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White matter microstructure mediates the relationship between cardiorespiratory fitness and spatial working memory in older adults

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Abstract

White matter structure declines with advancing age and has been associated with a decline in memory and executive processes in older adulthood. Yet, recent research suggests that higher physical activity and fitness levels may be associated with less white matter degeneration in late

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life, although the tract-specificity of this relationship is not well understood. In addition, these prior studies infrequently associate measures of white matter microstructure to cognitive outcomes, so the behavioral importance of higher levels of white matter microstructural organization with greater fitness levels remains a matter of speculation. Here we tested whether cardiorespiratory fitness (VO_{2max}) levels were associated with white matter microstructure and whether this relationship constituted an indirect pathway between cardiorespiratory fitness and spatial working memory in two large, cognitively and neurologically healthy older adult samples. Diffusion tensor imaging was used to determine white matter microstructure in two separate groups: Experiment 1, N=113 (mean age = 66.61) and Experiment 2, N=154 (mean age = 65.66). Using a voxel-based regression approach, we found that higher VO_{2max} was associated with higher fractional anisotropy (FA), a measure of white matter microstructure, in a diverse network of white matter tracts, including the anterior corona radiata, anterior internal capsule, fornix, cingulum, and corpus callosum ($p_{FDR\text{-}corrected} < .05$). This effect was consistent across both samples even after controlling for age, gender, and education. Further, a statistical mediation analysis revealed that white matter microstructure within these regions, among others, constituted a significant indirect path between VO_{2max} and spatial working memory performance. These results suggest that greater aerobic fitness levels are associated with higher levels of white matter microstructural organization, which may, in turn, preserve spatial memory performance in older adulthood.

Keywords

Aging; Memory; White Matter; Fitness

1.0 INTRODUCTION

The aging brain experiences both macro- and microstructural changes, including gray matter atrophy and degeneration of white matter tracts. Older adults are particularly susceptible to precipitous declines in white matter, with anterior tracts showing the most pronounced degradation (Burzynska et al., 2010; Pfefferbaum and Sullivan, 2003; Salat et al., 2005; Westlye et al., 2009). Age-related declines in white matter microstructure may lead to disruptions in neural communication, which, in turn, could lead to consequent declines in cognitive function. This notion is supported by studies combining behavioral measures of cognitive performance and diffusion tensor imaging (DTI), which demonstrate that age-related decline in cerebral white matter may be related to cognitive deficits associated with aging. In particular, white matter degeneration in older adults is associated with impaired performance on memory, executive function, and processing speed tasks (Bennett and Madden, 2014; Charlton et al., 2006; Gold et al., 2010; Grieve et al., 2007; Kennedy and Raz, 2009; Madden et al., 2009; Vernooij et al., 2009; Voineskos et al., 2012).

Fortunately, moderate intensity physical activity (PA) may prevent or reverse age-related changes in neural structure and function. For example, randomized controlled trials (RCTs) have demonstrated that 6-12 months of aerobic exercise improves cognitive performance and alters gray matter structural morphology and function in older adulthood (Colcombe and Kramer, 2003; Colcombe et al., 2006; Erickson et al., 2011; Niemann et al., 2014;

Ruscheweyh et al., 2011; Voelcker-Rehage et al., 2011; Voss et al., 2010b). Despite the wealth of data on associations between PA, fitness, and exercise on gray matter volume in older adults (Erickson et al., 2014) considerably less is known about these relationships with white matter microstructure. A handful of recent cross-sectional and prospective longitudinal studies provide preliminary evidence that self-reported regular PA may preserve white matter in older adulthood (Gons et al., 2013; Gow et al., 2012; Tian et al., 2014a). In addition, positive relationships between objectively measured PA using accelerometry and fractional anisotropy (FA), a measure of white matter microstructure, have been shown (Burzynska et al., 2014; Tian et al., 2015). Recent work has also examined associations between white matter microstructure and cardiorespiratory fitness (CRF) in older adulthood. CRF, often measured by VO_{2max}, a quantitative estimate of oxygen capacity and utilization during an exercise test, can be improved (increased) by aerobic exercise. Higher CRF levels have been associated with greater white matter microstructural integrity in several tracts including the cingulum, corpus callosum, superior corona radiata, and inferior longitudinal fasciculus (Hayes et al., 2015; Johnson et al., 2012; Marks et al., 2010; Tseng et al., 2013); however see Burzynska et al., 2014). Yet, despite this evidence for associations between PA, fitness, and white matter microstructure, many of these studies have been encumbered by methodological limitations that restrict the scope of interpretation. Most prior studies have had relatively small sample sizes ($n < 30$ healthy older adults) (Johnson et al., 2012; Marks et al., 2010; Tian et al., 2014b; Tseng et al., 2013), or have employed subjective measures of PA that are prone to social desirability bias and may not reflect actual PA patterns (Gons et al., 2013; Gow et al., 2012; Tian et al., 2014a). In addition, there is little agreement across studies on the particular white matter paths that correlate with PA or fitness. This may partially be due to the analytical approaches used in previous work, which in some cases has been limited to particular fiber bundles at the expense of other brain areas (Marks et al., 2010; Tian et al., 2014a). Thus, while previous research suggests a positive linear relationship between fitness and white matter microstructural integrity, regional specificity remains unclear, with small sample sizes and methodological limitations restricting interpretation.

There is also a dearth of knowledge on the role of white matter in the relationship between fitness and cognitive function. Higher levels of aerobic fitness are frequently associated with better cognitive performance and, as recent research suggests, greater white matter microstructural integrity (Hayes et al., 2015; Johnson et al., 2012; Tian et al., 2014b; Tseng et al., 2013). Given the association between white matter and cognition in healthy older adults, fitness-related variation in white matter microstructure may partially mediate the relationship between fitness and cognitive function, although this remains a matter of speculation. Only two studies have examined whether microstructural changes associated with fitness are also linked to elevated cognitive performance (Prakash et al., 2010; Voss et al., 2013b). Following a one-year aerobic exercise intervention ($n = 70$), Voss et al. (2013b) found that greater gains in CRF were associated with greater FA in prefrontal and temporal white matter. However, the increases in white matter FA post-intervention were not associated with memory improvement, although this may be a consequence of lack of statistical power for the cognitive measure employed (backward digit span) (Voss et al., 2013b). Similar patterns emerged in a study of multiple sclerosis (MS) (MS $n = 21$; Healthy controls n = 15), but in this study higher FA was associated with both greater fitness levels and faster processing speed (Prakash et al., 2010). Thus, while only a small number of studies have examined the link between fitness and white matter microstructure, even fewer have investigated the cognitive implications of this relationship.

Our primary aim was to further examine the relationship between CRF and white matter microstructure in older adulthood. Using diffusion imaging, we examined the relationship between CRF and tensor-based models of white matter structure in two large samples of healthy older adults (each with n > 110). Importantly, the two samples include an objective measure of aerobic fitness (CRF), collected using identical assessment methods, employed similar spatial memory tasks, and had similar demographic characteristics. Such similarities between two large, and independent, samples provided us with a unique opportunity to examine associations between fitness and white matter microstructure on a voxelwise basis to better characterize the tract-specificity of these associations. Based on studies of grey matter, fitness and exercise are most consistently associated with volume of the hippocampus and prefrontal cortex (Erickson et al., 2014; Erickson et al., 2009; Erickson et al., 2011; Weinstein et al., 2012). Therefore, we predicted that higher CRF would be associated with greater FA, particularly in tracts that facilitate communication between subcortical, hippocampal, and prefrontal regions. A secondary aim was to examine whether fitness and white matter associations would constitute significant indirect pathways to spatial working memory performance, a cognitive domain that is sensitive to aging (Schmiedek et al., 2009), and fitness effects (Erickson et al., 2009). In addition, performance on the spatial working memory paradigm used in the present investigation has been linked in prior studies to fitness and hippocampal volume (Erickson et al., 2009; Erickson et al., 2011) and resting connectivity (Voss et al., 2010b), making this measure well-suited for the purposes of the present study. Also, similar spatial working memory paradigms were used in both experiments described here, which allowed us to test associations between white matter and spatial memory performance in two different samples. To this end, quantitative mediation modeling was applied on a voxelwise basis to both samples to investigate whether white matter microstructure statistically mediated the relationship between fitness and spatial working memory performance. We predicted that white matter microstructure would be a significant indirect pathway by which higher CRF would be associated with superior spatial working memory performance. Characterizing such pathways provides insight into the putative mechanisms and neurocognitive implications of fitness-related variation in white matter microstructure in older adults.

2. 0 METHODS

We tested our hypotheses in two separate samples that are described below as Experiment 1 and 2. The data analysis and analytic procedures are described last as they were the same across both experiments.

Experiment 1

2.1 Participant Characteristics

One hundred and seventy-three participants between the ages of 60 and 81 (mean age 66.6 years; standard deviation = 5.6 years) were recruited to take part in a one-year, single-blind randomized controlled exercise intervention. Subjects were recruited through community advertisements and physician referrals in eastern Illinois. For the purposes of the present study, only data from baseline assessments were used. All subjects participated in three baseline sessions, which included cognitive and CRF assessments and the collection of magnetic resonance imaging (MRI) data. Of the 173 participants, 16 were excluded due to incomplete information on relevant behavioral data (cognitive, VO_2, or demographic). An additional 12 subjects did not complete the baseline MRI scan. Finally, 32 participants were excluded due to problems with their diffusion imaging data including 1) failure to finish the entire scan ($n = 2$) 2) poor orientation during image acquisition ($n = 4$), and 3) excessive noise/field distortion ($n = 24$). Thus, the final sample consisted of 113 participants. Excluded participants did not differ in age, gender, years of education, or fitness levels from those included in the analysis (all $p>.10$).

Participants were required to score ≥ 51 on the modified Mini Mental Status Examination (high score of 57) to rule out clinically present cognitive impairment (Stern et al., 1987). Additionally, to rule out depression, individuals that scored > 3 on the Geriatric Depression Scale were excluded (Sheikh et al., 1986). Participants were required to have normal color vision, a visual acuity of at least 20/40, and no history of neuropsychiatric conditions or neurological diseases or infarcts including Parkinson's disease, multiple sclerosis, Alzheimer's disease, or stroke. Further, participants were excluded if they reported a history of Type II diabetes and cardiovascular disease. Also, participants had to be free of any metal implants or other contraindications for MRI. In order to participate, subjects needed to obtain consent from their physician to engage in an exercise intervention, as well as a maximal graded exercise test (VO_2 max). Finally, participants were required to be 60+ years of age and physically inactive as defined by engaging in 30 minutes or less each week within the six months prior to baseline examination (Erickson et al., 2011). All participants signed an informed consent approved by the University of Illinois.

2.2 Cardiorespiratory Fitness Assessment

Maximal oxygen uptake (VO_{2max}) was used as an objective measure of cardiorespiratory fitness. As detailed by Voss et al., (2010b), assessment of CRF was conducted using graded maximum exercise testing on a motor-driven treadmill with continuous monitoring of respiration, heart rate, and blood pressure by a cardiologist and nurse (Voss et al., 2010b). During the assessment, subjects walked at a speed slightly faster than their normal walking pace with increasing graded increments of 2% every 2 minutes. Oxygen uptake was measured at 30 second intervals until a max VO_2 was attained or to the point of test termination due to exhaustion. VO_{2max} was defined as the highest recorded VO_2 value when two of three criteria were satisfied: 1) a plateau in VO_2 peak between two or more workloads 2) a respiratory exchange ratio > 1.00 or 3) a heart rate equivalent to their age predicted maximum (i.e., 220-age).

VO_{2max} scores are expressed in units of milliliters per kilogram per minute (ml/kg/min). VO_{2peak} was used as a proxy for VO_{2max} among the ~20% of participants in each sample that did not meet two of the three criteria for VO_{2max}.

2.3 Spatial Working Memory Paradigm

The spatial memory paradigm was administered as part of a larger battery of cognitive tests. However, as mentioned in the introduction, we focused our hypotheses on the spatial memory task as it has been associated in previous work to fitness and hippocampal volume (Erickson et al., 2009; Erickson et al., 2011) and resting connectivity (Voss et al., 2010b), and a similar task was used for assessing cognitive performance in Experiment 2. At the beginning of the task, participants were shown a fixation crosshair for 1 second, followed by the appearance of one, two or three dots placed in random locations on the screen for 500 ms. Then a 3 second fixation crosshair appeared, during which time participants were asked to try to remember where the previous dot(s) were located. Following the 3-second delay, a red dot appeared on the screen. Subjects had to indicate whether the red dot displayed was in the same location (match) or a different location (non-match) than one of the previously presented black dot(s) by pressing a designated key on a computer keyboard (x = nonmatch; m = match). There were 40 trials per set size (1, 2, and 3 black dots), with 20 match and 20 non-match trials in each, totaling to 120 trials. Prior to task administration, several practice trials were conducted to familiarize the participant with the task, during which time they were directed to respond as quickly and accurately as possible. Accuracy rates were recorded separately for the 1, 2, and 3 dot conditions and were then averaged to create mean accuracy scores.

2.4 Diffusion Tensor Imaging

Diffusion weighted images were acquired using a 3 T Siemens Allegra head-only scanner. The echo time (TE) was 94 ms, with repetition time (TR) = 4,200 ms. Twenty-eight 4 mm slices positioned according to the AC-PC line were obtained along the anterior-posterior commissural plane. The protocol involved a T2-weighted acquisition followed by a 12-direction diffusion-weighted echo planar imaging scan (b-value = 1,000 s/mm^2), which was repeated six times.

Experiment 2

2.5 Participant Characteristics

The participants were a pre-intervention cross-sectional subsample from a 6-month single-blind randomized controlled exercise trial (ClinicalTrials.gov; NCT01472744). Two hundred forty seven community-dwelling healthy, older adults were randomized into four intervention groups; baseline assessments from this intervention are reported here. 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 ≥23 on the Mini-Mental State Exam (MMSE) and >21 on a Telephone Interview of Cognitive Status (TICS-M) questionnaire, (4) scored <10 on the geriatric depression scale (GDS-15), (5) scored ≥75% right-handedness on the Edinburgh Handedness Questionnaire, (6) demonstrated normal or corrected-to-normal vision of at least 20/40 and no color blindness, (7) 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, (8) reported no participation in exercise-related physical activity (maximum of two moderate bouts per week) in the past six months. Therefore, this sample is described as low active and low fit, although capable of performing exercise (i.e., no physical disability that prohibits mobility). In addition, all participants needed to be cleared by their physician to participate in an exercise intervention and CRF testing. Out of 247 participants, 216 completed the baseline MRI imaging session with successful collection of DTI data. Of the 216 participants, 32 were excluded due to missing cognitive, VO_{2max}, or demographic data. An additional 27 participants were excluded due to insufficient diffusion data quality including 1) suboptimal placement of the field-of-view resulting in insufficient brain coverage ($n = 23$) and 2) image artifacts that affected more than 2 volumes/scan ($n = 4$). Finally, 3 subjects scored < 24 on the Modified Mini Mental Status Exam-II, and were therefore excluded. Thus, the final sample consisted of 154 participants. Those excluded from analysis did not differ from included participants on demographic factors or fitness levels (all $p>.10$).

2.6 Cardiorespiratory Fitness Assessment

As in Experiment 1, maximal oxygen uptake was used as an objective measure of CRF. Similar methods as described in Experiment 1 were used in this study (see section 2.2).

2.7 Spatial working memory paradigm

Participants completed a spatial working memory task similar to that described in Experiment 1. At the start of the task, participants were shown a fixation crosshair for 1 second, followed by the appearance of 2, 3, or 4 dots placed in random locations on the screen. Thus, the set sizes differed slightly between Experiment 1 (1, 2, and 3 dot trials) and Experiment 2 (2, 3, and 4 dot trials). Two and 3-dot trials were displayed for 500 milliseconds, and sets of 4 dots were displayed for 1 second. Then, a 3 second fixation crosshair appeared, followed by the appearance of a red dot, which displayed for 2 seconds. Similar to Experiment 1, participants had to indicate whether the red dot displayed was in the same location (match) or a different location (non-match) than one of the previously presented black dots, by pressing a designated key on a computer keyboard. Participants completed a total of 120 trials, 40 trials per set size (2, 3, and 4 black dots), each containing 20 match and 20 non-match trials. Participants completed 12 practice trials (6 match, 6 non-match) prior to task administration to familiarize them with the task. Accuracy rates were recorded separately for the 2, 3, and 4 dot conditions and were averaged to created a mean accuracy score.

2.8 Diffusion Tensor Imaging

Diffusion images were acquired on a 3T Siemens Trio Tim system with 45 mT/m gradients and 200 T/m/sec slew rates (Siemens, Erlangen, Germany) with a twice-refocused spin echo single-shot Echo Planar Imaging sequence (Reese et al., 2003) to minimize eddy current-induced image distortions. The protocol consisted of a set of 30 non-collinear diffusion-weighted acquisitions with b-value = 1000s/mm^2 and two T2-weighted b-value = 0 s/mm^2 acquisitions, repeated two times (TR/TE = 5500/98 ms, 128 × 128 matrix, 1.7×1.7 mm^2 in-plane resolution, FA = 90, GRAPPA acceleration factor 2, and bandwidth of 1698 Hz/Px, comprising 40 3-mm-thick slices), obtained parallel to the anterior-posterior commissure plane with no interslice gap. Visual checks were performed on every volume of the raw data of every participant by AZB. If excessive noise/field distortions were detected, up to 2 volumes/scan were removed with the corresponding b-vectors and b-values before further processing.

2.9 Image Processing

Identical image processing procedures were used on diffusion data in Experiment 1 and Experiment 2. In both studies, diffusion data was processed using tools in the FMRIB Software Library (FSL v5.0.1) (Image Analysis Group, FMRIB, Oxford, UK; http://www.fmrib.ox.ac.uk/fsl/; (Smith et al., 2004). Using FMRIB's Diffusion Toolbox (v.3.0; http://fmrib.ox.ac.uk/fsl/fdt/index.html), each participant's data was eddy current corrected by affine registration to the first *Bo* image. This was followed by the removal of non-brain tissue using the Brain Extraction Tool (BET). Next, DTIfit was used to calculate the diffusion tensor at each voxel. Specifically, this step computes the voxelwise eigenvalues and eigenvectors of the diffusion tensor from each participant's image, calculating various diffusion parameters, including FA. FA is a commonly used measure of white matter derived from DTI, and represents overall anisotropy within a voxel (Jones et al., 2013). FA values fall between 0 and 1, indicating the degree of microstructural organization, with higher values indicating greater directionality of diffusion.

FA data was fed into the FSL (v4.1.8) tract-based spatial statistics toolbox (TBSS; v1.2, http://www.fmrib.ox.ac.uk/fsl/tbss/index.html; (Smith et al., 2006) pipeline. TBSS is used frequently in DTI processing, and its algorithms for alignment of FA images across multiple subjects into a standard space have been tested and validated (Smith et al., 2006). First, FA images were eroded to remove likely outliers from the diffusion tensor-fitting step. Then, FA images were normalized to 1 X 1 X 1 mm MNI152 standard space via alignment to a common registration target. As considerable atrophy occurs in older adulthood, it is standard to compute a study specific template when using older adult populations, as the standard FSL FA template (FMRIB58_FA) reflects an average of young to middle aged adults. Therefore, in each experiment, a study-specific template was created and was used as the target for registration. To create the study specific templates, we first registered all native-space FA images to the FA template in MNI space using an affine warp, then averaged the registered images across subjects to generate the study-specific templates. Registration to the study-specific template is done by combining two transformations: 1) a non-linear transformation of each subject's FA image to the study specific template and 2) an affine registration of the template to MNI152 standard space. Following the MNI transformation for all subjects, a mean FA image was computed and an average skeleton was generated that represented major tracts common across participants. The skeleton was thresholded at an FA value of 0.2 (Smith et al., 2007) to ensure that major white matter tracts were included and to exclude regions that may contain multiple types of tissue. Then, in order to account for any residual misalignments not corrected for during registration, each participant's normalized FA image was projected onto the mean FA skeleton. These images were then used in the statistical analyses described below.

2.10 Statistical Analyses

We tested for differences across samples in age, gender, years of education, CRF, and spatial working memory performance using chi-squared tests and two-tailed t-tests. Within each study sample, the relationships between CRF and nuisance variables were explored using independent samples t-tests and bivariate correlations. In addition, the relationship between CRF and average spatial working memory accuracy was assessed using linear regression analyses. These analyses were conducted using SPSS (v. 21) (Spss, 2012). Sex, age, and years of education were entered as covariates in the regression models due to their association with the cognitive outcome variable.

We tested the association between CRF and white matter microstructure using a permutation-based regression tool within the Bootstrap Regression Analysis of Voxelwise Observations (BRAVO) toolbox. Documentation and tutorials for this toolbox are available at https://sites.google.com/site/bravotoolbox. For each regression model, a permutation approach evaluated statistical significance at each voxel. A total of 500 permutation tests were performed at each voxel, and for each iteration, the values in the model variables (covariates, CRF, FA) were independently scrambled. The significance of the association between CRF and fractional FA at each voxel (a path) was determined by comparing the distribution of the bootstrapped values with the distribution of the original values using a bias-corrected and accelerated method (DiCiccio and Efron, 1996) (see Verstynen et al., 2013 and Gianaros et al., 2012 for more details). Clusters that contained $k \geq 20$ contiguous voxels with a p threshold of < 0.05 after controlling for voxelwise multiple comparisons (false-discovery-rate) were considered significant.

In the mediation model, we examined whether white matter integrity served as an indirect pathway through which CRF predicted cognitive performance after adjusting for nuisance variables including age, gender, and years of education. A regression approach with permutation-based confidence interval estimation was employed to evaluate the indirect effect, with 500 permutation tests performed at each voxel. This resulted in a statistical parametric map of the indirect relationship between CRF and cognitive performance through voxelwise estimates of white matter microstructure (FA). In particular, mean spatial working memory accuracy rate, created by calculating the average accuracy scores across the 3 set sizes in each study, was our dependent variable. We chose to examine average accuracy rather than accuracy rates within each set size to 1) reduce the number of multiple comparisons and challenges with interpreting multiple mediator models, as this approach would have required at least 6 separate mediation models and 2) accuracy rates for set sizes were highly correlated with each other in both samples (Experiment 1: $r > 0.730$ for correlations between set sizes; $p < .001$; Experiment 2: $r > 0.754$ for correlations between set sizes; $p < .001$). In order to correct for multiple comparisons, we used a false-discovery-rate at the voxel-level threshold of 0.05 and isolated clusters using a spatial extent threshold of $k \geq 20$ contiguous voxels. Finally, we overlaid the Johns Hopkins University atlas to the voxel-based results to provide anatomical labels and to extract FA values for purposes of plotting. FA values for significant clusters within these regions were used to create the scatterplots shown in Figures 1 and 2. Identical analytical approaches were applied in Experiment 1 and Experiment 2.

3.0 RESULTS

A series of two-tailed t-tests compared age, education, fitness, and cognitive performance data across the two samples. These analyses revealed a significant difference in CRF between Experiment 1 (VO_{2max} mean = 21.40) and Experiment 2 (VO_{2max} mean = 20.17) ($t = -2.139$; $p = .033$). There were no significant differences in age, years of education, or cognitive performance (average accuracy) between the two samples (all $p > .10$). In addition, there was a similar distribution of males and females across samples (χ^2 (1) = 0.767; $p = 0.381$). Results are presented separately for each sample, below.

Experiment 1

3.1 Fitness and cognitive performance

Experiment 1 comprised of 113 low-active older adults (mean age = 66.61; 36.3% male). Demographic information and average accuracy for the spatial memory task are presented in Table 1. CRF differed by gender in this sample, with men (mean (SD) VO_{2max} = 24.22 (5.27)) demonstrating higher average fitness levels relative to women (mean (SD) VO_{2max} = 19.79 (3.85)) ($t = -4.71$; $p < .01$). Those with higher fitness levels also tended to be younger ($r = -0.460$; $p < .01$) and have greater years of education ($r = 0.291$; $p < .01$) (Table 2). In addition, older age was correlated with poorer spatial working memory accuracy, even after adjusting for gender and years of education ($r = -0.318$; $p = .001$).

After controlling for age, gender, and years of education, there was not a significant association between CRF and spatial working memory performance in this sample ($\beta = 0.171$; $p = 0.151$) (Table 2). Due to the differences in set size on the spatial working memory task across samples, a second average accuracy score was calculated using the 2-dot and 3-dot conditions, excluding the 1-dot condition. Follow-up analysis with this measure revealed a trending, but non-significant, association between CRF and average accuracy on the 2-dot and 3-dot conditions ($\beta = 0.228$; $p = 0.052$).

3.2 Cardiorespiratory fitness is associated with white matter microstructure

Consistent with our hypotheses, a voxelwise analysis revealed that higher CRF was associated with greater FA values across multiple regions of the white matter skeleton. These associations remained significant after adjusting for age, gender, and years of education. Regions in which significant associations between CRF and FA were located include the genu, body, and splenium of the corpus callosum; the fornix; bilateral anterior corona radiata, and bilateral anterior internal capsule ($P_{FDR\text{-}corrected} < 0.05$). A statistical map of the voxels within the white matter skeleton that were associated with CRF can be found in Figure 1A. For visualization purposes, the relationship between CRF and FA within several *post hoc* ROIs are plotted in Figure 1B. One small cluster in the right posterior thalamic radiation demonstrated a negative relationship between fitness and CRF.

3.3 CRF, white matter microstructure, and cognition

To determine whether white matter microstructure served as an indirect mediating pathway between CRF and spatial working memory performance, we employed a permutation-based regression approach. Consistent with our prediction, mediation analysis showed significant indirect associations between CRF and spatial working memory performance through distributed white matter regions, highlighted in cyan in Figure 1C. These regions included clusters in the genu and body of the corpus callosum, bilateral anterior corona radiata, superior corona radiata, and anterior internal capsule. Effects going in the positive direction indicate that higher levels of CRF were associated with greater FA in these clusters, which in turn, were associated with better performance (fewer errors) on the spatial working memory task. Only one small cluster in the right posterior white matter demonstrated a negative indirect effect.

Experiment 2

3.4 Fitness and cognitive performance

Experiment 2 included 154 low-active older adults (mean age = 65.66; 31.2% male). Subject demographics along with fitness and cognitive performance data can be found in Table 1. Whereas bivariate correlations showed no association between CRF and years of education ($r = 0.107$; $p = 0.187$), age was inversely correlated with CRF in this sample ($r = -0.247$; $p < .01$). In addition, an independent samples t-test revealed that females (mean (SD) $VO_{2peak} = 19.32$ (3.97)). tended to have lower fitness levels relative to males (mean (SD) $VO_{2peak} = 22.03$ (5.02)) ($t = -3.292$; $p < .01$) (Table 2). In addition, age was inversely correlated with accuracy rates ($r = -0.210$; $p = .009$) after adjusting for gender and years of education.

After accounting for age, gender, and years of education, multilevel linear regression analysis revealed a significant association between CRF and spatial working memory performance, such that higher CRF levels predicted better accuracy on the task ($\beta = 0.237$; $p = 0.006$) (Table 2).

3.5 Cardiorespiratory fitness is associated with white matter microstructure

Consistent with Experiment 1, we found that higher CRF was associated with higher FA within multiple white matter regions, even after controlling for age, gender, and education. Figure 2A shows the spatial distribution of statistically significant positive (red) and negative (blue) clusters. Among the regions containing significant clusters are the genu, body, and splenium of the corpus callosum, the left cingulum, bilateral segments of the superior longitudinal fasciculus, the anterior internal capsule, and the superior and anterior corona radiata ($P_{FDR\text{-}corrected} < 0.05$). A few small clusters showed a negative relationship between CRF and FA when controlling for covariates, with the largest clusters found bilaterally in the posterior limb of the internal capsule.

3.6 Fitness, white matter microstructure, and cognition

After controlling for age, gender, and education, mediation analyses revealed a large number of clusters distributed throughout white matter voxels that were indirect pathways between CRF and spatial working memory performance. Clusters showing a positive indirect association are highlighted in cyan in Figure 2C, and include clusters within the genu of the corpus callosum and the left cingulum segment. The positive values observed in these regions suggest that CRF is associated with greater FA in these clusters, which, in turn, are associated with greater accuracy on the spatial working memory task. The distribution of voxels showing significant negative indirect path effects are highlighted in brown in Figure 2C. Aside from bilateral clusters in the most rostral segment of the anterior thalamic radiation, negative indirect path voxels were primarily localized in the posterior white matter.

3.7 Conjunction Analyses

A post-hoc conjunction analysis was used to identify overlapping regions associated with CRF across Experiment 1 and Experiment 2. For this analysis, the results from the association with CRF in Experiment 1 were overlaid with the results from the association between CRF and FA in Experiment 2. Then, a white matter atlas was used to identify regions in which overlap was observed. Regions containing clusters commonly associated with fitness across samples included the body and splenium of the corpus callosum, the fornix, bilateral anterior corona radiata, anterior internal capsule, and sagittal stratum (Figure 3A).

A similar method was employed to examine common indirect path voxels across the two samples. Regions of significant overlap between the two samples included the body of the corpus callosum, fornix, right anterior corona radiata, right anterior internal capsule, and bilateral sagittal stratum (Figure 3B). Thus, in Experiment 1 and Experiment 2, higher CRF was related to greater FA in the aforementioned regions, which, in turn, were associated with better spatial memory performance.

4.0 DISCUSSION

Higher CRF has been associated with greater gray matter volume in the prefrontal cortex (Colcombe et al., 2006; Weinstein et al., 2012) and hippocampus (Erickson et al., 2009; Erickson et al., 2011) as well as greater task-evoked and intrinsic resting functional connectivity between hippocampal and frontal regions (Burdette et al., 2010; Smith et al., 2012; Voss et al., 2010a) in older adults. Consistent with this literature, here we found in two large, independent samples of older adults, that greater CRF was associated with greater FA across a diverse network of white matter tracts. White matter was significantly associated with CRF across both samples included tracts that facilitate intrahemispheric communication between the medial temporal lobe and the prefrontal cortex, but also included a distributed network of regions throughout the brain. These results are important as they demonstrate that the benefits of higher CRF may be relatively widespread across many areas of white matter, but with some regional specificity to areas that support communication between prefrontal and medial temporal lobe regions. Further, consistent with our prediction, we found that white matter microstructure constituted an indirect path between CRF and spatial working memory performance. This indicates that white matter plays an important role in elevated spatial memory performance in older adults.

The associations we report here between CRF and white matter are consistent with a growing literature on the beneficial associations of PA, exercise, and fitness with white matter structure. Prior studies have reported that greater amounts of PA (Gons et al., 2013; Gow et al., 2012; Tian et al., 2014a), greater fitness levels (Hayes et al., 2015; Johnson et al., 2012; Marks et al., 2010), and changes in fitness levels after participation in an exercise intervention (Voss et al., 2013b) are positively associated with white matter microstructure. Results from the present study complement the associations observed in earlier work, including some overlap in the regions identified. For instance, consistent with previous reports, we observed associations between fitness and FA in the cingulum bundle (Marks et al., 2010; Tian et al., 2014b) and segments of the corpus callosum (Johnson et al., 2012). Our findings also expand these associations to other white matter tracts not previously identified including the anterior internal capsule, fornix, and anterior corona radiata. These differences may be due to variations in sample characteristics, fitness measures, and analytical approaches between this work and others. For example, many of the prior studies examining associations with CRF have had small sample sizes (Hayes et al., 2015; Johnson et al., 2012; Marks et al., 2010; Tseng et al., 2013) or have examined relationships using *a priori* regions of interest (Marks et al., 2010; Tian et al., 2014a), which provides only a limited understanding of these relationships across neural circuits throughout the brain. In addition, some previous studies have explored these associations in older adult samples with various conditions known to affect microstructural white matter integrity including cerebral small vessel disease (Gons et al., 2013), multiple sclerosis (Prakash et al., 2010), and chronic conditions including cardiovascular disease and diabetes (Tian et al., 2014a; Tian et al., 2014b). Therefore, the relationship between fitness and white matter in these populations may differ in strength and regional-specificity relative to healthy older adult samples. In order to directly address these limitations, we conducted a voxelwise analysis of white matter in two large samples (N = 113; N = 154) of healthy older adults, without neurological or cardiovascular conditions.

Further, few studies have examined whether fitness-related variation in white matter microstructure translates to differences in cognitive performance. Across the two samples, we found indirect associations between fitness and spatial working memory performance in various white matter tracts including the genu of the corpus callosum and the anterior corona radiata. This is consistent with previous work demonstrating associations between performance on similar working memory tasks and FA in the genu as well as the anterior corona radiate (Kennedy and Raz, 2009). We selected to use spatial working memory as our outcome variable because 1) spatial working memory processes decline with age (Schmiedek et al., 2009) 2) this task has been found to be sensitive to fitness (Erickson et al., 2009) and exercise effects (Erickson et al., 2011) and 3) to ensure consistency across samples, as this task was administered in both Experiments. While memory performance has been linked to white matter fiber integrity, other cognitive domains have been more strongly and consistently correlated with fiber architecture, including processing speed and executive function (Bennett and Madden, 2014; Grieve et al., 2007; Madden et al., 2009; Vernooij et al., 2009). Although this is an important first step in exploring these relationships, our results may not reflect white matter tracts that may mediate the relationship between fitness and other cognitive domains including processing speed, verbal memory, and attentional control processes. Nonetheless, our results suggest widespread relationships between CRF and white matter microstructure, and that these relationships constitute important indirect associations with spatial working memory performance.

In contrast with our hypotheses, as well as the results from Experiment 2, there was not a direct association between fitness and cognitive performance in Experiment 1. This may, in part, be due to subtle differences in the spatial working memory task across samples. Using an average accuracy score including only the 2-dot and 3-dot conditions, which is more consistent with the set size in Experiment 2, strengthened the association, although it remained non-significant. In addition, Experiment 2 had a larger sample size and thus may have been more adequately powered to detect an association. Notably, a direct relationship between the independent and predictor variable is not required for a significant indirect pathway to be present (Hayes, 2009; Preacher and Hayes, 2004). For instance, in Experiment 1, it may be that fitness is related to spatial working memory performance through its association with white matter microstructure, which in turn is associated with better task performance.

Also in contrast with our hypotheses, we found several negative relationships between CRF, FA, and spatial working memory performance. In both studies, negative associations between CRF and FA were present, albeit negligible, and limited to a few small clusters. The bulk of the negative associations, however, emerged from the mediation model. As a majority of the CRF-FA associations were in the positive direction, the comparably larger presence of negative associations in the indirect paths suggests that this may be driven by negative associations between white matter integrity and spatial working memory performance. This is an interesting trend, and we can only speculate about why this might be occurring. It may be that greater white matter microstructural integrity in some paths is associated with a particular strategy preference that is suboptimal. Functional MRI studies have shown that older adults tend to differ both in terms of regional specificity and level of regional recruitment relative to younger adults during neurocognitive tasks (Huang et al., 2012; Kennedy et al., 2015). Further, these age-related differences in neural recruitment patterns have been linked to poorer task performance in older adults (Zhu et al., 2013), suggesting that effective versus non-effective strategies are related to both variation in performance and variation in neural circuits associated with performance. Similarly, greater white matter microstructure in certain brain areas may facilitate particular strategies that are associated with less effective task performance.

Despite these general conclusions, there were some important qualitative differences between the results from the two samples in our study. While both studies demonstrated widespread associations between fitness and FA in multiple neural pathways, the results from these associations in Experiment 1 were more focused in anterior segments (e.g., genu) than Experiment 2, which were more diffuse and consistently spread throughout white matter (see Figure 1A and 2A). Further, as seen in the scatter plots of Figures 1B and 2B, the variability in FA in Experiment 2 was greater than that found in Experiment 1 and the mediation results with spatial memory performance were also more diffuse in Experiment 2 than in Experiment 1. The differences in variability may be a consequence of the fact that diffusion data were collected using 12 gradient directions in Experiment 1 and 30 gradient directions in Experiment 2, with the latter providing a more reliable tensor estimate. Although we did not conduct a quantitative comparison between the two samples, there are several factors that could explain some of these differences. For example, there were significant differences between the samples in fitness levels, with subjects in Experiment 2 having a slightly lower mean VO_{2max} than the participants in Experiment 1. Although the effects we report appear linear, there could be regional differences in the degree of linearity with some areas becoming less associated with CRF at lower fitness levels. In addition, the MR sequence parameters and the machine type were different between studies as were some of the general demographic characteristics (e.g., slightly younger sample in Experiment 2). Furthermore, the spatial memory task used in both experiments was very similar, but differed in one key aspect: in Experiment 1 the task used a set size of 1, 2, or 3 to-be-remembered locations whereas in Experiment 2 the set sizes ranged from 2, 3, or 4 to-be-remembered locations. Such a difference was not associated with differences in performance, but could have contributed to strategy differences or the extent to which different brain areas supported task performance. Nonetheless, despite these qualitative differences between the results shown in Figures 1 and 2, the results from both samples clearly demonstrate that higher CRF was associated with greater white matter microstructural integrity, which was, in turn, related to better spatial working memory performance.

We can only speculate about the physiological mechanisms underlying these associations. In studies of gray matter volume, changes in hippocampal volume after participation in an exercise intervention were correlated with changes in serum brain-derived neurotrophic factor (BDNF) levels (Erickson et al., 2011). Other studies have also identified BDNF as an important potential mediator of resting state changes (Voss et al., 2013a), and these results in humans have been supported by non-human animal research showing increased expression of BDNF in the hippocampus and cortex after several weeks of exercise (Gómez-Pinilla et al., 2002; Vaynman et al., 2004). Rodent work has also identified associations between BDNF and brain white matter. For example, a recent study found that administration of BDNF predicted remyelination following induced subcortical damage in a rodent model of ischemic stroke (Ramos-Cejudo et al., 2015). These findings are complemented by recent advancements in the human literature, including a longitudinal study of healthy older adults, which found that lower levels of plasma BDNF were associated with a steeper decline in white matter volume among females (Driscoll et al., 2012). However, other mechanisms are also likely at play. For example, exercise reduces pro-inflammatory cascades, which have been shown to negatively impact white matter microstructure (Wersching et al., 2010). Fitness-related associations with insulin pathways, gene expression patterns, or cerebrovasculature may also be underlying mechanisms for the effects we report here.

There are several limitations to our study. First, we report results from a cross-sectional analysis of fitness and white matter. Currently there is an absence of studies examining white matter changes resulting from randomized trials of exercise (however see Voss et al., 2013b). Thus, we cannot determine a causal association between increased aerobic fitness and white matter. Both of the studies reported here were baseline assessments from two randomized trials of exercise, with the results from Experiment 2 still being collected and analyzed. Future reports will be able to more adequately describe the changes in white matter microstructure resulting from the intervention. Both samples consisted of healthy, cognitively intact older adults that were physically inactive at the time of baseline assessment. Due to these inclusion criteria, it is unclear whether similar results would be found in either a more impaired or more fit older adult sample. These results might be more robust in a more physically active sample with a greater range of CRF. Further, genetic factors play a role in determining fitness levels, and it is impossible in our study to rule out the contribution of genetic variants compared to the contribution of physical activity in determining fitness. Additionally, the diffusion data in Experiment 1 was collected using only 12 gradient directions, which limits the precision of the tensor estimation. Further, a FLAIR sequence was not run in either sample so we could not control for white matter hyperintensities. A final limitation is that our MR parameters between the two samples were quite different which limits our ability to validly conduct direct quantitative comparisons between samples. As the field of white matter imaging is quickly evolving, it will be important for studies to consider the consistency of the parameters to more accurately compare results across time and samples.

In summary, we find that higher CRF levels in two separate samples of older adults is positively related to white matter in widely distributed neural networks and that many of these associations statistically mediate the association between CRF and spatial working memory performance. Remaining aerobically fit might be an important approach for maintaining white matter health and working memory function into late adulthood.

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Highlights

- Fitness was associated with white matter (WM) microstructure in multiple WM tracts
- Regions of association included tracts that connect the medial temporal and prefrontal cortices
- The fitness-WM relationship was further associated with working memory performance

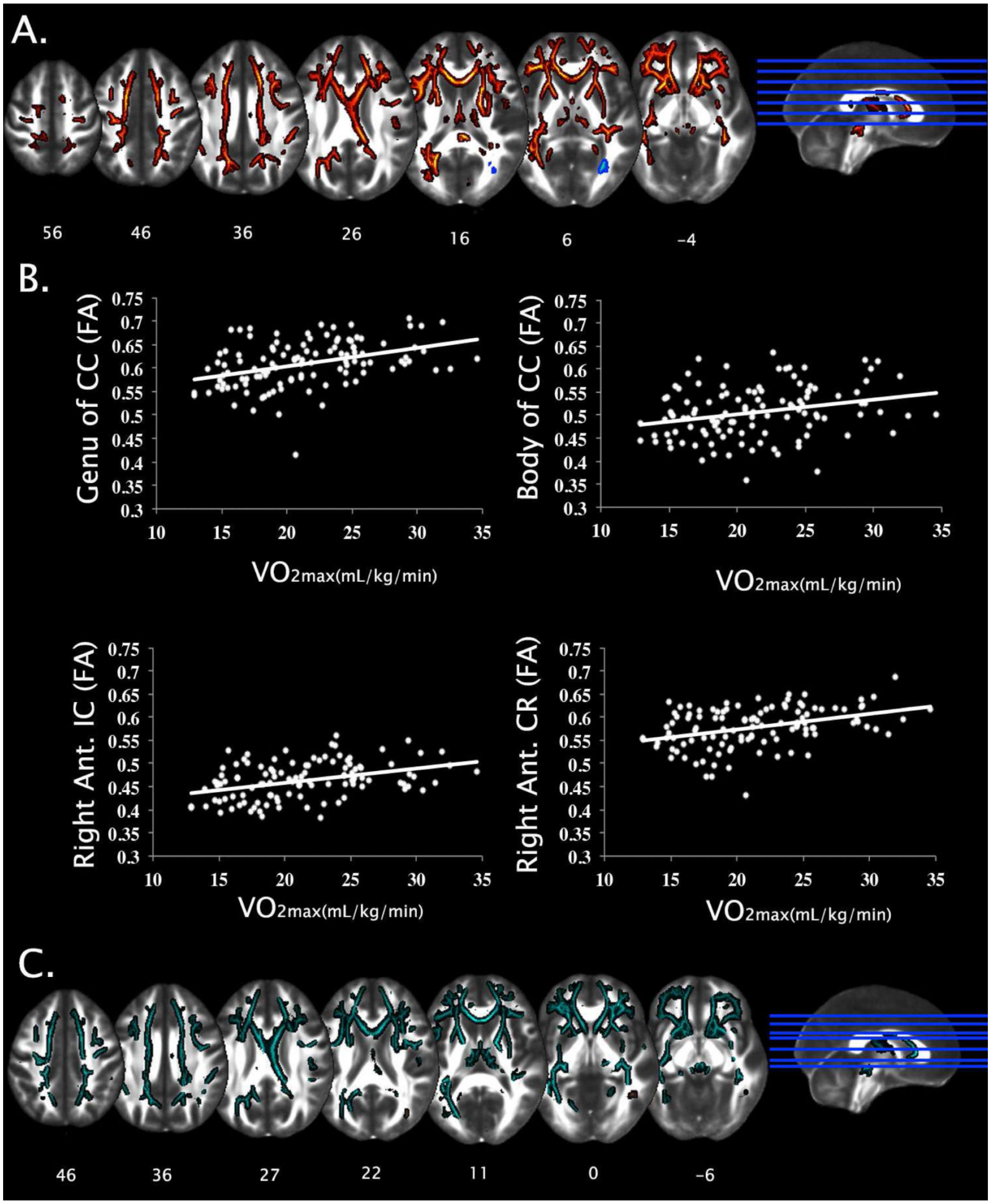

Figure 1.

Shown are significant associations between cardiorespiratory fitness (CRF), fractional anisotropy (FA), and mean spatial working memory performance from Experiment 1. (A) Clusters of voxels where fitness was significantly associated with FA. Warm-colored voxels show positive associations, cool-colored voxels demonstrate negative associations. Side panels indicate slice placements. Age, gender, and years of education were included as covariates. Clusters are thresholded to k≥20 contiguous voxels and a false discovery rate of 0.05. The z-plane coordinates of each slice, in MNI space, are presented at the bottom. For visualization purposes, tbss_fill was used to dilate statistical maps. (B) For illustration purposes, scatterplots and best-fit lines for the relationship between CRF and FA in selected regions. (C) Spatial distribution of indirect path voxel clusters. Cyan-colored voxel clusters show a positive indirect effect, brown voxel clusters indicate a negative indirect effect. Ant, anterior; IC, internal capsule; CC, corpus callosum; Ext, external; FA, fractional anisotropy.

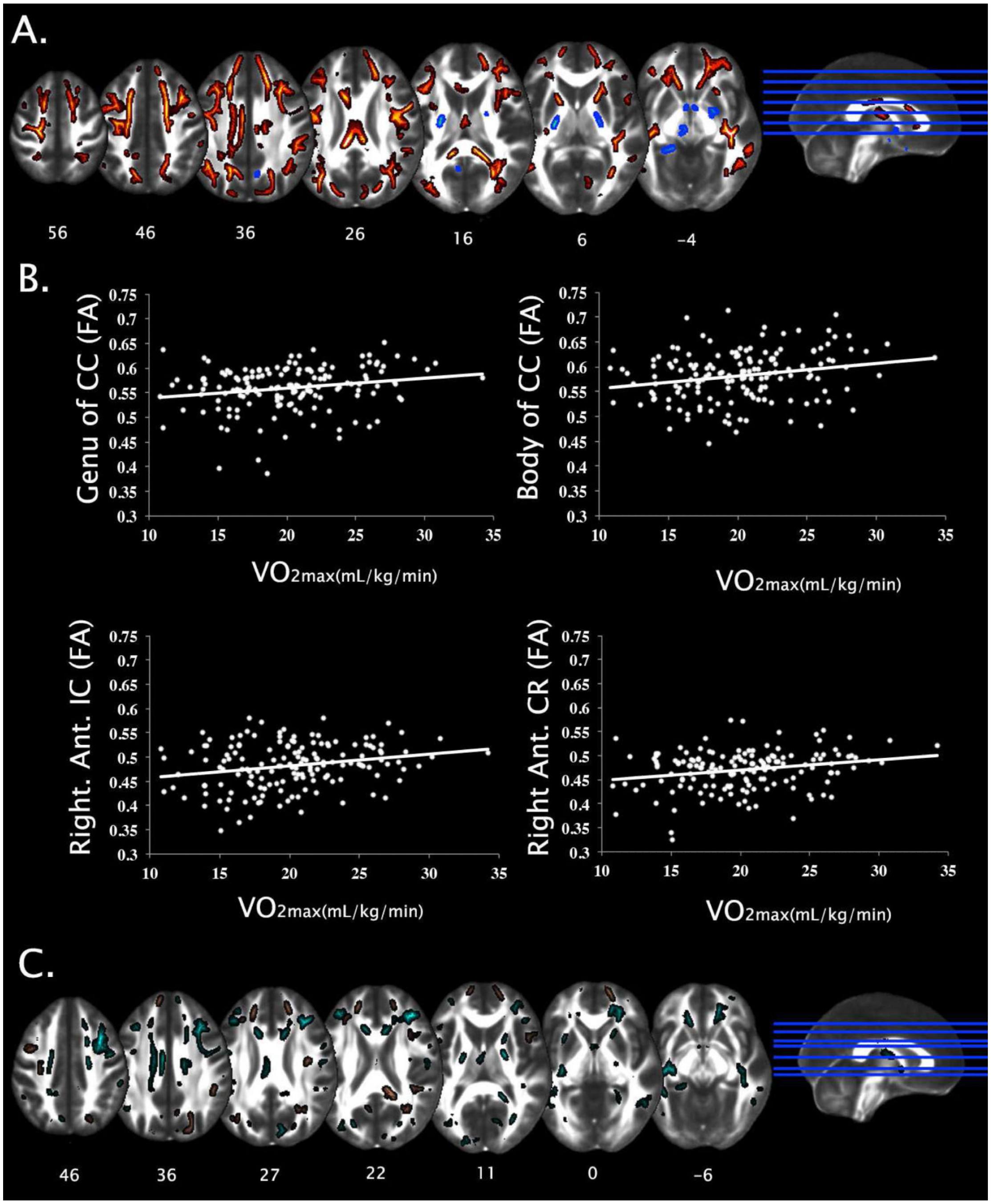

Figure 2.

Shown are associations between cardiorespiratory fitness (CRF), fractional anisotropy (FA), and spatial working memory performance from Experiment 2, with the same plotting conventions as Figure 1. Ant, anterior; FA, fractional anisotropy; CC, corpus callosum; IC, internal capsule; CR, corona radiata.

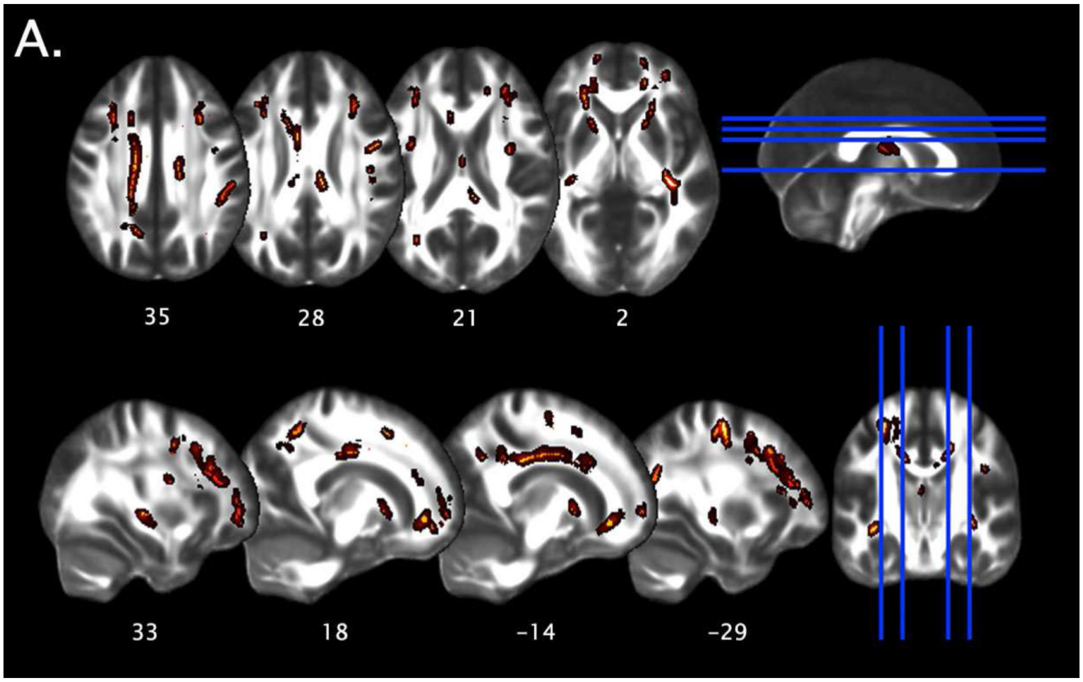

Figure 3A.

Shown are results from the conjunction analysis of the relationship between fitness and FA in Experiment 1 and Experiment 2.

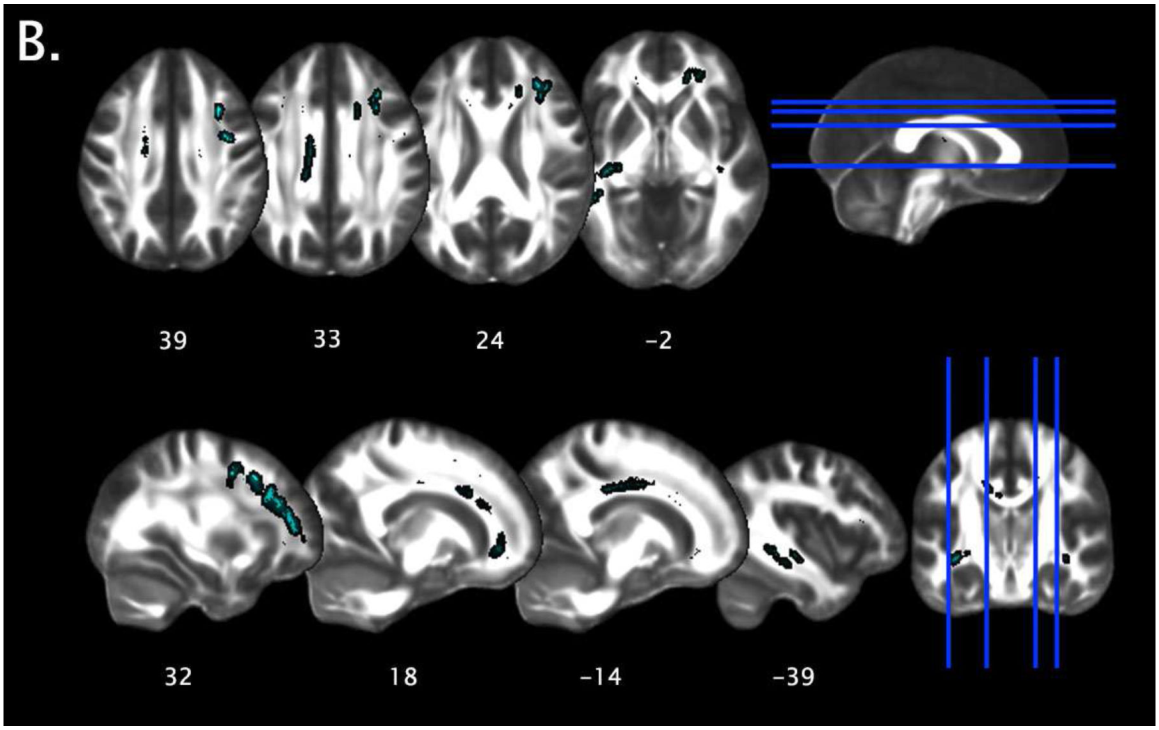

Figure 3B.

Shown are the results from the conjunction analysis of the indirect path voxels in Experiment 1 and Experiment 2.

Table 1

Demographic characteristics, CRF, and task performance for Experiment 1 and Experiment 2.

	Experiment 1 (*n = 113*)	**Experiment 2 (*n = 154*)**
Age (years)	66.61 (5.65)	65.66 (4.55)
Gender (% Male)	36.30%	31.20%
Education (years)	15.48 (2.92)	15.62 (2.85)
Cardiorespiratory Fitness (VO_{2max}, ml/kg/min)	21.40 (4.89) *	20.17 (4.49)
Spatial Working Memory Accuracy (%)	81.81 (12.86)	81.02 (11.66)

Values are mean (standard deviation).

* indicates a significant difference across studies at $p < .05$

Table 2

Relationships between fitness, demographic characteristics, and task performance for Experiments 1 and 2.

	Experiment 1 (*n = 113*)	**Experiment 2 (*n = 154*)**
Age[a] (r, *P* value)	−0.460[**] < .001	−0.247[**] .002
Gender[b] (t, *P* value)	−4.710[**] < .001	−3.292[**] .002
Education[a] (r, *P* value)	0.291[**] .002	0.107 0.187
Spatial Working Memory Accuracy[c] (β; *P* value)	0.171 0.151	0.237[**] 0.006

[a] Results of bivariate correlations examining the association between fitness and each variable. Values are Pearson's *r*; *p*-value.

[b] Results from independent samples t-test demonstrating the difference in VO_{2max} between males and females. Values are *t*; *p*-value. Females were coded 0; men coded 1.

[c] Results from multilevel linear regression analysis of the relationship between fitness and spatial working memory performance after adjusting for age, gender, and years of education. Values are beta; *p*-value.

[**] indicates significance at $p < .01$.