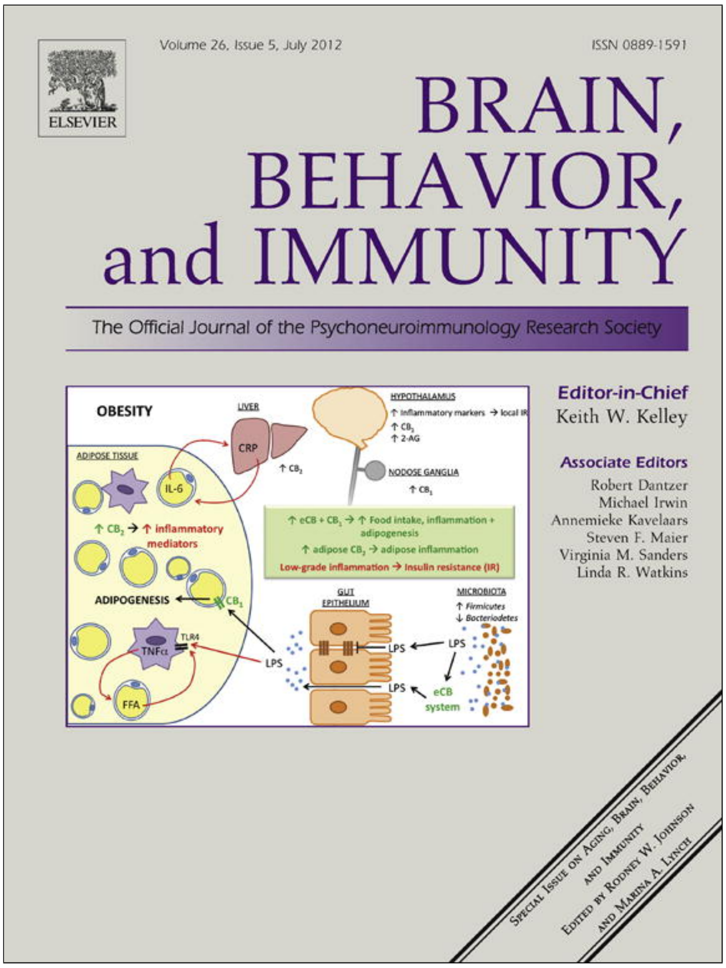

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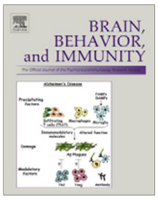

The association between aerobic fitness and executive function is mediated by prefrontal cortex volume

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ABSTRACT

Aging is marked by a decline in cognitive function, which is often preceded by losses in gray matter volume. Fortunately, higher cardiorespiratory fitness (CRF) levels are associated with an attenuation of age-related losses in gray matter volume and a reduced risk for cognitive impairment. Despite these links, we have only a rudimentary understanding of whether fitness-related increases in gray matter volume lead to elevated cognitive function. In this cross-sectional study, we examined whether the association between higher aerobic fitness levels and elevated executive function was mediated by greater gray matter volume in the prefrontal cortex (PFC). One hundred and forty-two older adults (mean age = 66.6 - years) completed structural magnetic resonance imaging (MRI) scans, CRF assessments, and performed Stroop and spatial working memory (SPWM) tasks. Gray matter volume was assessed using an optimized voxel-based morphometry approach. Consistent with our predictions, higher fitness levels were associated with: (a) better performance on both the Stroop and SPWM tasks, and (b) greater gray matter volume in several regions, including the dorsolateral PFC (DLPFC). Volume of the right inferior frontal gyrus and precentral gyrus mediated the relationship between CRF and Stroop interference while a non-overlapping set of regions bilaterally in the DLPFC mediated the association between CRF and SPWM accuracy. These results suggest that specific regions of the DLPFC differentially relate to inhibition and spatial working memory. Thus, fitness may influence cognitive function by reducing brain atrophy in targeted areas in healthy older adults.

1. Introduction

Healthy adults lose approximately 15% of their neocortical tissue between ages 30 and 90, with disproportionately higher losses in areas supporting executive control, such as the dorsolateral prefrontal cortex (DLPFC) (Jernigan et al., 2001; Raz, 2000). These volumetric losses in late adulthood often precede, and lead to, decline in executive function (Raz et al., 2005).

Despite this disheartening view of the aged brain, there is promising evidence that age-related cognitive decline might not be as immutable as previously thought. For example, both high cardiorespiratory fitness (CRF) and physical activity levels have been consistently associated with the maintenance of cognitive function across the lifespan, including a reduced risk for developing Alzheimer's disease and a slower progression of cognitive problems in cognitively impaired patients (Andel et al., 2008; Heyn et al., 2004; Kramer et al., 1999; Podewils et al., 2005; Yaffe et al., 2009). Cross-sectional neuroimaging studies have supported these results, finding that higher CRF and/or physical activity levels are associated with greater prefrontal cortex (PFC) volume (Colcombe et al., 2003; Gordon et al., 2008) and greater hippocampal volume (Bugg and Head, 2011; Erickson et al., 2009) in older adults. Randomized clinical trials have demonstrated that participation in moderate intensity aerobic exercise improves cognitive function in older adults, with the greatest benefits occurring on measures of executive control including inhibition, task-switching, and the coordination of multiple tasks in working memory (Colcombe and Kramer, 2003; Colcombe et al., 2004; Hawkins et al., 1992; Kramer et al., 1999; see Hillman et al. (2008) for a review). Likewise, randomized interventions have shown that moderate aerobic exercise results in increased gray matter volume in the PFC after just 6 months of exercise (Colcombe et al., 2006)

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and increased hippocampal volume after 1 year of exercise (Erickson et al., 2011), compared to a non-aerobic exercise control group.

Despite the relatively consistent benefits of CRF on cognitive function and gray matter volume in late adulthood, the link between enhanced cognition and increased gray matter volume in healthy older adults is under-explored. For instance, Floel and colleagues found a link between physical activity, bilateral PFC volume, and memory encoding in healthy older adult participants, but did not investigate tasks of executive function (Floel et al., 2010). Similarly, Peters and colleagues found a relationship between CRF, gray matter density in the right anterior insula, and memory performance, but only used 33 healthy young subjects and did not investigate tasks of executive function (Peters et al., 2009). Gordon and colleagues found an association between CRF and gray matter volume in the inferior frontal lobes of forty healthy older adult participants, but did not directly relate these findings to cognitive performance (Gordon et al., 2008). In fact, only two studies have directly examined the links between volume and cognition as a function of fitness or exercise in healthy older adults (Erickson et al., 2009, 2011) and both of these studies only examined the size of the hippocampus in relation to spatial working memory performance. These studies found that hippocampal volume partially mediated a relationship between CRF and spatial working memory performance. However, to our knowledge, an analogous investigation between fitness, brain volume, and executive function has not yet been published. Hence, we do not yet know whether PFC volume mediates the link between CRF and executive function in a manner similar to the link between CRF, hippocampal volume, and spatial working memory.

In the current study, we examined whether the association between higher CRF and executive functioning would be mediated by greater gray matter volume in the PFC. We had *a priori* interest in the PFC for several reasons. First, functional neuroimaging studies have verified that the DLPFC supports successful inhibition and attentional control in the Stroop task with left prefrontal regions playing a role in maintaining an attentional set in working memory and right prefrontal regions supporting response selection processes (Milham et al., 2002; Prakash et al., 2009). Therefore, we examined the relationship between CRF and inhibitory control as measured by the Stroop task and examined the extent to which variation in DLPFC volume mediated this association. On the other hand, spatial working memory performance has been associated with the size of the hippocampus (Erickson et al., 2009), but is also considered to be dependent on right prefrontal and parietal cortices, both of which have dominant roles in supporting non-verbal memory processes (Braver et al., 1997; Curtis and D'Esposito, 2004). In addition, hippocampal volume has been shown to only partially mediate the relationship between CRF and spatial working memory performance, leaving open the question of whether other brain regions are important in this relationship (Erickson et al., 2009). Given these associations, we predicted that the volume of areas within the DLPFC would also mediate the effect of aerobic fitness on spatial working memory. Therefore, this study examined the hypothesis that CRF effects on both Stroop and spatial working memory performance would be mediated by DLPFC volume such that higher fitness levels would be associated with larger DLPFC volume, which would, in turn, be associated with better cognitive performance.

2. Methods

2.1. Participants

One hundred and seventy-nine participants were recruited to participate in a 1-year randomized, controlled trial examining the effect of aerobic training on brain and cognition. Subjects were recruited through community advertisements and physician referrals. Potential subjects were initially screened over the phone for inclusion and exclusion criteria. Upon passing the initial phone screening, subjects were invited to a group orientation to receive study details and ask questions regarding the program. Three subsequent baseline sessions were performed after the group orientation. The current study focused on the cross-sectional baseline data from participants that had high-resolution magnetic resonance imaging (MRI) data and completed the cognitive assessments described below. One hundred and forty-two participants had high-resolution MRI scans without excessive motion artifacts that were acceptable for VBM analyses. All of these participants were included in the Stroop mediation analysis. Three of these individuals were missing data in the spatial working memory (SPWM) paradigm, so only 139 participants were included in the SPWM analyses.

Participants were between the ages of 58 and 81 at time of testing (mean age = 66.6 years; standard deviation = 5.6 years; see Table 1 for participant characteristics). Investigations of the full sample and sub-samples of this trial have been described in several studies (e.g. Erickson et al., 2009, 2011; McAuley et al., 2011a,b; Prakash et al., 2011; Voss et al., 2010b). Inclusion criteria for the study were as follows: 60+ years of age during the trial, capability to perform physical exercise, medical consent to perform physical exercise from a personal physician, successful completion of the VO_2 max test (described below), absence of cognitive impairment as assessed by the modified Mini Mental Status Examination, normal or corrected to normal vision, absence of clinical depression (as measured by the Geriatric Depression Scale; Sheikh and Yesavage, 1986), and a low-active lifestyle at time of baseline assessment. The low-active lifestyle requirement for this trial reduces the potential confound that people with better cognitive abilities have a higher propensity to exercise. A low-active lifestyle was defined as participating in no more than one 20-minute physical activity per week for the past 6 months, as assessed by the Physical Activity Scale for the Elderly (PASE) (Washburn et al., 1993). In addition, all participants met safety criteria for participating in an MRI study. These criteria included no previous history of head trauma, head or neck surgery, diabetes, neuropsychiatric or neurological conditions including brain tumors, or having any ferrous metallic implants that could cause injury due to the magnetic field.

Exclusion criteria for the study included: self-reported regular physical activity (2 or more times per week), physical disability that prohibited mobility, non-consent of a physician, evidence of cardiac abnormalities during the VO_2 max test, clinical depression, presence of implanted devices that would be an MRI safety concern, chronic steroidal treatment, and claustrophobia. Participants with severe asthma or other severe respiratory problems were excluded from the initial HALT trial since these conditions would impair ability to perform the VO_2 max assessment. All participants received a physician's consent to engage in a maximal graded exercise test (VO_2 max) and signed an informed consent approved by the University of Illinois institutional review board.

Table 1
Participant characteristics. For spatial working memory accuracy in the 3-Item condition, descriptive statistics were conducted on the 139 participants with complete data.

Characteristic	All participants (*N* = 142)
Age (mean years [sd])	66.4 (5.5)
Education (mean years [sd])	15.7 (3.0)
Sex (%female)	64.1
Fitness level (mean ml/kg/min [sd])	21.3 (4.8)
Stroop percent interference (mean [sd])	11.5 (12.9)
SPWM 3-Item accuracy (mean [sd])	76.9 (14.6)

SPWM = spatial working memory paradigm; sd = standard deviation.

2.2. Cognitive and fitness assessments

2.2.1. Stroop task

The Stroop task is a classic test of two key components of executive function: attention and inhibitory control (MacLeod, 1991, 1992). The modified Stroop task utilized in the current study involved three types of trials: congruent, neutral, and incongruent. In this task, participants named the ink color in which a word was printed by pressing one of three buttons. Neutral trials were those in which the word was a non-color word (e.g. "LOT"). Congruent trials were those in which the word named the ink color in which it was printed (e.g. "RED" in red ink). Incongruent trials were those in which the word named a different color than the ink (e.g. "RED" in green ink). There were both eligible incongruent trials and ineligible incongruent trials. The incongruent-eligible condition presented words that matched one of the potential responses (e.g. BLUE in red ink, if 'blue' was one of the potential responses). The incongruent-ineligible condition presented words that did not match the set of potential responses (e.g. PURPLE in blue ink if 'purple' is not one of the potential responses). One hundred and forty-four stimuli were presented, 36 of each trial type. Each stimulus was displayed for 1 s with a 1.5 s response window and a fixed 3 s stimulus onset asynchrony. A crosshair (+) was presented on the screen during all inter-stimulus intervals. An event-related stimulus design with 40% jitter was employed, such that the timing between trials varied, in order to optimize the stimulus sequence and timing for fMRI. Stimulus sequence and jitter for each participant was generated using OptSeq2 (http://surfer.nmr.mgh.harvard.edu/optseq/). Participants were instructed to respond as quickly and as accurately as possible during the task and reaction time (RT) was recorded.

The outcome measure of interest for this study was percent interference. Percent interference was calculated as the average incongruent-eligible trial RT minus the average congruent trial RT, divided by average congruent trial RT, then multiplied by 100. Given this calculation, percent interference scores could be both negative and positive. Interference provides a measure of how much additional executive processing is needed to respond to an incongruent trial above and beyond the processing needed for a congruent trial; thus, a higher interference score indicates worse performance on the task. Only eligible trials were used to calculate interference because they are the more traditional Stroop incongruent trial type and the most robust to the effects of cardiorespiratory fitness (Prakash et al., 2011).

The Stroop task was conducted during functional MRI scanning, the results of which are described elsewhere in relation to cardiorespiratory fitness levels (Prakash et al., 2011). The analyses we report here are limited to the behavioral results from the task.

2.2.2. Spatial working memory assessment

Participants performed a SPWM paradigm that has been previously associated with aerobic fitness and hippocampal function in older adults (Erickson et al., 2009, 2011). The SPWM task provided a measure of the ability to form and retain memories of spatial locations over a short delay. First, a fixation crosshair appeared for 1 s upon which participants were instructed to maintain fixation. Immediately following fixation 1, 2, or 3 dots appeared at random locations for 500 ms. The dots then disappeared for 3 s, during which time participants were instructed to remember the dot locations. At the end of the delay, a red dot appeared either in one of the same locations as the original targets (match) or at a different location (non-match). Participants had 2 s to decide whether the red test dot matched or did not match the spatial location of one of the initially presented dots, and were asked to indicate their response by pressing the designated key on a computer keyboard ('x' = non-match, 'm' = match). Participants were instructed to respond as to whether the new dot was in the same location or in a different location as any of the target dots. Each set size condition (1-Item, 2-Item, and 3-Item) included forty trials: half of the trials matched the probe dot location and half did not match the probe dot location. Participants first completed several practice trials in order to become acquainted with the protocol. Consistent with prior studies using this paradigm (Erickson et al., 2011; McAuley et al., 2011b; Szabo et al., 2011; Voss et al., 2010b), we used accuracy rates (percent correct) for the more difficult 3-Item condition as the primary outcome measure in the current study.

2.3. Cardiorespiratory fitness assessment

CRF was assessed by a maximal graded exercise test (VO_2 max) on a motor-driven treadmill within 2 weeks after the cognitive testing session. The VO_2 max test is the "gold standard" measurement of CRF (American College of Sports Medicine, 1991). To complete the VO_2 max test, participants walked at a speed slightly faster than their normal walking pace (approximately 3 mph) with increasing grade increments of 2% every two minutes, expiring air samples at 30-s intervals. A cardiologist and nurse continuously monitored measurements of oxygen uptake, heart rate, and blood pressure to ensure patient safety. The test continued until either there was objective evidence that the VO_2 max had been attained (as determined by an exercise physiologist), or the participant volitionally terminated the test due to exhaustion. VO_2 max 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; and (3) a heart rate equivalent to their age predicted maximum (i.e. 220 – age). Final VO_2 max scores consist of the maximum gases expired, adjusted for height and weight of the person, measured in units of milliliters per kilogram per minute (ml/kg/min).

2.4. Structural magnetic resonance imaging (MRI)

MRI scanning was conducted within 2.5 weeks of the initial cognitive assessment. All participants underwent structural MRI scanning on a 3 Tesla Siemens Allegra scanner with an echo time (TE) of 3.87 ms, repetition time (TR) of 1800 ms, field of view (FOV) of 256 mm, and an acquisition matrix of 192 × 192 mm. High resolution T1 weighted brain images were collected using a 3D Magnetization Prepared Rapid Gradient Echo Imaging (MPRAGE) protocol, collecting 144 contiguous slices in an ascending manner (see Erickson et al. (2009) for further scanning details).

2.5. Statistical analyses

2.5.1. MRI data analysis

MR data were processed using tools in the FMRIB Software Library (Image Analysis Group, FMRIB, Oxford, UK; http://www.fmrib.ox.ac.uk/fsl/; Smith et al., 2004). An optimized voxel based morphometry (VBM) protocol was used to analyze structural MRI data (FSL-VBM). This optimized procedure reduces error resulting from registration and multiples each voxel by the Jacobian determinant to allow for the estimation of partial volumes for each voxel. VBM allows for a whole-brain volumetric analysis in a semi-automated fashion, making it easily reproducible and accessible to researchers with more varied levels of anatomical knowledge. VBM analysis computes the probability that each voxel in a structural MR image is cerebrospinal fluid, gray matter, or white matter and yields statistical maps for each voxel type (see Ashburner and Friston (2000) for a detailed description of VBM methods). Voxels are then classified into the structural category with the highest probability and can be statistically analyzed between subjects. Separate statistical maps are created for gray matter voxels and white matter voxels, which can then be used for volumetric analysis. For the current study, we limited our investigation to DLPFC regions found in the gray matter statistical maps. VBM is a reliable method for analyzing gray matter data from healthy older adults (Colcombe et al., 2003; Good et al., 2001) and provides estimates that are similar to manual tracing in this population (Kennedy et al., 2009).

All images were processed in the following manner: (1) removal of non-brain matter that would obscure registration and statistical analyses using the brain extraction technique in FSL (Smith, 2004). All brain-extracted images were visually inspected for residual skull and non-brain matter that could affect registration - any residual skull was removed at this stage; (2) 12-parameter affine registration to the Montreal Neurological Institute template (Jenkinson and Smith, 2001) followed by non-linear registration (Andersson et al., 2007), using a b-spline representation of the warp field, to a study-specific template created from those 142 participants with both MRI and Stroop data; (3) partial volume estimation describing the proportion of cerebrospinal fluid, gray matter, or white matter in each voxel using FSL's automated segmentation technique (Zhang et al., 2001). Partial volume correction was conducted by dividing the partial volume images by the Jacobian determinant, which was calculated from the transformation matrices. These modulated images account for any changes in the warping taking place during registration; (4) smoothed the segmented, registered, and modulated gray matter images with an isotropic Gaussian kernel with a 3 mm sigma. Statistical analyses were conducted on these segmented, registered, modulated, and smoothed gray matter images. The final map of p values for each voxel in the brain in relation to CRF when controlling for age, sex, and education (family-wise-error corrected at $p < .05$) was generated by the *randomise* function in FSL. This function is a non-parametric permutation program that estimates a sampling distribution by generating bootstrapped samples, with replacement, from the VBM data. We used 5000 samples in our bootstrap analysis. This non-parametric approach works similarly to the more traditional general linear model in that all variables are entered into a regression-like design matrix. Non-parametric approaches for analyzing VBM data are preferred because intensity distributions of structural images frequently deviate from normality.

Using the Threshold-Free Cluster Enhancement (TFCE) technique (Smith and Nichols, 2009), brain regions were identified that were significantly correlated with CRF when controlling for the variance associated with age, sex, and education (Fig. 1A). The TFCE technique is completed without using *a priori* thresholds, such as those used in cluster-based thresholding (Smith and Nichols, 2009). Typical cluster and voxel thresholding techniques rely on arbitrarily defined thresholds that can be sensitive to variations in the data near the threshold level. In addition, cluster-based thresholds are sensitive to the amount of spatial smoothing applied before analysis. The TFCE method avoids these limitations by taking the raw statistical image and producing an output image where each value represents the weighted sum of the local clustered signal (see Smith and Nichols (2009) for further details). This method is most optimal when applied to unsmoothed or minimally smoothed data; therefore, we used an isotropic Gaussian kernel with a 3 mm sigma for smoothing, rather than a larger smoothing kernel. TFCE has been validated in several studies and has been shown to be more sensitive than cluster-based or voxel-based thresholding and is the recommended approach for most VBM analyses (Giorgio et al., 2010; Hayasaka et al., 2004; Salimi-Khorshidi et al., 2011; Smith and Nichols, 2009).

2.5.2. Region of interest selection

Five regions of interest (ROIs) were identified based upon the overlap between the main effect of fitness (Fig. 1A) with both cognitive variables. We focused our analyses to ROIs within the DLPFC. Given that performance on SPWM and Stroop tasks are dependent upon different cognitive processes, we predicted that these cognitive variables might be associated with volume in different areas within the DLPFC. Therefore, we identified separate DLPFC regions associated with both cognitive variables and used these areas to test for mediation effects.

We first found DLPFC regions associated with fitness when controlling for age, sex, and education. We next identified overlapping DLPFC regions sensitive to both fitness and cognition by using a conjunction analysis. For the conjunction analysis, both of the gray matter maps created from an association with one of the cognitive variables (Stroop percent interference and SPWM 3-Item percent accuracy) were separately overlaid with the gray matter map found from the main effect of CRF on brain volume. From these overlapping maps, five ROIs were identified within the DLPFC using standard calculations (Fig. 1B and C) (Poldrack, 2007). ROIs were not restricted to a certain number of voxels because: (A) we wanted to explore the entire DLPFC region for possible mediating areas, and (B) clusterwise thresholds are not needed with the TFCE approach. Three ROIs within the DLPFC were identified based on the conjunction of CRF and Stroop percent interference. The final two DLPFC ROIs were identified from the conjunction of CRF with SPWM accuracy in the 3-Item condition. Gray matter volume within these five statistically defined regions was extracted and used in a mediation analysis to determine if volume mediated the association between fitness and cognitive performance.

2.5.3. Mediation analysis

A mediating variable is a variable that is part of the causal pathway by which an independent variable affects a dependent variable. The main requirement for mediation is that the *indirect effect* of the independent variable (CRF) through the mediator (DLPFC volume) on the dependent variable (Stroop percent interference or SPWM 3-Item accuracy) be significant (Gelfand et al., 2009; Zhao et al., 2010).

Mediation analyses were conducted using the *indirect* macro designed for SPSS (Preacher and Hayes, 2008). This macro uses bootstrapped sampling to estimate the indirect mediation effect of volume on the relationship between fitness and executive function. In this analysis, 5000 bootstrapped samples were drawn with replacement from the dataset to estimate a sampling distribution for the indirect mediation pathway (i.e. the pathway from CRF to DLPFC volume to executive function). Indirect effects and 95% confidence intervals are reported. Mediation indirect effects can be interpreted as the strength of the relationship between the independent variable (CRF) and dependent variable (Stroop or SPWM performance) when accounting for the mediating pathway (Hayes, 2009). We report adjusted R^2 values for an estimate of the effect size of each mediator on the CRF and executive function relationship. The dependent variables were computed from both of the cognitive assessment scores: Stroop percent interference and SPWM accuracy in the 3-Item condition. Each assessment outcome measure was used as a dependent variable in a separate mediation analysis, rather than a composite executive function index, as these tests measure different aspects of executive function. Mediation models controlled for the variance from age, education, and sex. Effect sizes are reported using adjusted R^2 values for each significant model.

3. Results

3.1. Correlations between CRF and cognitive performance

Consistent with our hypotheses, higher CRF was associated with better performance on both the Stroop and SPWM tasks. Specifi-

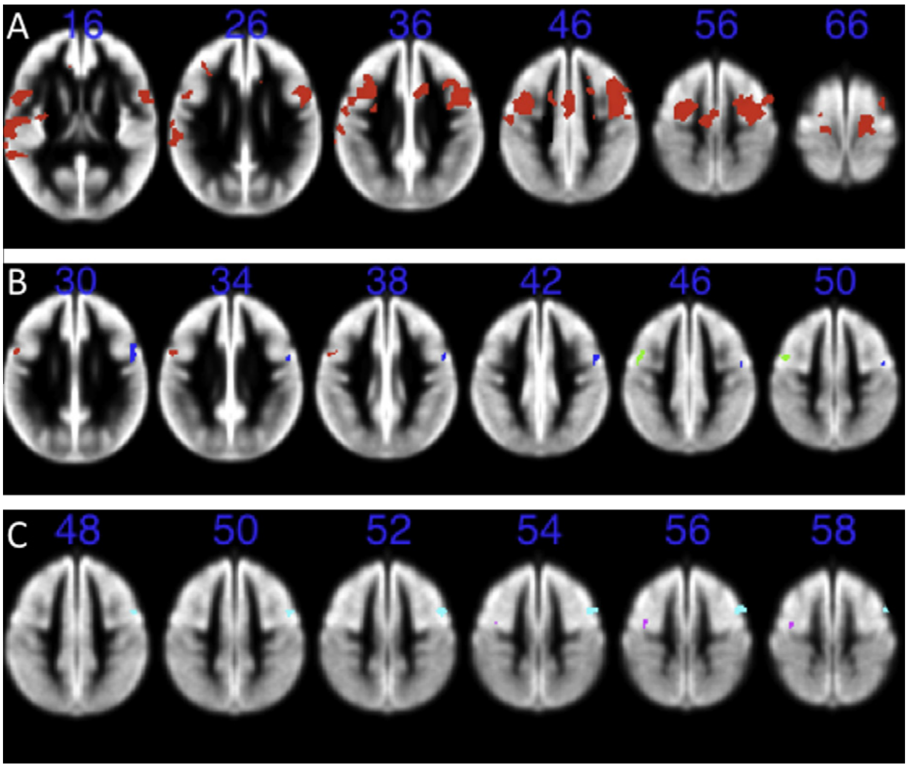

Fig. 1. Regions of interest (ROIs) chosen from overlapping brain regions that showed correlations with fitness and cognitive measures. All images are in neurological coordinates (right on the right side). Coordinates are *Z*-coordinates for each slice. (A) Brain regions exhibiting a significant relationship with fitness after controlling for age, sex, and education. Image is familywise-error corrected at $p < 0.05$. (B) Three ROIs chosen from a conjunction between the main effect of fitness and correlations with Stroop percent interference. These ROIs correspond to Table 2 as follows: blue corresponds to ROI #1; red corresponds to ROI #2; green corresponds to ROI #3. (C) Two ROIs chosen from a conjunction between the main effect of fitness and correlations with spatial working memory accuracy in the 3-Item condition. These ROIs correspond to Table 2 as follows: purple corresponds to ROI #4; cyan corresponds to ROI #5.

Table 2
Mediation indirect effects and 95% confidence intervals for the Stroop and spatial working memory task variables. The first three regions of interest were chosen from the overlapping brain regions that showed a main effect of fitness and a correlation with Stroop percent interference. The second two regions of interest were chosen from the overlapping brain regions that showed a main effect of fitness and a correlation with SPWM 3-Item accuracy.

	ROI description	Number of voxels (MNI space)	MNI coordinates			Stroop percent interference indirect effect (95% CI)	SPWM 3-Item accuracy indirect effect (95% CI)
			X	*Y*	*Z*		
1	Right IFG and PCG	122	56	6	30	−.128* (−0.336:−0.011)	0.077 (−0.074:0.288)
2	Left IFG and PCG	56	−44	10	40	−0.097 (−.327:−0.075)	0.042 (−0.141:0.254)
3	Left MFG	58	−48	2	48	−0.062 (−.272:0.100)	0.024 (−0.176:0.275)
4	Left MFG and PCG	14	−36	−8	60	−0.03 (−0.19:0.05)	0.114* (0.007:0.343)
5	Right MFG and PCG	75	42	4	54	−0.06 (−0.22:0.07)	0.116* (0.010:0.307)

CI = confidence interval; ROI = region of interest; SPWM = spatial working memory paradigm; IFG = inferior frontal gyrus; PCG = precentral gyrus; MFG = middle frontal gyrus.

* Indicates significant mediation effects determined by CIs that do not include 0.

cally, higher CRF was associated with less Stroop percent interference ($r = -.195$; $p < .05$) and higher accuracy on the 3-Item condition ($r = .348$; $p < .001$). Older age was associated with lower accuracy rates on the SPWM 3-Item task ($r = -.277$; $p < .01$) but not higher Stroop interference ($p > .75$). Stroop percent interference was not significantly correlated with SPWM accuracy on the 3-Item condition ($r = -.137$; $p = .11$). Accounting for age as a covariate in linear regression analyses, higher CRF values significantly predicted less Stroop percent interference ($F(2,139) = 2.77$; $\beta = -.202$; $p < .05$) and better SPWM 3-Item accuracy ($F(2,136) = 13.91$; $\beta = .273$; $p < .01$). Education and sex were not related to any of the cognitive variables (p's >.30).

3.2. CRF is related to gray matter volume in healthy older adults

Consistent with our hypotheses, we found that higher CRF levels were associated with greater gray matter volume in several brain regions including the prefrontal cortex, motor cortex, cingulate gyrus, anterior parietal lobe, and temporal lobe, when controlling for age, sex, and education (Fig. 1A). These associations remained significant after familywise-error correction of $p < .05$. CRF explained a significant portion of the variance in volume for each DLPFC ROI. Specifically, CRF accounted for 16.8% of the variance in right inferior frontal gyrus (IFG) and precentral gyrus (PCG) volume, (ROI 1 in Table 2), 13.0% of the variance in left IFG and PCG volume (ROI 2 in Table 2), and 12.8% of the variance in left middle frontal gyrus (MFG) volume (ROI 3 in Table 2). With regards to the ROIs chosen from a conjunction between CRF and SPWM accuracy, CRF accounted for 7.6% of the variance in left MFG and PCG volume (ROI 4 in Table 2) and 10.8% of the variance in right MFG and PCG volume (ROI 5 in Table 2).

3.3. The fitness–Stroop performance relationship is mediated by DLPFC volume

Mediation analyses were conducted to test the hypothesis that gray matter volume in the DLPFC would mediate the association between CRF and Stroop performance. After controlling for the variance associated with age, sex, and education, gray matter volume in the right IFG and PCG significantly mediated the relationship between CRF and Stroop interference (ROI 1 in Table 2; Fig. 2A). This mediation effect explained 3.5% of the variance in the CRF and Stroop relationship. For simplicity, we report a full list of these results in Table 2. This region was not a significant mediator of the fitness–SPWM association.

3.4. The fitness–SPWM relationship is mediated by DLPFC volume

Mediation analyses were conducted to test the hypothesis of whether gray matter volume in the DLPFC would mediate the association between CRF and SPWM accuracy in the 3-Item condition. When controlling for the variance associated with age, sex, and education, bilateral gray matter volume in the MFG and PCG mediated the association between CRF and SPWM accuracy in the 3-Item condition (ROIs 4 and 5 in Table 2; Fig. 2B and C). Volume in the left MFG and PCG (ROI 4) explained 17.5% of the variance in the CRF and SPWM relationship. Volume in the right MFG and PCG (ROI 5) explained 16.1% of the variance in the CRF and SPWM relationship. When both regions were included in the same multiple mediator model, the total mediation effect explained 17.7% of the variance in the CRF and SPWM 3-Item accuracy relationship. The regions that mediated SPWM accuracy were not significant mediators of the fitness–Stroop association (Table 2).

4. Discussion

Consistent with our predictions and with the extant literature, higher CRF levels were associated with greater gray matter volume throughout much of the cortex, including regions that typically show extensive age-related losses of tissue such as the DLPFC (Colcombe et al., 2003; Erickson et al., 2009, 2010, 2011; Floel et al., 2010; Gordon et al., 2008). In addition to replicating these prior results, we extend them by demonstrating, for the first time, that greater gray matter volume in the DLPFC mediates the association between aerobic fitness levels and executive function, including inhibitory control and spatial working memory.

Interestingly, the prefrontal brain region that mediated the CRF association with Stroop performance failed to mediate the CRF association with SPWM accuracy in the 3-Item condition, while those regions that mediated SPWM accuracy failed to mediate Stroop performance. A region in the right IFG and PCG mediated a fitness and Stroop relationship, whereas bilateral regions in the MFG and PCG mediated a fitness and SPWM relationship. All of these mediation pathways were such that higher fitness was associated with greater gray matter volume, which was in turn associated with better cognitive performance. This suggests that higher fitness levels are associated with better executive function by means of greater DLPFC volume and that the DLPFC regions that support attentional control and inhibition may differ from those that support spatial working memory.

Most studies (e.g. Colcombe et al., 2003, 2006; Erickson et al., 2009, 2011) have argued that greater gray matter volume is beneficial, especially to older adults for whom atrophy is relatively widespread. But how and why greater gray matter volume leads to better cognitive function remains unclear. In fact, the size of particular regions might have little to do with the processing capabilities of that region or the connectedness of the region with other brain areas (Burdette et al., 2010), both of which might be better predictors of cognitive performance than volume. For example, Colcombe et al. (2004) found that task-evoked activity in the prefrontal cortex differed as a function of CRF levels, but these differences occurred independently of any differences in gray matter volume. Similarly, Erickson et al. (2009) reported that greater hippocampal volume mediated the association between fitness and spatial working memory, but that it only accounted for less than 10% of the total variance in spatial memory performance. It may be the case that networks of brain regions are better predictors of cognitive performance than isolated areas (Voss et al., 2010a,b). Nonetheless, brain atrophy is common in older adults and greater volume is often indicative of less atrophy and better cognitive performance (Raz et al., 2004). Our results support this position since we demonstrate that greater DLPFC volume is associated with better Stroop and SPWM performance.

While the molecular pathways by which higher fitness augments DLPFC volume remain unknown, research with animals provides several possible biological explanations. Physiologically, aerobic exercise enhances learning and memory by inducing the proliferation of cells and by enhancing neural and synaptic plasticity (van Praag et al., 1999). Exercise has also been associated with widespread angiogenesis in the cortex, cerebellum, striatum, and hippocampus of adult rodents (Ding et al., 2006; see Cotman et al. (2007) and Hillman et al. (2008) for review). Besides creating new cells and blood vessels in the brain, rodent research shows that exercise also affects synaptic structure by inducing long-term potentiation in the hippocampus (Black et al., 1990; Farmer et al., 2004; Schmidt-Hieber et al., 2004; van Praag et al., 2005). Exercise may be influencing neural proliferation and vascularization by increasing the release of neurotrophins, improving insulin signaling in the brain, and/or reducing inflammation. Physical activity and fitness increases the production of insulin-like growth factor-1 and brain-derived neurotrophic factor, which facilitate neural and vascular proliferation (Cotman et al., 2007). In addition, insulin can cross the blood–brain barrier and bind to receptors throughout the brain (Carro et al., 2000; Plum et al., 2005). Insulin inhibits apoptosis and clears β-amyloid from brain tissue, promoting memory formation and overall cognitive health (Plum et al., 2005). Exercise also reduces circulating pro-inflammatory cytokines (Petersen and Pedersen, 2005) and has been associated with reduced β-amyloid in mouse models of Alzheimer's disease (Weisman et al., 2006). It is likely that reduction of inflammatory markers is one of the most prominent effects of exercise (see Cotman et al. (2007) for a review). This research suggests that aerobic exercise and CRF not only improve cognitive performance but also enhance and expand the neural circuitry supporting cognitive processing in areas known to be susceptible to age-related deterioration. While fitness is likely having widespread effects on the brain, the specific regional volumes that are important for a given task are likely to differ depending on the cognitive processes being investigated (Cotman et al., 2007; Hillman et al., 2008). For example, Stroop performance measures inhibition and executive control while spatial working memory measures

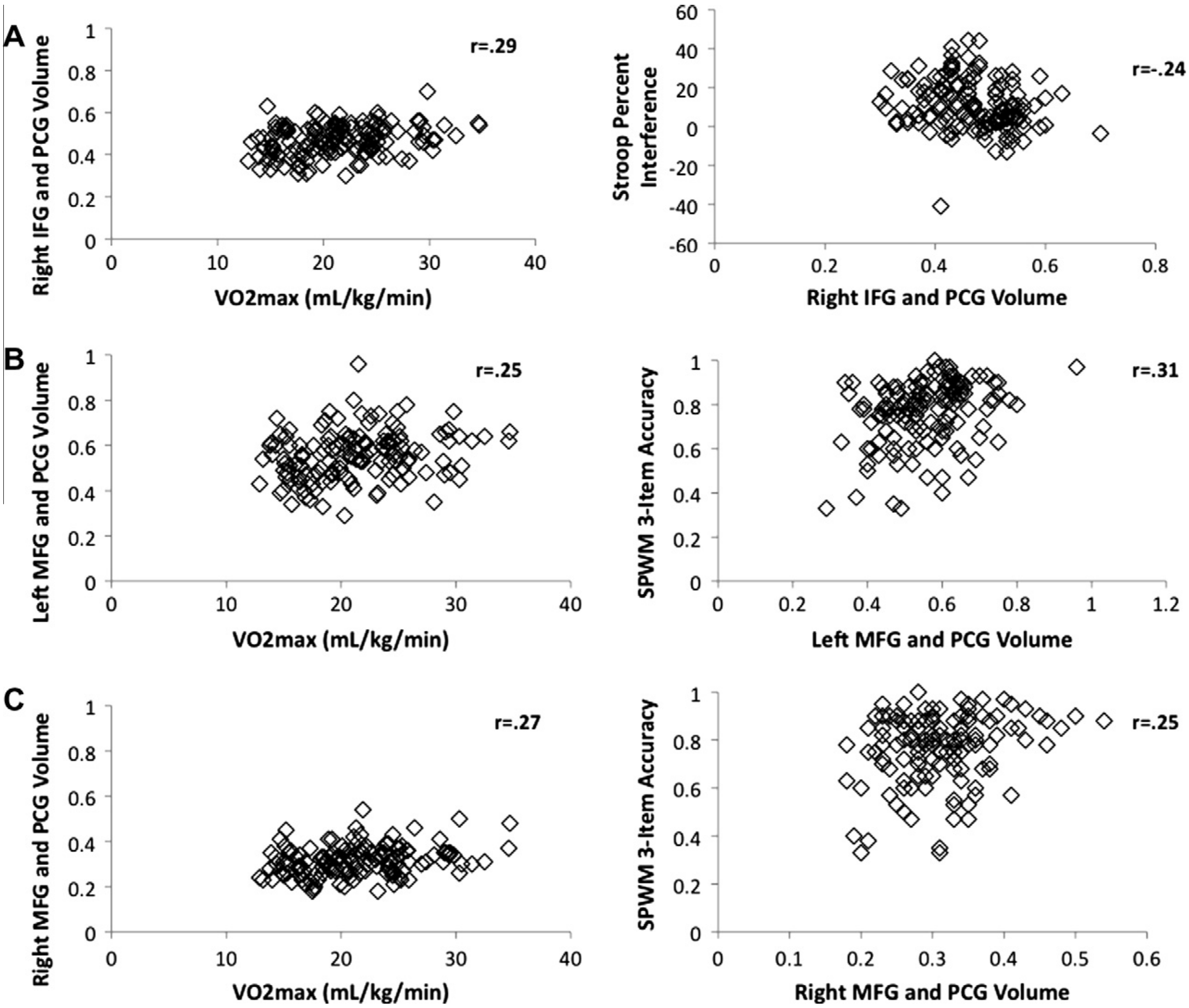

Fig. 2. Scatterplots of the relationship between fitness, brain volume, and executive function for the three significant mediation pathways. Spearman's correlations are reported for each bivariate relationship. IFG = inferior frontal gyrus; MFG = middle frontal gyrus; PCG = precentral gyrus; SPWM = spatial working memory. (A) *Left panel:* the relationship between fitness and volume in the right IFG and PCG region (ROI 1 in Table 2) found to significantly mediate the fitness–Stroop relationship. *Right panel:* the relationship between volume in the right IFG and PCG region and Stroop percent interference. (B) *Left panel:* the relationship between fitness and volume in the left MFG and PCG region (ROI 4 in Table 2) found to significantly mediate the fitness–SPWM relationship. *Right panel:* the relationship between volume in the left MFG and PCG region and SPWM 3-Item accuracy (percent correct). (C) *Left panel:* the relationship between fitness and volume in the right MFG and PCG region (ROI 5 in Table 2) found to significantly mediate the fitness–SPWM relationship. *Right panel:* the relationship between volume in the right MFG and PCG region and SPWM 3-Item accuracy (percent correct).

executive control and short-term/working memory function. There is likely some overlap and some separation of brain regions supporting performance on these two tasks. However, the anatomy of supporting brain regions, whether overlapping or not, may all be structurally affected by fitness in similar ways, even if there are functional dissociations. Which of these molecular processes contribute to the enhanced volume found in the current study remains a matter of speculation.

Our results should be interpreted in light of a recent functional MRI study that was conducted in a subset of this sample to examine the functional brain circuits associated with cardiovascular fitness during the Stroop task (Prakash et al., 2011). Prakash et al. found that higher fit older adults exhibited greater functional activity in the left and right MFG and PCG during the incongruent-eligible trials, as compared to neutral trials. These areas were similar to the regions we report here, suggesting that there might be some important links between increased brain function and increased volume with higher fitness levels. It will be important for future studies to examine the function–structure–cognition associations and the extent to which changes in volume occur independently from changes in functional responses during the task.

There are several limitations to the current study. First, while mediation analyses provide hypotheses regarding pathways of causation, true causal models can only be determined through experimental manipulations. Thus, the cross-sectional design limits any strong causal conclusions, although these results do create a foundation for future experimental research. Second, it is likely that there are other unmeasured factors that would improve the explanation of the relationship between CRF and cognition over and above DLPFC volume. For example, individual genetic variations also affect the production and efficacy of neurotrophins. However, these higher-level models are more easily tested once a formal link between physical activity, brain volume, and cognitive functioning has been firmly established.

It may be the case that spatial working memory relies more heavily upon neural circuitry outside of the DLPFC, such as in hippocampal and motor pathways (Erickson et al., 2009, 2011). However, this study focused directly on the role of the DLPFC in executive function, as the role of the hippocampus in SPWM processing has been investigated elsewhere with this same sample (Erickson et al., 2009, 2011). Future analyses would benefit from examining the network of brain regions mediating the link between fitness and executive function. Examining functional connectivity between brain regions associated with these tasks, such as the hippocampus and DLPFC, along with structural connectivity using diffusion tensor imaging, would elucidate the complex neural network involved in fitness–cognition relationships. In addition, future advances in statistical models that could examine voxel-wise mediation effects across the whole brain might reveal large-scale brain networks which mediate a fitness–cognition relationship.

Despite these limitations, this study had several strengths in comparison to previous research. First, the current investigation utilized a sample substantially larger than similar studies in the literature (e.g. Gordon et al., 2008; Peters et al., 2009). This larger sample size allowed us to reliably investigate associations with cognitive performance and to formally test mediation models. In addition, the creation of a study-specific template reduced registration error that often occurs when using atypical populations, such as older adults experiencing age-related atrophy. All subjects were registered to the study specific template using an optimized method that was not available at the time of many previous VBM investigations of fitness–brain relationships (e.g. Colcombe et al., 2003). In this optimized procedure, all brains were corrected for warping by using the Jacobian determinant, thus reducing registration error even further. Finally, our study used the "gold-standard" metric of CRF, two validated cognitive tasks, and a well-characterized and homogeneous sample to test our hypotheses.

In sum, we have demonstrated, in a large sample of healthy older adults, that higher fitness levels are associated with better cognitive function by the way of increased gray matter volume in the DLPFC. Additionally, it is not the volume of the DLPFC as a whole, but rather specific regions of the DLPFC, that differentially relate to inhibition and spatial working memory. These results indicate that aerobic fitness influences cognitive function by reducing brain atrophy in targeted areas in healthy older adults.

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