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# Chapter 4

## The effects of sustained cognitive task performance on subsequent resting state functional connectivity in healthy young and middle-aged male school teachers

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## **ABSTRACT**

Previous studies showed that functional connectivity (FC) within resting state (RS) networks is modulated by previous experience. In this study the effects of sustained cognitive performance on subsequent RS FC were investigated in healthy young (25 - 30 years; n = 15) and middle-aged (50 - 60 years; n = 14) male school teachers. Participants were scanned (functional MRI) after a cognitively demanding and a control intervention (randomised tester-blind within-subject design). Independent component analysis was used to decompose the data into spatially independent networks. This study focused on the executive control (ExN), the left and right fronto-parietal (FPN) and the default mode network (DMN). The effects of cognitive performance and age were calculated with a full factorial ANOVA.

A main effect of Age was found in the left inferior frontal gyrus for the ExN and in the middle frontal gyrus for the DMN with middle-aged teachers having reduced RS FC. Sustained cognitive performance increased subsequent RS FC between the ExN and a lingual/parahippocampal cluster, and between the left FPN and a right calcarine/precuneus cluster. In these clusters, FC strength correlated positively with the perceived amount of effort during the intervention. Furthermore, sustained cognitive performance affected subsequent RS FC between the ExN and the right temporal superior gyrus differently in young and middle-aged men. The results suggest that effects of age on RS FC are already present at middle age. Sustained cognitive performance increased RS FC between task-positive networks and other brain regions, although a change in RS FC within the networks was not found.

\* This chapter is based on data from fMRI study 1.

## INTRODUCTION

Over the last years the number of studies that have investigated functional connectivity (FC) of brain networks during resting state (RS) has grown substantially. These RS networks (RSNs) can be identified by examining the low frequency fluctuations of the blood oxygen level dependent response, which correlates between regions of a network (Biswal et al., 1995; Greicius et al., 2003). Advantages of studying brain functioning by investigating RS FC are: data collection last only a couple of minutes, the functioning of a large number of brain networks can be studied, and data is not confounded by differences in cognitive performance.

One of the networks than can be studied during RS is the default mode network (DMN). The DMN consists of regions that exhibit decreased activity during cognitive task performance (a so called task-negative network) and is thought to mediate processes that are important for the RS, such as attending to internally driven cognitive processes (Damoiseaux et al., 2006; Fox et al., 2005; Greicius et al., 2003; Raichle and Snyder, 2007). The functioning of the DMN has been associated with the amount of task-related activation (Greicius and Menon, 2004) and behavioural performance (Boly et al., 2007; Hampson et al., 2006). For example, attention lapses have been associated with reduced task-related deactivation of the DMN (Weissman et al., 2006). In addition to the DMN, RS studies have found patterns of FC between brain regions known to be involved in cognitive task performance, so called task-positive networks (e.g., Damoiseaux et al., 2006, Smith et al., 2009). Examples of task-positive networks are the executive control, the sensorimotor and the auditory network (Smith et al., 2009). Also RS FC within task-positive networks has been associated with the amount of stimulus induced activity (Fox et al., 2006) and behaviour (Fox et al., 2007).

RS FC is considered a relatively stable baseline activity of the brain based on structural connectivity (Damoiseaux and Greicius, 2009). In line with findings of reduced white matter integrity in old age (Madden et al., 2009; Sullivan and Pfefferbaum, 2006), it has been shown that RS FC is compromised in old age. Previous studies have shown decreased RS FC within the DMN (Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Grady et al., 2010; Koch et al., 2010; Sambataro et al., 2010), reductions in the extent of the DMN and reduced task-induced deactivation of the DMN (e.g. Andrews-Hanna et al., 2007; Sambataro et al., 2010) in older compared to young adults. In addition, previous studies found reduced FC within the dorsal attention system in elderly (Andrews-Hanna et al., 2007; Tomasi and Volkow, 2011). Although Grady and colleagues (2010) did not find reduced FC within the task-positive network in older subjects, they did show an increase in the extent of the task-positive network and changes in FC between task-positive network regions and other parts of the brain.

Although RSNs have been shown to be consistent across individuals and measurements (Damoiseaux et al., 2006), previous studies have reported that task performance can shape subsequent RS FC in both the default mode and task-positive networks (Albert et al., 2009; Hasson et al., 2009; Lewis et al., 2009; Northoff et al., 2010; Stevens et al., 2010; Tambini et al., 2010; Waites et al., 2005). For example, Waites et al. (2005) investigated the effect of performing a language task on subsequent RS FC within four language-

related brain networks. They showed that RS FC between the left and right middle frontal cortex and between the posterior cingulate and medial frontal cortex was increased. In the present study, the effects of sustained cognitive performance on subsequent RS FC were investigated.

A factor that is likely to interact with the effects of sustained cognitive performance is age (Avlund et al., 2007). Previous studies have shown that cognitive performance has already started to decline during middle age (Park et al., 2002; Van der Elst et al., 2005). Reduced cognitive abilities may change the effects of cognitive performance on subsequent RS FC. Adding a group of middle-aged participants to the present study enabled us to investigate whether 1) effects of ageing on RS FC are already present at middle age and 2) whether sustained cognitive task performance affects RS FC differently in middle-aged and young participants.

In the present study, the effects of performing highly cognitively demanding tasks for 1.5 h on RS FC were investigated in healthy young and middle-aged school teachers. Participants performed tasks that required working memory (N-back task), cognitive control (Stroop Task), and solved brain teasers and arithmetic during the experimental intervention and read a book or watched a DVD during the control intervention (within-subject design). Smith and colleagues (2009) described what cognitive domains load on what brain networks. This information, combined with the nature of the cognitively demanding tasks participants performed, was used to select the task-positive RSNs the present study focused on: the executive control (ExN), and the left and right fronto-parietal network (FPN). The DMN was added because of its importance for cognition and because effects of ageing have been found on FC within the DMN. Previous studies have shown that FC strength is moderately to highly reliable when measured across sessions on separate days (Damoiseaux et al., 2006; van Dijk et al., 2010; Zuo et al., 2010). Considering the exploratory nature of the present study and the suggestion that independent component analysis (ICA) might be a better method than the seed-based approach to detect subtle changes in FC (Koch et al., 2010), ICA was used to identify the selected RSNs.

Based on previous studies that examined the effects of cognitive performance on subsequent RS FC (Albert et al., 2009; Jolles et al., 2011; Waites et al., 2005), it was hypothesised that sustained cognitive task performance increases FC within task-positive networks that have been recruited during the cognitive intervention. Furthermore, reduced RS FC was expected in middle-aged compared to young participants. Finally, based on the finding that white matter integrity has already decreased in middle age (Salat et al., 2005), it was hypothesised that in brain regions sensitive to effects of ageing, sustained cognitive performance increases FC less in middle-aged than in young participants.

## **MATERIALS AND METHODS**

### **Participants**

Healthy right-handed male school teachers aged 25 - 35 (young;  $n = 15$ ) and 50-60 years (middle-aged;  $n = 19$ ) were tested. It was decided to test young and middle-aged male school teachers for the following reasons. Firstly, school teachers experience the same

amount and type of mental workload in their daytime jobs, resulting in a homogeneous research population. Secondly, school teachers might benefit from more insight into their response to high mental workload because the incidence of burnout is large among schoolteachers (Farber, 1991). Thirdly, only males were tested to avoid confounding effects of differences in oestrogen levels on brain functioning (e.g. Dietrich et al., 2001). Researchers involved in this project recruited participants by placing advertisements in school bulletins, and by giving short presentations and distributing flyers at schools to make teachers enthusiastic about participating in this study.

Men who suffered from significant past or present physical or psychiatric illnesses (Epilepsy, stroke, Parkinson's disease, Multiple Sclerosis, brain surgery, brain trauma, electroshock therapy, kidney dialysis, treatment by a neurologist or psychiatrist, diabetes, heart disease, migraine, Meniere's disease, hypertension, brain infections), who received medication at the moment of inclusion, or who had MRI contraindications (brain surgery, pacemaker, metal objects/part in body, claustrophobia, large parts of the body tattooed), were not included into this study.

This study was approved by the Dutch local Medical Ethical Committee of the Maastricht University Medical Centre and Maastricht University, and was carried out in accordance with the declaration of Helsinki (Seoul 2008). Participants were informed in advance about the goal of the study and the nature of the experimental procedures, and gave informed consent before the start of the study. They were compensated for their voluntary participation.

## Procedures

Participants were tested twice: after a highly cognitively demanding (high load) and after a less demanding control intervention (control). The test sessions took place during weekends, starting at 0900 h, 1100 h or 1300 h. Before the test sessions, participants received a training session in which the cognitive tasks that were performed in the scanner and some additional neuropsychological tests were carried out (not reported in this article). The order of the test sessions, high load or control first, was randomised and between the test sessions there was at least a week (max 9 weeks). The researcher, who operated the MRI system and gave instructions to participants during scanning, was blind to the condition they were in.

During the high load intervention participants performed the following cognitive tasks: 2 and 3-N-back task (in total for 30 min), Stroop task with incongruent trials only (in total for 20 min), mental arithmetic (in total for 20 min), and brain teasers/puzzles (in total for 20 min). The N-back and Stroop task were programmed in E-prime (Version 1.2; Psychology Software Tools, Pittsburgh) and presented on a laptop computer; arithmetic and brain teasers/puzzles were paper and pencil versions. Participants were engaged in these tasks for 1.5 h without a break. During the control intervention participants watched a documentary style DVD or read a magazine (e.g., the National Geographic) for 1.5 h.

After the intervention the participants were placed in the MRI scanner. Firstly, localiser scans, secondly, the RS scan, and thirdly, a T1 scan was made. These scans were followed by functional scans during which participants performed a working memory and a word-

learning task (results will be presented elsewhere).

## Questionnaires

To assess the subjective effects of the intervention, participants completed the NASA Task Load Index (NASA TLX: Hart & Staveland, 1988) after both interventions. The NASA TLX targets the subjective experience of workload and provides an indication of the costs of maintaining task performance. The following subscales were analysed: Mental Demand (“How mentally demanding was the task?”), Temporal Demand/Time Pressure (“How hurried or rushed was the pace of the task?”), Own Performance (“How successful were you in accomplishing what you were asked to do?”), Effort (“How hard did you have to work to accomplish your level of performance?”) and Frustration (“How insecure, discouraged, irritated, stressed, and annoyed were you?”). In addition, participants completed a short visual analogue version of the Profile of Mood State (POMS) questionnaire (McNair et al., 1988) after both interventions, of which the subscale ‘Fatigue’ was analysed for this study. This subscale examines the ‘mood of fatigue’ described as “feelings of having a reduced capacity to complete mental or physical activities” (O’Connor, 2004, pp S7).

Analysis of variance (OpenStat Jan. 31, 2012 <http://www.statprograms4u.com/>), with Load (2 levels: control and high load) as within-subject, and Age (young or middle-aged) as between-subject variable, was used to test the effects of Load and Age, and their interaction.

## MRI data acquisition

RS scans were acquired with a 3 Tesla whole body MRI system (Philips Achieva, Philips Medical Systems, Best, The Netherlands). The body coil was used for RF transmission and an 8-element SENSE head coil (SENSE-factor 2) for signal detection. In total 250 whole brain EPI images were acquired (TR = 2.0 s; TE = 35 ms; field of view = 224 x 112 x 256 mm; oblique orientation; interleaved slice acquisition; 32 slices; 3.5 mm slice thickness; 0.0 mm slice gap; 64 x 64 matrix; 4 x 4 x 3.5 mm voxels). During RS scanning, participants were asked to keep their eyes open, not to fall asleep and to lie as still as possible. Van Dijk and colleagues (2010) showed that when subjects rested with their eyes open (whether fixating or not) correlations in the default mode and attentional network were stronger than when participants rested with their eyes closed or performed a cognitive task. We chose for the ‘eyes open not fixation’ condition because this is in practice the easiest option and less likely to be confounded by differences in the ability to attend to a fixation cross that might exist between conditions and age groups. Furthermore, a 3D T1-weighted anatomical scan was acquired (256 x 256 matrix, 150 slices, 1 x 1 x 1 mm voxels).

## MRI data analysis

Preprocessing: SPM5 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, Institute of Neurology, University College London) was used to preprocess the RS images. The following preprocessing steps were applied: slice-time correction (corrected to the middle slice with SPM5’s Fourier phase shift interpolation), motion correction (registered to the first image with 2nd Degree B-spline interpolation),

unwarping (to correct for susceptibility-distortion-by-movement interactions), spatial normalisation (the mean EPI image of each session was matched to SPM5's EPI template in Montreal Neurological Institute (MNI) space) where after the parameters were applied to all images of that session). During normalisation voxel size was rescaled to 2 x 2 x 2 mm. Finally, the data were smoothed with a FWHM 6 mm Gaussian kernel. The RS time-series data was detrend (removal of linear trend) and low band pass filtered (between 0.01 and 0.08 Hz) with the 'RESTing-state fMRI data analysis toolkit' (REST version 1.3, by Song Xiaowei, <http://restfmri.net/forum/?q=rest>).

Independent component analysis: The Matlab toolbox, Group ICA of functional MRI Toolbox (GIFT v1.3h: <http://icatb.sourceforge.net>) was used for ICA. ICA was carried out (algorithm: Infomax) on the entire dataset and the number of components was restricted to 20. Out of these 20 components, the ExN and the left and right FPN were selected by visual inspection. The DMN was selected based on the spatial correlation with the DMN template provided by the GIFT (DMN\_ICA\_REST.nii: the DMN extracted with ICA from a dataset of 42 subjects). Subject and session specific images and the associated time courses (both converted to z-scores) were produced by back reconstruction. Thereafter for each network the individual networks were used as input for a full factorial ANOVA (factor 1: Age with 2 independent levels, and factor 2: Load with 2 dependent levels) carried out in SPM5. The effects of Age and Load were firstly calculated with whole brain (WB) analysis, and secondly, with a small volume correction (SVC) based on the size of the network. For the second analysis, each network was built by combining the functional clusters that make up the network ( $p$ FWER-corrected  $< .05$ ).

For all analysis we controlled for multiple comparisons by applying a family wise error rate (FWER) correction on the group statistics. In SPM5 this correction is carried out with the use of random field theory (RFT). In this approach both the volume of search and the smoothness of the data are taken into account. FWER correction combined with a  $p$ -value  $< .05$  yielded a 95% confidence level that there are no false positives in our results (Bennett et al., 2009). Effects of Age, Load and their interaction were considered significant when  $p$ FWER-corrected of the peak voxel(s) is smaller than .05 and the cluster size is larger than 5 voxels. Coordinates were labeled with MRICron's (Chris Rorden, 2007; <http://www.mccauslandcenter.sc.edu/mricron/>) anatomical templates (i.e., the AAL template (Tzourio-Mazoyer et al., 2002) and the Brodmann template).

Total grey matter volume (TGMV): To be able to control for differences in grey matter volume, TGMV was calculated. Structural T1 images were segmented (segmentation tool SPM5) into white matter, grey matter and cerebrospinal fluid images. Grey matter images were normalised to MNI space (SPM5; template: the average T1 image of 152 MNI brains) and TGMV was computed, following a procedure described in Pernet et al. (2009). A one-way ANOVA ( $F = 21.6$ ;  $p < .001$ ) showed that TGMV was lower in middle-aged (mean = 102,462 voxels [819.7 cm<sup>3</sup>]; SD = 3,619 voxels) than in young participants (mean = 110,378 voxels [883.0 cm<sup>3</sup>]; SD = 5,316 voxels). TGMV was added as a covariate to the full factorial ANOVA, to statistically correct for the difference in TGMV.



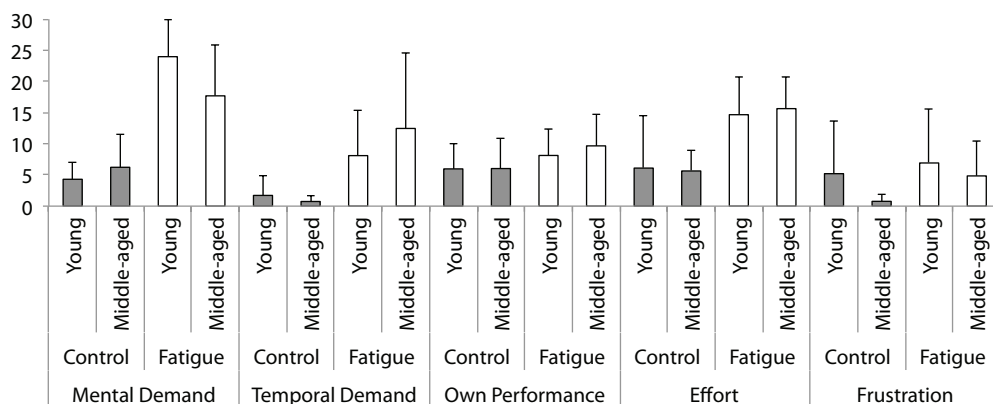
## RESULTS

### Participants

Data was complete for 15 young and 17 middle-aged participants. To assess data quality, raw MRI images were visually inspected. In six datasets, artefacts were seen. To check whether these artefacts affected the quality of the RSNs, we calculated the spatial correlation between the DMN found in these datasets (the result of ICA analysis with all 64 datasets) and a spatial DMN template provided by the GIFT (DMN\_ICA\_REST.nii). Datasets were included into the analysis, when the spatial correlation was less than 2 standard deviations from the mean (mean spatial correlation = .36; SD = .07) and the DMN looked good when inspected visually. Based on these criteria, data of three middle-aged participants was excluded. So data of 15 young (mean age = 30.7; SD = 3.0) and 14 middle-aged men (mean age = 54.9; SD = 3.7) was used for analysis. A chi-squared-test indicated that the number of participants in each group was statistically balanced ( $\chi^2 = .03$ ;  $p = .85$ ). Seven young participants started with the control and eight started with the high load intervention. Six middle-aged participants started with the control and eight started with the high load intervention. A chi-squared-test indicated that the order of the interventions was statistically balanced ( $\chi^2 = .04$ ;  $p = .84$ ). A one-way ANOVA ( $F = 2.0$ ;  $p = .17$ ) showed no difference in the number of years of education following after primary school between the groups. Young teachers followed on average 12.4 (SD = 1.4) and middle-aged teachers on average 13.6 years (SD = 3.1) of education after primary school.

### Questionnaires

See Figure 1 for the average scores on the questionnaire subscales (continuous variables). For the Frustration subscale, no main effect of Load or Age (categorical variables), and no interaction between Load and Age was found. A main effect of Load was found for the NASA TLX Temporal Demands, ( $F(1,27) = 20.6$ ;  $p < .001$ ), Own Performance ( $F(1,26) = 8.8$ ;  $p = .006$ ), and Effort ( $F(1,27) = 28.8$ ;  $p < .001$ ) subscale, indicating that the high load intervention was perceived as temporally more demanding, more effortful, and leading to a lower judgment of own performance than the control intervention. For these three subscales no main effect of Age and no interaction between Load and Age was found. For the NASA TLX Mental Demand subscale, a main effect of Load ( $F(1,27) = 108.9$ ;  $p < .001$ ) and an interaction between Load and Age ( $F(1,27) = 6.4$ ;  $p = .018$ ) was found. A one-way ANOVA with Load as factor showed that both the middle-aged ( $F(1,26) = 21.0$ ;  $p < .001$ ) and young participants ( $F(1,28) = 91.8$ ;  $p < .001$ ) experienced the high load intervention as more mentally demanding than the control. Furthermore, a one-way ANOVA with Age as factor, showed that the young participants experienced the high load intervention as more mentally demanding than the middle-aged participants ( $F(1,27) = 6.5$ ;  $p = .02$ ). No difference between age groups was found after the control intervention. For the POMS subscale Fatigue, a significant effect of Load ( $F(1, 27) = 7.3$ ;  $p = .012$ ) was found. Participants were more fatigued after the high load than after the control intervention. No main effect of Age and no interaction between Load and Age were found.



**Figure 1** | Average scores with standard deviations on the NASA TLX and POMS subscales. A higher score on the NASA TLX Mental Demand, Temporal Demand, Effort and Frustration subscales and on the POMS Fatigue subscale, indicates that more mental demand, temporal demand, effort, frustration and fatigue was experienced. For the subscale Own performance a higher score indicates that participants experienced their own performance as worse.

## Imaging results

The ExN, the left and right FPN, and the DMN could be clearly identified and the overall average networks were very similar to networks reported in previous studies (Damoiseaux et al., 2006; Damoiseaux et al., 2008; Smith et al., 2009). The four RSNs are shown in Figure 2.

**Effects of Age:** See Table 1 for the effects of Age (categorical variable) on RS FC (continuous variable). For the ExN, a main effect of Age was found in a left inferior frontal cluster (WB and SVC analysis: cluster falls within the ExN) with middle-aged teachers having reduced FC. At the peak voxel of this cluster, the mean Z-value = .90 (SD = .61) for young, and .04 (SD = .67) for middle-aged participants. For the DMN, a main effect of Age was found in a middle frontal cluster (SVC analysis: cluster falls within the DMN) with middle-aged teachers having reduced FC: mean Z-value = 1.83 (SD = .98) for young and .99 (SD = .85) for middle-aged participants. No main effects of Age were found in the left and right FPN.

**Effects of Load:** See Table 1 for the effects of Load (categorical variable) on RS FC (continuous variable). For the ExN, Load increased FC in a right lingual/parahippocampal cluster (WB analysis only: cluster falls outside the ExN): mean Z-value control condition = -.31, SD = .55; mean Z-value high load condition = .51, SD = .57. In a post-hoc analysis the individual Z-scores for the peak voxel of the right lingual/parahippocampal cluster were correlated with the individual scores on the NASA TLX Effort and Mental Demand subscales and the POMS Fatigue subscale (both groups, both conditions). Individual scores on the Mental Demand ( $r(56) = .49$ ;  $T = 4.2$ ;  $p < .001$ ) and Effort ( $r(56) = .39$ ;  $T = 3.1$ ;  $p < .001$ ) subscale correlated positively with the individual Z-values. No correlations were found for the POMS Fatigue subscale ( $r(56) = -.03$ ;  $T = 0.2$ ;  $p = .85$ ).

An interaction between Load and Age was found in a right superior temporal cluster

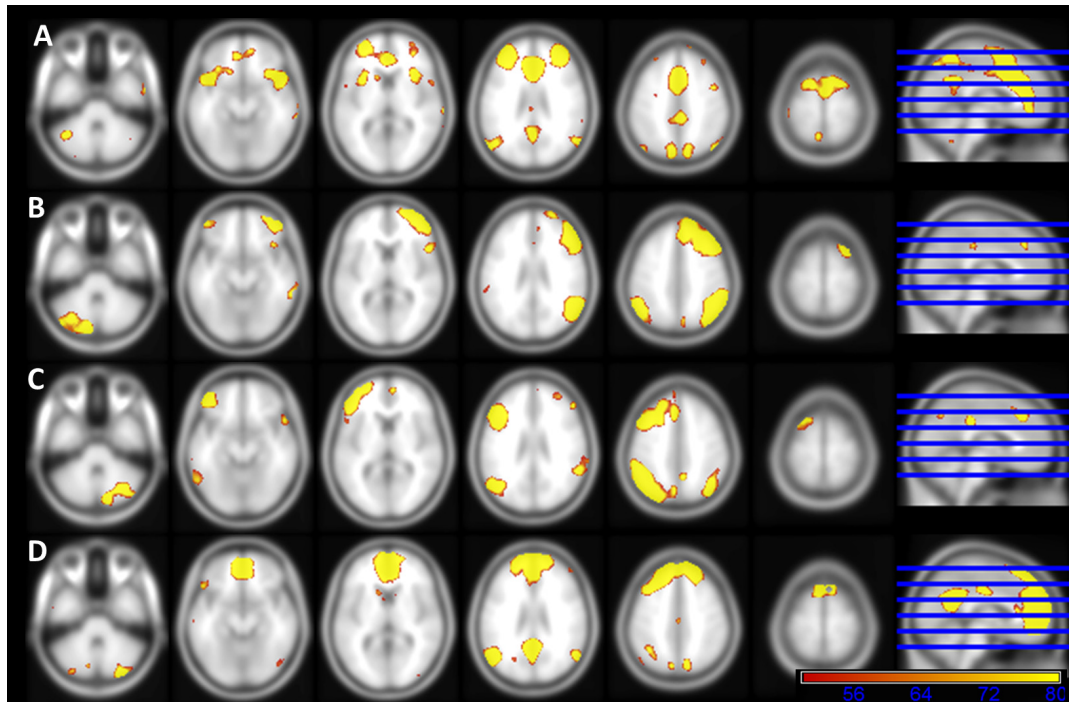
(WB analysis only: cluster falls outside the ExN): load increased FC in young participants (mean Z-value control =  $-.41$ ; SD =  $.75$ ; mean Z-value high load =  $.52$ ; SD =  $.84$ ), but decreased FC in middle-aged participants (mean Z-value control =  $.31$ ; SD =  $.75$ ; mean Z-value high load =  $-.56$ ; SD =  $.84$ ). For young participants, the individual Z-values of the peak voxel of the right superior temporal cluster correlated positively ( $r(56) = .63$ ;  $T = 4.3$ ;  $p < .001$ ) with the individual scores on NASA TLX Effort subscale. No correlation with the scores on the NASA TLX Mental Demand subscale ( $r(56) = .28$ ;  $T = 1.5$ ;  $p = .14$ ) and with the POMS Fatigue subscale ( $r(56) = .21$ ;  $T = 1.1$ ;  $p = .27$ ) was found. For middle-aged participants, the individual Z-values of the peak voxel of the right superior temporal cluster correlated negatively with the individual scores on the NASA TLX Effort subscale ( $r(56) = -.50$ ;  $T = 2.9$ ;  $p = .01$ ) and positively ( $r(56) = .40$ ;  $T = 2.2$ ;  $p = .037$ ) with the individual scores on the POMS Fatigue subscale. No correlation ( $r(56) = -.28$ ;  $T = 1.5$ ;  $p = .15$ ) with the scores on the NASA TLX Mental Demand subscale was found.

For the left FPN, load increased FC in a right calcarine/precuneus cluster (WB analysis only: outside the left FPN):  $Z = -.53$  (SD =  $.63$ ) after the control and  $Z = .09$  (SD =  $.51$ ) after the high load condition. Individual scores on NASA TLX Effort subscale ( $r(56) = .29$ ;  $T = 2.3$ ;  $p = .03$ ) correlated with the individual Z-values of the peak voxel of the left inferior frontal cluster. No correlation with the scores on the NASA TLX Mental Demand subscale ( $r(56) = .25$ ;  $T = 1.9$ ;  $p = .06$ ) and with the POMS Fatigue subscale ( $r(56) = .06$ ;  $T = .4$ ;  $p = .68$ ) was found. No effects of Load were found for the default mode and right FPN.

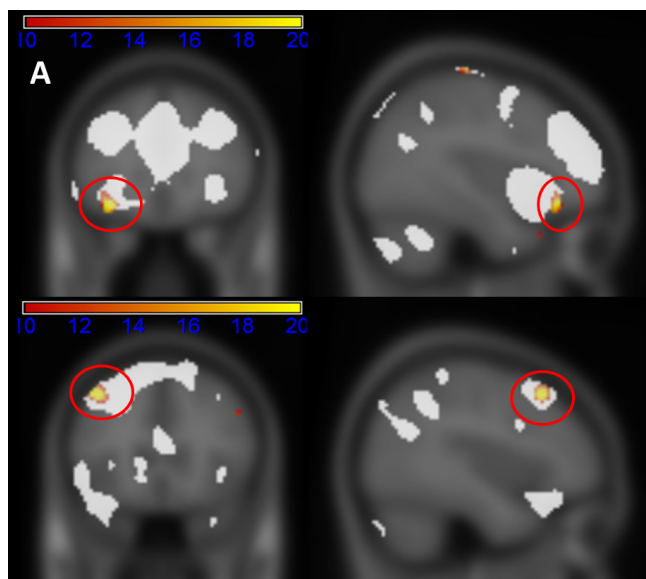
**Table 1.** Overview of neuroimaging results. The effects of sustained cognitive performance (LOAD) and age (AGE) on resting state functional connectivity are shown.

Brain region	BA	N voxels	F-value	pFWE-corrected	MNI coordinates
<i>Executive Network</i>					
Main effect of AGE (WB): decreased FC					
Left inferior frontal gyrus	47	60	34.4	.028	-32, 32, -14
Main effect of AGE (SVC): decreased FC					
Left inferior frontal gyrus	47	32	32.2	.008	-32, 30, -14
Main effect of LOAD (WB): increased FC					
Right lingual/parahippocampal gyrus	37	87	32.8	.043	26, -50, -4
Interaction AGE and LOAD (WB): FC increased in young, decreased in middle-aged					
Right superior temporal gyrus	22	59	34.2	.030	54, -12, 6
<i>Left Fronto-parietal Network</i>					
Main effect of LOAD (WB): increased FC					
Right calcarine/precuneus	17	192	41.8	.004	18, -58, 14
<i>Default Mode Network</i>					
Main effect of AGE (SVC): decreased FC					
Left middle frontal gyrus	9/46	81	26.6	.031	-40, 22, 46

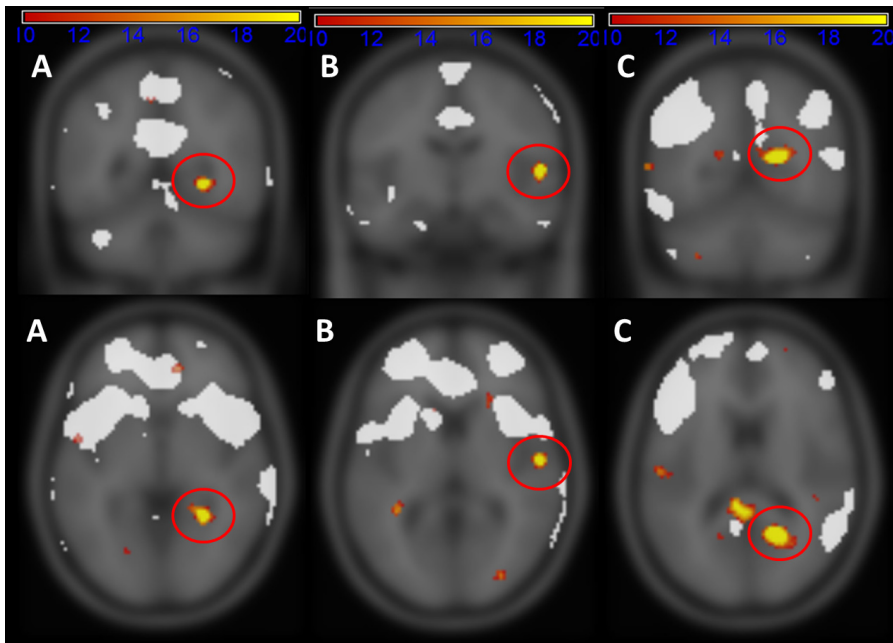
Abbreviations: WB = whole brain; SVC = small volume corrected.



**Figure 2** | Resting state networks: the executive control (A), the right frontoparietal (B), the left frontoparietal (C), and the default mode network (D). The average networks (across groups and conditions) are superimposed on a T1 MNI 152 standard space template. Network regions with an F-value > 50 are shown.



**Figure 3** | Effects of age on resting state functional connectivity. Brain regions are superimposed on a MNI 152 standard space T1 template. Network regions are shown in white (F-values > 30). Age effects are shown in red/yellow (F-values > 10). A) In the executive control network, reduced functional connectivity was found in the left inferior frontal gyrus in middle-aged compared to young schoolteachers (BA 47). B) In the default mode network, reduced functional connectivity was found in the left middle frontal gyrus in middle-aged compared to young schoolteachers (BA 8).



**Figure 4** | Effects of sustained cognitive performance on resting state functional connectivity (FC). Brain regions are superimposed on a MNI 152 standard space T1 template. Network regions are shown in white (F-values > 30). Effects of sustained cognitive performance (load) are shown in red/yellow (F-values > 10). A) In the executive control network, load increased FC in the right lingual/parahippocampal gyrus (BA 37). B) In the executive control network, load increased FC in young, but decreased FC in middle-aged participants, in the right temporal superior gyrus (BA 22). C) In the left frontoparietal network, load increased FC in a right calcarine/precuneus cluster (BA 17).

## DISCUSSION

### Age effects

In the present study, the effects of sustained cognitive task performance on subsequent RS FC were investigated in healthy young and middle-aged male schoolteachers. Tomasi and Volkow (2011), who evaluated datasets from 913 healthy subjects, showed that age reduced long-range FC in the default mode (most strongly in the precuneus) and dorsal attention network. Allen et al. (2011), who investigated datasets from 603 healthy adolescents and adults, showed age-related decreases in FC across all networks (except the basal ganglia). The results of the present study show reduced FC in the left inferior (part of ExN) and middle frontal gyrus (part of DMN). Although Tomasi and Volkow (2011) reported the strongest effects of ageing in the precuneus and posterior cingulate, they also reported reduced short-range FC in the inferior frontal gyrus, and reduced short and long-range FC in the middle frontal gyrus.

Both the inferior and the middle frontal gyrus are part of the prefrontal cortex, which is one of the regions most affected by ageing (Jurasca and Lowry, 2011; Fjell and Walhovd, 2010). Previous studies have shown substantial grey matter loss (Kalpuozos et al., 2009;

Raz et al., 1997) and white matter degradation (Pfefferbaum et al., 2005; Salat et al., 2005; Yoon et al., 2008) in especially the prefrontal cortex in healthy ageing. Reduced white matter integrity has been related to decreased cognition in older age (Carlton et al., 2010; Schiavone et al., 2009; Vernooij et al., 2009).

The results of the present study suggest that some effects of ageing on RS FC are already present at middle age, which is in line with studies that have shown that white matter integrity (Salat et al., 2005) and cognitive performance (Bopp & Verhaeghen, 2005, Park et al., 2002; Van der Elst et al., 2005) is already reduced at middle age.

### Effect of sustained cognitive performance

Sustained cognitive performance modulated FC for two task-positive networks: it increased FC between a right lingual/parahippocampal cluster and the ExN, and between a calcarine/precuneus cluster and the left FPN. In contrast with the effects of age, effects of sustained cognitive performance were found outside the examined RSNs.

In line with the findings of Lewis et al. (2009), it is possible that the negative correlation between task-positive networks (i.e. the ExN and left FPN) and the DMN (the parahippocampal gyrus and precuneus have been reported as DMN regions: for review see Buckner et al., 2008) has been reduced due to sustained cognitive performance. In the present study, the lingual/parahippocampal and calcarine/precuneus cluster were, however, not part of the identified DMN. Two post-hoc analyses were carried out to investigate whether sustained cognitive performance changed the relationship between RSNs. Firstly, it was investigated whether sustained cognitive performance changed the average correlation between the individual time courses of any other cognitive RSN (out of the 20 components revealed by ICA) which contained or bordered the lingual/parahippocampal and calcarine/precuneus clusters, and the individual time courses of the ExN and left FPN (functional network connectivity: see Jafri et al., 2008). Two RSNs were identified: the first, which could be described as a posterior DMN, consisted of a large bilateral precuneus cluster, bilateral mid occipital, cuneus and cerebellar clusters, bilateral inferior and middle frontal clusters, and a bilateral fusiform/parahippocampal cluster; the second, which could be described as primary visual areas, consist of one large occipital cluster extending into the lingual gyrus and the calcarine. No changes in the average correlation between the individual time courses of these networks and those of the ExN and left FPN were found. The second post-hoc analysis showed that the average correlation between the individual time courses of every possible pair formed from the four examined networks did not change after sustained cognitive task performance. These post-hoc analyses suggest that sustained cognitive performance did not change the relationship between the examined task-positive networks and the DMN.

Another possible explanation for the increased FC in regions outside the ExN and left FPN is that participants who had to put in more effort recruited additional brain regions during task performance. Since increased FC might represent a memory of repeated coactivation (Fox and Raichle, 2007), the recruitment of additional brain regions could have resulted in increased FC between these regions and the relevant task-positive RSN. The hypothesis that effortful processing was associated with increased lingual/parahippocampal and calcarine/precuneus activation cannot be tested by the present study. The tasks performed

during the high load intervention were not performance in the MRI scanner.

Previous research has shown that the lingual gyrus is important for word recognition (Borowsky et al., 2007) and the parahippocampal gyrus for object recognition (Borowsky et al., 2007; Murray et al., 2000). Furthermore, the calcarine, in which most of the calcarine/precuneus cluster is located, is part of the primary visual cortex. Based on the functions of these regions it could be speculated that participants who had to put in more effort, recruited more brain regions associated with stimulus recognition to perform the cognitively demanding tasks.

For the ExN, an interaction between load and age was found in the right superior temporal gyrus: sustained cognitive performance increased FC in young, but decreased FC in middle-aged participants. The increased FC in young participants is in line with the effects of sustained cognitive performance discussed in the previous paragraph. The right superior temporal cortex is involved in spatial awareness (Karnath, 2001), speech perception (Price, 2010) and visual search (Gharabaghi et al., 2006). Increased effort might be associated with increased recruitment of these functions. However, the effect in middle-aged participants showed the opposite pattern: decreased FC that correlated negatively with effort. The superior temporal gyrus has been shown to be susceptible to age-related changes (e.g., Fjell and Walhovd, 2010; Ohnishi et al., 2001). If increased FC in young participants is the result of coactivation of this region with the ExN, then a decrease in FC in middle-aged participants might be the result of reduced coactivation due impaired functionality of this region.

Previous studies have shown that learning can increase RS FC within the trained network (Albert et al., 2009; Jolles et al., 2011; Waites et al., 2005). In the present study, in contrast to our hypothesis, no effects of sustained cognitive performance were found on RS FC within task-positive RSNs. In contrast to other studies into the effect of cognitive performance or training on subsequent RS FC, participants performed a variety of tasks for 1.5 h. The tasks performed during the intervention required cognitive control, working memory, problem solving and making calculations. This intervention did not train one cognitive network, but has induced sustained effortful cognitive performance which is likely to have recruited a number of networks. The effects found in the present study correlated positively with the amount of effort participants required during task performance. The results of this study suggest that sustained cognitive performance does not change FC within the recruited task-positive networks but increases FC between task-positive networks and brain regions that might have been recruited when more mental effort is required.

## Limitations

Firstly, the number of participants in each group was low, which reduced the statistical power to detect effects of sustained cognitive performance and age. Secondly, only on a limited number of RSNs were examined. However, the investigated networks were the most relevant for the cognitive intervention used in this study. Further increasing the number of examined RSNs would have required a correction for multiple testing, which might have further reduced the detection power. Thirdly, the statistical threshold used for the functional MRI analysis might be considered conservative. However, we consider

it necessary to correct for the number of tests and thereby properly limit the number of false positives (as argued by Bennet et al., 2009). Fourthly, between the end of the high load intervention and the start of the RS scan, participants had a short break. It would have been too expensive if they had performed the cognitive tasks in the MRI scanner. Participants therefore performed the cognitive tasks in a room next to the scanner room. After the end of the intervention they were placed in the MRI scanner as quickly as possible. This took about 10 minutes: they were given the possibility to use the restroom, were put into the scanner and two short localiser scans were made. Although short, this break might have given participants the opportunity to recover to certain extent from the cognitive intervention. Finally, since in the present study a very specific sub- population was tested, male school teachers, the results of this study cannot be generalised to the general population. School teachers might be more used to cognitively demanding and stressful situations but on the other hand have an increased risk of burnout (e.g., Farber, 1991). They might therefore respond differently to sustained cognitive task performance than non-teachers. However, the results might be generalisable to other people who have jobs that require a high level of education and are mentally demanding, such as medical doctors.

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