Title: Resting state brain network segregation is associated with walking speed and working memory in older adults

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ABSTRACT

Older adults exhibit larger individual differences in walking ability and cognitive function than young adults. Characterizing intrinsic brain connectivity differences in older adults across a wide walking performance spectrum may provide insight into the mechanisms of functional decline in some older adults and resilience in others. Thus, the objectives of this study were to: (1) determine whether young adults and high- and low-functioning older adults show group differences in brain network segregation, and (2) determine whether network segregation is associated with working memory and walking function in these groups. The analysis included 21 young adults and 81 older adults. Older adults were further categorized according to their physical function using a standardized assessment; 54 older adults had low physical function while 27 were considered high functioning. Structural and functional resting state magnetic resonance images were collected using a Siemens Prisma 3T scanner. Working memory was assessed with the NIH Toolbox list sorting test. Walking speed was assessed with a 400 m-walk test at participants' self-selected speed. We found that network segregation in mobility-related networks (sensorimotor, vestibular, and visual networks) was higher in younger adults compared to older adults. There were no group differences in laterality effects on network segregation. We found multivariate associations between working memory and walking speed with network segregation scores. Higher right anterior cingulate cortex network segregation was associated with higher working memory function. Higher right sensorimotor, right vestibular, right anterior cingulate cortex, and lower left anterior cingulate cortex network segregation was associated with faster walking speed. These

results are unique and significant because they demonstrate higher network segregation is largely related to higher physical function and not age alone.

Keywords: Functional connectivity, resting state, aging, fMRI, behavior, segregation

Highlights:

- Segregation is lower in mobility-related networks in older adults vs younger adults.
- Older adults with high and low physical function have segregation differences.
- Laterality of functional network segregation is not different between age groups.
- Higher network segregation is associated with faster walking speed.

1. INTRODUCTION

In 2015, persons aged 65 years or older represented ~ 9% of the world population; by 2050 this number is expected to increase to ~17% (He et al., 2016). Many adults over the age of 65 have difficulty walking, which can decrease their quality of life and result in a variety of complications such as increased risk of cardiovascular disease and decreased bone health (Inouye et al., 2007; Shafrin et al., 2017). However, some older adults (i.e., “super agers” or “master athletes”) maintain their function and outperform their age-matched peers (Geard et al., 2017; Klinedinst et al., 2023). Characterizing differences in brain function across a wide performance spectrum may provide insight into the mechanisms of walking decline in some older adults and resilience in others.

Measures of how functional networks in the brain are connected at rest can inform us about intrinsic brain characteristics, including the capacity for communication within and across networks. Resting-state functional connectivity is quantified by fluctuations in the BOLD signal during rest (i.e., with participants lying in the magnetic resonance (MR) scanner without performing a task) (Biswal et al., 1995). By using graph theory approaches, we can model the brain as nodes (regions of interest; ROIs) and edges (connections identified by correlation of fluctuations in the BOLD signal across different brain regions). This approach has allowed the identification of networks of nodes that are functionally but not necessarily monosynaptically connected.

Neural de-differentiation (or more diffuse brain activity) has been observed in task-based functional neuroimaging studies of older adults (e.g., greater bilateral

prefrontal activation during cognitive tasks) (Cabeza, 2002; Park et al., 2004). In healthy young adults, there is a balance between within- and between-network connectivity. In typical aging, functional brain networks are less connected within-network, but more connected between networks, leading to less segregated functional networks (Chan et al., 2014; Deery et al., 2023). This example of age-related neural dedifferentiation describes decreased functional specificity of localized brain regions and broader connectivity between-networks (Koen et al., 2020; Li and Lindenberger, 1999). Importantly, lower sensorimotor network segregation is associated with lower sensorimotor function in older adults (Cassady et al., 2020; Cassady et al., 2019). Reduced network segregation reflects more overlapping or interacting processes in the aging brain, which is potentially less efficient. A better understanding of associations between physical and cognitive function with age differences in network segregation is important, and may help identify therapeutic target(s) for age-related neurological impairment. Less segregated networks in Alzheimer's disease and mild cognitive impairment are associated with poorer cognitive function and greater tau burden (Ewers et al., 2021; Fu et al., 2022; Iordan et al., 2022; Steward et al., 2023). In addition, higher network segregation is predictive of greater intervention-related cognitive gains (Cohen and D'Esposito, 2016; Gallen et al., 2016; Gallen and D'Esposito, 2019).

In addition to reduced network segregation with age, the human brain also undergoes lateralization changes with aging (Sugiura, 2016). Reduced lateralization in functional brain activation has been shown during cognitive task performance for older adults. This finding has led to the development of the hemispheric asymmetry reduction in older adults (HAROLD) model, which proposes that aging is associated with a more

bilateral distribution of brain activity (Cabeza, 2002). This model has also been supported in studies of motor control. Young adults generally show higher activation in the sensorimotor cortex contralateral to the moving limb. In contrast, there is increased brain activation in the hemisphere ipsilateral to the moving limb in older adults, resulting in brain activation that is more bilaterally symmetrical and diffuse (Bernard and Seidler, 2012; Seidler et al., 2010). Independent component analysis has demonstrated that resting-state functional connectivity in the sensorimotor networks has both left and right dominant components, but lateralization decreases with increasing age (Agcaoglu et al., 2015). This finding suggests that the age-related changes in lateralization are not limited to cognitive processing, but rather reflect a more general shift towards a more bilateral distribution of brain activity. The factors that contribute to age-related changes in lateralization are still not fully understood, but it is thought that both genetic and environmental factors play a role (Liu et al., 2009; Yoon et al., 2010). Age-related changes in resting state brain network segregation have been speculated to play a role in cognitive decline in older adults (Deery et al., 2023), but the role of hemisphere-specific network segregation on sensorimotor function has not been investigated. Investigating the hemispheric-specific role in cognitive and walking function has the potential to identify new neural mechanisms underlying mobility variability in older adults.

The objectives of this study were to: (1) determine whether young adults and older adults with high and low physical function show group differences in resting state brain network segregation, and (2) determine whether resting state brain network segregation is associated with working memory and walking speed in these groups.

Neural de-differentiation is associated with age-related decline in cognition (Koen et al., 2020; Li and Lindenberger, 1999; Li et al., 2001). In this study we specifically assessed working memory performance, since working memory is a key function driving various cognitive behaviors (Dang et al., 2014; Engle et al., 1999; Kane et al., 2004). We also assessed walking speed; walking speed is associated with overall health, independence level, fall risk, cognition, and quality of life in community-dwelling adults (Abellan van Kan et al., 2009; Kim et al., 2016; Middleton et al., 2015). We recruited young adults and older adults who were categorized into high and low function according to their performance on a standardized battery of physical tasks. We hypothesized that: (1a) older adults would demonstrate lower functional sensorimotor network segregation compared to younger adults, and that older adults with lower mobility function would demonstrate greater differences than older adults with higher mobility function when compared to healthy younger adults, (1b) older adults with lower mobility function would demonstrate less lateralized functional connectivity compared to older adults with higher mobility function and younger adults, and (2) higher brain network segregation would be associated with faster walking speed and better working memory performance in older adults.

2. MATERIAL AND METHODS

2.1. Participants

This study enrolled community-dwelling volunteers with 23 young adults, and 87 older adults. Participants recruited for this study were part of a larger NIH U01 study at the University of Florida (U01AG061389). Briefly, all participants were required: to be able to walk 400 m within 15 minutes without sitting or assistance; be English speaking; to have no significant medical event within the past six months; to have no major neurological injuries (e.g., stroke, vestibular dysfunction, traumatic brain injury, dementia); and to meet MR imaging eligibility (Full exclusion and inclusion criteria outlined: Clark et al., 2019). Older adults were further categorized into their physical function level, based on their Short Physical Performance Battery score. The battery included a short distance usual walk speed test, balance test and chair stand speed. Each test was graded according to normative values and tallied for a total score that ranged from 0-12 with 12 serving as the highest physical function (Guralnik et al., 1994). The study screened in those who scored > 10 for the high functioning (n = 27) group and those who scored ≤ 10 for the low functioning (n = 54) group. The sample size for the older adults with lower physical function was the largest because it was the target sample of interest; in most studies of human aging, the older adult cohort is relatively fit due to inclusion/exclusion criteria (Brach et al., 2023). Therefore, we especially wanted to study the variability in resting state brain mechanisms in older adults with lower physical function. In addition, the sample size between older adults with lower function and higher function came out unequal because our data collection was disrupted due to the COVID-19 pandemic. Group characteristics for participants who were included in the final analysis of this study are presented in Table 1. Participants who identified as left-handed were excluded from the analyses to investigate laterality (2 younger adults,

2 older adults with higher physical function, and 4 older adults with lower physical function were excluded; participants included in final analysis included 21 younger adults, 27 older adults with higher physical function, and 54 older adults with lower physical function). Participants' written informed consent was obtained prior to the study, which was approved by the Institutional Review Board of the University of Florida, Gainesville, FL, USA (Protocol# 201802227).

2.2. Brain Imaging

Brain images were collected using a Siemens Prisma 3T scanner (Siemens Healthcare, Erlangen, Germany) with a 64-channel head coil. Structural T1-weighted images were collected using a magnetization-prepared rapid gradient echo (MPRAGE) sequence with the following parameters: repetition time (TR) = 2000 ms, echo time (TE) = 2.99 ms, flip angle = 8°, FOV = 167 mm × 256 mm × 256 mm, voxel size = 0.80 mm^3. Whole-brain resting-state functional MR images were collected using a multiband, interleaved echo planar imaging (EPI-BOLD) sequence with the following parameters: TR = 1500 ms, TE= 30 ms, flip angle= 70°, FOV= 240 x 240 x 165 mm, matrix=64x64, voxel size= 2.5 mm^3, 66 axial slices, slice thickness = 2.5 mm, acceleration factor = 3, duration = 8 minutes.

2.3. Neuroimaging preprocessing

Neuroimaging data analyses were performed using the Statistical Parametric Mapping 12 software (SPM12; www.fil.ion.ucl.ac.uk/spm) and the CONN toolbox version 19 (https://www.nitrc.org/projects/conn) (Whitfield-Gabrieli and Nieto-Castanon, 2012) in MATLAB 2020b (Mathworks, Natick, MA, USA). Preprocessing steps included: slice-time correction, realignment and unwarping, movement correction, bias correction, co-registration of functional and anatomical images and normalization to a Montreal Neurological Institute 152 (MNI152) template using Advanced Normalization Tools (ANTs, https://github.com/ANTsX/ANTs) (Avants et al., 2011), then spatial smoothing (Gaussian smoothing kernel 4mm full width at half maximum). We used the Artifact Detection Tool (ART, www.nitrc.org/projects/artifact_detect/) to detect and correct for movement artifacts. Within a run, a volume was considered an outlier and covaried out if the participant's composite movement was equal to or greater than 2.5 mm. Additional denoising was done with CONN; we filtered the data using a temporal band-pass filter of 0.008-0.09 Hz to examine the slower frequency band of interest and to exclude higher frequency sources of noise.

To improve cerebellar normalization to MNI space, the cerebellum from each anatomical image was segmented from the brain using the CERES (CEREbellum Segmentation) (Romero et al., 2017) pipeline. They were then normalized to the spatially unbiased infra-tentorial template (SUIT) cerebellar template (Diedrichsen, 2006).The SUIT cerebellar template shows greater anatomical detail than the whole brain MNI152 template, allowing for more accurate cerebellar normalization (Diedrichsen, 2006). We applied the CERES-generated cerebellar masks to the co-registered functional images, allowing extraction of the cerebellum. The extracted fMRI

cerebellums were then normalized to the SUIT cerebellar template using the warp fields generated for the T1 scans.

2.4. Connectivity analysis

We used the CONN (v19b) toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012) for all connectivity analyses. For identification of our networks we used a seed-to-voxel analysis with seed regions of interest (ROIs). We derived the seed ROI coordinates from previous findings that identified brain regions to be associated with mobility (Left and right sensorimotor (Zhao et al., 2019) , left and right vestibular (zu Eulenburg et al., 2012), default mode (Schmidt et al., 2017), and visual (Shen et al., 2019)). The left and right dorsolateral and left and right anterior cingulate cortex seed ROIs, were identified using custom ROIs for this study. The seed regions were identified using the Neurosynth meta-analysis with “working memory” search terms for the dorsolateral prefrontal cortex ROI and “anterior cingulate” for the anterior cingulate cortex ROI (Yarkoni et al., 2011). We had a priori hypotheses regarding the dorsolateral prefrontal cortex and the anterior cingulate cortex, therefore we wanted to identify brain networks associated with each of these regions of interest, and also preserve the typical brain networks used in other whole brain network analyses (e.g., Gordon et al., 2016; Power et al., 2011)). We then performed the seed-to-voxel analyses on 110 subjects from the Washington University 120 dataset (data were obtained from the OpenfMRI database, accession number: ds00243) – First seed-to-voxel analysis was performed with the MNI normalized whole brain images as the target (with the cerebellum masked), then a

second seed-to-voxel analysis was performed with the SUIT normalized cerebellum images as the target. For each seed-to-voxel analysis performed, we implemented a second level analysis using the default statistical thresholds for cluster-based inferences (Worsley et al., 1996). The resulting T map images with a-values over 12, and local maxima separated by 8 mm were identified. A 4 mm radius sphere was created around each cluster. We combined the cerebellar coordinates with the whole brain coordinates for each respective seed. In total, we identified 279 nodes in 10 networks for analysis in this study: 1. Left sensorimotor network (26 nodes), 2. Right sensorimotor network (22 nodes),3. Left vestibular network (26 nodes), 4. Right vestibular network (26 nodes), 5. Left dorsolateral prefrontal cortex network (21 nodes), 6. Right dorsolateral prefrontal cortex network (22 nodes), 7. Left anterior cingulate cortex network (27 nodes), 8. Right anterior cingulate cortex network (30 nodes), 9. Default mode network (48 nodes), and 10. Visual network (31 nodes), (Figure 1A; MNI coordinates for the ROIs are in Supplementary table 1).

We applied a node-to-node first-level functional connectivity analysis to the current dataset using the 10 networks described above. Resting-state time series within each node were extracted from the unsmoothed functional images and the mean time series was computed for each node (averaged across voxels). Then, we computed a correlation analysis for all nodes, producing a 279 x 279 cross-correlation matrix for each participant. The correlation coefficients (i.e., graph edges) were converted into z-values using Fisher's r-to-z transformation. Negative-weighted edges were set to zero in each participant's correlation matrix to avoid potential misinterpretation of negative edge weights (Chan et al., 2014).

We quantified network segregation for each network as the difference of the mean within-network connectivity and the mean between-network connectivity divided by the mean within-network connectivity (Eq. 1, Figure 1B).

$$Network\ segregation = \frac{\bar{Z}_w - \bar{Z}_b}{\bar{Z}_w} \qquad \text{(Eq. 1)}$$

Within-network connectivity ($\bar{Z}_w$) is the mean of Fisher z-transformed correlations between nodes within each network and between-network connectivity ($\bar{Z}_b$) is the mean of Fisher z-transformed correlations between nodes of one network to all nodes in all other networks (Chan et al., 2014).

2.5. Walking function

To measure walking speed (m/s), we conducted a 400 m-walk test where participants were asked to walk around a pair of traffic cones set 20 meters apart. We instructed participants to complete 10 laps (each lap being 40 meters long) by walking at their preferred pace. Participants could stop for up to 1 minute if they were feeling fatigued or unable to continue.

2.6. Cognitive Function

We used a subset of tests from the NIH toolbox cognitive battery to assess cognitive function as part of the larger study. The list sorting test assessed working memory

capacity and recall, presenting the participants with a series of visual and aural stimuli using an iPad (Apple Inc., Cupertino, CA) (Tulsky et al., 2014). Each stimulus was displayed on the iPad screen for two seconds followed by an audible stimulus (e.g., an image of a horse was shown on the screen while a computerized voice read the word “horse”). We instructed participants to remember each stimulus, arrange them mentally by size, and then verbally list them in that order. We used the raw score for the working memory test, which ranges from 0 to 26. These scores were chosen over age-corrected standard scores to assess the participant's absolute performance in each test, as opposed to their performance relative to the NIH Toolbox normative sample. This approach to interpreting computed and raw scores aligns with the NIH Toolbox Scoring and Interpretation guide's recommendations.

2.7. Statistical analysis

Group differences in segregation scores, between-network, and within-network connectivity were assessed through one-way ANCOVA, with biological sex as a covariate. Post-hoc pairwise comparisons were conducted with Bonferroni-Holm corrections.

The relationship between functional measures and network segregation scores was assessed with a canonical correlation analysis. Canonical correlation analysis is a multivariate analytical approach that measures the relationship between two datasets. We identified a “Function variable dataset” with walking speed, working memory, age/physical function group, and biological sex, and a “Network segregation dataset”

with network segregation scores from the 10 identified networks. Canonical correlation analysis was conducted twice: first with the age groups (Older adult = -1 | Younger adult = 1), second with the physical function groups, with the younger adults omitted from the analysis (Lower function = -1 | Higher function = 1). We chose to do canonical correlation analysis twice with a maximum of two groups in our categorical variable to maintain simplicity and transparency in the inference. All variables were standardized before canonical correlation analysis to make the canonical coefficients comparable across the variables. Canonical correlations range from 0 to 1, 0 being no relationship and 1 being a perfect relationship between the two datasets.

As a post-hoc analysis, we tested two multiple linear regression models with age groups: 1. Walking speed and 2. Working memory, both with biological sex covaried out. Similarly, for functional groups we tested multiple linear regression models with older adults only. Due to missing data, one young adult was excluded from the linear regression with walking speed, and 3 young adults, 4 older adults with high physical function, and 19 older adults with lower physical function were excluded from the regressions with working memory scores. Univariate linear regressions between the dependent variable and segregation scores and age groups for each network determined the variables and network-age group interactions that were considered in the step-wise multiple regression model.

All statistical significance was established with an alpha level = 0.05. We used RStudio (R version 4.3.0) for analysis. For canonical correlation analysis results, we applied the “CCA” package (González et al., 2008) which includes a regularization approach to handle missing data.

3. RESULTS

3.1. Segregation scores

There were significant group effects for the sensorimotor network ($F(2, 98) = 5.14$, $p = 0.008$) and vestibular network ($F(2, 98)= 21.47$, $p <0.001$), but not for dorsolateral prefrontal cortex ($F(2,98)=2.67$, $p = 0.074$) or anterior cingulate cortex networks ($F(2,98)=1.97$, $p =0.145$) (Figure 2, Table 2). Sensorimotor network segregation was lower in older adults with lower physical function compared to younger adults and older adults with higher physical function, but sensorimotor network segregation was not significantly different between young adults and older adults with higher physical function. The left sensorimotor network was less segregated compared to the right ($F(1, 98) = 64.58$, $p < 0.001$); there were no Group x Laterality interaction effects ($F(2,98) = 0.96$, $p = 0.385$). Vestibular network segregation was higher in young adults compared to both older adult groups with higher and lower physical function. Older adults with higher physical function had higher vestibular network segregation compared to older adults with lower physical function. Left vestibular network was less segregated compared to the right ($F(1,98) = 158.73$, $p < 0.001$), but there were no Group x Laterality interaction effects ($F(2,98) = 0.34$, $p =0.711$).

In contrast to the sensorimotor and vestibular networks, dorsolateral prefrontal cortical network was more segregated in the left compared to the right ($F(1,98) = 14.88$, $p < 0.001$). Anterior cingulate cortex network was less segregated in the left compared

to the right ($F(1,98) = 36.62$, $p < 0.001$). Group x Laterality interaction effects were not statistically significant for these two networks (Dorsolateral prefrontal cortex: $F(2,98) = 1.09$, $p = 0.339$; Anterior cingulate cortex: $F(2,98) = 0.31$, $p = 0.735$)

We included the visual network in the analyses because of its role during walking navigation, and we included the default mode network as a 'control' network (we did not expect that default mode network segregation would be associated with working memory performance or walking speed). Since these two networks have medial seed regions, they do not contain laterality outcomes; thus, we present them separately in Figure 3. The visual network was more segregated in younger adults compared to older adults with high physical function, but not significantly different compared to older adults with low physical function (Group effect: $F(2,98)=3.27$, $p = 0.042$). Default mode network connectivity was not significantly different between groups ($F(2,98)=0.41$, $p = 0.668$).

3.2. Within- and between-network connectivity

To parse out the root of segregation score differences, we also examined group and laterality differences in within-network connectivity and between-network connectivity, both of which make up the network segregation scores (Figure 3 C-F and Figure 4, Tables 3 and 4). In general, within-network connectivity was higher in younger adults compared to older adults. Sensorimotor within-network connectivity was lower in older adults with lower physical function compared to younger adults and older adults with higher physical function, but was not significantly different between younger adults

and older adults with higher physical function (Group effect: $F(2,98) = 9.79$, $p<0.001$). Left and right sensorimotor within-network connectivity was not significantly different ($F(1,98) = 2.00$, $p = 0.161$). Vestibular and anterior cingulate cortex within-network connectivity was significantly different between all groups (Vestibular: $F(2,98) = 21.98$, $p<0.001$; Anterior cingulate cortex: $F(2, 98) = 18.06$, $p<0.001$); within-network connectivity was higher in younger adults compared to both older adult groups, and within-network connectivity was higher in older adults with higher physical function compared to older adults with lower physical function. Dorsolateral prefrontal cortex within-network connectivity was higher in young adults compared to both older adult groups, but dorsolateral prefrontal cortex network within-network connectivity was not significantly different between older adults with higher and lower physical function (Group effect: $F(2, 98) = 15.59$, $p < 0.001$).

Group x Laterality interaction effect on within-network connectivity was significant for the vestibular network, but not for sensorimotor, dorsolateral prefrontal cortex, and anterior cingulate cortex networks (Interaction effect, Vestibular: $F(2,98) = 9.08$, $p<0.001$; Interaction effect, Sensorimotor: $F(2,98) = 0.19$, $p = 0.824$; Interaction effect, Dorsolateral prefrontal cortex: $F(2,98) = 0.30$, $p = 0.740$; Interaction effect, Anterior cingulate cortex: $F(2,98) = 0.57$, $p = 0.566$). Post-hoc tests on within-group differences in left and right vestibular within-network connectivity showed that all groups had lower connectivity in the left network compared to the right (Supplementary table 2; all $p<0.010$); Left and right within-network connectivity differences were largest in the younger adult group for the vestibular network (Young adults group estimate = -0.049;

Older adults with higher physical function group estimate = -0.016; Older adults with lower physical function group estimate= -0.017).

Default mode within-network connectivity was different between all groups ($F(2, 98) = 10.58$, $p < 0.001$; Figure 3 C-D); younger adults had higher within-network connectivity compared to both older adult groups, and older adults with higher physical function had higher within-network connectivity compared to older adults with lower physical function. Visual within-network connectivity was higher in younger adults compared to both older adults groups, but group differences between older adults with higher and lower physical function was not statistically significant (Group effect: $F(2,98) = 10.80$, $p < 0.001$).

In general, between-network connectivity was higher in younger adults compared to older adults. Sensorimotor, vestibular, dorsolateral prefrontal cortex, and anterior cingulate cortex between-network connectivity were lower in older adults with lower physical function compared to young adults and older adults with higher physical function, but within-network connectivity was not significantly different between younger adults and older adults with higher physical function (Group effect, Sensorimotor: $F(2,98) = 9.41$, $p < 0.001$; Group effect, Vestibular: $F(2,98) = 10.45$, $p < 0.001$; Group effect, Dorsolateral prefrontal cortex: $F(2,98) = 10.01$, $p < 0.001$; Group effect, Anterior cingulate cortex: $F(2,98) = 11.27$, $p < 0.001$). Between-network segregation was higher in the left compared to the right for sensorimotor, vestibular, dorsolateral prefrontal cortex, and anterior cingulate cortex networks (Sensorimotor: $F(1,98) = 267.32$, $p < 0.001$; Vestibular = $F(1,98) = 282.99$, $p < 0.001$; Dorsolateral prefrontal cortex: $F(1,98) = 84.15$, $p < 0.001$; Anterior cingulate cortex: $F(1,98) = 10.39$, $p = 0.002$). Group x

Laterality interaction effect on between-network connectivity were significant for the sensorimotor, vestibular, and dorsolateral prefrontal cortex networks, but not for the anterior cingulate cortex network (Interaction effect, Sensorimotor: $F(2,98) = 4.68$, $p = 0.011$; Interaction effect, Vestibular: $F(2,98) = 5.80$, $p = 0.004$; Interaction effect, Dorsolateral prefrontal cortex: $F(2,98) = 4.08$, $p = 0.020$; Interaction effect, Anterior cingulate cortex: $F(2,98) = 0.93$, $p = 0.397$). Post-hoc tests on between-network connectivity showed that all groups had higher between-network connectivity in the left network compared to the right (Supplementary table 3; all p's < 0.001); Left and right between-network connectivity differences were largest in the younger adult group (Sensorimotor: Young adults group estimate = 0.026; Older adults with higher physical function group estimate = 0.024; Older adults with lower physical function group estimate = 0.016; Vestibular: Younger adults group estimate = 0.017; Older adults with higher physical function group estimate = 0.016; Older adults with lower physical function estimate = 0.010; Dorsolateral prefrontal cortex: Younger adults group estimate = 0.002; Older adults with higher physical function group estimate = 0.002; Older adults with lower physical function group estimate = 0.001).

Default mode between-network connectivity was different between all groups ($F(2,98)= 11.98$, $p < 0.001$; Figure 3 E-F); younger adults had higher between-network connectivity compared to older adults with higher and lower physical function, and older adults with higher physical function had higher between-network connectivity compared to older adults with lower physical function. Visual between-network connectivity was lower in older adults with lower physical function compared to younger adults and older adults with higher physical function, but between-network connectivity was not

significantly different between younger adults and older adults with higher physical function (Group effect: $F(2,98) = 9.72$, $p < 0.001$).

3.3. Association with walking speed and working memory performance: Age groups

Canonical correlation analysis with age groups showed that only the first canonical correlation was statistically significant ($r = 0.629$, $p < 0.001$) as shown in Table 5. Figure 5 presents the standardized canonical coefficients for the first dimension. In the Function variable dataset the canonical correlation was most strongly influenced by age groups (Coef. = -0.735) and walking speed (Coef. = -0.441). For network segregation, the canonical correlation was most strongly influenced by right vestibular (Coef. = -0.620), left sensorimotor (Coef. = -0.491), right anterior cingulate cortex (Coef. = -0.435), and left anterior cingulate cortex (Coef. = 0.407). This observation demonstrates that younger adults with faster gait speed had higher network segregation in right vestibular, left sensorimotor, and right anterior cingulate cortex networks, and lower network segregation in the left anterior cingulate cortex network.

We tested two multiple linear regression models with age groups: 1. Walking speed and 2. Working memory, both with biological sex covaried out. For walking speed, univariate linear regressions determined the left and right sensorimotor, left and right vestibular, left and right dorsolateral prefrontal cortex, left and right anterior cingulate cortex, default mode, and visual network segregation, and left anterior cingulate cortex network segregation x Age group interaction to be considered in the step-wise multiple regression model (Supplementary figure 1). Multiple linear regression

with walking speed was statistically significant ($F(6,94) = 7.04$, $p < 0.001$, $r^2 = 0.310$). Right sensorimotor ($p = 0.006$), right vestibular ($p = 0.005$), and left anterior cingulate cortex network segregation ($p = 0.012$), and interaction of left anterior cingulate cortex network segregation x Age group ($p = 0.002$) were significant predictors. Age group ($p = 0.151$) and right anterior cingulate cortex network segregation ($p = 0.080$) were not significant predictors, but contributed to the model (Figure 6A, Table 6).

For working memory, univariate linear regressions determined left and right sensorimotor, left and right vestibular, left and right dorsolateral prefrontal cortex, left and right anterior cingulate cortex, and right anterior cingulate cortex x age group interaction to be considered in the step-wise multiple regression model (Supplementary figure 2). Multiple linear regression with working memory was statistically significant ($F(3,72) = 11.60$, $p < 0.001$, $r^2 = 0.326$); however only right anterior cingulate cortex network segregation ($p = 0.324$), age group ($p = 0.049$) and the interaction of right anterior cingulate cortex network segregation x age group ($p < 0.001$) were contributors to the model (i.e., the model was a univariate model) (Figure 6B, Table 7).

3.4. Association with walking speed and working memory: Physical function groups

Canonical correlation analysis with physical function groups showed that the first ($r = 0.571$, $p = 0.002$) and second canonical correlations ($r = 0.495$, $p = 0.045$) were significant. Dimension reduction showed that the third and fourth dimensions did not significantly explain shared variance (Table 8). Figure 7 presents the standardized canonical coefficients for the first and second dimensions. For the first canonical

dimension, canonical correlation was most strongly influenced by physical function groups (Coef. = -0.687), walking speed (Coef. = -0.429), and working memory performance (Coef. = 0.452) from the function dataset. From the network segregation dataset, left dorsolateral prefrontal cortex (Coef. = 0.558), visual (Coef. = 0.477), and left sensorimotor networks (Coef. = -0.398) most strongly influenced the canonical correlation. This observation demonstrates that older adults in the lower physical function group with slower walking speed had lower network segregation in the left sensorimotor network and higher segregation in the left dorsolateral prefrontal cortex and visual networks.

In the second dimension, canonical correlation was most strongly influenced by walking speed (Coef. = 1.045) along with moderate influences from group (Coef. = -0.640) and sex (Coef. = -0.524) from the function variable dataset, and left anterior cingulate cortex (Coef. = -1.165), right anterior cingulate cortex (Coef. = 0.668), left dorsolateral prefrontal cortex (Coef. = 0.576), and right vestibular (Coef. = 0.512) network segregation data. This observation demonstrates that older adults in the lower physical function group with faster walking speed who were more likely to be females had lower network segregation in left anterior cingulate cortex, and higher network segregation in the right anterior cingulate cortex, left dorsolateral prefrontal cortex, and right vestibular networks.

For walking speed with older adults only, univariate linear regressions determined the left and right sensorimotor, left and right vestibular, left and right dorsolateral prefrontal cortex, left and right anterior cingulate cortex, default mode, and visual network segregation to be considered in the step-wise multiple regression model

(Supplementary figure 3). Post-hoc multiple linear regression analysis with walking function was statistically significant ($F(5,75) = 10.27$, $p < 0.001$, $r^2 = 0.406$). Right vestibular ($p = 0.009$), left anterior cingulate cortex ($p < 0.001$), and right anterior cingulate cortex network segregation ($p = 0.008$), and functional group ($p < 0.001$) were significant predictors. Right sensorimotor network segregation ($p = 0.064$) was not a significant predictor but it contributed to the model. The correlation coefficient estimate for the left anterior cingulate cortex was negative, while all other correlation coefficients were positive (Figure 6, Table 9). For working memory, none of the network segregation scores were significantly associated in the univariate analysis, so no multiple linear regression model was tested (Supplementary figure 4).

4. DISCUSSION

We found that network segregation in mobility-related networks (sensorimotor, vestibular, and visual networks) was higher in younger adults than older adults (Aim 1a). We observed laterality differences in segregation scores for some networks, but no group by laterality interactions (Aim 1b). We observed multivariate associations between working memory and walking speed with network segregation scores (Aim 2). For young and old age groups, canonical correlation analysis demonstrated that right vestibular, left sensorimotor, and right and left anterior cingulate cortex network segregation largely influenced the variability in walking speed observed. Working memory was associated with right anterior cingulate cortex network segregation. For older adults, higher walking speed were largely influenced by left dorsolateral prefrontal

cortex, visual, and left sensorimotor network segregation, although the relationship is complex and may be influenced by other networks as demonstrated by the multiple significant dimensions in the canonical correlation analysis.

4.1. Older adults have lower segregation in mobility-related networks

We observed that older adults with higher physical function had significantly higher segregation in the sensorimotor and vestibular networks than their peers with lower physical function (as determined with the short physical performance battery score). Importantly, while aging studies have consistently demonstrated that older adults have decreased network segregation compared to younger adults (Cassady et al., 2019; Chan et al., 2014; Damoiseaux, 2017; King et al., 2018), we observed that sensorimotor network segregation was not significantly different between younger adults and older adults with high physical function. Whether the network segregation differences between the older adult subgroups reflect cause or effect of physical function differences is unknown and outside the scope of this study. However, sensorimotor and vestibular network segregation appear to be key correlates of physical function in older adults.

In line with our hypothesis and previous studies (Cassady et al., 2020; Cassady et al., 2019), sensorimotor network segregation was higher in younger adults compared to older adults. When we examined several different networks, networks that are important for mobility function such as sensorimotor, vestibular, and visual networks demonstrated group differences in network segregation, but not networks that are

typically involved in more cognitive functions- the dorsolateral prefrontal cortex, anterior cingulate cortex, and default mode networks. Our results suggest that mobility-related networks are more vulnerable to age-related changes in network segregation compared to cognitive function networks. This is in line with behavioral studies that showed that mobility declines precede cognitive decline in older adults and neurodegenerative disease such as Alzheimer's and mild cognitive impairment (Buracchio et al., 2010; Merchant et al., 2021; Skillbäck et al., 2022). Moreover, recent studies on age differences in brain structure reveal the largest age effects in the pre and post central gyri, the thalamus, striatum, and cerebellum (Hupfeld et al., 2022; Taubert et al., 2020).

We expected within-network connectivity to be higher and between-network connectivity to be lower in older adults compared to younger adults. However, in general, younger adults had higher connectivity in both within-network and between-network connectivity. This suggests that the lower network segregation scores in older adults are driven more by within- than between-network connectivity differences.

4.2. Laterality in network segregation is similar between younger and older adults

We showed group x laterality interactions in both the within-network connectivity and between network connectivity. Left and right connectivity differences were largest in the younger adults (as shown by the post-hoc estimates) for vestibular network within-network connectivity, and sensorimotor, vestibular, and dorsolateral prefrontal cortex between-network connectivity. The right vestibular network (which is the dominant side network) had stronger within-network connectivity compared to the left side. This is in

line with expectation and previous work in task-based functional MRI showing reduced lateralization in older adults. Reduced lateralization of the cortex has been shown in seminal work in cognitive processing, resulting in the hemispheric asymmetry reduction in older adults conceptual model (HAROLD) (Cabeza, 2002; Cabeza et al., 2002). However, the group x laterality interactions were not present in the segregation scores. This may suggest that the between-network connectivity may be compensating for the changes in within-network connectivity. It may also be that decreased lateralization with increased age is primarily evident in task-based functional MRI data rather than resting state network segregation scores.

4.3. Resting state network segregation may have differential roles in function

Our canonical correlation analysis demonstrated that not all resting state networks are positively correlated to walking speed and working memory. We expected network segregation to be positively correlated to behavioral function as studies have consistently demonstrated lower network segregation in older adults compared to younger adults, suggestive of decreased efficiency of functional networks (Deery et al., 2023). Studies of cognitive aging have supported the scaffolding theory of aging and cognition (STAC), which suggests that the brain adapts and reorganizes to recruit additional neural resources to preserve cognitive function, which is a result of functional and structural challenges that occur with aging (Park and Reuter-Lorenz, 2009; Reuter-Lorenz and Park, 2014). The networks with negative canonical correlation coefficients in

our model support that STAC may also apply to our multi-function model including walking and cognition.

Anterior cingulate cortex also houses important connections with other brain regions that influence planning, attention, and error correction of movement control. In the working memory specific model, right anterior cingulate cortex network segregation entered the model and had a positive correlation coefficient. This is in line with previous studies that found positive associations between network segregation with episodic memory (Chan et al., 2014) and global cognitive ability (Pedersen et al., 2021). In our model with walking speed, there was a significant age group x left anterior cingulate cortex network segregation interaction, in which the correlation coefficient was positive in younger adults, while the correlation coefficient was negative in older adults (Also shown in Supplementary figure 1G). This is in contrast to our initial hypothesis and provides novel insight that not all lower segregation is related to decreased function. Specifically, the left anterior cingulate cortex network segregation may be playing a compensatory role in older adults with regards to walking function. The left anterior cingulate cortex plays crucial roles in cognition, emotional processing, and error monitoring (Gwin et al., 2011; Peterson and Ferris, 2019; Sipp et al., 2013). The negative correlation of left anterior cingulate cortex may demonstrate that older adults recruit additional neural resources for the left anterior cingulate cortex network for diffuse connectivity to compensate for the lower network segregation in right sensorimotor, right vestibular, and right anterior cingulate cortex networks underlying walking function.

In general, (other than the left anterior cingulate cortex segregation score in multiple linear regression models with walking speed) our step-wise multiple regression models demonstrated a positive relationship with segregation and walking speed, and segregation and working memory performance. This is in support of previous brain-behavior relationships that showed sensorimotor network segregation positively correlated with general sensorimotor function in both younger and older adults (Cassady et al., 2020; Cassady et al., 2019), and higher network segregation is predictive of greater intervention-related cognitive gains (Cohen and D'Esposito, 2016; Gallen et al., 2016; Gallen and D'Esposito, 2019). This may suggest potential interventions to modulate resting state network segregation to increase functional performance in walking or working memory. Recently, transcranial direct stimulation has been shown to increase resting state network segregation (Iordan et al., 2022). Clinical trials to modulate resting state network segregation have mainly been with cognitively impaired populations (i.e., Alzheimer's disease and related dementias and mild cognitive impairment) as they have reduced network segregation (Ewers et al., 2021; Fu et al., 2022; Iordan et al., 2022; Steward et al., 2023). Our results demonstrating association with walking speed and resting state network segregation may suggest that network segregation may serve as a potential therapeutic target to maintain or increase walking speed in typical older adults.

4.4. Limitations

As mentioned in the methods section, this study was part of a larger NIH U01 study at the University of Florida (U01AG061389) (Clark et al., 2019). Due to how the larger study was designed, the brain scans and behavioral tests took place on different days (Average number of days between brain scans and behavioral tests: 81.5 ± 94.8 days). In addition, data collection for the study was interrupted due to the COVID-19 pandemic. This may be a limitation in our current study because there is a variability in the time between the brain scan collection date and the behavioral tests collection date. However, as outlined in our protocol paper (Clark et al., 2019), we had strict exclusion/inclusion criteria, and all participants were required to be free from any major neurological injuries. Therefore, we expect little to no variability in resting state brain network segregation due to the time between the brain scan collection date and the behavioral tests.

Another limitation of the study is that the older adult cohort who participated in this study represent a relatively high functioning population. Even those in the low functioning group who scored 10 or less on the short performance battery score were required to be able to walk 400 m without sitting or assistance of a person or a walking device as part of the inclusion criteria. A challenge of interpreting studies in aging is the wide variability in function of older adults. To facilitate interpretation of results, we included a table on participant characteristics. Readers should take caution in generalizing our findings to a broader population for these reasons.

4.5. Conclusion

Resting state brain network segregation is emerging as a metric that is sensitive to age, mobility and cognitive function, and is predictive of cognitive gains from various interventions (Cohen and D'Esposito, 2016; Gallen et al., 2016; Gallen and D’Esposito, 2019). A greater understanding of this metric and its behavioral correlates may help identify biomarkers related to function. In this study, we demonstrated age and physical function group differences in network segregation and laterality in network segregation, and we identified a multivariate brain-behavior model that showed differential roles of network segregation specific to function. This multivariate model is novel and significant because we show that predominantly higher network segregation is associated with higher walking speed and working memory. These findings highlight the importance of resting state brain network segregation to walking and working memory function in older and younger adults.

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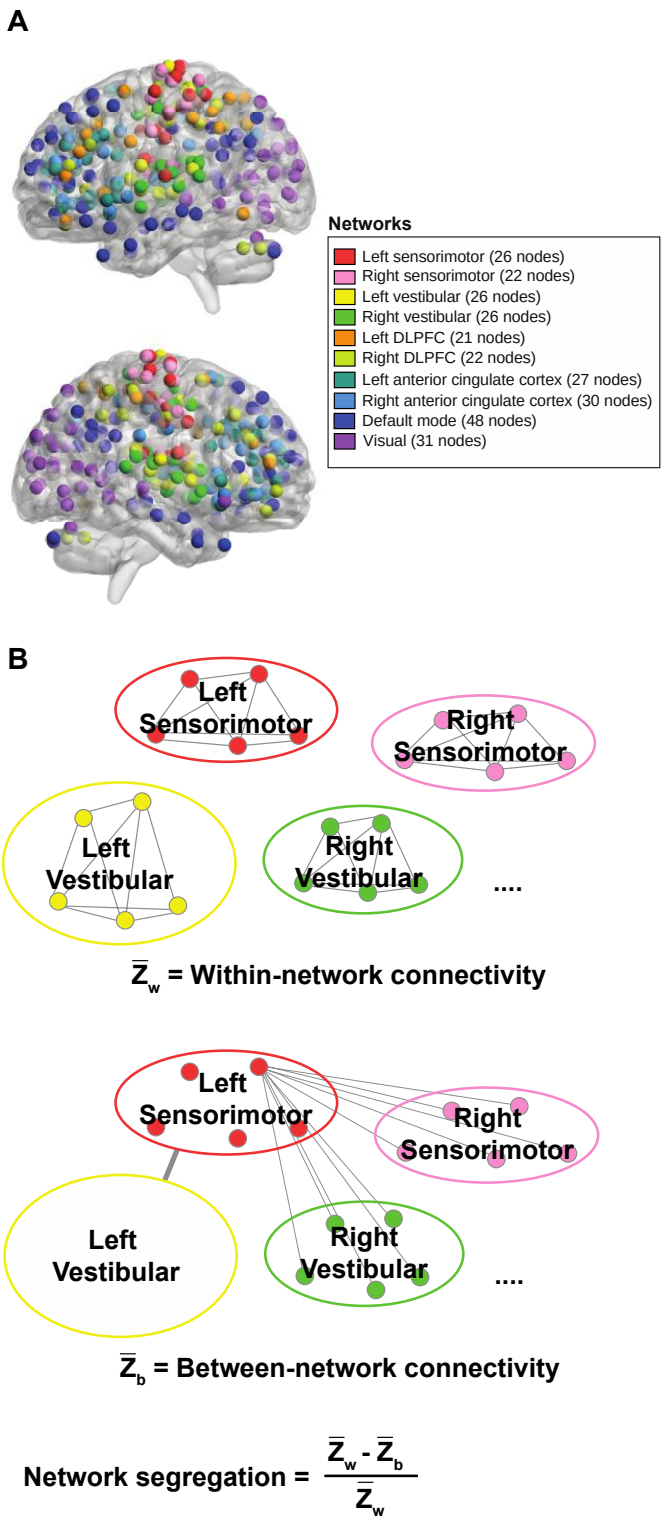

Figure 1. Network segregation analysis. A. Network connectivity nodes included in the study. In total, 279 nodes in 10 networks were identified for analysis. BrainNet Viewer (https://www.nitrc.org/projects/bnv/; Xie et al., 2013) was used to illustrate the nodes. B. For each network, mean within-network connectivity was calculated as the mean z-values between the nodes within the network (i.e., Left sensorimotor within-network connectivity = mean z-value of all nodes in the network to all other nodes in the left sensorimotor network). Between-network connectivity was calculated as the mean of z-values between each node in the network and all the nodes in other networks (i.e., Left sensorimotor between-network connectivity = mean z-value between all nodes in left sensorimotor network and all other nodes in the brain). Network segregation score for each network was quantified as the difference of the mean within-network connectivity and the mean between-network connectivity divided by the mean within-network connectivity.

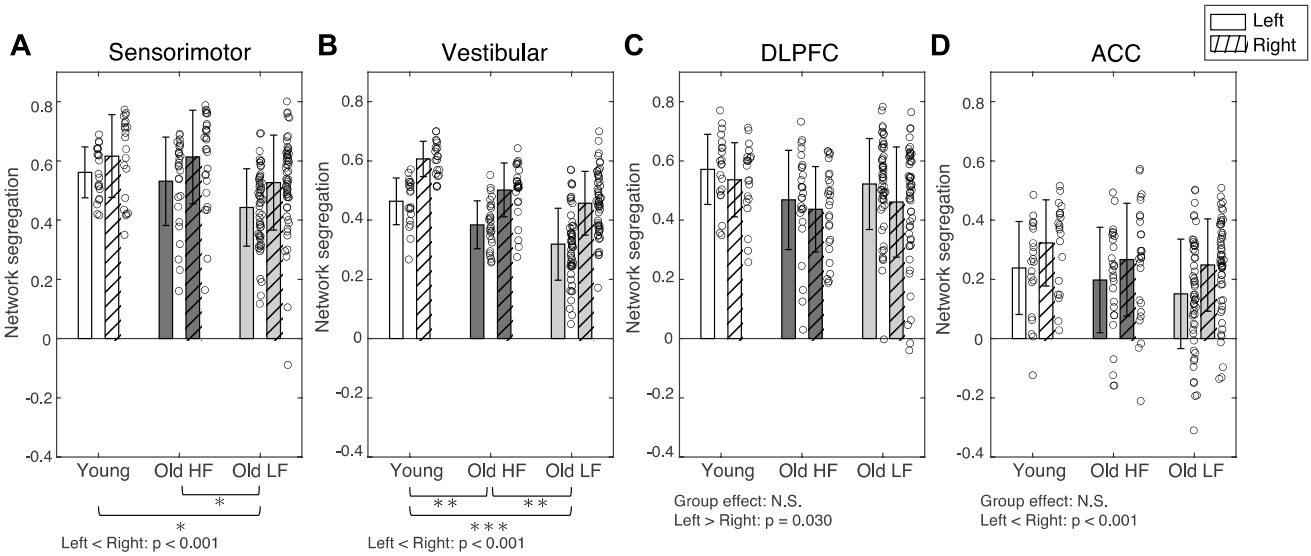

Figure 2. Network segregation score for left and right sensorimotor (A), vestibular (B), dorsolateral prefrontal cortex (C) and anterior cingulate cortex (D) networks. Brackets indicate statistically significant ($p < 0.05$) between groups comparisons from post-hoc tests. DLPFC = Dorsolateral Prefrontal Cortex. ACC = Anterior Cingulate Cortex. HF = High physical function. LF = Low physical function. * = p-value $0.010 \leq p < 0.050$. ** = p-value $0.001 \leq p < 0.009$. ***= $p < 0.001$

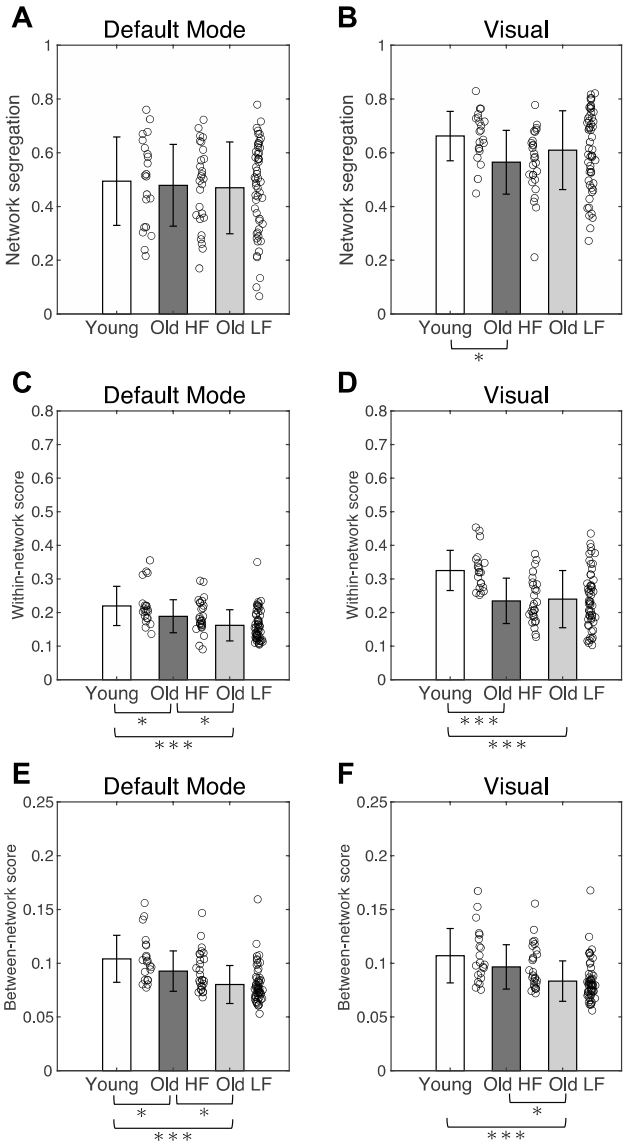

Figure 3. Network segregation (A-B), within-network connectivity (C-D) and between-network connectivity (E-F) for default mode (A, C, E), visual (B, D, F) networks. Brackets indicate statistically significant ($p < 0.05$) between groups comparisons from post-hoc tests. DLPFC = Dorsolateral Prefrontal Cortex. ACC = Anterior Cingulate Cortex. HF = High physical function. LF = Low physical function. * = p-value $0.010 \leq p < 0.050$. ** = p-value $0.001 \leq p < 0.009$. ***= $p < 0.001$

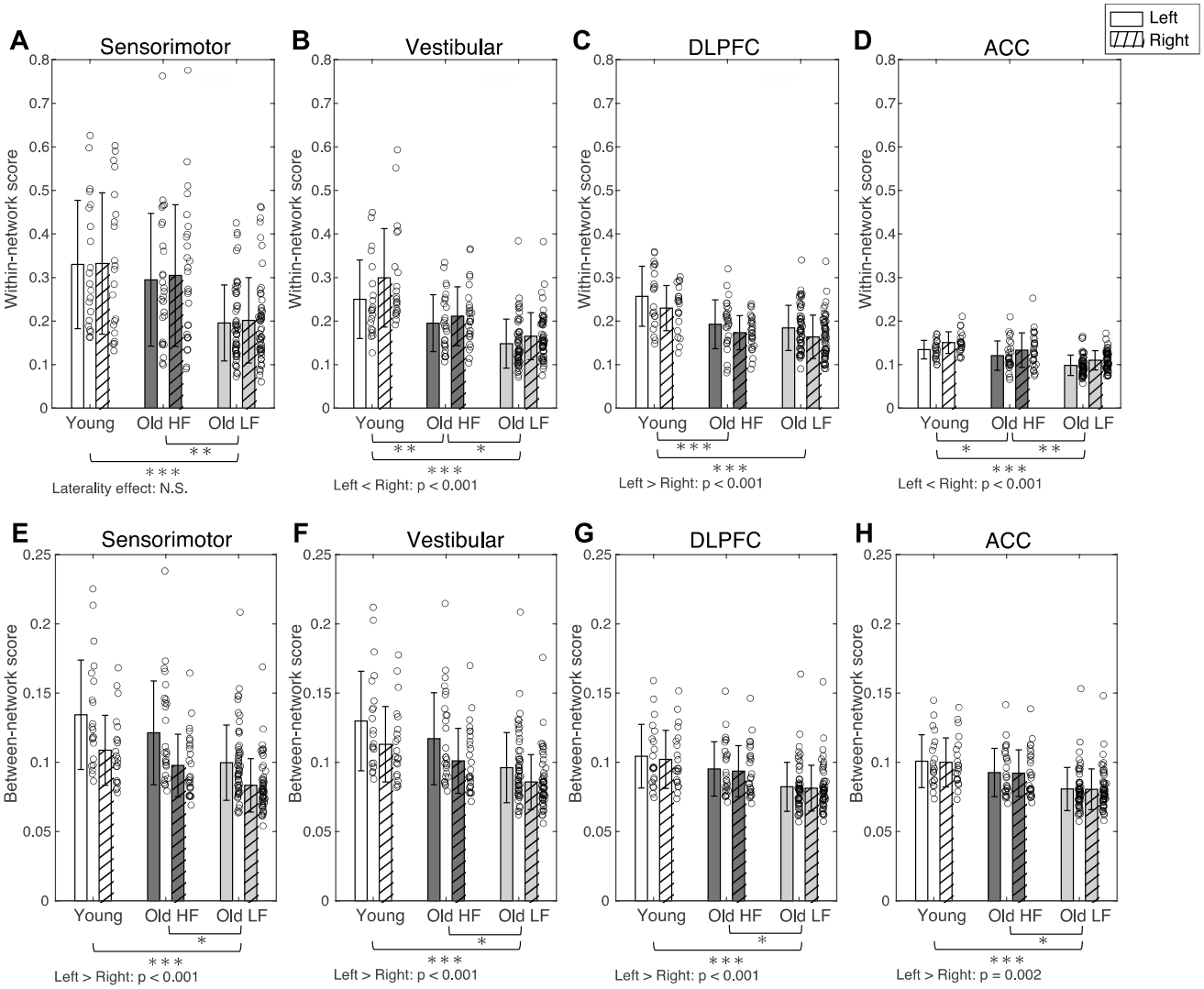

Figure 4. Within-network connectivity (A-D) and between-network connectivity (E-H) for sensorimotor (A, E), vestibular (B, F), dorsolateral prefrontal cortex (C, G) and anterior cingulate cortex (D, H) networks. Brackets indicate statistically significant ($p < 0.05$) between groups comparisons from post-hoc tests. DLPFC = Dorsolateral Prefrontal Cortex. ACC = Anterior Cingulate Cortex. HF = High physical function. LF = Low physical function. * = p-value $0.010 \leq p < 0.050$. ** = p-value $0.001 \leq p < 0.009$.

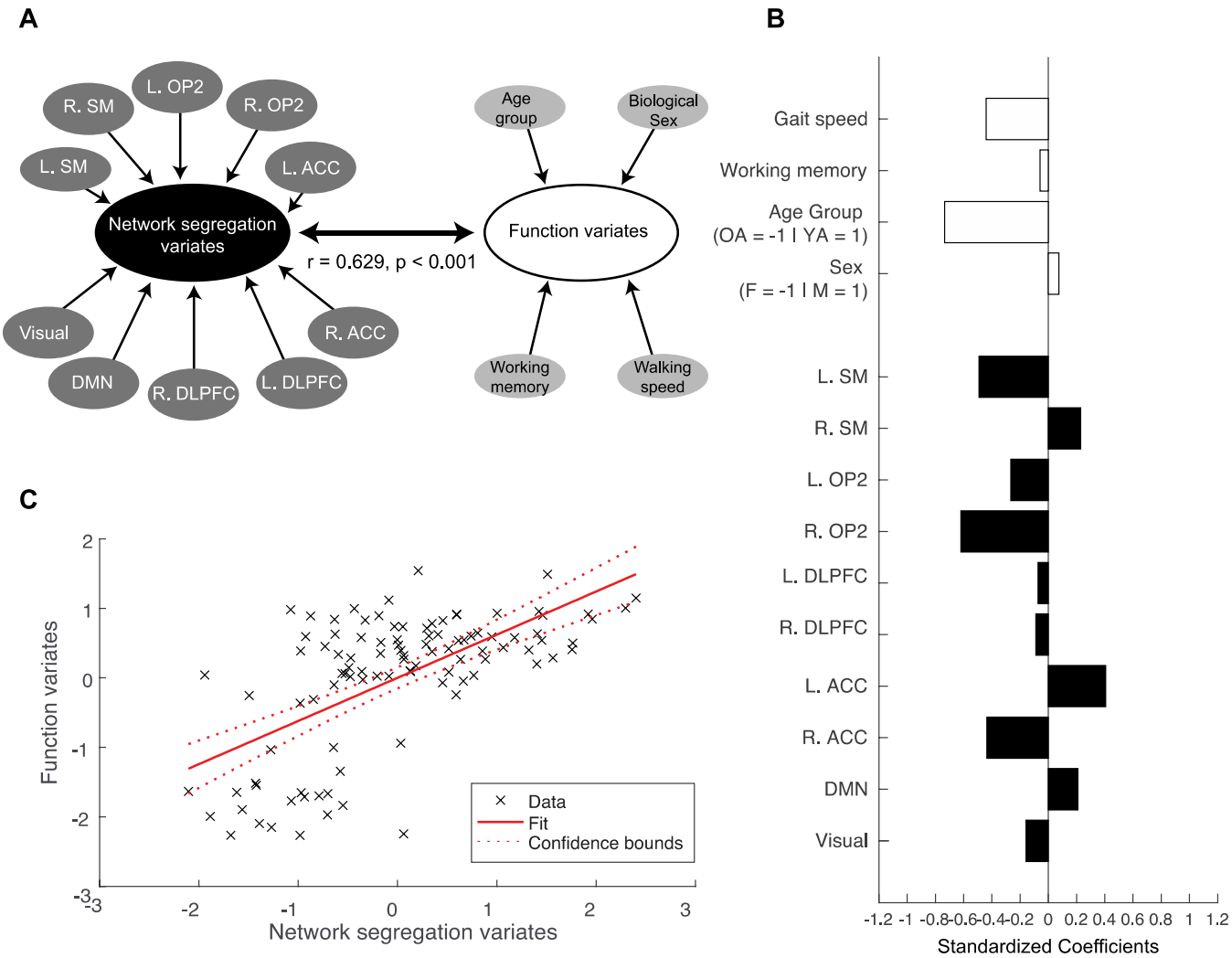

Figure 5. Canonical correlation analysis for age groups. A. Canonical correlation analysis (CCA) was conducted to assess the relationship between the 'function dataset', which included walking speed, cognitive function, age group, and biological sex, and the 'network segregation dataset', which included network segregation scores from the 10 identified networks. First dimension showed significant association of variance between the two datasets. B. Standardized correlation coefficient for the first dimension. White bars = Coefficients for the Function dataset; Black bars = Coefficients for the Network segregation dataset. C. Canonical variates for the first dimension. Canonical variates are values of the canonical variables which is computed by multiplying the normalized variables by the canonical coefficients. L = Left; R = Right; SM = Sensorimotor; OP2 = Vestibular; DLPFC = Dorsolateral prefrontal cortex; ACC = Anterior cingulate cortex; DMN = Default mode network.

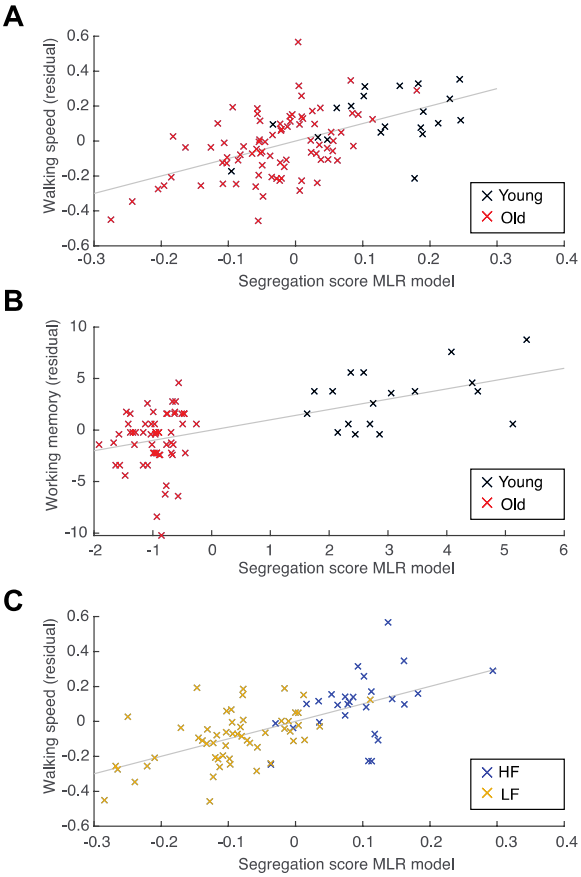

Figure 6. Step-wise multiple linear regression models for age groups (A, B) and older adults with different function (C). Tables with statistical output is shown in tables 7-9. A. Multiple linear regression with walking speed with biological sex covaried out and segregation scores from young and older adults. B. Multiple linear regression with working memory with biological sex covaried out and segregation scores from young and older adults. C. Multiple linear regression with walking speed with biological sex covaried out and segregation scores from older adults with high and low physical function. MLR = Multiple linear regression. HF = High physical function. LF = Low physical function.

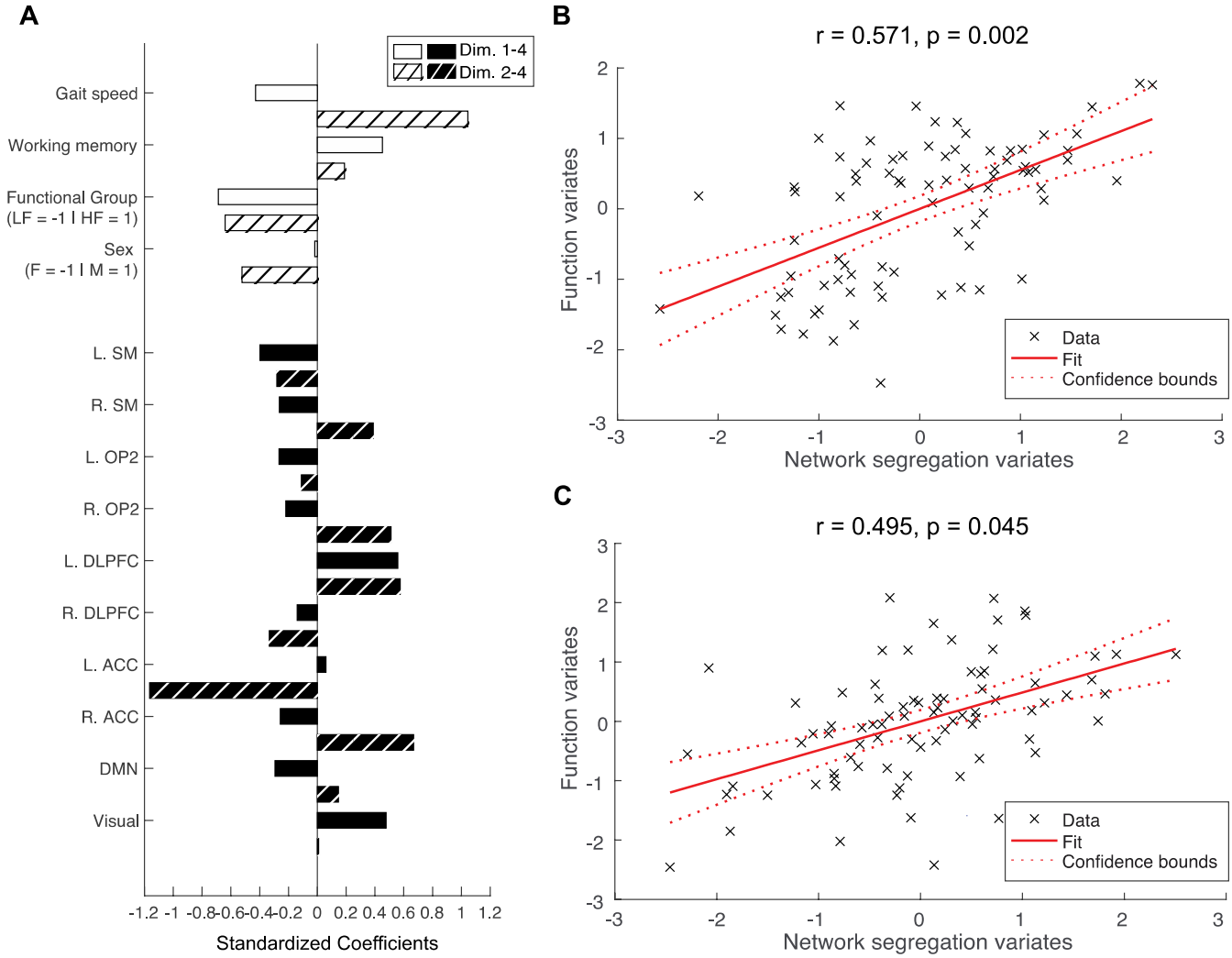

Figure 7. Canonical correlation analysis for physical function groups. Canonical correlation analysis (CCA) was conducted to assess the relationship between the 'function dataset', which included walking speed, cognitive function, physical function group, and biological sex, and the 'network segregation dataset', which included network segregation scores from the 10 identified networks. First and second dimensions showed significant association of variance between the two datasets. A. Standardized correlation coefficient for the first and second dimensions. White bars = Coefficients for the Function dataset; Black bars = Coefficients for the Network segregation dataset; Solid bars = First dimension; Striped bars = Second dimension. B. Canonical variates for the first dimension. Canonical variates are values of the canonical variables which is computed by multiplying the normalized variables by the canonical coefficients. C. Canonical variates for the second dimension. L = Left; R = Right; SM = Sensorimotor; OP2 = Vestibular; DLPFC = Dorsolateral prefrontal cortex; ACC = Anterior cingulate cortex; DMN = Default mode network.

Table 1. Participant characteristics. HF = Older adults with high physical function. LF = Older adults with low physical function. There was one young adult with missing gait speed. For working memory, 3 young adults, 4 older adults with high physical function, and 19 older adults with lower physical function were excluded due to missing data. SPPB = Short Physical Performance Battery (Max score = 12; Higher score = higher physical function). Working memory was assessed with the list sorting test on the NIH Toolbox (Max score = 26; Higher score = higher working memory).

	Young (n = 21)	Old HF (n = 27)	Old LF (n = 54)
Age	23.1 ± 3.4	73.5 ± 5.0	76.0 ± 7.2
Sex (M:F)	9:12	14:13	17:37
SPPB	--	11.1 ± 0.8	7.8 ± 1.2
Walking speed (m/s)	1.2 ± 0.2	1.1 ± 0.2	0.9 ± 0.2
Working memory	20.1 ± 2.7	16.7 ± 1.7	15.5 ± 3.3

Table 2. Statistical output for post-hoc pairwise group comparisons in network segregation scores. P-values are adjusted for multiple comparisons using the Holm-Bonferroni method. Y = Young adults. OA HF = Older adults with high physical function. OA LF = Older adults with low physical function. DLPFC = Dorsolateral Prefrontal Cortex. ACC = Anterior Cingulate Cortex. DMN = Default Mode Network.

Network	Contrast	Estimate	SE	t-ratio	p-value
Sensorimotor	Y - OA HF	0.022	0.039	0.566	0.573
	Y - OA LF	0.097	0.035	2.814	0.018
	OA LF - OA- HF	0.075	0.032	2.346	0.042
Vestibular	Y - OA HF	0.091	0.026	3.560	0.001
	Y - OA LF	0.148	0.023	6.518	< 0.001
	OA LF - OA- HF	0.057	0.021	2.696	0.008
DLPFC	Y - OA HF	0.096	0.043	2.246	0.081
	Y - OA LF	0.070	0.038	1.836	0.139
	OA LF - OA- HF	-0.027	0.035	-0.754	0.452
ACC	Y - OA HF	0.048	0.047	1.035	0.606
	Y - OA LF	0.081	0.041	1.969	0.155
	OA LF - OA- HF	0.033	0.038	0.863	0.606
DMN	Y - OA HF	0.009	0.047	0.182	1.000
	Y - OA LF	0.033	0.042	0.800	1.000
	OA LF - OA- HF	0.025	0.039	0.642	1.000
Visual	Y - OA HF	0.097	0.038	98.000	0.036
	Y - OA LF	0.052	0.034	98.000	0.248
	OA LF - OA- HF	-0.045	0.031	98.000	0.248

Table 3. Statistical output for post-hoc pairwise group comparisons in within-network connectivity scores. P-values are adjusted for multiple comparisons using the Holm-Bonferroni method. Y = Young adults. OA HF = Older adults with high physical function. OA-LF = Older adults with low physical function. DLPFC = Dorsolateral Prefrontal Cortex. ACC = Anterior Cingulate Cortex. DMN = Default Mode Network.

Network	Contrast	Estimate	SE	t-ratio	p-value
Sensorimotor	Y - OA HF	0.036	0.036	1.012	0.314
	Y - OA LF	0.126	0.032	3.991	< 0.001
	OA LF - OA HF	0.090	0.029	3.072	0.006
Vestibular	Y - OA HF	0.073	0.020	3.708	0.001
	Y - OA LF	0.116	0.018	6.607	< 0.001
	OA LF - OA HF	0.042	0.016	2.612	0.010
DLPFC	Y - OA HF	0.060	0.014	4.193	< 0.001
	Y - OA LF	0.070	0.013	5.501	< 0.001
	OA LF – OA HF	0.010	0.012	0.828	0.410
ACC	Y - OA HF	0.016	0.007	2.163	0.033
	Y - OA LF	0.038	0.007	5.725	< 0.001
	OA LF - OA HF	0.022	0.006	3.541	0.001
DMN	Y - OA HF	0.031	0.015	2.114	0.049
	Y - OA LF	0.058	0.013	4.508	< 0.001
	OA LF - OA HF	0.027	0.012	2.288	0.049
Visual	Y - OA HF	0.092	0.022	4.125	< 0.001
	Y - OA LF	0.084	0.020	4.254	< 0.001
	OA LF - OA HF	-0.008	0.018	-0.435	0.665

Table 4. Statistical output for post-hoc pairwise group comparisons in between-network connectivity scores. P-values are adjusted for multiple comparisons using the Holm-Bonferroni method. Y = Young adults. OA-HF = Older adults with high physical function. OA-LF = Older adults with low physical function. DLPFC = Dorsolateral Prefrontal Cortex. ACC = Anterior Cingulate Cortex. DMN = Default Mode Network.

Network	Contrast	Estimate	SE	t-ratio	p-value
Sensorimotor	Y - OA HF	0.013	0.008	1.706	0.091
	Y - OA LF	0.029	0.007	4.178	< 0.001
	OA LF - OA HF	0.015	0.006	2.429	0.034
Vestibular	Y - OA HF	0.014	0.007	1.810	0.073
	Y - OA LF	0.029	0.007	4.406	< 0.001
	OA LF - OA HF	0.016	0.006	2.548	0.025
DLPFC	Y - OA HF	0.010	0.005	1.798	0.075
	Y - OA LF	0.020	0.005	4.319	< 0.001
	OA LF - OA HF	0.011	0.004	2.469	0.031
ACC	Y - OA HF	0.009	0.005	1.846	0.068
	Y - OA LF	0.019	0.004	4.565	<0.001
	OA LF - OA HF	0.010	0.004	2.676	0.018
DMN	Y - OA HF	0.012	0.005	2.306	0.038
	Y - OA LF	0.023	0.005	4.810	<0.001
	OA LF - OA HF	0.010	0.004	2.381	0.038
Visual	Y - OA HF	0.011	0.006	1.882	0.063
	Y - OA LF	0.023	0.005	4.287	< 0.001
	OA LF - OA HF	0.011	0.005	2.333	0.043

Table 5. Statistical output for canonical correlation analysis with age groups. Dimension reduction results from Wilks lambda are presented.

Dimension	Corr.	Wilks	F	df1	df2	p
1-4	0.629	0.432	2.08	40	335.54	< 0.001
2-4	0.382	0.715	1.17	27	260.57	0.259
3-4	0.326	0.838	1.04	16	180.00	0.418
4-4	0.250	0.938	0.86	7	91.00	0.538

Table 6. Statistical output for step-wise multiple regression with walking speed (controlled for biological sex) in young and older adults ($F(6, 94) = 7.04$, $p < 0.001$, $r^2 = 0.31$). Analysis included 20 young adults and 81 older adults (1 young adult excluded due to missing walking speed data). Y = Young adults. ACC = Anterior Cingulate Cortex.

	Estimate	**Standardized**	**SE**	**t-value**	**p-value**
Intercept	-0.437	--	0.102	-4.301	< 0.001
Right Sensorimotor	0.293	0.250	0.104	2.819	0.006
Right Vestibular	0.500	0.296	0.175	2.862	0.005
Left ACC	-0.367	-0.350	0.143	-2.573	0.012
Right ACC	0.263	0.230	0.149	1.770	0.080
Age group (Y)	-0.112	-0.239	0.078	-1.449	0.151
Left ACC x Age group (Y)	0.826	0.513	0.257	3.212	0.002

Table 7. Statistical output for step-wise multiple regression with working memory (controlled for biological sex) in young and older adults ($F(3, 72) = 11.60$, $p < 0.001$, $r^2 = 0.33$). Analysis included 18 young adults and 58 older adults (3 young adults and 23 older adults excluded due to missing working memory scores). Y = Young adults. ACC = Anterior Cingulate Cortex.

	Estimate	**Standardized**	**SE**	**t-value**	**p-value**
Intercept	-1.595	--	0.731	-2.180	0.033
Right ACC	2.354	0.111	2.369	0.994	0.324
Age group (Y)	7.170	0.950	1.724	4.160	< 0.001
Right ACC x Age group (Y)	-10.021	-0.483	4.994	-2.006	0.049

Table 8. Statistical output for canonical correlation analysis with physical function groups (older adult participants only). Dimension reduction results from Wilks lambda are presented.

Dimension	Corr.	Wilks	F	df1	df2	p
1-4	0.571	0.384	1.84	40	255.91	0.002
2-4	0.495	0.571	1.56	27	199.24	0.045
3-4	0.474	0.756	1.29	16	138.00	0.209
4-4	0.160	0.975	0.26	7	70.00	0.967

Table 9. Statistical output for step-wise multiple regression with walking speed (controlled for biological sex) in older adults with high and low physical function (younger adults were excluded from this analysis) ($F(5, 75) = 10.27$, $p < 0.001$, $r^2 = 0.41$).). Analysis included 27 older adults with high physical function and 54 older adults with low physical function. HF = Older adults with high physical function. ACC = Anterior Cingulate Cortex.

	Estimate	**Standardized**	**SE**	**t-value**	**p-value**
Intercept	-0.418	--	0.096	-4.330	< 0.001
Right Sensorimotor	0.195	0.174	0.104	1.883	0.064
Right Vestibular	0.440	0.250	0.165	2.669	0.009
Left ACC	-0.495	-0.494	0.133	-3.731	0.000
Right ACC	0.383	0.349	0.141	2.705	0.008
Functional group (HF)	0.165	0.426	0.036	4.531	< 0.001