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Multi-modal characterization of rapid anterior hippocampal volume increase associated with aerobic exercise

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Abstract

The hippocampus has been shown to demonstrate a remarkable degree of plasticity in response to a variety of tasks and experiences. For example, the size of the human hippocampus has been shown to increase in response to aerobic exercise. However, it is currently unknown what underlies these changes. Here we scanned sedentary, young to middle-aged human adults before and after a six-week exercise intervention using nine different neuroimaging measures of brain structure, vasculature, and diffusion. We then tested two different hypotheses regarding the nature of the underlying changes in the tissue. Surprisingly, we found no evidence of a vascular change as has been previously reported. Rather, the pattern of changes is better explained by an increase in myelination. Finally, we show hippocampal volume increase is temporary, returning to baseline after an additional six weeks without aerobic exercise. This is the first demonstration of a change in hippocampal volume in early to middle adulthood suggesting that hippocampal volume is modulated by aerobic exercise throughout the lifespan rather than only in the presence of age related atrophy. It is also the first demonstration of hippocampal volume change over a period of only six weeks, suggesting gross morphometric hippocampal plasticity occurs faster than previously thought.

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Inline Supplemental Methods and Results

Insert Supplementary Methods and Results sections here. To appear inline.

Keywords

neurogenesis; angiogenesis; dentate gyrus; hippocampus; fitness; exercise; aging; environmental enrichment; myelin; plasticity

1. Introduction

Habitual aerobic exercise has been widely associated with greater cognitive performance in older adults as well as adolescents (Kramer and Erickson, 2007; Anderson-Hanley et al., 2012; Benedict et al., 2012; Aberg et al., 2009; Young et al., 2015). A recent longitudinal study has demonstrated that one year of aerobic exercise has a direct causal role in increasing the volume of the hippocampus in sedentary older adults (Erickson et al., 2011). However, there is little evidence on the effects of exercise on the brain in early to middle adulthood, despite clear relevance of this question to lifelong cognitive health (Voelcker-Rehage and Niemann, 2013). For example, epidemiological studies have shown that lifestyle during middle age has significant consequences for cognitive performance decades later (Wilson et al., 2002; Karp et al., 2006; Belsky et al., 2015). It is also unknown whether brain plasticity in humans requires exercise interventions measured in months or years, although see Sagi et al. (2012) for an example of a more rapid structural change in the hippocampus with a non-exercise intervention and Tavor et al. (2013) for an exploration of the time course of this change. Measures of cardiovascular fitness have been shown to increase after as little as one week of exercise training (Hickson et al., 1981). The current study aims to test whether just six weeks of exercise is sufficient to elicit an increase of hippocampal volume in young to middle aged adults.

However, simply detecting volume change does not reveal the nature of the underlying biological processes. Animal and human studies provide a number of candidate mechanisms for exercise-driven or experience-dependent change in brain structure Voss et al. (2013). For example, it has been shown that aerobic exercise increases the rate of neurogenesis in the dentate gyrus region of the rodent hippocampus and increases cerebral blood volume (CBV) in the same region in humans (Pereira et al., 2007; van Praag et al., 1999a,b). Environmental enrichment, a less specific manipulation, has been shown to increase myelination of callosal fibres (Markham et al., 2009; Zhao et al., 2012). In rats, some efforts have been made to relate the morphometric measurements commonly used in neuroimaging to underlying changes in the neural tissue elicited by other behavioral training paradigms (Lerch et al., 2011; Sampaio-Baptista et al., 2013), but little is known about what drives these changes in humans (Zatorre et al., 2012).

Our goals in designing this experiment were threefold. First, to explore hippocampal volume change with increased aerobic exercise in a younger population and on a shorter time scale than previously shown. Second, to determine if this volume change is maintained in the absence of continued aerobic exercise. And third, to attempt to use multi-modal MRI to explore what might be driving the hippocampal volume change. Based on prior histological studies, we specifically chose to explore potential changes in both vasculature and myelination.

2. Materials and Methods

2.1. Participants and experimental design

We tested a total of 62 sedentary adult subjects (35 women and 27 males, mean age 33.7, S.D. 11.9). All subjects were right-handed, with no history of psychiatric or neurological disease. Informed written consent was obtained from all subjects in accordance with ethical approval from the Central Office for Research Ethics Committees. Using a standard crossover design, 54 of the 62 participants were pseudo-randomly assigned to one of two groups: “rest first” or “exercise first” (Figure 1). In addition to these randomly assigned participants, we made use of 8 subjects who had been recruited for a single scan. We refer to this group as the “baseline only” group. On the first visit, all participants were given a cognitive and fitness assessment ($\dot{V}O_2$ max test) and an MRI scan. Participants in the rest first group were then asked to maintain their normal level of physical activity for six weeks, after which they returned for a second cognitive and fitness assessment and MRI scan. They then began a six-week exercise program consisting of thirty minutes of supervised aerobic exercise on a stationary bicycle, five days per week. At the end of the six weeks, they were given a final cognitive and fitness assessment and MRI scan. Participants in the exercise first group started the same exercise program immediately after their baseline assessment. At the end of the exercise program they were scanned and assessed again, and allowed to return to their normal level of activity. After six more weeks they were scanned and assessed a third time. Participants in the baseline only group received a single cognitive and fitness assessment and MRI scan.

Thirty participants were assigned to the exercise first condition. In this group, one participant dropped out after the first scan, four dropped out after two scans (pre- and post-training), and 25 completed all three scans. Twenty-four participants were assigned to the rest first condition. In this group, one participant dropped out after the fist scan, eight dropped out after two scans, and fifteen completed all three scans. Eight participants recruited into the Baseline Only group complete a single scan, as expected. A total of 151 scans were acquired. See supplementary figure S1 for more information.

2.1.1. Cognitive assessment—A battery of cognitive tests was administered including the Rey Osterrieth Complex Figure Test (ROCF), the Rey Verbal Learning Test (RVLT), the Center for Epidemiological Studies Depression Inventory (CES-D), Letter and Semantic Fluency, and forward and backward digit span. All tasks were administered using paper and pencil except for the digit span test which was administered by computer using the PEBL software package (Mueller and Piper, 2014) in a stepwise fashion as described in Woods et al. (2010).

2.2. Fitness Measures

Physiological tests included a standard $\dot{V}O_2$ max test in which the rate of Ventilatory Oxygen ($\dot{V}O_2$) consumption was measured while the participant peddled on a stationary bike. Resistance on the bike was increased every two minutes. As many participants did not reach their maximal heart rate and oxygen consumption, estimated $\dot{V}O_2$ max was calculated by performing a linear fit between Heart Rate (HR) and $\dot{V}O_2$ scores during the test and

projecting the $\dot{V}O_2$ value at the participant's age-predicted heart rate maximum (208 − 0.7 * *Age*, Tanaka et al., 2001; American College of Sports Medicine, 2006). Additional details are provided in supplemental materials.

2.2.1. Exercise training—Participants were required to attend monitored training sessions five days a week, for six consecutive weeks. The training consisted of thirty minutes of continuous cycling at a cadence between 60 – 70 Revolutions Per Minute (RPM), on an upright exercise bike, and to maintain a heart rate consistent with their aerobic training zone of between 55% – 85% of their maximum HR. Training zones were calculated using each subject's age-predicted heart rate maximum and were monitored by an experimenter every five minutes using digital heart rate monitoring hand sensors. Rating of Perceived Exertion (RPE) was also monitored (Borg, 1970). Participants were started at the lower end of the aerobic zone for the first few sessions and were encouraged to progress by increasing their maintained heart rate as the weeks progressed.

2.3. Imaging methods

Each MRI scanning session was conducted using a 3T Siemens Verio scanner (Erlangen, Germany) and a 32-channel head coil. All scans were acquired using "Auto Align" to minimize variability in slice positioning (van der Kouwe et al., 2005). A battery of different imaging sequences were collected including:

1. One multi-echo susceptibility weighted (SWI) scan (1.3 mm isotropic voxels, TE = 6.72 ms & 24.60 ms, TR = 30 ms, matrix size = 192 × 192, and GRAPPA factor = 2 Haacke et al., 2004).
2. The DESPOT1 protocol which consisted of a series of spoiled gradient recalled echo (SPGR) images (1.7 mm isotropic voxels, TR = 5.6 ms, TE = 2.6 ms, flip angles = 3, 4, 5, 6, 7, 9, 13, 18 degrees, matrix size = 128 × 128), and an inversion-recovery prepared SPGR scan for correction of flip angle inhomogeneity (1.7 × 1.7 × 3.4 mm voxels, TR = 5.6 ms, TE = 2.6 ms, flip angles = 5 degrees, matrix size = 128 × 128, TI = 450 ms; Deoni, 2007).
3. The DESPOT2 protocol consisted of a series of balanced steady state free procession (bSSFP) images (1.7 mm isotropic voxels, TR= 4.6ms, TE= 2.3ms, flip angles = 10, 13, 16, 20, 23, 30, 43, 59 degrees, matrix size = 128 × 128 acquired with phase-cycling increments of 0 and 180 (for correction of off-resonance effects; Deoni, 2009).
4. A series of diffusion weighted scans used 2 mm isotropic voxels, 60 directions, b-value = 1000 s/mm^2, 65 slices, TR = 9.6 sec, TE = 87 ms, Phase encode = Anterior to Posterior. Eight additional volume were acquired with a b-value = 0 s/mm^2.
5. Two ME-MPRAGE scans with and without contrast agent (1 mm isotropic voxels, TR = 2530 ms, TI = 1200 ms, TE = 1.69, 3.55, 5.41 and 7.27 ms, matrix size = 256 × 256 × 176, and GRAPPA factor = 2; van der Kouwe et al., 2008).

2.3.1. T1 and T2 map calculation—T1 and T2 maps were calculated using DESPOT1-HIFI (Deoni, 2007) and DESPOT2-FM (Deoni, 2009). Details of this calculation have been

previously described (Deoni, 2007, 2009). In short, the acquired signal at multiple flip angles was fit to the signal equations for the steady state images, with corrections for flip angle inhomogeneity and off-resonance effects.

2.3.2. CBV Calculation—Cerebral Blood Volume (CBV) is a measure of the percentage of blood (both arterial and venous) in a given voxel or region of interest. There are two prevalent techniques of measuring CBV using MRI, one known as the “dynamic” or “bolus-tracking” method and the other as the “static” or “steady-state” technique (Lin et al., 1999; Speck et al., 1999; Barbier et al., 2001). For our study we opted to use the static method in order to maximize our resolution and to maintain similar methods to previous studies investigating exercise and angiogenesis (Pereira et al., 2007). After all other scans had been acquired, an ME-MPRAGE scan was collected with the parameters described above. The participant then received an injection of contrast agent (Dotorem, 0.1 mg per kg) via a previously inserted venous catheter in the arm. A second ME-MPRAGE scan was then conducted with identical parameters. These two images are used to produce an image of cerebral blood volume using the static method. Specifically, the pre- and post contrast scans were rigidly aligned and subtracted. A ROI containing only blood was automatically identified by selecting the 1000 brightest voxels on the post contrast agent scan within the sagittal sinus near the center of the brain immediately above the cerebellum. The mean difference between the post-contrast and pre-contrast signal intensity inside this region was used to normalize the subtracted image:

$$rCBV = \frac{\mathrm{Brain}_{\mathrm{post}} - \mathrm{Brain}_{\mathrm{pre}}}{\overline{\mathrm{Bloodmask}_{\mathrm{post}}} - \overline{\mathrm{Bloodmask}_{\mathrm{pre}}}}$$

2.3.3. QSM Calculation—Quantitative susceptibility maps were computed from the SWI sequence described above. Phase data was computed from data combined over all channels for subsequent QSM processing. Phase maps were unwrapped using Phase Region Expanding Labeller for Unwrapping Discrete Estimates (PRELUDE) (Jenkinson, 2003). Background phase was estimated using the Morphology Enabled Dipole Inversion (MEDI) technique (Liu et al., 2011); residual phase maps representing underlying variations due to microstructural inhomogeneities in susceptibility were produced by subtracting the estimated background from the unwrapped phase maps. QSM maps were then computed by implementing the highly computationally efficient thresholded k-space division (TKD) (Shmueli et al., 2009; Schweser et al., 2013) modification of the dipole-inversion approach (Salomir et al., 2003; Marques and Bowtell, 2005).

2.3.4. Segmentation and alignment—Grey and white matter segmentations were performed using FMRIB’s Automated Segmentation Tool (FAST) (Zhang et al., 2001) without smoothing. Grey Matter (GM) and White Matter (WM) were determined in the subject’s native space so no spatial normalization was used.

Segmentation of the hippocampus for each visit was performed on individual subject MPRAGE images (without contrast) in native space using the Freesurfer hippocampal subfield segmentation pipeline (Van Leemput et al., 2009). Each volume was visually

inspected for segmentation errors. No manual correction was required. The Freesurfer segmentation pipeline produces a probabilistic map of each subfield in each subject. In order to create an ROI for the whole hippocampus in each subject, the probability distributions of all hippocampal subfields were combined, thresholded at 50%, and binarized. Thus a mask was created in the subject's native space in which voxels with a 50% likelihood of being contained within the hippocampus were included. As previous studies have shown that hippocampal plasticity tends to be focused in the anterior head portion (Erickson et al., 2011; Sagi et al., 2012), we focused our analysis on the anterior quarter of the hippocampus. The posterior-most 75% of the full hippocampal mask was removed, leaving just the anterior-most 25% of the hippocampus on which volume and ROI measures were based. The volume of this ROI was calculated for each subject and each time point. Note that no correction for intracranial volume was necessary as our analysis only explored differences within subject.

For modality measurements within the hippocampal ROI, whole brain voxelwise maps of T1, T2, Mean Diffusivity (MD), Fractional Anisotropy (FA), Quantitative Susceptibility Mapping (QSM), CBV, and Susceptibility Weighted Imaging (SWI) were linearly aligned to the MPRAGE scan collected on the same subject in the same session. Boundary Based Registration (BBR) was use for alignments of EPI images to MPRAGE scans Greve and Fischl (2009). Spline interpolation was used to minimize blurring. For each measure, the average across all voxels within the anterior hippocampus ROI was then determined in the subject's native space. All ROIs were calculated in the subject's native space so none of the spatial normalization typically used for Voxel-Based Morphometry (VBM) were needed.

2.4. Statistical analyses

Changes in fitness, cognitive, and anterior hippocampal volume were analyzed using a longitudinal Linear Mixed Effects (LME) approach implemented in the nlme package of the R statistical programming language.(R Core Team, 2013; Pinheiro et al., 2013; Verbeke and Molenberghs, 2009; Fitzmaurice et al., 2011). Longitudinal LME modelling has been demonstrated to be a be superior technique to more common alternatives such as repeated measure ANOVA or simple paired T-test in that it provides more statistical power and is robust in the face of missing data (Bernal-Rusiel et al., 2013). It is similar to hierarchical linear regression in that explanatory variables are sequentially added and tested to determine if they explain a statistically significant amount of the variance to warrant their inclusion in the final model. There are two important differences between the hierarchical linear regression approach and longitudinal LME. The first is that LME separately and effectively models variance due to within and between subject factors (Fitzmaurice et al., 2011; Verbeke and Molenberghs, 2009). Second, the baseline model (or the null hypothesis) in longitudinal LME is typically a linear change with time. Many incidental factors, such as practice, aging, and instrument drift, can cause the dependent variable to change with time. Longitudinal LME allows one to ask if some other variable, exercise in this case, explains a significant amount of variance in a measure, over and above the effects of time. For all of our LME analyses, a linear change with time was used as the null hypothesis.

It is common practice in the imaging literature to analyze each modality collected separately. However there are well established statistical tools that can be used to combine different modalities into a single statistical test of a given biological prediction (Stouffer et al., 1949). In this study, inference on multimodal imaging parameters were conducted using a non-parametric, permutation-based version of Stouffer's method implemented in MATLAB (MATLAB, 2013; Stouffer et al., 1949). Stouffer's Method is a meta-analytic method for combining a set of Z-scores when the consistency of the sign of the effect is important. (Compare to Fisher's method, Mosteller and Fisher (1948), where significance can be obtained even if the signs are inconsistent). If there are K independent Z-scores to combine, the method simply consists of computing the average Z-score and scaling by $\sqrt{k}$; the scaling is necessary so that the Stouffer's measure is once again a Z-score under the null hypothesis.

$$Z=\frac{\sum_{i=1}^{k} Z_i}{\sqrt{k}}$$

Here, as we don't have independent studies but K dependent tests, it is essential that we use a non-parametric permutation procedure to obtain P-values for this measure. The permutation method randomly flips the signs of each subject's data, but does so synchronously over subjects to preserve the dependence.

3. Results

3.1. Baseline correlations between fitness, hippocampal volume, and cognitive

At baseline, we found no significant differences between the exercise-first and rest-first groups in fitness, anterior hippocampal volume or cognitive scores. No significant correlations were found between cognitive scores and fitness after accounting for age, sex, and total brain volume and correcting for multiple comparisons.

3.2. Exercise training improves fitness but does not not change cognitive scores

Out of thirty possible aerobic exercise training sessions, the average attendance was 29.05 sessions (SD 1.05). Change in fitness was evaluated using the LME approach discussed above (Bernal-Rusiel et al., 2013). We constructed a model to explain variation in estimated $\dot{VO}_2$ max across all subjects and all sessions using three explanatory variables: subject ID, time (visit number, 1–3), and a variable indicating whether the fitness test occurred immediately after completion of the six week exercise training program (postEx, 1 or 0). This model provided a significantly better fit for the variance in $\dot{VO}_2$ max than the baseline model which included only subject ID and time (log likelihood ratio=13.25, $P < 0.001$), suggesting that exercise caused significant change in fitness. Inspection of the time course of change in $\dot{VO}_2$ max (Figure 2 and Table 1) reveals that fitness is increased immediately post training. In order to determine if $\dot{VO}_2$ max remained elevated six weeks after the completion of training we added an additional within-subject explanatory variable to the model indicating whether the fitness test occurred six weeks after the completion of exercise training (6wksPostEx, 1 or 0). This model did not provide a significantly better fit than the previous simpler model containing subject ID, time, and the postEx variable (log likelihood=0.01,P=0.92). Thus, $\dot{VO}_2$ max is not statistically different from baseline at the

twelve-week visit, six weeks after the end of the exercise training program. Figures 2 and S2 summarizes these results and Table 1 shows absolute $\dot{V}O_2$ max scores for the rest-first and exercise-first groups separately. No significant changes with exercise were observed in any of the cognitive tasks.

3.3. Anterior Hippocampal Volume Increase

To test whether the six-week exercise intervention had a significant effect on the volume of the anterior hippocampus, we again used a longitudinal LME approach and constructed a model to explain the variance in anterior hippocampal volume across all visits and subjects. Explanatory variables were subject ID, visit number (1–3), and a binary variable indicating whether the hippocampal volume measure was acquired immediately after completion of the six-week training regime (postEx, 1 or 0). This model provided a significantly better fit for the anterior hippocampal volume as compared to a model containing just subject ID and time (Log Likelihood Ratio=6.95, P=0.0084), demonstrating that the exercise intervention significantly changed anterior hippocampal volume. Inspection of the time course of volume change (Figure 3 and S2) reveals that hippocampal volume is increased immediately post-training. We then asked whether hippocampal volume remains elevated in the six-week post-training scan. We again addressed this question by adding an additional within-subject explanatory variable for the scan six weeks after the end of the exercise training program (6wkPostEx, 1 or 0) to the model. This variable provided no significant additional power to the model (Log Likelihood Ratio=0.38, P=0.54), suggesting that six weeks after the completion of the exercise training program, anterior hippocampal volume was not statistically different from baseline. A similar pattern was observed for the whole hippocampus however the effect was not significant (Log Likelihood Ratio=1.31, P=0.25). To test the specificity of these changes we also looked at volume changes in the thalamus which is located near the hippocampus and easily segmented. Thalamus volume showed no significant effects of exercise (Log Likelihood Ratio=0.0007, P=0.98).These data are summarized in Figures 3 and S2 and Table 1. To verify that these volume changes were not due to an acute effect of training we looked at the time between the last exercise session and the MRI scan. We found no correlation between this interval and the change in anterior-hippocampal volume (R=−0.07, P=0.64).

3.4. Exploratory multimodal measurements of change within anterior hippocampus

After having demonstrated an aerobic exercise-mediated volume increase in anterior hippocampus we sought to use alternative imaging modalities and measures to explore potential biological drivers of volume change. Our motivation was to generate hypotheses that could then be tested by future studies using direct measurements of the underlying biology. One potential substrate for exercise-mediated volume increases that is relatively accessible with neuroimaging methods is increased vasculature (i.e. angiogenesis), possibly accompanied by neurogenesis (Black et al., 1990; Pereira et al., 2007). Recent evidence also suggests that axonal activity can promote increase in myelination (Gibson et al., 2014), which provides an alternative substrate, also amenable to measurement through MR. We hypothesized that these two underlying mechanisms would produce predictable but distinct changes in T1, T2, QSM, SWI, WM, and CBV, due to the different sensitivities of these MR

measures to different tissue components (Deistung et al., 2013; Haacke et al., 2004; Lutti et al., 2013; Whittall et al., 1997).

Specifically, we predicted that an increase in vasculature would be reflected by an increase in T1, T2, and CBV and a decrease in SWI. Similarly, we predicted an increase in myelination would be reflected by a decrease in T1, T2, and QSM with an increase in segmented white matter (WM). These predictions are based on a significant body of literature demonstrating that these parameters vary with differing tissue concentrations of vasculature and myelin. More specifically:

- The T1 of blood is high whereas that of myelin is low, compared to average T1 in gray matter (Stanisz et al., 2005; Wansapura et al., 1999), thus an increase in T1 would be consistent with an increase in vasculature, while a decrease would be consistent with a shift toward myelin.
- T2 is similarly significantly higher in vasculature than in myelin-rich regions (Stanisz et al., 2005; Wansapura et al., 1999), thus a similar prediction applies.
- CBV is a direct measure of blood volume (Wenz et al., 1996; Lu et al., 2005; Barbier et al., 2001), thus it would obviously increase if vasculature increased, but a myelin change would not be predicted to change CBV.
- SWI images show lower intensity in highly vascularized regions (Haacke et al., 2004), thus an decrease in SWI would support an increase in vasculature. SWI signal in myelinated regions depends on a number of factors, such as the orientation of the fibers with respect to the field (Deistung et al., 2013; Lutti et al., 2013; Lee et al., 2012), thus no clear prediction with respect to myelin change can be made.
- Brain segmentation algorithms attempt to determine the amount of white matter, gray matter, and CSF in each voxel of a T1-weighted image using an expectation maximization algorithm. An increase in the white matter compartment would be consistent with a increase in myelin. Segmentation provides no obvious information regarding vascualture.
- QSM has been shown to be dark in myelin-rich regions and bright in blood-rich regions (Deistung et al., 2013; Stuber and Turner, 2010), thus making clear predictions for both a myelin and vasculature change.

We also measured MD and FA which have been used in other studies to draw conclusions about axonal orientation, integrity, and myelination within white matter regions where fibers are bundled with coherent orientation (Scholz et al., 2009) though aspects of this interpretation are controversial (Mädler et al., 2008). In gray matter, where the orientation of fibers is much more heterogeneous, it is not clear what effect an increase in myelination or vasculature would have on MD or FA. As the anterior hippocampus is primarily a gray matter region, MD and FA have been left out of the predictive tests but the change values is still reported. The prediction for the direction in which segmented GM would change in the face of an increase in vascualture or myelination is similarly ambiguous. GM was therefore also left out of the predictive tests but reported below.

The two predictions were tested using a non-parametric version of Stouffer's method to assess changes in relevant measures within the anterior hippocampal ROI (Stouffer et al., 1949). Similar to Fisher's method (Mosteller and Fisher, 1948), Stouffer's method allows several tests to be combined into a single Z-score. The permutation-based version employed here addresses potential interdependence between the tests. Testing the vascular change hypothesis failed to reach significance on the Stouffer's test ($Z=-1.05$, $P=0.78$), while the myelin change hypothesis was significant ($Z=2.64$, $P=0.01$). Some measures showed greater change than others. The top of Figure 4 shows all of the paired Z-statistics (before vs. after training, non-parametrically derived) for every measure collected. It is apparent from this graph that WM, T2, and QSM are all showing relatively large shifts consistent with a myelination change, while T1 and CBV show little change at all (Figure 4).

4. Discussion

In this study we have provided the first demonstration that volume increase in the anterior hippocampus occurs in early to middle adulthood after just six weeks of aerobic exercise. This increase is not permanent, returning to baseline after six further weeks without regular aerobic exercise. Using multimodal neuroimaging to quantify tissue properties within this region, we have also presented exploratory analyses which suggest that changes in WM, T1, T2, QSM, CBV, and SWI are not consistent with an increase in vasculature as might be predicted by the existing literature. Rather, the pattern of changes are better explained by an increase in myelin.

Although a number of studies have reported change in brain volumes with exercise interventions in older adults (Erickson et al., 2011; Anderson-Hanley et al., 2012), effects in young to middle aged adults have not been well studied. In addition, interventions in most prior longitudinal studies have been six to twelve months in duration (Erickson et al., 2011), far longer than the six week intervention employed here. This study therefore provides novel evidence that just six weeks of exercise results in increases in hippocampal volume in young to middle aged adults. This result is particularly relevant given the growing recognition of the importance of lifestyle during middle age for lifelong cognitive health (Wilson et al., 2002; Karp et al., 2006; Young et al., 2015). The apparent transient nature of the myelin increase also suggests that continuing an active lifestyle may be important for maintaining improvement in brain structure.

Despite the extensive cognitive battery used in our study, we did not find significant changes in any cognitive measure after exercise. Possible reasons for the lack of change may include the short duration of our training intervention, our choice of cognitive tests, or the age of our participants. Previous studies have shown longitudinal change in performance on a spatial memory task in older adults that correlates with change in hippocampal volume after a year-long exercise intervention (Erickson et al., 2011). Our battery did include spatial tasks such as the ROCF test, however its sensitivity to subtle changes in spatial ability is limited by the fact that the scoring technique is relatively coarse and that healthy subjects often perform near ceiling. It is also possible that older adults have more capacity for recovering performance degraded by cognitive decline.

Previous studies have shown volume changes in the entire hippocampus which appeared to be driven by the anterior half of the structure (Erickson et al., 2011). Histological examination of the hippocampus reveals three obvious subdivision along the longitudinal axis: the head, the body, and the tail (Duvernoy, 2005; Strange et al., 2014), with the body being somewhat longer than the other two segments. Distinguishing these three segments with 3T MRI images is difficult thus we have adopted a quartering strategy (as used in Sagi et al., 2012) to provide a transparent and replicable way of approximating the hippocampal head. Future studies might employ higher resolution, different contrasts, and new segmentation algorithms to more precisely segment the hippocampal head from the body and tail.

We note that it has been recently demonstrated that an increase in head motion could result in artifactual changes in segmented volumes (Reuter et al., 2014). It's possible that exercise training could increase or decrease participants' tendency to move in the scanner. Although we do not have motion parameters for the MEMPRAGE scan, we evaluated the motion parameters from our DTI sequence and found no evidence of a difference in absolute or relative subject motion before and after exercise ($P > 0.15$). We also found no correlation between motion parameters and anterior hippocampus volume or total gray matter volume ($P > 0.47$).

In light of several recent critical reviews of longitudinal neuroimaging approaches (Bernal-Rusiel et al., 2013; Thomas and Baker, 2012; Thomas et al., 2009; Aarts et al., 2014), all longitudinal analyses in this study have been con- ducted in the subject's native space, avoiding potential confounds due to biased longitudinal alignments. We have also used well established Linear Mixed Effects models for statistical inference. These approaches remove the possibility of spurious results due to biased alignments, properly model both between and within subject sources of variance, and control for potential time-related confounds such as scanner drift and practice.

In our multi-modal analysis, we tested whether changes in our MR measures supported a vascular change or a myelin change as a potential substrate for the observed volume change in anterior hippocampus. These two types of change were chosen as they have been shown to occur after exercise or neuronal activity, and also because they modulate MR properties in predictable ways. We did not test for other changes such as neuronal density, dendritic spines, or synaptogenesis as these micro-structural changes are not known to produce predictable changes in MR properties. We cannot rule out the possibility that these or other micro-structural changes, that we have not tested here, could also contribute.

Myelination is typically associated with white matter whereas here we are examining myelin change in a gray matter structure: the anterior hippocampus. In T1-weighted MRI scan, myelin is most apparent in the white matter, however there are also a considerable number of myelinated fibers within hippocampal grey matter (Benes et al., 1994). Growth of mossy fibers in the rat hippocampus with physical exercise has been previously reported (Toscano-Silva et al., 2010), however these fibers are not myelinated. Neuronal activity-dependent changes in myelination have been observed in cell culture (Wake et al., 2011; Fields, 2010) as well as in recent *in vivo* experiments (Gibson et al., 2014). It is possible that such

mechanisms explain the changes in myelin associated with experience that have been reported in several recent imaging studies (Blumenfeld-Katzir et al., 2011; Sampaio-Baptista et al., 2013). These and other studies have shown that myelination changes can occur in a time frame much less than the six-week intervention used here (Yeung et al., 2014; Gibson et al., 2014).

Although the majority of our imaging measures supported an increase in myelination within anterior hippocampus, the lack of an increase in T1 is a surprising and notable exception as many investigators consider R1 (1/T1) to be the best MR measure of myelin content (Stüber et al., 2014; Lutti et al., 2013). One possible explanation for this discrepancy could be noise in our T1 measurement. The method we used to quantify T1 has been recently criticized as suffering from inaccuracies due to RF field inhomogeneities (Lankford and Does, 2013; Stikov et al., 2014). So it is possible that artifactual noise in our T1 measures prevented the detection a underlying change in T1. It is also possible that other biological changes within the anterior hippocampus had an opposing effect on T1, washing out the net change. However, another measure that has been used to measure myelin, the T1/T2 ratio (Glasser and Van Essen, 2011), did show an increase in the anterior hippocampus ($Z = 1.54$). Future studies might attempt to replicate our findings using more accurate methods of measuring T1 that have recently become available (Stikov et al., 2014; Lutti et al., 2013).

It is interesting to note that our results differ from previously published studies in rodents that have shown a robust vascular change within the hippocampus after a prolonged period of exercise (Pereira et al., 2007; Van der Borght et al., 2009; van Praag et al., 2005). In contrast, we found no evidence of an increase in vasculature in the anterior hippocampus. There are several possible reasons for this difference. It is possible that the histological technique is more sensitive to subtle effects than the imaging measures used here. However, the disadvantage of histology is that only a small portion of a given region is sampled, whereas imaging allows the signal to be averaged across the entire region of issue. Histology also requires fixation which, depending on the precise procedure used, can cause shrinkage and dehydration, potentially distorting volume measurements (Overgaard and Meden, 2000).

A previous human study by Pereira et al. (2007) reported a significant increase in CBV specifically in the dentate gyrus region of the hippocampus after three months of regular aerobic exercise in a group of eleven healthy adults. We were unable to replicate this result in our study even when our CBV analysis was restricted to the dentate gyrus region of the hippocampus. While our study had more participants completing the exercise intervention (41 vs. 11), the intervention in Pereira et al. (2007) is twice as long (3 months vs. 6 weeks). It is possible therefore that our intervention was not long enough to elicit robust vascular effects even though effects on hippocampal volume and aerobic fitness could already be observed. Our study also differed in hippocampal segmentation technique. Whereas the present study used automated of hippocampal segmentation on isotropic voxels, Pereira et al. (2007) used manually drawn subfields on a limited number of 4 mm thick coronal slices.

A number of rodent studies have focused on how exercise alone vs. exercise with environmental enrichment produces different structural modifications in the brain. In the hippocampus, exercise alone leads to increased production of new neurons while exercise

paired with environmental enrichment leads to greater survival of new neurons (van Praag et al., 2000, 2005). Similarly, in cerebellum, exercise alone has been shown to increase capillary density while exercise paired with environmental enrichment leads to an increase in synaptic density (Black et al., 1990). It is difficult to address this dichotomy in a human study, as the daily lives of humans are constantly enriched compared to that of a rodent housed in a laboratory setting. Although this study was designed to assess the effects of cardiovascular exercise, we cannot rule out the possibility that other features of the intervention, such as social interaction and experience of a novel environment, could contribute to the effects we observed.

In conclusion, this study has provided important insight into the time course and mechanism of exercise-mediated plasticity in the anterior hippocampus, but further work is needed. Future studies should assess whether longer term interventions in this age-group can elicit improvements in cognition and growth of vasculature. Future imaging studies might employ higher resolution imaging of the hippocampus to explore the laminar specificity of changes in volume and myelination. Finally, animal studies are needed to determine the biological drivers by which increased aerobic exercise triggers structural expansion in specific brain regions such as the hippocampus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Human anterior hippocampal volume can be increased by only six weeks of regular aerobic exercise.
- This increase in anterior hippocampal volume returns to baseline in the absence of continued aerobic exercise.
- These changes can occur in middle adulthood, not only in the presence of age related atrophy.
- Multimodal neuroimaging techniques support a change in myelination but not a change in vasculature within anterior hippocampus.

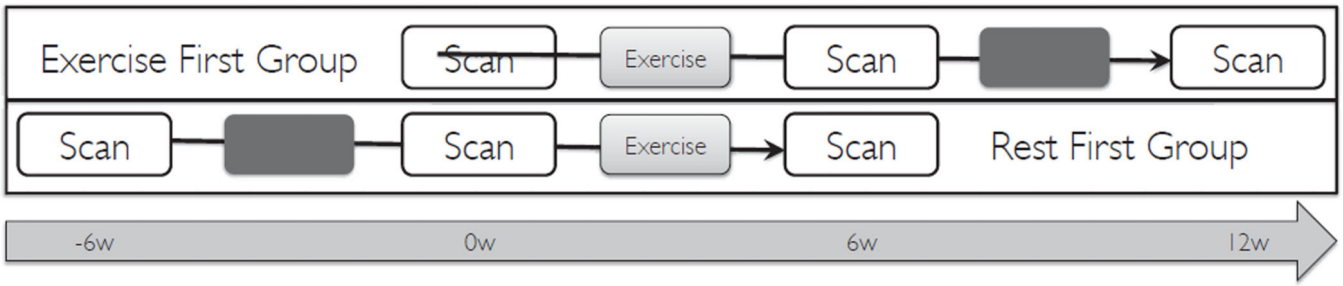

Figure 1.

Sixty-two participants were recruited and pseudo-randomly assigned to one of three conditions. In the “Exercise First Group” (top) participants completed six weeks of aerobic exercise (five times per week, thirty minutes per day) and were scanned again. These participants were then allowed to return to their baseline activity levels for six weeks and then scanned a third time. For participants in the “Rest First Group” condition (bottom), the order of the exercise and rest periods was reversed. The third “Baseline Only Group” (not shown) were only scanned once. Note that both “Rest First” and “Exercise First” groups were scanned both before and after the exercise intervention. See supplementary figure S1 for more details.

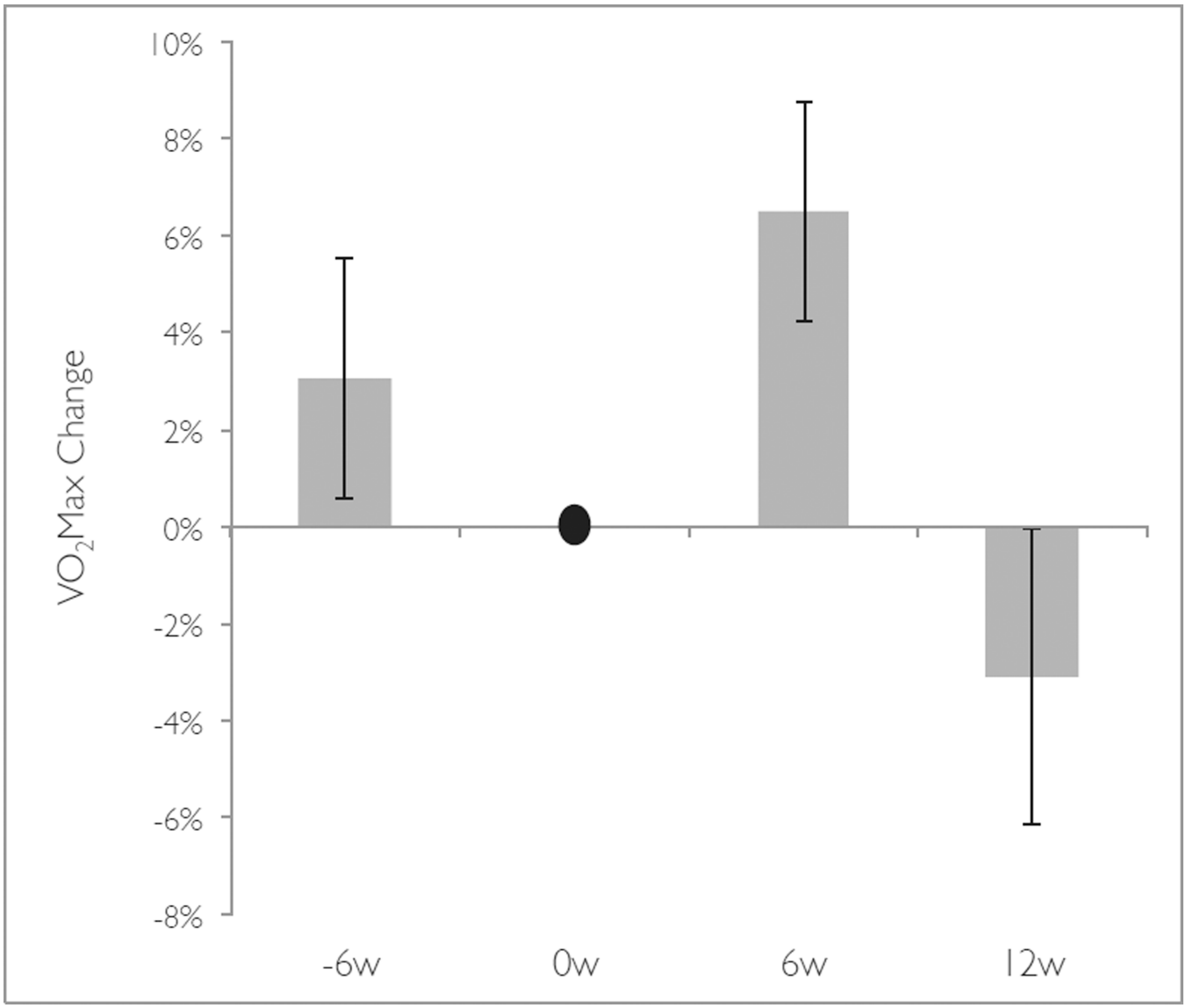

Figure 2.

Change in estimated $\dot{VO}_2$ max relative to the 0w visit for both groups before and after the six-week exercise intervention. A linear-mixed effects analysis demonstrates a significant increase in est. $\dot{VO}_2$ max immediately after exercise that returns to near baseline after six weeks without training. See also supplementary figure S2.

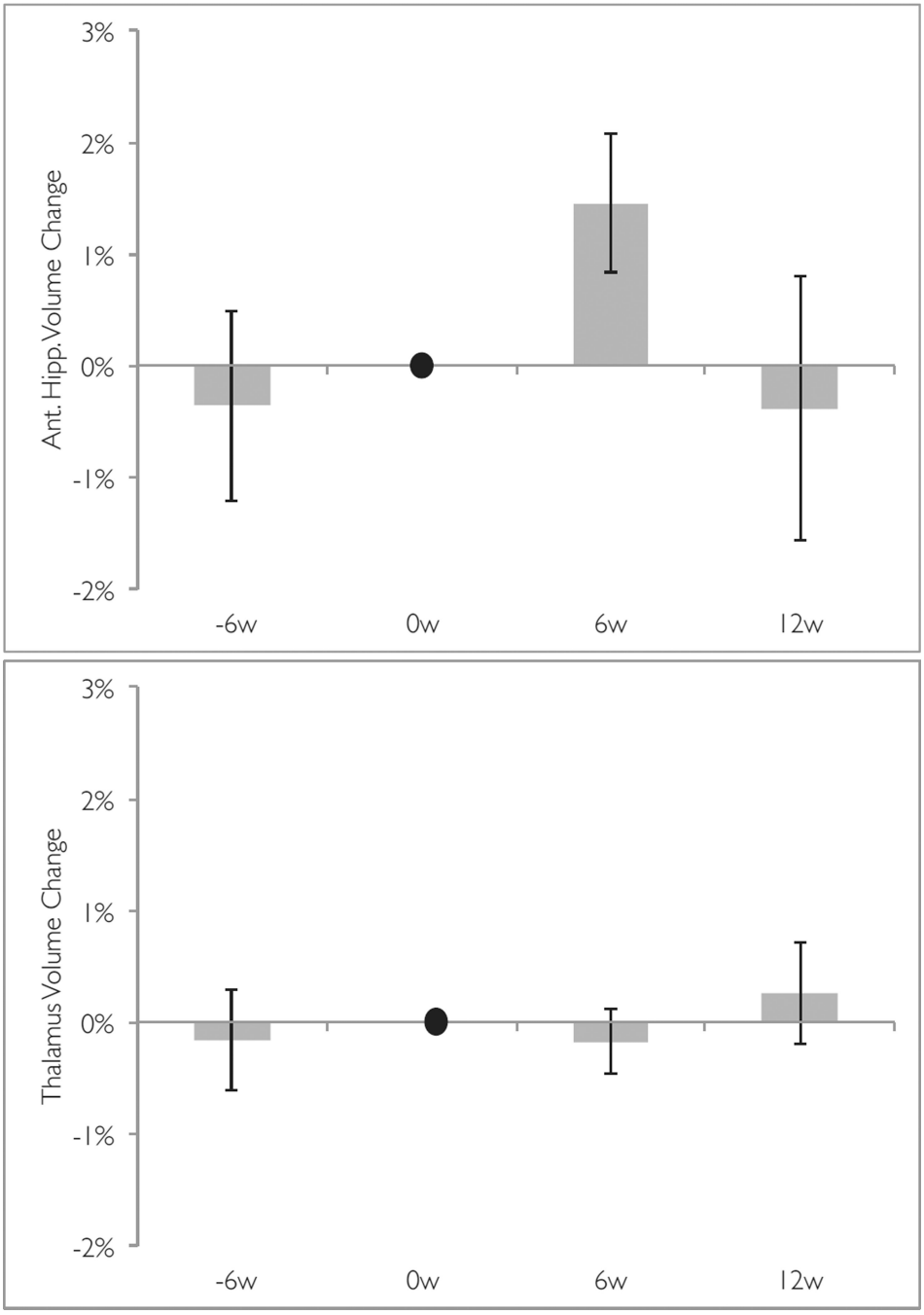

Figure 3.

Top: Change in the volume of of anterior hippocampus volume relative to the 0w visit for both groups before and after the six-week exercise intervention. A linear-mixed effects analysis demonstrates a significant increase in volume immediately after exercise that does not persist in a follow up scan six weeks later. Bottom: No significant volume changes were observed in thalamus volume with exercise. See also supplementary figure S2.

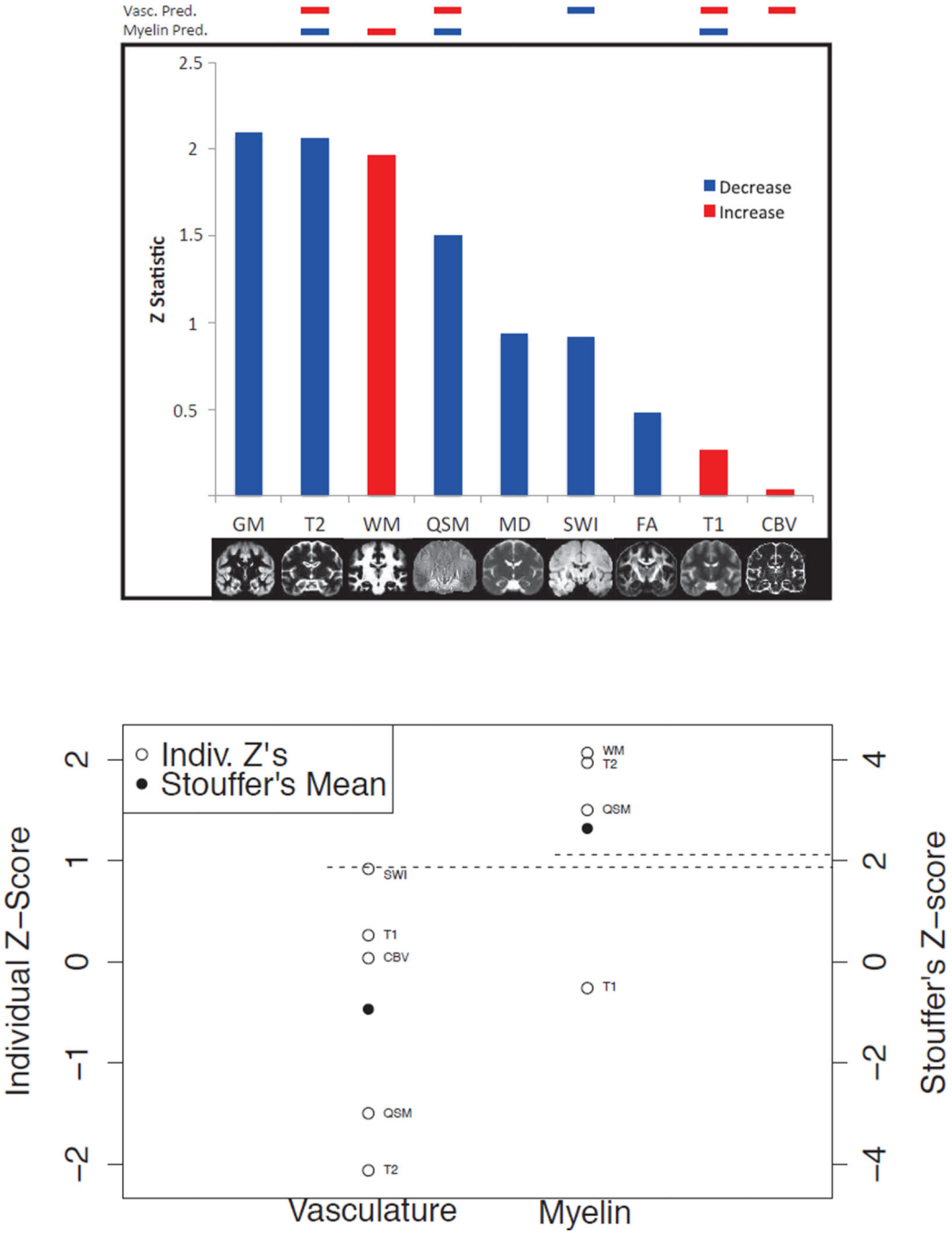

Figure 4.

Top: Z-scores of change in each measure from before to after exercise in anterior hippocampus. Decreases in blue and increases in red. Top bars illustrate predicted direction of change with myelin and vascular change. Bottom: Plot of Z-scores from individual tests (open circles) predicted in the case of an increase of vasculature vs. a predicted increase in myelination (scale for both is shown on left Y-axis). These values are equivalent to those plotted in the top, but the sign of the score depends on whether an increase or decrease was predicted and observed (i.e., a positive Z-score is assigned when the observed change is in

the predicted direction). For each prediction, the combined Stouffer's Z-score is plotted in filled circles (scale shown on the right Y-axis). This Z-score is significant for the myelin test ($Z=2.64$) but not for the vasculature test ($Z=-1.05$). The dotted lines show the one-tailed $P < 0.05$ significance level for the four and five modality permutation tests. GM = grey matter, WM = white matter, QSM = quantitative susceptibility map, MD = mean diffusivity, SWI = susceptibility-weighted image, FA = fractional anisotropy, CBV = cerebral blood volume.

Table 1

Raw values for $V\dot{O}_2$ max and volumes of anterior hippocampus, whole hippocampus, and thalamus at each visit for both groups.

	Rest First Group			**Exercise First Group**		
	−6w	**0w**	**6w**	**0w**	**6w**	**12w**
$V\dot{O}_2$ max (ml/kg-min)	35.07 (1.59)	33.42 (1.47)	36.74 (2.28)	35.71 (1.63)	37.21 (1.72)	34.49 (1.83)
Anterior Hippocampus (cm^3)	2.27 (0.05)	2.28 (0.04)	2.31 (0.05)	2.25 (0.04)	2.29 (0.05)	2.24 (0.050
Whole Hippocampus (cm^3)	6.98 (0.14)	6.97 (0.13)	7.05 (0.15)	6.90 (0.13)	6.92 (0.13)	6.89 (0.14)
Thalamus (cm^3)	16.06 (0.28)	16.09 (0.29)	16.20 (0.41)	15.85 (0.34)	15.84 (0.34)	15.93 (0.34)