**Increased Neural Differentiation after a Single Session of Aerobic Exercise in Older Adults.**

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**Abstract**

Typical aging is associated with decreased cognitive function. One prominent theory posits that this cognitive decline is in part due to multiple neural systems becoming de-differentiated in old age. To counteract age-related decline, it has been reported that exercise can improve cognition in older adults – even after only a single session. However, the neural mechanisms of this improvement are unclear. We hypothesized that one mechanism of improvement immediately after exercise is a re-differentiation of neural systems that become dedifferentiated due to age induced decline. This would involve detecting differentiation levels in older individuals that shift to levels observed in relatively younger individuals immediately after exercise. To test this, we used a within-subject, cross-over design involving two sessions: either 30-minutes of moderate intensity aerobic Exercise or 30-minutes of seated Rest (n=32; ages 55-81 years). Both fMRI and behavioral tests (i.e., Stroop performance) were acquired after the Exercise and Rest conditions. We quantified local neural differentiation in functional Magnetic Resonance Imaging (fMRI) data using a novel General-Heterogeneity Regression analysis. There were three main findings. First, following exercise participants were significantly better at reducing Stroop interference. Second, following exercise there was greater neural differentiation within the hippocampal formation and multiple cerebellar regions. Third, the increases in neural differentiation in both cerebellar Crus 1 and 2 and the parahippocampal and entorhinal cortex were more pronounced in the relatively older ages. These results suggest that one contributing neural mechanism of improved cognition after acute exercise in healthy older adults is neural re-differentiation to a *relatively* younger level of neural differentiation.

**1. Introduction**

The decline of cognitive and neural systems in old age is one of the most important public health issues of our day. It has been reported that 1 in 5 older adults (>60 years old) in the U.S. report some form of cognitive decline (Plassman et al., 2008), and that such cognitive decline is a major risk factor for neurodegenerative diseases such as Alzheimer’s disease (AD) (Albert et al., 2001; Small, 2000, 2001). Given cognitive decline can negatively impact quality of life and there is no cure for AD, it is of paramount importance to employ preventions or early interventions to preserve cognitive health for as long as possible. As such, there is growing interest in understanding lifestyle factors, including exercise, which can help to improve cognitive and neural function in old age (Livingston et al., 2020).

Such work has reported that over the long term (i.e., weeks to years) physical exercise does improve cognition and brain health (Angevaren et al., 2008; Cassilhas et al., 2007; Erickson et al., 2011; Fernandes et al., 2017; Firth et al., 2018a; Maass et al., 2015; Voss et al., 2019), and does help to reduce the risk of cognitive and neural decline in old age (Angevaren et al., 2008; Colcombe and Kramer, 2003; Gomez-Pinilla and Hillman, 2013; Heyn et al., 2008; Hillman et al., 2008; Yaffe et al., 2009). Fundamental to these long-term impacts of exercise on cognitive and brain health is the question of how single sessions of exercise contribute to these longitudinal benefits. Long-term adaptations in the brain following exercise training are hypothesized to result from the accumulation of physiological adaptations driven by numerous consecutive sessions of exercise (El-Sayes et al., 2019; Loprinzi et al., 2021), so it is important to determine how single sessions of exercise impact both neurophysiological and cognitive changes. Current research indicates that single sessions of exercise have been associated with many benefits to improved neural and cognitive function, particularly improvements to executive functions and episodic memory (Suwabe et al., 2017a; Won et al., 2019a).

Episodic memory involves the ability to encode and retrieve memories of events (Dickerson and Eichenbaum, 2010) and has also been found to improve after a single session of exercise (Coelho-Júnior et al., 2021; Loprinzi, 2019; Sng et al., 2018; Suwabe et al., 2018; Weinberg et al., 2014). Of relevance is the pattern separation task which involves discriminating between learned and similar un-learned items (Berron et al., 2016; Knierim and Neunuebel, 2016; Stark et al., 2013; Yassa and Stark, 2011). Not only are older adults relatively poor at this task, but this decline is associated with hippocampal signal de-differentiation (Koen and Rugg, 2019; Stark et al., 2015, 2013; Toner et al., 2009). Given that hippocampal formation neural differentiation (and performance on the pattern separation task) improves immediately after aerobic exercise in young adults (Callow et al., 2022; Suwabe et al., 2018, 2017b), it is predicted that hippocampal neural differentiation will also increase immediately after exercise in older adults.

Executive function, on the other hand, is a family of different functions including information updating (associated with working memory) and inhibitory control (associated with selective attention) (Diamond, 2013; Miyake et al., 2000), both of which have been shown to improve with long term exercise as well as immediately after exercise. Improved *Information updating* (i.e., working memory) in older adults has been shown to improve immediately after exercise on an indoor bike (Voss et al., 2020).  *Inhibitory control* was also reported to be increased immediately following aerobic exercise as measured with the Flanker task (Eriksen and Eriksen, 1974; Kamijo et al., 2009a), or the Stroop task (Barella et al., 2010; Chang et al., 2019; Hogervorst et al., 1996; Lichtman and Poser, 1983; Sibley et al., 2006; Stroop, 1935).

Turning to the brain, the focus of this work is to identify possible neural mechanisms associated with the aforementioned executive function and memory improvements associated with a single session of exercise. One possible neural mechanism pertains to the ability of healthy neural systems to encode information in a well-differentiated, heterogeneous manner with relatively distinct neural signals. This idea is based on the findings that health of a neural system is in part based on its capacity to differentiate amongst similar stimuli and task states, which is an indicator that information is well-encoded within neural networks (e.g., Favila et al., 2016; Glezer et al., 2015; Jiang et al., 2017; Perez-Nieves et al., 2021; Purcell et al., 2019; Purcell and Rapp, 2018). Critically, a prominent theory of the neurobiology of aging is that, in general, cognitive and neural decline in old age is because multiple neural systems become more de-differentiated with age (Carp et al., 2011b; Dennis and Cabeza, 2011; Koen et al., 2020; Koen and Rugg, 2019; Park et al., 2004). One method used to quantify this de-differentiation involves demonstration of the relative lessening of distinctness of spatial patterns of fMRI signal responses for different stimuli (Carp et al., 2011b; Srokova et al., 2020). Another popular method to examine de-differentiation is to use fMRI repetition suppression (fMRI-RS) effects, which measure the relative reduction in neural responses for the same items relative to similar items, and can be used to quantify neuronal selectivity (Bakker et al., 2008; Berron et al., 2016) and the aforementioned pattern separation of similar memory representations in the hippocampal system (Bakker et al., 2008). Such studies reported reduced fMRI-RS effects in visual association areas in old age (Goh et al., 2010), and that there is reduced neural pattern separation in the hippocampal system in old age (Reagh et al., 2018; Yassa et al., 2011). One disadvantage of these methods is they necessitate quantifying fMRI responses to task conditions or trials, and therefore require task-based fMRI.

Based on the aforementioned theory of neural de-differentiation in aging (Koen et al., 2020) and findings that a single session of exercise in young adults can increase hippocampal system activation during pattern separation performance (i.e., better differentiation of memory representations) (Suwabe et al., 2018), we hypothesized that one consequence of a single session of exercise is that it promotes the re-differentiation of neural systems in older adults. This hypothesis has not yet been tested, as most studies that have examined fMRI signal changes after a single session of exercise in older adults have relied on and have reported the average response to an fMRI task, and as such the results have been constrained by the cognitive demands of the task. Such work has reported mean signal changes in both frontal, temporal, and hippocampal areas in a working memory task (Li et al., 2019) and semantic memory and executive function tasks (Won et al., 2019b, 2019a; previous study using a subset of the data reported here). However, previous studies have not examined whether there are changes in local differentiation of the fMRI responses that are independent of task demands.

Rather than relying on fMRI task-based analyses, our current approach estimates the (de)-differentiation of local neural responses by directly quantifying the relative (dis)-similarity or *differentiation* of adjacent fMRI signals – independent of specific task responses. Quantifying local neural differentiation is rooted in the assumption that memory representations span more than one voxel (Haxby et al., 2001), and that if you directly compare the neural signals across these adjacent voxels, one can quantify the relative similarity or dissimilarity (termed differentiation). This approach is motivated by sparse coding theory which posits that well-learned (encoded) neural representations have sparsely distributed neural codes (i.e., high activation in a relatively small number of neurons (Rolls and Tovee, 1995), and these sparser codes require locally heterogeneous neural activity (Vinje and Gallant, 2000). Previous studies using such approaches have shown that the magnitude of this differentiation in fMRI signal is related to the integrity of the underlying representations in both hippocampal *and* cortical memory systems (Jiang et al., 2017; Purcell et al., 2019; Purcell and Rapp, 2018).

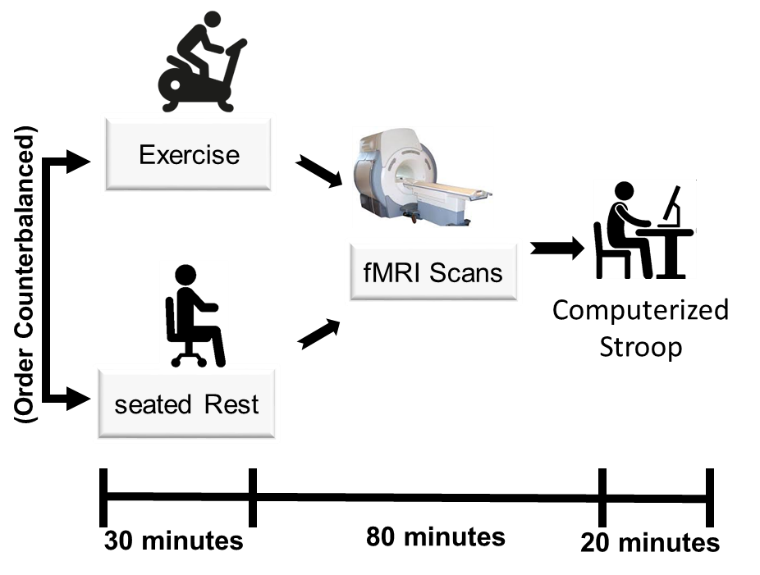
Here we build upon this previous work by introducing a novel implementation of this approach termed General Differentiation Regression (General-Hreg), which can be applied to any fMRI dataset irrespective of its task design, and can be used to examine neural differentiation across multiple neural systems. One advantage of this approach is that it does not require a complex task design, e.g., as required for spatial pattern-based analyses (e.g., Carp et al., 2011b), and can be used to examine effects in the entire brain, i.e., not just specific to a single region, such as the hippocampal formation (e.g., Reagh et al., 2018).

In this study we applied the General-Hreg method to fMRI data from a within-subject cross-over design involving either 30-minutes of rest or moderate-intensity aerobic exercise on a cycle ergometer (Figure 1) in a sample of healthy older adults (Table 1). We tested three key hypotheses. First, in confirmation of previous work (Chang et al., 2015), we tested whether 30 minutes of aerobic exercise improves executive function (i.e., inhibitory control/selective attention) as measured with the Stroop interference effect. Second, we tested whether a single exercise session increases levels of neural differentiation in the brain, including hippocampal, cortical, and cerebellar regions. Finally, to confirm that the changes in neural differentiation are coupled with improvements in cognitive function, we tested whether differences in executive function between exercise and rest were related to differences between the conditions in differentiation.

**2. Materials and Methods**

2.1 Participants: A total of thirty-two older adults (ages 55-81 years) were selected to participate in the study after meeting the eligibility criteria (SI Appendix Figure S1). Participants were recruited from local senior fitness classes, recreation centers, and swimming clubs. Prior to selection, participants completed a phone interview to determine eligibility based on the following exclusion criteria: any contraindications of MRI scanning (e.g., ferromagnetic metallic implants or claustrophobia), a history of heart attack; stroke; diabetes; high blood pressure; neurological disease; major psychiatric disturbance; hypertension; seizures; neurological disorder; or taking psychotropic prescription medications. Next, participants attended an in-person screening session in which they provided informed consent approved by the Institutional Review Board at The University of Maryland. Participants were further excluded if they had a score less than 24 on the Mini-Mental Status Examination (Folstein et al., 1975), were left-handed using the Edinburgh Handedness Inventory (<0) (Oldfield, 1971), were severely obese (body mass index *≥*40 kg/m2), or had low levels of physical activity (<30 min of physical activity three times per week during the past 6 months) as per the 7-Day Physical Activity Recall (Paffenbarger et al., 1978) used to estimate physical activity energy expenditure. All eligible participants obtained physician approval before engaging in moderate-intensity exercise. This study was conducted according to the Helsinki Declaration of 1975. Demographic, physical, and cognitive data for all participants are illustrated in Table 1. Further details about the participant recruitment process are illustrated in our prior work (Alfini et al., 2020; Won et al., 2019a).

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| Table 1 Participant Information (N = 32) | |  |  |
| Demographics | | Mean (Min-Max) | (n, %) |
|  | Age (years) | 66.3 (55-81) |  |
|  | Gender (n, %) |  |  |
|  | Male |  | 8 (25%) |
|  | Female |  | 24 (75%) |
|  | Education (n, %) |  |  |
|  | High School |  | 2 (6.25%) |
|  | College or higher |  | 30 (93.75%) |
| Physical Characteristics | |  |  |
|  | Height (cm) | 166.6 (147.3-188) |  |
|  | Weight (kg) | 71.3 (45.4-103.4) |  |
|  | BMI (kh/m2) | 25.6 (18.2-36.8) |  |
|  | Physical Activity Recall Score (kJ/kg/day) | 132.7 (105.6-175.2) |  |
|  | Resting HR (BPM) | 73.3 (49-89) |  |
| Cognitive Performance | |  |  |
|  | MMSE | 29.2 (26-30) |  |
| Notes: BMI = body mass index; Physical Activity Recall Score = kilojoule per kilogram per day (kJ/kg/day) derived from the approximate number of hours spent in light to very hard physical activities over the past 7 days; Resting HR = resting heart rate measured in average beats per minute (BPM) at the start of each visit (averaged across visits); MMSE = Mini-Mental Status Exam | | | |



**Figure 1**: Data Acquisition Summary. Each participant performed the Exercise and seated Rest condition on separate days (order was counterbalanced).

2.2 Exercise and Rest Experimental Conditions: Participants came in for a scanning session during which they either were at Rest prior to scanning or Exercised (see summary in Figure 1). This was carried out using a within-subject cross-over design where each participant underwent two experimental visits (Exercise and Rest) on separate days (mean of 12 days between visits; min-max = 1-91 days). Prior to participation for either visit, participants were fitted with a heart rate (HR) monitor (Polar Electro, Kempele, Finland) and were provided with standard instruction using the Borg 6-20 Ratings of Perceived Exertion (RPE) scale (Borg, 1970); scaled between 6 to 20 (6-7 = “very, very light”; 15 = “hard”; 19-20 = “very, very hard”).

During the Exercise visit condition participants sat on a mechanically-braked stationary bike ergometer (Monark 828E, Varbro, Sweden), and proceeded to cycle as follows: a 5-min warm-up session with self-selected intensity, 20-min continuous moderate intensity cycling, a 5-min cool-down, and a 5-min recovery period. Participants were instructed to maintain a pedal cadence of 60 to 80 rpm during exercise and instructed to select a flywheel resistance that maintained their intensity at an RPE of 15 (associated with the verbal anchor “hard”). HR and RPE were recorded every five minutes.

During the Rest condition visit, participants were instructed to sit in a chair for a total of 35 mins, including a 5-min recovery period. Participants remained seated and awake in a chair while HR and RPE were collected during each 5-min interval. Participants were instructed not to read, write, use technology (e.g., phones), or talk excessively during the Rest session.

After either condition participants underwent eighty minutes of structural and functional MRI data collection, and then participated in a computerized version of the Stroop task.

2.3 fMRI Scans: The protocol involved the acquisition of four different functional runs during the scanning session. Three were obtained during performance of a task, while one was a Resting State scan. As discussed in the analysis section, it is worth noting that the task components were not directly examined in this work because only the fMRI signal residuals for each of the runs were used in the below analysis (i.e., task components of the time series were included as regressors but ignored in the analyses). Thus, only a brief description of the tasks is included ([SI Appendix, Text S1](https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2112783118/-/DCSupplemental)). For discussion of in-scanner task performance see SI Appendix, Text S2.

2.4 fMRI Data Acquisition**:** Whole-brain, fMRI was conducted on a Siemens 3.0 Tesla MR scanner (Magnetom Trio Tim Syngo, Munich, Germany). A 32-channel head coil was used for radio frequency transmission and reception. Foam padding was positioned within the head coil to minimize head movement within the coil. A high-resolution T1-weighted anatomical image was acquired for co-registration with the following sequence parameters: Magnetization Prepared Rapid Acquisition of Gradient Echo (MPRAGE), matrix = 256, field of view (FOV) = 230 mm, voxel size = 0.9 × 0.9 × 0.9 mm, slices = 192 (sagittal plane, acquired right to left), slice thickness = 0.9 mm, repetition time (TR) = 1900 ms, echo time (TE) = 2.32 ms, inversion time (TI) = 900 ms, flip angle = 9°, and sequence duration = 4:26 min.

All of the fMRI runs were acquired using the same following sequence parameters: single-shot gradient echo planar images, matrix = 64, FOV = 192mm, voxel size = 3×3×3 mm, slices = 36, slice thickness = 3.0 mm, TR/TE = 2000/24 ms, volumes = 175, flip angle = 70°, bandwidth = 2232 Hz/Px, and multi-slice mode = Interleaved. The scan durations are noted in SI Appendix Table S1. Although most (75%) of the participants received all four of these runs, some runs were missed for a few participants due to technical issues. The scan session began approximately 15 minutes after the completion of either experimental condition (Rest or Exercise).

2.5 Behavioral Stroop Task and Analysis: Approximately 80 minutes after each experimental condition, a computerized version of the color-naming Stroop task was administered using E-Prime 2.0 (Psychology Software Tools, Pittsburgh, PA) which took a total of approximately 6.5 minutes. Other brief neuropsychological tests were also administered before the Stroop task but are not discussed in this study. Participants sat at a desk in front of a computer monitor and were instructed to press one of four keyboard keys re-labeled to represent each color (e.g., ‘B’ for blue, ‘Y’ yellow, ‘G’ for green, and ‘R’ for red). Participants were instructed to use their left and right index and middle fingers to indicate the color of the stimulus as quickly and accurately as possible. A total of 64 color-word trials consisting of 28 congruent (font color and written word refer to the same color), 28 incongruent (font color and written word conflict), and 8 neutral (written word does not refer to a color) conditions were presented. Before beginning the test, a short (2-min) practice session consisting of 20 trials (8 congruent, 8 incongruent, and 4 neutral stimuli) was administered. To minimize practice effects, two versions of the Stroop task comprising unique orders of color-word stimuli were counterbalanced across experimental visits. Of the 32 total participants who completed the entire study protocol, 2 participants were excluded from analyses involving the Stroop data due to a technical failure at the time of data acquisition.

The Stroop reaction time (RT) in ms. and percent accuracy (% ACC) were calculated for the conditions of interest including the congruent and incongruent trials. The primary measure of interest is the Interference measure for both the RT and the % ACC which compares the behavior on the congruent trials relative to the incongruent trials. The Interference effect was obtained from the % accuracy (% ACC) and reaction time (RT) scores. Estimates of the Interference effect are typically obtained from the comparison of performance on the congruent and incongruent trials; relatively low performance (% accuracy or RT) on the incongruent relative to the congruent trials. This is thought to quantify the ability to selectively attend to task-relevant information (color) while excluding irrelevant information (written word) (Algom and Chajut, 2019). The measure of interest for this study though is the interaction between trial type and that of the Exercise and Rest condition. To measure this we ran mixed effects models including fixed effects for condition and trial type (congruent and incongruent) along with random effect intercepts and slopes. An example R (r-project.org) code mixed-model used for the Stroop ACC is as follows:lmer(Stroop\_Accuracy ~ Rest\_Exercise \* Congruent\_Incongruent + (1 + Rest\_Exercise + Congruent\_Incongruent | Subject\_ID). In this formula, Rest\_Exercise is a categorical variable for the experimental conditions and Congruent\_Incongruent is a categorical variable for the Stroop trial type. Interference Score ACC statistics (t and p values) are based on *Rest\_Exercise \* Congruent\_Incongruent* interaction effect estimate.

2.6 MRI Data Preprocessing**:** First, both anatomical and functional images were converted into 3D space using the Analysis of Functional NeuroImages (AFNI)’s Dimon program (Cox, 1996). Second, the anatomical images were processed using Freesurfer’s (version 5.3.0) cortical reconstruction process for cortical parcellation and subcortical segmentation (Fischl et al., 2002). The functional data were realigned using Slice-Oriented Motion Correction (SLOMOCO), a highly effective method to reduce motion artifact effects in a slice-wise manner (i.e., relative to the more traditional volume-wise motion correction) (Beall and Lowe, 2014). The functional time series data were then aligned with the Freesurfer-processed anatomical images using AFNI’s anatomical and echo-planar imaging alignment function (align\_epi\_anat). The aligned anatomical and functional data were visually inspected and submitted to the AFNI’s preprocessing program (proc.py). Functional data were despiked (3dDespike) and each volume of the time series was time-shifted to the beginning of the TR (3dTshift). TRs with excessive motion (> 5mm scan to scan motion) were indexed, and if a run had greater than 25% time points with excessive motion that run was excluded from analysis (1 run of the IAPS task was excluded). Non-linear transformation of the anatomical images to the standard space (AFNI’s MNI152\_T1\_2009c) was performed (3dQwarp). The resulting non-linear transformation matrices were used to normalize and resample the functional data. The resolution of the final image was 3x3x3 mm (corresponding to the original functional acquisition resolution).

We also ensured that there were equal numbers of runs at both the Rest and Exercise sessions for all participants. That is, if one participant had a run at the Rest session missing, that equivalent run was ignored at the Exercise session for that participant (and vice versa).

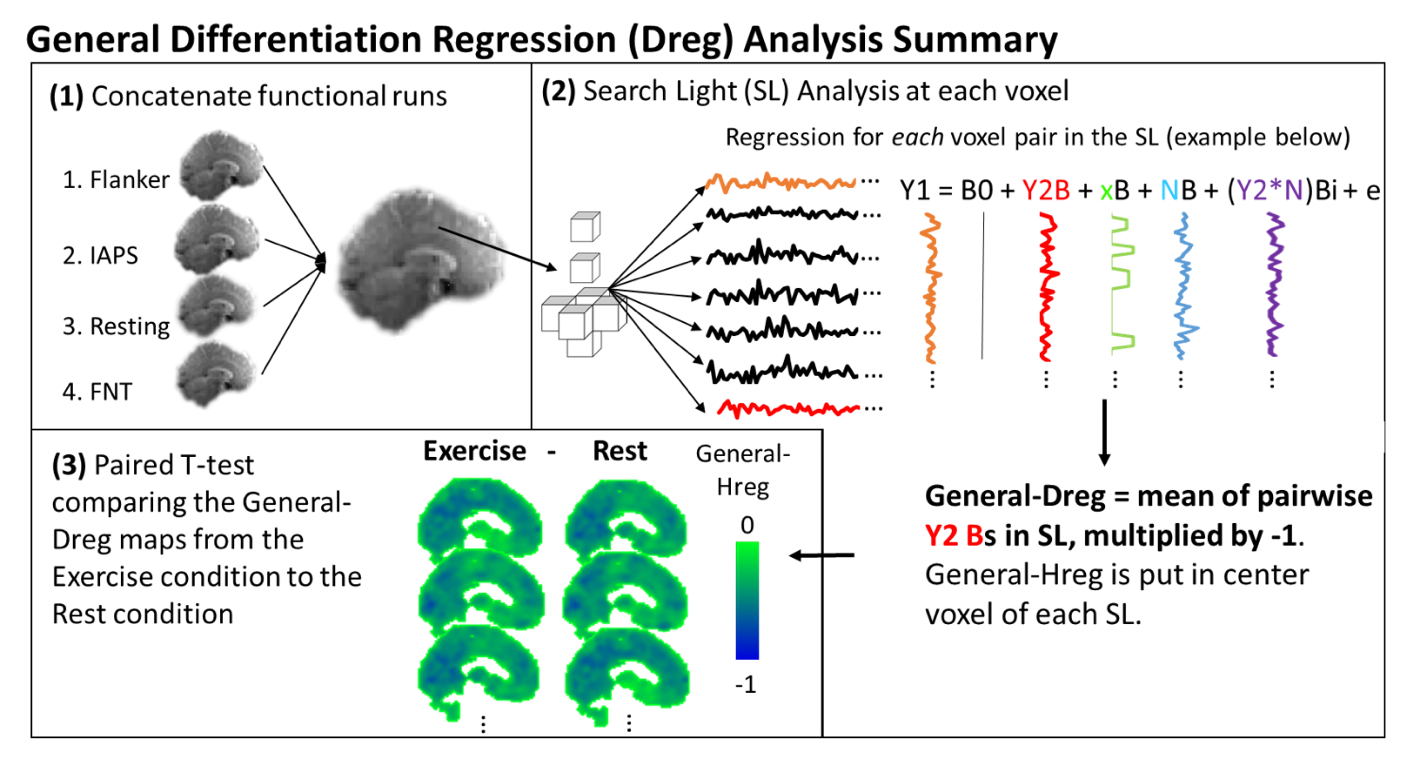
2.7 General-Heterogeneity Regression (General-Hreg) Analysis**:** Heterogeneity regression (Hreg) was initially developed to examine task specific differentiation in the brain (Purcell and Rapp, 2018). In the present study, it was adapted to examine the local differentiation of Resting State and Task-residuals (i.e., the fMRI time series after task effects have been regressed out). This analysis quantifies the relative similarity of adjacent fMRI signals while accounting for similarity due to both within and cross-voxel noise. See Figure 2 for a schematic summary of the analysis. Hreg is specifically a searchlight analysis performed on un-smoothed, pre-processed, MNI normalized data. Each searchlight contains 7 voxels: a central voxel and the 6 shared-face voxels. This approach essentially takes each pair of voxels in the search-light and uses a regression analysis to identify the similarity of two adjacent time series. It also uses the general psychophysiological interaction approach (McLaren et al., 2012) to account for BOLD signal similarity due to noise across adjacent voxels (e.g., the similarity of signal *across adjacent voxels* due to motion). In more detail, for each combination of voxel pairs the following equation was used*:* . is the voxel time series of one voxel.  is the predictor voxel time-series; corresponds to the condition regressors (, e.g., Famous Names) convolved with the hemodynamic function; N corresponds to the nuisance regressors (e.g., motion parameters). The interaction term is used to estimate and control for the interaction between the time series and nuisances regressors (e.g., if adjacent signals change in sync due to motion). In this manner, the unstandardized B estimate quantifies the relative similarity between the voxel () and this adjacent voxel while accounting for the mean task-related BOLD response, noise, and task by noise interaction terms. The Hreg value is simply the inverse of the mean of all of the unstandardized B coefficients for each of the unique 42 pair-wise voxel-voxel combinations (7!/(7-2)!=42) in each searchlight (i.e., the mean of these 21 pairwise values multiplied by negative one). In this way, higher Hreg values correspond to higher relative differentiation, while lower Hreg values correspond to lower relative differentiation. These values are then assigned to the searchlight center voxel.

In this work, we advanced upon this Hreg measure by developing a General Heterogeneity Regression (General-Hreg). This approach builds directly from the General Functional Connectivity approach that was recently introduced that concatenates both task and resting state data in order to leverage as much data as possible in fMRI connectivity analyses (Elliott et al., 2019). The key difference is that the General-Hreg approach examines *local* adjacent voxel-voxel similarity, instead of long distance connectivity as discussed in Elliott et al. (2019).

A General-Hreg analysis was run using a whole brain gray matter cortical, subcortical, and cerebellar mask based on the Harvard Oxford (HO; 0.25 probability) (Desikan et al., 2006) and Cerebellar atlas (Diedrichsen et al., 2009). The General-Hreg models discussed above included the concatenated time series from all of the functional runs for each participant (see SI Appendix Table S1 for a summary of the amount of data used). Some runs were excluded due to technical issues at the time of acquisition or due to excessive motion. Most (75%) of the participants had the full 933 timepoints (31:06 minutes of data) for the analysis prior to censoring. Post-censoring there was an average of 28:15 minutes for the Rest condition and 28:26 minutes for the Exercise condition used for analysis; there was no significant difference between the amount of data used across conditions (t(31) = -1.1, p=0.299). Besides including the concatenated voxel time series, condition regressors for each task (which were subsequently ignored), and the following noise covariates were included. First, a single motion repressor recommended for use with SLOMOCO was included – termed slomoco.volumetric.TDzmetric and quantifying the average voxel displacement generated from the slicewise out-of-plane motion parameters (Beall and Lowe, 2014). In addition, the top 5 principal components from the cerebral spinal fluid mask defined via the HO Atlas (Desikan et al., 2006). Thus, there were 6 noise covariates (this is along with the corresponding 6 noise interaction term covariates as discussed above).

Group-level maps were generated by smoothing the General-Hreg maps using an 8 mm FWHM, and then running a paired t-test comparing the Exercise minus the Rest condition maps (Figure 2). A regression-based paired t-test was used as described here (Hedberg and Ayers, 2015) since it is more efficient than the traditional paired t-test. Two additional subject-level noise nuisance covariates were included in the paired t-test. First, the average whole brain (i.e., gray matter mask) General-Hreg value was extracted for each participant and included to ensure that no global differentiation signal or noise was accounting for the regional differences between the experimental conditions. Second. the number of total timepoints post-censoring was also included as a noise covariate to avoid differences that could be driven by the amount of data or excessive motion. A stringent threshold-free cluster enhancement (Smith and Nichols, 2009) was used to correct for multiple comparisons using a two-tailed alpha level of 0.05. Results are reported in MNI space as peak coordinates and use anatomical labels either from the Harvard Oxford Atlas (Desikan et al., 2006) or a standard cerebellar atlas (Diedrichsen et al., 2009).

Whole brain split half intra-class correlation (ICCs) were calculated in order to quantify the group reliability of the General-Hreg maps. See SI Appendix Text S3 for more details.

 **Figure 2**: General Heterogeneity Regression (Hreg) Analysis Summary. (1) After standard pre-processing, concatenate all functional runs for each participant into a single timeseries. (2) Run a search light (SL) analysis on each voxel with 7 voxels in each SL. For each SL, run pair-wise regression analyses where one voxel is the dependent variable and in turn each other is an independent variable. The General-Hreg value is the mean of the pairwise unstandardized B coefficients for each dependent variable time series, multiplied by -1; thus higher General-Hreg equals greater local neural differentiation. (3) Run group-level paired T-tests to generate group Exercise minus Rest maps. The average whole brain General-Hreg value for each participant was included as a covariate to account for any individual variation or condition differences in neural differentiation that could be driven by unaccounted for noise.

2.8 Exercise by Age Interaction in Regions of Interest Analysis: The main focus of this work was to test for differences in neural differentiation between Exercise and Rest conditions. An added focus was to determine if any of those effects were age-dependent. In order to do this, we planned to identify whether any of the regions which had significant differences between Exercise and Rest, also then interacted with age via a set of post hoc analyses. Given this analysis is dependent on the main comparison of the Exercise and Rest conditions, it is necessarily post hoc. The goal was to estimate the interaction between the effect of age and Exercise versus Rest Condition comparison. Given this was a planned approach with a limited number of tests, no correction for multiple comparisons are included.

The focus of these analyses was to estimate the interaction between the effect of age and Exercise versus Rest Condition comparison. As with the whole brain analysis described above the whole brain General Hreg and the total number of time points were included as nuisance covariates. Here we provide an example R (r-project.org) model with fixed and full set of random intercepts and slopes by-subjects: Hreg ~ Rest\_Exercise \*Age \* Hemisphere + WholeBrainHreg + TimePoint# + (1 + Rest\_Exercise + hemisphere | Subject Identification). This formula indicates that both the effect of Condition (rest versus exercise) and Hemisphere were allowed to vary across participants. Although all fixed main and interaction effects were obtained, only the fixed and interaction effects involving Age and the Condition (Rest\_Exercise) are of interest and reported in section 3.5 below.

**3. Results**

3.1 Overview: In 3.2 we report that the experimental manipulation of Exercise showed high internal validity based on the expected increase heart rate and perceived exertion. In 3.3 we report better Stroop performance after Exercise compared to Rest. In 3.4 we report increased neural differentiation in the brain after Exercise compared to Rest. Finally, in 3.5 we report exercise-related difference in neural differentiation was moderated by age.

3.2 Experimental Manipulation Check: In order to confirm the integrity of the Exercise manipulation, both Heart Rate (HR) and Ratings of Perceived Exertion (RPE) were recorded during the exercise and rest sessions. As expected, there was minimal change in HR by the end of the Rest session (decrease of ~4 BPM (SEM = 1), and there was a marked increase in HR by the end of the Exercise session (increase of ~61 BPM (SEM = 3.3). Note that all paired t-tests reported in this work use the regression based approach as described here (Hedberg and Ayers, 2015). A paired t-test revealed a significantly greater increase in HR after the Exercise condition relative to the Rest condition (*t*(31) = 19.8, *p <* .00001, Cohen’s *d* = 3.5). Further, there was a markedly greater increase in RPE by the end of the Exercise session relative to the Rest session (*t*(31) = 31.3, *p <* .00001, Cohen’s *d* = 5.6). The mean RPE during exercise corresponded to a rating between the verbal anchors “Somewhat Hard” and “Hard” (RPE Borg 6-20 scale = 13-15) consistent with a moderate intensity exertion. These expected effects support the internal validity of the experimental manipulation (Exercise and Rest) in this study; see summary in SI Appendix, Table S2).

3.3 Stroop Performance: The primary measure of interest is the Interference for both the response time (RT) and the percent accuracy (% ACC), which compares the behavior on the congruent trials relative to the incongruent trials. This was obtained as the interaction effect in a mixed effects model with a 2x2 design which included trial type (incongruent or congruent) and condition (exercise or rest). An interaction effect between trial type and condition would suggest that exercise impacts the amount of Stroop interference. As reported in Table 2 (SI Appendix, Figure S2), there was a significant interaction effect for the experimental condition and trial type for % accuracy (t(29) = 2.49, p =0.016, Cohen’s d = 0.38). However, there was no significant corresponding interaction effect in reaction time (t(29) = -0.13, p = .956, Cohen’s d = -0.13). This interaction effect for % accuracy indicates that participants were noticeably better at selectively attending to the color naming task after aerobic Exercise relative to seated Rest. This finding is particularly notable because the Stroop performance was obtained approximately 80 minutes after the Exercise or Rest conditions, suggesting a prolonged improvement in executive function due to exercise.

**Table 2:** Stroop Performance (N = 30)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Rest | Exercise | Exercise minus Rest | | Interference effect: Trial Type by Condition Interaction | | |
|  | mean (SEM) | mean (SEM) | Raw Difference | Cohen's d | t-stat | p | R ² Full Model |
| % Accuracy |  |  |  |  |  |  |  |
| Congruent | 99.3% (.3%) | 99% (.7%) |  |  |  |  |  |
| Incongruent | 95.8% (1.1%) | 97.9% (.9%) |  |  |  |  |  |
| **Interference** | **-3.5% (.98%)** | **-1.2% (.6%)** | **2% (1.1 - 3.5)** | **0.38** | **2.487** | **0.016** | **0.689** |
| RT (ms) |  |  |  |  |  |  |  |
| Congruent | 1125 (55.5) | 1146 (49.2) |  |  |  |  |  |
| Incongruent | 1317 (54.4) | 1336 (56.9) |  |  |  |  |  |
| Interference | 19.2 (2.6) | 17 (2.5) | -2 ( -6.2 - 2.7) | -0.13 | -0.056 | 0.956 | 0.958 |
| Notes: SEM = Standard Error of the Mean; ACC = percent accuracy; RT = reaction time; Interference Measure RT = Incongruent RT - Congruent RT)/Congruent RT; Interference Measure ACC = Incongruent % ACC – Congruent % Acc. Estimates of the interference effect due to exercise was obtained from mixed effects models including trial type (congruent, incongruent) and condition (rest, exercise). Main and interaction fixed effects were included in the model along with random effect intercepts and slopes. An example R mixed-model used for the Stroop ACC is as follows: lmer(Stroop\_Accuracy ~ Rest\_Exercise \* Congruent\_Incongruent + (1 + Rest\_Exercise + Congruent\_Incongruent | Subject\_ID) where Rest\_Exercise is a categorical variable for the experimental conditions and Congruent\_Incongruent is a categorical variable for the trial type. Interference Score ACC statistics (t and p values) are based on Rest\_Exercise \* Congruent\_Incongruent interaction effect. | | | | | | | |

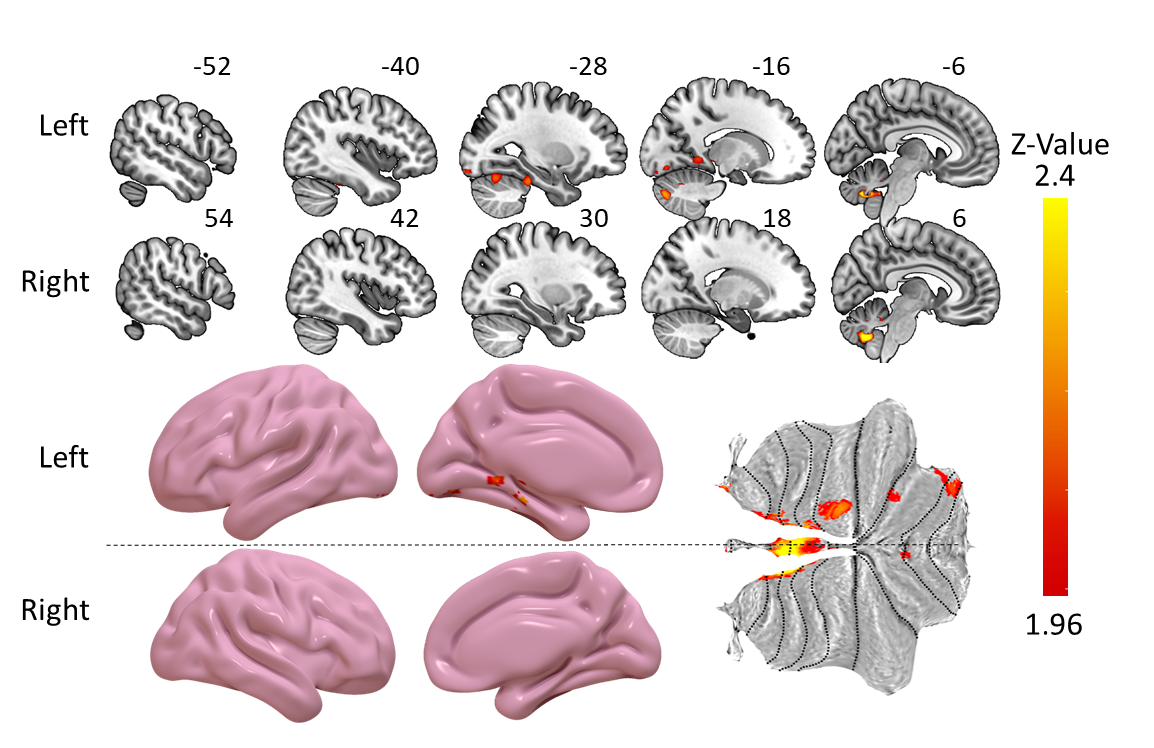
3.4 General-Hreg and Intra-Class Correlation (ICC) Results: Whole-brain General Heterogeneity Regression (Dreg) values were obtained for each participant, followed by a paired t-test to compare the values between the Rest and Exercise conditions. See Table 3 and Figure 3 for the significant General-Hreg clusters within-subject Exercise minus Rest condition contrasts. The Intra-Class correlation values are also reported for these results ([SI Appendix](https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2112783118/-/DCSupplemental) Figure S3).

There were six significant clusters which demonstrated greater neural differentiation following 30 minutes of exercise relative to 30 minutes of rest (two-tailed corrected p < 0.05; see Table 3 and Figure 3). These included both the cerebellum and cerebrum. In the cerebellum there were three clusters that included peaks in both medial and lateral cerebellum regions including the Vermis, Crus 2 and Lobule 6. In the cerebrum the most prominent effect was in the parahippocampal gyrus and hippocampus. There was a relatively weaker effect in the occipital pole and association cortices.

Overall, the within-condition reproducibility was primarily good across the local maxima (average for the Exercise and Rest was 0.66 and 0.64 respectively). Thus the underlying within-condition reproducibility was good to strong in many of the reported regions. The most reproducible regions with good reproducibility (ICC > 0.6 in both Exercise and Rest conditions) were in the Vermis, Crus 2, Lobule 6, PHG and hippocampus. This good reproducibility provides support for using these values in the region of interest analyses to examine the relationship between age and the effects of exercise on neural differentiation. Finally, although the within condition ICC values are most important metric of reliability, we also report ICC values for the Exercise minus Rest contrast some of which had fair reproducibility (ICC > 0.4) such as Lobule 6 and the hippocampal formation; see Table 3 and SI Appendix Figure S3.

No clusters were observed which had significantly greater neural differentiation following 30 minutes of rest compared to 30 minutes of exercise.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 3:** General-Hreg Whole Brain Results for the Exercise - Rest Contrast (N=32; two-tailed corrected for multiple comparisons at an alpha = 0.05 using permutation-based threshold free cluster enhancement). | | | | | | | | | |
|  |  |  | **MNI Coordinates** | | | | **Mean Intra-Class Correlations (Min-Max)** | | |
| **Hemisphere/Region** | | **Cluster mm3** | **x** | **y** | **z** | **Peak z-value** | **Exercise Mean** | **Rest Mean** | **Exercise-Rest Mean** |
| **Cerebellum** | |  |  |  |  |  |  |  |  |
| R | Vermis 8 | 196 | 5 | -65 | -41 | 2.707 | .83 (.79-.86) | .75 (.68-.84) | .19 (.01-.54) |
| L | Crus2 |  | -17 | -83 | -38 | 2.267 | .71 (.64-.79) | .72 (.65-.79) | .32 (.22-.4) |
| L | Lobule 6 | 40 | -17 | -65 | -29 | 2.016 | .73 (.68-.82) | .75 (.68-0.81) | .52 (.43-.59) |
| L | Lobule 6 |  | -29 | -68 | -23 | 2.152 | .51 (.40-.63) | .60 (.48-.74) | .28 (.20-.35) |
| L | Vermis 4/5 | 34 | -2 | -56 | -23 | 2.03 | .72 (.66-.77) | .80 (.76-.82) | .28 (.05-.42) |
| **Cerebrum** | |  |  |  |  |  |  |  |  |
| L | PHG | 142 | -26 | -32 | -26 | 2.203 | .76 (.65-.90) | .63 (.56-.69) | .40 (.20-.53) |
| L | Hippocampus |  | -20 | -26 | -14 | 2.2 | .71 (.62-.78) | .66 (.51-.77) | .45 (.27-.56) |
| L | Occipital Pole | 35 | -29 | -95 | -17 | 2.077 | .69 (.62-.75) | .56 (.44-.62) | .10 (.01-.17) |
| L | Occipital FG | 27 | -20 | -80 | -14 | 2.195 | .27 (.15-.43) | .26 (.17-.37) | 0 (0-.002) |
|  |  |  | Mean | | | 2.14 | **0.66** | **0.64** | **0.28** |
|  |  |  | Min | | | 2.02 | **0.27** | **0.26** | **0.00** |
|  |  |  | Max | | | 2.27 | **0.83** | **0.80** | **0.52** |
| Notes: A minimum cluster threshold 20 voxels was used (each voxel is 3x3x3mm); the top 2 peaks from each significant cluster are reported if they are at least 10 mm apart and are in different anatomical structures. Cerebrum regions are defined using the Harvard Oxford atlas while the cerebellum regions are defined using a standard cerebellum atlas (Diedrichsen et al., 2009); the Intra-Class Correlations (ICC) values of the participant General-Hreg values were obtained separately for the Exercise minus Rest contrast, the Exercise minus implicit baseline and the Rest minus implicit baseline maps. On average the ICC values ranged *mostly* from fair to strong in the significant peaks using the following ICC scale: [0-0.4]=poor; [0.4-0.6]=fair; [0.6-0.75]=good; [0.75-1]=strong. Peak z-stat = local maximum z statistic; R = Right; L = Left. PHG = Parahippocampal Gyrus; FG = Fusiform Gyrus | | | | | | | | | |



**Figure 3**: Group-level paired t-test comparing the General-Hreg after 30 minutes of Exercise compare to 30 minutes of seated Rest (Exercise – Rest). A permutation-based threshold free cluster enhancement approach was used to correct for multiple comparisons; the color scale depicts the z value from the minimum (z = 1.96 which is equivalent a two-tailed alpha level =0.05) to a maximum of 4. As reported in detail in Table 4 the most prominent regions which show a significant difference are in the cerebellum and left hippocampus. Top are sagittal slices with MNI slice # (generated in MRIcroGl: https://www.nitrc.org/projects/mricrogl), and bottom are surface projections for the cortex (generated in Surf ICE: www.nitrc.org/projects/surfice/) and a flattened map of the cerebellar surface (generated in SUITE flatmap: www.diedrichsenlab.org/imaging/suit\_flatmap.htm). Note, images in Figures 4 and 5 below also used these tools.

3.5 Exercise by Age Interaction in Regions of Interest: We report on the results from a planned set of post hoc region of interest mixed linear effects models to determine if the effects of Exercise on neural differentiation are dependent on age. See section 2.8 for more details.

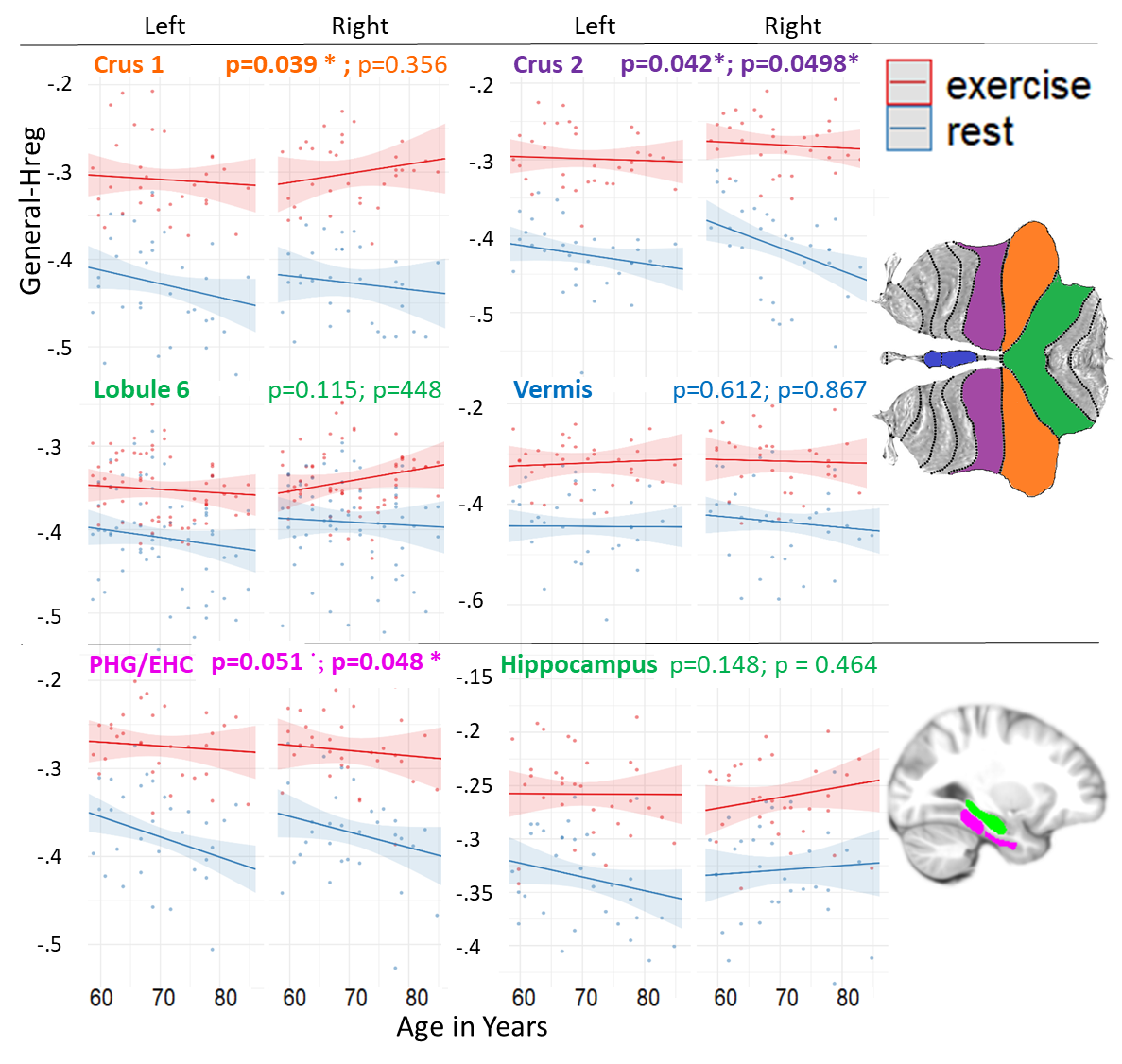
3.5.1: Region of Interest Selection: In an effort to limit the number of post-hoc tests, and focus the analyses on the most relevant areas, we used two criteria for region selection. First, regions were selected if they were identified to be associated with the clusters with a significance difference in Exercise versus Rest, and which had good replicable clusters (i.e., ICC values greater than 0.6 for both Rest and Exercise conditions) (Figure 3, Table 3); these include the vermis, Crus 2, Lobule 6, and the parahippocampal gyrus/entorhinal cortex, and hippocampus). Second, they were also selected based on whether there is literature based evidence suggesting dedifferentiation in old age. Notably, both the parahippocampal/entorhinal cortex (combined) and the hippocampus proper have been associated with lower or decreased neural differentiation in old age and are thought to be associated with complimentary memory declines in old age (Koen et al., 2020; Koen and Rugg, 2019; Rabipour et al., 2020). These hippocampal formation regions were defined based on standard atlas regions of the hippocampal formation developed in the lab C. Stark (e.g., 115). The cerebellum has also been notably associated with age-related dedifferentiation thought to be associated with motor and cognitive declines in old age (Bernard and Seidler, 2014; Carp et al., 2011a). Given that the lateral cerebellum including both the Crus 1 and Crus 2 are reported to have network level dedifferentiation in old age (Hausman et al., 2020), the Crus 2 was also incorporated into the analysis. The cerebellar regions were defined from a standard atlas (Diedrichsen et al., 2009). These set of 6 regions are visually depicted in right side of Figure 4.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table 4: Fixed effects outputs from the 6 separate mixed effects models used to quantify the interaction between age and the Experimental conditions (Rest and Exercise).** | | | | |
| Regions of Interest | Fixed Effects of Interest | t | p (Sig.) | R ² Full Model |
| Cerebellum Crus 1 | Age | -0.473 | 0.6397 | 0.9640 |
| Rest\_Exercise:Age | 2.16 | **0.0390 \*** |  |
| Rest\_Exercise:Age:Region:Hemisphere | -0.936 | 0.3566 |  |
| Cerebellum Crus 2 | Age | -1.449 | 0.1576 | 0.9572 |
| Rest\_Exercise:Age | 2.123 | **0.0422 \*** |  |
| Rest\_Exercise:Age:Region:Hemisphere | -2.045 | **0.0498 \*** |  |
| Cerebellum Lobule VI | Age | -0.201 | 0.8423 | 0.5487 |
| Rest\_Exercise:Age | 1.582 | 0.1154 |  |
| Rest\_Exercise:Age:Region:Hemisphere | -0.76 | 0.4483 |  |
| Vermis | Age | -0.173 | 0.8635 | 0.9023 |
| Rest\_Exercise:Age | 0.513 | 0.6120 |  |
| Rest\_Exercise:Age:Region:Hemisphere | -0.169 | 0.8670 |  |
| Parahippocampal Gyrus /Entorhinal Cortex (PHG/EC) | Age | -1.638 | 0.1118 | 0.9656 |
| Rest\_Exercise:Age | 2.031 | **0.0512 ˙** |  |
| Rest\_Exercise:Age:Region:Hemisphere | 1.998 | **0.048 \*** |  |
| Hippocampus | Age | 0.032 | 0.9749 | 0.8982 |
| Rest\_Exercise:Age | 1.486 | 0.1480 |  |
| Rest\_Exercise:Age:Region:Hemisphere | 0.742 | 0.4636 |  |
| Notes: Tabulated results from the 6 separate post hoc region of interest mixed effect models; see Figure 4 for plotted results. The model regions are listed on the left. The regions were primarily selected in a post hoc manner based on brain areas with significantly different General-Hreg values between the Rest and Exercise Conditions (Table 3 and 4). For each left and right region the General-Hreg values were extracted from the voxels with the top 20% difference between Rest and Exercise for each individual subject. These General-Hreg values were set as the dependent variables in each of the 6 models. This resulted in 6 mixed effects models that quantified the fixed effects of age, condition, the two hemispheres and all interactions between them and also a full set random effect slopes and intercept. Whole brain General Hreg and the total number of time points were included as nuisance covariates, and ignored. Effects of Age and Rest/Exercise Conditions interactions are of interest and reported here; the effect of Rest/Exercise condition is not reported due to explicitly selecting voxels that are different for these conditions. Significance: \* significant at alpha = 0.05; **·** approaching significance at p<0.6. The cerebellar Crus 1 and 2 and PHG/EC each showed a significant interaction effect between age and exercise. | | | | |

3.5.2 Region of Interest Results: The focus of these analyses was to estimate the interaction between the effect of age and Exercise versus Rest Condition comparison. For each left and right region selected for this post-hoc analysis, the General-Hreg values were extracted from the voxels with the top 20% difference between Rest and Exercise for each individual subject. This approach was used because it is the voxels of greatest differentiation difference between Rest and Exercise, which we would predict to interact the most with the effects of age. For completeness and qualitative comparison, we still did include the model results from a region of interest analysis that uses the mean General-Hreg values for each bilateral region (see SI Table S3). For each of these mixed effects models the interaction effect of primary interest between Age and the Rest & Exercise conditions were derived from 6 separate models with General-Hreg as the dependent variable of interest and independent variables of Rest & Exercise, Age, and Brain Hemisphere (and associated interactions).

There was a significant interaction effect between age and the effects of exercise in the Cerebellum Crus 1 and Crus 2 as well as the Parahippocampal Gyrus/Entorhinal Cortex (PHG/EC; see Table and Figure 4). In the Crus 1 region there was a significant interaction between age and the effects of exercise (t (30) = 2.216, p = 0.039). In the Crus 2 there was not only a significant interaction between Age and the Rest & Exercise (t (30) = 2.13, p = 0.042), but also a significant 3-way interaction between age, the effect of exercise, and hemisphere (t(30) = -2.045, p = 0.049). As depicted in Figure 4, this 3-way interaction was driven by the greater interaction effects of age on exercise effects in the right Crus 2 relative to the left. Finally, there was also an approaching-significant interaction between age and exercise effects difference (t (30) = 2.031, p = 0.051), as well as a significant 3-way interaction between age, the effect of exercise, and hemisphere (t(30) = 1.998, p = 0.048). As depicted in Figure 4, this 3-way interaction appears to be driven by the greater interaction effects of age on exercise effects in the left PHG/EC. There were no significant interaction effects between the regions, hemisphere, age, and condition (Exercise, Rest) in the Cerebellum Lobule 6, Vermis, or Hippocampus proper (see Table and Figure 4). This suggests that the reported significant interaction effects of age are relatively selective.

Importantly, the plots from the regions demonstrating significant interactions show patterns that *pivots* at the lower ages such that the slope associated with the Exercise condition is less negatively sloped compared to for the Rest condition. That is, the effects of exercise on local neural differentiation in these regions brings the differentiation levels in the *older* participants closer to that of the levels observed in the relatively younger - although still defined as senior - participants. This is most clearly depicted in the right Crus 2 and left PHG/EC plots. Overall, these finding suggests a re-differentiation of local fMRI signals in older age after a single session of exercise in both hippocampal formation and cerebellar regions.



**Figure 4**: Neural differentiation interacts with Age and the experimental conditions (Exercise and Rest). Plotted results from the six separate post hoc region of interest mixed effect modes. Each region is color coded to match the brain images on the far right. The top four models are associated with cerebellum regions and include Crus 1, Crus 2, Lobule 6, and the Vermis (colored orange, purple, green, and blue respectively). The bottom two models are associated with the parahippocampal gyrus (PHG)/entorhinal cortex EHC) and the hippocampus (colored in violet and green respectively). The regions were selected in a post hoc manner based on the results in Figure and Table 3. For each left and right region the General-Hreg values were extracted from the voxels with the top 20% difference between Rest and Exercise for each individual subject. This resulted in six mixed effects models that quantified the fixed effects of age, condition, the two hemispheres and all interactions between them and also a full set random effect slopes and intercepts. Whole brain General Hreg and the total number of time points were included as nuisance covariates, and ignored. Each plot contains the estimated effect lines and associated raw data points for each set of regions. Data points are the raw General-Hreg values at the corresponding age for each region. The p-values reported at the top of each model output plot are from the interaction effects of interest; the left p-value is the interaction of Rest\_Exercise\*Age, while the right p-value is the interaction of Rest\_Exercise\*Age\*Hemisphere. Significance: **\*** significant at alpha = 0.05; **·** approaching significance at p<0.6. The cerebellar Crus 1 and 2 and PHG/EC each showed a significant interaction effect between age and exercise.

**4. Discussion**

There were three main findings. First, behaviorally, participants were better at suppressing Stroop interference after the Exercise condition, which confirms previous reports of improved cognitive function after aerobic exercise. Second, following exercise there was significantly greater neural differentiation primarily within the cerebellum and hippocampal formation. This suggests that aerobic exercise induces more distinct neural signals within these systems. Third, the increases in neural differentiation in the hippocampal formation and cerebellum were greater as age increased, suggesting that these local neural responses were functionally “re-differentiated” in the older participants. Together, these results suggest that a single session of aerobic exercise leads to increased neural differentiation that is both more pronounced in older ages and related to improvements in executive function.

4.1 Acute Exercise and Executive function*:* The first finding was a significant reduction in the Stroop interference effect in accuracy and thus improved inhibition or selective attention after 30 minutes of Exercise. This finding is in line with previous literature reporting improvements in Stroop interference effects immediately after aerobic exercise (Barella et al., 2010; Chang et al., 2019; Hogervorst et al., 1996; Lichtman and Poser, 1983; Sibley et al., 2006). The findings in this study are particularly noteworthy because performance was measured after an fMRI session that immediately followed the Exercise (or seated Rest) conditions, and thus indicates that these improvements in executive function may persist at least 80 minutes after exercise. This particular finding is supported by work showing an improvement on executive function performance (i.e., a composite score of multiple tasks including the Stroop task performance) lasted up to 2 hours after aerobic exercise (Basso et al., 2015). These findings support the idea that the specific executive function associated with inhibiting prepotent responses (also referred to as selective attention) is improved up to 80 minutes following 30 minutes of exercise. This general conclusion is in line with other work which indicates that Flanker task performance is better immediately following aerobic exercise in young (Coleman et al., 2018) and older adults (Kamijo et al., 2009b).

4.2 Exercise Induced Greater Differentiation in Neural Signals: The main novel finding of this study was that there was higher neural differentiation in multiple brain regions immediately after 30 minutes of Exercise relative to seated Rest. The most prominent findings are that there is greater differentiation in the hippocampal formation and in the cerebellum. Generally, these findings of improved local differentiation are associated with better differentiated local neural signals, and thus considered a beneficial functional outcome of aerobic exercise. This is of particular importance to the older population studied here, which are considered to undergo age-related de-differentiation in multiple neural systems (Carp et al., 2011b; Dennis and Cabeza, 2011; Park et al., 2004). Thus, a single session of aerobic exercise appears to promote local neural differentiation. Further work is required to determine if this acute effect accumulates longitudinally over repeated exercise sessions, and in doing so counteracts the effects of age-induced de-differentiation in the brain.

Regarding specific neural findings, to start, we address the hippocampus, as it is the brain region most reported on in the exercise neuroscience literature, and is particularly important to the effects of exercise on the aging brain (Bettio et al., 2017; Erickson et al., 2011; Voss et al., 2019). The largest cluster in the cerebrum, and the region with the highest reproducibility (both for the Exercise-Rest contrast and within each of the Exercise and Rest conditions), was the left hippocampus. This speaks to the selectivity of this increase in differentiation to the hippocampus relative to a whole-brain response. This finding supports the interpretation that aerobic exercise leads to acute increases in the severability of local neural signals within the hippocampus, which is known to be associated with episodic memory performance and memory declines in old age (Yassa et al., 2011).

These findings line up with both structural and functional neuroimaging data indicating exercise-induced changes in the hippocampal formation after a single session of aerobic exercise. In particular, the function of the hippocampus and surrounding cortex (parahippocampal and entorhinal cortices) has been shown to improve after acute aerobic exercise as measured with an fMRI pattern separation task (Suwabe et al., 2018), a working memory fMRI task (Li et al., 2019), semantic memory task (Won et al., 2019b; previous study using a subset of the data reported here), and flanker task (Won et al., 2019a; previous study using a subset of the data reported here). These findings also fit with studies finding microstructural changes in the hippocampus in older adults after a single session of aerobic exercise (Callow et al., 2021; previous study using a subset of the data reported here), which may be associated with changes in the extracellular space and glial, synaptic, and dendritic processes within the hippocampus. Furthermore, several studies in younger adults have indicated that hippocampal cerebral blood flow improves after a single session of exercise (Smith et al., 2010; Steventon et al., 2019). Although we did not predict a difference in responsiveness to exercise between the right and left hippocampus, our findings were limited to the left hippocampus. This finding is in line with a recent meta-analysis of long term effects of aerobic exercise on hippocampal volume which reported bilateral effects, but a notably more consistent and stronger effect in the left hippocampus relative to the right (Firth et al., 2018b). Finally, given the prominent role of the hippocampal formation in episodic memory performance, it is predicted that aerobic exercise will not only lead to improved behavioral episodic memory performance (as reported by several other labs: (Bernstein and McNally, 2019; Coelho-Júnior et al., 2021; Labban and Etnier, 2011; Sng et al., 2018; Suwabe et al., 2017b)), but that the magnitude of improvements in memory will be related to the magnitude of increased neural differentiation in the hippocampus. This was not examined in this study because episodic memory behavior was not acquired; but this will be addressed in future studies on the impact of aerobic exercise on hippocampal system processing.

Next, the largest cluster of differences due to Exercise with most robust effects was in the cerebellum. Although differences in task-based fMRI (i.e., working memory task) immediately after exercise have been reported in the cerebellum for both prepubescent children (Chen et al., 2016) and young adults (Li et al., 2019), this is the first work to report local differences in the cerebellum in older adults. Generally, the cerebellum is known to be associated with both more automatic sensory or motor processing as well as higher cognitive function (Koziol et al., 2014). Computationally the cerebellum is thought to act as a co-processor with specific cerebrum centers to which it is connected (D‘Angelo and Casali, 2013). With respect to higher cognitive functions, the cerebellum is thought to be critical for sensory discrimination, associative learning (Cayco-Gajic and Silver, 2019) and cognitive control (Abrahamse et al., 2016). Generally, the more medial regions, including the Vermis, are thought to be associated with the more automatic sensory and motor functions, while the more lateral regions are thought to be associated with higher level cognition functions (Klein et al., 2016). In one recent study, increased functional connectivity between the cerebellum and the precuneus and inferior parietal lobule after 12-weeks of exercise training in patients diagnosed with mild cognitive impairment were associated with improved verbal fluency performance (Won et al., 2021). The current findings support the notion that the acute effects of exercise on executive function networks involve cerebellar circuits.

An interesting observation is that the predominance of significant differences in neural heterogeneity after exercise were observed in brain regions associated with the separation of neural signals (i.e., pattern separation) in the brain. As described by Cayco-Gajic and Silver (2019), both the hippocampus (specifically the dentate gyrus) and the cerebellar cortex contain circuitry known to perform pattern separation, and both regions were observed to show significant differences in this study. The critical computation performed by this circuitry relates to separating overlapping input neural signals. Whereas for the hippocampal this computation is critical for encoding distinct memory representations, for the cerebellum this is critical for sensory discrimination, associative learning (Cayco-Gajic and Silver, 2019) and cognitive control (Abrahamse et al., 2016). This leads to an interesting hypothesis that exercise particularly affects those circuits which are suited for pattern separations, a key mechanism of which is divergent excitatory projections with feedback inhibition.

4.2 Age Interacts with the Effects of Exercise on Neural Differentiation: The third finding a repoted interaction effect between age and the effect of exercise within the Crus 1, Crus 2 and PHG/EC. These effects were comparatively selective in that they were not observed in other adjacent areas such as the hippocampus proper or cerebellar vermis/lobule 6. It is important to note that all of these interaction effects with age demonstrated a pattern that fit with the “exercise induced neural re-differentiation hypothesis”. This hypothesis posits that exercise leads to an increase in neural differentiation at older ages such that the older brain regions become functionally younger, i.e., re-differentiated. The pattern that supports this hypothesis the best is when the relationship between age and neural differentiation has a negative slope during Rest, and the relationship between age and neural has a horizontal slope during Exercise. Although this pattern is approximately present in each region with a significant interaction effect, it is most pronounced in the right Crus 2 and left PHG/EHC (See Figure 4).

One important question pertains to what the consequences of this pattern of neural re-differentation would be. One interpretation that suggests multiple future studies is that this exercise induced increase in neural differentiation in the lateral cerebellum and PHG/EHC would lead to improved performances that rely on these areas. For instance, given that Exercise did lead to improvements in Stroop task performance in this work, and that the lateral cerebellum function (Taylor et al., 1997), it is reasonable to hypothesize that the magnitude of Stroop task improvement after exercise may be related to the magnitude of differences in the lateral cerebellar General-Hreg. We did not examine this here given concerns over exploring individual differences in brain-behavior relationships with a limited number of subjects (Marek et al., 2022). This hypothesis is compelling because the Crus 1 and 2 are known to be functionally connected to the prefrontal cortex (Dorsal Lateral Prefrontal Cortex including Brodmann’s Area 46) (Buckner, 2013; Krienen and Buckner, 2009; Stoodley and Schmahmann, 2009), a cortical region important for mediating cognitive control via increased selective attention to task-relevant information (Vanderhasselt et al., 2009). Furthermore, it is predicted that performance on cognitive tasks that utilize the PHG/EC such as declarative memory tasks involving associative memory, source memory, and recollection tasks (Davachi et al., 2003; Diana et al., 2010). Thus, acute neural re-differentiation of hippocampal formation and lateral cerebellar areas provide an intriguing hypothesized mechanism for acute improvements in executive function (Barella et al., 2010; Kamijo et al., 2009b) and memory (Coelho-Júnior et al., 2021; Suwabe et al., 2017b; Voss et al., 2019) after a single session of exercise.

Given we did hypothesize that such age and exercise effect interactions would be observed in the hippocampus proper, is worth considering why we did not observe this. One possibility is that the hippocampus is not only impacted by pattern separation processes - sourced in the dentate gyrus - but also by pattern completion levels - sourced in CA 2/3 (Knierim and Neunuebel, 2016; Lee et al., 2015; Riphagen et al., 2020; Rolls, 2013). That is, a mixture of effects driven by pattern separation and pattern completion neural circuitry may confound any age interaction effects reported here for the hippocampus. Therefore, the hippocampus proper may require a more detailed investigation (i.e., that separates the dentate gyrus from the CA 3) in order to better pry apart the interaction effects of exercise and age.

4.3 Intra-class Correlation (ICC) of General-Hreg: In examining the split half ICCs we report that the General-Hreg method provides highly reliable estimates of neural differentiation within each condition (Exercise: mean ICC = .66; Rest: mean ICC = 0.64). This within condition reliability was much higher than typically reported in a recent meta-analysis from Nobel et al., (2019) (overall functional connectivity ICC = .29). Comparing the ICC values in the current study to that of previous functional connectivity studies is appropriate given that the General-Hreg measure is essentially a functional connectivity measure applied across adjacent voxels. Furthermore, it was also higher than that reported in a recent task-functional MRI meta-analysis of ICC values from Elliot et al. (2020) (overall task-fMRI ICC= .397). Overall, this helps to confirm that the General-Hreg measures within each condition as ‘good’ to ‘strong’ in many regions, and thus provides support for the utility of this measure in studies looking at individual differences in local neural differentiation (Elliott et al., 2020).

4.4 Conclusion: Overall, these findings support the hypothesis that after just 30 minutes of moderate intensity aerobic exercise there is an increase in local neural signal differentiation within brain regions reported to play a role in the separation of neural signals that include the hippocampal formation and the cerebellar cortex (Cayco-Gajic and Silver, 2019). Further, these effects interact age in a manner that suggests that one mechanism of functional improvement due to exercise is a “neural re-differentiation” of local neural signals in the hippocampal formation and cerebellum. One interesting hypothesis for further studies is that acute exercise would lead to improvements in any cognitive function underlying such neural circuitry including memory (PHG/EC) or a wide array of sensory, motor, and cognitive tasks underlying the lateral cerebellum. Future work is required to explore these possibilities, and also determine whether these acute effects of exercise on neural differentiation actually stimulate long-term, beneficial re-differentiation of the aging brain.

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