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Cardiorespiratory fitness and virtual navigation in healthy older adults

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CARDIORESPIRATORY FITNESS AND VIRTUAL NAVIGATION IN HEALTHY OLDER ADULTS

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DEDICATION

I dedicate this work to my best friend and partner in crime, Amara, and to my loving family for their endless support.

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ABSTRACT

One of the earliest symptoms of Alzheimer's disease (AD) and age-related cognitive decline is topographical disorientation or impairment to spatial navigation. Furthermore, aging and AD are associated with cortical gray-matter thinning, particularly in the medial temporal and posterior cingulate regions, which have been associated with spatial navigation. Aerobic exercise has been well-established as a beneficial intervention to curtail the neurodegenerative effects of aging. This study aims to explore the relationship between cardiorespiratory fitness (CRF), and two markers of AD and cognitive aging, virtual navigation ability and cortical thickness of the entorhinal, parahippocampal and retrosplenial regions. Cross-sectional data utilized in this study was collected from 23 healthy older adults (60-80 years). Measures included in our analyses consisted of estimated VO_2max, T1-weighted structural MR images, and behavioral performance on a virtual navigation task, measured as numbers of objects located during recall. Cortical thickness of the regions of interest (ROIs) was determined by processing T1-weighted MR images in FreeSurfer. We hypothesized that greater CRF would correlate with improved virtual navigation performance and greater cortical thickness of ROIs. Our analyses did not reveal statistically significant relationships between CRF and navigation performance or CRF and cortical thickness. However, Pearson's correlations found right retrosplenial cortical (RSC) thickness and navigation performance to be

significantly related. Multiple regression models of right RSC thickness and navigation performance were performed controlling for age, sex, education and task version. These analyses revealed that greater right RSC thickness predicted navigation performance. Additionally, this model showed that older age predicts decline in navigation performance. Our findings did not survive multiple comparisons correction; nonetheless, the results provide promising insight to the relationship between cortical thickness and navigation performance in healthy aging. Further cross-sectional and longitudinal investigations with a larger sample size are required to assess the impact of CRF and exercise on cortical thickness and navigation abilities in healthy aging. Understanding these relationships would contribute to the expansive body of literature that has linked CRF and exercise to neuroprotective mechanisms in the aging brain.

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LIST OF ABBREVIATIONS

AD ..... Alzheimer's Disease

AHN ..... Adult Hippocampal Neurogenesis

BDNF ..... Brain Derived Neurotrophic Factor

CRF ..... Cardiorespiratory Fitness

DG ..... Dentate Gyrus

EC ..... Entorhinal Cortex

FDR ..... False Discovery Rate

fMRI ..... Functional Magnetic Resonance Imaging

HC ..... Hippocampus

HR ..... Heart Rate

IGF-1 ..... Insulin-Like Growth Factor 1

IQR ..... Interquartile Range

MCI ..... Mild Cognitive Impairment

MR ..... Magnetic Resonance

MTL ..... Medial Temporal Lobe

MWM ..... Morris Water Maze

PHC ..... Parahippocampal Cortex

PHG ..... Parahippocampal Gyrus

PPA ..... Parahippocampal Place Area

PRC ..... Perirhinal Cortex

ROI(s) ..... Region(s) of Interest

RPE ..... Rate of Perceived Exertion

RSC ..... Retrosplenial Cortex

VEGF ..... Vascular Endothelial Growth Factor

vMWM.....Virtual Morris Water Maze

VR .....Virtual Reality

INTRODUCTION

Normal aging is characterized by structural and functional brain changes, with associated cognitive decline. A significant cognitive aspect impaired by aging is spatial cognition, which includes spatial navigation (Klencklen et al., 2011). Spatial navigation is a complex, multisensory, cognitive skill involving a range of processes contributing to movement through space, route learning and locating objects in the environment (Moffat, 2009). During early stages of Alzheimer's disease (AD), patients display topographical disorientation, characterized by wandering and difficulty navigating outside of familiar environments (Pai & Jacobs, 2004). Assessing spatial navigation deficits in older adults may distinguish early markers of AD from normal cognitive aging (Lithfous et al., 2013). Additionally, navigation may serve as an outcome measure for determining the efficacy of behavioral interventions intended to slow cognitive aging, such as exercise (Moffat, 2009). Exercise has been increasingly explored as a therapeutic strategy for slowing cognitive decline and brain deterioration in the growing elderly population, with aerobic physical activity proving to be an effective, accessible intervention (Intlekofer & Cotman, 2013). However, physical activity is not universally accepted as a therapeutic for age-related neurodegeneration due to conflicting evidence (Voss et al., 2019). By investigating navigation task performance and cortical thickness in healthy older adults and their attenuation through exercise, we can perhaps translate attained knowledge to reinforce physical activity recommendations and promote successful aging.

1.1 Conceptual Framework of Spatial Navigation

Spatial navigation is a complex process requiring a variety of cognitive and perceptual abilities to integrate spatial information, ultimately guiding wayfinding and route planning in complex environments (Lithfous et al., 2013; Wolbers & Hegarty, 2010). Given the complex nature of navigation, it is best studied when broken down into components. Investigating navigation may be facilitated by employing spatial reference frames or representations to describe behavior. The two primary spatial reference frames are egocentric representations – guided by proximal cues from the navigators' perspective – and allocentric representations – guided by a flexible survey perspective of the environment, independent of the navigators' position (Lithfous et al., 2013). Allocentric and egocentric strategies may be used interchangeably or in combination to navigate a given environment and successful navigation is dependent on this flexibility (Burgess, 2006). Investigating navigation in humans is challenging due to constraints of exploring the real-world environment in the laboratory setting, thus virtual reality (VR) provides a controlled alternative to effectively study some of these processes. Given the difficulty of controlling a large-scale real-world environment, most studies examine navigation by using virtual environments or imagined wayfinding. Spatial navigation task performance has been correlated between VR and real environments; furthermore, virtual environments are closely related to and assess real-world navigation (Cushman et al., 2008).

1.2 Brain Basis of Spatial Navigation

Entorhinal-hippocampal communication is critical for navigation. The entorhinal cortex (EC) has direct projections to the hippocampus (HC) and the dentate gyrus (DG), a subregion of the HC (Witter et al., 1989). The EC, HC, parahippocampal cortex and perirhinal cortex compose the medial temporal lobe (MTL) memory system (Squire & Zola-Morgan, 1991). The EC relays sensory information about the environment from parahippocampal and perirhinal cortices to the HC (Suzuki & Amaral, 1994). The MTL is a region involved in declarative learning and memory, including aspects of spatial memory (Squire et al., 2004). Spatial memory is supported by hippocampal place cells, thought to form a cognitive, spatial map that may guide navigational processes (O'Keefe & Nadel, 1978). O'Keefe & Nadel (1978) discovered place cells in the rat HC, providing early evidence for the cognitive map hypothesis, which suggests hippocampal place cells encode a mental map of their environment to allow for flexible, allocentric navigation. Since the discovery of place cells, spatial navigation has been extensively studied in rodents, especially using the Morris Water Maze (MWM) task. Morris (1981) describes a task where rats must swim in opaque water in a circular pool and find a hidden platform, which allows exploration of spatial localization and directionality. Despite the absence of proximal or visible cues, the rats were able to learn the location of the platform, indicating that spatial encoding is possible even when only distal cues are available (Morris, 1981). When comparing normal rats to rats that have undergone hippocampal lesions, lesioned rats' performance on the MWM task was profoundly impaired, but lesioned rats eventually located the platform and learned that escape is possible (Morris

et al., 1982). Eichenbaum (2017) argues that the HC does not play a direct role in navigation, but rather is needed for memory organization via the cognitive map. Eichenbaum (2017) cites MWM impairments in rats with hippocampal lesions to be deficits in memory or retrieving the cognitive map, rather than deficits to navigation, as the lesioned rats must navigate in order to escape the task although they have not learned the location of the platform.

After the discovery of place cells in the HC, another group of cells was identified in the EC and termed 'grid cells' due to a grid-like firing pattern that contributes to spatial mapping (Hafting et al., 2005). Hafting et al (2005) reported a maintenance of grid cell firing pattern in rats despite visual deprivation, suggesting involvement in path integration, a mechanism critical for allocentric navigation. Path integration is a process where self-motion cues are monitored to keep track of one's location as they travel. For example, as one travels down a hallway their sensory systems provide information about the distance and direction travelled, allowing estimation of one's position in space. The EC has been further implicated in navigation, in which lesions to the EC of rats led to impairments in path integration (Parron & Save, 2004). Evidence of grid cells has been reported in the human EC from both functional magnetic resonance imaging (fMRI) and single-neuron recording (Doeller et al., 2010; Jacobs et al., 2013).

Human neuroimaging research has supported the roles of the HC and EC in navigation. Studies utilizing functional magnetic resonance imaging (fMRI), have shown activation of the HC and various MTL structures during virtual navigation (Hartley et al., 2003; Grön et al., 2000). Hartley et al. (2003) reported an association between increased

hippocampal activity and greater accuracy in wayfinding. In terms of structural MRI findings, Bohbot et al. (2007) linked greater grey matter density in the HC to use of allocentric strategies during virtual navigation. In contrast, a study exploring mental or imagined navigation of familiar environments in humans found no fMRI activation of the HC, instead activation was seen in regions including the parahippocampal and retrosplenial cortices (Rosenbaum et al., 2004). In regards to the EC, an fMRI study in humans demonstrated posterior medial EC activation while navigating through unfamiliar mazes (Brown et al., 2014). Furthermore, evidence of fMRI activation has been demonstrated in the human EC during both virtual navigation and imagined navigation, with the latter suggesting a role in route planning (Horner et al., 2016). The literature demonstrates the complexity of navigation and its structural counterparts. In summary, the general consensus is the HC and EC are vital for spatial memory as they encode map-like spatial codes, whereas other cortical regions such as the parahippocampal and retrosplenial cortices contribute to various aspects of navigation (Epstein et al., 2017).

1.3 Cortical Regions in Spatial Navigation

Beyond the cellular underpinnings of the cognitive map and the role of the EC-HC system, spatial navigation has been observed in various cortical regions. The parahippocampal cortex (PHC) and retrosplenial cortex (RSC) are two regions consistently shown to be vital for spatial navigation (Epstein, 2008). Evidence from non-human primates shows that the PHC is strongly connected to the RSC, which is located in the posterior cingulate region; further, both regions provide crucial input to the EC and

HC (Suzuki and Amaral, 1994, 1990). Lesions to these regions are linked to topographical disorientation in humans and deficits in wayfinding – finding one's way to a location (Aguirre & D'Esposito, 1999). In rats, lesions to the parahippocampal gyrus (PHG), which encompasses the entorhinal and perirhinal cortices, led to deficits in place learning, the spatial localization of a target without local cues (Nagahara et al., 1995). In humans, evidence from an fMRI study demonstrates activity of the PHC during topographic learning of virtual mazes (Aguirre et al., 1996). Further exploration of the PHC led to the discovery of a specific, posteriorly located region termed the parahippocampal place area (PPA), due to its activation during scene/place viewing, principally scenes with spatial context (Epstein & Kanwisher, 1998). In an fMRI study, mental navigation tasks showed PPA activity, likely due to mental imagery of the locations being visualized (Rosenbaum et al., 2004). Collectively, these studies demonstrate the PHC's essential role in navigation and scene processing.

The RSC has several unique properties with its contribution to navigation, one being its strong activity in response to familiar environments during scene processing (Epstein et al., 2007a), a contrast to the PPA which is unaffected by familiarity (Epstein et al., 1999). It is important to note that the RSC is occasionally defined beyond the anatomical limits of Brodmann's area 29 and 30 and is instead viewed as a functional region including the posterior cingulate cortex (Epstein et al., 2007b; Latini-Corazzini et al., 2010). An fMRI study requiring participants to mentally navigate in a familiar environment from one location to another, revealed a strong RSC activation (Ino et al., 2002). As reported in other anatomical regions, lesions to the RSC in rats have been

consistently shown to cause navigation deficits (Aggleton & Vann, 2004). Similarly, RSC damage in humans was repeatedly implicated in topographical disorientation and difficulty establishing a direction of travel, with the notable feature of recognizing familiar scenes but being unable to utilize them for orientation (Maguire, 2001). Additionally, the RSC is thought to mediate between egocentric and allocentric reference frames (Epstein et al., 2017; Latini-Corazzini et al., 2010). In sum, the RSC is a key region supporting the navigational network by enabling directional orientation and scene processing.

1.4 Aging, Neurodegeneration & Navigation

Deterioration in normal aging is widespread, however, the magnitudes of volumetric shrinkage are variable and particularly accelerated in hippocampal and entorhinal regions (Raz et al, 2005). Evidence from the Baltimore Longitudinal Study has shown that brain atrophy in normal aging to manifest in several ways, one of which is diffuse declines in cortical thickness, a morphometric measure of gray matter determined by the distance between white matter and pial surfaces (Thambisetty et al., 2010). Declines in cortical thickness during normal aging were observed in an anterior-posterior gradient, in which frontal and parietal regions are affected prior to temporal and occipital regions; moreover, the precuneus and cingulate regions also exhibited substantial thinning (Thambisetty et al., 2010). Lemaitre et al. (2012) observed global cortical thinning in normal aging, as well as robust, regional reductions to several regions including the posterior cingulate, precuneus, precentral, paracentral and frontal gyri.

While the literature is not in agreement, some studies have shown EC atrophy to occur prior to HC atrophy, in preclinical stages of AD (Killiany et al, 2002). Regions with significant inputs to the entorhinal-hippocampal memory system such as the retrosplenial and parahippocampal cortices have also exhibited atrophy in early AD (Villain et al, 2008). Buckner (2004) suggested that memory deficits in AD may be driven by the disruption of the MTL and precuneus, extending into the posterior cingulate and retrosplenial cortex, given that these regions are some of the first to be affected in AD progression. Collectively, numerous anatomical regions evidenced to profoundly degenerate in normal aging are also known to be involved in navigation.

Aging rodents exhibit impairments in spatial navigation and place learning. Aging rats have been reported to perform poorly on place learning tasks, suggesting deficits in spatial memory (Barnes et al., 1980). A study investigating the firing patterns of hippocampal place cells found that older rats in comparison to younger rats show deficits in place cell firing (Barnes et al., 1983). Studies in transgenic mouse models of AD also provide evidence for impaired spatial learning in various behavior tasks including MWM (Walker et al., 2011; O'Leary & Brown, 2009). Evidence of spatial memory deficits in aging and AD rodent models provides a foundation that may guide exploration of navigation in aging humans.

Studies investigating navigation across age in humans have repeatedly found significant differences when comparing younger and older adults. One of the first studies to explore virtual navigation in aging found that older adults exhibit impairments in navigation performance as evidenced by a greater number of errors, distance traveled and

amount of time needed for maze completion (Moffat et al., 2001). Further evidence of navigation impairment in older adults was reported by Moffat & Resnick (2002), in which age effects on place learning were present in a virtual version of the MWM task (vMWM). The vMWM task is thought to require greater allocentric processing than a task with the same starting and ending location across trials (Moffat & Resnick, 2002). Exploration of fMRI activity during a virtual navigation task that encourages allocentric encoding, found reduced activation of the HC, PHC and RSC in older compared to younger adults (Moffat et al., 2006). Although much lesser in severity, these findings are not surprising given the wandering behavior and impairments in wayfinding that present in AD and other dementias (Klein et al, 1999; Pai & Jacobs, 2004).

1.5 Effects of Aerobic Fitness and Exercise on Brain Structure

Several rodent studies have shown that aerobic exercise promotes adult neurogenesis in the dentate gyrus region of the hippocampus and angiogenesis in many brain areas, leading to improvements in learning and memory (Voss et al., 2011). Adult neurogenesis, the birth of new neurons in the adult brain, is a process that is almost exclusive to the dentate gyrus of the hippocampus (Eriksson et al, 1998); this process is often referred to as adult hippocampal neurogenesis (AHN). Several studies utilizing rodent models have linked voluntary wheel running to AHN across the lifespan and in AD-like pathology (Voss et al., 2019). A study comparing running mice to controls found that physical activity enhances adult neurogenesis in the dentate gyrus, MWM learning and long-term potentiation, a measure of synaptic plasticity (van Praag et al., 1999)

thought to underlie learning and memory (Bliss & Collingridge, 1993). The positive effects of exercise are not limited to young adult mice, as aged mice that underwent voluntary wheel running for 45 days exhibited enhancement in hippocampal neurogenesis and spatial learning (van Praag et al., 2005).

Other structural changes may also occur as a result of aerobic exercise. For example, a study exploring running in rats found increases in dendritic spine density in the HC and EC (Stranahan et al., 2007). Environmental enrichment is often utilized as an intervention in rodent studies to explore structural and functional attenuation of the brain. An enriched environment is one supplemented by stimulating objects such as balls, ladders, tunnels and running wheels, in contrast to an impoverished environment where the animals are housed separately in small cages (Mohammed et al., 2002). As summarized by Mohammed et al. (2002), enriched environments have been shown to have various beneficial effects on the rodent brain including greater brain weight, thicker cerebral cortices and increased glial cells. Rodents exposed to enriched environments exhibit greater entorhinal cortical thickness when compared to rodents in an impoverished environment (Diamond, 1988; Mohammed et al., 2002). Importantly, aerobic exercise or the presence of a running wheel was determined to be the driving component of enhanced neuroplasticity in an enriched environment (Mustroph et al., 2012).

Research from animal models provides a neurobiological foundation for human subject research by identifying exercise-induced mechanisms of change; however, findings from animal models may not necessarily translate to humans. Human subject

research is limited in its ability to explore neurogenesis and structural changes at the cellular level without donation of post-mortem tissue, although some MRI methods claim to image neurogenesis (Sierra et al., 2011). Neuroimaging research has produced robust literature surrounding structural and functional changes to the human brain in response to various interventions. Aerobic exercise in particular has been repeatedly shown increase the size of cortical structures and improve cognition. A meta-analysis of the literature exploring aerobic exercise and hippocampal volume, determined that aerobic exercise had a significant positive effect on left hippocampal volume (Firth et al., 2018). Previous work from our laboratory linked greater cardiorespiratory fitness (CRF) in young adults to greater EC volume (Whiteman et al., 2016). More recent work from our laboratory has shown improved CRF and increased DG/CA3 volume in the hippocampal head following exercise training to be positively correlated in young adults (Nauer et al., 2019). These findings are not limited to younger adults, as Erickson et al. (2009) demonstrated that older adults with higher aerobic fitness levels showed greater preservation of hippocampal volume. Additionally, a one-year aerobic exercise intervention increased hippocampal volume in a cohort of older adults (Erickson et al., 2011). Limited research has linked these structural changes to improvements in spatial learning as seen in rodents.

The beneficial effects of aerobic exercise on brain structure and function are likely the result of several mechanism. One mechanism thought to play a vital role, is the effect of brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF) which are upregulated in response to aerobic exercise (Carro et al., 2001; Fabel et al, 2003; Neeper et al., 1995). These factors

have been linked to improvements in learning, as well as promotion of angiogenesis and AHN. However, limited evidence is available from human studies to support these factors' role in neural plasticity (Cotman et al., 2007). Compellingly, Erickson et al. (2011) reported a positive association between resting serum levels of BDNF and hippocampal volume in older adults.

Evidence from a randomized controlled trial has shown aerobic exercise to be associated with increased volume of grey and white matter in older adults primarily in prefrontal and temporal cortices (Colcombe et al, 2006). Similar findings indicated that greater physical activity is associated with greater grey matter volume, in prefrontal and temporal regions which include the HC and EC (Erickson et al., 2010). Studies have also linked aerobic fitness and exercise to increased cortical thickness in several brain regions including the parahippocampal, cingulate and entorhinal cortices (Stern et al., 2019; Scheewe et al., 2013; Williams et al., 2017). A study exploring a twelve-week balance training intervention also found increases in cortical thickness in the superior temporal cortex, posterior cingulate cortex and superior frontal sulcus (Rogge et al., 2018). Collectively, this evidence suggests a role for aerobic exercise and physical activity in modulating brain volume and cortical thickness.

1.6 Effects of Aerobic Fitness on Spatial Cognition & Navigation

Aerobic exercise is associated with a broad spectrum of improvements in cognition with supporting evidence from both human and animal models (Voss et al., 2019; Cassilhas et al., 2016; Barak et al., 2015). Rodent literature shows improvements to

spatial memory in response to wheel running, as demonstrated by tasks involving navigation and wayfinding such as the MWM, Y-maze and radial arm maze tasks (van Praag, 2008). Human subject data from a cohort of middle-aged adults, revealed that those with greater CRF at baseline, showed greater fMRI activation of the HC, RSC, PHG and several other regions, during spatial learning of a virtual environment (Holzschneider et al., 2012). However, participants' spatial learning on the virtual maze task did not improve after the intervention (Holzschneider et al., 2012). Herting & Nagel (2012) conducted a study in an adolescent human cohort to investigate aerobic fitness, hippocampal volume and virtual MWM performance. Results from this study provided evidence for an association between aerobic fitness and spatial learning on vMWM, although memory recall did not correlate with CRF (Herting & Nagel, 2012). Although aerobic exercise is linked to improvements in various aspects of cognition, limited research provides insight to its effect on spatial navigation.

1.7 Hypotheses & Goals

The current study explores the role of CRF as a modulator of spatial navigation and cortical thickness in healthy older adults. Insights from this investigation may be valuable to countering neurodegeneration and cognitive decline in the aging population. The first aim was to examine the relationship between CRF and navigation performance, assessed by estimated VO_2max and recall of object locations in a virtual navigation task adapted from the Moffat lab (Moffat et al., 2006). Rodent and human literature demonstrate that aerobic exercise improves spatial learning, suggesting that spatial

navigation may be modulated by CRF. Given existing findings, I hypothesize that CRF will predict navigation performance, after controlling for the covariates of age, sex, and education. The second aim was to examine the relationship between CRF and cortical thickness in brain regions associated with navigation, and whether cortical thickness in these regions is associated with navigation performance. MR images were processed to automatically parcellate cortical regions and estimate cortical thickness. Several studies in both rodent and human models have demonstrated greater cortical thickness to be associated with aerobic exercise or CRF. Guided by previous literature, I hypothesize that greater CRF will be positively correlated with cortical thickness of the entorhinal, parahippocampal and retrosplenial cortices, and that greater cortical thickness will correlate with improved navigation performance.

METHODS

2.1 Participant Recruitment

Participants included in the analyses were recruited from the greater Boston area primarily using advertisements, for a larger study entitled *The entorhinal cortex and aerobic exercise in aging*, run by the Brain Plasticity and Neuroimaging Lab at Boston University School of Medicine. Eligibility was established through telephone screening and followed by an in-person visit for those meeting inclusion and exclusion criteria. Participants were cognitively intact, non-smokers between 60 to 80 years of age, who live in the greater Boston area and are fluent English speakers, defined by attending elementary school and higher in English. Participants were required to be physically inactive or sedentary for three months prior to joining the study; sedentary status was defined as performing less than 30 minutes of moderate intensity physical activity, three times per week (The American College of Sports Medicine [ACSM], Pescatello et al., 2014). Moderate intensity physical activity commonly includes brisk walking, dancing, tennis doubles and heavy cleaning such as vacuuming or washing windows (ACSM, 2014). Individuals with cardiovascular, respiratory, metabolic, musculoskeletal and neurological or psychiatric conditions, were excluded to reduce risk of harm and potential effects on outcomes of interest, such as the known effect of depression on hippocampal volume. Additionally, use of cardioactive and psychoactive medications was exclusionary due to possible alterations to heart rate (HR) response and other outcome measures (ACSM, 2014). To ensure safety during MRI procedures, participants with ferromagnetic materials in their body, history of severe claustrophobia or motion sickness

were excluded. Prior to engaging in study procedures, eligible participants were presented with informed consent forms and signed consent was obtained from those who wished to continue.

2.2 Participant Demographics

Baseline data from twenty-three participants (18 female, 5 male) will be included in the analyses. Six participants were excluded, from the possible twenty-nine, due to discovery of incidental findings (n=2) or an inability to reach 85% of predicted maximum HR during CRF testing (n=4). Participants ranged from 60 to 80 years of age, with a mean of 68.12 and a standard deviation of 5.86 years. Education was also self-reported with a mean of 17.26 years and a range of 12 to 20 years (Table 1).

2.3 Cardiorespiratory Fitness Testing

To determine CRF, participants underwent a submaximal incremental treadmill test intended to estimate maximum oxygen uptake (VO_2 max), which took place at the Boston University Fitness and Recreation Center. To maintain participant safety, a submaximal modified Balke protocol was used as opposed to a maximal graded exercise test with gas exchange (Cooper & Storer, 2001). Estimation of VO_2max was based on the linear relationship between HR and oxygen uptake (Wasserman et al., 2012). The test was conducted on a treadmill at a fixed, comfortable speed selected for each individual, correspondingly, the incline was increased each minute. Gradient increases were chosen to terminate the test in 8-12 minutes, in which participants had to reach 85% of their age-

predicted maximum HR (Buchfuhrer et al., 1983). To calculate age-predicted maximum HR, we followed an equation as previously described (Tanaka et al., 2001). We then multiplied the age-predicted maximum HR by 85% to establish a target HR for each participant. Designated warm-up and recovery periods were included before and after testing, furthermore, blood pressure and HR were recorded before, during and after the test. During the test, HR was recorded at the last 5-10 seconds of each minute interval using a Polar HR monitor fitted to the participant's chest and an associated HR watch, which was wirelessly connected to the monitor. To assure participant comfort and safety, Borg scale ratings of perceived exertion (RPE; Borg, 1982) and blood pressure were recorded every three minutes. CRF assessment was terminated when participants reached 85% of age-predicted maximum HR, if requested by the participant or if they experienced adverse symptoms. Speed (*S*) and percent treadmill grade expressed as a fraction (*G*) were used to estimate a VO_2max score using a metabolic equation for walking assessments (ACSM, 2014):

$$VO_2 = 0.1\,\text{ml/kg}\cdot\text{min} \times S + 1.8\,\text{ml/kg}\cdot\text{min} \times S \times G + 3.5\,\text{ml/kg}\cdot\text{min}$$

Estimated VO_2max scores were converted to percentiles to adjust VO_2max for age and sex differences (ACSM, 2014); means, standard deviations and ranges of estimated VO_2max scores and percentiles were reported (Table 1).

Table 1. ***Participant Demographics***

There are no significant differences in age ($t_{21} = 1.234$, $p = 0.231$), education ($t_{21} = 1.946$, $p = 0.082$) and VO_2max Percentile ($t_{21} = 1.811$, $p = 0.089$) between men and women. A significant difference was found for VO_2max across sex ($t_{21} = 4.454$, $p = 0.0004$).

Variable	**Total** *N = 23*	**Female** *n = 18*	**Male** *n = 5*
Age Range, years	60 – 80	60 – 76	65 – 80
Mean Age ± SD	68.17 ± 5.86	67.39 ± 5.79	71.00 ± 5.79
Education Range, years	12 – 20	12 – 20	16 – 20
Mean Education ± SD	17.26 ± 2.56	16.83 ± 2.62	18.8 ± 1.79
VO_2max Range, mL/kg/min	17.68 – 40.99	17.68 – 39.52	33.88 – 40.99
Mean VO_2max ± SD***	30.18 ± 6.64	28.35 ± 6.25	36.78 ± 2.65
VO_2max Percentile Range	2 – 97	2 – 97	50 – 92
Mean VO_2max Percentile ± SD	54.87 ± 32.13	50.67 ± 34.55	70.00 ± 15.43

***p < .001

2.4 MR Image Acquisition

MR images were acquired using a 3-Tesla Siemens MAGNETOM Prisma scanner with a 64-channel head coil, located in the Cognitive Neuroimaging Center at Boston University. Prior to scanning all participants were thoroughly screened for potential contraindications. Several scans were conducted for *The entorhinal cortex and aerobic exercise in aging* intervention study; however, only structural T1-weighted images from baseline will be used in the analyses described here. High-resolution whole-brain T1-weighted scans were acquired with a magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence (TR = 2200 ms, TE = 1.67 ms, TI = 1100 ms, flip angle = 7°, field of view = 230 x 230 mm, frames = 176, voxel resolution = 1.0 mm x 1.0 mm x 1.0 mm).

2.5 Allocentric Navigation Task

The VR allocentric navigation task conducted during the MRI visit of the study was designed by Dr. Scott Moffat. This task was adapted from an established Moffat lab task that showed reduced activation in HC, PHG, RSC and parietal regions in older adults compared to younger adults during VR navigation (Moffat et al., 2006). The Moffat lab VR task, displayed on a computer, consisted of several rooms connected by hallways with various objects distributed in the virtual environment (Moffat et al., 2006). The task involved three stages: (1) training using a practice environment different than the testing environment, (2) encoding of the testing environment, and (3) recall of the testing environment. At the start of the visit, the task was briefly described and participants were instructed on how to use a button box, allowing movement in four directions, to navigate the practice environment. Participants completed extensive training to become comfortable with using the button box and navigating through the environment. Following training, participants explored one of two testing environments (version 1 & 2) and learned the location of six common objects. The instructions emphasized learning the virtual environment and location of objects during encoding. Additionally, participants were encouraged to construct a mental map of the environment from a bird's eye view. Following encoding, the participants underwent a recall phase, where memory of navigating to a target object was tested. During the recall phase, the participants were prompted to locate a target object in a 60 second interval. If the object was successfully approached, participants received a point and were prompted to locate a different object; however, if the incorrect object was approached, this was noted as an attempt and a new

prompt was provided. Performance was assessed by the experimenter who manually scored each target object, without indicating to the participant whether they were successful or not. The final scores were quantified as 'objects located' and 'objects attempted' for each participant. Number of 'objects located' during recall will be used as the measure of navigation task performance for these analyses.

2.6 Measurement of Cortical Thickness

Processing of MR images was performed using the FreeSurfer version 6.0 software package on Boston University's Shared Computing Cluster, in which cortical parcellations were used to calculate an average distance between the pial and white matter surfaces of various cortical regions. Processing consisted of automated computational techniques, summarized in Dale et al. (1999) and Fischl (2012), that provided data such as regional cortical thickness. Briefly, cortical surface reconstruction includes Talairach registration, intensity normalization, skull-stripping, segmentation of white matter and sub-neocortical structures, and surface tessellation (Dale et al., 1999). Automated reconstruction of cortical surfaces and high-resolution surface-averaging of T1-weighted MR images generated models of the pial and white matter surfaces (Fischl & Dale, 2000). Cortical thickness is then calculated as an average distance between the pial and white matter surfaces of a given cortical region along each point of the white matter surface in three-dimensions. Cortical regions were defined based on probabilistic information from manually labelled training sets, allowing the software to estimate and label neuroanatomical regions of a given MRI (Desikan et al., 2006). For the current

analyses, cortical thickness outputs were taken from two automated labelling atlases that define different cortical regions. The Desikan-Killiany atlas subdivides the cortex into gyral based regions of interest (Desikan et al., 2006); this atlas was used to parcellate the EC and PHC. The Destrieux atlas uses gyri and sulci to divide the cortex into various ROIs (Destrieux et al., 2010), notably the posterior-ventral cingulate region which will be considered an approximation of the RSC for these analyses.

2.7 Statistical Analyses

Statistical analyses were performed using RStudio (version 1.2.1335). All variables demonstrated normal distribution, except for number of objects located. Normally distributed continuous variables were reported as means ± standard deviation (Table 1, Table 4). Two-sample t-tests were used to assess differences in mean age, education, and CRF by sex and differences in mean cortical thickness by hemisphere. Participants were also divided into higher and lower fitness groups based on a median split of estimated VO_2max. Median splits were conducted separately for males and females to account for sex differences in VO_2max. Females and males below their respective medians were categorized as low-fit, whereas those greater than or equal to the median were considered high-fit. Two-sample t-tests were used to assess differences in mean age, education and CRF between high-fit and low-fit participants. Number of objects located were reported as medians and interquartile ranges (IQR) due to their non-normal distribution (Table 2). Wilcoxon rank sum tests were used to assess differences in navigation performance by sex and fitness level. A multiple linear regression model was

used to assess the relationship between CRF and objects located with age, sex, education and task version as covariates (Table 3). Since there were two distinct virtual environments of the navigation task randomized to each participant, we included task version as a covariate to control for potential confounding. One version of the task may have been more difficult than the other, and this may confound a significant relationship. Pearson's correlations were used to elucidate relations between CRF and cortical thickness (Table 5). Although the number of objects located was not normally distributed, diagnostic plots revealed that the data was sufficiently linear. Accordingly, Pearson's correlations were conducted to determine associations between ROI cortical thickness and objects located (Table 6). Significant correlations were explored in multiple linear regression models that included age, sex, education and task version as covariates (Table 7, Figure 3). Significant findings were adjusted for multiple comparisons using the false discovery rate (FDR) method, with the *p.adjust* function in R.

RESULTS

3.1 Participant Descriptive Statistics and VR Navigation Task Performance

Baseline data from twenty-three participants were included in these analyses. Participant demographics are reported for the total and stratified sample, based on sex (Table 1). Participants had a mean age of 68.17 years (5.86), a mean 17.26 years (2.56) of education, and a mean VO_2 max of 30.18 mL/kg/min (6.64). There were no significant differences in age, education and VO_2 percentile between females and males ($p > 0.05$). However, a two-sample t-test revealed a statistically significant difference in mean VO_2max in women (28.35 ± 6.25 mL/kg/min) and men (36.78 ± 2.65 mL/kg/min); males had a greater mean VO_2max than women ($t_{21} = 4.454$, $p<0.001$). Figure 1 displays boxplots of estimated VO_2max distribution by sex to provide a visual demonstration of the difference. As expected given the use of a median-split, a two-sample t-test of estimated VO_2max in high-fit (34.85 ± 3.46 mL/kg/min) and low-fit (25.09 ± 5.41 mL/kg/min) participants showed a significant difference in means ($t_{21} = -5.0959$, $p<0.001$). Figure 2 displays boxplots of estimated VO_2max by fitness level.

Figure 1. ***Distribution of VO_2max by Sex***

Boxplots of estimated VO_2max distribution by sex. There is a statistically significant difference in mean estimated VO_2max between females and males ($p<0.001$).

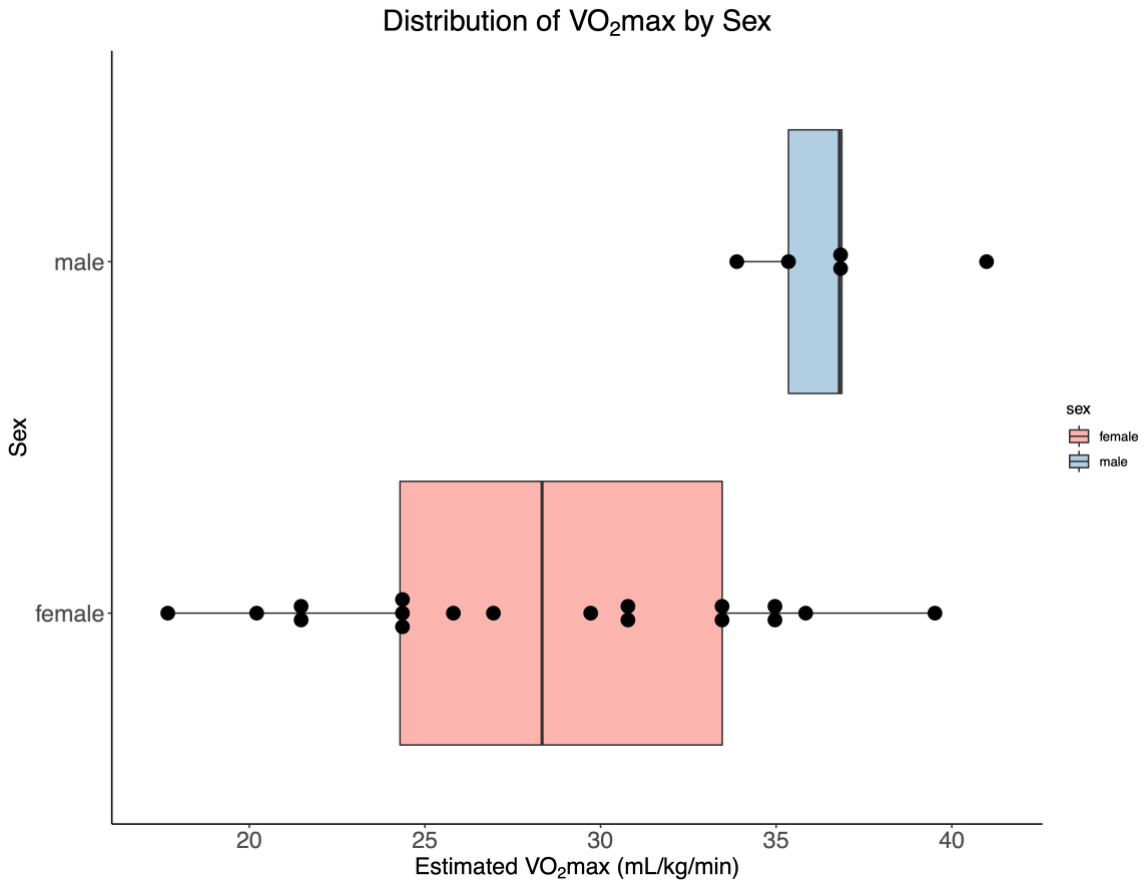
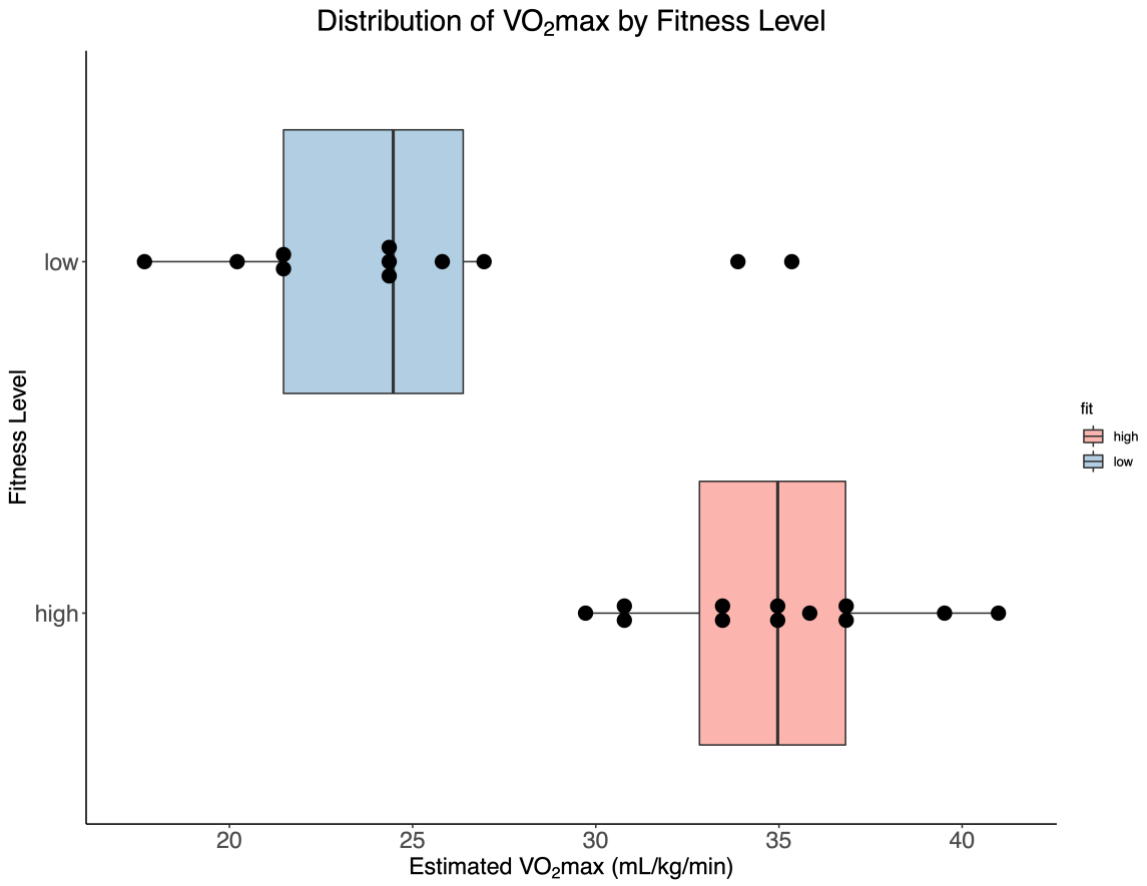


Figure 2. ***Distribution of VO2max by Fitness***

Boxplots showing distribution of estimated VO2max by fitness level.



Navigation task performance is summarized by the median number of objects located during recall for the total group and stratified by sex and fitness (Table 2). To explore potential differences in the cohort, Wilcoxon rank sum tests were conducted for males and females, as well as for the high-fit and low-fit participants. Although higher fitness participants had a greater median and IQR for objects located (4.5, 2-6) than lower fitness participants (2, 1-2.5), no significant differences were found in the number of objects located between high-fit and low-fit participants (U_{21}= 37.5, p= 0.0803) or males and females (U_{21}= 51.5, p= 0.650).

Table 2. ***Navigation Task Performance***

Median number of objects located during recall phase of VR task in the total sample and stratified subsamples based on sex and fitness level.

Objects Located	**Total** (N = 23)	**Sex**		**Fitness**	
		Female (n = 18)	Male (n = 5)	High (n = 12)	Low (n = 11)
Median	2	2.5	2	4.5	2
IQR	1.5 – 5	1.25 – 4.75	2 – 9	1 – 2.5	2 – 6

3.2 Cardiorespiratory Fitness and Navigation Task Performance

To assess whether CRF predicts navigation performance, we used a multiple linear regression model of estimated VO_2max as the primary predictor of number of objects located with age, sex, education and task version as covariates (Table 3). Contrary to the hypothesized relationship, we did not find a significant association for CRF predicting navigation performance (B = 0.16, t = 1.4, p = 0.180) (Table 3). None of the other predictors were significantly associated with predicting navigation performance.

Table 3. ***Multiple Regression of Navigation Performance and VO_2max***

Multiple regression model of VO_2max and navigation performance, controlling for age, sex, education and task version (*Adjusted* R^2 = 0.227, p = 0.0917)

Predictors	**Regression Coefficient** *(B)*	***t*-statistic**	**p-value**
VO_2max, mL/kg/min	0.1548	1.400	0.1795
Age, years	-0.1617	-1.360	0.1917
Sex (1 = male, 2 = female)	0.6852	0.355	0.7268
Education, years	0.5487	2.043	0.0568
Task Version (1= ver1, 2 = ver2)	-1.2452	-0.892	0.3847

Ver1, version 1; ver2, version2

3.3 Descriptive Statistics of ROI Cortical Thickness

Cortical thickness of each region of interest are reported as a mean ± standard deviation (Table 4). Participants had a mean EC thickness of 3.42 *mm* (±0.25) in the right and 3.63 *mm* (0.33) in the left hemispheres; a mean PHC thickness of 2.73 *mm* (0.23) in the right and 2.75 *mm* (0.26) in the left hemispheres; and a mean RSC thickness of 2.59 *mm* (0.17) in the right and 2.55 *mm* (0.23) in left hemispheres. There are no significant differences in mean thickness of EC (*t = -0.699, p= 0.488*), PHC (*t = 0.293, p= 0.771)*, and RSC (*t = -0.699, p= 0.488*) between the right and left hemispheres. Additional exploratory t-tests were run for each cortical region by sex and by fitness level; however, no differences were found (p>0.05) (Table 4).

Table 4. ***ROI Cortical Thickness***

Summary of cortical thickness (mm) for the EC, PHC and RSC by laterality in the total sample and stratified subsamples based on sex and fitness level.

	Mean ± *SD*				
		Sex		**Fitness**	
ROI	**Total** (*N* = 23)	Female (*n* = 18)	Male (*n* = 5)	High (*n* = 12)	Low (*n* = 11)
L EC	3.36 ± 0.33	3.36 ± 0.28	3.38 ± 0.53	3.36 ± 0.34	3.37 ± 0.35
R EC	3.42 ± 0.25	3.44 ± 0.20	3.35 ± 0.41	3.43 ± 0.31	3.42 ± 0.19
L PHC	2.75 ± 0.26	2.77 ± 0.28	2.70 ± 0.22	2.68 ± 0.26	2.83 ± 0.24
R PHC	2.73 ± 0.23	2.76 ± 0.23	2.62 ± 0.23	2.70 ± 0.27	2.77 ± 0.19
L RSC	2.55 ± 0.23	2.54 ± 0.25	2.60 ± 0.19	2.57 ± 0.23	2.53 ± 0.24
R RSC	2.59 ± 0.17	2.59 ± 0.19	2.61 ± 0.13	2.58 ± 0.18	2.61 ± 0.17

L, left; R, right; EC, entorhinal cortex; PHC, parahippocampal cortex; RSC, retrosplenial cortex

3.4 Cardiorespiratory Fitness and Cortical Thickness

To determine whether CRF and cortical thickness are correlated, we first used Pearson's correlation (Table 5). Findings did not support the hypothesis that greater CRF will positively correlate with cortical thickness of the EC (Left: r = 0.0626, p = 0.777; Right: r = -0.0164, p = 0.941), PHC (Left: r = -0.382, p = 0.0721; Right: r = -0.383, p = 0.0709), and RSC (Left: r = 0.0114, p = 0.959; Right: r = -0.0335, p = 0.879). All of the 95% confidence intervals (CI) contained the null value of zero.

Table 5. ***Pearson's Correlations of VO2max and Cortical Thickness***

Correlations of VO2max and cortical thickness of ROIs in the total sample (N = 23)

ROI	Correlation Coefficient *(r)*	95% CI	p-value
L EC	0.0626	-0.359, 0.463	0.777
R EC	-0.0164	-0.426, 0.399	0.941
L PHC	-0.382	-0.686, 0.036	0.0721
R PHC	-0.383	-0.687, 0.0341	0.0709
L RSC	0.0114	-0.403, 0.422	0.959
R RSC	-0.0335	-0.44, 0.384	0.879

L, left; R, right; EC, entorhinal cortex; PHC, parahippocampal cortex; RSC, retrosplenial cortex

3.5 Cortical Thickness and Navigation Task Performance

To examine if cortical thickness and navigation task performance are correlated, we conducted Pearson's correlations (Table 6). Correlations for the EC (Left: r = -0.216, p = 0.322; Right: r = 0.00396, p = 0.986) and PHC (Left: r = -0.201, p = 0.358; Right: r = -0.120, p = 0.587) were not significant. However, right RSC thickness (Left: r = 0.306, p = 0.155; Right: r = 0.486, p = 0.0188) was significantly correlated to navigation performance. To correct for multiple comparisons, p-values were adjusted using false discovery rate for the right RSC ($p_{adjusted}$ = 0.113).

Table 6. ***Pearson's Correlations of Cortical Thickness and Navigation Performance***

Correlations of cortical thickness and navigation performance in the total sample (N = 23)

ROI (mm)	**Correlation Coefficient** *(r)*	**p-value**	**Adjusted p-value**
L EC	-0.216	0.322	0.537
R EC	0.00396	0.986	0.986
L PHC	-0.201	0.358	0.537
R PHC	-0.120	0.587	0.704
L RSC	0.306	0.155	0.465
R RSC	0.486	0.0188*	0.113

L, left; R, right; EC, entorhinal cortex; PHC, parahippocampal cortex; RSC, retrosplenial cortex
*$p < 0.05$

Given the significant correlations found for right RSC and navigation performance, prior to multiple comparisons correction, we explored whether thickness of the right RSC predicts navigation performance when controlling for covariates. We used a multiple linear regression model of right RSC thickness as the primary predictor of objects located with age, sex, education and task version as covariates (Table 7). Greater right RSC thickness was shown to predict number of objects located (B = 9.9450, p = 0.0110), and older age negatively predicted number of objects located (B = -0.2595, p = 0.0209). However, these associations became insignificant after correcting for multiple comparisons using the false discovery rate method ($p>0.05$). The association of right RSC thickness and navigation task performance is displayed in a scatterplot (Figure 3).

Table 7. ***Multiple Regression of Navigation Performance and Right RSC Thickness***

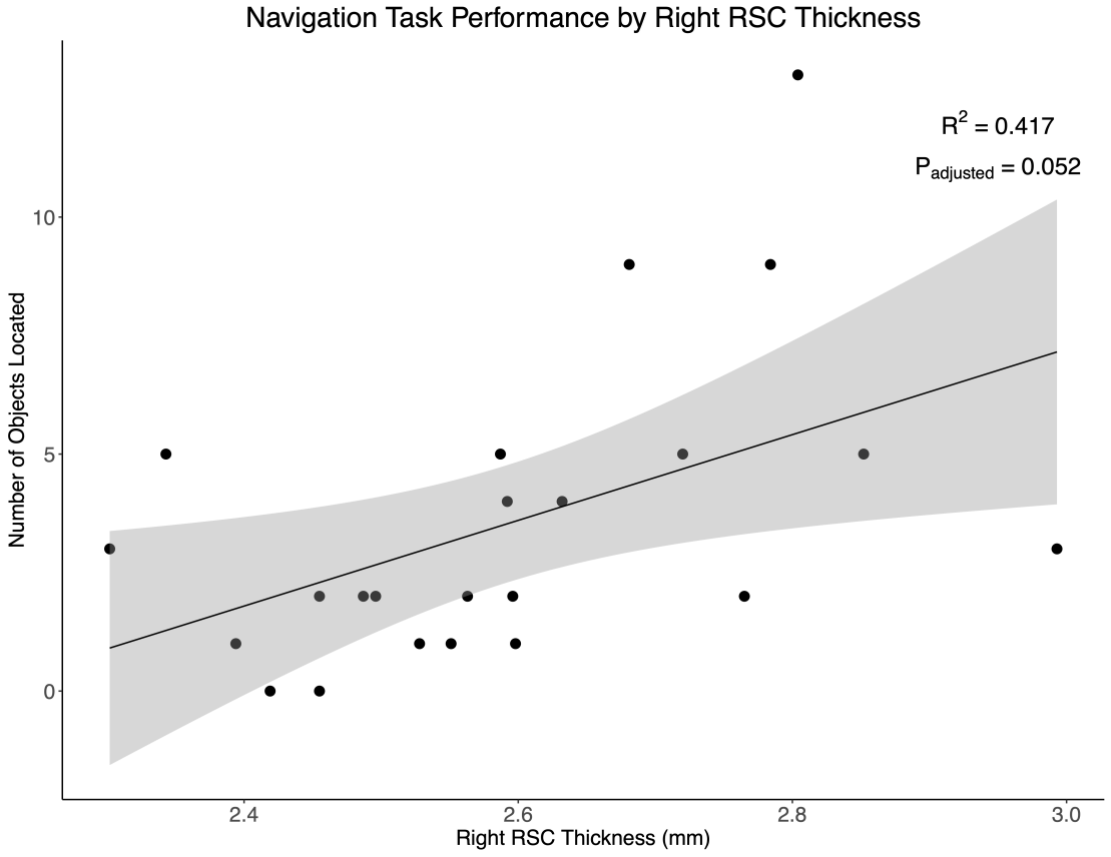
Multiple regression model of right RSC thickness and navigation performance, controlling for age, sex, education and task version (*Adjusted R_2= 0.4172, p= 0.01202)*

Predictors	**Regression Coefficient** *(B)*	***t*-statistic**	**p-value**	**Adjusted p-value**
R RSC Thickness, mm	9.9450	2.855	0.0110*	0.05225
Age, years	-0.2595	-2.546	0.0209*	0.05225
Sex (1 = male, 2 = female)	-1.2901	-0.889	0.3864	0.38640
Education, years	0.2821	1.105	0.2846	0.38640
Task Version (1= ver1, 2 = ver2)	-1.1411	-0.942	0.3595	0.38640

Ver1, version 1; ver2, version2
*$p < 0.05$

Figure 3. Association between Right RSC Thickness and Navigation Performance

Scatterplot of the association between right RSC thickness and navigation performance, controlling for age, sex, education and task version (*N* = 23).



DISCUSSION

The aim of this study was to assess the relationship between CRF, spatial navigation task performance and cortical thickness of brain regions involved in navigation. We did not find any significant associations when testing the hypothesized relationship that CRF will predict navigation performance. Examining CRF and cortical thickness of the regions of interest did not reveal any significant linear relationships to support our hypothesis. However, exploring correlations between cortical thickness and navigation performance showed a positive correlation between right RSC thickness and navigation performance, prior to multiple comparisons correction. Multiple regression analysis revealed that greater thickness of the right RSC significantly predicts improved navigation performance. However, this finding was no longer significant after multiple comparisons correction. Aging was also shown to negatively predict navigation performance in the regression model, but the significance of the association was also reduced by multiple comparisons correction.

Previous literature has linked greater aerobic exercise or CRF to improved spatial learning and greater cortical thickness (Herting & Nagel, 2012; Williams et al. 2017). This study did not replicate previously observed relationships between CRF and cortical thickness or navigation performance. Nonetheless, the results of this study provide intriguing insights to potential relationships between outcomes measures, especially in regards to RSC thickness and navigation recall performance.

4.1 Cardiorespiratory Fitness and Spatial Navigation

Limited research has explored the relationship between CRF and spatial navigation. We hypothesized that CRF would positively predict navigation performance during recall. Our findings did not demonstrate a significant association between VO_2max as a predictor of navigation recall in a multiple regression model. Previous work from our laboratory demonstrated that greater CRF attenuates age-related decline in a virtual navigation task requiring mnemonic discrimination (Nauer et al., 2020). The aforementioned study investigated a much larger sample of participants with a broad age range, allowing for observation of age-associated declines in cognition and fitness. Perhaps if the current investigation explored the same outcomes across the lifespan, associations would be more apparent. In line with the findings of Nauer et al. (2020) regarding CRF attenuating spatial cognitive decline, we noticed that participants with higher fitness levels had a greater median number of objects located than those with lower fitness levels; however, the observed difference was not statistically significant. A larger sample size may have supported a significant difference in this finding, as concluded in Nauer et al. (2020), which retained a sample of 62 participants from across the adult lifespan. Evidence from other literature is not conclusive, with an array of significant and non-significant reports. For example, a study in adolescents showed that aerobic fitness significantly predicts spatial learning on a virtual MWM (Herting & Nagel, 2012). Spatial learning was measured as the difference of percent distance travelled in the target quadrant between the first and sixth learning trials. In addition to spatial learning, Herting & Nagel (2012) explored delayed memory recall as an outcome

measure for the virtual MWM. The navigation recall score in their study was not significantly predicted by aerobic fitness, which is consistent with the results of the present analyses. However, it is important to acknowledge the limitations of comparing our findings to those in Herting & Nagel (2012) due to the vast age difference in the samples. Similarly, an aerobic exercise intervention in a cohort of 47 middle-aged adults did not observe variation in spatial learning performance between baseline and follow-up, using a virtual maze task similar to the one in the current study (Holzschneider et al., 2012). The authors also indicated that they did not find a significant correlation between CRF and spatial learning, likely due to low variance in correct responses on the task, suggesting a floor effect. However, Holzschneider et al. (2012) did find a significant increase in fMRI activity during the spatial navigation task in several ROIs including the HC, PHG and RSC. Further exploration of CRF and spatial navigation with larger sample sizes is needed to elucidate the cognitive significance of the structural and functional brain changes that occur in response to aerobic exercise.

4.2 Cardiorespiratory Fitness and Cortical Thickness

CRF and aerobic exercise are established modulators of brain structure and function. We hypothesized that greater CRF will be positively correlated with cortical thickness in regions involved in navigation and susceptible to neurodegeneration in aging. However, our analysis did not reveal any significant correlations between CRF and cortical thickness of the ROIs. Several published studies have reported significant associations between CRF and cortical thickness in various populations. A study in

young to middle-aged adults with schizophrenia and matched healthy controls reported a positive association for both study groups between CRF and cortical thickness of the frontal, temporal and cingulate cortices in the left hemisphere (Scheewe et al., 2013). Another study determined that greater CRF and cortical thickness are positively associated in older adults, particularly in regions known to exhibit neurodegeneration (Williams et al., 2017). Notably, several initially significant ROIs did not survive multiple comparisons including the left paracentral gyrus extending into the posterior cingulate and the left parahippocampal gyrus (Williams et al., 2017). The present study is inconsistent with previous work, as the referenced studies had greater sample sizes and included a broader age range of participants. We are not aware of studies that have significantly correlated CRF or aerobic exercise to cortical thickness of the EC, PHC and RSC. It is possible that observable differences across fitness level may have been found in our sample if different ROIs were examined.

4.3 Cortical Thickness and Spatial Navigation

We hypothesized that cortical thickness of regions involved in navigation will positively correlate with performance on a navigation recall task. EC and PHC thickness did not significantly correlate with number of objects located during navigation task recall. However, greater right retrosplenial cortical thickness significantly correlated with the number of objects located during navigation recall. This correlation was no longer significant after multiple comparisons correction. These findings led us to exploring right retrosplenial cortical thickness as a predictor of navigation performance with the

covariates of age, sex, education and task version to control for potential confounding effects. Greater right RSC thickness significantly predicted navigation task performance after the addition of covariates. The regression model also revealed age as a significant negative predictor of navigation task performance. These findings did not survive multiple comparisons correction, but it is possible that the associations would have been significant with a greater sample size. Our findings may be explained by the putative role of RSC in mediating between egocentric and allocentric reference frames, which would provide the flexibility needed for successful navigation (Epstein et al., 2017; Latini-Corazzini et al., 2010). Furthermore, the observed age-related declines in navigation performance may be related to the reported declines in allocentric, but not egocentric representations, observed in older adults (Rodgers et al., 2012; Moffat & Resnick, 2002). Investigation of cortical thickness and spatial navigation is relatively scarce, to our knowledge; however, one study explored these associations in a cohort of younger and older adult participants who participated in a 42-session navigation training intervention, using a task similar to the one used in the present study (Wenger et al., 2012). The task used in Wenger et al. (2012) required participants to navigate through a virtual zoo to locate target animals and performance was measured by the number of targets found within 50 minutes of navigation. Although Wenger et al. (2012) did not find significant correlations between navigation task performance and cortical thickness in older adults, young navigators showed a significant increase in cortical thickness of the precuneus, superior parietal lobule and paracentral lobule of the left hemisphere. Notably, the training intervention improved navigation task performance in both younger and older

adults, suggesting that older adults have a diminished potential for training-dependent cortical changes (Wenger et al., 2012). Future studies should attempt to validate the correlation between cortical thickness and navigation performance as the relationship remains elusive.

4.4 Limitations and Future Directions

The primary limitation of this study was a small sample size, which limited statistical power and the probability of finding significant relationships other than those with very large effect sizes. Given the cross-sectional design of this study, we were unable to establish cause and effect relationships. Despite the wide range of estimated VO_2max in our sample, the small sample size was likely not sufficient to correlate CRF to brain structure and spatial cognition. Cross-sectional studies have successfully correlated CRF with structural or cognitive measures (Williams et al., 2017; Herting & Nagel, 2012; Nauer et al., 2020). However, causal relationships cannot be inferred without longitudinal observation. We also did not consider exercise history as a covariate or predictor in our analyses, which may have been impactful to our outcomes despite the participants' sedentary status. Sedentary status was defined by ACSM (2014) guidelines as performing less than 30 minutes of moderate intensity physical activity, three times per week. Selection of participants by sedentary status likely limited the variability needed for correlational analyses. Future studies should obtain an appropriately powered sample size, include exercise history in analyses and use a longitudinal approach to assess changes in CRF, cortical thickness and behavioral performance.

In regards to our navigation protocol, exclusively examining performance during the recall phase may have been limiting. Previous studies have explored the correlation between spatial learning and CRF with some success (Herting & Nagel, 2012; Holzschneider et al., 2012). We are not aware of studies that have linked CRF to navigation recall; thus, future studies should consider including a measure of spatial learning across the learning trials. Several studies utilize other parameters such as distance travelled and time to completion, to measure virtual navigation (Moffat et al., 2001; Head & Isom, 2010). Future studies should explore measures beyond object recall as significant relationships may be found with more nuanced outcomes such as distance travelled, which would inform us of spatial memory deficits if longer distances than necessary are travelled to arrive at a target. Finally, the task may have been too difficult to show variability in navigation performance, which is a limitation that has been suggested by other authors (Holzschneider et al., 2012). This is of particular concern with an older adult population as embracing virtual environments is likely more challenging to them than younger populations. A solution to this challenge is to modify the difficulty of existing tasks in future investigations.

Structural outcomes examined in the present study have been correlated to aerobic fitness and cognition; however, there are other brain measures that may provide insight. Ziegler et al. (2010) conducted a study not only examining grey matter cortical thickness but also white matter integrity as they relate to cognition. Although neurodegeneration occurred in both grey and white matter, their findings suggested that only white matter integrity correlated to cognitive performance (Ziegler et al., 2010). Few studies have

examined aerobic exercise or fitness and white matter in aging. Colcombe et al. (2006) conducted a six-month aerobic training intervention in older adults and found increases in white matter volume in prefrontal and temporal cortices. Furthermore, preliminary evidence from younger and older adults shows a positive correlation between aerobic exercise and white matter integrity of the cingulum and uncinate fasciculus (Marks et al., 2007). In light of these findings, future studies should examine diffusion-weighted imaging methods to assess white matter metrics, aerobic exercise and cognition in aging.

CONCLUSION

The work conducted in this study provides an evidence-guided exploration of CRF, spatial navigation and cortical thickness. Our findings suggest a significant correlation between right RSC thickness and allocentric navigation performance in healthy older adults. Subsequent multiple regressions models with age, sex and education as covariates, maintained the association of right RSC thickness and navigation performance. However, multiple comparisons correction mitigated our findings. We believe that in a larger sample size, right RSC thickness would have significantly predicted navigation performance after controlling for covariates. We did not find any other significant relationships in our data; however, with a larger sample size these relationships may have been significant, especially given prior evidence of cardiorespiratory fitness modulating cortical thickness and improved cognition. Future research with an adequately powered sample size is needed to investigate the brain-behavior relationships explored in this study, particularly to understand the role of improved CRF in ameliorating age-related deficits in spatial navigation.

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