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Acute Cycling Exercise and Hippocampal Subfield Function and Microstructure in Healthy Older Adults

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Abstract

Aging is associated with deterioration in Dentate Gyrus (DG) and CA3, both crucial hippocampal subfields for age susceptible memory processes such as mnemonic discrimination (MD). Meanwhile, a single aerobic exercise session alters DG/CA3 function and neural activity in both rats and younger adults and can elicit short-term microstructural alterations in the hippocampus of older adults. However, our understanding of the effects of acute exercise on hippocampal subfield integrity via function and microstructure in older adults is limited. Thus, a within subject-design was employed to determine if 20-minutes of moderate to vigorous aerobic exercise alters bilateral hippocampal subfield function and microstructure using high-resolution functional magnetic resonance imaging (fMRI) during an MD task (n=35) and high angular resolution multi-shell diffusion imaging (n=31), in healthy older adults, compared to seated rest. Following the exercise condition, participants exhibited poorer MD performance, particularly when their perception of effort was higher. Exercise was also related to lower MD-related activity within the DG/CA3 but not CA1 subfield. Finally, after controlling for whole brain gray matter diffusion, exercise was associated with lower neurite density index (NDI) within the DG/CA3. However, exercise-related differences in DG/CA3 activity and NDI were not associated with differences in MD performance. Our results suggest moderate to vigorous aerobic exercise may temporarily inhibit MD performance, and suppress DG/CA3 MD-related activity and NDI, potentially through neuroinflammatory/glial processes. However, additional studies are needed to confirm whether these short-term changes in behavior and hippocampal subfield neurophysiology are beneficial and how they might relate to long-term exercise habits.

Keywords

NODDI; fMRI; Aging; Diffusion-Weighted Imaging; Pattern Separation

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

1 Introduction

Across the world, the number of older adults is expanding rapidly as advances in medicine, public health, and education prolong the human lifespan (Beard et al., 2016; World Health Organization (WHO), 2021). This extended lifespan is unfortunately accompanied by a growing number of individuals developing dementia and memory impairments (Geneva: World Health Organization, 2019). However, growing evidence suggests that modifiable lifestyle factors, such as exercise, may help delay or prevent the onset of cognitive impairment in older adults (Livingston et al., 2020). In particular, exercise training preserves memory and can preferentially protect age-susceptible and memory-critical structures such as the hippocampus (Voss, Soto, et al., 2019). However, although aerobic exercise training protects various memory networks and reduces age-related cognitive decline and dementia risk, the benefits appear to vary greatly across individuals, and several randomized controlled trials (RCTs) have failed to show positive effects (Barnes et al., 2013; Sink et al., 2015; Voss, Soto, et al., 2019). While RCTs are a gold standard for determining the effects of exercise interventions on the aging memory system, they have high costs concerning time and money, limiting their use (Hariton & Locascio, 2018). Additionally, inconsistencies and variability in previous RCTs may result from a lack of consistency in cognitive domains tested, a failure to tailor exercise protocols and track physiological adaptions and responses, and an inability to control for other lifestyle factors (sleep, diet, environmental enrichment, and socialization) (Voss, Soto, et al., 2019).

Notwithstanding, identifying the short-term behavioral and neurophysiological responses of a single (acute) aerobic exercise session on specific age-susceptible memory processes and structures can provide more nuanced insight into the relationship between exercise and memory. Within-subject acute exercise study designs generally occur over a shorter period of time and allow subjects to act as their own control, providing an opportunity to better attribute behavioral and neurophysiological changes specifically to exercise. Acute exercise adaptations may also predict outcomes for training programs and long-term physical activity habits (El-Sayes et al., 2019; Voss, Soto, et al., 2019). Therefore, characterizing acute exercise effects may help inform more individualized and optimized exercise interventions and health promotion guidelines. Thus, acute exercise interventions that specifically probe age-susceptible memory constructs and structures are crucial for better addressing how exercise affects the memory system of older adults.

Growing evidence indicates acute aerobic exercise can alter hippocampal integrity, function, and memory performance (Callow, Won, Alfini, et al., 2021; Callow, Pena, et al., 2022; El-Sayes et al., 2019; Loprinzi et al., 2021). However, these effects may depend on the duration, timing, and intensity of the exercise, the age of the participants, and the specific memory construct tested (Loprinzi et al., 2021). For example, a short bout of light to moderate-intensity aerobic exercise can lead to better mnemonic discrimination (MD) on highly similar stimuli in college-aged younger adults (Suwabe et al., 2017, 2018). Meanwhile 20 minutes of moderate-intensity exercise can preserve pre to post MD performance in healthy older adults compared to seated rest (Callow, Pena, et al., 2022). MD is a memory construct that engages the Dentate Gyrus (DG) and CA3 subfields of the hippocampus by placing a high demand on pattern separation (Stark et al., 2019; Yassa & Stark, 2011) and represents

a process by which individuals accurately discern between previously viewed (old) stimuli and newly presented but visually similar (lure) stimuli. Prior studies investigating acute exercise effects on MD have been predominantly limited to younger adults (Bernstein & Mcnally, 2019; Suwabe et al., 2017, 2018), but MD performance is of particular interest for understanding aging memory systems. This is because MD and the ability to behaviorally separate similar stimuli often declines earlier and more rapidly during aging compared to other cognitive processes, potentially due to declines in neurogenesis within the DG (Nakashiba et al., 2012; Sahay et al., 2011; Stark et al., 2015; Stark & Stark, 2017).

Various mechanisms may underlie age-related memory decline, such as increased oxidative stress and neuroinflammation, as well as alterations in plasticity, connectivity, excitability, and neurogenesis within hippocampal subfield circuitry (Bettio et al., 2017; Leal & Yassa, 2015). Research supports that wheel running-induced upregulation of neurogenesis within the DG directly improves MD-related performance in mice and rats (Creer et al., 2010; Vivar et al., 2016). Meanwhile, human studies in older adults suggest that improving fitness through exercise training may preserve hippocampal volume and memory performance (Firth et al., 2018; Frodl et al., 2019; Sexton et al., 2016). Understanding of short-term effects of acute exercise that may compound over time to elicit long-term adaptations in hippocampal subfield-dependent memory and structure in healthy older adults remains limited. Acute exercise effects on memory may be due to changes in arousal, hippocampal subfield neural activity, and synchrony, neurotransmitter and neurotrophic release, and hippocampal neurogenic inflammatory mediators (Basso & Suzuki, 2016; Packer & Hoffman-Goetz, 2015; Soya et al., 2007; Suwabe et al., 2018; Whitney et al., 2009). It is thus essential to employ sensitive and specific neurophysiological biomarkers to characterize acute exercise-related alterations to the integrity of the aging memory system.

MD-related DG/CA3 functional magnetic resonance imaging (fMRI) hyperactivity is associated with age- and neurodegeneration-related memory impairment in older adults and those with mild cognitive impairment (Yassa, Stark, et al., 2010; Yassa et al., 2011). Indeed, a drug-induced reduction of DG/CA3 hyperactivity has been associated with better MD performance, suggesting that hyperactivity reflects neural distress rather than adaptive compensation (Bakker et al., 2012). Animal studies have also shown that DG/CA3 hyperactivity may be due to a loss of inhibitory neurons within the CA3 (Leal & Yassa, 2015). However, the relationship between MD performance and MD-related DG/CA3 fMRI activity appears to be age-dependent (Riphagen et al., 2020). DG/CA3 hyperactivity represents enhanced network integrity in younger but network dysfunction in older adults.

In addition to MD-related hippocampal subfield activation, indices of hippocampal microstructure may also be valuable biomarkers for understanding acute exercise-related changes in age-susceptible memory circuitry. While diffusion-weighted imaging has predominantly been employed to ascertain the microstructural integrity of white matter tracts (Walhovd et al., 2014), improvements in multi-shell diffusion imaging sequences and modeling approaches now allow researchers to better probe gray matter integrity with more biophysically relevant measures, particularly within the hippocampus (Assaf, 2019; Schilling et al., 2018; Venkatesh et al., 2020; H. Zhang et al., 2012). For example, it has been reported that hippocampal microstructure may better predict mild cognitive impairment

and dementia than hippocampal volume (Fellgiebel & Yakushev, 2011; Kantarci et al., 2005) and is more closely related to age and hippocampal-dependent memory processes (Callow et al., 2020; Radhakrishnan et al., 2020; Venkatesh et al., 2020; Wolf et al., 2015; Yassa et al., 2011). Furthermore, short-term changes in hippocampal diffusion have been linked to learning (Blumenfeld-Katzir et al., 2011; Sagi, Tavor, Hofstetter, et al., 2012), as well as the expression of hippocampal brain derived neurotrophic factor (BDNF), synaptophysin, and glial related activity and morphological changes (Blumenfeld-Katzir et al., 2011; Sagi, Tavor, Hofstetter, et al., 2012; Yi et al., 2019). Thus, coupling hippocampal subfield fMRI activity and hippocampal microstructural diffusion in healthy older adults could provide independent and complimentary insight into the impacts of short-term perturbations and alterations to the hippocampal memory system in older adults.

Unfortunately, most of the evidence for the effects of acute exercise on memory-related circuits is limited to animal studies, behavioral measures, or samples of younger adults in humans. Animal studies have found that a single session of low to moderate-intensity wheel running increases hippocampal activity and promotes neurogenesis (Lee et al., 2003; Soya et al., 2007), while higher-intensity wheel running may upregulate hippocampal neuroinflammatory mediators that are neurogenic (Nogueira et al., 2019; Packer & Hoffman-Goetz, 2015; Pervaiz & Hoffman-Goetz, 2012; Whitney et al., 2009). Meanwhile, younger adults exhibited higher MD-related DG/CA3 activity following 10 minutes of light-intensity exercise (Suwabe et al., 2018). Furthermore, we recently reported that 20 minutes of moderate to vigorous aerobic exercise elevated whole hippocampal diffusion compared to seated rest in healthy older adults (Callow, Won, Alfini, et al., 2021). However, the effects of acute aerobic exercise on highly age-susceptible hippocampal function and microstructure in healthy older adults are currently unknown. Thus, this study aimed to determine how 20 minutes of moderate to vigorous aerobic exercise affects MD performance, MD-related DG/CA3 activity, and DG/CA3 microstructural integrity compared to seated rest, using a within-subject counterbalanced crossover design and high-resolution hippocampal subfield specific imaging approaches. We hypothesized that following the acute exercise session participants would perform better on the MD task, would exhibit reduced MD-related DG/CA3 activity (consistent better network function in older adults), and have elevated DG/CA3 extracellular diffusion (consistent with lower NDI and higher ODI and indicative of an upregulation of neurotrophic factors and neuroinflammatory mediators).

2 Materials and Methods

2.1 Subjects

Forty-one cognitively healthy and physically active older adults (ages, 60–89 years) were recruited from the local community to participate in the study in accordance with the Helsinki Declaration. Participants were excluded if they reported a history of stroke, diabetes, untreated high blood pressure, neurological disease, major psychiatric disorder, contraindications to undergoing an MRI scan (claustrophobia/ferromagnetic metal in body), and any contraindications to exercising on a bike. All participants completed a phone screening, baseline session, rest session, and an exercise session (order of Rest and Exercise sessions were counterbalanced across participants).

2.2 Baseline visit

Prior to the two experimental day visits participants attended a baseline visit in which they first provided written informed consent approved by the Institutional Review Board. Participants then completed the Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005), a 30 point questionnaire used to screen for cognitive impairment (participants had to score ≥ 26 to participate in the study). Next, participants completed a battery of questionnaires to determine health history, as well information about sleep habits (PSQI), physical activity (Stanford 7-day Physical Activity Recall questionnaire; (Sallis et al., 1985)), anxiety symptoms (Geriatric Anxiety Scale; (Segal et al., 2010)), and depression symptoms (Geriatric Depression Scale; (Yesavage et al., 1983)). Then, participants performed a submaximal stress test to determine baseline cardiorespiratory fitness levels. Finally, participants watched a video with standardized instructions and then performed Set 1 of the continuous MST to allow for familiarization with the task and to minimize practice effects during the following two experimental visits (Stark et al., 2015).

2.3 Submaximal Exercise Stress Test

Participants performed a submaximal stress test on a cycle ergometer (Corival, Lode, Netherlands) and respiratory gases were monitored via open-circuit spirometry (True One 2400 integrated metabolic system). A staged ramp protocol (Cress & Meyer, 2003) was employed where, following a two-minute warm-up at 25W, an initial 30W resistance was set and increased by 10W/min until termination criteria was reached. Throughout the test heart rate (Polar H9, Polar) and measures of ventilation including rate of oxygen (O2) consumption, rate of carbon dioxide (CO2) production, and the respiratory exchange ratio (RER; CO2 production/O2 consumption) were collected, while the ratings of perceived exertion (RPE; 6–20 scale administered with instructions consistent with (Borg 1982; Cook et al., 1997)) scale was used to monitor subjective effort every minute. Tests were terminated upon attainment of 85% of participant's age predicted maximal heart rate response (220-Age), participant's request, or observations of exercise contraindications.

2.4 Mnemonic Similarity Task

Participants completed the continuous version of the Mnemonic Similarity Task (MST) at 3 different time points. Participants completed the continuous version of the MST on the computer at the baseline visit and in the mock scanner and scanner during the two experimental visits. During each task, participants were shown 140 colored images of everyday objects, one at a time, for 2.4 seconds (.3s Interstimulus Interval) and then asked to indicate whether the item was an "old", "similar", or "new" image. The 140 items consisted of 80 new (foils), 30 similar (lures), and 30 old (repeat) images. Trial types were presented pseudo-randomly and separate images were used for each visit. Each participant completed Set 1 for practice at the baseline visit and an abbreviated version of Set 6 (30 total images) for practice in the mock scanner at the beginning of each experimental day to familiarize participants with the task. Then, each participant completed Set 2 and 3 in the scanner during their first experimental visit and Set 4 and 5 in the scanner during their second experimental visit. A total of 6 Sets were used, with each set being equivalent in terms of the mnemonic similarity of their lures. Specifically, each lure image varied in its degree of

similarity and was previously empirically ranked by assessing the false alarm rates (% old response) in a separate population (Lacy et al., 2011). These lures were then divided into 5 lure bins based on false alarm rates and each set was given an equal number of lures for each bin (Stark et al. 2013). Furthermore, as previously conducted by Suwabe et al. 2018, the lag order (how far apart lures or repeated images were displayed after they were initially displayed) was consistent across Sets and all lures and repeats occurred between 10–30 images following initial presentation to limit use of working memory and to keep a consistent difficulty of the task (Suwabe et al., 2018). The MST provides two primary behavioral measures. First, a traditional object recognition memory measure was calculated as rate of "Old" responses minus "Old" responses that were foils (Old | Target – Old | Foil) to account for response bias to the "Old button". Second, the lure discrimination index (LDI) was calculated, a measure of MD calculated as the rate of "Similar" responses to lures minus Similar responses to new objects (Similar | Lure – Similar | Foil) to control for response bias of the choice "Similar". LDI is a quantitative measure that operationally defines MD and is closely linked to DG/CA3 function (Stark et al., 2019; Yassa & Stark, 2011).

2.5 Exercise and Rest Conditions

Using a within-subject design, participants performed two experimental conditions (exercise and rest) on separate days (spaced 1 to 7 days apart) in counterbalanced order. Participants completed both experimental sessions at the Maryland Neuroimaging Center within a week of each other and completed the exercise and rest sessions in the room adjacent to the scanner to minimize time between completing conditions and initiating scanning. Due to scheduling restrictions, some of the participants performed the experimental conditions at slightly different times of day for each condition; however each participants two scans were obtained at most two hours apart of the same time of day. Before both conditions, participants were provided standardized instructions for the Borg 6–20 Ratings of Perceived Exertion (RPE) and Self Assessment Manikin (SAM) scale (Borg, 1982; Bradley & Lang, 1994). Additionally, on both days participants would again watch the standardized video of MST directions and would then practice another abbreviated version (30 images) of MST Set 6 using identical button boxes to those used in the scanner to provide familiarity and limit practice effects.

For the exercise condition, participants completed a continuous bout of cycling on a Monark cycle ergometer (Varberg, Sweden) located outside the MRI scanner. They were free to adjust the resistance of the bike while maintaining a cadence between (60–80 rpm) to achieve the target RPE. Participants performed a 5-minute warm-up at a self-selected pace, followed by a 20-minute bout of cycling at a target RPE of 13–15 on the Borg 6–20 RPE scale (corresponding to moderate-vigorous intensity and associated with the verbal anchor of "somewhat hard" to "hard"), and finished with a 5-minute cooldown. Heart Rate, RPE, and a subjective valence (pleasantness) and arousal measure via the SAM scale were collected every five minutes. They received water *ad libitum* during both conditions, and after the exercise condition, they were provided with a towel and clean and dry clothing for the scan. During the rest condition, participants were seated on the same cycle ergometer and asked to sit quietly for 30 minutes, while HR and RPE were measured every five minutes. Participants did not have access to cell phones, and excessive talking was discouraged

during both conditions. Following the cooldown, participants changed, had an opportunity to go to the bathroom, and then immediately entered the scanner room to prepare for scanning. Approximately 10–15 minutes elapsed from the end of each condition to the initiation of the first MRI scan.

2.6 MRI Acquisition

Immediately following the rest and exercise condition, participants were prepared for MRI scanning on a Siemens Prisma 3.0 Tesla MR scanner. A 32-channel head coil was used for radiofrequency transmission and reception, and foam padding was positioned within the head coil to minimize head movement. Furthermore, for each individual scan, the MRI operator was trained to specify the imaging prescriptions (brain coverage, slice orientation, etc.) as uniformly as possible across all participants, particularly within subjects, to minimize the variability. High-resolution T1-weighted anatomical images were acquired with the following sequence parameters: Magnetization Prepared Rapid Acquisition of Gradient Echo (MPRAGE), field-of-view (FOV) = 256 mm × 256 mm^2, voxel size = 0.8×0.8×0.8 mm, repetition time (TR) = 2400 ms, echo time (TE) = 2.32 ms, inversion time (TI) = 1060 ms, flip angle = 8°, and parallel acceleration factor=2 were used to achieve a scan duration = 6:36 min. Following the functional and diffusion scans, a high-resolution medial temporal lobe (MTL) T2-weighted fast spin echo scan, and then a whole brain T2-weighted scan with the same FOV and resolution settings were also collected for each session to improve hippocampal subfield segmentation and registrations for later analysis.

Following the anatomical T1 scan, the Mnemonic Similarity Task (MST) event-related data were acquired using the following sequence parameters; A Simultaneous Multi-Slice (SMS) echo planar imaging sequence with a multi-slice acceleration factor of 4 combined with a parallel imaging factor of 2, FOV = 210 mm × 210 mm^2, voxel size = 1.5×1.5×1.5 mm, TR/TE = 2000/32 ms, Bandwidth = 2298 Hz/Px, sequence duration = 6 min 40 sec per run (2 runs per scan).

Then, high angular resolution diffusion-weighted images were acquired with a twice-refocused spin-echo single-shot sequence using Multi-Band Echo Planar Imaging at an acceleration factor of 4. The protocol included two sets of 64 non-collinear diffusion-weighted acquisitions collected, using phase-coding in the AP and PA directions, respectively. Each set included 2 diffusion weightings (b = 1500, and 3000 s/mm^2) and 4 single T2-weighted b = 0 s/mm^2 acquisition (TR/TE = 3500/102 ms, 1.7×1.7×1.7 voxel size, flip angle = 90°, and a bandwidth of 1698 Hz/Px).

2.7 Functional Image Processing

Functional analysis results included in this manuscript come from preprocessing performed using fMRIPrep 21.0.2 (Esteban et al., 2019), which is based on Nipype 1.6.1 (Gorgolewski et al., 2011). In short, functional images were corrected for susceptibility induced distortions using FSL's topup function. Anatomical images were then skull stripped and a subject specific anatomical template was created with freesrufer's mri_robust_template function using each subject's 2 T1-weighted and 2 T2-weighted scans. Next a one step volume based registration was calculated between T1 weighted images, subject template space, and our

target template in MNI space (ICBM 152 Nonlinear Asymmetrical template version 2009c 1mm^3 resolution) and was then executed with ANTS (Avants et al., 2008). For all distortion corrected functional images the following preprocessing steps were then performed: skull stripping, head motion parameter estimation with FSL's mcflirt, slice time correction with AFNI's 3dTshift, coregistration to subject T1 with bbregister, and frame wise displacement, global signal, and physiological regressor estimation based on freesurfer segmentation masks. Within each BOLD time series, volumes before, during, and after a frame wise displacement > 0.5mm were identified and flagged for statistical removal via dummy coding. BOLD time-series were resampled into standard space, generating preprocessed BOLD runs in MNI152NLin2009cAsym space. A reference volume and its skull-stripped version were generated using a custom methodology of fMRIPrep. All resamplings were performed with a single interpolation step by composing all pertinent transformations (i.e., head-motion transform matrices, susceptibility distortion correction when available, and co-registrations to anatomical and output spaces) with ANTS.

2.8 Functional Image Analysis

MD-related activity was first assessed within the hippocampal region of interest as described below and similarly to previous papers (Klippenstein et al., 2020; Suwabe et al., 2018). Specifically, analyses were limited to an anatomical mask of the bilateral hippocampus (>40% probability in FSL's Harvard-Oxford subcortical atlas). First level analysis was conducted for each subject and time point with behavioral vectors (i.e., correct or incorrect identification of targets, foils, or lures) using a deconvolution approach based on multiple linear regression (3dDeconvolve). Deconvolution of the hemodynamic response was achieved with tent functions covering stimulus onset to 16 s after onset with 9 estimator functions across the time window. Motion parameters and global signal regressors for CSF and WM were further entered into the model to suppress their effect on task-related parameters. Statistical fit coefficients were estimated from the regression analysis and represented differences in activity between trial types and baseline (response of new for novel foil) at a given time point in a voxel. The sum of the fit coefficient taken over the expected hemodynamic response (3–11 s after trial onset) was used for the model's estimate of the relative response to each trial type.

Next, group-level analyses were performed on all scans to determine voxels within the hippocampus that were sensitive to MD by using a linear mixed effects model (3dLME). Participant's MD-related activity (i.e., activity modulated by our MD-related contrast of interest (correct lure identification vs incorrect "old" response to a lure) irrespective of exercise or rest condition) was thresholded at $P<.05$, with a cluster corrected threshold of 20 voxels to create a mask of MD active voxels within the hippocampus. Note, our goal here is not to determine whether reliable activity, that survives a full correction for multiple comparisons exists for this contrast within the hippocampus as other papers have done as much. Our goal is to first identify task-relevant voxels (or remove task-irrelevant voxels) at the group level and determine whether these are altered by exercise. Thus, we intentionally use a more liberal threshold in this initial pass to reduce voxel selection biases and set our final alpha at the end, when examining the effects of exercise. With these task-selective voxels, identified, we next collapsed any sub-clusters that fell within the same

hippocampal subfield ROIs (bilateral DG/CA3, CA1, Subiculum), based on a previously used hippocampal subfield template (Stark et al., 2021). This resulted in a bilateral DG/CA3 ROI (derived from 5 clusters) and a bilateral CA1 ROI (derived from 7 clusters), see Supplementary Table 1. The average BOLD response within these two ROIs was then extracted for all scans for second-level analysis to determine the difference between exercise and rest conditions on MD-related hippocampal subfield activity. This approach and keeping the hippocampal subfield ROIs as bilateral helped improve SNR and reduce the number of multiple comparisons as we had no a priori reason to separate left from right for this task. fMRI processing scripts for this analysis can be found in the following GitHub repository (https://github.com/CallowBrainProject/Acute-Cycling-Hippocampal-Subfields).

2.9 Diffusion-Weighted Image Processing

Diffusion-weighted images were processed using MRtrix3 scripts (Tournier et al., 2019). First, physiological noise due to water molecules' thermal motion was removed, followed by eliminating Gibbs ringing artifacts, bias field correction, and brain extraction. Images were then corrected for b0 inhomogeneities and eddy currents. We then used the AMICO software (Daducci et al., 2015) to fit the Neurite Orientation Dispersion Density Imaging (NODDI) model for each subject. NODDI is arguably the most popular and widely used multi-compartment modeling technique. It attempts to parameterize the diffusion signal into three microstructural compartments: intracellular diffusion (restricted diffusion within axons and dendrites), extracellular diffusion (hindered diffusion outside of axons and dendrites, such as within cell bodies, glial cells, extracellular matrices, and vascular structures)), and isotropic diffusion (i.e., free water). More specifically, the NODDI model provides three primary scalar values: the neurite density index (NDI; the proportion of intraneurite diffusion relative to extraneurite diffusion), the orientation dispersion index (ODI; 0 for perfectly parallel and aligned neurites and 1 for completely isotropic neurites), and the volume fraction of isotropic diffusion (ISO; the proportion of free water such as CSF). Thus, higher NDI indicates greater intracellular than extracellular diffusion and, thus, greater neural density. Meanwhile, higher ODI in gray matter may indicate a more complex dendritic and synaptic architecture. Importantly, NODDI has excellent test-retest reliability, and unlike DTI, accounts for partial volume effects and has undergone histological validation in animals and humans (Grussu et al., 2017; Kamiya et al., 2020; McCunn et al., 2019). Next, a subject specific anatomical template was created using T1w-reference map after registration of 2 T1w and 2 T2w images (after INU-correction) using mri_robust_template (FreeSurfer 6.0.1, Reuter, Rosas, and Fischl 2010). A nonlinear warp was calculated with the ANTS program (Avants et al., 2008) between the b0 diffusion image and each subjects T1 and then anatomical template image. Finally, each NODDI map was then registered to subject specific T1 space using the previously calculated warps in one step.

2.10 Hippocampal and Subfield ROI analysis

Automated hippocampal subregion segmentation was performed for each participant's T1 and high resolution hippocampal T2 scan using the Automatic Segmentation of Hippocampal Subfields (ASHS) software (Yushkevich et al., 2015). Using ASHS, hippocampal Subfield ROIs for the Dentate Gyrus and CA3 (DG/CA3), CA1, and Subiculum were created for each subject in native T1 space. To further threshold and make

sure that diffusion values were extracted from hippocampal gray matter, a whole brain gray matter mask was created using FSL's FAST tissue segmentation algorithm and hippocampal and hippocampal subfields ROI were further thresholded based on only keeping voxels that were present within the gray matter mask (Y. Zhang et al., 2001). These subfield ROIs and gray matter mask segmentations were then warped into subject template space using previously calculated nonlinear warps with ANTS. At which point, both ODI and NDI values were extracted from the whole brain gray matter mask, bilateral DG/CA3, CA1, and whole hippocampal ROIs.

2.11 Statistical Analyses

Group level statistical analyses were performed with R (R Core Team, 2018). First, we ran paired t-tests to determine whether there were statistically significant differences in measures of effort between the final 10 minutes of the exercise condition compared to the final 10 minutes of the rest condition. Additionally, to determine the independent effect of exercise on hippocampal subfield function and microstructure we employed a linear mixed effects model with Condition (exercise vs rest), Order (which condition was performed first), Condition by Order interaction, days apart (number of days between two experimental visits), and age as fixed effects and subject as a random effect for all additional behavioral and neuroimaging analysis. Using likelihood ratio tests we did not find any significant improvements in our model with the inclusion of additional random slopes and therefore did not include them in our analysis to reduce the likelihood of overfitting given our limited number of timepoints for each subject. Linear mixed effects were performed using maximum likelihood estimation with the lme4 R package (Bates et al., 2015). We further tested whether there was a significant fixed effect of Condition on overall MD performance, followed by testing whether there was an interactive effect of Lure Similarity Bins (1 most similar – 5 least similar) on the effect of Condition. Next, we identified MD-related voxels within the hippocampal subfields and tested for Condition's independent effect on DG/CA3 and CA1 activity. We then tested for a main effect of Condition on extracted ODI and NDI values from the hippocampus and DG/CA3 and CA1, while again controlling for the Order of the Conditions, a Condition by Order interaction effect, the number of days between Conditions, and the age of participant. Furthermore, to determine specificity to the subfield regions, we then added whole brain gray matter ODI and NDI values as an additional covariate in the diffusion analysis. Finally, partial correlations controlling for age, Order and number of days apart were performed to determine relationships between differences in HR, RPE, valence, and arousal with differences in MD performance and neuroimaging measures, as well as to determine if their were significant relationships between MD performance and neuroimaging measures. Statistical significance was set based on a two-tailed alpha $<.05$ and Bonferroni family wise error rate (FWER) correction within each behavioral and neuroimaging analysis.

3 Results

3.1 Participants

Of the 41 participants who completed all study protocols, one participant (female) was excluded from the behavioral analysis due to exceptionally poor performance (below 0.5

for object recognition) and one more (female) was excluded due to failure to use the "similar" response button at least ten times. This criteria has been similarly employed to remove participants who failed to follow task instructions (Callow, Pena, et al., 2022). An additional three subjects (females) were excluded due to MRI goggles that fogged and prevented completion of the fMRI task at one of the two time points. An additional one subject (male) was removed from the fMRI and diffusion analysis due to claustrophobia and failure to complete the scanning sessions. Furthermore, 9 subjects' diffusion scans were excluded from the analysis due to protocol sequence errors. The final samples were 36 participants for the behavioral analysis, 35 for fMRI analysis, and 31 for the diffusion analysis. Participants were cognitively healthy (MoCA ≥ 26), had an average age of 67.1 years and were predominantly female (30 Female, 6 Male) (see Table 1).

3.2 Experimental Check

As expected, HR ($t(35)=15.9$, $p<.001$), RPE ($t(35)=35.9$, $p<.001$), and arousal ($t(35)=3.86$, $p<.001$) were significantly higher during the exercise condition compared to the seated rest condition. Valence (pleasantness) was not found to be significantly different between the two conditions ($t(35) = 1.3$, $p = .195$) (see Table 2).

3.3 Behavioral Performance

While controlling for Order of Condition, Condition by Order interactions, age, and days between the two visits, participants had significantly worse LDI scores after exercise compared to after seated rest ($F(1,35) = 4.73$, $\eta^2=.12$, $p = .036$). Additionally, age was negatively related to LDI scores ($F(1,35) = 5.35$, $p = .027$) and there was a significant Condition by Order interaction ($F(1,35) = 12.15$, $p = .001$), in which participants who performed exercise first had significantly poorer LDI scores following exercise versus rest ($t(34) = -3.79$, $p=.005$), compared to no significant difference for those completing the rest condition first ($t(38) = .93$, $p = .360$), see Supplementary Figure 1. More specifically, when controlling for similar covariates participants responded old when the object was a lure (Old | Lure) at a significantly higher rate than following the rest condition ($F (1, 35) = 4.39$, $\eta^2=.09$, $p = .043$). There was no significant Condition by Lure Similarity Bin interaction effect for LDI scores ($F (4, 324) = 1.05$, $p = .380$). There was no significant difference between Conditions for traditional object recognition performance ($F(1,35) = 3.53$, $\eta^2=.09$, $p = .068$), and no significant relationship with age or a significant Order by Condition interaction effect, see Figure 1 and Supplementary Table 2. Differences in valence ($r(33) = .02$, $p=.892$), arousal ($r(33) = .11$, $p=.559$), and HR ($r(33) = -.05$, $p=.754$) were not associated with differences in LDI. However, differences in RPE between conditions were negatively associated with differences in LDI ($r(33) = -.40$, $p = .022$; uncorrected). Furthermore, a post hoc analysis indicated that greater differences in RPE were related to a higher likelihood of a false alarm when viewing a lure after exercise compared to after rest ($r = .52$, $p = .002$).

3.4 MD related fMRI activity

While controlling for Order of the conditions, Order by Condition interaction, age, and days between the visits, MD-related DG/CA3 activation was significantly lower in the exercise condition compared to the rest condition ($F(1,33) = 9.02$, $\eta^2=.21$, $p = .005$). There was

also a significant positive relationship between age and DG/CA3 activity, but not for other covariates. There was no significant difference in MD-related CA1 activity between the exercise and rest conditions ($F(1,33) = 0.06$, $\eta^2<.01$, $p = .806$) or for any other covariates, see Figure 2 and Supplementary Table 2.

3.5 Subfield NODDI analysis

Controlling for Condition Order, Order by Condition interaction, age, and days between visits, whole hippocampal ODI was significantly higher ($F(1,31) = 6.44$, $p = .016$), while NDI was lower following exercise compared to rest ($F(1,31) = 8.93$, $p = .005$). Additionally, DG/CA3 ODI was significantly higher ($F(1,31) = 6.67$, $p = .015$), and NDI was significantly lower following exercise compared to rest ($F(1,31) = 8.52$, $p = .006$). Finally, CA1 ODI ($F(1,31) = 3.60$, $p = .067$) and NDI ($F(1,31) = 4.59$, $p = .040$) and Subiculum ODI ($F(1,31) = 4.48$, $p = .041$) and NDI ($F(1,31) = 4.56$, $p = .042$) were not significantly different between exercise and rest after accounting for multiple comparisons.

However, we also found that exercise was related to higher global gray matter ODI ($F(1,31) = 38.70$, $\eta^2=.56$, $p <.001$) but was not associated with significant differences in global gray matter NDI ($F(1,31) = 2.19$, $\eta^2=.07$, $p = .149$). Thus, we re-ran the analysis to determine whether exercise-related differences in hippocampal NDI and ODI were specific to the DG/CA3 or were driven by global differences in these metrics while controlling for whole brain NDI and ODI values. Following the addition of global diffusion to the model, we found that DG/CA3 ODI was no longer significantly different between the two conditions ($F(1, 31) = 0.55$, $\eta^2=.01$, $p = .464$), but exercise-related suppression of DG/CA3 NDI remained robust ($F(1,31) = 7.10$, $\eta^2=.18$, $p = .012$). Meanwhile, neither CA1 ODI ($F(1,31) = 5.32$, $\eta^2=.11$, $p = .030$) and NDI ($F(1,31) = 2.62$, $\eta^2=.08$, $p = .116$), or Subiculum ODI ($F(1,31) = 0.15$, $\eta^2<.01$, $p = .700$) and NDI ($F(1,31) = 2.63$, $\eta^2=.08$, $p = .115$) differences between conditions were significant following correction for global diffusion and multiple comparisons. Furthermore, no covariate fixed effects were significantly related to our hippocampal subfield diffusion measures, see Supplementary Table 2. This suggests that the effects of acute exercise on DG/CA3 ODI may be attributed to a more global effect of exercise on gray matter ODI, while the exercise-related effects on NDI may be more specific to the DG/CA3, see Table 3.

3.6 Exercise-Related Behavioral and Hippocampal Subfield Neuroimaging Associations

Differences in both DG/CA3 ($r(33) = -.19$, $p = .354$) and CA1 ($r(33) = -.24$, $p = .232$) MD-related activation were not associated with differences in LDI performance. Additionally, differences in DG/CA3 ODI ($r(24) = -.09$, $p = .653$) and NDI ($r(24) = -.17$, $p = .418$), CA1 ODI ($r(24) = -.12$, $p = .57$) and NDI ($r(24) = -.05$, $p = .801$), and Subiculum ODI ($r(24) = -.17$, $p = .409$) and NDI ($r(24) = -.36$, $p = .071$), were not associated with differences in LDI performance. Finally, differences in valence, HR, RPE, and arousal were not related to differences in DG/CA3 MD related activity, or with differences in DG/CA3 ODI or NDI measures (all $p > .05$).

4 Discussion

Our results are the first to show that an acute 20-minute bout of moderate to vigorous intensity aerobic exercise can alter DG/CA3 specific function, activity, and microstructural diffusion in healthy older adults. More specifically, we found that moderate to vigorous acute exercise led to lower MD performance, and a higher false alarm rate. Additionally, MD-related DG/CA3 activation, but not CA1 activation, was lower following exercise than seated rest. Finally, exercise was associated with greater extracellular diffusion within the DG/CA3, but not CA1 or Subiculum. These findings suggest that an acute bout of moderate to vigorous intensity aerobic exercise can lead to short-term alterations in DG/CA3 function and microstructure in healthy older adults, indicating that higher-intensity acute exercise may elicit neurophysiological perturbations to age-susceptible hippocampal networks.

Acute Exercise on Mnemonic Discrimination

We report moderate to vigorous aerobic exercise may suppress MD performance in healthy older adults. Furthermore, we found a higher response rate of false alarms to lures (responding ‘old’ when presented with a dissimilar lure image) following the exercise condition and that poorer MD and higher false alarm rates were related to a higher subjective perception of effort during the exercise versus rest condition. We also report a significant Order-by-Condition effect in which participants performing the exercise condition first saw significantly lower MD performance following the exercise condition, but not for those performing the rest condition first. Accounting for this significant interactive effect and controlling for the main effect of Order, we report that, on average, participants had lower MD performance following exercise than rest.

Several smaller studies in younger adults show that acute exercise improves MD performance. For example, Suwabe et al. (2017 & 2018) report that 10 minutes of light and moderate-intensity aerobic exercise in college-aged adults was associated with better MD on highly similar lures. We have recently reported that moderate-intensity exercise in active older adults was associated with reduced MD interference seen from pre to post-seated rest; however, MD did not specifically improve from pre to post-exercise (Callow, Pena, et al., 2022). While our current finding of reduced MD performance was not in the direction we hypothesized or reported in Suwabe et al. (2017 & 2018), there are several reasons why a longer bout of acute moderate to vigorous intensity exercise in healthy older adults may have led to lower MD performance.

Notably, the effect of acute exercise on hippocampal memory depends on the timing, length, and intensity of the exercise bout and the cognitive task tested (Chang et al., 2012; Loprinzi et al., 2019, 2021; Marchant et al., 2020; Sng et al., 2018). Although moderate to vigorous intensity acute aerobic exercise has generally been shown to provide benefits for cognition and executive function in older adults (Chang et al., 2012; Moreau & Chou, 2019), the effects of acute exercise for hippocampal-specific MD and general memory function are far less established (Callow, Pena, et al., 2022; Etnier et al., 2021; Griebler et al., 2022). In fact, several studies suggest that higher-intensity exercise may be stressful, elevating cortisol and lactate levels and specifically interfering with hippocampal-dependent memory (Basso et al., 2015; Marchant et al., 2020; Soya et al., 2007). Consistent with this argument, our results

suggest that individuals whose effort was perceived as a greater proportion of their maximal capacity saw a greater decline in MD and a higher false alarm rate following exercise compared to rest (although this relation for MD performance did not survive correction for multiple comparisons).

Importantly, in the current study, participants performed a time-restricted continuous version of the MST in the MRI scanner, exercised at a slightly higher percentage of age-predicted maximum heart rate, and only completed the task post-intervention. While the continuous version of the MST shows good consistency with the originally developed time-unrestricted study test version (Stark et al., 2015), the time-restricted and forced choice nature of the continuous MST performed in the scanner may relate to poorer performance, particularly for older adults (Stark et al., 2015). Specifically, we found that acute aerobic exercise appears to result in poorer MD, particularly for the participants performing the exercise condition before their first time completing the MST in the scanner. This might suggest that our reported effects of acute exercise on MD are specific to less familiar, potentially more stressful environments, as this was many participants first time undergoing MRI scanning of the brain. As we report in this sample, a shift to poorer MD is generally seen with aging (Stark et al., 2015) and is associated with compensation or dysfunction within the hippocampal circuitry (Leal & Yassa, 2015; Yassa et al., 2011). In our older adults sample, we also report that age was negatively associated with MD performance, but not object recognition. However, acute exercise could also facilitate a compensatory mechanism in older adults when performing such a time-restricted task and within an unfamiliar environment (such as an MRI scanner) by promoting more efficient encoding and recall of the “gist” of viewed objects instead of the greater detail needed to distinguish between old and new yet similar objects accurately. Notably, the acute exercise-related MD suppression we observed does not indicate that higher-intensity exercise will lead to adverse long-term outcomes for hippocampal memory function. As a recent study found that 12 weeks of higher-intensity exercise training in older adults led to greater benefits for MD performance than lighter-intensity exercise and a control condition (Kovacevic et al., 2019). This suggests that short-term perturbations to MD following a single moderate to vigorous aerobic exercise session may stimulate network adaptations that improve MD and do not indicate longer-term network dysfunction. However, additional studies that pair acute exercise and exercise training interventions are needed to determine how short-term perturbations to MD may relate to longer-term behavioral trajectories (Voss, Weng, et al., 2019).

Acute Exercise and MD-related activity

Our findings suggest that 20 minutes of moderate to vigorous exercise in healthy older adults is associated with lower MD-related activity within the DG/CA3, but not CA1. Meanwhile, Suwabe et al. (2018), report that 10 minutes of very light-intensity aerobic exercise in 16 college-aged adults was associated with elevated MD-related hippocampal subfield activity, including in the DG/CA3 (Suwabe et al., 2018). However, similar to our analysis, they did not find differences in hippocampal subfield activity associated with differences in behavioral MD performance. This suggests that exercise-related DG/CA3 activity differences may not be sensitive or specific to short-term differences in MD

performance. Instead, Suwabe and colleagues argue that light-intensity exercise modulated MD-related activity via increased arousal and cholinergic neurotransmission, which may help facilitate hippocampal networks in proper memory storage and recall (Hasselmo et al., 1995). However, while we did find exercise was associated with a higher level of subjective arousal via the SAM, differences in arousal were not associated with differences in behavioral performance or MD-related DG/CA3 activation, indicating these exercise-related differences are likely due to different underlying factors.

Lower-intensity acute exercise has been shown to elicit higher DG/CA3 activity in rats compared to more stressful moderate and vigorous intensity exercise (Soya et al., 2007) and Suwabe et al. (2018), showed that acute low-intensity exercise elevated MD-related hippocampal activity in younger adults (Suwabe et al., 2018). However, we report moderate to vigorous exercise suppressed MD-related DG/CA3 activity in healthy older adults. Interestingly, in younger adults, MD-related DG/CA3 hyperactivity is associated with better hippocampal function and integrity (Riphagen et al., 2020), while in older adults, DG/CA3 hyperactivity is associated with poor hippocampal function and integrity (Bakker et al., 2012; Riphagen et al., 2020; Yassa, Stark, et al., 2010). In fact, hippocampal hyperactivity is observed in conditions of elevated risk for Alzheimer's disease (Putcha et al., 2011; Yassa & Stark, 2011), and a previous clinical trial has shown that lowering MD-related DG/CA3 activity with levetiracetam in older patients with mild cognitive impairment can improve cognitive performance compared to those receiving a placebo (Bakker et al., 2012). This MD-related DG/CA3 hyperactivity is also associated with poorer hippocampal function in non-demented healthy older adults (Yassa, Lacy, et al., 2010) and is linked to a loss of inhibitory neurons and an inability of these CA3 neurons to encode new information in aging animals (Leal & Yassa, 2015). We also report that DG/CA3 but not CA1 MD-related activity was positively associated with age in our older participants. Therefore, given that DG/CA3 hyperactivity indicates hippocampal network distress, our finding of acute exercise-related reduction of MD-related DG/CA3 activity in healthy older adults suggests short-term therapeutic and beneficial effects for hippocampal subfield network function. Unfortunately, no previous research has determined exercise training-related effects on MD-related DG/CA3 activity in healthy older adults. Thus, additional studies will be needed to confirm and determine the long-term nature of these acute exercise-related changes in DG/CA3 activity.

Acute Exercise and DG/CA3 Microstructure

In addition to acute exercise-related functional and behavioral changes, our study provides new evidence for acute exercise-related changes to hippocampal subfield microstructure using an advanced high angular resolution diffusion imaging technique. Specifically, we found moderate to vigorous aerobic exercise was related to higher ODI (greater neurite dispersion) and lower NDI (higher extracellular diffusion) in the hippocampus. Importantly, we found a global effect of acute exercise on whole brain gray matter ODI, but not NDI. And after controlling for global diffusion values, only the effect of acute exercise on hippocampal NDI remained significant. Furthermore, the effect of acute exercise on hippocampal NDI was specific to the DG/CA3, but not the CA1 or Subiculum. This suggests acute exercise may lead to elevated extracellular water diffusion, particularly within the

DG/CA3. Indeed, we have previously reported a similar effect of acute exercise leading to high diffusion and dispersion within the whole hippocampus in healthy older adults (Callow, Purcell, et al., 2022). However, the current study uses high-resolution multi-shell diffusion scans that allow for the use of the NODDI model, which better accounts for partial volume effects and is critical for accurately ascertaining microstructural differences in the aging hippocampal structure (Henf et al., 2018). Our analysis approach also allowed us to control for whole brain global gray matter diffusion metrics and better attribute these microstructural effects of acute exercise to the DG/CA3 structure. The current results largely replicate those we have previously reported and further support the interpretation that acute exercise leads to higher diffusion of water within the hippocampal gray matter. Our use of high-resolution multi-shell diffusion scans and the NODDI model provide additional confidence that this increased extracellular diffusion is not associated with cerebrospinal fluid or free water and is independent of more global differences in gray matter diffusion. Furthermore, the higher resolution of our diffusion scan (1.7mm isotropic) and our hippocampal-specific structural scans allowed us to further delineate these microstructural effects to the DG/CA3 subfield specifically. This is particularly noteworthy given that previous animal research indicates that exercise-related benefits for the hippocampus may be specific to the DG/CA3 structure due to its importance for neurogenesis (Bekinschtein et al., 2011; Creer et al., 2010; Pereira et al., 2007; Voss, Soto, et al., 2019).

We have previously shown that cardiorespiratory fitness and exercise training are related to mean diffusion and NDI in the hippocampus of healthy younger adults and cortical regions in individuals diagnosed with MCI, respectively (Callow, Purcell, et al., 2022; Callow, Won, Pena, et al., 2021). Furthermore, Kleemeyer et al. (2016) found that exercise training-related fitness changes were associated with decreased hippocampal mean diffusion via increased neural density (Kleemeyer et al., 2016). Meanwhile, lower DG/CA3 NDI mediates age-related decrements in episodic memory performance (Radhakrishnan et al., 2020). DG/CA3 extracellular diffusion can also distinguish between healthy individuals and those with MCI and AD and is associated with neurofilament light chain, a plasma biomarker for neuroaxonal damage (Shahid et al., 2022). Greater extracellular diffusion and dispersion are also associated with elevated glial cell count, glial activity, and neuroinflammation within the hippocampus (Radhakrishnan et al., 2022; Sone et al., 2020; Yi et al., 2019). While this novel diffusion imaging approach allows for greater spatial resolution and provides more biophysically relevant information about hippocampal subfield microstructure than our previous study (Callow, Won, Alfini, et al., 2021), it is still not possible in humans to determine the effects of acute exercise on neurophysiological changes at the cellular level. However, given the nature of the intervention and the time scale of these effects, they are unlikely to result from previously reported neurogenesis-mediated benefits of exercise (Creer et al., 2010; Sahay et al., 2011).

Rather, our finding of acute exercise-related elevation of extracellular diffusion in the DG/CA3 is more likely to reflect several underlying neurophysiological changes that may prove to be neurogenic through repeated bouts. For example, several studies have found that short-term learning, on a similar time scale as a single session of acute exercise, is associated with diffusion changes in the hippocampus that relate to glial activity, remodeling, and promotion of neurotrophic and inflammatory factors (Blumenfeld-Katzir

et al., 2011; Sagi, Tavor, & Assaf, 2012; Tavor et al., 2020). Meanwhile, higher-intensity wheel running is also associated with acutely increased hippocampal neuroinflammatory and neurogenic mediators (Basso & Suzuki, 2016; Ferris et al., 2007; Nogueira et al., 2019; Packer & Hoffman-Goetz, 2015; Pervaiz & Hoffman-Goetz, 2012). While chronic levels of neuroinflammation are generally maladaptive and associated with reductions in hippocampal integrity and neurogenesis, the short-term elevation of hippocampal neuroinflammatory mediators promotes neurotrophic factor expression (Belarbi & Rosi, 2013; Fan & Pang, 2017; Pervaiz & Hoffman-Goetz, 2012; Whitney et al., 2009). Therefore, acute exercise-related DG/CA3 elevation of extracellular diffusion could indicate increased glial activity associated with the expression of neuroinflammatory mediators and neurotrophic factors which may in turn upregulate neurogenesis and promote long-term adaptations that benefit older adults' brain health (El-Sayes et al., 2019). However, additional studies that link short-term diffusion changes to long-term exercise training adaptations are still needed to determine if short-term differences in NDI are beneficial and can promote a healthy aging memory system.

Limitations

This study provides novel evidence of acute exercise-related functional and structural neuroplasticity in the hippocampal subfields of healthy older adults. However, when interpreting these results, it is important to note several limitations. Our convenience sample consisted of predominantly female, well-educated Caucasian participants, limiting generalizability to the broader population. Our sample included physically active older adults who could complete a 20 minute bout of moderate to vigorous intensity cycling exercise. While we did not find that cardiorespiratory fitness moderated the effects of acute exercise on any of our behavioral or neuroimaging measures, sedentary individuals may have a different response to an acute bout of moderate to vigorous intensity exercise. Because a 20 minute bout of higher intensity cycling exercise could be challenging to complete in sedentary older adults, future studies should include sedentary individuals and potentially implement shorter and/or lower intensity interventions. Neuroimaging and behavioral measures were only collected post-exercise and rest, limiting our ability to infer changes in performance between the two conditions. However, participants confirmed similar pre-test day routines, the Order of Conditions was counterbalanced across participants, and condition Order was controlled for in all analyses, providing greater confidence in attributing differences to the acute exercise intervention. Additionally, given safety limitations in performing maximal effort stress tests in older adults, it was impossible to tailor acute exercise intensity based on individual maximal heart rates or percentage of maximal oxygen uptake. Instead, we asked participants to exercise based on subjective ratings of perceived exertion, which has been shown to control the relative exercise intensity among individuals who may vary in their absolute maximal capacity to perform cycle ergometer work (Dishman, 1994; Dunbar et al., 1994). Finally, while our novel imaging techniques provide new insight into differences in hippocampal subfield function and microstructure, they are not specific to any underlying neurophysiological mechanism. Additionally, neither MRI measure was associated with differences in MD performance between the exercise and rest conditions. Thus, it is impossible from this study to link these structural and functional network effects t behavioral changes.

5 Conclusion and Future Direction

We provide new evidence that 20 minutes of moderate to vigorous intensity acute cycling exercise, compared to a seated rest control condition, is associated with differences in DG/CA3 function and microstructure in healthy older adults. Specifically, we found acute aerobic exercise reduced MD performance, MD-related DG/CA3 fMRI activation, and DG/CA3 NDI, but the differences in these measures were not associated. These findings suggest that a single session of higher-intensity aerobic exercise may rapidly alter the DG/CA3 hippocampal subfield through several different mechanisms and pathways. Future studies are needed to determine whether different durations, intensities, and/or types of acute exercise might modify the effects of acute exercise on the aging memory system and link these shorter-term alterations in the hippocampal subfields to longer-term training-induced adaptations and health habit benefits.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability

Data available on request from the authors.

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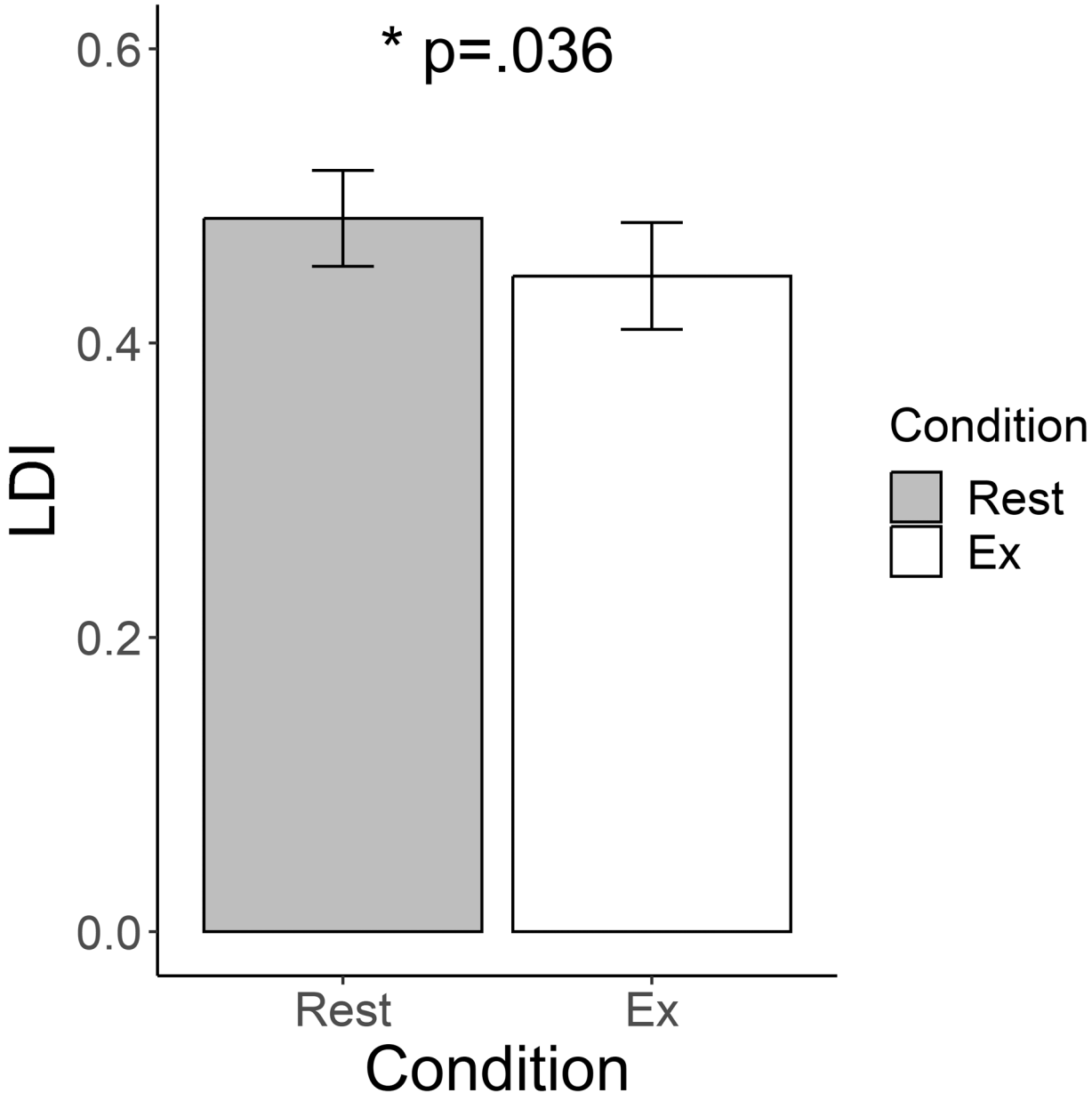

Figure 1.

Acute exercise effects on Lure Discrimination performance. LDI = Lure Discrimination Index. Comparison of post Rest vs post Ex (Exercise) LDI performance while accounting for Order of Conditions, Condition by Order effect, age, and number of days apart.

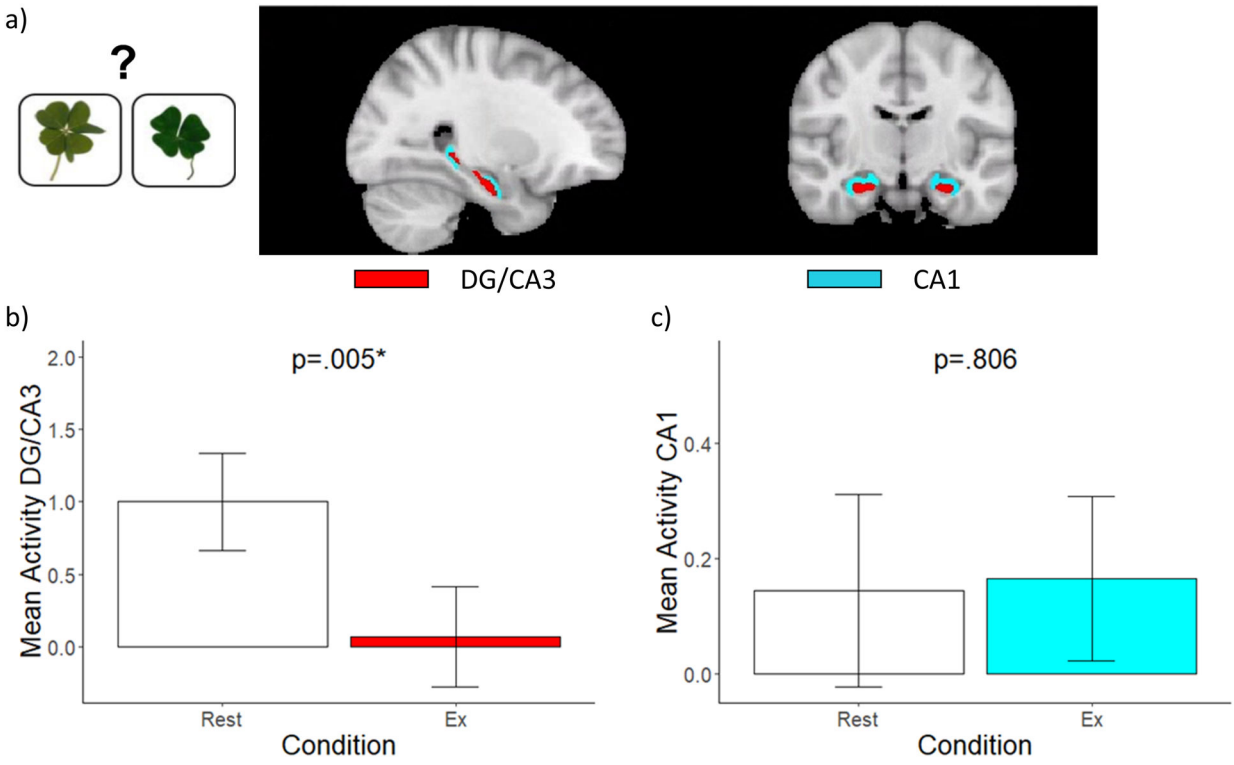

Figure 2:

a) Hippocampal Subfield segmentation of the DG/CA3 (red) and CA1 (blue) and an example of a lure pair image condition under which mean activity was extracted. b) Significant difference of mnemonic discrimination related DG/CA3 activity following exercise (Ex) and rest (Rest) while controlling for order of conditions, age, and number of days apart. c) Nonsignificant difference of mnemonic discrimination related CA1 activity following exercise and rest. p = p-value. * $p < .05$.

Table 1.

Participant Demographic Information (n = 36).

		Total sample (n=36)
		Mean (SD)
Demographics		
	Age (years)	67.1 (4.3)
	Sex	30 Female, 6 Male
	Education (n,(%), ≥ Graduate School)	24 (67%)
Health		
	BMI (kg/m^2)	25.7 (4.3)
	$HR_{resting}$ (bpm)	68.7 (10.9)
Cardiorespiratory Fitness and Leisure-Time Physical Activity		
	VO_{2peak} (ml/kg/min)	22.7 (7.3) [a]
	7-day Physical Activity Energy Expenditure (MET/week)	94.7 (39.4)[b]
Cognitive Status, Depression, and Anxiety		
	MoCA	28.0 (1.3)[c]
	Geriatric Depression Score	2.8 (2.7)[d]
	Geriatric Anxiety Score	6.8 (7.5)[e]

Notes: bpm = beats per minute; RHR = Resting Heart Rate; HR_{max} = Maximum Age predicted heart rate; MoCA = Montreal Cognitive Assessment. ml/kg/min = kilogram per milliliter per minute. MET = ratio of working metabolic rate relative to energy at rest. 7-day Energy Expenditure = the total MET- hours completed in the last 7 day period. MET is a unit of energy expenditure relative to the resting metabolic rate with 1 MET = 1kcal/kg/hour. VO_{2peak} = Peak oxygen consumption estimated from submaximal exercise stress test.

[a] American College of Sports Medicine 50th percentile for peak oxygen consumption of older adults aged 60+ is approximately 30 (male) & 27 (female).

[b] American Heart Association physical activity guidelines suggest at least 10 MET/week for significant health benefits.

[c] MMSE scores below 27 indicate potential mild cognitive impairment.

[d] Geriatric Depression Scores between 9–15 indicate moderate to severe depression symptoms.

[e] Geriatric Anxiety Scores between 16–63 indicate moderate to severe anxiety symptoms.

Table 2.

Experimental condition outcomes and manipulation check.

Measure	Mean (SD)		
	Rest	Exercise	p
HR (BPM)	68.7 (15.2)	125.6 (36.4)	**<.001**
RPE (Borg 6–20 scale)	6.2 (0.5)	13.1 (1.6)	**<.001**
Valence	7.1 (2.5)	6.7 (2.4)	0.195
Arousal	4.6 (2.4)	5.8 (2.5)	**<.001**

Notes: SD = Standard Deviation. p = p-value from paired t-tests performed between Exercise and Rest conditions. Measures of HR = heart rate; BPM = beats per minute; RPE = rating of perceived exertion. Valence = subjective measure of valence; Arousal = subjective measure of arousal; All measures were averaged and compared over the final 10 minutes of the moderate to vigorous intensity exercise session (minutes 15–25 of the experimental conditions). Average participant heart rate in the final 10 minutes of the exercise condition was approximately 82% (SD 17%) of age predicted maximal heart rate. This is consistent with a moderate to hard intensity rating based on ACSM guidelines (American College of Sports Medicine, 2013).

Table 3:

Linear mixed effects analysis looking at the effect of acute aerobic exercise on ODI and NDI measures for whole brain gray matter, whole hippocampus and DG/CA3 and CA1 hippocampal subregions.

Region	Diffusion Measure	Exercise Effect (F-value)	p-value	Effect Controlling for Whole Brain GM Diffusion (F-value)	Effect Size (η^2)	Corrected p-value
Whole Brain GM	ODI	38.70			**+ .56**	<.001 *
	NDI	2.19			− .07	.149
Whole Hippocampus	ODI	6.44	**.016** *	2.09	+ .05	.156
	NDI	8.93	**.005** *	6.28	− **.17**	**.018** *
DG/CA3	ODI	6.67	**.015** *	0.55	+ .01	.464
	NDI	8.52	**.006** *	7.10	− **.18**	**.012** *
CA1	ODI	3.60	.067	5.07	+ .11	*.030*
	NDI	4.59	*.040*	2.62	− .08	.116
SUB	ODI	4.48	*.041*	0.15	+ <.01	.700
	NDI	4.56	*.042*	2.63	− .08	.115

Notes: For effect sizes + = higher following exercise. – = lower following exercise. GM= Gray Matter. ODI= Orientation Dispesion Index, NDI=Neurite Density Index. Bold and

* indicate significant effect of exercise in linear mixed effect model, while controlling for age, Order of Condition (exercise or rest first), Order by Condition interaction effect, and number of days apart between two visits based on a FWER corrected p-value <.05. Corrected F and p-values indicate the effect of exercise when additionally controlling for whole brain gray matter diffusion values in the model. Italicized p-values indicate not significant after multiple comparison correction.