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Reduced Specificity of Functional Connectivity in the Aging Brain During Task Performance

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Abstract: The importance of studying connectivity in the aging brain is increasingly recognized. Recent studies have shown that connectivity within the default mode network is reduced with age and have demonstrated a clear relation of these changes with cognitive functioning. However, research on age-related changes in other functional networks is sparse and mainly focused on prespecified functional networks. Using functional magnetic resonance imaging, we investigated age-related changes in functional connectivity during a visual oddball task in a range of functional networks. It was found that compared with young participants, elderly showed a decrease in connectivity between areas belonging to the same functional network. This was found in the default mode network and the somatomotor network. Moreover, in all identified networks, elderly showed increased connectivity between areas within these networks and areas belonging to different functional networks. Decreased connectivity within functional networks was related to poorer cognitive functioning in elderly. The results were interpreted as a decrease in the specificity of functional networks in older participants. *Hum Brain Mapp 35:319–330, 2014.* © 2012 Wiley Periodicals, Inc.

Key words: aging; functional connectivity; dedifferentiation; neural noise; fMRI

INTRODUCTION

Recent research has shown that connectivity between brain areas is crucial for effective cognitive functioning [Biswal et al., 2010; Kelly et al., 2008; Spreng and Schacter, in press; Wen et al., 2012]. At the same time, it is becoming clear that aging affects connectivity in the brain on a large scale [Andrews-Hanna et al., 2007; Damoiseaux et al., 2008]. Therefore, changes in connectivity might be an important factor underlying the level of cognitive decline a person will experience with advancing age. Research on connectivity in the brain has identified a number of functional networks; groups of brain areas that show a strong correlation in their activation patterns [Sporns et al., 2004]. Most research on age-related changes in connectivity has focused on one of these networks; the default mode network (DMN).

The DMN is a network of brain areas that is more active while participants are not engaging in a specific task (i.e. in a resting state) than during task performance [Buckner et al., 2008; Greicius et al., 2003; Raichle et al., 2001]. Brain areas belonging to the DMN are the precuneus, the medial

Additional Supporting Information may be found in the online version of this article.

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prefrontal cortex, the superior frontal gyrus, the angular gyrus, the hippocampus, and the middle temporal gyrus. Over a range of studies, during both task execution and resting state conditions, it has consistently been shown that connectivity within the DMN is significantly decreased with advancing age [Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Grady et al., 2010; Sambataro et al., 2010]. Moreover, this decrease in connectivity within the DMN has been linked to a deterioration in performance on processing speed and working memory tasks in elderly [Andrews-Hanna et al., 2007, Sambataro et al., 2010].

In addition, there is evidence, although less consistent, of age-related changes in other networks. Frontoparietal connectivity within both the dorsal attention network (DAN) and the frontoparietal control network (FPCN) was found to be reduced with aging [Andrews-Hanna et al., 2007; Rieckmann et al., 2011], whereas connectivity between frontal areas was increased [Rieckmann et al., 2011]. These networks were argued to be involved in cognitive control [FPCN; Spreng et al., 2010; Vincent et al., 2008] and in overt and covert spatial attention and the creation of motor plans based on sensory inputs during task execution [DAN; Fox et al., 2005; Toro et al., 2008]. Connectivity within the motor network was also found to be decreased during resting state [Wu et al., 2007].

Connectivity between networks also appears to be important for task performance. For example, research has shown that the DMN and the dorsal attention network (DAN) generally tend to be anticorrelated; when activity in one network increases, activity in the other network decreases. This is in line with the function ascribed to these specific networks; while the DMN is less active during cognitive tasks, the DAN becomes more active during task execution [Fox et al., 2005]. Two studies linked the anticorrelation between these networks to behavioral performance; participants with a stronger negative correlation, had less variable behavioral performance and performed better on a working memory task [Hampson et al., 2010; Kelly et al., 2008].

Although multiple functional networks have been discerned, most aging studies mainly focused on functional connectivity in one or a limited number of prespecified networks. Instead, we were interested in the commonalities between connectivity changes in different networks; are the observed age-related changes in network connectivity characteristic for specific networks or is it possible to identify common patterns which are present in all or most of the networks? In addition, due to the focus on specific networks, most studies have looked at changes within this functional network, without taking into account connectivity between different networks. However, as previous studies have suggested [Park and Reuter-Lorenz, 2009; Rieckmann et al., 2011], the increase in neural activation that is often found with aging might be a sign of neural reorganization, which might affect the functional organization of networks identified mainly in young adults. Therefore looking at connectivity *between* networks is of crucial importance and will be the second focus of the present study.

The age-related changes that have been found so far, were observed during task execution, as well as, during resting state. In the current study, seed regions for the functional connectivity analysis were selected based on areas involved in task performance. Participants performed a visual oddball task which included novel stimuli. For the current purpose this task has several advantages. First of all, task difficulty is low, so both older and younger participants are able to perform well and to reach accuracy levels at ceiling. Second, adequate task performance in the oddball task requires continuous attentional control. Third, performance of visual oddball tasks generally recruits widespread areas in the occipital, parietal, and frontal lobes [Kiehl et al., 2005]. This enables us to use a broad range of areas that are involved in task performance and, more importantly, that are related to different functional networks, as a starting point for functional connectivity analysis.

In sum, in the present study we used areas involved in task performance to identify a range of functional networks and compared connectivity within these networks between young and elderly participants. Based on previous studies we expected to find age-related reductions in connectivity within functional networks, especially within the DMN. Moreover, we examined age-related changes in functional connectivity between networks.

METHODS

Participants

Twelve young participants (10 females, mean age 24.1, SD = 2.9) and 30 older participants[1] (10 female, mean age 63.9, SD = 6.2) without a history of head injury or other neurological conditions participated in this study. All participants had normal or corrected to normal vision and a normal score (19 or 20) on the unabbreviated cognitive screening test for dementia [de Graaf and Deelman, 1991]. Four of the elderly participants indicated that they used medication for high blood pressure, two elderly participants took medication against high cholesterol and one elderly participant took medication for diabetes. The study protocol was approved by the medical ethical committee of the University Medical Center Groningen and all participants gave written informed consent.

Task and Stimuli

Participants performed a visual oddball task. They were instructed to press a button with their right thumb when a target (letter "X") appeared on the screen. No response was required when a standard (the letter "O") or a novel (any of the other letters in the alphabet and digits 1–9) appeared on the screen. Three task versions were used. In

[1]A larger number of older than younger participants were included in this study in order to evaluate effects of performance level in the elderly group. However, this issue will not be discussed here.

the first version, only standard stimuli were presented; in the second version standard ($P = 0.85$) and target ($P = 0.15$) stimuli were presented and in the third version, standard ($P = 0.70$), target ($P = 0.15$), and novel ($P = 0.15$) stimuli were presented. Each novel stimulus did not occur more than once per block. Three blocks of each task version were presented in a balanced random order, with the restriction that the task versions of consecutive blocks were dissimilar. Alternation of different task blocks was used to motivate participants to keep attention focused on the task. Participants were asked to fixate on the fixation cross during task performance and rest periods and to react as fast and accurately as possible.

Stimuli were generated on a Personal Computer using E-prime (Psychology Software Tools Inc., Pittsburgh). They were presented on a screen positioned at the head end of the MRI scanner, which participants saw via a mirror attached to the head coil. Stimuli were presented in white, on a black background with a vertical visual angle of approximately 2°, and a horizontal visual angle between 0.5 and 2°, varying for the different symbols. Stimulus duration was 150 ms and interstimulus interval varied randomly between 1,050 and 1,450 ms (mean 1,250 ms), resulting in approximately 128 stimuli per task block of three minutes. Task blocks were alternated with rest periods of 45 s.

Behavioral Data

In addition to the fMRI experiment, all older participants completed a series of neuropsychological tests on a previous day. These tasks related to visual–motor sequencing [Trail making test A and B; Reitan, 1958; Tombaugh, 2004], executive functioning [Stroop interference test; Stroop, 1935; Rule shift test; Behavioral Assessment of the Dysexecutive Syndrome; Wilson et al., 1996], working memory and incidental recall [forward and backward digit span; Wechsler Adult Intelligence Scale—Revised; Wechsler, 1981], and verbal learning [Dutch version of the Rey Auditory Verbal Learning Test; Lezak et al., 2004].

Data of one younger and one older participant, were excluded from the analysis because they did not comply with the task instructions. For four participants data of only two of the three task blocks were included in the analysis. For one participant, this was due to technical problems during data acquisition, the other three participants did not comply with the task instructions in one of the blocks. For each participant median reaction times (RT_{med}) were calculated only for correct trials. Trials with RTs faster than 150 ms were regarded as fast guesses and were removed from the data. Differences between groups were assessed using the Mann-Whitney *U* test, because the data were not normally distributed.

Recordings

Functional images were acquired using a 3T Philips Intera MRI scanner (Best, The Netherlands), using a standard transmit/receive head coil. The following pulse sequence parameters were used: fast field echo (FFE) single shot echo planar imaging (EPI); 46 slices; slice thickness 3.5 mm; no gap; field of view 224 mm; scanning matrix 64 × 64; transverse slice orientation; repetition time (TR) = 3 s; echo time (TE) = 35 ms; flip angle 90°. In addition, T1-weighted three-dimensional FFE anatomical images of the entire brain were obtained with the following pulse sequence parameters: field of view 256 mm; scanning matrix 256 × 256; 120 slices; slice thickness 1 mm; transverse slice orientation; TE = 4.6 ms; TR =25 ms; flip angle 30°.

fMRI Data Analysis

Functional imaging data were analyzed using Statistical Parametric Mapping software (SPM8; available at: http://www.fil.ion.ucl.ac.uk/spm) implemented in Matlab 7.1.0 (The MathWorks, Natick, MA). Functional images were corrected for motion artifacts, coregistered to the T1 image, normalized to the Montreal Neurological Institute (MNI) standard template, and smoothed with an 8 mm full-width at half-maximum (FWHM) Gaussian kernel. For the first-level statistical analysis of the fMRI data, the onsets of standard, target and novel trials were entered as separate regressors. Preliminary analysis of the data indicated that there were no differences in activation patterns between task versions, therefore stimuli were collapsed over task versions in order to optimize parameter estimation. Onsets of trials with incorrect responses were modeled as a separate regressor. Additionally, the realignment parameters and the first derivatives thereof were entered as covariates to correct for the effects related to head motion [Friston et al., 1996]. No high-pass filter was applied because of the low-frequency cycling of conditions.

The task-related regressors were convoluted with the canonical hemodynamic response function (HRF), the temporal derivative, and the dispersion. When comparing BOLD signal changes in older and younger participants, changes in the timing and the shape of the HRF are a source of concern [Steffener et al., 2010]. Therefore, we used an approach which combines the contribution of the three HRF terms by calculating the total area under the curve [see also Kokal et al., 2009]. The area under the curve was calculated by reconstructing the fitted bold response for each of the stimulus categories, which were each subsequently integrated over time. This area under the curve value was fed into a factorial design in a second-level analysis, containing a subject factor, the three stimulus categories, and the age groups (young and older participants). A family wise error correction (FWE) of 0.05 and a cluster extent of 20 voxels was used to identify regions in which there was a main effect of stimulus type. Interactions between stimulus type and participant group were investigated using an FWE cluster threshold of 0.05 (initial threshold $P < 0.001$). Interpretation of results was restricted to gray matter areas.

Functional Connectivity Analysis

The procedure for the functional connectivity analysis mostly followed the approach described by Van Dijk et al. [2010]. Seed regions were defined by a 4 mm sphere around voxels displaying peak activation in the F-contrast examining main effect of stimulus type over all groups. These seed regions were used for all participants. Because of the larger number of older, compared with younger participants this approach could lead to a bias toward the older participants in the selection of seed regions. To control for this possibility, we created separate *F*-contrasts for the young and older groups. Our aim was to find out how many significant voxels in the original *F*-contrast could be explained by voxels in the *F*-contrast for the younger or the older group, respectively. Therefore, we lowered the *F*-threshold for both the older and the younger maps until together they explained 98% of the voxels in the original map ($F > 10$). Following this procedure, 39% of the significant voxels could be attributed to voxels in the *F*-map of young participants, 44% to the *F*-map of older participants, and 14% to both. This demonstrated that there is no bias in the selection of seed regions due to unequal group sizes.

Maps of functional connectivity were obtained by regressing the first eigenvariate of the time course from the seed region (corrected for the effects of stimulus onset) against the time courses of all acquired voxels. To minimize the effects of noise caused by the cardiac and respiratory cycles, scanner drifts, and motion, the following nuisance regressors were included in the first-level model: the realignment parameters and the first derivative of the realignment parameters, average white matter- and cerebral spinal fluid (CSF) signals, and the mean whole brain signal. In addition, stimulus onsets were included as nuisance regressors. White matter and CSF voxels were defined using the a priori probability maps for various tissue types included in the SPM8 package. These were turned into binary maps by applying a threshold of 95% and 75% probability for white matter and CSF, respectively. From these binary maps the average time courses for white matter and CSF signals were extracted. Separate first-level analyses were constructed for each of the seed regions. The regressors containing the first eigenvariate of the time course of the seed region were included in the second-level models. All second-level models included age group as independent variable. Main effects between groups were examined at a FWE cluster corrected threshold of 0.05 (initial threshold $P < 0.001$).

Clustering of Functional Connectivity Maps

Cluster analysis was used to group seed regions according to the similarity of their functional networks. This enabled us to compare our results with functional networks previously presented in the literature and thereby clarify the interpretation of age-related differences. For each seed region, a *t*-map was constructed representing the functional connectivity of that region with all voxels in the brain averaged over all participants. To reduce the dimensionality of the data, the Euclidian distance (or L2 norm) between the *t*-maps of each seed was calculated. The resulting distance matrix was fed into a *K*-means cluster analysis with 5,000 repetitions and random starting points. A solution with six clusters was chosen based on the knee in the scree plot [Ding and He, 2004]. Solutions with more clusters explained less than 5% additional variance. The clusters that were found were in accordance with functional networks as presented in the literature. In order to make one map on second level representing the functional connectivity within each resulting cluster, an additional functional connectivity analysis was performed on first level using the first eigenvariate extracted from the activation in the combined seed regions per cluster as the time course. For each cluster, a *t*-test was used to construct a functional connectivity map over groups. These maps were only used for comparing the functional networks with those in the literature; the assessment of group differences was done separately for each seed. To improve interpretability of the results and alignment with previous research, cluster membership of each seed is used in the presentation and discussion of the results. Membership as part of a functional network was determined for each area of which functional connectivity to the seed region changed depending on age group, through comparison with the cluster maps.

Corrections for Gray Matter Volume

Additional analyses were carried out to determine whether the observed differences in functional connectivity could have been influenced by underlying differences in gray matter density or registration error [Oakes et al., 2007]. First, voxel based morphometry (VBM) was used to identify regional brain volume of gray matter for each of the participants [Ashburner and Friston, 2000]. Structural T1 images for each participant were segmented into gray matter (GM), white matter (WM), and cerebral spinal fluid (CSF) using tissue probability maps provided by SPM8. Diffeomorphic Anatomical Registration using Exponentiated Lie algebra (DARTEL) was used to increase the accuracy of intersubject alignment [Ashburner, 2007]. Images were then normalized and modulated by the Jacobian determinants derived from the normalization step in order to adjust for the resulting volume changes due to normalization. Modulated normalized images were smoothed with a 10 mm FWHM Gaussian kernel. For statistical analysis, the two groups were combined in a one-way ANOVA, with the total intracranial volume (TIV) as a covariate to correct for individual differences in brain volume. TIV was calculated by summing volumes of GM, WM, and CSF derived from non-normalized segmented images. Because TIV in the present study includes CSF, it represents total brain volume and does not reflect atrophy

of either GM or WM. Differences between young and elderly participants were examined with a threshold of $P_{FWE} < 0.05$, and a cluster extent of 15 voxels.

Second, the fMRI data were reanalyzed using gray matter density information of each participant as a voxel-dependent covariate. This was done using the robust regression procedure which is less sensitive to the effects of outliers [Yang et al., 2011] as implemented in the biological parametric mapping toolbox [Casanova et al., 2007]. Although this robust procedure reduces the effects of outliers, we also noticed that it increased the voxel to (neighboring) voxel variability in the resulting *t*-map. For each of the areas that originally showed an effect of age on functional connectivity, we identified the number of voxels within that area that remained significant ($P < 0.001$) if gray matter density was taken into account.

Connectivity and Task Performance

To evaluate the relation between changes in connectivity and cognitive functioning in the elderly group, the connectivity estimates were correlated with the performance on neuropsychological tests. Each score was converted into a *z*-score and reversed if required so that higher scores indicate better performance. The Spearman rank correlation coefficient was used instead of the Pearson correlation coefficient to cope with deviations from normality, linearity and to reduce effects of possible outliers. Because of the large number of multiple comparisons (9 test scores $\times$ 28 connectivity estimates), we used a Monte Carlo resampling procedures to evaluate whether the number of significant correlations we detected was higher than could be expected by chance. The scores of participants were randomly permuted, and correlations with connectivity estimates were recomputed. The number of significant ($P < 0.05$) positive correlations was stored. This procedure was repeated 5,000 times to generate a null distribution. When the actual number of significant results was in the 5% tail of this distribution, we concluded that the number of significant differences was larger than could be expected based on chance. This was done separately for areas where older participants compared with young participants showed (a) decreased connectivity and (b) increased connectivity to the seed region. A subsequent question was whether the number of significant correlations differed significantly between regions with increased connectivity and regions showing decreased connectivity with age. Therefore, the number of significant clusters was compared between these two types of areas using a similar procedure.

RESULTS

Behavioral Results

Older participants showed a tendency to respond slower (O, $RT_{med} = 473$, IQR = 82) than younger participants, although this difference was not significant (Y, $RT_{med} = 427$, IQR = 34; $U(40) = 215$, $z = 1.68$, $P = 0.098$). The proportion of misses and false alarms was below 0.01% in both groups.

Effects of Task and Age on fMRI Activations

The first step in the analysis was to identify areas showing an effect of stimulus category (*F*-contrast); these areas were used in the functional connectivity analysis. Additionally, interactions between age group and stimulus category on brain activation were tested.

Over all groups, increased activation in target and novel trials compared with standard trials was observed in the right precentral gyrus (BA44), the left and right inferior parietal lobules, the right middle frontal gyrus, and the right and left fusiform gyri. The right cerebellum pars 7 and the right inferior orbitofrontal gyrus showed more activation in target than standard trials. In addition, in particular the left inferior frontal operculum, the left middle occipital, and the right inferior occipital gyrus, were more active in novel than in standard trials (see Table I and Fig. 1a). Furthermore, we found a number of areas where activation was decreased in target compared with standard and novel trials. These areas included the left postcentral gyrus, the right precentral gyrus (BA4), the mid cingulum, the left rolandic operculum, the right superior parietal gyrus, the left precuneus, and the left calcarine sulcus. The right middle orbitofrontal gyrus and the left anterior cingulate were specifically less active in target than standard trials.

Young participants showed decreased activation in target compared with standard trials in the left postcentral gyrus, the middle cingulum, the right fusiform gyrus, and the left and right superior temporal gyri, while there was no significant difference between these stimulus categories in the older participants (age group*stimulus category, see Supporting Information Table 1).

Functional Connectivity

In the second part of the analysis, we used functional connectivity analyses to examine differences in functional networks between older and younger participants. Areas showing a main effect of stimulus category were used as seed regions in this analysis. To facilitate comparisons with functional networks in the literature, functional connectivity maps of all seed regions were clustered. Seeds in each cluster were taken together to generate one functional connectivity map for each cluster on second level. Cluster membership of seed regions is presented in Table I. Below we will discuss the networks identified.

Default mode network (DMN)

The functional connectivity maps of the first and second clusters that were identified, closely resembled the DMN [see e.g. Raichle et al., 2001]. In the first cluster,

TABLE I. MNI coordinates for areas that show a main effect of stimulus category ($P_{FWE} < 0.05$, k20)

Area	K	F	*x*, *y*, *z*	Cluster	Effect
L Precuneus (BA7)	228	23.68	−6, −56, 34	DMN-p	S>T, N>T
L Calcarine (BA30)	167	24.76	−6, −52, 8	DMN-p	S>T, N>T
R Middle orbitofrontal (BA10)	85	23.45	10, 46, −2	DMN-a	S>T
L Anterior cingulate (BA10)	63	26.10	−10, 48, −2	DMN-a	S>T
R Precentral3 (BA44)	26	18.12	40, 4, 32	FPCN	N>S, N>T
R Precentral2 (BA44)	54	20.38	50, 12, 42	FPCN	N>S, N>T
L Inferior frontal operculum (BA44)	45	23.88	−50, 10, 30	FPCN	N>S, N>T
R Middle frontal (BA45)	198	27.49	44, 48, 20	FPCN	T>N, T>S
L Inferior parietal (BA7)	726	33.06	−30, −54, 46	FPCN	N>T, T>S
R Inferior parietal (BA40)	782	42.61	54, −42, 48	FPCN	T>N, N>S
R Superior parietal (BA2)	60	20.78	18, −44, 70	DAN-SMN	S>T,N>T
L Rolandic operculum (BA48)	25	22.44	−38, −20, 18	DAN-SMN	S>T,N>T
R Precentral (BA4)	456	34.82	46, −14, 58	DAN-SMN	S>T,N>T
L Postcentral (BA3)	1150	44.28	−34, −30, 62	DAN-SMN	S>T,N>T
L Mid cingulum (BA23)	1184	53.57	−8, −22, 46	DAN-SMN	S>T,N>T
R Cerebellum 7	20	20.31	8, −72, −44	DAN-VAN	T>N, T>S
L Middle occipital (BA19)	72	25.23	−34, −84, 14	DAN-VAN	N>S, N>T
R Fusiform (BA19)	186	27.57	38, −70, −20	DAN-VAN	N>S,T>S
R Inf occipital (BA19)	67	31.12	34, −86, −8	DAN-VAN	N>S, N>T
L Fusiform (BA37)	1705	44.74	−44, −62, −16	DAN-VAN	N>T, T>S
R Inferior orbitofrontal (BA38)	88	23.34	50, 22, −6	COCN	T>N, T>S

L = left, R = right, BA = Brodmann's area, *x*, *y*, *z* = stereotactic coordinates, k = cluster extent, p = posterior, a = anterior, DMN = default mode network, VAN = visual attention network, FPCN = frontoparietal control network, DAN = dorsal attention network, SMN = somatomotor network, COCN = cingulo-opercular control network, S = standards, T = targets, N = novels.

connectivity to posterior parts of the DMN was most pronounced while in the second cluster connectivity to anterior parts was predominant. In both DMN clusters, seed regions showed functional connectivity to a network consisting of the precuneus (bilateral), superior (medial) frontal gyri extending on the left side into the middle frontal gyrus, the angular gyri, the left and right hippocampus and the middle temporal gyri (see Fig. 1b).

Frontoparietal control network

The third cluster closely resembled the frontoparietal control network [FPCN, Vincent et al., 2008]. Areas belonging to this network were the right and left inferior parietal lobules, the right and left middle frontal gyri, the right and left inferior frontal operculum and inferior frontal triangular areas, the superior medial frontal gyrus, the supplementary motor area, and left and right caudate.

Dorsal attention network

The networks of the fourth and fifth cluster contained areas involved in vision, attention, and somatomotor processing which together closely resemble the DAN. The network of the fourth cluster contained the somatomotor parts of the DAN and closely resembles the previously identified somatomotor network [DAN-SMN, Beckmann et al., 2005; Damoiseaux et al., 2006]. Functional connectivity from these seeds was found to the right and left superior temporal gyri, the right and left pre- and postcentral gyri, the left and right superior parietal lobules, the left and right posterior insula, the paracentral lobule, the supplementary motor area, and the mid cingulum. Primary and secondary visual processing areas, as well as inferior and superior parietal areas were the main components of the fifth cluster, subsequently called DAN-visual attention network (DAN-VAN). Seeds in this cluster showed functional connectivity to the left and right middle and inferior occipital gyrus, the left and right calcarine and lingual gyrus, bilateral cerebellar areas 6, 7, and vermis 7 and 8, and the bilateral inferior and superior parietal lobules.

Cingulo-opercular control network

Cluster 6 consisted of only one seed region, the right inferior orbitofrontal gyrus. The network of this seed region closely resembles the lateral parts of the cingulo-opercular control network [COCN, Dosenbach et al., 2007]. Connectivity was found to the left and right inferior orbitofrontal gyri, the right and left inferior frontal opercula, the right and left superior temporal gyrus, the bilateral insula, and the right middle temporal gyrus. Contrary to the findings of Dosenbach et al. [2007] we did not observe any connectivity with the anterior cingulate.

Effects of Age on Functional Connectivity

For each seed region separately, effects of age on functional connectivity were examined. For reasons of clarity,

the observed effects are grouped according to cluster membership of the seed.

The pattern that became clear from Figure 1(c) and Supporting Information Table 2 is that elderly show more connectivity to areas that do not belong to the functional network of the respective seed region than young participants. From seed regions in the DMN clusters, older participants showed more connectivity to areas belonging to the DAN-SMN (right and left rolandic operculi, supplementary motor area, and anterior cingulate). From seeds in the FPCN elderly showed increased connectivity to areas belonging to the DMN, DAN-VAN, and the DAN-SMN (precuneus, cuneus, and middle cingulum, respectively). Seeds in the DAN-SMN showed increased connectivity to areas in the DMN and FPCN (precuneus, superior medial frontal gyrus, and left inferior parietal lobule). The left middle occipital gyrus (DAN-VAN) seed showed increased connectivity to the left inferior parietal sulcus (FPCN). The COCN seed showed increased connectivity to the right insula (DAN-SMN) and the right calcarine sulcus (DMN). An exception to this pattern was two seed regions in the DAN-SMN which showed increased connectivity to the middle cingulum (within the DAN-SMN).

In addition, elderly showed reduced connectivity to areas within the network of the seed region, especially for the DMN and the DAN-SMN [see Fig. 1(c)]. From DMN seeds, there was reduced connectivity to the precuneus and the angular gyrus. From seeds in the DAN-SMN we found reduced connectivity to the right insula, the left pre- and postcentral gyri and the right rolandic operculum. From DAN-VAN seeds, reduced connectivity was found to the left fusiform gyrus. Exceptions to this pattern were (a) the reduced connectivity in elderly to the right superior temporal gyrus from two DAN-SMN seeds; this area was not within any of the functional networks identified in the current study, and (b) reduced connectivity in elderly from the right inferior occipital gyrus (DAN-VAN) to the left superior frontal gyrus (DMN).

Correction for Gray Matter Density

VBM analyses showed significant decreases in gray matter in the older group compared with young participants, most notably in the anterior cingulate, the middle cingulum, and the medial superior frontal gyrus (see Supporting Information Fig. 1). Additionally, gray matter losses were identified in the left middle frontal gyrus and the left frontal inferior and rolandic operculum. To try and disentangle the connectivity changes from the age-related changes in gray matter density, connectivity analyses were repeated, using gray matter density as a voxelwise covariate [Oakes et al., 2007]. Within each area that initially showed a significant effect of age on connectivity with one of the seed regions, the number of voxels which stayed above threshold [$P < 0.001$] after gray matter correction is reported in Supporting Information Table 3. Although a general decrease in the number of above-threshold voxels was observed, we found for all but two of the areas, significant differences between the two age groups in functional connectivity between different brain areas. The proportion of remaining voxels tended to be larger for areas showing an age-related increase in connectivity compared with areas showing an age-related decrease in connectivity with the seed region.

Correlations Between Functional Connectivity and Neuropsychological Test Scores

All significant Spearman correlations between connectivity and neuropsychological test scores (NTs) are presented in Supporting Information Table 2. In areas where older adults showed more connectivity than young adults, we observed three positive and one negative correlations between NTs and connectivity. That is, connectivity correlated positively with the digit span backwards (in two areas) and Stroop interference tests and negatively with the Rule shift task. Moreover, we found eight positive correlations in areas where elderly showed less connectivity than young adults. Here, connectivity correlated positively

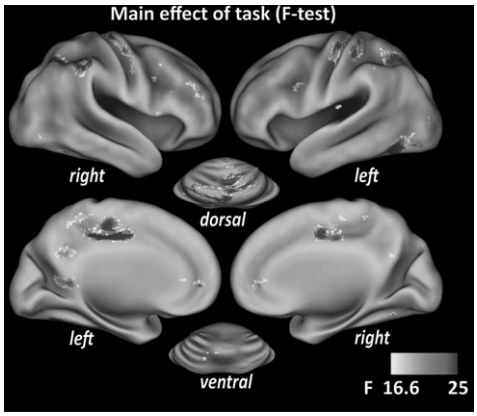

Figure 1.

(a) Areas showing a main effect of stimulus category are displayed ($P_{FWE} < 0.05$). The top row shows the lateral view and the bottom row shows the medial view of the brain. (**b**) For each cluster the functional connectivity averaged over all participants, from the combined seed regions (using the first eigenvariate of all the seed voxels) to other voxels in the brain is displayed ($P_{FWE} < 0.05$). (**c**) Effects of age on functional connectivity. Left, areas where elderly show more functional connectivity to the seed region than young participants (color represents cluster membership of the seed). Right, areas where elderly show less functional connectivity to the seed region than young participants ($P_{FWE\text{-}cluster} < 0.05$). Activations are displayed on an inflated surface rendering of the human brain using the CARET program [Van Essen et al., 2001]. Note that an area in a single color can indicate connectivity to one or multiple seeds within a cluster.

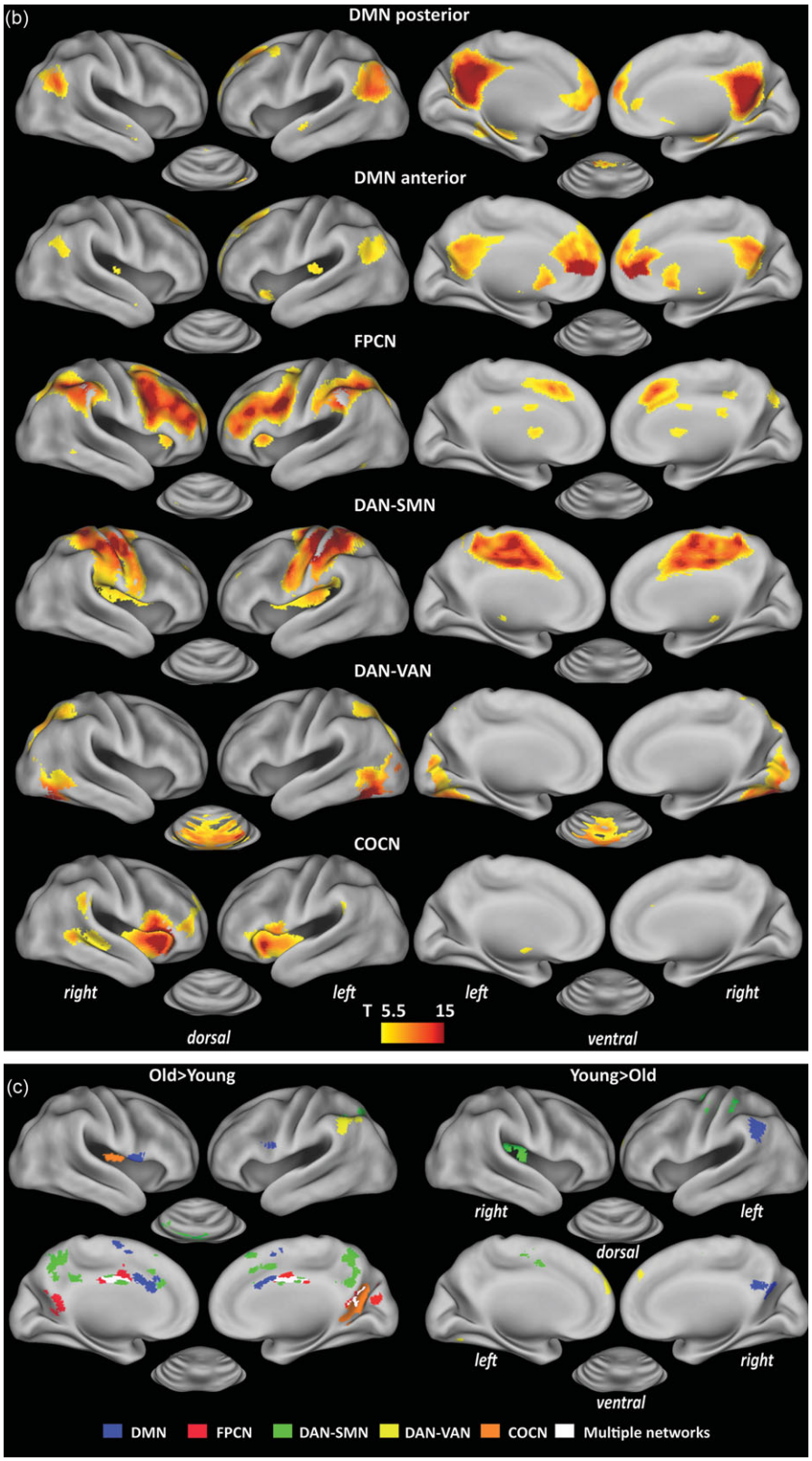

Figure 1.
Continued

with the Rule shift task (in three areas), the digit span forward and backward, the Rey Auditory Verbal Learning Task, and part A of the Trail making test (in two areas).

The Monte Carlo resampling procedure was used to indicate whether the number of significant positive correlations between connectivity and NTs exceeded the amount expected by chance. This analysis revealed that connectivity correlated significantly with test scores for connections that were decreased in elderly compared with young participants. The number of correlations was not significant for the connections where elderly showed more connectivity than the young participants. In addition, we found that the difference between the number of significant correlations in the two types of areas was significantly larger than expected by chance.

DISCUSSION

Although the present study used methods which were different from previous studies, the functional networks that were identified closely matched the networks that have previously been identified in the literature (see Fig. 1b). This was true for studies using a priori defined seeds in seed based regression analyses [Fox et al., 2005; Vincent et al., 2008; Voss et al., 2010], as well as studies using independent component analysis [Beckmann et al., 2005; Damoiseaux et al., 2006; Rosazza and Minati, 2011]. The fact that we were able to find the same networks using a more exploratory seed based approach along with a clustering method is a strong indication of the robustness of the networks that were identified.

Although seed based approaches usually identify the DAN as a single network [Fox et al., 2005; Vincent et al., 2008], we identified it in two subcomponents within this network. One component consisted of the somatomotor network, including the pre- and postcentral gyri and the supplementary motor areas, while the other consisted of a visual attention network, including the occipital, calcarine and lingual gyri, and the inferior and superior parietal lobules. These networks we identified closely resemble the identified networks in studies using independent component analyses [see for example Fig. 6b,d in Beckman et al, 2005]. The reason for the discrepancy with other seed based correlation studies might be related to specific task requirements; in our task the link between visual input, visual attention, and response depended on the stimulus category. All three stimulus types provided the participants with visual input, while especially standard and novel stimuli captured attention and only target stimuli required a response.

The approach adopted in the present study allowed us to identify a consistent pattern of differences between older and younger participants in connectivity within and between the different functional networks identified. In general, older participants showed positive correlations between seed regions and areas which were located outside the functional network of that seed region. Moreover, in the DMN and the DAN-SMN, connectivity to areas within the functional networks of the seed region was reduced in older participants. These results suggest a general decrease in the specificity of functional networks in elderly.

Voss et al. [2010] found similar patterns of reductions in specificity of functional networks in elderly in the DMN, the FPCN, and the DAN. They used a seed based approach with one a priori defined seed region for each of the networks. In their study, participants performed a series of passive viewing tasks. From the seed regions in all three networks, they found decreased connectivity in elderly to areas within the network and increased connectivity to areas outside the network. The similarity to our findings suggests that the pattern we identified was not specific to the visual oddball task that was used in the current study. Moreover, their results taken together with the current results, confirm that changes in connectivity in functional networks are not limited to specific networks but appear to be present in all networks identified. However, note that not all networks need to be affected to the same degree. At the moment it is not clear how differences between the tasks that participants perform affect the age-related changes in connectivity that are detected. Therefore, it would be interesting to see whether these findings can be replicated in resting state data or in more demanding cognitive tasks.

One possible explanation for the observed changes in connectivity in older individuals compared with our group of younger adults might be the decline in gray matter that is generally observed with age. In our elderly group, we observed age-related declines in gray matter density mainly in the anterior cingulate, the middle cingulum, and the rolandic operculum. This is in line with the gray matter changes observed in previous studies [Good et al., 2001]. The functional connectivity analysis using voxelwise gray matter density as a covariate, remained to show connectivity differences similar to the differences originally identified, except for two areas. The resulting number of significant voxels was larger for areas showing an age-related increase in connectivity compared with areas showing an age-related decrease in connectivity with the seed region. These results show that the observed age-related connectivity changes cannot be fully explained by the changes in gray matter.

An important question concerns the mechanism underlying the observed reduction in specificity of network connectivity. Dedifferentiation theory suggests that areas in the older brain may become less functionally distinct [Baltes and Lindenberger, 1997]. This idea has been supported by a range of studies. It was, for example, found that distinct categories of visual stimuli activate less selective areas in elderly in the visual cortex [Park et al., 2004], as well as, in the parietal and prefrontal cortex [Carp et al., 2011b]. Similarly, representations of distinct movements in the motor system, such as tapping the right and

the left finger, are less selective in the elderly [Carp et al., 2011a]. In addition, during visual imagery, the distinction between areas related to motion or faces was reduced in elderly [Kalkstein et al., 2011] and the specificity of the connections between the prefrontal cortex and the visual cortices during imagery was reduced in elderly. The current findings of reduced specificity in functional networks could be interpreted as a confirmatory evidence of dedifferentiation.

Changes in neural specificity can in some cases be related to changes in performance level; neural specificity in elderly, for example, was found to be a good predictor of fluid intelligence, but not crystallized intelligence [Park et al., 2010]. The relation with performance level was partly confirmed in the current study. We found that decreased connectivity within the DAN-SMN was related to poorer performance on neuropsychological tests of visual–motor sequencing, executive functioning, working memory, incidental recall, and verbal memory. However, we found no evidence of a negative or positive effect of increased connectivity between areas in different functional networks on task performance.

Overactivations caused by dedifferentiation might be related to reduced specificity of functional networks. A recent study provided evidence for this idea; Langan et al. [2010] showed that reduced interhemispheric connectivity in elderly was related to a decreased ability to inhibit activity in the nondominant hemisphere during unilateral motor task performance. It would be interesting to see if future studies in other domains will be able to link the decrease in specificity of neural representations to the specificity in functional networks, as well. Note however, that there are also a lot of studies showing positive relations between overactivation in elderly and cognitive performance [for a review, Park and Reuter-Lorenz, 2009]. In addition, we have shown in a recent electroencephalogram (EEG) study, using a selective attention task, that specific increases in connectivity in elderly can have a compensatory function [Geerligs et al., in press]. Therefore, we agree with other researchers that aging theories need to incorporate both dedifferentiation and compensation to fully account for the age-related changes on the neural level and their relation to cognitive performance [Carp et al., 2010].

Li et al. [2001] proposed that neural noise, defined as haphazard neuronal activity, might be the cause the reduction in the specificity of neural representations with age. An increase in neural noise with age might not only affect the specificity of neural representations within specific brain areas, but also the specificity of functional networks. This is in line a recent simulation study showing that there seems to be an optimal level of neural noise, at which correlation within networks and anticorrelations between networks are highest. An increase or a decrease in noise with regard to this optimum reduces both correlations and anticorrelations [Deco et al., 2009]. Therefore, we suggest that the increased levels of neural noise with age could be a plausible mechanism underlying both decreased connectivity within functional networks and increased connectivity between networks along with less specific neural representations.

In conclusion, we have shown a widespread decrease in the specificity of functional networks in older compared with younger participants. This was expressed in both an increase in connectivity between areas belonging to different functional networks and a decrease in connectivity between areas belonging to the same functional network. Specifically decreased connectivity within functional networks was related to poorer cognitive functioning in elderly.

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