

A Brain-Wide Study of Age-Related Changes in Functional Connectivity

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Aging affects functional connectivity between brain areas, however, a complete picture of how aging affects integration of information within and between functional networks is missing. We used complex network measures, derived from a brain-wide graph, to provide a comprehensive overview of age-related changes in functional connectivity. Functional connectivity in young and older participants was assessed during resting-state fMRI. The results show that aging has a large impact, not only on connectivity within functional networks but also on connectivity between the different functional networks in the brain. Brain networks in the elderly showed decreased modularity (less distinct functional networks) and decreased local efficiency. Connectivity decreased with age within networks supporting higher level cognitive functions, that is, within the default mode, cingulo-opercular and fronto-parietal control networks. Conversely, no changes in connectivity within the somatomotor and visual networks, networks implicated in primary information processing, were observed. Connectivity between these networks even increased with age. A brain-wide analysis approach of functional connectivity in the aging brain thus seems fundamental in understanding how age affects integration of information.

Keywords: aging, dedifferentiation, fMRI, functional connectivity, graph theory

Introduction

Performance in various domains of cognitive functioning has been found to decline with age (Grady 2012). There is evidence that these deteriorations are partly related to changes in communication between different brain areas (Andrews-Hanna et al. 2007; Sambataro et al. 2010). We previously found the first evidence that aging not only affects functional connectivity within specific functional networks, implicated in particular cognitive functions, but communication between different functional networks as well (Geerligs et al. 2012). In the current study, we investigated how aging affects the integration of information across the whole brain, that is, within as well as between functional brain networks.

Whole-brain analysis requires a novel approach. So far, effects of aging on functional connectivity have mainly been assessed using seed-based functional connectivity and independent component analysis (Biswal et al. 1995; van de Ven et al. 2004; Fox et al. 2005). Both methods have a limited capability of providing a complete view of the characteristics of connectivity between and within functional networks. Seed-based connectivity requires a hypothesis regarding the chosen seed region, while independent component analysis has the inherent limitation that only connectivity within functional networks can be examined. In the current study we apply

complex network measures, to assess connectivity within a brain-wide graph of functional areas (Rubinov and Sporns 2010; Power et al. 2011).

In the context of a graph, brain areas are referred to as nodes and connections between nodes are referred to as edges. Nodes that are directly connected through one edge are referred to as neighbors, whereas a series of edges connecting distant nodes are referred to as a path. After a network has been defined in such a way, it is possible to extract different complex network measures that characterize the connectivity structure of the network. This approach has major advantages over a mass-univariate approach in which all connections between all areas are tested independently. First of all, graph theory avoids the large number of multiple comparisons that accompany a mass-univariate approach. Second, by examining complex network measures, specific features that are important for the functioning of the network can be assessed.

Studies in younger adults have shown that the brain consists of a number of separate functional networks. There are dense connections within these networks whereas connectivity between different networks is sparse. This organization is thought to benefit specialized or segregated information processing in different brain networks (Bullmore and Sporns 2012). The extent to which such an organization is present can be measured with the complex network measure modularity (Newman 2004). There are indications that functional brain networks in elderly become less distinct, due to an increase in internetwork connections along with a decrease in intranetwork connections (Geerligs et al. 2012). Previous studies have already shown that brain areas become functionally dedifferentiated with advancing age (Baltes and Lindenberger 1997; Park et al. 2004; Carp et al. 2011; Dennis and Cabeza 2011). Geerligs et al. (2012) extended these findings by showing that dedifferentiation might also occur on the level of large-scale functional brain networks. One of the goals of the current study was to lend more support to these findings, using complex network measures. If functional networks indeed become dedifferentiated with age, we would expect a reduction in modularity. A reduction in modularity with age might be driven by global changes throughout the networks or by decreased connections within or increased connections between specific functional networks. To examine these possibilities, we additionally examined the participation coefficient (Guimerà and Amaral 2005), which is a local measure of the proportion of inter- and intra-network connections.

The trajectory between the input and the output of the brain, that is, between perception and overt and covert behavior, requires the integration of information within, as well as between, different functional networks. The capacity to integrate information across all brain areas can be assessed with

global efficiency (Latora and Marchiori 2001). Higher level functions, such as executive functions, that require integration of information from different sources, benefit from global efficiency across the whole network (Bullmore and Sporns 2012). In addition, primary processing functions, such as visual information processing benefit from clustered connections between neighboring nodes. This can be measured using local efficiency, which quantifies connections between neighboring nodes (Latora and Marchiori 2001). If neighboring nodes are well connected, information exchange will be more segregated as well as more efficient and the networks will be more resilient to disruptions in connectivity.

Previous studies using graph theory in fMRI data have shown that aging is accompanied by a reduction in global and local network efficiency (Achard and Bullmore 2007). Modularity on the other hand was reported to be similar in older and younger participants (Meunier et al. 2009). In the current paper, we extend these previous findings by examining age-related effects on complex networks measures within different functional networks. Because each functional network tends to be related to specific cognitive functions, this approach allows for a more direct link between the changes in complex graph measures and the changes in cognitive functioning. In this paper, we used the network measures described above to provide a coherent whole-brain view of age-related changes in functional connectivity. To extract functional networks in the older and younger groups, we used data driven methods which did not require any a-prior hypotheses about specific features of these networks. We found that, the balance between intra- and inter-network connections shifted with age, as reflected by decreased modularity. Changes in local efficiency varied across networks. In the visual and somatomotor networks, subserving more elementary cognitive functions, efficiency was maintained in elderly, whereas a sharp decrease in efficiency was found in higher level processing networks, the default mode network (DMN, Raichle et al. 2001; Greicius et al. 2003; Buckner et al. 2008), fronto-parietal control network (FPCN, Vincent et al. 2008; Spreng et al. 2010), and the cingulo-opercular network (Dosenbach et al. 2007).

Methods

Participants

Forty older adults (24 males, $M_{\text{age}} = 64.9$ years, age range: 59–74 years) and 40 younger adults (21 males, $M_{\text{age}} = 20.6$ years, age range: 18–26 years) participated in this experiment. All participants were right handed and had no history of neurological or psychiatric disorders. Older participants had a score of 26 or higher on the mini mental status examination (MMSE, Folstein et al. 1975) and <16 on each of the subscales of the hospital anxiety and depression scale (HADS, Zigmond and Snaith 1983). All participants had normal or corrected-to-normal visual acuity. The study adhered to the Declaration of Helsinki and was approved by the local ethics committee of the University Medical Center Groningen, the Netherlands. Informed consent was obtained from all participants. Data of one older participant was lost due to technical problems. One older participant was excluded because a brain abnormality was detected.

Data Acquisition and Preprocessing

fMRI scans were obtained during 10 min of resting state with a 3-T MR scanner (3T Achieva, Philips Medical Systems, Best, the Netherlands), with echo planar imaging (EPI) capability and an 8-channel SENSE head coil. Participants were instructed to keep their eyes closed and not fall asleep. Functional images were obtained with the following pulse sequence parameter settings: single shot EPI; 37 slices; slice thickness 3.5 mm; no gap; field of view 224 mm; matrix scan size 64 by

62; transverse slice orientation; repetition time (TR) = 2000 ms; echo time (TE) = 30 ms; minimal temporal slice timing (1836 ms); flip angle 70°. A 3D T_1 -weighted anatomical scan of the entire brain was obtained for each participant using the following pulse sequence parameters: field of view 256 mm; matrix scan size 256 by 256; 170 slices; slice thickness 1 mm; transverse slice orientation; TE = 3.6 ms; TR = 9 ms; flip angle 8°. Offline processing was performed using the statistical parametric mapping software package (SPM 8; <http://www.fil.ion.ucl.ac.uk/spm/software>). First, for each participant, the functional images were motion-corrected and coregistered to the anatomical scan. Coregistration was checked visually and adjusted manually when required. Smooth signal intensity variations due to field inhomogeneities were reduced in both structural and functional images by applying bias regularization as implemented in SPM. For functional images, the regularization was initially applied only to the first and the last functional scan. Based on these 2 corrections, an average correction factor was computed for each voxel, which was applied to all scans. A study-specific anatomic template was created (for young and elderly participants together), using Diffeomorphic Anatomical Registration Exponentiated Lie algebra (DARTEL), to optimize interparticipant alignment (Ashburner 2007). Data were smoothed with an 8-mm full-width half maximum (FWHM) Gaussian kernel.

For the functional connectivity analyses, additional preprocessing steps were used to remove spurious variance from the time courses. One of these steps was global signal regression. The global signal is assumed to reflect a combination of resting-state fluctuations, physiological noise (e.g., respiratory and cardiac noise), and other noise signals (Birn et al. 2006). It has been shown that (physiological) noise in the BOLD signal increases with advancing age (D'Esposito et al. 1999; Makedonov et al. 2013). Therefore, in the current study, we applied global signal regression to reduce these effects of noise differences between groups on estimates of the correlation coefficient. Using SPM routines, a multiple regression approach was used which included regression of the time-courses from the white matter, cerebrospinal fluid, and the whole brain (global signal) and regression of motion parameters (for details of this procedure see Geerligts et al. 2012). First derivatives of these signals were also regressed out. In addition, a high pass filter (time constant of 111 s) was applied.

It has been shown that participant motion can have large effects on functional connectivity estimates (Power et al. 2012). To minimize such effects, scans which might have been affected by movement were excluded from the analysis. The first step in this correction was to calculate the total displacement per scan. Rotations and translations were combined by computing the square root of the summed squared values (<http://cibsr.stanford.edu/tools/human-brain-project/artrepair-software.html>). The absolute scan to scan differences in both the rotational and translational displacement were summed to represent the total displacement per scan. Scans in which the displacement compared with the previous scan was larger than 0.5 mm were flagged. The second step in the correction was to identify scans which could have been affected by participant motion by examining changes in the intensity of the functional image. For each voxel (within the participant-specific brain mask) a temporal derivative of the signal was calculated, by computing the intensity difference between subsequent scans. Subsequently, the root mean square (RMS) intensity change over all voxels was calculated as index of total intensity change. Scans in which the RMS was >3 standard deviations above the average were flagged (Smyser et al. 2010; Shannon et al. 2011). For functional connectivity analysis, all flagged scans were excluded, as well as the scan before and 2 scans after the flagged scan. Two younger and 2 older participants with <200 remaining scans were excluded. In the remaining younger participants, an average of 10.4% of all scans was removed based on this procedure, in the older participants the proportion of removed scans was 7.2% on average. Before, as well as after this motion correction procedure, there was no significant difference between age groups in the average total displacement per scan.

Functional Connectivity Analysis

For functional connectivity analysis the brain-wide graph of 264 putative functional areas (10 mm diameter spheres) created by Power et al. (2011) was used. The functional areas in this graph were defined

based on meta-analysis and functional connectivity mapping so that each area represents an element of brain organization. To make sure that the graph only included areas that did not suffer from susceptibility artifacts, a group mask was created. First, participant-specific binary images were created by thresholding functional images at 70% of *mean* signal intensity. A group mask was created by multiplying the binary images of all participants. If the group mask overlapped <50% with a functional area, this area was excluded from analysis (i.e., 29 functional areas). Average time courses were extracted for the remaining 235 functional areas. Pearson correlation coefficients were computed between the time courses of all functional areas in each participant separately. To remove connections which might be due to re-slicing or motion-induced artifacts, correlations between areas <20 mm apart were set to zero (Power et al. 2011). The diagonal of the correlation matrix was set to zero to remove correlations between an area and itself.

Thresholding

Based on the correlation matrix, graphs were constructed for each participant. Graph characteristics, such as modularity and global efficiency are affected by the number of nodes, but also by the number of edges in a graph (van Wijk et al. 2010). For each participant, the correlation matrix was thresholded to enhance the contrast between relevant (strong) and irrelevant (weak) connectivity values. This was done in such a way that the number of edges in the graph was constant. A threshold was selected using the method below, in order to maximize the amount of information obtained about the network on the group level.

For a range of thresholds (selecting between 1 and 50% strongest connections), and for both age groups separately, we applied the following procedure. For each participant, the correlation matrix was binarized by setting the connections above the predefined threshold to 1 and all other connections to zero. Subsequently these binarized matrices were averaged over all participants within each group. This averaged matrix is referred to as the “actual” matrix. Information theory was applied to compute the entropy over the actual matrix (Shannon 1948). The threshold at which the entropy is lowest, is the threshold at which the actual matrix contains the least disorder and therefore the largest stability over participants. However, the entropy also depends on the number of elements taken into account for each participant; at a threshold of 100% the entropy will be zero. Therefore, a correction was applied to account for these changes by comparing the entropy in the actual matrix to the entropy in a randomized matrix. We created 50 randomized matrices per participant, per threshold, preserving the number of nodes and the degree distribution (Maslov and Sneppen 2002). These random graphs were used to construct 500 new average graphs, by randomly sampling one of the 50 randomized networks per participant. The entropy was computed for each of these average random matrices and averaged. Then, the difference between the entropy in the actual and the entropy in the random matrices was computed. Once this procedure was performed for all thresholds, the optimal threshold was defined as the threshold at which the difference between the entropy in the actual matrices and the entropy in the randomized matrices is maximal. The optimal threshold is found when the information in the actual matrix is as unique as possible (i.e., highest stability across subjects), and more importantly, least resembles the result for a random network. More details of this method, including simulations, are presented in the Supplementary materials. Applying the procedure described above to both age groups separately, resulted in a threshold set at the 2.8% strongest connections in the network for the younger participants and 2.6% for the older participants. Therefore, a threshold of 2.7% was selected (see Supplementary Fig. 2).

Graph Analysis

Network measures were calculated using functions implemented in the Brain Connectivity Toolbox (Rubinov and Sporns 2010, www.brain-connectivity-toolbox.net). Modularity is the extent to which a graph can be divided into modules with a large number of within module connections and a minimal number of between module connections (Girvan and Newman 2002). For fMRI data, such modules are similar to the functional networks that can be identified using seed-

based correlations or independent component analysis (Power et al. 2011). Network modularity estimates were computed using the algorithm by Blondel et al. (2008), using the average modularity across 50 runs of the algorithm. In addition, local and global efficiency were assessed (Latora and Marchiori 2001). Global efficiency is the inverse of the average shortest path length in the network and is suitable for use in disconnected networks. Local efficiency is the inverse of the average shortest path length between all immediate neighbors of a node. Local efficiency tends to be related to modularity; networks which have dense local connections tend to have a more modular organization (Bullmore and Sporns 2012). Local efficiency was averaged over all nodes to estimate the mean local efficiency for the complete graph or specific networks.

The graph was partitioned into modules separately for younger and older participants. As input to the partitioning algorithm, we computed averages of the binary matrices of all participants (correlation matrices thresholded at 2.7%) in each age group. To achieve the optimal module division, we adopted a 2-step procedure, similar to the one applied by Rubinov and Sporns (2011). An initial partition into modules was created using the algorithm by Blondel et al. (2008), which attempts to maximize within module connections and minimize between module connections. As the approach is susceptible to the occurrence of local maxima, this procedure was repeated 500 times. Subsequently, all of these partitions were refined, using a modularity fine-tuning algorithm (Sun et al. 2009) which randomly assigns nodes to different modules or randomly creates a separate module. Changes that led to an increase in modularity were retained. The fine-tuning algorithm was applied repeatedly until the modularity of the partitioning no longer increased, and the partitioning with the highest modularity was used for further analyses.

To compare the module decompositions in older and younger participants, we used normalized mutual information (NMI). NMI measures how much information is provided by one set of assignments about another set of assignments (Strehl and Ghosh 2003) and varies from 0 (no mutual information) to 1 (identical node assignments). Statistics on differences in module decomposition between age groups (NMI <1) was obtained using permutation testing. In the permutation procedure, participants were randomly divided into 2 groups (retaining original group sizes). Subsequently, the optimal module decomposition was calculated for each group and their NMI was calculated as described above. This procedure was repeated 1000 times to get a distribution of NMI values under the null hypotheses. If the actual NMI between age groups was smaller than the fifth percentile of this distribution, the difference between groups was considered significant.

To find modules which were representative for both the older and the younger participants, we used the intersection of the modules defined in the 2 groups. Only nodes that belonged to a specific module in both groups, were taken as representative of that module for both groups. Additional details on how common networks were constructed are reported in the Results section. For each of the 5 large networks defined in this manner, we computed the average local efficiency and participation coefficient. The participation coefficient is an index of the number of between module connections versus the total number of connections of a certain node (Guimerà and Amaral 2005).

To examine the connectivity within and between all the different modules we developed a specific procedure, which was performed separately for both negative and positive connections. For this analysis the original weighted graph was used. Correlations with $P < 0.05$ after false discovery rate correction (FDR, Benjamini and Hochberg 1995) were retained, while all other correlations were set to zero. For each pair of modules and within each module, we then computed the sum of all correlations and divided these by the number of possible correlations. Group comparisons were performed with Mann Whitney U tests.

Correlation with Behavioral Measures

To assess how the observed changes in network properties affected the functioning of older participants, the relation with cognitive performance was examined. All participants were tested on an extensive neuropsychological battery, consisting of visual-motor sequencing (Trail making test A and B, Reitan 1958; Tombaugh 2004), executive

functioning (Stroop task, Stroop 1935), working memory and incidental recall (digit span test forward and backward, Wechsler Intelligence Scale—Revised, Wechsler 1981), verbal learning (Dutch version of the Rey Auditory Verbal Learning Test, Lezak et al. 2004), and a simple reaction time test. In addition, an estimation of crystallized intelligence (Dutch version of the National Adult Reading Test, Schmandt et al. 1992) and fluid intelligence (matrix reasoning test, Wechsler Intelligence Scale—Revised, Wechsler 1981) was obtained. One younger participant was excluded from the analysis because neuropsychological data were not available. All neuropsychological test scores were transformed to z -scores and scaled such that a higher value indicates better performance. Because some of the neuropsychological test scores were highly correlated, we first performed factor analysis on the neuropsychological tests using maximum likelihood estimation and varimax rotation. Four factors, with an eigenvalue >1 , were chosen based on the interpretability of the results. Subsequent correlations with complex network measures were performed using participant factor scores. Only complex network measures that showed a significant difference between the age groups were related to behavioral performance.

Correlation with Structural Measures

To assess whether complex network measure differences between younger and older adults were related systematic gray matter volume differences between the groups, the determinant of the Jacobian matrix was used. This determinant is the local expansion factor, which results from the DARTEL procedure and represents differences in local volume between the individual images and the template brain. Values of the Jacobian determinant that are >1 , indicate volume expansion relative to the group template, whereas values <1 indicate contraction (Lee et al. 2007). For each functional area that was used in the graph analysis, the corresponding average Jacobian determinant was extracted for each participant. Subsequently, Spearman rank correlations were computed between complex networks measures and the Jacobian, both averaged across all functional areas as well as for each module separately.

Results

Functional Networks in Old and Young

Functional networks were identified separately in the older and the younger group, by using module decomposition algorithms (Rubinov and Sporns 2011). The modules we identified were similar to the functional brain networks described in the literature (Damoiseaux et al. 2006) and to the modules described by Power et al. 2011 (see Fig. 1A). To examine the similarities between the node–module assignments (i.e., which nodes are assigned to which functional networks) of older adults and younger adults, we used normalized mutual information (NMI). Subsequently, permutation testing was used to test whether this similarity was significantly below chance level. Over all nodes and all modules, the NMI between older and younger participants was 0.6, which was significantly lower than expected by chance ($P = 0.006$). Tests per module revealed significant differences between younger and older participants in the visual module (NMI = 0.63, $P = 0.001$), whereas no significant differences between age groups were observed in the somatomotor and cingulo-opercular network (NMI = 0.66, $P = 0.07$, and NMI = 0.60, $P = 0.69$, respectively). In addition, age-related differences were observed in the FPCN and the DMN. While the FPCN and the DMN were separate modules in younger participants, they were identified as one module in the older participants. The NMI expressing the extent to which DMN/FPCN node assignment in the older group was predicted by node assignments of both the DMN and the FPCN in the younger group was 0.39. This similarity

was significantly below chance level ($P = 0.009$). The null distributions of the permutation tests per module are shown in Supplementary Figure 6. To test whether the observed age-differences are specific to the threshold of 2.7% applied here, we repeated the analysis presented above for a range of thresholds between 2 and 10%. At all of the thresholds, a significant difference in module decomposition between younger and older participants was observed (see Fig. 3B).

To compare characteristics of networks between the younger and older groups, we first derived common networks. The nodes belonging to the same network in both groups were taken as representatives of that network (see Fig. 1C and Table 1). The DMN and FPCN modules were based on the node assignments in young participants but included only nodes that belonged to the DMN/FPCN in older participants. In Figure 1B, the average graphs of the younger and older participants are presented using a force atlas layout. To illustrate age-related differences in labeling, the graphs of older and younger participants are presented both with the labeling of their own group and with the labeling of the other group.

Age-Related Changes in Network Distinctiveness: Modularity and Participation Coefficient

Segregation of functional networks was reduced in the older (mean modularity old [M_{old}] = 0.61) compared with the younger participants ($M_{young} = 0.67$; $z = 5.3$, $P < 0.001$), see Figure 2A. Additional correlation analyses between modularity and age within the older group revealed no significant correlation ($r = -0.21$, $P = 0.22$). The difference between the age groups can also be observed in Figure 1B; nodes within functional networks are less clustered in older than in younger participants. Particularly, the visual network shows more pronounced local isolation in younger than in older participants. Confirming these findings, the participation coefficient was increased in older compared with younger participants in the visual and the somatomotor networks ($z = 4.53$, $P < 0.001$; $z = 4.04$, and $P < 0.001$, respectively), indicating that a larger proportion of the connections of the nodes in these networks are directed to nodes outside the network (see Fig. 2B). Similar to Power et al. (2011), we observed that the FPCN was the network with the highest proportion of inter-network connections (the highest participation coefficient). This is in agreement with its central role in cognitive control, requiring communication with other networks (Vincent et al. 2008; Spreng et al. 2010).

Age-Related Changes in Efficiency of Connectivity: Global and Local Efficiency

While global efficiency was similar in older and younger participants ($M_{young} = 0.20$, $M_{old} = 0.20$, $z = 0.25$, $P = 0.80$), local efficiency was significantly reduced in the older compared with the younger participants ($M_{young} = 0.35$, $M_{old} = 0.39$, $z = 4.7$, $P < 0.001$). These results were independent of the chosen connectivity threshold (see Fig. 3). Separate analyses in each functional network showed an age-related decrease in local efficiency in the DMN ($z = 2.87$, $P = 0.004$), the FPCN ($z = 2.51$, $P = 0.012$) and the cingulo-opercular network ($z = 3.53$, $P < 0.001$, see Fig. 2B). Correlations between local or global efficiency and age within the older group, did not show any significant effects ($r = 0.22$, $P = 0.20$; $r = 0.05$, $P = 0.78$, respectively). Additional analyses with more stringent movement correction criteria (0.3 mm) and additional low-pass filtering (0.08 Hz) did not change the effects of age

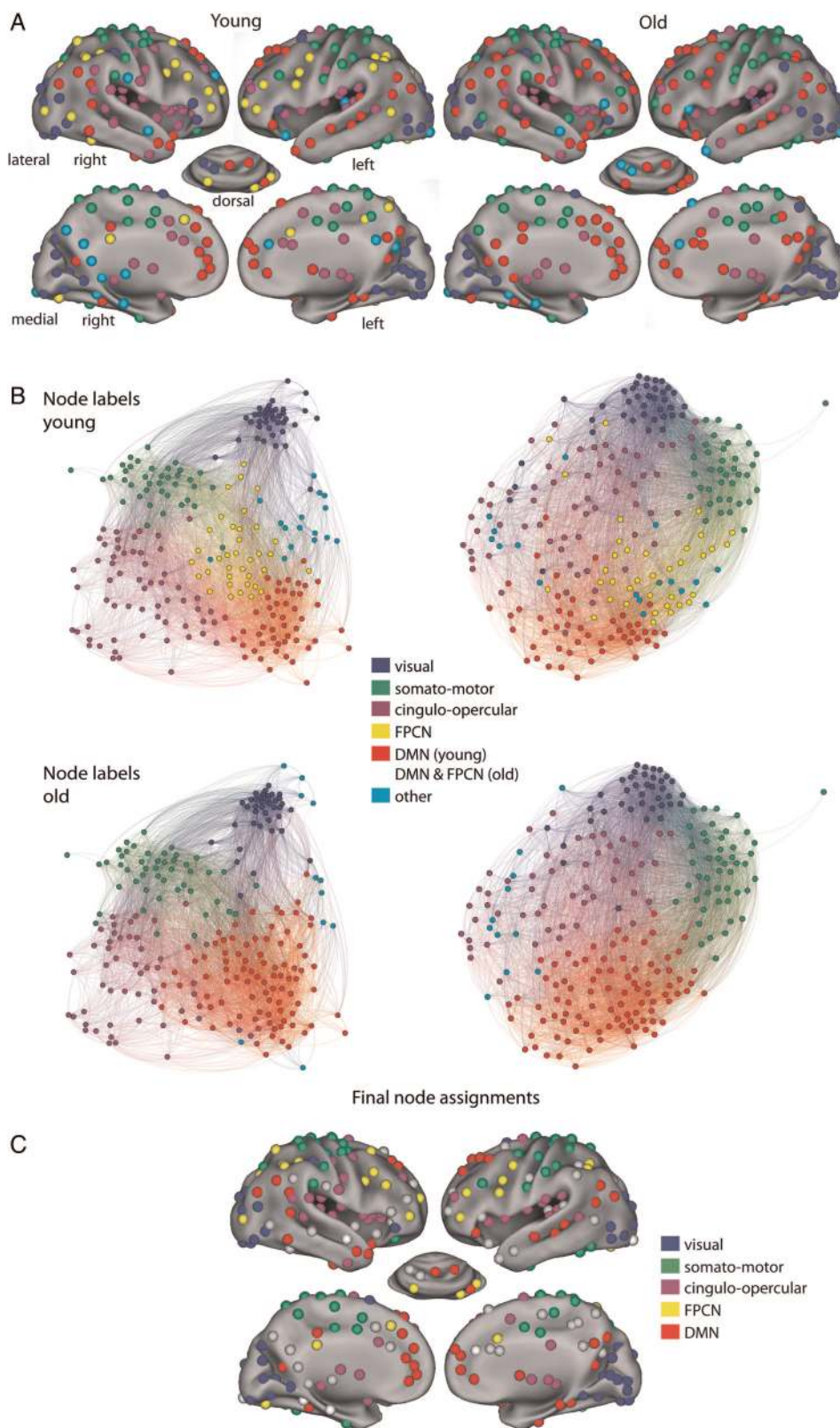


Figure 1. (A) The different modules are shown separately for older (right) and younger (left) participants. The colors indicate the nodes that belong to each module. Nodes are pasted on an inflated surface rendering of the human brain using the CARET program (Van Essen et al. 2001). (B) The graphs for younger (left) and older (right) participants are visualized using a force atlas layout implemented in Gephi (Bastian et al. 2009). The top row shows the graphs of younger and older participants with the node assignments of the younger participants. The bottom row shows the graphs for both groups with the node assignments of the older participants. (C) Final node–module assignments based on the intersection of node assignments in both groups. Gray nodes were not assigned to any of the modules.

Table 1
Nodes assigned to each module in younger and older participants: overlap and differences

	Young						Total (count)	% Nodes in common
	Visual	Somatomotor	Cingulo-opercular	DMN	FPCN	Other		
Old								
Visual	33	0	0	4	0	1	38	87%
Somatomotor	0	37	3	6	0	1	47	79%
Cingulo-opercular	0	0	47	0	2	1	50	94%
DMN and FPCN	1	1	9	26	44	8	89	79%
Other	4	0	0	0	2	5	11	
Total (count)	38	38	59	36	48	16	235	
% Nodes in common	87%	97%	80%	72%	92%			

Total (count) refers to the number of nodes in each module. % Nodes in common refers to the percentage of nodes assigned to a specific module that ended up in the same module in the final node-module assignments.

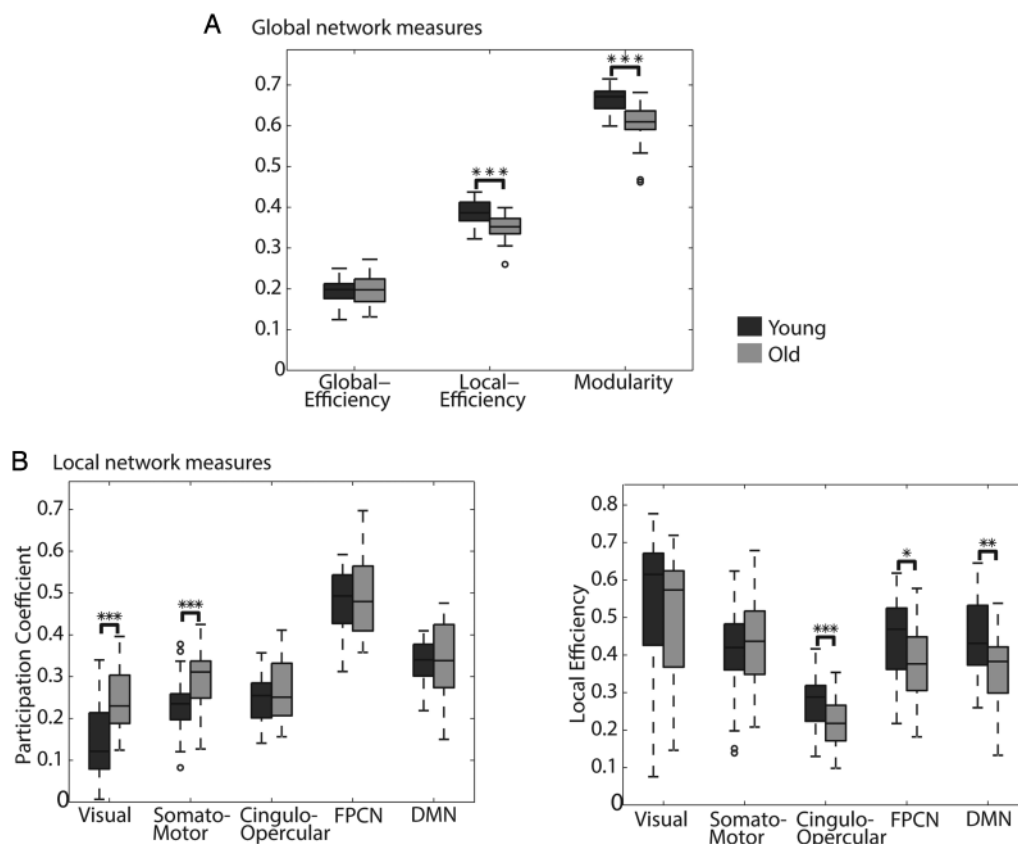


Figure 2. (A) Global network measures are presented in boxplots for older (lighter) and younger (darker) participants. From left to right, global efficiency, local efficiency, and modularity. Stars indicate a significant difference between the older and younger participants ($***P < 0.001$). (B) For each of the functional networks (modules), participation coefficient, and local efficiency are displayed in boxplots for younger and older participants. The darker boxplots represent the younger participants, the lighter boxplots the older participants. Difference between the older and younger groups; $*P < 0.05$, $**P < 0.005$, and $***P < 0.001$.

group on the network measures described above (global and local efficiency, modularity and participation coefficient, see Supplementary Figs 3 and 4). As a final check, we investigated the effect of global signal regression on the results. In the literature, it has been shown that global signal regression can have both positive and negative effects on the analysis of functional connectivity (Murphy et al. 2009; Weissenbacher et al. 2009; Song et al. 2012). Therefore, we have repeated the analyses without global signal regression. Although these analyses point to the same pattern of differences between older and younger participants, some differences were observed compared with

the original analyses. These are presented and discussed in the Supplementary materials (see Supplementary Fig. 5).

Age-Related Changes in Functional Connectivity Within and Between Networks

Functional connectivity is reflected in the strength of the correlations between all functional brain areas. To examine the effect of age on overall functional connectivity, we compared the correlation distribution of the unthresholded correlation matrix between younger and older participants. The number of negative correlations (between -0.25 and -0.15) and the

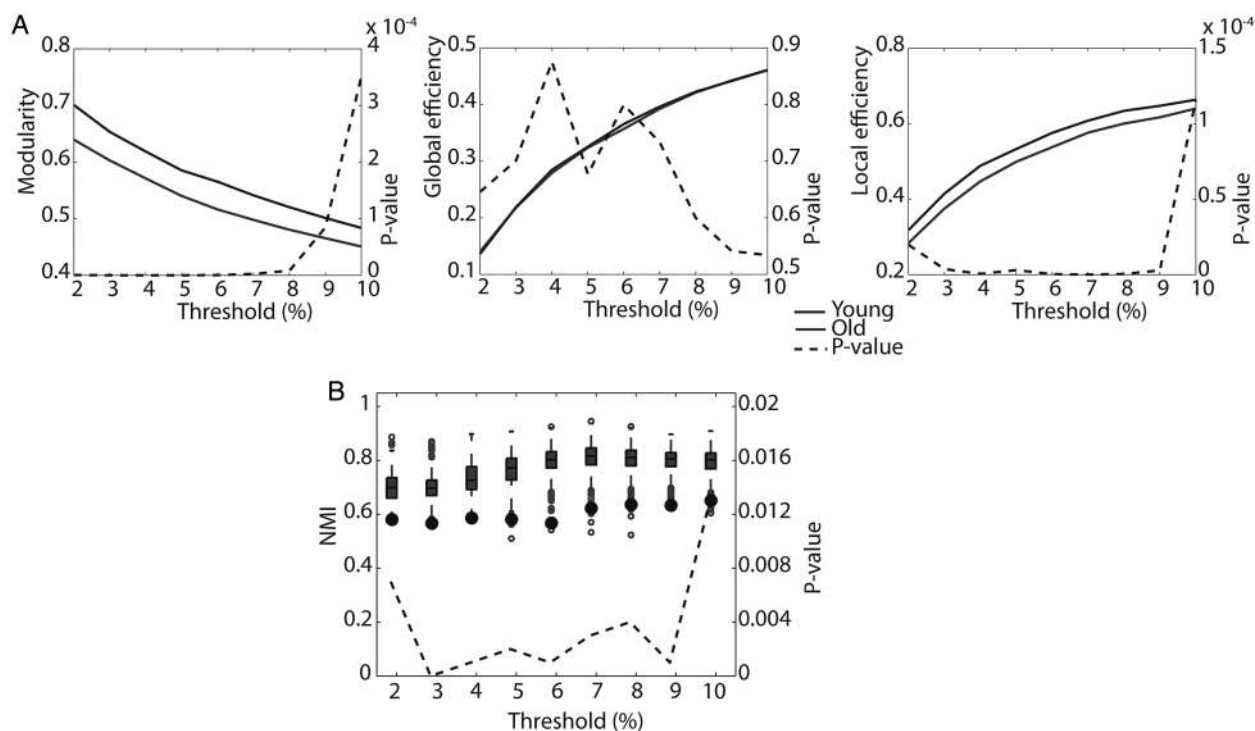


Figure 3. (A) Differences between old and young participants in modularity, global efficiency and local efficiency are plotted for thresholds between 2% and 10% of all possible connections. The dashed line indicates the P -values corresponding to the difference between the 2 groups, these values are presented on the right y -axis. (B) A test of age-related differences in module decomposition is shown over a range of thresholds. The null distribution of NMI values resulting from the permutation testing procedure is shown in the boxplots. The black dot represents the actual NMI value of the correspondence between the 2 age groups.

number of strong positive correlations (between 0.4 and 0.8) was reduced in elderly ($P < 0.05$, see Fig. 4A). Correlations between 0.08 and 0.18 were more pronounced in elderly. Taken together, overall functional connectivity decreased with age.

In addition, functional connectivity within and between each of the functional networks was examined. To distinguish effects of aging on positive and negative correlations (also referred to as anti-correlations Fox et al. 2009), these were examined separately (see Fig. 4B). The strength and number of correlations was combined in a single measure (total positive correlation or total negative correlation, respectively) and compared between younger and older participants. To select relevant connections an FDR-threshold was applied ($P < 0.05$ FDR corrected) to the correlation matrix of each participant. Subsequently node–module assignments were used to identify correlations within and between specific networks. The connectivity within the cingulo-opercular control network ($z = 4.59$, $P < 0.001$), the FPCN ($z = 2.64$, $P = 0.008$), and the DMN ($z = 4.23$, $P < 0.001$) was reduced with age, as was the connectivity between the cingulo-opercular network and the somatomotor network ($z = 2.02$, $P = 0.04$). The connectivity between the visual network and the somatomotor networks ($z = 3.04$, $P = 0.002$) and between the visual and the cingulo-opercular network ($z = 2.62$, $P = 0.009$) increased with age.

Negative correlations were reduced in elderly between the somatomotor network and the visual network ($z = 2.48$, $P = 0.013$), between the cingulo-opercular network and the FPCN ($z = 2.01$, $P = 0.044$) and between the DMN and the FPCN ($z = 2.07$, $P = 0.038$). An increase in negative correlations was observed between the cingulo-opercular network and the somatomotor

network ($z = 2.04$, $P = 0.042$) and within the cingulo-opercular network ($z = 4.24$, $P < 0.001$).

Uddin et al. (2009) argued that the negative correlations between the DMN and FPCN result from a unilateral influence of the DMN on the FPCN. Therefore, we tested whether decreased intra-network connectivity in either the FPCN or DMN was indeed related to a decrease in negative correlations between the DMN and FPCN. We found that correlations within the DMN were associated with negative correlations between the DMN and FPCN in both younger ($r = 0.33$, $P = 0.044$) and older participants ($r = 0.47$, $P = 0.005$). However, correlations within the FPCN were not predictive of negative correlations between the DMN and FPCN in younger ($r = -0.01$, $P = 0.95$) nor older participants ($r = -0.06$, $P = 0.72$).

Age-Related Changes in Network Measures Related to Cognitive Performance

Cognitive performance was assessed by neuropsychological tests in all participants. Since some of the neuropsychological test scores showed high collinearity, we first performed factor analysis on the data of all participants (younger and older) to cluster the tests. Four factors were identified: *verbal learning*, loading high on the Rey auditory verbal learning test direct recall (0.77) and recognition (0.98); processing speed, with high loadings on the trail making tests A (0.84) and B (0.58), symbol substitution test (0.53) and matrix reasoning test (0.47); working memory, loading high on the forward (0.52) and backward digit span (0.95); crystallized intelligence with high loadings on the trail making B (0.45) and adult reading test (0.61). Older participants showed a significant decline of

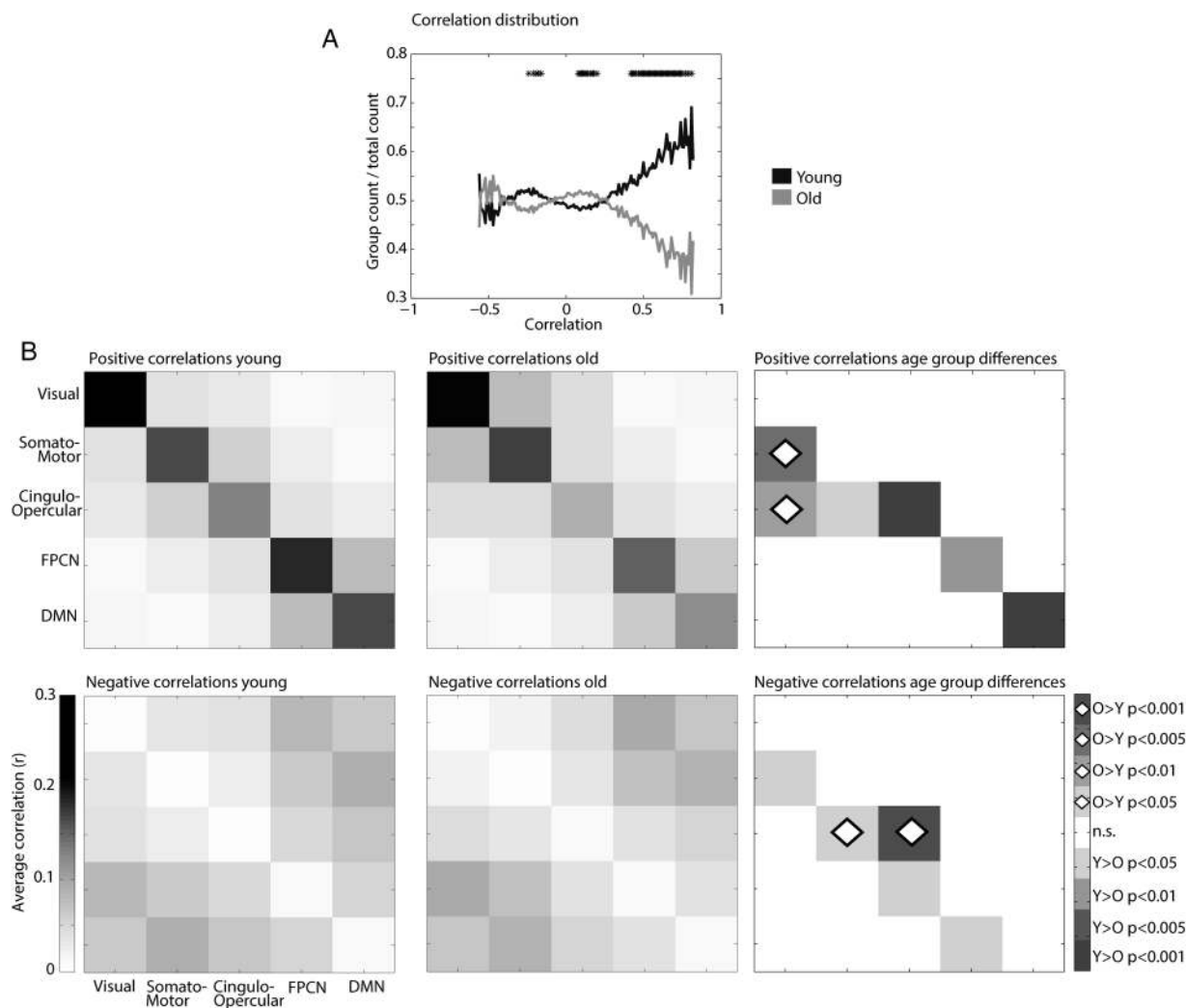


Figure 4. (A) The distribution of correlations shown for older (lighter) and younger (darker) participants. The number of correlations was counted in the separate bins with a size of 0.01. On the y -axis, the average number of correlations within the group, divided by the average number of correlations across both groups is depicted. A bin was included if at least half of the participants had one or more correlations in that particular bin. Stars indicate bins showing a significant difference between older and younger participants ($*P < 0.05$). (B) Average total functional connectivity (representing both strength and number of correlations) within and between networks is shown for younger (left) and older (middle) participants. The upper row demonstrates the changes in positive correlations, whereas the bottom row demonstrates the changes in negative correlations. The right panel shows significant differences in functional connectivity between both age groups. A fully filled square indicates decreased total correlations with age, a filled square with a diamond shape indicates increased total correlations with age.

performance in verbal learning ($z = 3.36$, $P < 0.001$) and processing speed ($z = 3.22$, $P = 0.001$) but not on working memory or crystallized intelligence.

For each of the network measures that showed an effect of age, we tested whether it was related to any of the 4 neuropsychological test factors, using *partial* Spearman correlations, *controlling for the effects of age*. Within the younger group, we found that reduced modularity was related to better verbal learning ($r = -0.37$, $P = 0.025$), while increased local efficiency in the full graph was related to increased processing speed ($r = 0.37$, $P = 0.028$). Modularity and local efficiency did not show significant relations with performance in the older group.

Within the younger group, we found that better verbal learning was associated with increased local efficiency and intra-network connections within the DMN ($r = 0.40$, $P = 0.015$ and $r = 0.40$, $P = 0.015$, respectively). In addition, better verbal learning was related to increased negative correlations between the cingulo-opercular network and the DMN ($r = 0.39$, $P = 0.019$ and $r = 0.42$, $P = 0.01$, respectively). Increased working

memory was associated with decreased local efficiency within the cingulo-opercular network ($r = -0.33$, $P = 0.044$) and decreased negative correlations between the cingulo-opercular network and the FPCN ($r = -0.35$, $P = 0.037$). In elderly, increased intra-network correlations in the FPCN were related to increased working memory ($r = 0.34$, $P = 0.043$) and crystallized intelligence ($r = 0.39$, $P = 0.019$). In addition, increased crystallized intelligence was associated with increased negative correlations within the cingulo-opercular network ($r = 0.35$, $P = 0.042$). It should be noted that the correlations between behavior and network measures do not survive an FDR correction for multiple comparisons. Therefore, these results should be interpreted with caution.

Age-Related Changes in Network Measures Related to Structural Differences

The Jacobian determinant was used as a measure of local gray matter volume differences between the individual images and the DARTEL template. In the young, the Jacobian determinant (local expansion factor) tended to be larger (1.058) than in

the older participants (0.978 ; $z = 1.72$, $P = 0.085$), indicating that on average, the local volume was larger in younger than older participants. The same pattern was observed in all 5 separate modules (visual, $z = 3.09$, $P = 0.002$; somatomotor, $z = 1.92$, $P = 0.055$; cingulo-opercular, $z = 2.99$, $P = 0.003$; FPCN, $z = 3.99$, $P < 0.001$, DMN, $z = 4.84$, $P < 0.001$).

We found that the average Jacobian determinant over the whole brain correlated significantly with the whole-brain local efficiency ($r = 0.31$, $P = 0.006$) as well as modularity ($r = 0.32$, $P = 0.005$) but not with the global efficiency ($r = 0.18$, $P = 0.12$). When we examined the correlations for each specific network, we found that some of the network measures that changed with age, were correlated with the Jacobian determinant. For the local efficiency per module, we found no significant correlations with the Jacobian determinant (visual, $r = 0.14$, $P = 0.22$; somatomotor, $r = 0.04$, $P = 0.77$; cingulo-opercular, $r = 0.21$, $P = 0.076$; FPCN, $r = 0.07$, $P = 0.56$; DMN, $r = 0.16$, $P = 0.16$). For the participation coefficient, we found a significant correlation with the Jacobian determinant ($r = -0.35$, $P = 0.002$) only in the somatomotor network. In the other networks no significant relationship was observed (visual, $r = -0.06$, $P = 0.59$; cingulo-opercular, $r = -0.10$, $P = 0.41$; FPCN, $r = -0.06$, $P = 0.61$, DMN, $r = 0.02$, $P = 0.85$).

Discussion

Using complex network measures, we identified clear differences in the organization of connections within and between functional networks with age. Brain networks in the elderly showed decreased modularity and decreased local efficiency within the DMN, FPCN and cingulo-opercular networks. Conversely, local efficiency in the visual and somatomotor networks was not affected by age while the participation coefficient of these networks was increased in elderly. Additional analyses showed that this increase in participation coefficient was due to increased connectivity between the visual and somatomotor network, as well as, between the visual and cingulo-opercular network.

In younger adults, functional brain networks were found to be highly modular, as reflected in high intranetwork connectivity along with few internetwork connections (Ferrarini et al. 2009; Meunier et al. 2010). In this study we have shown that this modularity is reduced in elderly, indicating that functional brain networks become less differentiated or less specific with age. These findings are in accordance with our previous study, where we used seed-based correlation analyses to demonstrate an increase in internetwork connections along with decreased intra-network connections during task performance (Geerligts et al. 2012). The present study extended these findings in 2 ways. First, the current findings demonstrate that age-related changes in functional connectivity are general and not restricted to performance during specific tasks. Second, the use of graph theory allowed us to quantify the effects of age on modularity. These effects are large, there is only little overlap in the modularity values of younger and older participants (see Fig. 2A).

The dedifferentiation theory suggests that overactivation of brain areas in elderly might be due to a decrease in functional distinction between brain areas (Baltes and Lindenberger 1997; Park et al. 2004; Carp et al. 2011; Dennis and Cabeza 2011). In line with our previous study (Geerligts et al. 2012), the current findings show that dedifferentiation also occurs on the level of functional networks. Functional networks show increased internetwork connections in older age along with

decreased intra-network connections, which makes them less distinct. These age-related changes in functional connectivity could be related to a dedifferentiation of activation patterns. Although the term dedifferentiation has often been used to indicate a link with age-related declines in performance, the increase in internetwork connections might also have a compensatory role.

Along with reduced modularity, the local efficiency across the whole network was reduced in elderly, while global efficiency was not affected by aging. The latter finding might be related to the increase in internetwork connections with age. Our findings are partly in accordance with previous results (Achard and Bullmore 2007), that have shown a reduction in local efficiency and global efficiency with age, while modularity was reported to be stable across age groups (Meunier et al. 2009). Differences between those results and the current findings may be related to the regional parcellation of the brain that was used in the previous study for graph construction (90 vs. 235 nodes in the present study), which has a limited ability to represent functional networks due to coarse nodes which encompass different functional areas (Power et al. 2011).

It has been suggested that overactivations in elderly are caused by less efficient use of neural resources (reduced cost efficiency); this theory has related over-recruitment of brain areas to less efficient performance in elderly (Morcom et al. 2007; Rypma et al. 2007; Stevens et al. 2008). The decline in local efficiency can be interpreted as a sign of reduced cost efficiency in the elderly brain, that is, with the same number of connections (cost) the efficiency is decreased. Because of the large metabolic costs of supplying the brain with resources, minimizing these costs is likely one of the selection pressures during evolution (Chen et al. 2006). Minimal metabolic costs can be achieved through high clustering of connections in brain networks (i.e., high local efficiency) along with sparse long range connections which are more costly but greatly increase the speed of information transfer (Buzsáki et al. 2004; Kitzbichler et al. 2011; Bullmore and Sporns 2012). Even though for older and younger participants the analyzed graphs contained the same number of connections, the local efficiency in the resulting network was smaller in the older participants. Furthermore, we found an increase in the number of intermodular connections in older compared with younger participants. Intermodular connections tend to be longer and therefore more costly, than intramodular connections (Meunier et al. 2010). Together, these findings indicate a decrease in the cost efficiency of functional networks of elderly. Note that we have shown that the reduction in cost efficiency is not only present during task performance (Morcom et al. 2007; Rypma et al. 2007; Stevens et al. 2008) but also during resting-state conditions.

In addition to comparisons between age groups, we also studied the correlations between the global network measures (modularity and global and local efficiency) and chronological age within the older group. No significant correlations were observed, which suggests that the changes in functional connectivity are not linearly related to chronological age. This fits with the model presented in a recent review article of Grady (2012), which illustrates how the effect of aging on functional connectivity could be mediated by many different (environmental) factors. These mediating variables (e.g., such as stress, education, exercise, genes, life experiences and diet (Milgram et al. 2002; Kramer et al. 2004; Pesonen et al. 2013)) might obscure a linear relation between aging and functional connectivity.

Besides age-related changes in global network properties, we showed changes in connectivity within and between specific functional networks in the older brain. Internetwork connections increased with age, primarily between the visual, somatomotor and cingulo-opercular networks. Local efficiency and intra-module correlations within the cingulo-opercular network, the FPCN and the DMN decreased with age. These results are in line with a recent study by Tomasi and Volkow (2012). They examined functional connectivity in relation to aging and showed that long range connectivity decreased from areas within the DMN and FPCN, while long range connectivity increased from areas in the somatomotor network, thalamus and cerebellum.

Previous research has linked age-related decreases in connectivity within the DMN to decreased memory, executive functioning and processing speed (Andrews-Hanna et al. 2007; Sambataro et al. 2010; Geerligts et al. 2012). Decreased connectivity within the FPCN with age has also been shown before (Andrews-Hanna et al. 2007; Madden et al. 2010; Rieckmann et al. 2011) and was associated with more efficient semantic retrieval in both younger and older participants (Madden et al. 2010). In line with these findings, we found that both connectivity within the DMN and local efficiency within the DMN correlated positively with verbal learning in younger participants. In addition, we found that connectivity changes were related to cognitive functioning; higher connectivity within the FPCN was associated with better working memory and crystallized intelligence in elderly. Although these findings are in line with the results in the literature, the correlations with behavior did not survive corrections for multiple comparisons and should therefore be interpreted with caution. Note that the decreases in intra-network connections occurred in 3 networks involved in higher level functions, while the networks involved in primary sensory and motor processing maintained intra-network connections with age. The findings in the literature as well as the observations in the current study suggest that the decreased connectivity within the DMN and FPCN might be related to cognitive decline in the aging brain.

The FPCN and the DMN formed one functional network in elderly in the module decomposition, while they formed separate networks in younger participants. In addition, decreased negative correlations between the FPCN and the DMN were observed with age. In several studies, it has been shown that older participants show reduced suppression of the DMN during performance of cognitive tasks (Lustig et al. 2003; Grady et al. 2006; Persson et al. 2007; Sambataro et al. 2010). In addition, the ability to flexibly decouple the FPCN from the DMN in tasks requiring an external focus was shown to be reduced with age (Spreng and Schacter 2011). These findings were argued to reflect a decline in neuromodulation at the level of larger-scale brain networks due to deficits in executive control. The current results suggest that the reduced integrity of both the DMN and the FPCN, as well as the decreased negative correlations between the 2 networks, result in reduced differentiation of these 2 networks. This dedifferentiation might underlie the reduced ability of elderly to modulate the 2 networks separately during task performance.

The correlation with behavior suggests that increased negative correlations between the DMN and the FPCN in the elderly might be related to improved verbal learning. This is in agreement with previous studies that have shown an association between negative correlations between FPCN and DMN and

better working memory and flanker task performance in younger participants (Kelly et al. 2008; Hampson et al. 2010). It has been suggested that the negative correlations between the DMN and FPCN are due to a unilateral influence of the DMN on the FPCN (Uddin et al. 2009). Supporting this idea, we have shown in the present study that the negative correlations between DMN and FPCN are related to the connectivity strength within the DMN but not to the connectivity strength within the FPCN, in older as well as younger participants. As aging is related to a decrease in intra-DMN connectivity as well as a decrease in DMN–FPCN negative correlations, the current findings suggest that both phenomena might be related to the reduction in intra-DMN connectivity.

Limitations

We found some interesting relations between performance on neuropsychological tests and complex network measures. Whereas some of the results are well in line with previous literature, suggesting that decreased connectivity within functional networks is related to reduced levels of task performance, for other results the interpretation is less straightforward. It is important to note that correlations between cognitive performance and network measures did not survive correction for multiple comparisons, therefore, they should be interpreted with caution to avoid speculation. However, they do provide a starting point for future studies.

Possibly, older and younger participants had different levels of arousal during the scanning session. However, there are a number of reasons why it is unlikely that such differences were the cause of the age-related effects on functional connectivity we observed. First of all, none of the participants mentioned that they had fallen asleep during the debriefing. Second, previous studies, that have examined functional connectivity differences in awake versus sleeping participants, showed only minor changes in functional connectivity (Horowitz et al. 2008; Larson-Prior et al. 2009). Furthermore, these changes were very different from the effects of aging that we observed in the current study (i.e., only a small increase in connectivity was observed during sleep within the DAN and no change was observed in the DMN in Larson-Prior et al. 2009).

In addition to functional changes, aging is known to be related to changes in underlying brain structure (Park and Reuter-Lorenz 2009). The functional networks in which we identified an age-related decrease in local efficiency in the present study show overlap with areas that generally show age-related reductions in gray or white matter. Reduced white matter is generally observed in frontal areas of the aging brain, whereas gray matter reductions are mainly found in frontal and parietal cortices, as well as in the insula and hippocampus (Good et al. 2001; Resnick et al. 2003; Raz et al. 2005; Gunning-Dixon et al. 2009; Madden et al. 2009). We therefore performed additional analyses to examine the relation between complex network measures and structural differences. The observed correlations between whole-brain local efficiency and modularity with the Jacobian determinant indicated that for these measures, it was not possible to disentangle the effects of aging on structural differences from the effects on functional connectivity. However, for the measures of local efficiency per module, we found no significant correlation with the Jacobian determinant. In addition, only for the somatomotor network, but not for the visual network, we observed a significant correlation between the Jacobian determinant and the participation

coefficient. These results demonstrate that not all of the observed differences in complex network measures can be attributed to age-related differences in brain structure. This is in line with the results of a previous study (Geerligs et al. 2012), in which we showed that changes in functional connectivity cannot be fully explained by changes in gray matter volume. Nevertheless, based on the results of the present study, it is difficult to conclude whether the reduction of gray matter in specific functional areas (nodes) and/or the reduced white matter integrity between functional areas (edges) is an underlying cause of the decline in intra-network connections. It would be important for future longitudinal studies to assess to what extent the changes in functional connectivity are indeed driven by the changes in gray and white matter.

Conclusions

In the current study we have shown that aging has pronounced effects on specific functional networks in the brain. In general, modularity and local efficiency were reduced and the distinction between the DMN and FPCN was diminished. Moreover, we have shown that the decreases in intra-network connections did not occur in primary processing networks, but were restricted to networks involved in higher order cognitive processes. Together with the increase in connectivity between visual and somatomotor networks, these results suggest a shift in the balance between intra- and inter-network connections. The results demonstrate that a brain-wide analysis approach of functional connectivity in the aging brain is fundamental to understand how age affects integration of information, both within and between networks.

Supplementary Material

Supplementary material can be found at: <http://www.cercor.oxfordjournals.org/>.

Notes

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