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Cardiorespiratory fitness is associated with enhanced hippocampal functional connectivity in healthy young adults

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Abstract

Consistent associations have been found between higher cardiorespiratory fitness (CRF) and indices of enhanced brain health and function, including behavioral measures of cognition as well as neuroimaging indicators such as regional brain volume. Several studies have reported that higher CRF levels are associated with a larger hippocampus, yet associations between volume and memory or functional connectivity metrics remain poorly understood. Using a multi-modal framework, we hierarchically examine the association between CRF and hippocampal volume and resting state functional connectivity (rsFC) in younger adults, as well as their relationship between with memory function. We conducted theoretically-driven analyses with seeds in the anterior and posterior hippocampus, as well as control seeds in the caudate nucleus. We tested whether (1) hippocampal connectivity with prefrontal cortical regions was associated with CRF in an adult sample much younger than traditionally tested, (2) associations between CRF and rsFC remain significant after adjusting for volume, and (3) volume and rsFC are related to memory. We found that higher CRF levels were associated with larger anterior hippocampal volume and more positive rsFC of the anterior hippocampus to several regions including the prefrontal cortex. rsFC also accounted for significant variance in CRF, above and beyond volume. CRF can thus be independently linked to increased anterior hippocampal volume, as well as stronger hippocampal rsFC in a population much younger than those typically tested, suggesitng it is critical to maintainig multiple aspects of brain health.

KEYWORDS

cardiorespiratory fitness, functional connectivity, hippocampal subfield, resting state

1 | INTRODUCTION

Consistent associations have been found between higher cardiorespiratory fitness (CRF) and indices of enhanced brain health and function that include behavioral measures of mood and cognition as well as neuroimaging indicators such as regional brain volume, evoked neuroelectric responses, and white matter microstructure (Erickson, Hillman, & Kramer, 2015; Erickson, Leckie, & Weinstein, 2014; Etnier et al., 1997; Sibley & Etnier, 2003). A great many of these studies have focused on the hippocampus, a brain region central for learning and episodic and relational memory, that is relatively easy to identify and segment, and that is sensitive both to wheel running in rodents and to numerous neurologic and psychiatric conditions in humans (e.g., depression, schizophrenia, Alzheimer's disease; Colcombe et al., 2006; Colcombe & Kramer, 2003; Erickson et al., 2009, 2011; Niemann, Godde, & Voelcker-Rehage, 2014; ten Brinke et al., 2015).

Despite recognition that the volume of the hippocampus is an important clinical marker for many conditions (e.g., pathological aging), it is often only weakly associated with behavioral outcomes (e.g., relational memory performance). In fact, while associations between CRF and hippocampal volume have been consistently observed (e.g., Chaddock-Heyman et al., 2014, 2015; Erickson et al., 2009, 2011), many studies do not examine, or report, the association between CRF-related variation in hippocampal volume with memory performance, and those that do assess these associations often report small effect sizes (e.g., Erickson et al., 2009, 2011; Chaddock et al., 2010; Honea et al., 2009). Such limited evidence for associations between behavior and hippocampal volume in the context of CRF, begs the scientific field

to look beyond traditional neuroimaging measures of volume and to approach the hippocampus (and the rest of the brain) from a multimodal neuroimaging perspective. By combining metrics from more than one neuroimaging technique into a single analytic model, we may improve our understanding of the association between CRF and hippocampal volume and function, and might also improve our understanding of the links between the hippocampus and the behaviors that it supports.

Functional connectivity is one such neuroimaging technique that could inform the associations between CRF and hippocampal function. In fact, several studies have now reported associations between higher CRF and increased functional connectivity (FC) between numerous cortical and subcortical nodes (Herting & Nagel, 2013; Voss, Erickson, et al., 2010). For example, in exploratory analyses of memory-related task-evoked activation patterns, Herting and Nagel (2013) found that high and low CRF groups differed in the connectivity between the hippocampus and several prefrontal and parietal regions comprising the default mode network (DMN) during memory tasks. Although these group differences in connectivity were not associated with differences in memory performance, these associations suggest that variation in CRF relates to connectivity measures of the hippocampus. However, Herting and Nagel (2013) did not control for variation in hippocampal volume, so it is unknown whether these associations were confounded by, or independent of, volume.

In addition, to assessing connectivity during a task, higher CRF has also been associated with the FC between brain regions during rest, referred to as intrinsic or resting state functional connectivity (rsFC) (Smith et al., 2009). Using a data-driven approach across the entire brain, Voss et al. found that higher levels of CRF were related to stronger connectivity amongst nodes making up the DMN, such that older adults with higher CRF had rsFC levels similar to that of younger adults within this network (Voss, Erickson, et al., 2010). Notably, these relationships held even after controlling for variance associated with gray matter volume, suggesting a unique contribution of CRF to brain FC. The data-driven approach taken in this article, however, did not isolate the hippocampus as a theoretically-derived seed region, so it remained unknown as to how rsFC of the hippocampus varied as a function of CRF and whether variation in hippocampal volume accounts for any CRF-related differences in rsFC.

Data-driven approaches have also shown that the rsFC of large-scale networks, including the DMN and Frontal Executive Network, increase following moderate-intensity exercise training in older adults (Burdette et al., 2010; Voss et al., 2016; Voss, Prakash, et al., 2010). Intervention-related changes in rsFC were specific to frontal and temporal connections of the DMN (Voss, Prakash, et al., 2010), and changes in the global efficiency of these largescale networks were related to an estimated doubling in hippocampal connectivity within the exercise group (Burdette et al., 2010). Thus, even using data-driven analytical approaches, the hippocampus emerges as a key region showing sensitivity to CRF.

As the evidence above suggests, prior rsFC work has relied predominately on data-driven, whole-brain, exploratory approaches to examine relationships between rsFC and CRF, or have focused on large-scale networks rather than specific regions of interest (but see Prakash, Patterson, Janssen, Abduljalil, & Boster, 2011 for one exception). While such exploratory approaches provide valuable opportunities for identification of whole-brain connectivity patterns, no rsFC studies have focused on the hippocampus as a seed region, despite overwhelming evidence for its unique sensitivity to CRF in both volumetric and morphometric domains of brain health. Further, only one prior rsFC study to our knowledge (Voss, Erickson, et al., 2010) has examined both volume and functional connectivity data in the same participants to examine the hierarchical nature of these metrics in relation to CRF. In the present study, we conducted theoretically driven rsFC analyses with seeds in the hippocampus. Our goals were to determine (1) whether hippocampal connectivity with prefrontal cortical regions is associated with CRF in an adult sample much younger than those traditionally tested, (2) whether associations between CRF and rsFC remains significant after adjusting for volume, and (3) to examine whether volume and rsFC are related to memory performance in a cognitively normal and healthy young adult sample. We conducted this analysis using anterior and posterior hippocampal seeds separately since several studies have reported stronger associations between CRF and anterior regions of the hippocampus than posterior regions (Chaddock-Heyman et al., 2015; Erickson et al., 2011; Killgore, Olson, & Weber, 2013). In addition to the hippocampal seeds of interest, we also included analyses of the left and right caudate as negative control seeds. We predicted that hippocampal (but not caudate) volume would be positively associated with CRF and memory and that this relationship would be specific to the anterior hippocampus. We also predicted that connectivity of the hippocampus, but not caudate, would vary as a function of CRF and that differences might be particularly salient for anterior hippocampal rsFC. Finally, we predicted that rsFC would explain unique variance in CRF and memory function, above and beyond that explained by the association between CRF and hippocampal volume.

2 | METHODS

2.1 | Subjects

A cross sectional sample of 50 young adults ranging in age from 20 to 38 ($M \pm SD = 25.22 \pm 5.11$; 28 female) was recruited from the University of Pittsburgh and surrounding community. All participants reported no neurological or health conditions that could affect central nervous system functioning, such as history of psychiatric disease, epilepsy, or metabolic disorder. In addition, all participants were deemed MRI-safe via screening prior to the start of the study and indicated that they were physically healthy enough to engage in PA. All protocols and procedures were approved by the University of Pittsburgh Institutional Review Board, and informed consent was obtained in accordance with the principles set forth in the Declaration of Helsinki.

2.2 | Fitness testing

CRF was assessed by graded maximal exercise testing on a motor-driven treadmill (VO_{2max}). All study participants were acclimated to the

general environment and test procedures. All participants began the test by walking at 3.0 mph and 0% incline as a warm-up. All study participants completed a modified Bruce protocol in which Stage 1 started at 3.5 mph and 2.0% incline. The modified Bruce protocol was chosen for this sample because it gives larger metabolic equivalents (MET) increases per stage compared to other protocols. The speed increased 0.50 mph and grade increased in increments of 2% every 2 min. This protocol was designed to increase exercise intensity in a linear format over time to achieve a workload that the participant would be unable to maintain within an 8- to 12-min duration. A trained exercise physiologist continuously monitored measurements of oxygen uptake, heart rate and blood pressure. Gas exchange values were measured from expired air samples averaged at 15 s intervals using a Parvo Medics metabolic cart. Expired air was collected via a mouth piece connected to a two-way valve. The mouthpiece was supported by a comfort fitted head gear. During the test, participants wore nose clips to ensure that all expired air was collected. All equipment was worn until a maximal VO_2 was attained either due to symptom limitation and/or self-reported exhaustion. VO_{2max} was defined as the highest recorded VO_2 value. A test was defined as maximal for each participant when they met one of the following two criteria: (1) a plateau in VO_2 peak between two or more workloads (.15 L/min or 2.0 ml/kg/min), or (2) two of the following three criteria were met: a respiratory exchange ratio >1.10, a heart rate within 10 beats of their age predicted maximum (i.e., 220 - Age), or a Rating of Perceived Exertion (RPE) equal to or greater than 17. Forty-nine of the 50 participants met one of the two criteria listed above, and one participant did not meet either but stopped the test from volitional exhaustion.

2.3 | Cognitive testing

Participants completed a relational memory task related to hippocampal functioning. The task used was a variant of the spatial reconstruction task developed by Watson, Voss, Warren, Tranel, and Cohen (2013) and described in detail by Monti et al. (2015). The task involves relational memory binding, and several different variants of the task, including the outcome measure "swaps" or "swap errors" (see below), have been shown to be highly sensitive to the structural integrity and/or volume of the hippocampus (Watson et al., 2013; Monti et al., 2015; Schwarb et al., 2017), as well as to be positively associated with CRF (Monti, Hillman, & Cohen, 2012). Briefly, on each trial of the task participants study the spatial arrangement of five novel line drawings and are told to remember the arrangement for a later test. Study time is self-paced, and participants are instructed to use the mouse to click on each stimulus. Following the study phase, there is a 4,000 ms delay in which participants see a blank screen; a self-paced test phase begins after this delay. In the test phase, stimuli appear aligned at the top of the screen, and participants use the mouse to click and drag them to where they were positioned during the study phase. Participants completed three practice trials and 15 experimental trials of the task (2,000 ms ITI).

Memory errors committed during the test phase were the primary outcome measures from the spatial reconstruction task. Errors were assessed using 4 metrics: (1) average item misplacement (in pixels), (2) edge resizing (in pixels), (3) edge displacement (in radians), and (4) swaps (proportion of all possible pairwise relationships). Detailed descriptions and examples of the various errors are provided in Watson et al. (2013). In all cases, higher values indicate worse memory performance. We created a single metric of relational memory performance by creating a composite score, computed as the sum of the normalized values of each type of possible error.

2.4 | Resting state MRI

2.4.1 | Acquisition

Each participant completed a functional magnetic imaging (MRI) scan, which included acquisition of resting state and structural images, within 2 weeks of VO_{2max} testing. All images were collected on a 3T head-only Siemens Allegra MRI scanner. High-resolution T1-weighted brain images were acquired using a 3D Magnetization Prepared Rapid Gradient Echo Imaging (MPRAGE) protocol with 176 contiguous axial slices, collected in ascending fashion parallel to the anterior and posterior commissures (echo time [TE] = 2.48 ms, repetition time [TR] = 1.4 s, field of view [FOV] = 256 mm, acquisition matrix 256 mm × 256 mm, flip angle = 8). The resting-state fMRI (rsfMRI) data consisted of a series of 180 scans acquired using a Gradient Echo Pulse Sequence with TR = 1.7 s while participants rested with eyes open, fixating on a centrally located crosshair inside the MRI scanner for 5:11 min (33 slices; TE = 25 ms; FOV = 205 × 205 mm; acquisition matrix 64 × 64 mm; 90 degree flip angle; voxel dimensions 3-mm isotropic).

2.4.2 | Preprocessing

After skull stripping, the structural image was spatially normalized to MNI space. All rsfMRI frames were aligned to correct for head motion during the scan, co-registered to each participant's structural image, and spatially normalized to MNI space. The rsfMRI time courses were then band-pass filtered (0.009–0.08 Hz) to attenuate respiration and other physiological noise. In addition, six affine transformation parameters from the alignment process, as well as the mean time courses from the brain parenchyma including white matter tissue and ventricles were included as covariates to further account for motion and physiological noise. The data were of high quality in this healthy young adult sample, and no subjects were eliminated due to excessive motion (mean framewise displacement ranged from .04 to .26 mm; $M \pm SD = .09 \pm .03$) or physiological noise. The residualized parameter estimate maps were converted to *z* scores (via Fishers *r* to *z* transform) to achieve normality and were entered into higher level analyses.

2.4.3 | Seed creation

For the functional connectivity and volumetric analysis of the hippocampus and control (caudate nucleus) seeds, we used FMRIB's Integrated Registration and Segmentation Tool (FIRST) in FMRIB's Software Library (FSL) version 5.0. FIRST is a semi-automated model-based subcortical segmentation tool which uses a Bayesian framework from shape and appearance models obtained from manually segmented images from the Center for Morphometric Analysis, Massachusetts General Hospital, Boston, MA, USA (see Patenaude, Smith, Kennedy, &

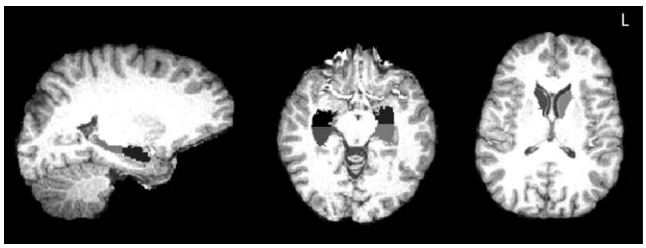

FIGURE 1 Location of the hippocampal and caudate seed regions in each hemisphere derived from FIRST segmentation. The seed masks are presented on a representative subject's MPRAGE. Black: posterior hippocampus; Gray: anterior hippocampus; Dark Gray: right caudate nucleus; Light Gray: left caudate nucleaus

Jenkinson, 2011 for further description of this method). Briefly, FIRST runs a two-stage affine registration to a standard space template (MNI space) with 1 mm resolution using 12 degrees of freedom and uses a subcortical mask to exclude voxels outside subcortical regions. Second, subcortical regions, including hippocampus, are segmented (both hemispheres separately). Manual volumetric region labels are parameterized as surface meshes and modeled as a point distribution model. The hippocampus segmentation from FIRST was then split based on the center of gravity of the region into anterior and posterior subregions (for each hemisphere separately). This resulted in separate anterior and posterior hippocampal seeds for each participant, for each hemisphere. This procedure for dividing the hippocampus shows differences as a function of CRF and exercise (Erickson et al., 2009, 2011). The final segmentations of both the hippocampus and caudate nucleus seeds were visually inspected for quality. The volume of each seed region was obtained from FIRST in mm3. Figure 1 shows the masks for all seeds on a representative participant's MPRAGE.

2.5 | Statistical analyses

First, we examined the relationship between (anterior and posterior) hippocampal volume, caudate volume, and CRF using six separate linear regressions. Results were corrected for multiple comparisons using the Benjamini Hochberg procedure implemented in SPSS with a false discovery rate (q) value of .10. This method and specific value of q was chosen because (1) we had a very specific a priori hypothesis regarding which seed volumes should relate to CRF and (2) this method is less susceptible to false negatives when a small number of pre-planned comparisons are made compared to other methods of controlling for false discovery (Benjamini & Hochberg, 1995).

Next, voxelwise functional connectivity network maps were then constructed for each seed, for each participant using the pre-processed rsfMRI data. These first-level seed maps were then entered into (separate) group-level linear regressions to identify regions where connectivity with the seed covaried with $V0_{2max}$ scores. Gender and mean framewise displacement (in mm) were included as nuisance regressors in the group-level analyses of functional connectivity, and gender was included in group analyses of volume. All variables were mean-centered prior to being entered into group-level models. Results were corrected for multiple comparisons at $p < .05$ using FSL's automatic FEAT cluster-based thresholding, which is a method of Family-Wise Error correction based on Gaussian Random Field Theory.

Finally, we conducted a hierarchical regression to compare the extent to which volume explains the variability in any rsFC–CRF relationships. Sex and seed volume were entered as explanatory variables in the first-wave model. CRF was subsequently entered in wave 2, to determine the unique variance in rsFC accounted for by each of these variables (i.e., volume vs. CRF). Finally, we examined associations between CRF, volume, rsFC, and memory performance.

3 | RESULTS

Consistent with previous research in older adults and patient populations, there was a wide range of variability in $V0_{2max}$ scores in our healthy young adult sample. Scores ranged from a minimum of 25.4 to a maximum of 60.4 ml/kg/min ($M \pm SD = 44.9 \pm 7.9$), corresponding to a CRF percentile range of 4.4 and 87.2, respectively. Such variability was well suited for examining individual differences in brain structure and function. Age was limited to 18–38 and was unrelated to any variable or outcome of interest and was, therefore, not included as a covariate in any of the analyses described below.

3.1 | Volume-fitness correlations

We observed a positive correlation between the volume of the left anterior hippocampus and CRF, $\beta = .01$, $p = .04$. This relationship was specific to the anterior seed (Figure 2). There were no other significant

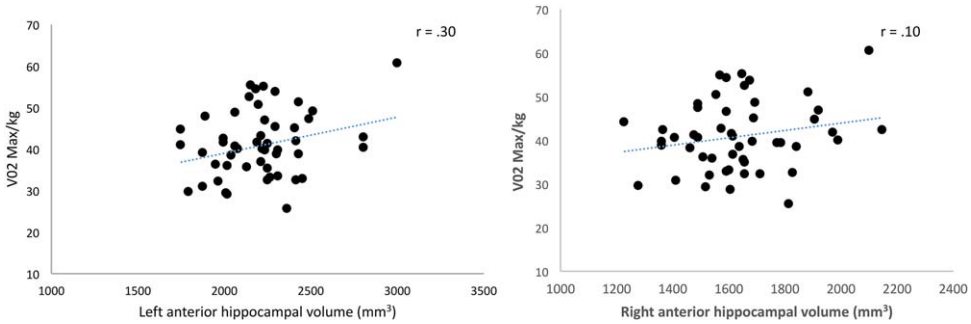

FIGURE 2 Positive correlation between left anterior hippocampal volume and cardiorespiratory fitness ($V0_{2max}$) in healthy younger adults [Color figure can be viewed at wileyonlinelibrary.com]

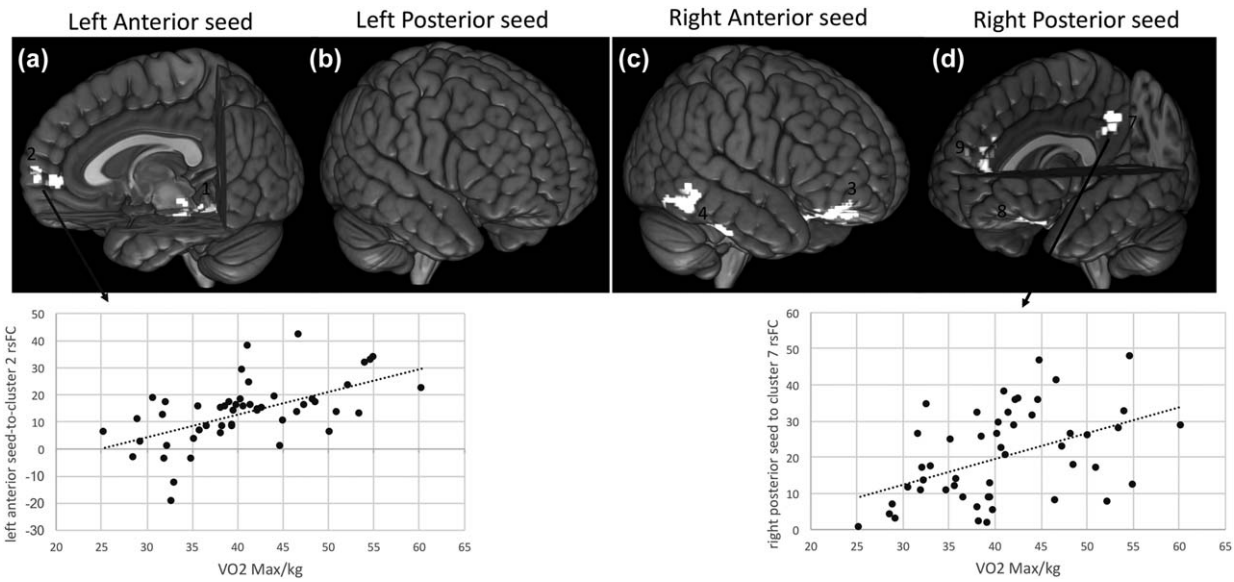

FIGURE 3 Positive correlations between resting state connectivity of the anterior and posterior hippocampus seeds and cardiorespiratory fitness ($VO_{2max\ score}$) in healthy younger adults. Panel (a) depicts results from the left anterior hippocampus seed. (b) depicts (NS) results from the left posterior hippocampal seed. Panel (c) depicts results from the right anterior seed, and (d) results from the right posterior hippocampal seed. Representative scatter plots are presented for clusters 2 and 7. Numbers within each panel correspond to the cluster labels in Table 1. Clusters 5 and 6 are not visible in panel (c). Results were corrected based on the voxel *z*-score and extent of activity given the correlated nature of the voxels. Specifically, the voxel-wise *z*-score was 2.3 ($p < .01$) and the clusters were significant at $p < .05$)

relationships between volume of the other hippocampal and caudate seeds and CRF (*p*s >.12, βs <.26). Because we did not detect a significant correlation in both hemispheres, we did a post hoc analysis to examine hemispheric differences in hippocampal volume and whether these differences varied as a function of CRF. While there was a main effect of hemisphere, such that hippocampal volume of the left anterior hippocampus was on average larger ($M \pm SD = 2{,}216.34 \pm 256.10$) than that of the right anterior hippocampus ($M \pm SD = 2{,}216.34 \pm 197.58$; $F(1,47) = 518.32$, $p < .001$), there was no evidence for a hemispheric interaction with CRF, $F(1,47) = .09$, $p = .76$.

3.2 | Connectivity-fitness correlations

3.2.1 | Left anterior hippocampus seed

Greater CRF was associated with greater connectivity between the left anterior hippocampus to clusters located in the frontal pole/middle frontal gyrus, as well as posterior hippocampus/brain stem (Figure 3; Table 1). Because MRI signal in brain stem regions is highly susceptible to physiological noise/artifacts (e.g., see Beissner, Schumann, Brunn, Eisenträger, & Bär, 2014), we do not further consider the CRF-related cluster that mostly includes brain stem regions in the analyses below. There were no significant negative relationships for this seed.

3.2.2 | Left posterior hippocampus seed

No CRF–rsFC relationships survived correction for the left posterior hippocampus seed.

3.2.3 | Right anterior hippocampus seed

Greater CRF was associated with greater connectivity of the right anterior hippocampus to three clusters located in the frontal pole extending to middle frontal gyrus, and posterior parahippocampal cortex (Figure 3; Table 1). In addition to these positive correlations, greater CRF was associated with less connectivity between the right anterior hippocampus seed to a cluster in the right superior frontal gyrus (Table 1).

3.2.4 | Right posterior hippocampus seed

Greater CRF was associated with greater connectivity of the right posterior hippocampus to three clusters in the frontal pole extending into anterior cingulate, paracingulate/superior frontal gryus, and precuneus. There were no negative relationships for this seed (Figure 3; Table 1).

Supplemental Analysis confirmed that the identified fitness-related hippocampal functional connections were included in baseline connectivity networks of the seeds.

3.2.5 | Left caudate (control) seed

No CRF–rsFC relationships survived correction for the left caudate seed.

3.2.6 | Right caudate (control) seed

No CRF–rsFC relationships survived correction for the right caudate seed.

3.3 | Hierarchical regression results

We next conducted a hierarchical regression to compare the extent to which CRF-related rsFC (detected only in the hippocampal seeds) could

TABLE 1 Significant clusters in which hippocampal connectivity positively covaries with cardiorespiratory fitness (CRF)

Seed	Nature of correlation	Cluster label	Cluster location	*k*	Peak *Z*	Peak MNI coordinates
Left Anterior Hippocampus	Positive	1	L brain stem/posterior parahippocampal gyrus	415	3.70	(−16, −30, −20)
	Positive	2	L frontal pole/medial prefrontal/anterior cingulate	242	3.41	(−10, 68, −4)
Right Anterior Hippocampus	Positive	3	R frontal pole	2037	4.41	(26, 46, −22)
	Positive	4	R inferior/middle temporal gyrus	865	3.59	(66, −46, −16)
	Positive	5	L inferior/middle temporal gyrus	239	4.31	(−34, −26, −30)
	Negative	6	R precentral gyrus	453	3.69	(36, 10, 62)
Right Posterior Hippocampus	Positive	7	R precuneus	365	3.82	(8, −60, 38)
	Positive	8	L frontal pole/anterior cingulate	244	3.17	(−30 42, −20)
	Positive	9	L superior frontal/paracingulate gyrus	229	2.26	(−2, 60, 14)

There were no significant CRF-related rsFC connections for the left posterior hippocampus seed.

be explained by independent vs. overlapping variability in hippocampal volume. Given that the volume-CRF correlation was specific to the left anterior hippocampus seed, we chose to focus on this seed in the hierarchical regression analysis. Wave 1 of the model, in which sex and seed volume were entered as the explanatory variables, was not significant, $F(2,49) = .70$, $p = .50$. Together, these variables only accounted for 2.9% of the variance in rsFC, suggesting volume does not share much overlapping variance with rsFC. Adding CRF into the model greatly improved model fit and accounted for an additional (and significant), 35.6% of variance in rsFC. A total of 38.5% of variance in rsFC was explained by the addition of CRF into wave 2, $F(3, 49) = 9.60$, $p < .0001$. These results are summarized in Table 2.

3.4 | Correlations with memory

Relational memory data were missing for two participants; we report data from the remaining 48 participants for this task. We first examined correlations between memory and hippocampal volume controlling for the confounding influence of gender. We focused on the left anterior hippocampus given the specificity of the volume-CRF correlation to this seed. There were no significant relationships between volume and performance on the relational memory task ($\beta = -11.2$, $p = .26$).

Next, we examined associations between memory and rsFC. As with volume, we focused specifically on CRF-related rsFC. However, no significant relationships between rsFC and relational memory performance were detected ($\beta = .32$, $p = .49$).

Finally, we examined correlations between CRF and memory performance. There was a marginal relationship between CRF and relational memory performance, such that those with higher CRF tended to commit fewer relational memory errors, $\beta = -.51$, $p = .06$.

4 | DISCUSSION

Consistent with our predictions and with previous literature in older adults and children (Chaddock-Heyman et al., 2014; Erickson et al., 2009, 2011; Niemann et al., 2014), higher CRF was associated with greater volume of the left anterior hippocampus in healthy young adults. There were no relationships between the volume of the other hippocampal seeds, nor caudate nucleus seeds, and CRF. In addition, higher CRF was associated with greater rsFC of the left anterior hippocampus seed to the frontal pole, middle frontal gyrus, and parahippocampus. These rsFC patterns appeared to be specific to the anterior hippocampal seeds in that a very similar pattern of CRF-related rsFC was observed for the right anterior seed, but not for the left or right posterior seeds. One major difference, however, between the left and right anterior seeds was the presence of a negative correlation between CRF and right anterior hippocampal connectivity to the right superior frontal gyrus. This association suggests CRF-related hemispheric differences of hippocampal connectivity and other brain areas. The negative correlation is difficult to interpret, but could be related to a shift in allocation of resources or attentional focus. As predicted, the caudate nucleus seeds showed no significant rsFC relationships in either direction to CRF. Finally, we demonstrated that CRF accounts

TABLE 2 Summary of hierarchical regression results

Model wave	Variables included	Standardized beta	SE beta	*R* squared	Model *p* value
Wave 1	Sex, **volume**	−.17, **−.06**	3.4, **.01**	.03	.50
Wave 2	Sex, volume, **CRF**	.17, −.21, **.71**	3.1, .01, **.20**	.39	<.0001

Results of wave 2 demonstrate that CRF accounts for significant portion of variance in rsFC, even after controlling for volume of the left anterior hippocampal seed. Wave 1 of the model (which controls for sex and volume only) was not significant. For each wave, all coefficients are reported, but the coefficient of interest (i.e., that for volume or CRF) is highlighted.

for a unique portion of variance in the rsFC of the left anterior hippocampus to the middle frontal gyrus, above and beyond the variance explained by the left anterior hippocampal volume.

4.1 | Results in the context of the broader field of exercise and brain

The specificity of the rsFC–CRF relationship to the anterior hippocampus extends findings of volumetric studies in the CRF/exercise literature by demonstrating that CRF–rsFC relationships are also largely confined to the anterior hippocampal subregion.

Individuals with a higher CRF exhibited stronger connectivity at rest between the anterior hippocampus and prefrontal and temporal cortical regions often implicated in supporting attention, declarative memory, and inhibition, namely the frontal pole, middle frontal gyrus, and parahippocampal gyrus. (Beaty, Benedek, Kaufman, & Silvia, 2015; Yang & Li, 2012; Zuo, Di Martino, et al., 2010; Zuo, Kelly, et al., 2010). Thus, the stronger rsFC of the anterior hippocampus to these regions suggests CRF may modulate the tonic intrinsic communication of specific networks supporting executive cognitive functions.

In addition to the positive rsFC–CRF relationships observed in the present study, one unexpected finding was that of a negative relationship between CRF and rsFC between the right hippocampal seed and right superior frontal gyrus. Such decreased connectivity in the context of increasing CRF is interesting and rarely, if ever discussed. Decreased connectivity in some cases is beneficial for behavior and long-term goals. In fact, this effect could be related to a difference in the brain circuits associated with attentional allocation or resources as a function of fitness, but in this context such an interpretation is highly speculative.

The results of our hierarchical regression demonstrate that the CRF–rsFC relationships we detected are not simply artifacts of the CRF-volume relationship. That is, once the variance in rsFC and volume is statistically accounted for, CRF was able to account for a significant portion of additional variance in rsFC. This suggests that CRF may have both overlapping and distinct influences on structural and functional brain health in this age group.

Finally, the CRF-anterior hippocampal volume correlation that we report here has been found numerous times in both cross-sectional and intervention studies (e.g., Erickson et al., 2009, 2011; Niemann et al., 2014). However, the present results are novel in that they are the first to replicate this relationship in a sample of healthy young adults not likely to be experiencing many developmental changes or age-related atrophy. Thus, our results suggest that CRF is a key component of brain health not only in youth and older adults but also in younger adults. For example, our results suggest that higher CRF may play a protective role in maintaining neural connections key to memory and other cognitive functions—in this case both structural integrity and functional connectivity of the anterior hippocampus at rest—in younger adults, as has been previously documented in older adults and children (Chaddock et al., 2010; Erickson et al., 2011; Monti et al., 2012; Schwarb et al., 2017). However, in terms of how CRF-related brain integrity may translate to behavior in younger adults, we found only very weak evidence that higher CRF was related to better relational memory performance. One interpretation of our results is that CRF has a larger effect on behavior in other age groups compared to healthy younger adults. However, at least one other study has previously reported a significant association between CRF and relational memory in a young adult sample using a different relational memory task with a more implicit measure of relational memory (looking time as opposed to explicit choice) (Baym et al., 2014). Thus, there is some ambiguity as to whether CRF is as strongly related to relational memory performance in younger adults as it is in older adults and children. More research on possible age group moderation of CRF is needed to clarify whether age group may be a moderator of CRF.

Interestingly, the association between CRF and anterior hippocampal volume in our young adult sample was only significant for the left hippocampus and did not reach statistical significance for the right hemisphere. Despite the nonsignificant hemisphere interaction term, the apparent laterality of the results suggests that the left hemisphere may be more sensitive to modifications by CRF. In fact, other cross-sectional and intervention studies have reported similar asymmetrical associations with CRF (Niemann et al., 2014), although some studies have reported bilateral effects (Erickson et al., 2009, 2011).

4.2 | Potential mechanisms

As is the case with hippocampal volume (e.g., Erickson et al., 2011), the mechanisms underlying associations between CRF and hippocampal rsFC are unknown. However, the fact that both anterior hippocampal volume and rsFC show associations with CRF suggests there might be some common mechanisms across these endpoints. These mechanisms may include cellular and molecular changes, such as neurogenesis or angiogenesis or changes in vasculature, myelination, or dendritic complexity. CRF may also induce higher-level changes, including changes in brain or socioemotional functioning (Stillman, Cohen, Lehman, & Erickson, 2016), which may contribute to CRF associations with behavior in humans.

Findings from the animal literature helps provide clues regarding the potential mechanisms of CRF-brain relationships in humans. The majority of the physical activity-related animal findings center on the hippocampus, which was the impetus for choosing hippocampal seeds in the present study. Experimental studies using animal models have established that animals with higher CRF (most typically manipulated through aerobic exercise training) show improved cognitive function compared to less fit animals, especially in cognitive domains dependent on the hippocampus, such as spatial or relational learning and memory, object recognition (e.g., Bechara & Kelly, 2013; Hopkins & Bucci, 2010), and avoidance learning (e.g., Baruch, Swain, & Helmstetter, 2004; Chen et al., 2008; see van Praag, 2008 for review). In addition, exercise increases long-term potentiation, a cellular analog of learning and memory, in a hippocampal subregion known as the dentate gyrus (e.g., van Praag, Christie, Sejnowski, & Gage, 1999). Animal models have been critical in establishing that the changes initiated by exercise extend beyond behavior into cognition, prompting further research into the mechanisms underlying exercise-induced synaptic, and downstream cognitive, changes.

Behavioral associations, however, were not observed in the present sample. We found no significant relationships between memory

and CRF. We also failed to detect significant associations between rsFC and memory, although we observed one relationship between CRF and relational memory in the hypothesized (positive) direction that did not meet statistical significance. The fact that there were few correlations with behavior is surprising. One possible explanation for the lack of associations between memory and CRF, rsFC, and volume in this sample could relate to the range of performance in this high-functioning young adult sample. For example, it is possible that given the typically high/ceiling performance of young adults on cognitive tasks and CRF is more strongly predictive of cognitive performance in older samples in which the range of variability is larger. Thus, the influence of individual-level (e.g., genetics) and environmental factors (e.g., PA) on behavior tends to be stronger in older age groups (McCormack, Shiell, Doyle-Baker, Friedenreich, & Sandalack, 2014; Woodard et al., 2012). Future CRF research should include a variety of cognitive tasks, including those with adaptive levels of difficulty, in studies of younger adults to examine whether the CRF–rsFC associations specifically support enhanced memory performance, or whether CRF-related rsFC may support enhanced performance in broader cognitive domains.

4.3 | Limitations

The results of the current study should be interpreted in the context of some limitations. First, we chose a seed-based approach to analyze our resting state data. We chose this approach because we had a theoretically-driven hypothesis focusing on the hippocampus and its subregions. However, by doing so we did not examine whether connectivity between other brain regions was associated with rsFC. There are many approaches for analyzing resting state data, including data-driven approaches that are more suited for capturing larger, network-level, whole-brain associations with CRF. Of course, the tradeoff is that data-driven approaches may be more atheoretical or miss regionally specific associations, which often makes the results of such approaches difficult to interpret in the context of the broader literature that focuses on particular brain areas. Second, we only included two brain metrics (volume and rsFC) in our test of shared versus distinct variance in hippocampal integrity accounted for by CRF. Future studies using multimodal techniques could expand on these findings by including additional measures of hippocampal structure or functioning (e.g., cerebral blood flow, white matter integrity). Finally, the present results are limited by the cross-sectional nature of the study design. Ideally, to demonstrate that exercise itself modulates anterior hippocampal structure and/or function across age groups, it would be ideal to include a more continuous age range, such as youth or older adults, in a randomized clinical trial that manipulated and controlled the levels of exercise and the magnitude of change in CRF.

4.4 | Conclusions

Despite these limitations, we can draw several broad and important conclusions from these data. The first is that higher CRF can be independently linked to increased anterior hippocampal volume, as well as stronger hippocampal rsFC. Second, these neurobiological markers of CRF can be observed in populations much younger than those typically tested. Finally, and more generally, CRF may be a critical factor for maintaining structural and functional brain health, even in young adults.

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SUPPORTING INFORMATION

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