Ageing and large-scale functional networks: White matter integrity, gray matter volume, and functional connectivity in the resting state

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Abstract

Healthy ageing is accompanied by neurobiological changes that affect the brain’s functional organization and the individual’s cognitive abilities. The aim of this study was to investigate the effect of global age-related differences in the cortical white and gray matter on neural activity in three key large-scale networks. We used functional-structural covariance network analysis to assess resting state activity in the default mode network (DMN), the fronto-parietal network (FPN), and the salience network (SN) of young and older adults. We further related this functional activity to measures of cortical thickness and volume derived from structural MRI, as well as to measures of white matter integrity (fractional anisotropy [FA], mean diffusivity [MD], and radial diffusivity [RD]) derived from diffusion-weighted imaging. First, our results show that, in the direct comparison of resting state activity, young but not older adults reliably engage the SN and FPN in addition to the DMN, suggesting that older adults recruit these networks less consistently. Second, our results demonstrate that age-related decline in white matter integrity and gray matter volume is associated with activity in prefrontal nodes of the SN and FPN, possibly reflecting compensatory mechanisms. We suggest that age-related differences in gray and white matter properties differentially affect the ability of the brain to engage and coordinate large-scale functional networks that are central to efficient cognitive functioning.

Keywords: ageing; resting state networks; white and gray matter; multimodal imaging; neural plasticity
Introduction

The brain at rest consistently yields activity in the default mode network (DMN), which includes areas in the posterior cingulate cortex, precuneus, medial prefrontal areas, and the medial temporal lobes (Greicius et al., 2003; Raichle et al., 2001). The DMN was initially considered to represent neural baseline activity until further investigations showed that activity within the DMN is functionally related to internally driven mental states, such as self-referential processing, long-term memory, and mentalizing, and that its deactivation plays a functional role during externally directed tasks (Anticevic et al., 2012; Buckner et al., 2008; Burianova, McIntosh, & Grady, 2010; Kelly et al., 2008; Mennes et al., 2010; Sambataro et al., 2010). In addition, an emerging view suggests that cognitive performance in general might rely on the dynamic interaction between the DMN and two other large-scale neural networks: the fronto-parietal task-positive network (FPN), which is associated with attention and cognitive control, and the salience network (SN) in anterior cingulate and fronto-insular cortex, which is involved in the selection of emotionally and motivationally relevant stimuli (Andrews-Hanna et al., 2014; Chen et al., 2013; Fox et al., 2005; Seeley et al., 2007; Sridharan et al., 2008; Spreng et al., 2013). These three neural networks are central to cognition, as they are engaged in a large number of functions, and their disruption has been associated with a variety of clinical syndromes, such as schizophrenia, traumatic brain injury, and Alzheimer’s disease (Manoliu et al., 2014; Sharp et al., 2014; Zhou et al., 2010). In addition, evidence suggests that the disruption of the dynamic coordination of these large-scale networks constitutes one of the main causes of cognitive decline associated with ageing (Andrews-Hanna et al., 2007; Sambataro et al., 2010), as shown by reduced neural activity in the DMN and SN at rest (Allen et al., 2011; Onoda et al., 2012) and
increased activity in the FPN of older adults during visual tasks (Grady et al., 2010). However, it is an open question as to why and how ageing affects the dynamic coordination of large-scale neural networks (Grady, 2012).

One possible reason for altered large-scale network activation with increasing age is that ageing leads to widespread neurobiological changes, which impact the structural organization and integrity on which large-scale networks critically depend (Greicius et al., 2009; Horn et al., 2013; Van den Heuvel et al., 2008; Teipel et al., 2010). Thus, structural changes related to ageing would, in part, account for the functional changes associated with cognitive decline. This view is supported by studies that found correlations between functional integration of anterior and posterior medial regions and fractional anisotropy (FA) of connecting white matter tracts (Andrews-Hanna et al., 2007), between activity in the DMN during fixation and age-related decreases in FA across the whole white matter skeleton (Burzynska et al., 2013), and between functional connectivity in bilateral prefrontal cortex and FA of corpus callosum (Davis et al., 2012). In addition, there is evidence that the white matter networks of older adults are organized less efficiently and with less functional connectivity within the DMN, FPN, and SN than those of younger adults (Achard & Bullmore, 2007; Geerligs et al., 2014; Zhu et al., 2012; for a recent review of this body of evidence, see Ferreira & Busatto, 2013). These studies show correlations between functional activity in the DMN and indicators of white matter integrity for specific brain regions. However, no study has comprehensively addressed the relationship between whole-brain structural changes related to healthy ageing and changes in functional connectivity across the three central large-scale neural networks. Therefore, the aim of this study was to investigate age-related differences in
global white and gray matter properties and their relationship to functional activity in three large-scale neural networks (DMN, FPN, and SN) in the resting state.

We hypothesized that age-related neurobiological changes related to the processing and transmission of information would affect functional connectivity, and that indicators of white and gray matter integrity, such as FA, MD, radial diffusivity (RD), axial diffusivity (AD), cortical thickness, volume, and surface area, would significantly correlate with neural activity as measured by fMRI in each of the three networks. Specifically, we predicted that global age-related decline in microstructural integrity of white matter tracts as measured by decreasing FA and increasing MD, RD, and AD, would be indicative of reduced global efficiency of long-distance connections and lead to less functional connectivity in all three networks of older adults. Similarly, we expected to find that age-related reduction in gray matter thickness, surface area, and volume would affect efficiency of neural information processing in nodes of each network and further contribute to altered large-scale network activity in older adults.

Methods

16 older participants (mean age = 66 years; range = 59 – 81 years; 9 males) and 16 young participants (mean age = 30 years; range = 23 – 37 years; 7 males) took part in the experiment. All participants were right-handed, with normal or corrected to normal vision, native English speakers, and received a comparable number of years of formal education (mean older adults = 17 years, mean younger adults = 18 years). All older participants were considered cognitively intact, scoring in the high range of the Mini-Mental State Examination (MMSE; average score = 29.3; range = 26 - 30). Only participants that met the following criteria were included in the study: no previous
head injury, no known neurological or psychological conditions, no history of alcohol or substance abuse, no blood-thinning medication.

All participants took part in a 6-minute eyes-closed resting state experiment, followed by a 13-minute diffusion weighted acquisition on a Siemens 3-T Magnetom Verio scanner with a standard 32-channel radiofrequency head coil at Macquarie University Private Hospital. The Human Research Ethics Committee at Macquarie University approved this study and written consent was obtained from all participants.

**Gray Matter Data Acquisition and Analysis**

For each participant, a T1-weighted volumetric anatomical MRI was acquired with the following parameters: 176 slices sagittal magnetization-prepared rapid acquisition with gradient echo (MP-RAGE); 0.94 x 0.94 x 0.94 mm isotropic volume; repetition time (TR) = 2110 msec; echo time (TE) = 3.52 msec; flip angle = 9°; FOV = 240 mm. T1 weighted images were analyzed with the default processing pipeline of Freesurfer 5.1 (http://surfer.nmr.mgh.harvard.edu/), which includes brain extraction, intensity normalization, segmentation, generation of white and pial surfaces, surface topology correction, inflation of surfaces to a sphere, and spherical registration to the average surface based on a measure of surface shape (Dale, Fischl, & Sereno, 1999; Fischl, Sereno, & Dale, 1999; Fischl et al., 1999, 2004; Fischl & Dale, 2000; Fischl, Liu, & Dale, 2001; Ségonne et al., 2004). Average whole-brain values of cortical volume, surface area, and thickness were extracted for each individual and compared statistically. To correct for differences in head size, volume measures were examined as percentage of total intracranial volume. Measures showing significant group differences between young and older adults were used as covariates for functional-structural covariance network analysis (see below).
**DWI Data Acquisition and Analysis**

Diffusion-weighted images were acquired along 64 gradient directions with the following parameters: TR = 11500ms, TE = 85ms, b-value = 1200s/mm², voxel size = 2 x 2 x 2 mm, 55 axial slices. To obtain diffusivity measures, images were analyzed using the FMRIB Software Library (FSL 5.0; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). After eddy-current correction, Gaussian diffusion tensor models were fitted to each voxel with DTIfit and eigenvectors and eigenvalues, as well as FA maps were computed (Behrens et al., 2007). Additionally, maps of different diffusivity measures, such as mean diffusivity (MD=(λ1 + λ2 + λ3)/3), radial diffusivity (RD=(λ2 + λ3)/2), and axial diffusivity (AD=λ1) were calculated for each participant.

Localization of group differences for these diffusivity measures was assessed using tract-based spatial statistics (TBSS; Smith et al., 2006). All diffusivity maps, were then aligned into a common space using the nonlinear registration tool FNIRT (Andersson 2007a, 2007b), which uses a b-spline representation of the registration warp field (Rueckert 1999). Next, the mean FA image was created and thinned to create a mean FA skeleton, which represents the centres of all tracts common to the group. Each subject's aligned diffusivity maps were then projected onto this skeleton and independent t-tests between the group maps were applied using FSL’s voxelwise permutation tool ‘randomise’ (Nichols & Holmes, 2002). Five thousand random permutations for each diffusivity measure and threshold-free cluster enhancement (TFCE) were used for statistical assessment (Smith & Nichols, 2009). The resulting statistical maps were thresholded at p < 0.05 and corrected for multiple comparisons using family-wise error (FWE) correction. Spatial locations of differences were
determined by using the John Hopkins University ICBM-DTI-81 white matter labels atlas (Mori et al., 2008; http://cmrm.med.jhmi.edu/). From the statistical maps, individual average values of global white matter integrity for each measure (FA, AD, MD, RD) were derived and statistically significantly different measures were used as covariates in the functional-structural covariance network analysis (see below).

fMRI Acquisition, Preprocessing, and Functional-Structural Covariance Network Analysis

Functional images were acquired using a T2*-weighted echo-planar image pulse sequence in ascending interleaved order with the following parameters: 40 slices; 2.5mm slice thickness with 0.5mm gap; voxel size = 2.0 x 2.0 x 2.5mm; TR = 3000msec; TE = 32msec; FOV = 240mm; flip angle = 90°. Brain activation was assessed using the blood oxygenation level dependent (BOLD) effect (Ogawa et al., 1990) with optimal contrast. For functional analysis, T2*-weighted images were preprocessed with Statistical Parametric Mapping software (SPM8; http://www.fil.ion.ucl.ac.uk/spm). Images were slice-time corrected, realigned to a mean image for head-motion correction, and then spatially normalized into a standard stereotaxic space with voxel size of 2 mm³, using the Montreal Neurological Institute (MNI) template. Head movement and rotation in the three dimensions did not exceed 1 mm and no dataset had to be excluded from analysis. Finally, the functional images were spatially smoothed with a 6-mm full width half maximum Gaussian kernel.

Resting-state functional connectivity was assessed with functional-structural covariance network analysis, which is a seed-based multivariate method using Partial Least Squares (PLS; http://www.rotman-baycrest.on.ca/index.php?section=84). PLS allows one to investigate the relation between whole-brain activity, behavioural or
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structural covariates, and the functional activity at a particular seed using a multivariate approach specifically suited to network analysis (McIntosh et al., 1997). In brief, PLS mean-centers and then decomposes the covariance matrix between brain activity and seed/covariate values for the whole group in a single analytic step into separate, mutually orthogonal latent variables (LVs), which describe patterns of brain activity related to the seed regions/covariates (McIntosh et al., 2004). This step maximizes covariance and generates a weight for each voxel, which designates its degree of covariance with the whole brain activity pattern. PLS then assesses the statistical significance of each LV using a permutation test (McIntosh et al., 1996) and the reliability of the brain activity patterns using a bootstrap procedure, resulting in a measure of reliability, the bootstrap ratio (BSR), for each voxel (Efron & Tibshirani, 1985). The sum of BSRs across the whole individual brain generates a brain score, which indicates how robustly an individual shows the overall brain activity pattern. Finally, brain scores are correlated with seed regions/covariates to assess the overall relationship between seed/covariate and brain pattern (see Fig. 1).

(INSERT FIGURE 1 HERE)

For functional-structural covariance network analysis, functional seed values were extracted from a spherical region of interest (ROI) with a neighborhood size of three voxels in the posterior cingulate cortex (PCC) at MNI coordinates [0 -48 28]. The PCC was chosen as seed region because it constitutes one of the central nodes of the DMN (Greicius et al., 2003), and because it has recently been suggested that PCC plays a crucial role in regulating the dynamic coordination of the DMN, FPN, and SN (Leech & Sharp, 2014). Then, functional PCC values were submitted to PLS for each
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group separately yielding one whole-brain pattern of resting state activity related to
the functional PCC seed for each group. Next, structural covariates for FA, MD, RD,
thickness, and volume were submitted to PLS for both groups together, resulting in
one activity pattern related the structural covariates across groups. All resulting brain
activity maps were thresholded at $p < 0.005$. Then, activity maps were converted to
network covariance plots, which show the level of covariation between a functional
seed or structural covariate and each node of the network and is therefore different
from other structural covariance analyses that assess how structural measures covary
across regions (e.g., Alexander-Bloch et al., 2013). ROIs covering the central nodes of
each of the three networks were defined a priori using the Harvard-Oxford cortical
and subcortical structural atlases supplied with FSL (Fox et al., 2005; Seeley et al.,
2007; see Supplementary Materials 1 for a list of ROIs). For each ROI in each
network, the percentage of active voxels was extracted from functional brain activity
maps that had been normalized to the mean image intensity. Finally, the percentages
were scaled to the maximum value across all ROIs for plotting purposes.

Results

The comparison of white and gray matter between young and older adults
resulted in significant group differences that are consistent with previous
investigations of age-related structural changes. We found reduced cortical volume
and thickness, as well as reduced FA and increased MD and RD values for older
adults compared with the younger group (Fjell et al., 2009; Westlye et al., 2010; see
Supplementary Materials 2 & 3 for detailed results).

*Functional-structural covariance network analysis*
To assess neural activity in the resting state, we first conducted a functional-connectivity analysis for each age group separately with functional activity in posterior cingulate cortex as the seed value. In young adults, the results showed activity in nodes of the DMN, the FPN, and the SN (one significant LV with $p=0.002$ that explains 100% crossblock covariance; brain images thresholded at $BSR = 3$, which approximates $p=0.0027$). In older adults, the results showed activity in nodes of the DMN only, which was also reduced in comparison to young adults (one significant LV with $p=0.064$ that explains 100% crossblock covariance; brain images thresholded at $BSR = 3$, which approximates $p=0.0027$). These results show (1) that older adults engage the DMN to a lesser extent than younger adults do, and (2) that only in young adults PCC is functionally connected to nodes of the DMN, FPN, and SN (see Figure 2).

To assess correlations between neural activity in the resting state and gray and white matter values, we conducted a second functional-structural covariance network analysis across both age groups, with global gray and white matter values as covariates. The analysis yielded one significant LV ($p=0.008$) explaining 41.3% crossblock covariance (brain images thresholded at $BSR = 3$, which approximates $p=0.0027$). The brain activation pattern of this LV showed activity mainly in frontal nodes of the SN and FPN related to global gray and white matter structural measures (see Figure 3). This pattern of activity was positively correlated with MD and RD for both young and older adults, but negatively correlated with FA only in young adults. Furthermore, activity in the frontal SN and FPN nodes was positively correlated with
cortical thickness in young, but negatively correlated with cortical thickness and volume in older adults. In summary, we found that: (1) nodes of the SN and FPN show more activation in young and older adults with high global MD and RD values; (2) in young adults, lower global FA values and higher global cortical thickness are associated with more activity in SN and FPN; and (3) older adults with more intact gray matter as indicated by high global values of cortical thickness and volume show less activity in SN and FPN.

(DIRECT FIGURE 3 HERE)

Discussion

The aim of this study was to assess the impact of global age-related differences in the cerebral white and gray matter on functional activity in three large-scale neural networks central to cognition: the default mode network (DMN), the fronto-parietal network (FPN), and the salience network (SN). We first replicated typical findings of age-related structural changes, with a loss in global cortical gray matter (reduced thickness and volume) and an overall degeneration of white matter integrity (reduced FA and increased MD and RD values) in older compared with younger adults. We then examined group differences in functional connectivity in the three key networks and investigated how age-related structural changes affect activity in these networks across the adult life-span using functional-structural covariance network analysis.

Ageing has previously been associated with reduced neural activity, and we expected to find a similar reduction in resting state activity for older adults in each of the three large-scale networks (Hafkemeijer et al., 2012; Koch et al., 2010; Wu et al.,
2011). Our results show reduced functional connectivity for older adults mainly in the FPN and SN and to a lesser degree in the DMN. This result confirms and extends previous findings in that it suggests that the reduced activity typically observed in older relative to younger adults is not entirely due to a general decrease in neural activity, but also relates to the extent to which large-scale networks are recruited, i.e., their functional connectivity. Our results suggest that older adults might engage the FPN and SN less consistently than younger adults do, leading to less reliable functional connectivity in all three networks. It has been proposed that the flexible recruitment of large-scale networks and the ability to readily switch between them relates directly to cognitive performance and aberrant processing (Chen et al., 2013; Fornito et al., 2012; Leech & Sharp, 2014). The lack of recruitment of FPN and SN in older adults might thus indicate that older adults do not switch between large-scale networks as readily and frequently as young adults do, which might contribute to their reduced cognitive performance. This finding is important for research on lifestyle choices and healthy ageing as it emphasizes the importance of engaging a variety of large-scale networks associated with physical, cognitive, and social activities (Grady, 2012).

In a similar vein, these results provide evidence against the notion that the observed reduction in BOLD activation in older adults during the resting state is due to age-related differences in cerebral vasculature alone (Bangen et al., 2009; D’Esposito, Deouell & Gazzaley, 2003; Kannurpatti et al., 2010). Such changes would presumably affect all three networks similarly, yet our results show a reduction in BOLD-based functional connectivity mainly in the FPN and SN and less in the DMN. This finding suggests that the observed age-related differences in functional connectivity during the resting state may be the result of not only vascular but also
cognitive and structural factors. This interpretation is further supported by recent evidence that confirms ageing effects on BOLD related fMRI activity in the FPN with non-BOLD related electroencephalography (EEG) (Balsters et al., 2013).

Functional networks are closely related to structural networks in the resting state (Greicius et al., 2009; Horn et al., 2013; Van den Heuvel et al., 2008; Teipel et al., 2010). We hypothesized that age-related functional changes would be related to neurobiological changes in the underlying white and gray matter. We used functional-structural covariance network analysis to correlate gray and white matter measures with functional activity in the three key networks across both groups. Our results show that across the adult lifespan, structural measures of gray and white matter covary differently with activity in the FPN and SN. In young adults, thicker cortical gray matter is associated with greater activity in the FPN and SN, whereas in older adults, the opposite was found: individuals with the most reduction in cortical gray matter thickness and volume engage the FPN and SN the most. These findings suggest that reduction of gray matter structure at a young age affects neural activity differently than such a reduction at an older age. Finally, activity in the FPN and SN was negatively correlated with fractional anisotropy (FA) in young adults and positively correlated with mean and radial diffusivity (MD and RD) in both groups. Together, a reduction in FA and an increase in MD and RD indicate lower microstructural integrity, suggesting that reduced integrity of long-distance white matter connections increases prefrontal activity in the FPN and SN (for a discussion of the appropriateness of DWI to assess white matter microstructure, see Jones et al., 2013).

These results reveal a differential contribution of age-related differences in gray and white matter properties to the flexible recruitment of key networks. Previous
studies have shown that age-related gray matter atrophy is related to activity in fronto-parietal and medial temporal regions (Kalpouzos, Persson, & Nyberg, 2012; Rajah, Languay, & Grady, 2011; for a review, see Maillet & Rajah, 2013). Similarly, it has previously been found that age-related differences in indexes of microstructural integrity of cortical white matter, such as FA, MD, or RD, are related to functional connectivity and efficiency within large-scale functional networks (Achard & Bullmore, 2007; Burzynska et al., 2013; Geerligs et al., 2014; Zhu et al., 2012), specifically, to the functional integration of anterior and posterior medial regions as well as bilateral prefrontal cortex (Andrews-Hanna et al., 2007; Davis et al., 2012). Our results support these findings by showing that across the adult life-span, activity in anterior medial and lateral prefrontal regions increases when cortical volume and white matter integrity are reduced, and that with increasing age, a decline in white and gray matter structure might be counterbalanced with compensatory reorganisation of functional connectivity. It has been suggested that older adults’ brains compensate for a reduced structural integrity through increased prefrontal activity (Grady, 2012; Park & McDonough, 2013). Thus, we interpret our results as further evidence for compensatory mechanisms in the ageing brain by showing that as gray and white matter structures decrease in integrity with age, functional connectivity with prefrontal cortex increases.

Our findings reveal that age-related differences in gray and white matter are mainly correlated with functional connectivity in the FPN and SN, which both show reduced connectivity in older adults. This reduced connectivity in the FPN and SN suggests that gray and white matter changes jointly affect the recruitment of those networks as well as the dynamic coordination of large-scale neural networks. However, the complexity of the ageing process suggests that gray and white matter
changes capture only part of the neurobiological changes affecting cognitive functioning in older adults. Further research investigating lifestyle choices, age-related neurotransmitter depletion, and genetic factors influencing the ageing brain are necessary (Grady, 2012). This point is illustrated by our finding that FA correlates with functional activity only in young adults but that other measures of white matter integrity (MD and RD) correlate with functional connectivity for both groups. This finding suggests that FA, in comparison to MD and RD, might not be a good predictor of the effects of white matter integrity on functional organization across the lifespan and that white and gray matter measures may only reveal part of the age-related differences in the structural-functional organization of the brain.

In sum, we demonstrate that the age-related structural changes in cerebral gray and white matter correlate with changes in the recruitment of functional brain networks. Unlike young adults who engage the SN and FPN in addition to the DMN, the resting state connectivity of older adults is largely confined to the DMN. Across the adult life-span, age-related gray matter atrophy and reduced white matter integrity relate to increased functional connectivity with prefrontal nodes of the FPN and SN possibly reflecting compensatory mechanisms. Taken together, our results suggest that measures of white matter diffusivity, such as FA, MD, and RD, and of gray matter structure, such as cortical volume and thickness, are related to the ageing brain’s ability to engage and coordinate large-scale functional networks that are central to efficient cognitive functioning and might underlie age-related cognitive decline.

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Figure Captions:

**Figure 1:** Functional-structural covariance network analysis using PLS. Behavioral covariates from FreeSurfer analysis (grey matter volume and thickness) and from FSL analysis (FA, MD, and RD values) were correlated with resting state fMRI data and decomposed using singular value decomposition (SVD) to yield latent variables that show how behavioural covariates, i.e., measures of brain structure, relate to functional activity in the resting state. Note that for group comparisons, additional ‘condition’ rows in the behaviour and data matrices are created.

**Figure 2:** A) Results of functional-structural covariance network analysis showing functional connectivity of whole brain resting state activity with posterior cingulate cortex (PCC) in young (blue pattern) and older adults (red pattern), all p < 0.01. Network covariance plots further specify these differences by plotting the co-variation between each node of the large-scale neural networks and the PCC seed as normalized and scaled percentage of active voxels within each ROI. While young adults (B) engage the default mode network (pink labels) as well as regions associated with the fronto-parietal network (blue labels) and the salience network (green labels), resting state connectivity is reduced and restricted to the default mode network in older adults (C), all p < 0.01 [Abbreviations: MPFC – medial prefrontal cortex; PCC – posterior cingulate cortex; PREC – precuneus; MTL – medial temporal lobe; ACC – anterior cingulate cortex; INS – insula; DLPFC – dorsolateral prefrontal cortex; IFG – inferior frontal gyrus; IPL – inferior parietal lobule].
Figure 3: A) Results of functional-structural covariance network analysis showing a covariance pattern between resting state connectivity and white and gray matter covariates that is common to both young and older adults, all $p < 0.01$. B) Scatter plots show how structural covariates correlate with pattern strength (normalized brain scores), which indicates how strong each individual expresses this resting state activity pattern. Young adults are plotted in blue, older adults in red. C) The network covariance plot shows that white and gray matter covariates are mainly correlated to connectivity with nodes of the fronto-parietal (blue labels) and salience networks (green labels). [Abbreviations: MPFC – medial prefrontal cortex; PCC – posterior cingulate cortex; PREC – precuneus; MTL – medial temporal lobe; ACC – anterior cingulate cortex; INS – insula; DLPFC – dorsolateral prefrontal cortex; IFG – inferior frontal gyrus; IPL – inferior parietal lobule].
Supplementary Materials

1. **ROIs used for network activity plots:**

   Default mode network (DMN):
   - Posterior cingulate cortex, PCC
   - medial prefrontal cortex / MPFC
   - precuneus / PREC
   - medial temporal lobe / MTL

   Salience network (SN):
   - anterior cingulate cortex / ACC
   - anterior insula / INS

   Fronto-parietal network (FPN):
   - inferior parietal lobe / IPL
   - dorsolateral prefrontal cortex / DLPFC
   - inferior frontal gyrus / IFG

2. **Results of Gray Matter Analysis: Thickness, Volume, and Surface Area**

   Global measures of gray matter thickness, volume, and surface area were examined using an ANOVA with age and gender as independent variables. This analysis showed a significant main effect of age for cortical thickness ($F(1,1) = 5.17, p < 0.05$) and volume ($F(1,1) = 14.38, p < 0.001$). In addition, the analysis revealed a main effect of gender for surface area ($F(1,1) = 13.66, p < 0.001$) and volume ($F(1,1) = 9.43, p < 0.01$), but no interactions between age and gender.

   Age differences for thickness and volume were explored independently using unpaired t-tests. These analyses revealed that young adults had thicker cortical gray matter than older adults ($t(29) = 2.29, p < 0.05$; mean / SD for young = 2.42 / 0.08 and for older = 2.35 / 0.08). In addition, young adults also generally had a larger gray matter volume than older adults ($t(29) = 3.38, p < 0.005$; mean /
SD for young = 0.39 / 0.031 and for older = 0.35 / 0.034). Analysis of correlation showed negative correlations of age with thickness (r = -0.46, p < 0.01) and volume (r = -0.55, p < 0.01).

Whole-brain analysis of gray matter thickness and volume showed significant differences between young and older adults in frontal and temporal regions. Cortical thickness was significantly reduced in older adults in left superior temporal, right lingual, and bilateral superior frontal gyri (all ps < 0.01). Similarly, cortical volume was significantly smaller in older adults in left inferior parietal and superior frontal cortex as well as right inferior frontal gyrus (all ps < 0.05).

3. Results of White Matter Analysis: AD, FA, MD, RD

Global measures of white matter integrity including AD, FA, MD, and RD were examined using an ANOVA with age and gender as independent variables. This analysis showed significant main effects of age for FA (F(1,1) = 5.28, p < 0.05), MD (F(1,1) = 6.18, p < 0.05), and RD (F(1,1) = 5.08, p < 0.05). The only significant main effect of gender was found for AD (F(1,1) = 4.33, p < 0.05). The analysis showed no significant interactions between age and gender.

The group differences between young and older adults were further investigated with unpaired t-tests. These analyses showed that young adults have significantly higher overall FA values than older adults (t(25) = 2.26, p < 0.05; mean / SD for young = 0.44 / 0.009 and for older = 0.43 / 0.014). Additionally, in comparison to older adults, young adults showed lower MD (t(28) = -2.36, p < 0.05; mean / SD for young = 0.00065 / 1.6e-05 and older = 0.00067 / 2.1e-05) and RD values (t(29) = -2.11, p < 0.05; mean / SD for young = 0.00079 / 1.7e-05 and older =
Further analysis showed that FA is negatively correlated with age ($r = -0.34, p = 0.05$), whereas MD is positively correlated with age ($r = 0.34, p = 0.06$).

Tract-based spatial statistics (TBSS) analysis revealed significant differences (at $p < 0.01$ FWE) between young and older adults for FA, MD, and RD measures (see Figure 3). Young adults showed significantly higher FA values throughout the white matter skeleton (percentage of white matter skeleton: 26.46 %). White matter tracts showing significantly higher FA values for young adults included bilateral anterior, superior, and posterior corona radiata, bilateral superior longitudinal fasciculus, the body of corpus callosum, right posterior limb and bilateral retrolenticular part of internal capsule, bilateral external capsule, bilateral posterior thalamic radiation, bilateral fornix, and bilateral sagittal stratum and inferior fronto-occipital fasciculus.

In contrast, young adults showed lower MD values in bilateral anterior, superior, and posterior corona radiata, body and genu of corpus callosum, bilateral superior longitudinal fasciculus and posterior corona radiata, bilateral external capsule and anterior limb of internal capsule, and left retrolenticular part of internal capsule (percentage of white matter skeleton: 16.69 %).

Young adults also showed lower RD values in the right anterior, superior, and posterior corona radiata, body and genu of corpus callosum, bilateral posterior thalamic radiation, bilateral superior longitudinal fasciculus, right anterior limb of internal capsule, and left retrolenticular part of internal capsule (percentage of white matter skeleton: 7.58 %).