The effects of regular aerobic exercise on hippocampal structure and function

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Abstract

Engaging in regular exercise has numerous benefits for brain health. In particular, a number of studies have associated exercise with improvements to the structure and function of the hippocampus, especially in older adults. This region plays a critical role in memory function, and is altered in a number of psychiatric disorders associated with memory impairments (e.g. depression and schizophrenia), as well as healthy ageing. Thus, exercise may provide an effective method of enhancing / ameliorating hippocampal integrity; however, an improved understanding of exercise-related effects in young to middle-aged adults is required. We assessed the effects of regular exercise and cardiorespiratory fitness on hippocampal structure and function in young to middle-aged adults. We recruited a sample of 40 healthy adults, comprised of individuals who self-reported as engaging in high (regular exercisers) or low (sedentary) levels of physical activity (PA) according to World Health Organisation guidelines (high PA n=20; low PA n=20). A multi-modal assessment of hippocampal structure and function was conducted using structural magnetic resonance imaging (sMRI), magnetic resonance (MR) spectroscopy, and hippocampal-dependent memory tasks. We observed evidence of increased N-acetyl-aspartate (NAA) concentration and associative memory performance in high PA individuals. However, no differences in hippocampal volume or pattern separation capacity were observed between groups. We found that cardiorespiratory fitness was positively associated with (left, right, and bilateral) hippocampal volume, NAA concentration, and pattern separation, but not associative memory. Therefore, we provide evidence that engaging in higher levels of exercise has demonstrable benefits for hippocampal integrity and function. Given that young and middle-aged adults are overrepresented in the diagnosis of psychiatric illnesses which affect hippocampal integrity (e.g. depression and schizophrenia), it is possible that exercise may provide a low-risk, effective method of remediating this dysfunction.

Keywords

exercise, NAA, hippocampus, declarative memory, pattern separation, neuroplasticity

1. Introduction

A physically active lifestyle is associated with a myriad of systemic health benefits, including a reduced risk of type 2 diabetes, cardiovascular disease, and certain forms of cancer (Bassuk & Manson, 2005; McTiernan, Schwartz, Potter, & Bowen, 1999). It has become increasingly clear that the benefits of physical activity (PA) are not limited to the periphery, but also extend to the brain (Voss, Vivar, Kramer, & van Praag, 2013). PA refers to forms of bodily movement that results in energy expenditure, and epidemiological studies have associated higher engagement in PA with greater preservation of cognitive capacity (Yaffe, Barnes, Nevitt, Lui, & Covinsky, 2001), and reduced incidence of dementia in later life (Laurin, Verreault, Lindsay, MacPherson, & Rockwood, 2001; Rovio et al., 2005). A number of meta-analyses have also reported improved cognitive performance following exercise interventions (Angevaren, Aufdemkampe, Verhaar, Aleman, & Vanhees, 2008; Smith et al., 2010), particularly within the memory domain (Smith et al., 2010).

Exercise is a subcategory of PA and refers to any deliberate, repetitive movement intended to maintain or improve physical fitness. Aerobic forms of exercise increase respiration and heart rate to increase delivery of energy substrates to working skeletal muscle. Aerobic exercise has emerged as a particularly promising intervention in several psychiatric conditions, such as depression (Kandola, Ashdown-Franks, Hendrikse, Sabiston, & Stubbs, 2019), anxiety (Kandola et al., 2018), and schizophrenia (Firth, Cotter, Elliott, French, & Yung, 2015). Thus, engaging in exercise (especially aerobic forms such as running) represents a practical method of mitigating age-related cognitive decline and improving cognitive performance, and may also offer an effective treatment alternative across a number of psychiatric disorders. However, to maximise clinical potential, a comprehensive understanding of the underlying neural mechanisms targeted by exercise is required.

The hippocampus plays a critical role in memory (Eichenbaum, Schoenbaum, Young, & Bunsey, 1996) and affective processing (Fanselow & Dong, 2010), and a number of studies

have associated exercise with changes to hippocampal structure and function (Kandola, Hendrikse, Lucassen, & Yücel, 2016). For example, increased hippocampal volume has been observed following exercise interventions in older adults (Colcombe et al., 2006; Erickson et al., 2011; Niemann, Godde, & Voelcker-Rehage, 2014), and a positive association between self-reported weekly exercise and right hippocampal grey matter volume has been demonstrated in young to middle-aged adults (Killgore, Olson, & Weber, 2013). Furthermore, higher cardiorespiratory fitness (CRF) level, reflecting physiological adaptation in response to regular PA/exercise, is associated with larger hippocampal volume in preadolescents (Chaddock et al., 2010) and older adults (Colcombe et al., 2003). At a micro-scale level, exercise also has demonstrable benefit on the biochemical composition of the hippocampus, with elevations in N-acetyl-aspartate (NAA; a marker of neuronal integrity/vitality) observed following aerobic exercise interventions in healthy adults (den Ouden et al., 2017) and individuals with schizophrenia (Pajonk et al., 2010). Overall, these studies suggest that exercise can influence hippocampal structure. However, the functional relevance of exercise-induced structural plasticity remains incompletely understood.

Exercise has been linked to enhancements of hippocampus-dependent forms of memory, such as declarative memory (Kandola et al., 2016). Declarative memory can be further sub-divided into associative and episodic memory (referring to the recollection of relationships between cues and autobiographical events respectively), and spatial memory (referring to spatial navigation/orientation in one's environment) (Kandola et al., 2016). Similarly, the hippocampus also supports the ability to select and distinguish similar spatial and temporal memory traces, a process referred to as 'pattern separation' (Kirwan & Stark, 2007; Stark, Yassa, Lacy, & Stark, 2013). Using cognitive tasks designed to assess these forms of memory, studies have shown positive associations between CRF and episodic/associative memory (Baym et al., 2014; Flöel et al., 2010) and pattern separation capacity following exercise interventions (Déry et al., 2013). However, while the evidence seems to support a role for exercise in the promotion of hippocampal function, a number of studies have failed to

demonstrate beneficial effects (for review see, Kandola, Hendrikse, Lucassen, & Yücel, 2016). Further, while some studies have examined the effects of exercise on the hippocampus of young to middle-aged adult samples (e.g. Déry et al., 2013; Killgore et al., 2013; Pajonk, Wobrock, Gruber, & et al., 2010; Thomas et al., 2016b; Wagner et al., 2015), the majority of studies have been conducted on older adults, i.e. > 65 years of age (Prakash, Voss, Erickson, & Kramer, 2015; Stillman, Esteban-Cornejo, Brown, Bender, & Erickson, 2020). This is a critical limitation of the current evidence-base, as lifestyle during young adulthood is associated with cognitive outcomes in later life (Belsky et al., 2015), and psychiatric disorders associated with hippocampal function are prevalent in young/middle-aged adults (e.g. schizophrenia, anxiety, depression) (Kessler et al., 2005). Development of psychiatric disorders in early life is also associated with increased symptom severity and illness duration (Korczak & Goldstein, 2009). Therefore, exercise may hold considerable clinical utility within these age groups. However, it remains unclear whether engaging in regular exercise to achieve a high level of PA (e.g. meeting current American College of Sports Medicine (ACSM) / World Health Organisation (WHO) guidelines of at least 150 minutes moderate intensity aerobic activity each week) is associated with improved hippocampal structure and function in these age groups. Thus, improved characterisation of the effects of exercise on the hippocampus at this stage of life is required.

The majority of past studies investigating the impact of exercise on hippocampal structure have utilised a single neuroimaging outcome measure (e.g. volume estimate from structural magnetic resonance imaging; sMRI) (Kandola et al., 2016). While such studies have undoubtedly advanced our understanding, multi-modal approaches may provide a more comprehensive account of the underlying mechanisms targeted by exercise (den Ouden et al., 2017; Thomas et al., 2016). For example, in comparing patterns of change across a number of MRI measures following a six-week aerobic exercise intervention, Thomas et al. (2016) concluded that an increase in anterior hippocampal volume was likely associated with changes to myelination, rather than increased vascularisation as previously reported (Maass

et al., 2015). However, volumetric increases are not uniformly observed following interventions of this duration. Wagner et al. (2015) observed hippocampal volume reduction in combination with stable hippocampal NAA concentration, indicating volumetric change was unlikely to be a consequence of neuronal loss (Wagner et al., 2015). Thus, the adoption of multi-modal neuroimaging facilitates a more nuanced investigation of the interactions between PA and hippocampal structure. Additionally, both of the aforementioned studies were conducted in young to middle-aged adult samples. The discrepancies in study outcomes suggest that the evidence of a beneficial effect of exercise on hippocampal structure within these age groups remains equivocal. Ultimately, the combination of several complimentary neuroimaging measures may serve to explain the heterogeneity observed across study outcomes in young to middle-aged adults thus far, and provide a more detailed account of the mechanistic effects of exercise to better inform therapeutic applications.

The aim of this study was to investigate the effects of exercise and CRF on hippocampal structure and function in young to middle-aged adults. We recruited a sample of 40 healthy adults, comprised of individuals who self-reported as engaging in a high level of exercise and met the WHO/ACSM guidelines (high PA, n = 20) and low levels of exercise below the guidelines (low PA, n = 20). A multi-modal assessment of hippocampal structure and function was conducted using sMRI, MR spectroscopy, and hippocampal-dependent memory tasks. CRF was also quantified by having participants complete an incremental exercise test to measure of maximal oxygen consumption i.e. VO_2max. We expected significant differences in hippocampal structure and function between high PA and low PA groups. Specifically, we expected significantly higher hippocampal volume and NAA concentration in the high PA group, relative to the low PA group. Similarly, we expected that the high PA group would demonstrate significantly higher performance on hippocampal-dependent memory tasks of associative memory (Wang et al., 2014) and pattern separation (Stark, Yassa, Lacy, & Stark, 2013). We also hypothesised positive associations between

CRF and measures of hippocampal structure and function (i.e. volume, NAA concentration, and pattern separation and associative memory performance).

2. Methods

2.1 Ethics approval

This study was approved by the Monash University Human Research Ethics Committee and all participants provided informed consent. The study confirmed to the standards set by the Declaration of Helsinki, and participants were remunerated $100 AUD for their participation as part of a larger study.

2.2 Participants

Participants were 40 right-handed healthy adults (52.5% female), aged 25.48 ± 9.35 years (mean ± SD; range 18 – 55), reporting no contraindications to MRI and no history of psychological or neurological disorders.

To investigate the influence of exercise on hippocampal structure and function, participants were recruited and selected on the basis of self-reported weekly exercise into a high PA or low PA group. The high PA group included individuals engaging in a minimum of 150 minutes of deliberate exercise each week (mean weekly exercise 428.5 minutes ± 215.6; mean ± SD, range = 150 – 900 minutes)**,** consisting primarily of sports/physical activities known to increase CRF level (e.g. running, swimming, and cycling). The low PA group included individuals engaging in no more than 60 minutes of deliberate exercise each week (mean weekly PA 13.8 minutes ± 25.0; mean ± SD, range = 0 – 60 minutes, Mann-Whitney test, $p=3x10^{-8}$, r = 1.00). All participants were required to complete the long format of the International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003) to verify self-reported exercise level prior to participation.

2.3 Experimental design

A between-subjects, cross-sectional design was used to investigate the effects of exercise on hippocampal structure and function. Participants completed an MRI scan (approximately 45 minutes in duration), followed by two assessments of memory function. Comparisons of hippocampal structure (left and right hippocampal volume and NAA concentration) and hippocampal-dependent memory tasks (face-word recall and mnemonic similarity task) were made between high and low PA groups. In a separate session, participants completed a VO_2max test to assess cardiorespiratory fitness level.

2.4 Cardiorespiratory fitness assessment (VO_2max)

To determine each subject's CRF level, maximal oxygen consumption (VO_2max) was assessed by a graded intensity maximal exercise test conducted on a motorised treadmill. VO_2max was conducted to provide an objective measure of individual fitness to correlate with measures of hippocampal structure and function. The starting pace was set at 6km/h with a 1% gradient, and workload was increased every three minutes until volitional exhaustion was reached. This was achieved via increasing speed by 2 km/h until 16 km/h, followed by incremental gradient increase of 2.5%. Heart rate, expired air volume, and concentration of oxygen and carbon dioxide were continuously recorded throughout the test duration. For determination of VO_2max on the basis of VO_2peak, at least two of the following indicators needed to be observed: (1) a plateau in VO_2ml/kg/min values irrespective of increased workload, (2) maximal respiratory exchange ratio of ≥ 1.1, (3) a heart rate within 10 beats of age-predicted heart rate max (Tanaka, Monahan, & Seals, 2001), or (4) a self-reported rating of perceived exertion (BORG scale; Borg, 1982) rating of perceived exertion > 17 out of 20. Three participants from the low PA group did not meet these criteria and recorded a submaximal test outcome. For these participants, individual regression equations were used (Mean R^2 = .77) to derive a predicted VO_2max value on the basis of age-predicted max heart rate (Tanaka et al., 2001).

2.5 Magnetic resonance imaging (MRI)

MRI data were collected from a Siemens 3T Skyra scanner with 32-channel head coil. T1-weighted structural images (Magnetization Prepared Rapid Gradient Echo, TR = 2.3 s, TE = 2.07 ms, voxel size 1mm^3, flip angle 9°, 192 slices) and GABA-edited Mescher-Garwood Point Resolved Spectroscopy (MEGA-PRESS) data were acquired. MEGA-PRESS data were acquired from a 2 x 3 x 1.5cm voxel localised to the left hippocampus (see figure 1) (96 ON-OFF averages, TE = 68 ms, TR = 1.5 s, editing pulses at 1.9 ppm and 7.5 ppm with bandwidth = 45 Hz). NAA concentration was estimated from the 'OFF' average spectra. Following each acquisition, 8 ON-OFF averages with unsuppressed water were also obtained using the same parameters.

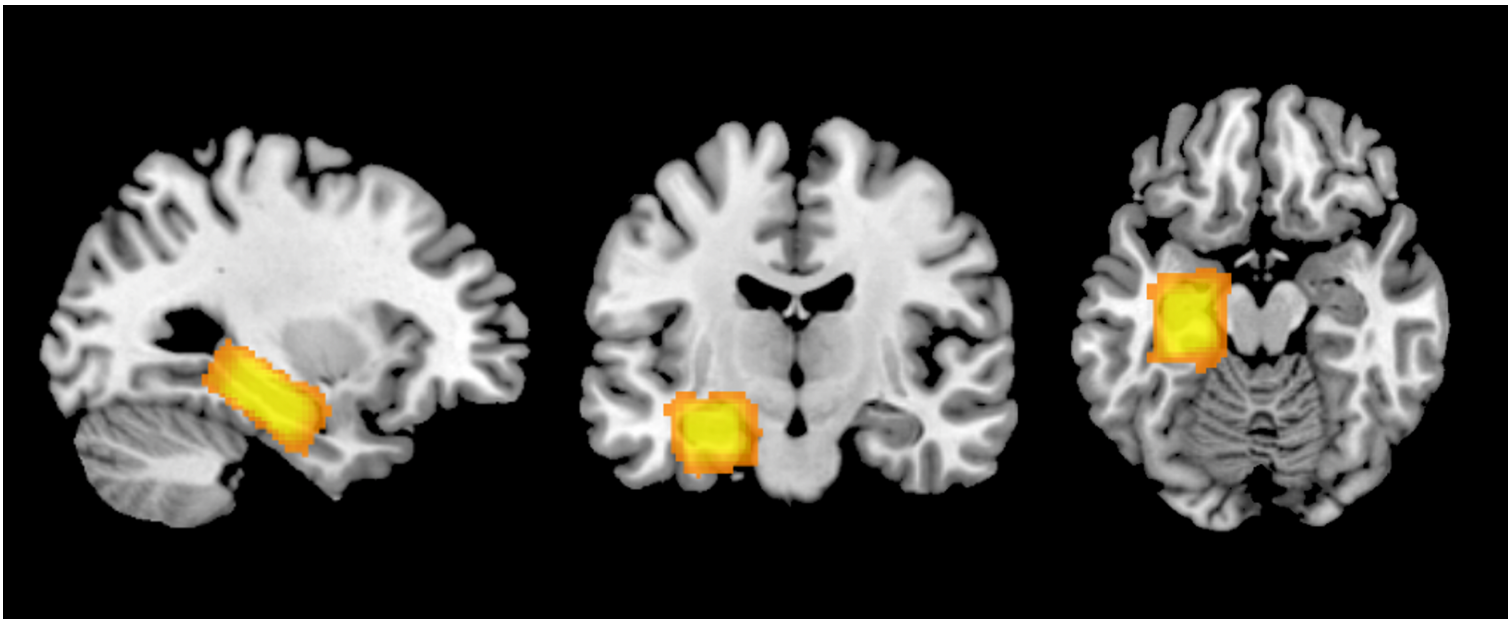

Figure 1. Positioning of MR spectroscopy voxel over left hippocampus. MEGA-PRESS data were acquired from 2 x 3 x 1.5 cm voxels localised to left hippocampus. NAA concentration estimates were derived from the unedited 'OFF' spectra and reported relative to the unsuppressed water signal. The overlap in voxel position across high and low PA participants is shown in orange/yellow, with greater spatial overlap represented by lighter colouration.

2.6 Memory assessment

2.6.1 Associative memory – Face-cued word recall

To assess the effects of PA on associative memory function, we employed a face-cued word recall task (Wang et al., 2014). Participants studied a set of 20 human face photographs

derived from a database of amateur model headshots (Althoff & Cohen, 1999), presented in greyscale on printed cards. Each card was placed on a table in front of the participant for three seconds, and a unique common English word was read aloud by the experimenter when each card was shown. The words were nouns between 3-8 letters in length, with Kucera-Francis written frequencies between 200 to 2000, and concreteness ratings of 300 to 700 (MRC Psycholinguistic Database; www.psych.rl.ac.uk). Participants were instructed to memorise the association between the face and word. Following presentation of the 20 face-word pairs, there was a filled delay of approximately one-minute duration. Following this, the same set of cards was represented to participants individually in a different randomised order, and participants were instructed to try and recall the word that accompanied each face. Word recall was scored as correct or incorrect, forgiving errors relating to pronunciation, including plurality. Five alternative versions of the task were used across participant groups (matched for word concreteness and frequency) and randomised across participants using a Latin square. To familiarise participants with the task and minimise practice effects, a sixth version of the task was employed as a practice set, whereby a smaller sub-set of 10 face-word pairs was administered prior to the main assessment.

2.6.2 Pattern separation - mnemonic similarity task

To investigate differences in behavioural pattern separation between PA groups, we used the mnemomic separation task developed by Stark, Yassa, Lacy, & Stark (2013). Performance on the task is correlated with increased BOLD activity in hippocampal sub-regions (Kirwan & Stark, 2007) and improvements have been reported following a six week aerobic exercise intervention (Déry et al., 2013). In the task, participants were required to complete an initial encoding phase in which they were presented with a series of 96 colour images of everyday objects in the centre of a screen on a white background. Participants were required to categorise each image as an ‘indoor’ or ‘outdoor’ item via button press, using first and third fingers of their right hand (V key for indoor items, N key for outdoor items). Each image was presented for 2.5 s with a 0.5 s inter-trial interval. After a short ~30 s

delay, participants were given instructions about a surprise recognition test during which a second series of 96 colour images were presented (2.5 s each, 0.5 inter-trial interval). Participants were required to identify whether the image was old, similar, or new via button press using first three fingers of their right hand (V key for old, B key for similar, N key for new). One third of images were identical repetitions of previously seen images (i.e. old/targets), one third were new images that had not been previously presented (i.e. new/foils), and one third were similar but not identical to those previously presented (i.e. similar/lures). Responses to each trial during the recognition phase were coded as correct or incorrect.

2.7 Data analysis

2.7.1 Exercise and cardiorespiratory fitness

We assessed differences in self-reported weekly exercise and CRF level (VO_2max) between PA groups. Two participants from the high PA group were lost to follow-up and did not complete the graded exercise test. Thus, analyses of CRF level were conducted with N = 38 (high PA, n = 18; low PA, n = 20). We also assessed the association between VO_2max and current self-reported weekly exercise using a Pearson's correlation.

2.7.2 Analysis of hippocampal volume

Left and right hippocampi were manually traced by an expert tracer (Y. C.) (Chye et al., 2019) using each subject's T1-weighted image and Analyze 12.0 software (AnalyzeDirect, Overland Park, KS) (see figure 2). Hippocampal tracing was conducted according to a validated protocol (Velakoulis et al., 1999), whereby the tracer was blinded to PA group categorisation and left/right structural image orientation. Posterior hippocampal boundaries were defined as the slice with the greatest length of continuous fornix; medial boundaries as the open end of the hippocampal fissure (posterior) and the uncal fissure (anterior); lateral boundaries as the temporal horn of the lateral ventricle; inferior boundaries as the parahippocampal white matter; and superior boundaries as the fimbria and alveus (posterior)

as well as the amygdala (anterior). To assess the intra-rater reliability of manual traced images, each subject's hippocampi were retraced from a T1-weighted structural image acquired one week after the experimental assessment. T1-images acquired at this follow-up time point were acquired in the context of a separate study investigating the effects of four days of repetitive transcranial magnetic stimulation on functional connectivity (see Hendrikse et al., 2020). The tracer was blinded to MRI assessment time points and hypotheses pertaining to this study. To our knowledge, there is no evidence that similar rTMS protocols influence hippocampal volume in the healthy brain, and there were no significant differences between hippocampal volume between time points (see supplementary figure S2). Therefore, intra-rater correlation coefficients are unlikely to be biased by the intervening TMS application. The intra-rater correlation coefficient between experimental and follow-up assessments was .89 for left hippocampus and .86 for right hippocampus, demonstrating consistent manual tracing of hippocampi (see supplementary materials for scatterplots). For each subject, total brain volume estimates were calculated using the 'fsl_anat' function (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). Total brain volume was used to control for differences in brain size across participants and normalise hippocampal volume estimates.

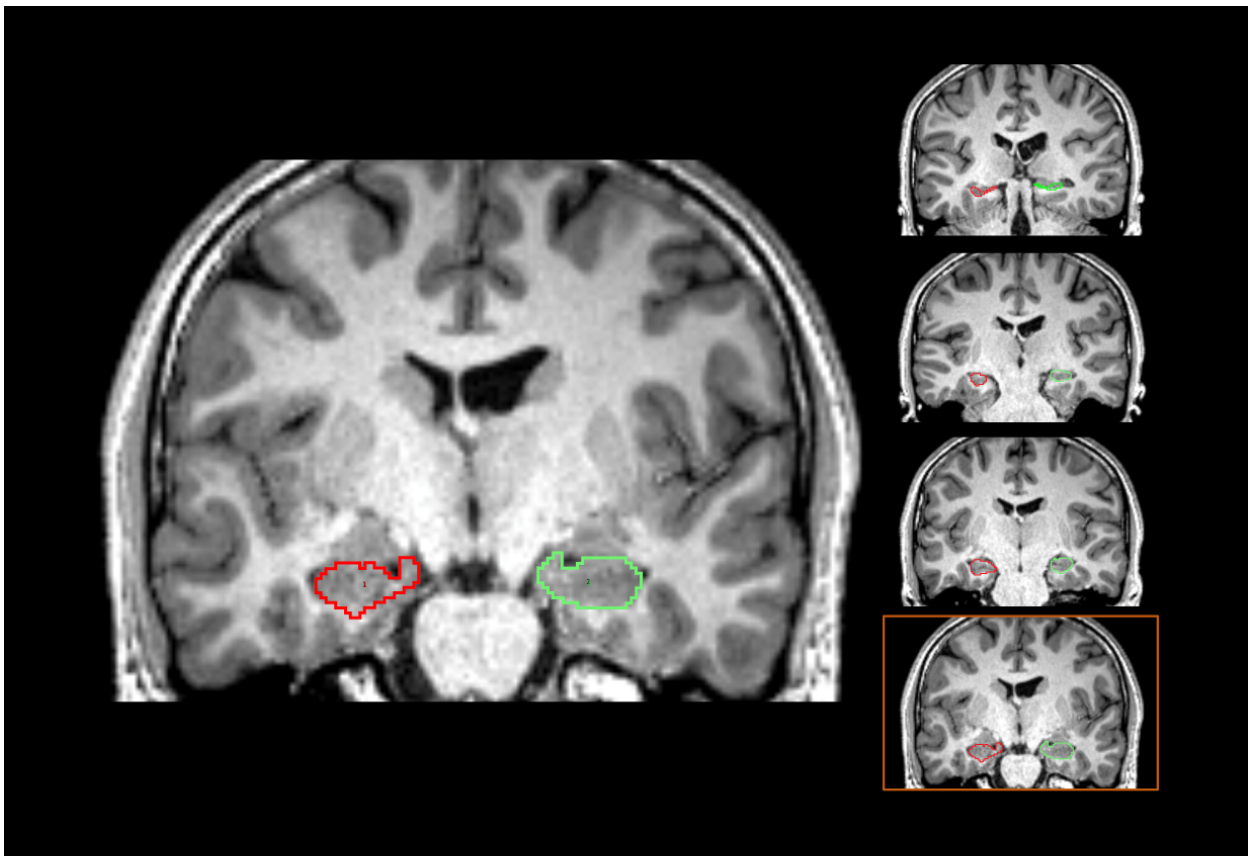

Figure 2. Manual tracing of the left and right hippocampus. Left and right hippocampi were manually traced by an expert tracer using each subject's T1-weighted image. Tracing was completed for left (red outline) and right (green outline) hippocampi according to a validated protocol. Figure shows represents the manual tracing process, with hippocampi traced across each slice of the T1-weighted image in the coronal plane.

2.7.3 NAA quantification

MEGA-PRESS data were acquired as part of a wider study assessing cortical GABA concentration. Relative to cortical regions, the hippocampus is associated with a lower signal-to-noise ratio, reducing the reliability of MRS GABA estimates. Therefore, in this study, we focussed on NAA concentration via the unedited PRESS spectra. NAA is present in much higher concentrations than GABA and produces the largest peak in (unedited) MRS scans of the healthy human brain (Moffett, Arun, Ariyannur, & Namboodiri, 2013), and is therefore one of the most reliable MRS signals (Moffett, Ross, Arun, Madhavarao, & Namboodiri, 2007). MRS data was not collected for two subjects due to scheduling issues, thus analyses were conducted with N = 38. Gannet (version 3) (Edden et al., 2014) was

used to estimate NAA concentration from the left hippocampus. The GannetLoad module was employed for phased-array channel combination of raw Siemens 'twix' files, exponential line broadening (3 Hz), Fourier transformation to yield time-resolved frequency-domain spectra, frequency and phase correction, outlier rejection, and time averaging. GannetFit module was employed to estimate the area of the NAA peak from the unedited 'OFF' acquisition using a single gaussian model and a nonlinear least-squares fitting approach. As an internal reference, NAA concentrations are expressed in institutional units, relative to the unsuppressed water signal, which was estimated with a Gaussian-Lorentzian model.

Partial volume segmentation of the T1-weighted anatomical image within each voxel was conducted using FSL's FAST(Jenkinson et al., 2012), and NAA:H_2O ratios were partial volume corrected by removing the contribution of cerebrospinal fluid fractions (NAA:H_2O / (1 - CSF%)). To assess the quality of NAA estimates, we compared the full width at half maximum (FWHM) values of NAA and water peaks, and NAA fit error (E_{NAA}) between PA groups. E_{NAA} is a measure reflecting the SD of model fit residual for the NAA peak, expressed as a percentage of peak amplitude. Spectra with an E_{NAA} value of > 20% or exceeding a z-score of 3.29 were labelled as outliers and removed from the analysis.

2.7.4 Face-cued word recall task

Performance on the face-cued word recall task was scored as the percentage of correctly recalled words out of a total of 20. Individual z scores > 2.58 were labelled as outliers and removed from subsequent analysis.

2.7.5 Mnemonic similarity task

Data from one subject (high PA) was lost due to computer malfunction during data acquisition, thus analyses are reported with N = 39. Performance on the mnemonic similarity task was calculated by a separation bias metric, which indexed subject's ability to discriminate 'similar' items from 'new' items, i.e. probability(respond 'similar' on 'similar'

image) – probability(respond ‘similar given ‘new’ image), and also the overall percentage of correct responses.

Statistical analysis of these data was conducted using JASP (v 0.10.2). An alpha level of .05 was adopted for all inferential statistics conducted under the null hypothesis significance testing framework. This approach was complemented with Bayesian analysis to assess the relative evidence in favour of the alternative hypothesis.

2.7.6 Statistical comparisons between high and low PA groups

To assess differences in CRF, self-reported exercise level, NAA: H_2O ratios, and hippocampal function (face-cued word recall, pattern separation), independent-samples t-tests were conducted between high and low PA groups (or non-parametric equivalent in the case of violations of normality). Left, right, and bilateral hippocampal total volume (mm^3) were analysed in separate ANCOVA models, with a dependent factor of Hippocampal Volume, fixed factor of PA Group (high PA, low PA), and Total Brain Volume (mm^3) as a covariate. Bayesian forms of these tests were conducted to quantify the relative evidence for the alternative vs null hypothesis (priors set in support of the alternative over null hypothesis (i.e. BF_{10}), and Cauchy parameter set to a conservative default value of 0.707 for independent-samples t-tests (Ly, Verhagen, & Wagenmakers, 2016; Rouder, Speckman, Sun, Morey, & Iverson, 2009).

2.7.7 Relationship between cardiorespiratory fitness and hippocampal structure and function

We assessed the relationship between CRF level and hippocampal structure (volume, NAA concentration) and function (i.e. associative memory, pattern separation). Upon inspection of the data, a relatively continuous distribution of VO_2max scores was found across PA groups (see figure 3). CRF/ VO_2max provides an indirect measure of habitual exercise levels (Carrick-Ranson et al., 2014) and is also influenced by a number of factors such as age, biological sex, and genetic/physiological predispositions. Thus, the continuous distribution of

VO_2max scores across PA groups is not particularly surprising, despite the deliberate selection of high and low PA individuals. Instead, this highlights that CRF and self-reported weekly exercise are different exercise parameters and may uniquely explain hippocampal outcomes. Given the continuous distribution of CRF levels, multiple linear regression was used to examine the associations between CRF (VO_2max) and hippocampal measures. To assess the relationship between VO_2max and hippocampal volume, separate multiple linear regression models were conducted on left, right, and bilateral volume estimates, with age, sex, and total brain volume as additional covariates. Multiple linear regression was also used to examine the relationship between VO_2max and NAA, with age and sex as additional covariates. Similarly, multiple linear regression was used to examine the extent to which CRF predicted hippocampal function (face-cued word recall and mnemonic discrimination performance), with age as an additional covariate. For each regression model, individual values exceeding a Cook's distance of three times the mean were labelled as outliers and removed from the analysis. Regression coefficients were derived from bootstrapped estimates with 5000 replicates. Bayesian correlations (two-tailed, BF_{10}) were conducted between VO_2max and outcome variables for each multiple linear regression analysis.
To assess the association between hippocampal structure and function, partial correlation analyses were also calculated between primary structural (left & right volume, NAA concentration) and functional (face-cued word recall and separation bias) measures, whilst accounting for the effect of age (Bonferroni-adjusted α-level = .008).

3. Results

3.1 Differences between PA groups

Data were collected from a sample of 40 participants (19 males, 21 females) with an average age of 25.48 (SD ± 9.35) years. There were no significant differences between PA groups in regards to age (high PA = 24.55 ± 8.77 years; low PA = 26.40 ± 10.04 years, t(1,38) = -0.62, p = .54, Cohen's d = 0.20), or sex (high PA = 40 % female; low PA = 65 % female, X^2(1, N = 40) = 2.51, p = .11, Cramer's V = .25).

We observed significantly higher VO_2max levels, and total test durations (mins) on the graded exercise protocol, in the high PA group relative to the low PA group (see Table 1, Figure 3). Overall, these findings provide strong evidence of higher CRF in the high PA group. Further, there was a significant positive association between self-reported weekly exercise and VO_2max, with increasing weekly exercise minutes associated with greater CRF ($r = .61$, $p = 4.29 \times 10^{-5}$, supplementary figure S1).

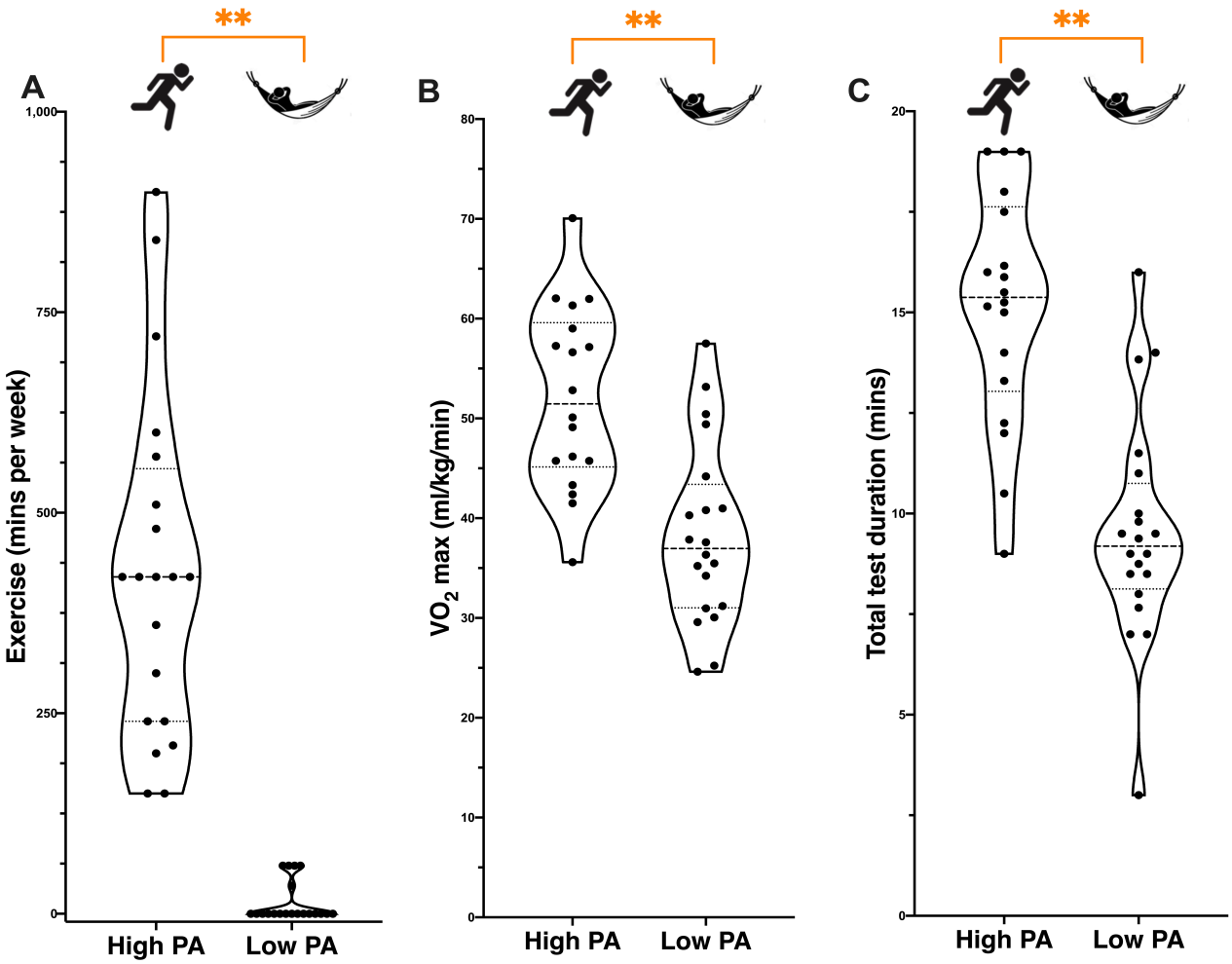

Figure 3. Comparison of self-reported weekly exercise and cardiorespiratory fitness between physical activity (PA) groups. (A) Self-reported weekly exercise. (B) Maximal oxygen consumption (VO_2max) following a graded maximal exercise test. (C) Total test duration on graded exercise test. For all plots, filled circles indicate individual participants. For each group, mean values are indicated by horizontal dashed lines, whereas higher and lower quartiles are indicated by dotted lines. ** $p < 5 \times 10^{-5}$.

In regard to hippocampal structure and integrity, we did not find evidence for differences in left, right, or bilateral hippocampal volume between PA groups (see Table 1, Figure 4). However, we did observe substantial evidence of higher NAA:H_2O concentration ratios in the High PA group, relative to the low PA group (Table 1; Figure 5A; see supplementary materials for data quality analysis).

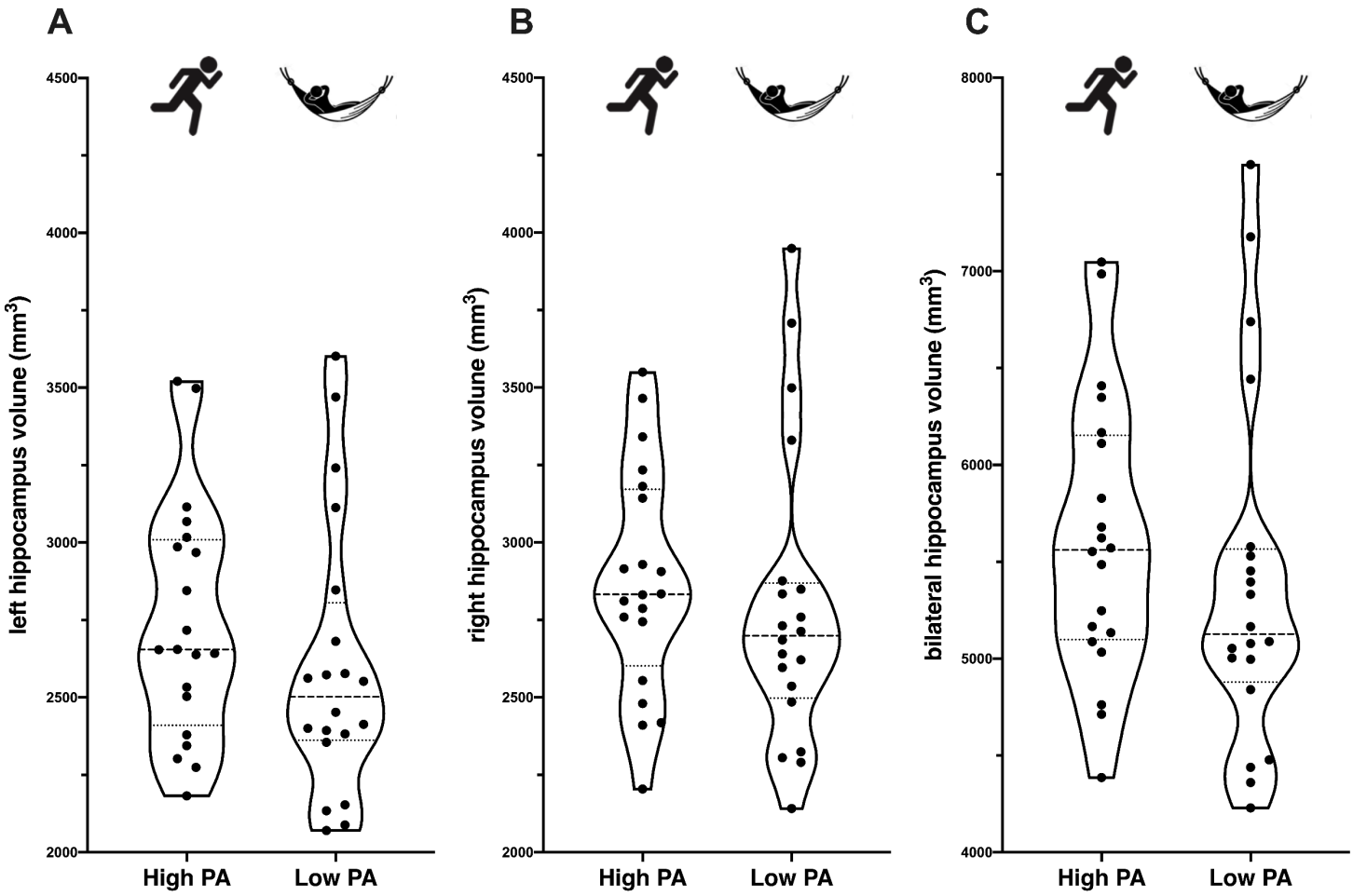

Figure 4. Comparison of hippocampal volume between PA groups. Manually traced estimates of left **(A)**, right **(B)**, and bilateral **(C)** hippocampal volume were compared between PA groups. There were no significant differences between PA groups in left, right, or bilateral hippocampal volume. For all plots, filled circles indicate individual participants. For each group, mean values are indicated by horizontal dashed lines, whereas higher and lower quartiles are indicated by dotted lines.

In regard to hippocampal function, we found moderate evidence of higher face-cued word recall performance in the high PA group, relative to the low PA group (Table 1; Figure 5B). However, we did not observe evidence of differences in pattern separation capacity between

PA groups (Table 1; Figure 5C-D). Together, these findings suggest that higher levels of PA are associated with increased hippocampal NAA concentration and associative memory, but not hippocampal volume or pattern separation.

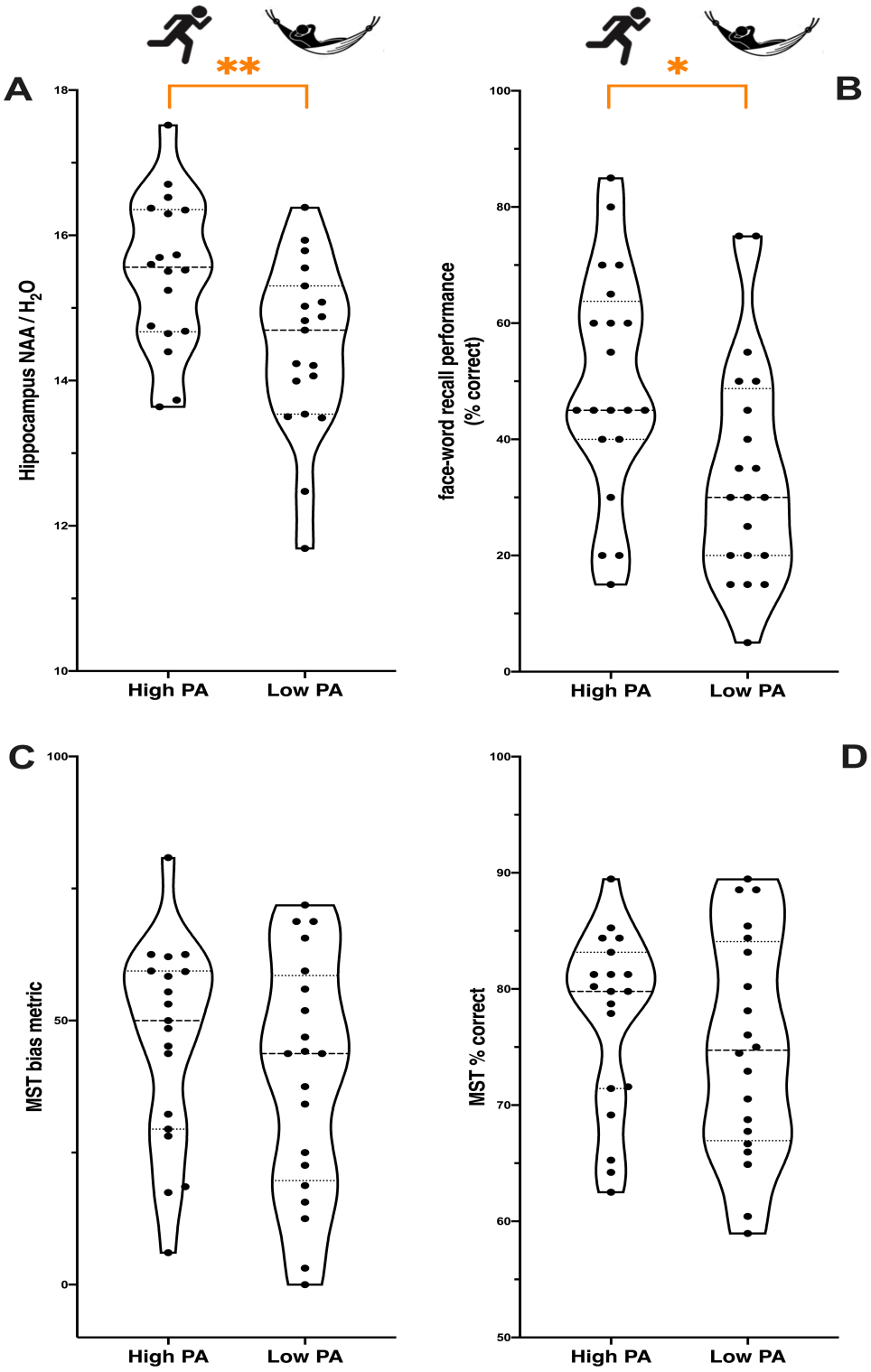

Figure 5. Comparison of NAA concentration and hippocampal function between PA groups.
A) Comparison of hippocampal NAA concentration between PA groups. **B)** Comparison of associative memory performance between PA groups. **C-D)** Comparison of pattern separation performance between PA groups. For all plots, filled circles indicate individual participants. For each group, mean values are indicated by horizontal dashed lines, whereas higher and lower quartiles are indicated by dotted lines. * $p < .05$; ** $p < .01$.

Table 1: Analysis of fitness and hippocampal structure and function between High and Low PA groups

Outcome Measure	Mean (SD) High PA	Mean (SD) Low PA	Statistic	df	p	Effect size	BF_{10}
VO_2max	52.11(9.11)	38.26 (9.06)	4.70[a]	1,36	**3.80×10^{-5}**	1.53[d]	**496.05**
Time VO_2	15.14 (2.90)	9.55 (2.82)	6.02[a]	1,36	**6.53×10^{-7}**	1.96[d]	**18218.50**
L HP vol	2742.0 (382.14)	2602.90 (442.16)	1.53[b]	1,37	.22	.04[c]	0.57
R HP vol	2874.80 (362.82)	2793.55 (480.35)	0.48[b]	1,37	.49	.01[c]	0.37
B HP vol	5616.85 (724.18)	5396.45 (916.71)	0.98[b]	1,37	.33	.03[c]	0.45
NAA	15.49 (1.05)	14.46 (1.19)	2.82[a]	1,35	**.008**	0.93[d]	**5.98**
FCWR	49.75 (19.50)	34.25 (19.35)	2.52[a]	1,38	**.016**	0.80[d]	**3.48**
MST bias	45.94 (19.21)	39.50 (22.38)	0.96[a]	1,37	.34	0.31[d]	0.45
MST % corr	77.42 (7.76)	75.01 (9.51)	0.86[a]	1,37	.39	0.28[d]	0.42

[a] *t* statistic (Independent-samples t-test); [b] *F* statistic (ANCOVA); [c] partial eta squared; [d] Cohen's d; Time VO_2 = total graded exercise test duration (mins); L HP vol = left hippocampus volume; R HP vol = right hippocampus volume (mm^3); T HP vol = bilateral hippocampus volume (mm^3); NAA = N-acetyl aspartate: H_2O concentration ratio; FCWR = face-cued word recall; MST bias = mnemonic pattern separation bias metric; MST % corr = mnemonic pattern separation task overall percentage correct

3.2 Relationship between cardiorespiratory fitness and hippocampal structure and function and fitness

We assessed whether CRF level (VO_2max) was positively associated with hippocampal volume. Two consistent outlier values (Cook's distance > 3 x mean) were identified in each regression model (i.e. left, right, bilateral volume analyses) and removed. A multiple linear regression model, including VO_2max, age, gender, and total brain volume as covariates, explained a significant proportion of the variance associated with left [R^2 = .59, Adjusted R^2 = .54, F(4,31) = 11.17, p = 9.99×10^{-6}], right [R^2 = .53, Adjusted R^2 = .47, F(4,31) = 8.70, p =

7.90 x 10^{-5}], and bilateral [R^2 = .57, Adjusted R^2 = .52, F(4,31) = 10.35, p = 1.92 x 10^{-5}] hippocampal volume. VO_2max was a significant variable in each model (Figure 6) [left, t(1,35) = 4.32, p = .0002, BF_{10} = 2.38; right, t(1,35) = 3.55, p = .001, BF_{10} = 1.47; bilateral, t(1,35) = 4.04, p = .0003, BF_{10} = 2.01] (see tables 1-3 in supplementary materials for corresponding regression coefficients). We next examined the relationship between CRF and hippocampal NAA concentration. One outlier value (Cook's distance > 3 x mean) was identified and removed from the analysis. A regression model, including VO_2max, age, and gender as covariates, explained a significant proportion of the variance associated with hippocampal NAA concentration, R^2 = .37, Adjusted R^2 = .31, F(3,31) = 6.12, p = .002. VO_2max was a significant variable in the model (Figure 7A), t(1,34) = 3.39, p = .002, BF_{10} = 0.61 (see table 4 in supplementary materials).

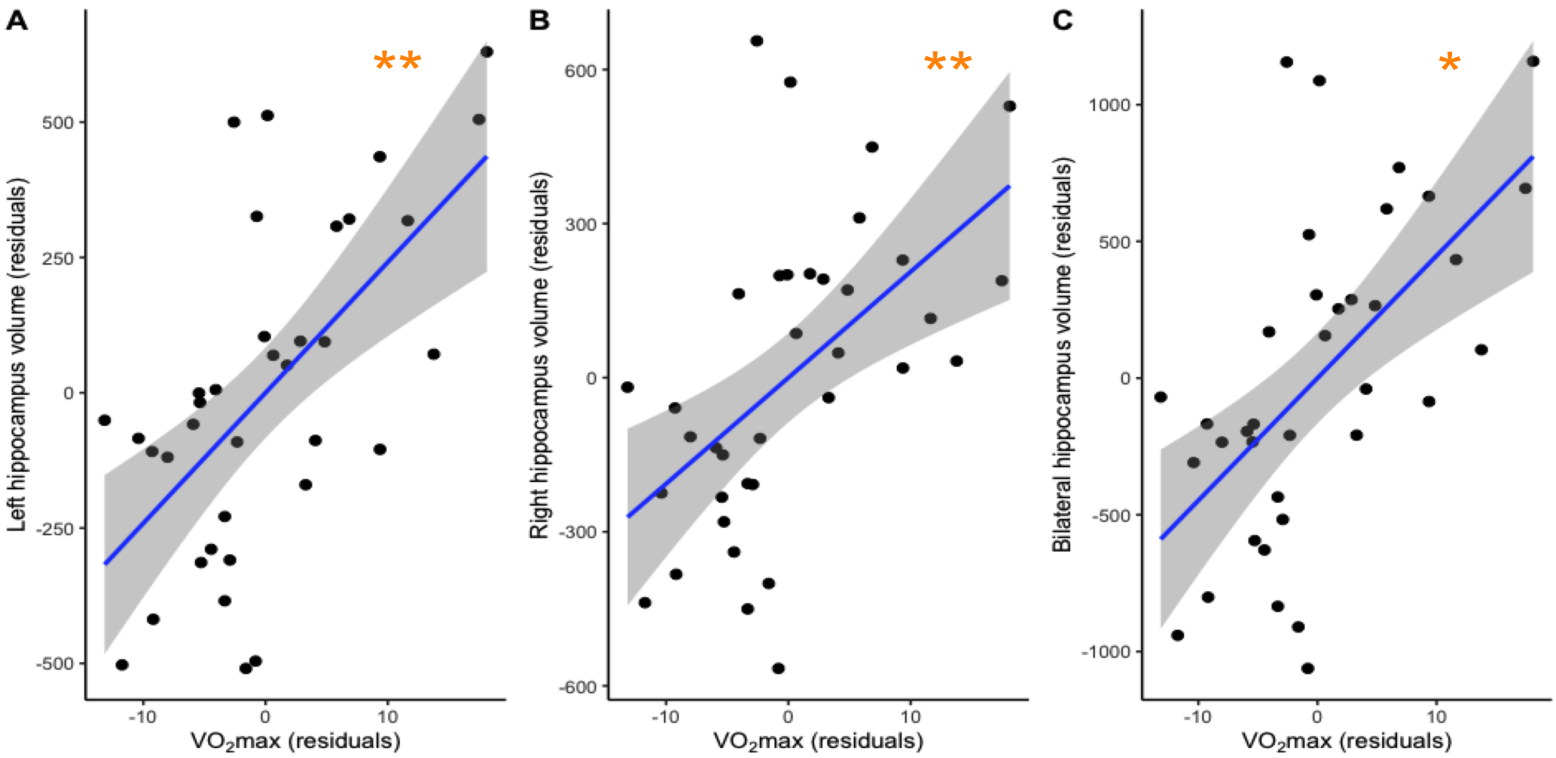

Figure 6. Associations between VO_2max and hippocampal volume. There were significant positive associations observed between VO_2max and left **(A)**, right **(B)**, and bilateral **(C)** hippocampal volume. Circles represent individual residual values, solid line shows regression line of best fit, grey shaded band depicts 95% confidence interval. Hippocampal volume residuals are represented on the y-axis and VO_2max residuals are represented on the x-axis. * p <.01; ** p <.001.

Multiple linear regression models were also conducted to assess the relationship between CRF and hippocampal function. One outlier value (Cook's distance > 3 x mean) was identified in the face-cued word recall regression and removed. A model including VO_2max and age as covariates explained a significant proportion of the variance associated with face-cued word recall performance, R^2 = .21, Adjusted R^2 = .21, F(2,34) = 4.54, p = .018. However, while age was a significant variable in the model, VO_2max was not significantly associated with face-cued word recall performance (Figure 7B), t(1,34) = 0.71, p = .48, BF_{10} = 0.41 (see table 5 in supplementary materials). The same regression model was also used to assess the relationship between VO_2max and pattern separation performance. The outcome measures from the MST task (separation bias metric and overall percentage correct) were analysed separately. There was weak evidence that this model explained the variance associated with separation bias, R^2 = .16, Adjusted R^2 = .11, F(2,33) = 3.19, p = .054. Similarly, there was some weak evidence that VO_2max was associated with separation bias (Figure 7C), t(1,33) = 1.87, p = .070, BF_{10} = 1.61. Further, this regression model accounted for a significant proportion of the variance associated with overall percentage correct on the MST task, R^2 = .19, Adjusted R^2 = .14, F(2,33) = 3.82, p = .032. VO_2max was a significant variable in the model (Figure 7D), t(1,33) = 2.40, p = .022, BF_{10} = 4.09.

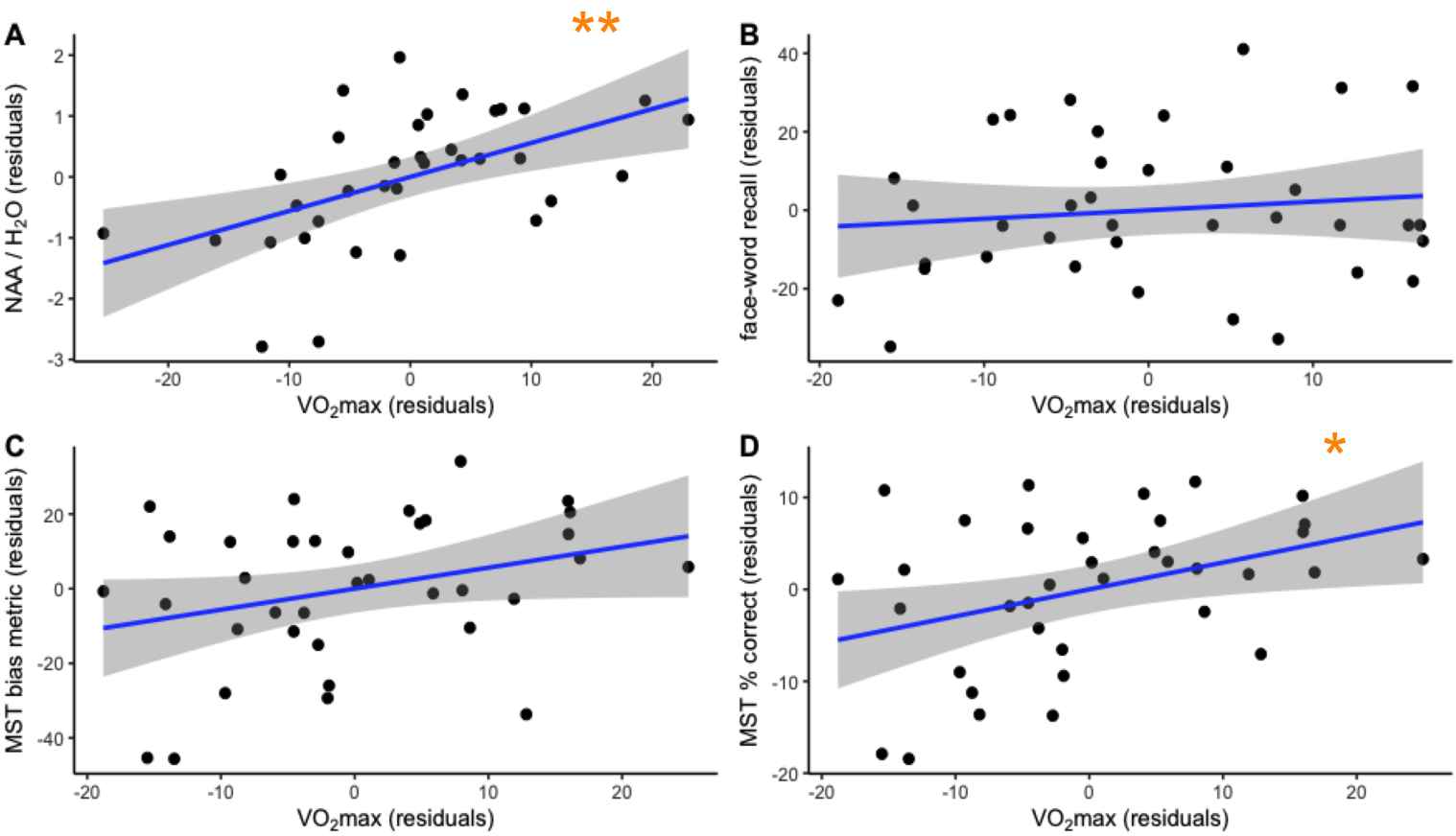

Figure 7. Associations between VO_2max, NAA concentration, and hippocampal function. There were significant positive associations observed between VO_2max and both NAA concentration and MST % correct. **(A)** Association between hippocampal NAA concentration and VO_2max residuals. **(B)** Association between associative memory and VO_2max residuals. **(C-D)** Association between pattern separation (**C** bias metric; **D** % correct) and VO_2max residuals. Circles represent individual residual values, solid line shows regression line of best fit, grey shaded band depicts 95% confidence interval. Hippocampal measures are represented on the y-axis and VO_2max is represented on the x-axis. * p <.05; ** p <.01.

We correlated hippocampal structural measures with each of the functional measures, controlling for the effect of age. There was a significant positive correlation between hippocampal NAA concentration and face-cued word recall performance (partial r = .46, p = .005, n = 36, see Figure 8). No other significant correlations were observed (all p values > .33).

In summary, our findings suggest that higher CRF is associated with increased hippocampal volume and NAA concentration independent of age and sex, but only weakly associated with hippocampal-dependent memory performance.

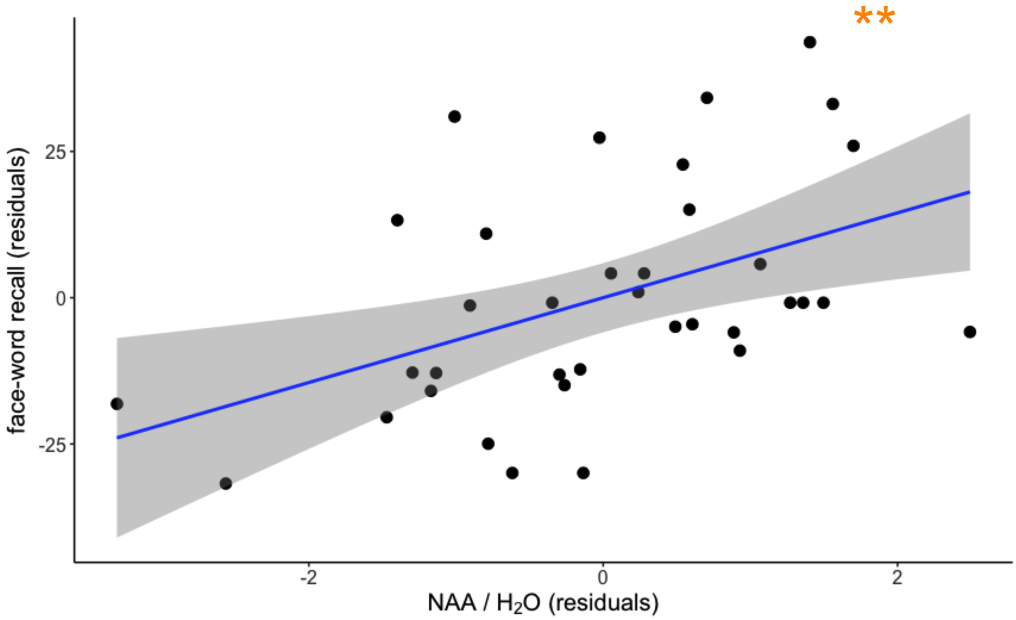

Figure 8. Associations between associative memory and NAA concentration. There was a significant positive association observed between associative memory and NAA concentration. Circles represent individual residual values, solid line shows regression line of best fit, grey shaded band depicts 95% confidence interval. Associative memory residuals are represented on the y-axis and NAA concentration residuals are represented on the x-axis. ** p <.01.

4. Discussion

In this study, we aimed to investigate the effects of exercise and CRF on hippocampal structure and function in young to middle-aged adults. Using a multi-modal assessment, we compared hippocampal structure and function, and hippocampal-related memory performance between groups of individuals engaging in high versus low levels of exercise. We observed evidence of increased NAA concentration and associative memory performance in the high PA group. However, no differences in hippocampal volume or pattern separation capacity were observed between PA groups. We also investigated whether CRF (VO_2max) was associated with measures of hippocampal structure and

function. We found that CRF was positively associated with (left, right, and bilateral) hippocampal volume, NAA concentration, and pattern separation, but not with associative memory. These findings highlight the utility of exercise in improving hippocampal structure and function in young to middle-aged adults - a group most at risk of developing psychiatric illnesses associated with hippocampal dysfunction, such as depression and schizophrenia (Kessler et al., 2005).

4.1 Effects of exercise and cardiorespiratory fitness on hippocampal structure and integrity

Contrary to our hypothesis, we did not observe differences in hippocampal volume (left, right, or bilateral) between high and low PA groups. Instead, using Bayesian statistics, we report anecdotal evidence in support of the null hypothesis. This finding is in contrast a previous study by Killgore et al. (2013) which demonstrated a significant positive association between weekly exercise level and hippocampal grey matter volume in young to middle-aged adults. The discrepancy between study outcomes may be partially attributable to methodological/sampling differences. In the present study, we investigated differences in volume between individuals engaging in high vs low amounts of weekly exercise (i.e. meeting vs not meeting ACSM/WHO PA guidelines). In contrast, Killgore et al., (2013) analysed associations between volume and exercise across a continuum of frequencies and durations (with 75% of individuals in their sample indicating some regular engagement in exercise). Therefore, it is possible that sampling a continuous distribution of exercise levels may be necessary to detect subtle changes to hippocampal volume related to recent exercise habits in these age groups. Indeed, a voxel-wise analysis of hippocampal regions of interest presented in the same study revealed no voxels surviving correction for multiple comparisons within the left hippocampus, and only a small cluster of 21 voxels surviving correction within an anterior portion of the right hippocampus (Killgore et al., 2013). Collectively, these findings suggest that the influence of current exercise engagement on hippocampal volume in young to middle-aged adults is likely subtle and requires further investigation in the context of well-powered experimental trial designs.

While we did not observe volumetric differences between PA groups, we found that individual CRF level was positively associated with left, right, and bilateral hippocampal volume. This finding aligns with two studies that have reported similar relationships in young to middle-aged adults. Stillman et al. (2018) demonstrated an association between CRF and anterior hippocampal volume in a cross-sectional study of healthy young adults, and Pajonk et al. (2010) reported an association between CRF and total hippocampal volume increases following an exercise intervention in young to middle-aged adults with schizophrenia and an age-matched healthy control group (Pajonk et al., 2010). Thus, the relationship between CRF and hippocampal volume reported adds to a limited number of studies across early-to-mid-adulthood. The finding of a relationship between CRF and hippocampal volume is important as these age groups are over-represented in the diagnosis of psychiatric illnesses associated with hippocampal dysfunction. Therefore, promoting CRF may provide an effective means of ameliorating hippocampal function. Furthermore, a number of studies have shown that higher CRF prevents age-related hippocampal deterioration (Firth et al., 2017). Together, these findings highlight that CRF likely benefits neuronal health across the adult lifespan, not just in older age.

The different pattern of results between CRF and self-reported exercise level may offer some insight into the timescales in which exercise influences hippocampal volume. Whilst there are multiple factors which contribute to individual differences in CRF (e.g. physiological/genetic predispositions), CRF also provides an indirect indictor of prior exercise history (Carrick-Ranson et al., 2014). An increase in CRF indicates physiological adaptation within the circulatory/respiratory systems in response to sustained, regular engagement in moderate/vigorous aerobic activity (which increase respiration and heart rate) (Caspersen, Powell, & Christenson, 1985). It is therefore possible that the positive relationship between CRF and hippocampal volume may reflect an accumulative effect of exercise. Namely, the repeated, sustained engagement in exercise required to increase

CRF may also be required for global changes to hippocampal volume. Engaging in aerobic exercise is known to upregulate a cascade of cellular and molecular factors within the hippocampus (e.g. BDNF, growth factors) which contribute to morphological effects (e.g. dendritic arborisation, synaptogenesis, vascularisation, and possibly neurogenesis) (Voss et al., 2013). It is possible that repeated triggering of these microscale mechanisms may be required before macroscale changes in hippocampal volume are detectable. Further, hippocampal blood flow is regulated by small, variably distributed arteries (ranging from 0.2 to 0.8 mm in diameter) branching off from the posterior cerebral and anterior choroidal arteries (Perosa et al., 2020; Spallazzi et al., 2019). It has been proposed that the effects of aerobic exercise on brain plasticity are primarily facilitated by angiogenesis and increased blood circulation (Stimpson, Davison, & Javadi, 2018). Given the variable and limited blood supply to the hippocampus, it is possible that increases in CRF (indicative of prolonged aerobic exercise over many weeks/months) are necessary for vascular plasticity and enduring structural changes within this region (Stimpson et al., 2018). Indeed, elevations in CRF following a 3-month aerobic exercise interventions are associated with increases in hippocampal blood flow, blood volume, and structural volume (Maass et al., 2015), suggesting that these mechanisms may respond over a longer timescale. While speculative, this explanation may also account for the absence of volumetric differences between PA groups (categorised on the basis of current weekly exercise level rather than longer-term exercise history). Future studies are required to characterise this dose-response relationship between exercise and hippocampal volume in young to middle-aged adults.

In line with our expectations, we observed evidence of increased hippocampal NAA concentration in the context of both higher weekly exercise levels and CRF. Our results are consistent with a study by Pajonk et al. (2010) which reported elevated hippocampal NAA concentration following an exercise intervention in young to middle-aged adults with schizophrenia/age-matched healthy controls. Interestingly, higher CRF also offsets age-related NAA decline in the prefrontal cortex, and prefrontal NAA concentration mediates the

positive association between CRF and working memory capacity (Erickson et al., 2012). NAA is predominantly localised to the cell body of neurons, where it plays a multi-factorial role in lipid synthesis/myelination and cell metabolism and is regarded as a marker of neuronal integrity/vitality (Moffett et al., 2007). Taken together, the results of our study and others indicate that higher CRF may confer neuroprotective effects in brain regions susceptible to age- and disease-related deterioration (i.e. hippocampus and prefrontal cortex). Furthermore, our results indicate enhanced NAA concentration in both high PA individuals, and those with higher CRF. Given the cross-sectional nature of this study, future longitudinal studies are required to examine the timescale of these exercise-related effects.

4.2 Effects of exercise and cardiorespiratory fitness on hippocampal function (associative memory, pattern separation)

Our findings provide some evidence of improved memory function in high PA individuals. We observed moderate evidence of increased associative memory performance in individuals engaging in high levels of weekly exercise. However, no relationship between CRF and associative memory was found. Interestingly, past studies have found positive associations between CRF and associative memory level in young to middle-aged adults (Baym et al., 2014; Schwarb et al., 2017). Collectively these studies support the notion that exercise confers beneficial effects for associative memory performance in young to middle-aged adults. The observed association between NAA and associative memory in this study also suggests that exercise-related improvements may relate to hippocampal neuronal vitality/integrity. However, it is unclear what factors have contributed to the discrepancies between study outcomes in relation to CRF and associative memory. It is possible that differences in associative memory task demands may have contributed to observed inconsistencies, with our study assessing associative memory via face-word pairs, while past studies have utilised associative memory tasks with spatial memory and scene recognition components (Baym et al., 2014; Schwarb et al., 2017). Retrieval of associative memories involving faces vs spatial positions is known to activate a topographically distinct

network of brain regions (Khader, Burke, Bien, Ranganath, & Rösler, 2005). Specifically, during retrieval of associative memories involving faces, several regions of the ventral visual pathway are preferentially activated (e.g. posterior fusiform gyrus/posterior cingulate). In comparison, associative spatial memory recall evokes stronger activation across dorsal visual regions (e.g. parietal cortex, dorsolateral prefrontal cortex, and precentral gyrus). In this sense, it is possible that current exercise levels and CRF may preferentially affect certain forms of associative memory (e.g. those with spatial component), though future research is required to confirm this hypothesis.

In contrast, our results suggest that pattern separation capacity is positively associated with CRF, but not current weekly exercise level. This finding is in line with Déry et al. (2013) who reported a correlated improvement in CRF and pattern separation capacity following an exercise intervention, using a high-intensity interval training protocol (periodic running at 95% of age-predicted heart rate maximum, i.e. sprinting). In animals, exercise-induced improvements in pattern separation capacity are specifically linked to enhanced neurogenesis within the dentate gyrus (Clark, Brzezinska, Puchalski, Krone, & Rhodes, 2009; Creer, Romberg, Saksida, Van Praag, & Bussey, 2010). Increased BDNF expression is an important determinant of hippocampal neurogenesis (Rossi et al., 2006) and synaptic plasticity (Vaynman, Ying, & Gomez-Pinilla, 2004), and is elevated by exercise in an intensity-dependent manner (Saucedo Marquez, Vanaudenaerde, Troosters, & Wenderoth, 2015). While the existence of neurogenesis in adult humans remains equivocal, it is possible that increases in CRF (from regular moderate/high intensity exercise) are required to promote neurogenesis, thereby improving pattern separation in young/middle age groups.

4.3 Future directions and conclusion

In this study, we highlight the beneficial effects of exercise on hippocampal structure and function in young and middle-aged adults. However, there are certain methodological limitations which should be acknowledged. Firstly, we utilised a cross-sectional design to

compare hippocampal integrity between individuals engaging in high vs low levels of exercise. Whilst we conducted a multi-modal characterisation of hippocampal structure and function, we are unable to draw causal links between exercise and observed outcomes. Future studies are encouraged to evaluate the replicability of these findings in the context of longitudinal, controlled trials. Further, we have reported evidence linking both higher weekly exercise engagement and individual CRF to improved hippocampal health. The measurement of both self-reported weekly exercise level and individual CRF has allowed an assessment of their relative associations with improved hippocampal integrity. However, we did not collect a detailed history of each participant's exercise history and thus the dose-response relationship between exercise and hippocampal outcomes cannot be determined.

In summary, we have provided evidence of exercise-related improvements of hippocampal structure and function in young to middle-aged adults. We demonstrate that high levels of exercise are associated with elevated hippocampal integrity, while higher CRF is associated with increases in both volume and integrity. Further, we show that high levels of exercise are also associated with enhanced associative memory function. These findings have important implications for our understanding of how exercise impacts brain health in young/middle-aged adults. While a number of studies have demonstrated the capacity for exercise to mitigate deterioration in hippocampal function in older adults, we provide evidence that engaging in higher levels of exercise has demonstrable benefits prior to the expected onset of age-related deterioration (Raz, Rodrigue, Head, Kennedy, & Acker, 2004). Further, young and middle-aged adults are particularly vulnerable to psychiatric illnesses which affect hippocampal integrity. It is possible that exercise may provide a low-risk, effective method of remediating this dysfunction. Future work using longitudinal/interventional designs is required to develop a comprehensive understanding of the effects of exercise on brain health across the lifespan.

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Material availability

All code used for cognitive and MRI analysis is available at https://github.com/jhendrikse/ex_rtms_code.

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Althoff, R. R., & Cohen, N. J. (1999). Eye-Movement-Based Memory Effect: A Reprocessing Effect in Face Perception. *Journal of Experimental Psychology: Learning Memory and Cognition*, *25*(4), 997–1010. https://doi.org/10.1037/0278-7393.25.4.997

Angevaren, M., Aufdemkampe, G., Verhaar, H. J., Aleman, A., & Vanhees, L. (2008). Physical activity and enhanced fitness to improve cognitive function in older people without known cognitive impairment. *Cochrane Database Syst Rev*, *3*(3).

Barnes, D. E., Yaffe, K., Satariano, W. A., & Tager, I. B. (2003). A Longitudinal Study of Cardiorespiratory Fitness and Cognitive Function in Healthy Older Adults. *Journal of the American Geriatrics Society*, *51*(4), 459–465. https://doi.org/10.1046/j.1532-5415.2003.51153.x

Bassuk, S. S., & Manson, J. A. E. (2005, September). Epidemiological evidence for the role of physical activity in reducing risk of type 2 diabetes and cardiovascular disease. *Journal of Applied Physiology*. American Physiological Society. https://doi.org/10.1152/japplphysiol.00160.2005

Baym, C. L., Khan, N. A., Pence, A., Raine, L. B., Hillman, C. H., & Cohen, N. J. (2014). Aerobic fitness predicts relational memory but not item memory performance in healthy young adults. *Journal of Cognitive Neuroscience*, *26*(11), 2645–2652. https://doi.org/10.1162/jocn_a_00667

Belsky, D. W., Caspi, A., Israel, S., Blumenthal, J. A., Poulton, R., & Moffitt, T. E. (2015). Cardiorespiratory fitness and cognitive function in midlife: Neuroprotection or neuroselection? *Annals of Neurology*, *77*(4), 607–617. https://doi.org/10.1002/ana.24356

Borg, G. A. V. (1982). Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise*.

Carrick-Ranson, G., Hastings, J. L., Bhella, P. S., Fujimoto, N., Shibata, S., Palmer, M. D., … Levine, B. D. (2014). The effect of lifelong exercise dose on cardiovascular function during exercise. *Journal of Applied Physiology*, *116*(7), 736–745. https://doi.org/10.1152/japplphysiol.00342.2013

Caspersen, C. J., Powell, K. E., & Christenson, G. M. (1985). Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Reports (Washington, D.C. : 1974)*, *100*(2), 126–131. https://doi.org/10.2307/20056429

Chaddock, L., Erickson, K. I., Prakash, R. S., Kim, J. S., Voss, M. W., Vanpatter, M., … Kramer, A. F. (2010). A neuroimaging investigation of the association between aerobic fitness, hippocampal volume, and memory performance in preadolescent children. *Brain Research*, *1358*, 172–183. https://doi.org/10.1016/j.brainres.2010.08.049

Chye, Y., Lorenzetti, V., Suo, C., Batalla, A., Cousijn, J., Goudriaan, A. E., … Solowij, N. (2019). Alteration to hippocampal volume and shape confined to cannabis dependence: a multi-site study. *Addiction Biology*, *24*(4), 822–834. https://doi.org/10.1111/adb.12652

Clark, P. J., Brzezinska, W. J., Puchalski, E. K., Krone, D. A., & Rhodes, J. S. (2009). Functional analysis of neurovascular adaptations to exercise in the dentate gyrus of young adult mice associated with cognitive gain. *Hippocampus*, *19*(10), 937–950. https://doi.org/10.1002/hipo.20543

Colcombe, S. J., Erickson, K. I., Raz, N., Webb, a. G., Cohen, N. J., McAuley, E., & Kramer, a. F. (2003). Aerobic Fitness Reduces Brain Tissue Loss in Aging Humans. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *58*(2), M176--M180. https://doi.org/10.1093/gerona/58.2.M176

Colcombe, S. J., Erickson, K. I., Scalf, P. E., Kim, J. S., Prakash, R., McAuley, E., … Kramer, A. F. (2006). Aerobic exercise training increases brain volume in aging humans. *Biological Sciences and Medical Sciences*, *61A*(11), 1166–1170. https://doi.org/10.1093/gerona/61.11.1166

Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., … Oja, P. (2003). International Physical Activity Questionnaire: 12-Country Reliability and Validity. *Medicine & Science in Sports & Exercise*, *35*(8), 1381–1395. https://doi.org/10.1249/01.MSS.0000078924.61453.FB

Creer, D. J., Romberg, C., Saksida, L. M., Van Praag, H., & Bussey, T. J. (2010). Running enhances spatial pattern separation in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(5), 2367–2372. https://doi.org/10.1073/pnas.0911725107

den Ouden, L., Kandola, A., Suo, C., Hendrikse, J., Costa, R., Watt, M. J., … Sabaroedin, K. (2017). The Influence of Aerobic Exercise on Hippocampal Integrity and Function: Preliminary Findings of a Multi-Modal Imaging Analysis. *Brain Plasticity*, 1–6. https://doi.org/10.3233/BPL-170053

Déry, N., Pilgrim, M., Gibala, M., Gillen, J., Wojtowicz, J. M., MacQueen, G., & Becker, S. (2013). Adult hippocampal neurogenesis reduces memory interference in humans: opposing effects of aerobic exercise and depression. *Frontiers in Neuroscience*, *7*(7 APR), 66. https://doi.org/10.3389/fnins.2013.00066

Edden, R. A. E., Puts, N. A. J., Harris, A. D., Barker, P. B., & C. John Evans. (2014). Gannet: A

Batch-Processing Tool for the Quantitative Analysis of Gamma-Aminobutyric Acid–Edited MR Spectroscopy Spectra Richard, *40*(6), 1445–1452.
Eichenbaum, H., Schoenbaum, G., Young, B., & Bunsey, M. (1996). Functional organization of the hippocampal memory system. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(November), 13500–13507. https://doi.org/10.1073/pnas.93.24.13500
Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., … Kramer, A. F. (2011). Exercise training increases size of hippocampus and improves memory. *Proceedings of the National Academy of Sciences*, *108*(7), 3017–3022. https://doi.org/10.1073/pnas.1015950108
Erickson, K. I., Weinstein, A. M., Sutton, B. P., Prakash, R. S., Voss, M. W., Chaddock, L., … Kramer, A. F. (2012). Beyond vascularization: aerobic fitness is associated with N-acetylaspartate and working memory. *Brain and Behavior*, *2*(1), 32–41. https://doi.org/10.1002/brb3.30
Fanselow, M. S., & Dong, H. W. (2010, January 14). Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? *Neuron*. Cell Press. https://doi.org/10.1016/j.neuron.2009.11.031
Firth, J., Cotter, J., Elliott, R., French, P., & Yung, A. R. (2015, May 28). A systematic review and meta-Analysis of exercise interventions in schizophrenia patients. *Psychological Medicine*. Cambridge University Press. https://doi.org/10.1017/S0033291714003110
Flöel, A., Ruscheweyh, R., Krüger, K., Willemer, C., Winter, B., Völker, K., … Knecht, S. (2010). Physical activity and memory functions: are neurotrophins and cerebral gray matter volume the missing link? *NeuroImage*, *49*(3), 2756–2763. https://doi.org/10.1016/j.neuroimage.2009.10.043
Hendrikse, J., Coxon, J., Thompson, S., Suo, C., Fornito, A., Yücel, M., & Rogasch, N. C. (2020). Multi-day rTMS exerts site-specific effects on functional connectivity but does not influence associative memory performance. *BioRxiv*, 2020.04.23.056655. https://doi.org/10.1101/2020.04.23.056655
Jenkinson, M., Beckmann, C. F., Behrens, T. E. J., Woolrich, M. W., & Smith, S. M. (2012). FSL. *NeuroImage*, *62*, 982–790. https://doi.org/10.1016/j.neuroimage.2011.09.015
Kandola, A., Hendrikse, J., Lucassen, P. J., & Yücel, M. (2016). Aerobic exercise as a tool to improve hippocampal plasticity and function in humans: Practical implications for mental health treatment. *Frontiers in Human Neuroscience*, *10*(JULY2016). https://doi.org/10.3389/fncel.2016.00373
Kandola, Aaron, Ashdown-Franks, G., Hendrikse, J., Sabiston, C. M., & Stubbs, B. (2019, December 1). Physical activity and depression: Towards understanding the antidepressant mechanisms of physical activity. *Neuroscience and Biobehavioral Reviews*. Elsevier Ltd. https://doi.org/10.1016/j.neubiorev.2019.09.040
Kandola, Aaron, Vancampfort, D., Herring, M., Rebar, A., Hallgren, M., Firth, J., & Stubbs, B. (2018). Moving to Beat Anxiety: Epidemiology and Therapeutic Issues with Physical Activity for Anxiety. *Current Psychiatry Reports*, *20*(8), 63. https://doi.org/10.1007/s11920-018-0923-x
Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime Prevalence and Age-of-Onset Distributions of. *Arch Gen Psychiatry*, *62*(June), 593–602. https://doi.org/10.1001/archpsyc.62.6.593
Khader, P., Burke, M., Bien, S., Ranganath, C., & Rösler, F. (2005). Content-specific activation during associative long-term memory retrieval. *NeuroImage*, *27*(4), 805–816. https://doi.org/10.1016/j.neuroimage.2005.05.006
Killgore, W. D. S., Olson, E. A., & Weber, M. (2013). Physical exercise habits correlate with gray matter volume of the hippocampus in healthy adult humans. *Scientific Reports*, *3*, 1–6. https://doi.org/10.1038/srep03457
Kirwan, C. B., & Stark, C. E. L. (2007). Overcoming interference: an fMRI investigation of pattern separation in the medial temporal lobe. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *14*(9), 625–633. https://doi.org/10.1101/lm.663507
Korczak, D. J., & Goldstein, B. I. (2009). Childhood Onset Major Depressive Disorder: Course of Illness and Psychiatric Comorbidity in a Community Sample. *Journal of Pediatrics*, *155*(1), 118–123. https://doi.org/10.1016/j.jpeds.2009.01.061
Laurin, D., Verreault, R., Lindsay, J., MacPherson, K., & Rockwood, K. (2001). Physical activity and risk of cognitive impairment and dementia in elderly persons. *Archives of Neurology*, *58*(3), 498–504. https://doi.org/10.1001/archneur.58.3.498
Ly, A., Verhagen, J., & Wagenmakers, E. J. (2016). Harold Jeffreys's default Bayes factor hypothesis tests: Explanation, extension, and application in psychology. *Journal of Mathematical Psychology*, *72*, 19–32. https://doi.org/10.1016/j.jmp.2015.06.004
Maass, A., Düzel, S., Goerke, M., Becke, A., Sobieray, U., Neumann, K., … Düzel, E. (2015).

Vascular hippocampal plasticity after aerobic exercise in older adults. *Molecular Psychiatry*, *20*(5), 585–593. https://doi.org/10.1038/mp.2014.114
McTiernan, A., Schwartz, R. S., Potter, J., & Bowen, D. (1999). Exercise clinical trials in cancer prevention research: A call to action. *Cancer Epidemiology Biomarkers and Prevention*, *8*(3), 201–207.
Moffett, J. R., Arun, P., Ariyannur, P. S., & Namboodiri, A. M. A. (2013, December 26). N-Acetylaspartate reductions in brain injury: Impact on post-injury neuroenergetics, lipid synthesis, and protein acetylation. *Frontiers in Neuroenergetics*. Frontiers Research Foundation. https://doi.org/10.3389/fnene.2013.00011
Moffett, J. R., Ross, B., Arun, P., Madhavarao, C. N., & Namboodiri, A. M. A. (2007, February 1). N-Acetylaspartate in the CNS: From neurodiagnostics to neurobiology. *Progress in Neurobiology*. Pergamon. https://doi.org/10.1016/j.pneurobio.2006.12.003
Niemann, C., Godde, B., & Voelcker-Rehage, C. (2014). Not only cardiovascular, but also coordinative exercise increases hippocampal volume in older adults. *Frontiers in Aging Neuroscience*, *6*(JUN), 1–24. https://doi.org/10.3389/fnagi.2014.00170
Pajonk, F.-G., Wobrock, T., Gruber, O., Scherk, H., Berner, D., Kaizl, I., … Falkai, P. (2010). Hippocampal plasticity in response to exercise in schizophrenia. *Archives of General Psychiatry*, *67*(2), 133–143. https://doi.org/10.1001/archgenpsychiatry.2009.193
Perosa, V., Priester, A., Ziegler, G., Cardenas-Blanco, A., Dobisch, L., Spallazzi, M., … Düzel, E. (2020). Hippocampal vascular reserve associated with cognitive performance and hippocampal volume. *Brain : A Journal of Neurology*, *143*(2), 622–634. https://doi.org/10.1093/brain/awz383
Prakash, R. S., Voss, M. W., Erickson, K., & Kramer, A. (2015). Physical Activity and Cognitive Vitality. *Ssrn*. https://doi.org/10.1146/annurev-psych-010814-015249
Raz, N., Rodrigue, K. M., Head, D., Kennedy, K. M., & Acker, J. D. (2004). Differential aging of the medial temporal lobe: A study of a five-year change. *Neurology*, *62*(3), 433–438. https://doi.org/10.1212/01.WNL.0000106466.09835.46
Rossi, C., Angelucci, A., Costantin, L., Braschi, C., Mazzantini, M., Babbini, F., … Caleo, M. (2006). Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. *European Journal of Neuroscience*, *24*(7), 1850–1856. https://doi.org/10.1111/j.1460-9568.2006.05059.x
Rouder, J. N., Speckman, P. L., Sun, D., Morey, R. D., & Iverson, G. (2009). Bayesian t tests for accepting and rejecting the null hypothesis. *Psychonomic Bulletin and Review*, *16*(2), 225–237. https://doi.org/10.3758/PBR.16.2.225
Rovio, S., Kåreholt, I., Helkala, E. L., Viitanen, M., Winblad, B., Tuomilehto, J., … Kivipelto, M. (2005). Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease. *Lancet Neurology*, *4*(11), 705–711. https://doi.org/10.1016/S1474-4422(05)70198-8
Saucedo Marquez, C. M., Vanaudenaerde, B., Troosters, T., & Wenderoth, N. (2015). High-intensity interval training evokes larger serum BDNF levels compared with intense continuous exercise. *Journal of Applied Physiology*, *119*(12), 1363–1373. https://doi.org/10.1152/japplphysiol.00126.2015
Schwarb, H., Johnson, C. L., Daugherty, A. M., Hillman, C. H., Kramer, A. F., Cohen, N. J., & Barbey, A. K. (2017). Aerobic fitness, hippocampal viscoelasticity, and relational memory performance. *NeuroImage*, *153*, 179–188. https://doi.org/10.1016/j.neuroimage.2017.03.061
Smith, P. J., Blumenthal, J. A., Hoffman, B. M., Cooper, H., Strauman, T. A., Welsh-Bohmer, K., … Sherwood, A. (2010). Aerobic exercise and neurocognitive performance: a meta-analytic review of randomized controlled trials. *Psychosomatic Medicine*, *72*(3), 239–252. https://doi.org/10.1097/PSY.0b013e3181d14633
Spallazzi, M., Dobisch, L., Becke, A., Berron, D., Stucht, D., Oeltze-Jafra, S., … Düzel, E. (2019). Hippocampal vascularization patterns: A high-resolution 7 Tesla time-of-flight magnetic resonance angiography study. *NeuroImage: Clinical*, *21*, 101609. https://doi.org/10.1016/j.nicl.2018.11.019
Stark, S. M., Yassa, M. A., Lacy, J. W., & Stark, C. E. L. (2013). A task to assess behavioral pattern separation (BPS) in humans: Data from healthy aging and mild cognitive impairment. *Neuropsychologia*, *51*(12), 2442–2449. https://doi.org/10.1016/j.neuropsychologia.2012.12.014
Stillman, C. M., Esteban-Cornejo, I., Brown, B., Bender, C. M., & Erickson, K. I. (2020). Effects of Exercise on Brain and Cognition Across Age Groups and Health States. *Trends in Neurosciences*, *43*(7), 533–543. https://doi.org/10.1016/j.tins.2020.04.010
Stillman, C. M., Uyar, F., Huang, H., Grove, G. A., Watt, J. C., Wollam, M. E., & Erickson, K. I. (2018). Cardiorespiratory fitness is associated with enhanced hippocampal functional connectivity in healthy young adults. *Hippocampus*, *28*(3), 239–247. https://doi.org/10.1002/hipo.22827

Stimpson, N. J., Davison, G., & Javadi, A.-H. (2018). Joggin' the Noggin: Towards a Physiological Understanding of Exercise-Induced Cognitive Benefits. *Neuroscience & Biobehavioral Reviews*, *88*(March), 177–186. https://doi.org/10.1016/j.neubiorev.2018.03.018

Tanaka, H., Monahan, K. D., & Seals, D. R. (2001). Age-predicted maximal heart rate revisited. *Journal of the American College of Cardiology*. https://doi.org/10.1016/S0735-1097(00)01054-8

Thomas, A. G., Dennis, A., Rawlings, N. B., Stagg, C. J., Matthews, L., Morris, M., … Bandettini, P. A. (2016). Multi-modal characterization of rapid anterior hippocampal volume increase associated with aerobic exercise. *NeuroImage*, *131*, 162–170. https://doi.org/10.1016/j.neuroimage.2015.10.090

Vaynman, S., Ying, Z., & Gomez-Pinilla, F. (2004). Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *European Journal of Neuroscience*, *20*(10), 2580–2590. https://doi.org/10.1111/j.1460-9568.2004.03720.x

Velakoulis, D., Pantelis, C., McGorry, P. D., Dudgeon, P., Brewer, W., Cook, M., … Copolov, D. (1999). Hippocampal volume in first-episode psychoses and chronic schizophrenia: A high-resolution magnetic resonance imaging study. *Archives of General Psychiatry*, *56*(2), 133–140. https://doi.org/10.1001/archpsyc.56.2.133

Voss, M. W., Vivar, C., Kramer, A. F., & van Praag, H. (2013). Bridging animal and human models of exercise-induced brain plasticity. *Trends in Cognitive Sciences*, *17*(10), 525–544. https://doi.org/10.1016/j.tics.2013.08.001

Wagner, G., Herbsleb, M., de la Cruz, F., Schumann, A., Brünner, F., Schachtzabel, C., … Bär, K.-J. (2015). Hippocampal structure, metabolism, and inflammatory response after a 6-week intense aerobic exercise in healthy young adults: a controlled trial. *Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *35*(10), 1570–1578. https://doi.org/10.1038/jcbfm.2015.125

Wang, J. X., Rogers, L. M., Gross, E. Z., Ryals, A. J., Dokucu, M. E., Brandstatt, K. L., … Voss, J. L. (2014). Targeted enhancement of cortical hippocampal brain networks and associative memory. *Science*, *345*(6200), 1054–1057. https://doi.org/10.1126/science.1252900

Yaffe, K., Barnes, D., Nevitt, M., Lui, L. Y., & Covinsky, K. (2001). A prospective study of physical activity and cognitive decline in elderly women women who walk. *Archives of Internal Medicine*, *161*(14), 1703–1708. https://doi.org/10.1001/archinte.161.14.1703

Supplementary materials

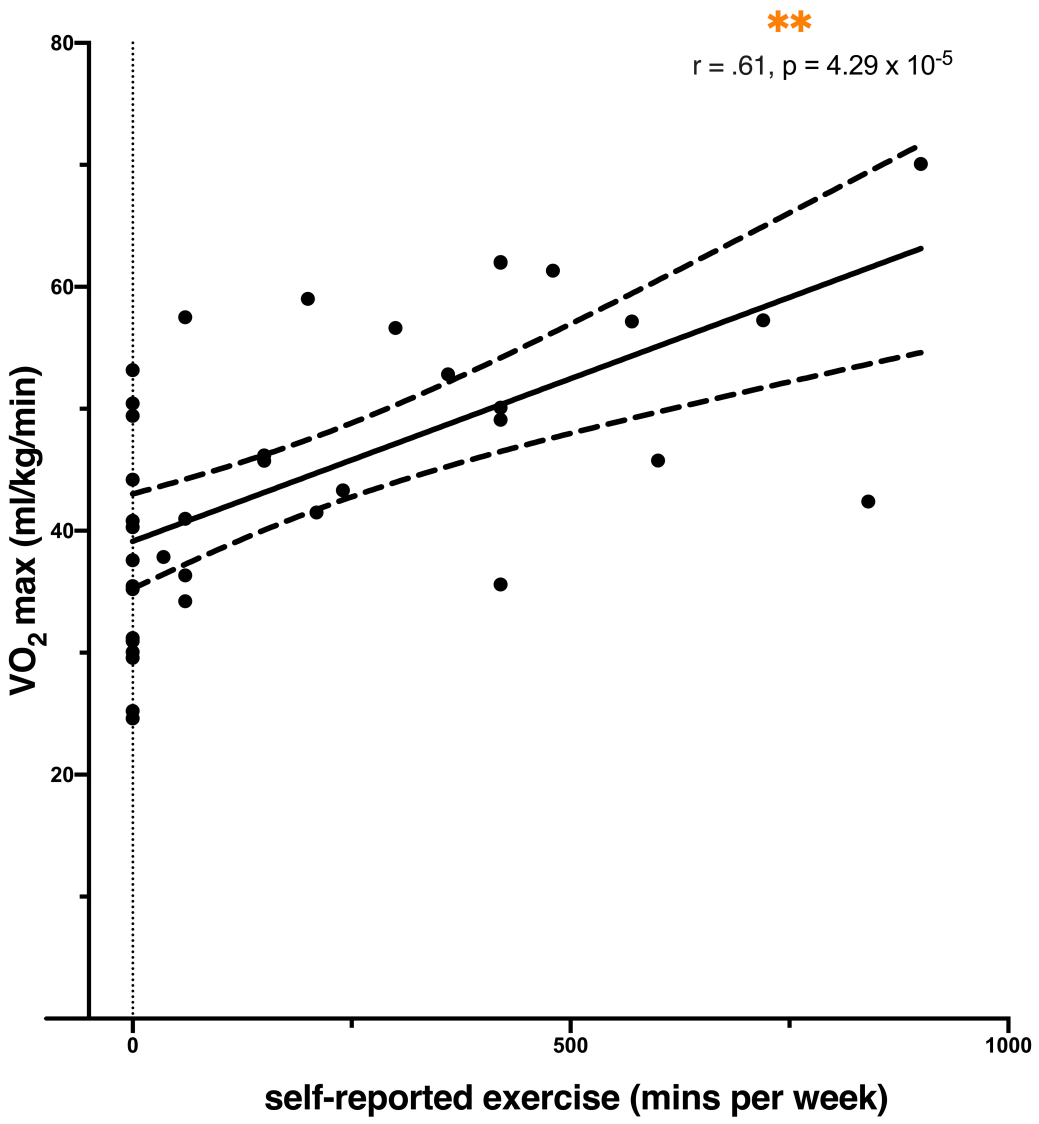

Figure S1. Association between self-reported exercise and VO_2max. There was a significant positive association observed between self-reported exercise and VO_2max (across PA groups). Circles represent individual scores, solid line shows regression line of best fit, dotted lines depict 95% confidence interval. VO_2max score is represented on the y-axis and exercise level (self-reported weekly minutes of exercise) is represented on the x-axis. ** $p < 5 \times 10^{-5}$.

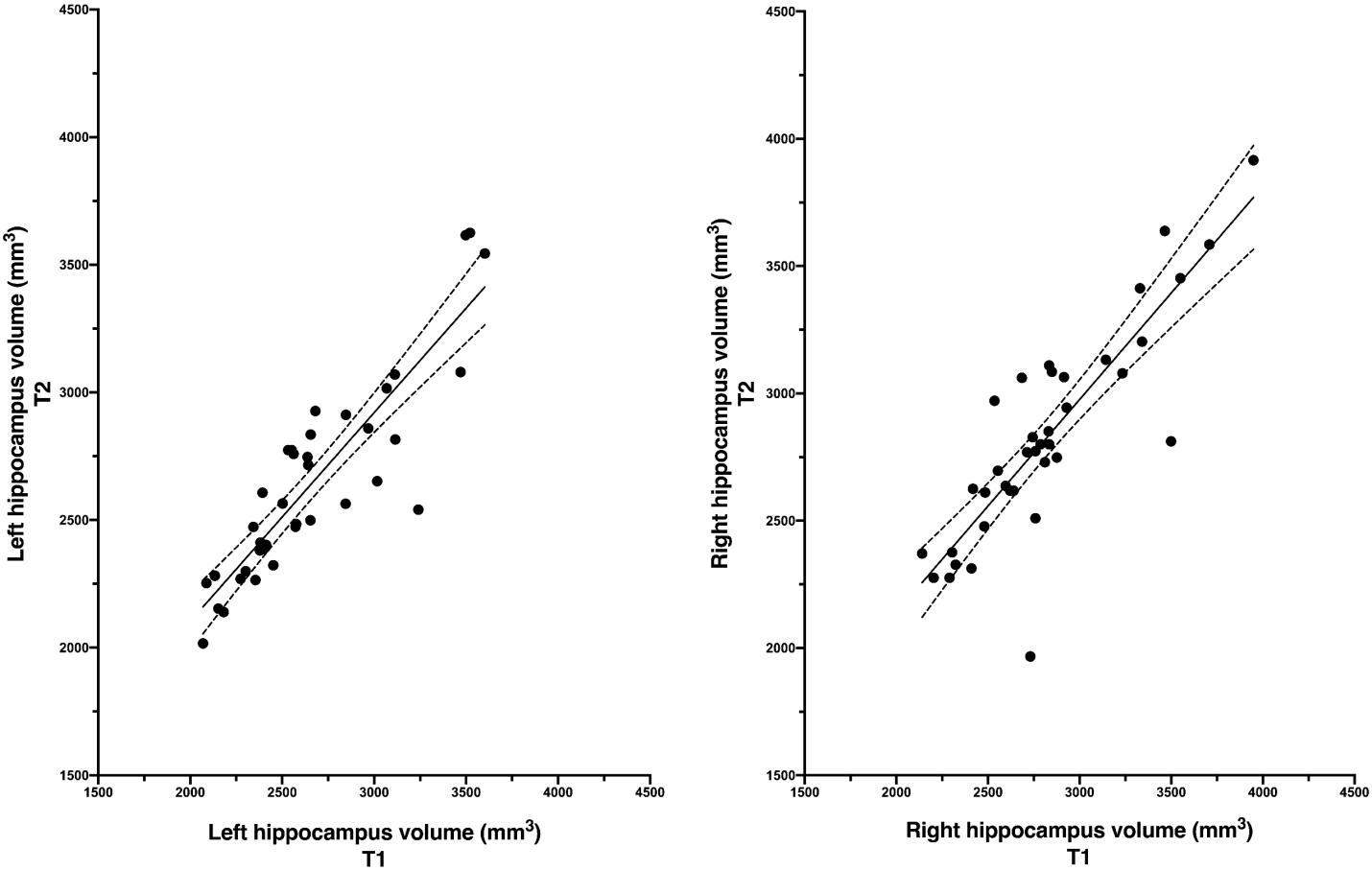

Figure S2. Intra-rater reliability of manual hippocampal tracing. To assess the intra-rater reliability of manual traced images, each subject's hippocampi were retraced from a structural T1-weighted structural image acquired one week after the experimental assessment. The intra-rater correlation coefficient between experimental and follow-up assessments was .89 for left hippocampus and .86 for right hippocampus. The left plot depicts the association between manually traced estimates of left hippocampus volume at experimental time point (x-axis) and manually traced estimates of left hippocampus volume at the follow-up time point (y-axis). The right plot depicts the association between manually traced estimates of right hippocampus volume at experimental time point (x-axis) and manually traced estimates of right hippocampus volume at the follow-up time point (y-axis). Circles represent individual scores, solid line shows regression line of best fit, dotted lines depict 95% confidence interval.

<u>Comparison of manually traced estimates of hippocampal volume between experimental and follow-up assessments</u>

To assess the intra-rater reliability of manual traced images, each subject's hippocampi were retraced from a structural T1-weighted structural image acquired one week after the

experimental assessment. To assess the consistency of hippocampal volume estimates across time points, separate 2 x 2 repeated-measures ANOVA was conducted on left and right hippocampal volume, with a within-subject factor of MRI Time Point (experimental, follow-up) and between-subject factor of PA group (high PA, low PA). In regard to left hippocampal volume, there was no significant main effect of MRI Time Point (experimental, follow-up; $F_{1,35}$ = 0.02, p = .90, η^2_p = .00), PA Group (high PA, low PA; $F_{1,35}$ = 2.47, p = .13, η^2_p = .07), or significant Time Point x PA Group interaction ($F_{1,35}$ = 0.00, p = .97, η^2_p = .00). Similarly, in regard to right hippocampal volume, there was no significant main effect of MRI Time Point (experimental, follow-up; $F_{1,35}$ = 1.48, p = .23, η^2_p = .04), PA Group (high PA, low PA; $F_{1,35}$ = 0.65, p = .43, η^2_p = .02), or significant Time Point x PA Group interaction ($F_{1,35}$ = 0.03, p = .86, η^2_p = .00). Overall, there were no evidence of significant changes to total hippocampal volume in the week period between MRI assessments, suggesting that intra-rater reliability estimates are unlikely to be biased by systematic changes to hippocampal volume across MRI assessment time points.

Analysis of NAA data quality

We assessed the consistency and reliability of NAA estimates between PA groups. The assumption of normality was violated for NAA FWHM, water FWHM, and E_{NAA} estimates. Therefore, these variables were compared between PA groups using non-parametric Mann-Whitney tests. There were no significant differences in NAA FWHM between high PA (M = 12.52, SD = 1.89) and low PA (M = 13.14, SD = 4.38) groups, U = 192, p = .74, r = .07. Similarly, there were no differences in water FWHM between high PA (M = 12.83, SD = 1.91) and low PA (M = 12.63, SD = 1.44) groups, U = 182, p = .95, r = .01. There was also no difference in E_{NAA} between PA groups (high PA M = 1.23, SD = 0.32; low PA M = 1.29, SD = 0.39), U = 168, p = .74, r = .07. Collectively, these results provide strong evidence of equivalent data quality and consistent NAA quantification between PA groups. One outlier

NAA:H_2O value was identified (Z-score > 3.29, low PA group) and removed from the analysis.

Tables 1-3: Regression statistics for hippocampus volume (DV) and VO_2max, Age, Gender, Total Brain Volume (IVs)

	Left hippocampus volume				
Measure	*B*	SE B	β	pr	p
Constant	-1285.72 (-2568.24, 74.63)	689.45			.13
VO_2max	24.37 (12.89, 32.79)	4.87	.68	.61	**.0002**
Age	-5.75 (-14.47, 6.14)	5.44	-.13	-.18	.30
Gender	316.27 (101.34, 529.34)	110.06	.41	.40	**.02**
Total BV	0.002 (0.002, 0.003)	0.0003	.74	.70	**5.91×10^{-6}**

	Right hippocampus volume				
Measure	*B*	SE B	β	pr	p
Constant	-897.93 (-2191.15, 327.85)	679.95			.33
VO_2max	21.29 (9.78, 29.61)	4.80	.60	.54	**.001**
Age	-0.56 (-14.44, 7.42)	5.53	-.024	-.03	.86
Gender	244.01 (10.98, 464.07)	118.69	.31	.29	.10
Total BV	0.002 (0.002, 0.003)	0.0003	.74	.67	**1.90×10^{-5}**

	Bilateral hippocampus volume				
Measure	*B*	SE B	β	pr	p
Constant	-2177.69 (-4727.08, 293.85)	1309.16			.20
VO_2max	45.78 (24.57, 61.10)	9.06	.65	.59	**.0003**
Age	-6.47 (-24.76, 14.61)	10.55	-.08	-.11	.54
Gender	561.10 (133.71, 975.04)	217.78	.37	.36	**.041**
Total BV	0.004 (0.003, 0.006)	0.0006	.75	.70	**6.83×10^{-6}**

B = unstandardised beta weight (values in brackets depict 95% bias corrected and accelerated confidence intervals); SE B = unstandardised beta standard error; β = standardised beta, pr = partial correlation (adjusting for other covariates in model), p = probability value (bolded values indicate $p < .05$); Total BV = total brain volume. Confidence intervals and standard errors based on 5000 bootstrap samples.

Table 4: Regression statistics for hippocampus NAA concentration (DV) and VO_2max, Age, Gender (IVs)

	Hippocampus NAA concentration				
Measure	*B*	SE B	β	pr	p
Constant	9.39 (5.14, 11.65)	1.53			**3.36×10^{-7}**
VO_2max	0.056 (0.029, 0.096)	0.016	.55	.52	**.002**
Age	0.029 (-0.010, 0.068)	0.020	.21	.24	.18
Gender	1.51 (0.86, 2.40)	0.39	.65	.57	**.0005**

B = unstandardised beta weight (values in brackets depict 95% bias corrected and accelerated confidence intervals); SE B = unstandardised beta standard error; β = standardised beta, pr = partial correlation (adjusting for other covariates in model), p = probability value (bolded values indicate p < .05). Confidence intervals and standard errors based on 5000 bootstrap samples.

Table 5: Regression statistics for face-cued word recall (DV) and VO_2max, Age (IVs)

	Face-cued word recall				
Measure	*B*	SE B	β	pr	p
Constant	58.05 (20.60, 94.77)	18.91			**.003**
VO_2max	0.19 (-0.36, 0.88)	0.31	.11	.12	.48
Age	-0.92 (-1.54, -0.26)	0.32	-.42	-.42	**.010**

Table 6-7: Regression statistics for pattern separation (DV) and VO_2max, Age (IVs)

	Separation bias metric				
Measure	*B*	SE B	β	pr	p
Constant	30.51 (-7.74, 68.87)	19.53			.085
VO_2max	0.58 (-0.06,1.19)	0.32	.30	.31	.070
Age	-0.48 (-0.97,0.37)	0.33	-.21	-.22	.20

Measure	Overall percentage correct				
	B	SE B	β	pr	p
Constant	66.86 (51.07, 83.79)	8.42			**7.4×10^{-11}**
VO_2max	0.30 (0.06,0.56)	0.13	0.38	.39	**.022**
Age	-0.13 (-0.32,0.17)	0.12	-0.14	-.15	.39

B = unstandardised beta weight (values in brackets depict 95% bias corrected and accelerated confidence intervals); SE B = unstandardised beta standard error; β = standardised beta, pr = partial correlation (adjusting for other covariates in model), p = probability value (bolded values indicate $p < .05$). Confidence intervals and standard errors based on 5000 bootstrap samples.