), we found that participants that peaked at both baseline and post-intervention showed an improvement in memory performance across time, whereas those that did not peak at least once, did not improve.

Many cross-sectional studies find that older adult females outperform males in episodic memory (Crook III et al., 1993). In this study we demonstrated that females improve memory performance over time, whereas males decline. Colcombe and colleagues' meta-analysis of the effects of aerobic exercise interventions on cognition came to a similar conclusion: when the group consisted of more females, the group as a whole showed greater cognitive benefits to exercise (Colcombe and Kramer, 2003).

Based on cross-sectional findings demonstrating a positive relationship between CRF and memory performance (McAuley et al., 2011; Baym et al., 2014; Hayes et al., 2014b), we hypothesized that in the longitudinal analysis, change in CRF would be positively associated with change in memory. However, we found no relationship between change in CRF and change in memory performance. Previous work using the same face-scene task in children found that after a 9-month aerobic exercise intervention, children demonstrated eye-movement patterns that indicated superior relational memory compared to the waitlist control group, despite no differences in behavioral performance (Monti et al., 2012). One limitation of our study is that we

did not record eye movements, which may be more sensitive to changes in fitness than behavioral measures. There is a paucity of literature investigating the relationship between change in CRF and change in task-related brain activation. One study found that changes in CRF positively correlated with changes in brain activation in the middle frontal gyrus and the cuneus during a spatial memory task, despite no relationship between CRF and memory performance (Holzschneider et al., 2012). We too found a positive association between change in CRF and change in hippocampal activation for 'hit' trials during retrieval. Furthermore, we found that change in hippocampal activation is highly positively correlated with change in memory performance. We suspected that hippocampal activation might act as a mediator between CRF and memory performance. However, we found no evidence for mediation by the hippocampus. Thus, CRF increases hippocampal activation and, in turn, increased hippocampal activation supports memory performance. There was also a strong positive relationship between change in DLPFC activation and change in memory.

4.3 Physical Activity as a Continuous Variable

One advantage of this study is that we measured physical activity using an accelerometer at baseline and post-intervention, whereas previous studies rely on self-reported measures of physical activity, which are notoriously noisy and unreliable. This is particularly the case for studies that ask participants to estimate their physical activities from previous decades (Bielak et al., 2014; Czajkowska et al., 2014). Yet, while most studies demonstrate positive relationships between physical activity and memory, we found that light PA is negatively related to memory performance at baseline. We found no relationship between MVPA and memory at baseline. We found that hippocampal activation mediated the relationship between MVPA and memory in an unexpected way: less MVPA may facilitate more hippocampal activation and more hippocampal

activation is associated with better memory performance. Longitudinally, we found no relationship between change in MVPA and change in memory performance. However, for both the hippocampus and DLPFC, we found a positive relationship with change in memory performance.

4.4 Limitations

Our episodic memory task may simply not have been sensitive enough to detect exercise-induced enhancements that exceeded the within-subject test-retest variability due to factors that are not of interest but can significant change memory performance, such as mood, sleep, and caffeine. Thus, one limitation is that we did not include eye movement data, which may have been more sensitive to underlying hippocampal function than memory performance. Because we found no group differences in memory performance we collapsed across group and used CRF at baseline and change in CRF as continuous variables. Correlation analyses reduce the ability to infer causality. It is possible that a confounding factor that we did not control for is actually driving the relationship.

4.5 Conclusions

In sum, we found no group differences in memory performance, no relationship between CRF and memory performance at a baseline, and no relationship between change in CRF and change in memory performance longitudinally. We did find that CRF is positively related to hippocampal activation and that greater hippocampal activation is associated with better memory performance, but there was no evidence of mediation by the hippocampus. Future research should investigate various imaging and behavioral measures of episodic memory may be more sensitive to detecting subtle changes in hippocampal functioning.

FIGURES AND TABLES

Table 1

Participant Demographics at Baseline

	Age	Sex	Education	Health	MMSE	VO2max
Control	65.76 ± 4.3	70%	16.33 ± 3.1	3.91 ± 0.8	28.64 ± 1.5	19.74 ± 4.0
Dance	65.70 ± 4.7	75%	15.33 ± 3.2	4.00 ± 0.7	28.63 ± 1.5	19.84 ± 4.0
Walking	64.66 ± 3.8	74%	16.23 ± 3.2	3.86 ± 0.6	28.66 ± 1.6	19.79 ± 4.4
Walking +	65.52 ± 4.3	76%	15.17 ± 2.1	3.93 ± 0.8	28.72 ± 1.3	19.81 ± 3.4
p-value	0.69	0.95	0.26	0.89	0.99	1.00

Note: Mini Mental Status Exam (MMSE), gold standard cardiorespiratory fitness (VO2max).

Table 2

	Control	Dance	Walking	Walking +
VO2max (Pre)	19.7 ± 4.0	19.8 ± 4.0	19.8 ± 4.4	19.8 ± 3.4
VO2max (Post)	20.0 ± 4.1	19.9 ± 5.1	21.2 ± 4.7	21.0 ± 3.4
Light PA (Pre)	289.0 ± 62.2	264.6 ± 68.0	269.0 ± 70.1	273.5 ± 62.4
Light PA (Post)	288.3 ± 60.5	269.3 ± 68.8	255.3 ± 63.1	264.7 ± 73.2
MVPA (Pre)	47.6 ± 26.0	43.5 ± 24.3	54.9 ± 43.7	40.6 ± 26.3
MVPA (Post)	51.3 ± 26.5	50.0 ± 24.8	66.9 ± 32.2	57.5 ± 21.7
D' (Pre)	1.13 ± 0.6	1.16 ± 0.6	1.05 ± 0.5	1.08 ± 0.4
D' (Post)	1.09 ± 0.6	1.21 ± 0.6	1.02 ± 0.5	1.15 ± 0.6

Table 2. Baseline and post-intervention data for fitness, physical activity, and memory performance.

Figure 1

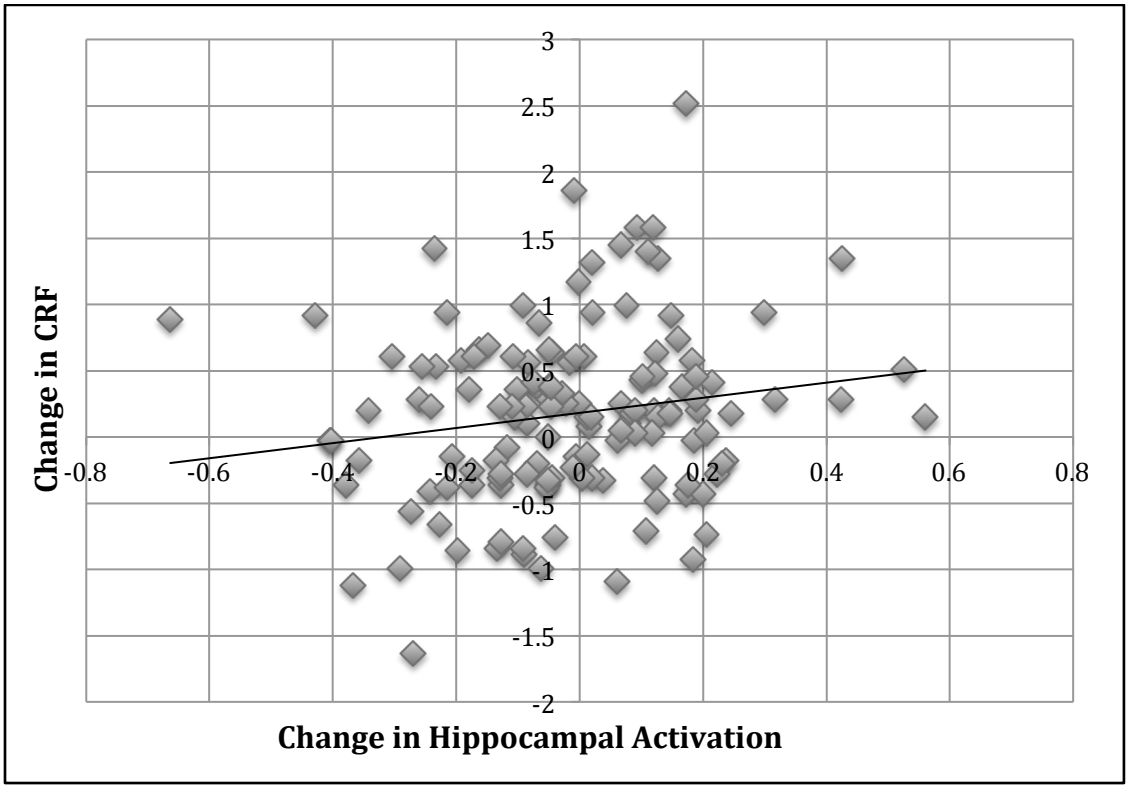

Figure 1. Positive correlation between change in CRF and change in hippocampal activation during 'hit' trials in retrieval task (R=0.18, p=0.04).

Figure 2

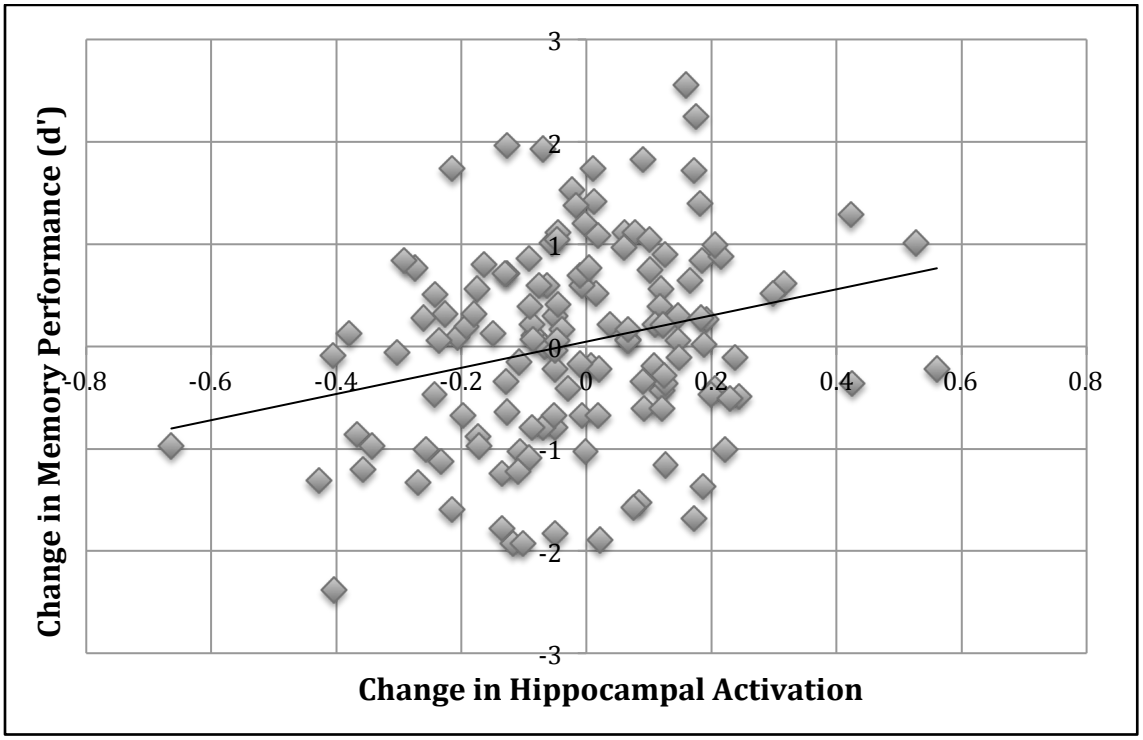

Figure 2. Positive correlation between change in hippocampal activation during 'hit' trials in retrieval task and change in memory performance (R=0.25, p=0.004).

Figure 3

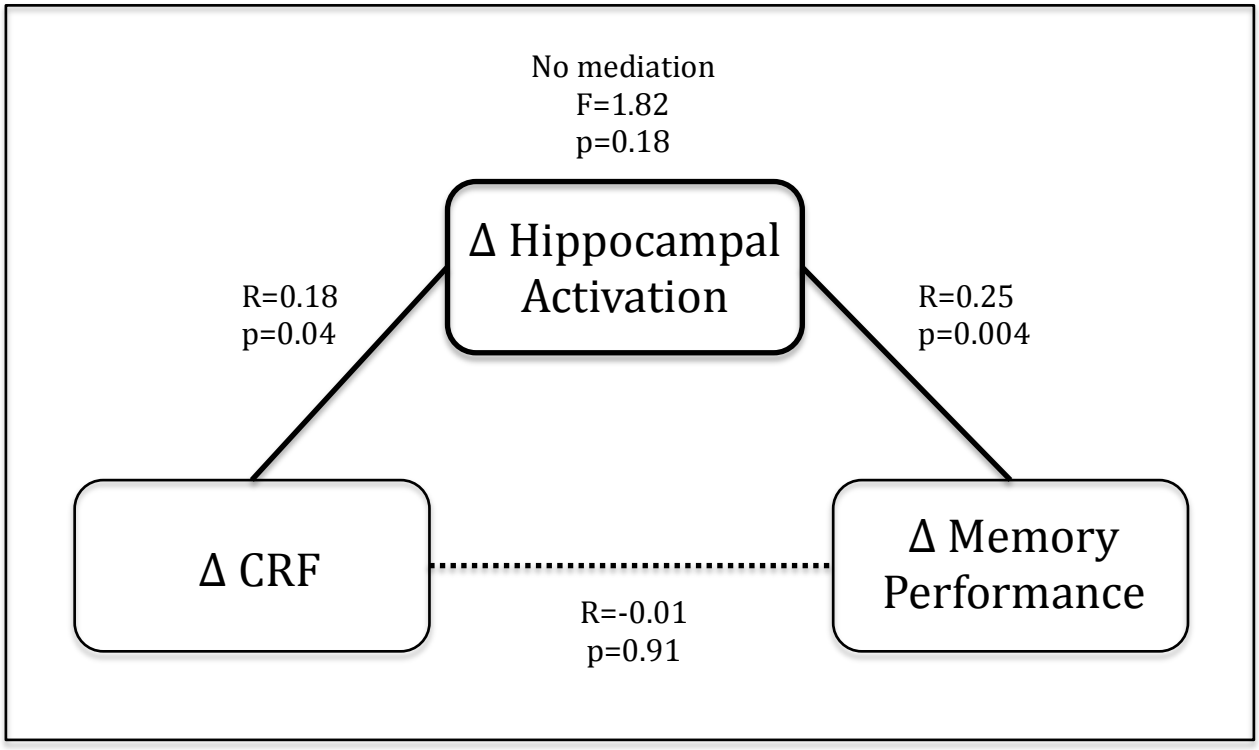

Figure 3. Graphical representation of correlations among change in CRF, change in hippocampal activation, and change in memory performance (d'). Although there were significant positive associations among (1) change in CRF and change in hippocampal activation and (2) change in hippocampal activation and change in memory performance, there was no mediation by the hippocampus on the relationship between change in CRF and change in memory performance.

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