Default Mode Resting-State Functional Connectivity of the Aging Brain

Athanasia Monika Mowinckel

Master of Philosophy in Psychology,
Cognitive Neuroscience discipline at the Department of Psychology.
UNIVERSITY OF OSLO
May 2011

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Acknowledgements

This thesis falls under a larger project (Regional Committee for Medical Health Research Ethics; South-East Norway; Project ID: S-03116) which seeks information on how genetics are associated with cognition, headed by Post Doctor Thomas Espeseth, (main supervisor). All participants have been part of this project for several years. All MRI images were inspected by Dr. Paulina Due-Tønnesen (M.D.) for screening of abnormal brain development. The analyses were run in collaboration with Post Doctor Lars Tjelta Westlye, (co-supervisor), who has been a major contributor to the analysis process. Behavioural data, including BDI, MMSE, CVLT and WAIS, were gathered prior to this thesis' inception, and have not been acquired by the author.

Due to the explorative nature of this project, it is hard to say who decided what. The procurement of data for the project, with sequences for MRI, had already chosen before I started on the project. However, I was not interested in changes of morphology or volume and wanted to explore the functional resting-state data. Organising the acquirement of the data (booking participants, negotiating work hours for radiographers, collection of the data) was done by me. Some help in the collection of the data was given by a paid assistant, Ms. Nina Westby.

The decision of which gene to focus on was debated frequently between Post Doctor Espeseth and I, and the choice to explore *APOE* was a mutual decision. I had early on found independent component analysis to be an intriguing method of analysis, and Post Doctor Westlye explained how the dual-regression approach, in combination with this, would be an interesting way to go. There were several choices to be made with the large dataset we had, and after many discussions we settled on the project as it is presented here. Since there has been an on-going dialog between us all, it is hard to pinpoint exactly who decided what. However, without my supervisors' dedicated involvement and unconstrained contribution of knowledge, I could not have completed this project. I am very thankful for this.

Additional thanks to Professors Edmund Sonuga-Barke and Xavier Castellanos for permission to use Figure 1. Lastly I want to thank my brother, Mr. Mikkel Mowinckel, for computer tech support and discussions concerning biology, and to Ms. Inger Tolleskoven for the layout of the paper.

Abstract

The term functional connectivity is used to describe which parts of the brain work together on a process, and might aid the understanding of how the processing systems in the human brain are fundamentally organised. The default mode network (DMN) is a constellation of cortical structures that has shown remarkable reliability as a resting-state network (RSN). It has often been referred to as a task-negative network, because it typically exhibits amplified activation patterns during rest. Numerous studies have documented DMN alterations in various clinical conditions, including mild cognitive impairment and Alzheimer's disease. However, little is known about the impact of normal aging on this network. The present study investigates age–differences in DMN functional connectivity and further, whether the effects of age are modulated by the allelic variation of the apolipoprotein E-gene, *APOE*. Based on current theories of cognitive aging and the few existing previous studies on resting-state patterns and *APOE*, we have two hypotheses: 1) an elevated co-activation in the DMN with increasing age, and 2) different effects for ε4-carriers compared to non-carriers in the MTL structures, including the hippocampus. We tested these hypotheses on resting-state functional magnetic resonance imaging (fMRI) data from 182 healthy participants aged 20-78 years, including 63 carriers of the ε4-allele. Using a combination of independent component analysis (ICA) and dual-regression, we document regionally specific escalations in DMN synchronicity with increased age, especially in frontal brain areas. Additionally, we observed a moderate negative effect of the ε4-allele in the posterior cingulate cortex (PCC) of the posterior parts of the DMN, indicating lower co-activity in carriers compared to non-carriers in areas spanning core parts of the DMN. These findings are discussed in light of theories of cognitive aging, and we argue that the amplified DMN functional connectivity with age is indicative of an age-related decrease in neural differentiation manifested as decreased decoupling between task-negative and task-positive brain networks during rest.

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Introduction

The brain is a complex neural network. This does not only pertain to the intricacy of its anatomical makeup, but also to the hierarchical functional organisation. The term functional connectivity is used to describe which parts of the brain work together on a process, and might aid the understanding of how the processing systems in the human brain are fundamentally organised (Cole, Smith, & Beckmann, 2010). Functional connectivity relates to the extent of synchronous fluctuations between different brain areas, e.g. the degree of temporal correlations between anatomical regions in the blood oxygen level dependency (BOLD) signal (Vincent et al., 2007). It has been proposed that studying the brain at rest will be the next frontier in neuroscience (Raichle, 2006). Raichle argues that only about 1% of the activity observed with fMRI is task induced, while the remaining activity reflects the tonic state of the brain. In order to increase the knowledge of the working brain, the functional significance of the "idle" brains' enormous energy consumption should be better characterized and understood (Raichle, 2006).

When investigating functional connectivity patterns of the brain, researchers often use so-called resting-state protocols. Instead of looking at task-evoked activations, resting-state data is purported to reveal the *intrinsic* activation patterns of the brain while the participants are scanned without performing any tasks, i.e. at "rest" (Biswal, Yetkin, Haughton, & Hyde, 1995). It is due to this rest condition that it is believed that this method elucidates the *fundamental* organisation of the brain, as neural activity is allowed to fluctuate unconstrained (Cole et al., 2010). Several studies have documented reliable brain networks during rest across various psychological states (Greicius et al., 2008; Horovitz et al., 2009; Horovitz et al., 2008), across species (Pawela et al., 2008; Rilling et al., 2007; Vincent et al., 2007), and across human subjects (Friston, 2009; Gusnard & Raichle, 2001; Shehzad et al., 2009). This strongly indicates that the functional hierarchical organisation of the brain is an evolutionary conserved trait. Furthermore, it has been shown that the correlation patterns of slow oscillations (< 0.1 Hz) spontaneously occurring at rest form widely distributed resting-state functional networks (RSNs), such as the sensory systems (De Luca, Beckmann, De Stefano, Matthews, & Smith, 2006), the motor cortex (Biswal et al., 1995), the attention systems (Fox, Corbetta, Snyder, Vincent, & Raichle, 2006), and the memory systems (Greicius, Krasnow, Reiss, & Menon, 2003). In a review of resting-state literature Fox & Raichle (2007) argue that inherent BOLD changes correlate with variability in human behaviour, and the authors propose that the intrinsic co-activation plays an important role in cognition and behaviour, perhaps through this fundamental functional organisation of the brain.

The default mode network

The default mode network (DMN) is a constellation of cortical structures that has shown remarkable stability as an RSN. The DMN comprises the posterior cingulate cortex (PCC), medial prefrontal cortex (MPFC), the inferior, medial and lateral parietal cortex, and

displays a consistent pattern of activation throughout rest and deactivation during goal directed activity (Buckner, Andrews-Hanna, & Schacter, 2008; Raichle et al., 2001; Shehzad et al., 2009). The initiation of a task leads to deactivation of the DMN, but the activity is not quenched; the commencement of a task attenuates the DMN rather than extinguishing it (Fransson, 2005; Greicius et al., 2003). Glahn et al. (2010) reported significant heritability of DMN resting-state functional connectivity (RSFC), suggesting a genetic contribution for activity of this network, thus supporting the use of resting-state functional connectivity in the field of imaging genomics (Hariri & Weinberger, 2003). It was further found that the genetic factors contributing to the heritability of the functional connectivity of the DMN are distinct from those influencing grey matter densities, indicating that different genes influence functional connectivity and neuroanatomy respectively.

The putative role of the DMN in modulating cognition and behaviour is not fully understood. Sonuga-Barke and Castellanos (2007) proposed a model termed the *default-mode interference hypothesis*, which emphasises four factors that interplay in the transition between rest and goal-directed activity. The balance between these four elements are purported to determine how successful the transition between resting-state DMN activity and task-induced
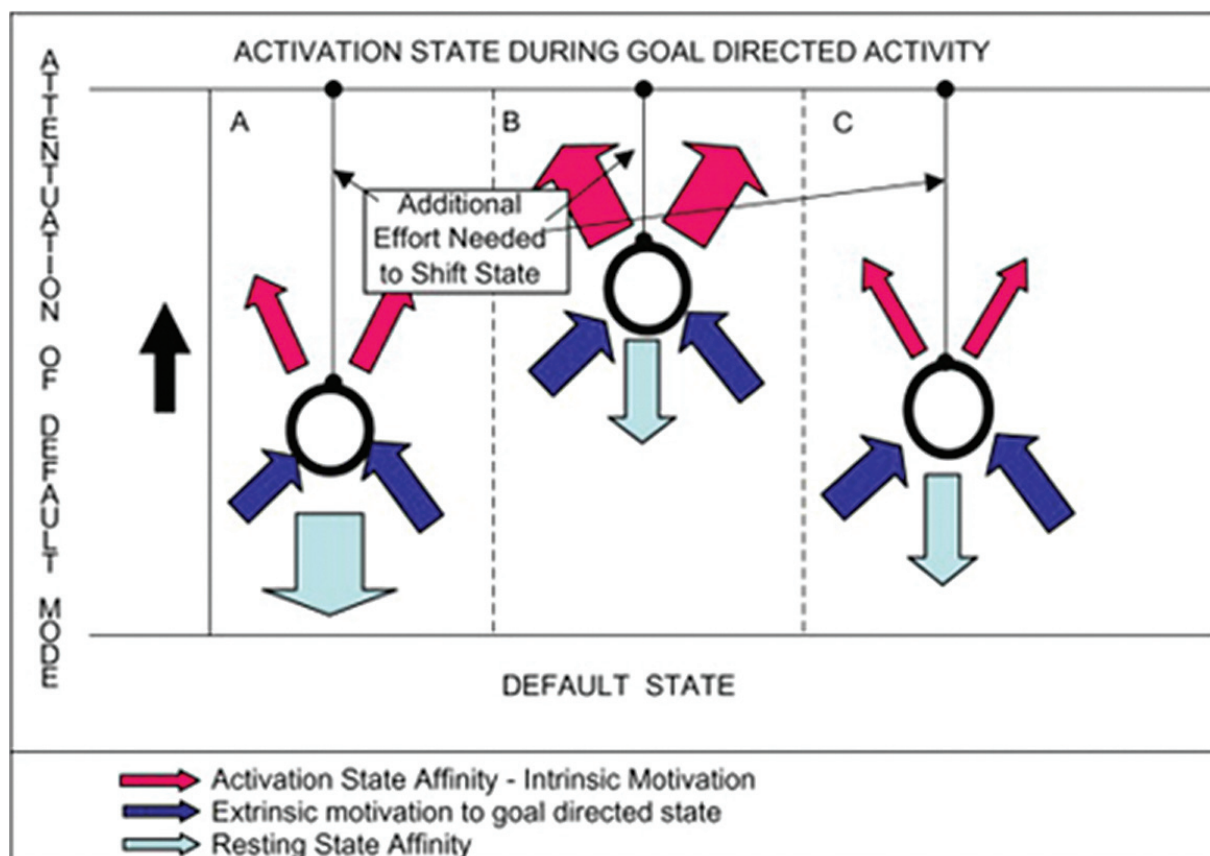
Figure 1. A diagram illustrating the operation of four factors that might affect transition from resting to goal-directed activity states; resting-state affinity, goal-directed state affinity, extrinsic motivation and cognitive effort. The thickness of the arrows indicates the strength of particular factors in each scenario and the position of the circle outlines in black the degree of attenuation of the default-mode and the associated transition from resting to goal-directed activity. Obtained with permission from Songua-Barke & Castellanos (2007).

action is. The four factors are: default-mode affinity (how active the DMN is), goal-directed affinity (intrinsic motivation), extrinsic motivation and availability and degree of cognitive effort. (Sonuga-Barke & Castellanos, 2007). Figure 1 gives a graphical representation of the interplay between these factors.

Aberrant DMN activity has been found in various clinical conditions, including schizophrenia (Garrity et al., 2007), ADHD (Uddin et al., 2008), epilepsy (Vanhatalo et al., 2004), and mild cognitive impairment (MCI) and Alzheimer's disease (AD; Celone et al., 2006; Sorg et al., 2007). According to the default-mode interference hypothesis, cognitive deficits associated with these pathologies might be due to increases in DMN affinity or inability to exert enough cognitive effort to attenuate the DMN. However, there has yet to be much focus on the DMN in a healthy aging population.

Theories of cognitive and brain aging

Aging is associated with cognitive decline and concurrent changes in the structural and functional makeup of the brain (Fjell & Walhovd, 2010; Raz & Rodrigue, 2006). Numerous reports have documented increased vulnerability of age-related changes in anterior parts of the brain (Davis, Dennis, Daselaar, Fleck, & Cabeza, 2008) and the incorporation of these in empirical findings by various theories of cognitive aging converge on a frontal aging hypothesis. Characteristics of this so-called posterior-anterior shift during the course of aging have been observed with morphometry (Fjell, Westlye, et al., 2009), diffusion tensor imaging (DTI; Salat et al., 2005), event-related potentials (ERP; Walhovd et al., 2006), and fMRI (Davis et al., 2008).

In addition to the general frontal vulnerability in aging, previous studies on functional changes in the aging brain have reported a hemispheric asymmetry reduction in old adults (HAROLD; Cabeza, Anderson, Houle, Mangels, & Nyberg, 2000; Dolcos, Rice, & Cabeza, 2002). There is an observed difference between how young adults and older adults functionally engage the brain during task execution: young adults seem to show a more lateralised pattern (i.e. activity relevant to the task is observed in only one hemisphere), whereas older adults seem to engage bilaterally (i.e. activating areas within both hemispheres).

The two main theories that try to explain frontal vulnerability and HAROLD are the *compensatory* and *dedifferentiation* theories of aging (Cabeza, 2001). The theory of compensation posits that older individuals need to engage both hemispheres to compensate for cognitive deficits and neural atrophy associated with old age (Cabeza, McIntosh, Tulving, Nyberg, & Grady, 1997). This notion of compensation for cognitive deficits is supported by studies showing higher bilateral activation during task execution for older adults than for younger participants (Cabeza et al., 2000; but see Nyberg et al., 2010). Also, there have been studies reporting recruitment of homologous regions in the healthy hemisphere in recovery after injury (Dancause et al., 2005; Kuhn, Palmer, & Fuchs, 2001), supporting potential plastic changes in the brain in response to injuries. Several researchers link the compensation theory

of cognitive aging with recent findings of plastic brain alterations in response to altered contextual demands, including physical and cognitive training interventions (Lövdén, Bäckman, Lindenberger, Schaefer, & Schmiedek, 2010). Hence, the brain and cognitive changes observed with aging are likely shaped by a dynamic relationship between a vast amount of contextual variables, in conjunction with the brains' on-going positive and negative plastic responses and compensatory mechanisms (see e.g. Greenwood, 2007; Lövdén et al., 2010; Park & Reuter-Lorenz, 2009).

The dedifferentiation theory suggests that the reduction of asymmetry observed with progressed age is due to the brains' inability to recruit specialized neural mechanisms (Cabeza, 2001). Supported by e.g. bilateral activation patterns during task-performance (Cabeza, Anderson, Locantore, & McIntosh, 2002) and functional dedifferentiation in the visual systems (Park et al., 2004) in older individuals, this theory is additionally buttressed by studies reporting stronger correlations between cognitive measures with increasing age; performance on different cognitive measures seem to be more inter-correlated in older adults than in young adults (de Frias, Lövdén, Lindenberger, & Nilsson, 2007). Dedifferentiation might also occur due to the frontal shift, where the availability of specialised circuits is reduced in frontal areas of the brain, resulting in a need to recruit other areas to perform the same task. In this way, dedifferentiation can itself be seen as a compensation mechanism. Even though the compensation and the dedifferentiation theories of cognitive aging provide two different frameworks from which to interpret and understand age-related changes in brain and cognition, they are not mutually exclusive (Cabeza, 2001). However, an important prediction of the compensation hypothesis is that the increased activation seen in older compared to younger subjects should have positive cognitive correlates, i.e. that the frontal over-recruitment helps maintain cognitive functions in response to e.g. neural loss. In contrast, the dedifferentiation hypothesis does not predict any positive cognitive effects of decreased neural specialisation.

Structural brain changes in aging

The cognitive and functional changes observed in the aging brain are likely to have a structural foundation. In order to investigate age-related volumetric changes of the brain, Good et al. (2001) performed voxel-based morphometric analyses on structural MRI scans from 465 individuals aged 18-79 years. Their seminal cross-sectional study showed that there was a loss of grey matter volume with age and larger build-up of cerebral spinal fluid (CSF) as indicated by expanding ventricles. Expanding on this, Fjell et al. (2009) conducted a longitudinal study and found marked cortical atrophy and ventricular expansion in their participants after only one year, and further that this effect increased with age. This indicates accelerating age-related changes with increasing age, even in healthy individuals. Along with several other studies showing similar effects of age on volumetric and morphometric measures (Salat et al., 2009; Walhovd et al., 2005; Westlye et al., 2009), these two studies together give convincing accounts of the morphometric changes associated with age.

Age-related structural brain changes seem to be different for white matter and grey matter. While grey matter seems to typically show a linear shrinkage during the adult life-span (Fjell, Westlye, et al., 2009; Westlye et al., 2009), white matter follows an inverted U-curve with age (Raz, 2005; Walhovd et al., 2011): young children and elderly have less white matter than young and middle-aged adults. Diffusion tensor imaging (DTI) measures the amount and direction of the diffusion of water along myelinated axonal sheaths, and has become widely used to explore white matter microstructure (Chanraud, Zahr, Sullivan, & Pfefferbaum, 2010).

Several studies have found that structural connectivity, measured by DTI, partly predicts functional connectivity (Greicius, Supekar, Menon, & Dougherty, 2009; Honey et al., 2009; Koch, Norris, & Hund-Georgiadis, 2002). Ystad et al. (2011) argue that subcortical nuclei and their widely distributed cortical connections, are essential for RSN connectivity. A review by Damoiseaux and Grecius (2009) on resting-state fMRI and DTI conclude that it is reasonable to assume, based on current data, that there is a link between functional and structural connectivity. If structural and functional connectivity were positively correlated, one would consequently expect that functional connectivity would show a developmental progression shaped like an inverted U, as indicated by volumetric and DTI studies of life-span age-differences of the human white matter (Westlye et al., 2010).

Functional connectivity and aging

In a study of functional connectivity in the aging brain, Andrews-Hanna et al. (2007) found that there was no difference in the topological pattern of the RSNs between a group of young (aged 18-34 years) and a group of old age (aged 60-93years) participants. However, the functional connectivity was more robust in the younger individuals compared to the older participants, as indicated by the temporal correlation between an anterior and posterior part of the DMN. Bluhm et al. (2008) found no significant age-differences in the DMN functional connectivity in a sample spanning 17-58 years of age, suggesting that changes in resting-state activity might only change markedly after the age of 60. Andrews-Hanna et al. (2007) found that functional connectivity was positively correlated with cognitive test scores: lower cognitive scores were associated with lower functional connectivity. Similarly, Ystad et al. (2011) revealed that age related decline in cognitive processing speed and executive functioning was associated with fibre integrity between subcortical nuclei and cortical RSNs. This suggests a link between functional connectivity and behavioural measures, though this correlation might also be due to other factors varying with age, like atrophy, demyelization, neurotransmitter availability etc. (Andrews-Hanna et al., 2007).

Dosenbach et al. (2010) found that in a sample of developing children, adolescents and young adults (until 30 years of age) the largest contributor in estimating brain maturity was the attenuation of functional connections as measured by resting-state fMRI: weaker functional connectivity indicating increased age. This suggests that brain maturation during development brings forth a differentiation of functional brain networks that reaches its peak approxi-

mately at age 22. Koch et al. (2010) found lower DMN co-activation in the anterior cingulate cortex (ACC) in older compared to younger participants. There was lower co-activation over-all for the DMN in the older group, but only decrease in the ACC reached significance. Studies of age-related changes in RSFC of adults have mainly been done between two age groups, but in order to investigate age differences properly one should conduct a study with a wide-spanning age-range.

There are reports of older individuals showing less attenuation of the DMN during goal-directed activity compared to younger persons, which have lead some researchers to believe that there is a decrease in the ability to effectively switch between a resting state and task-execution with increased age (Grady, Springer, Hongwanishkul, McIntosh, & Winocur, 2006). This attenuation problem can, according to the default-mode interference hypothesis, be due to heightened default mode affinity with age. One would expect, based on the compensatory theory, more bilateral co-activation in the PFC in older adults than in young adults. The dedifferentiation theory would be supported by augmented synchronicity in the PFC, irrespective of bilateral effects. Either of these findings would be in line with the default-mode interference hypothesis (Sonuga-Barke & Castellanos, 2007), where increases in RSFC would be believed to show higher default-mode affinity. This heightened affinity would further be purported to lead to difficulties in attenuating the DMN during goal-directed activity, ultimately leading to poorer cognitive test scores. Still accounting for age, however, there is great individual variability in the DMN functional connectivity patterns.

APOE

A considerable portion of the individual variability in DMN functional connectivity is likely attributed to genetic factors (Glahn et al., 2010). With the mapping of the human genome, more information becomes available about the underpinnings of our diversity. The *APOE*-gene on chromosome 19 has three allelic variants: ε2, ε3, and ε4, and codes for three isoforms of the apolipoprotein E (*APOE*; Mahley, 1988). This gene is probably most known for its association with Alzheimer's disease (AD), but is also connected with brain plasticity (Pearson-Fuhrhop, Kleim, & Cramer, 2009; Pearson-Fuhrhop & Cramer, 2010), neuronal development (Kuhn et al., 2001) and repair functions (Jordan, 2007) by the transportation of lipids in the central nervous system (Holtzman & Fagan, 1998). The ε3-allele is the most common in the population, with ε2 and ε4 being less common. This has allele-frequencies are roughly what is expected in Nordic countries (Gerdes, 2003). The ε4-allele has been shown to correlate with reduction in repair of damaged nerve cells and reduced brain plasticity, while the ε2-allele demonstrates the opposite trend (Mahley, 1988).

It is the ε4-allele that has been marked as a risk factor for the development of AD, with amplified risk of development with increased number of alleles (Corder et al., 1993). There have been reports that healthy carriers of the ε4-allele display decreases in the DMN that is correlated with increased accumulations of cortical fibrillary amyloid (Buckner et al., 2009;

Hedden et al., 2009), a substance which in large amounts is a neurobiological hallmark of AD (Braak & Braak, 1991; but see Herrup, 2010). In a review of the *APOE* literature, Mahley and Rall (2000) present data that might explain the link between *APOE* and amyloid β (Aβ) deposits in carriers of the ε4-allele. Studies have shown that apoE3 binds with Aβ with a higher affinity than apoE4 (LaDu et al., 1994). ApoE4 might then not be able to clear these deposits as effectively as apoE3, leaving Aβ to accumulate in the brain (Mahley, Huang, & Weisgraber, 2006). Additionally, apoE4 has been found to accelerate Aβ-*production*, which further reinforces apoE4's role in AD (Mahley, Weisgraber, & Huang, 2006). Mahley et al. (2006) additionally report the role of *APOE* in plasticity: apoE3 stimulates neurite outgrowth in the presence of a source of lipid, while apoE4 does not. This process does not, however, necessarily lead to dementia (though it would be a contributing factor). Carriers of ε4 might exhibit less neural plasticity than non-carriers, something that might be evident with age. Consistent with this, accelerated cortical thinning with age has been reported in healthy carriers of the ε4-allele compared to non-carriers (Espeseth et al., 2008).

Studies on the DMN and AD have found aberrant DMN patterns in AD (for a review see Broyd et al., 2009), and studies of DMN RSFC in AD patients report decreases of connectivity in AD patients compared to healthy controls. Rambouts, Barkhof, Goekoop, Stam and Scheltens (2005) have found decreased deactivation of the DMN during two different memory tasks for AD patients compared to healthy elderly controls. Dickerson and Sperling (2009) argue that observed hippocampal hyperactivation in MCI (a prodromal stage of AD) during rest is a monetary compensatory mechanism that might herald future hippocampal failure, subsequently leading to AD. This is consistent with the default-mode interference hypothesis. However, the role of *APOE*'s functional effect on the brain is still unclear.

Some previous studies have investigated the effects of *APOE* on the functional architecture of the brain. Using resting-state fMRI, Filippini et al. (2009) recently tested whether young ε4-carriers showed different resting functional patterns than young ε4 non-carriers. They found that carriers had an amplified degree of co-activation within the DMN in medial temporal regions, and that there were no brain regions where carriers had decreased co-activation compared to non-carriers. In line with this, and extending the findings to a middle aged and elderly sample, Westlye et al. (In press) found elevated hippocampal synchronisation with the posterior DMN in carriers of the ε4-allele. This rise in co-activation was additionally negatively correlated to memory performance: augmented RSFC was associated with poorer memory performance. In a second study, Filippini et al. (2011) compared samples of older and younger individuals, with or without the ε4 allele, using task-induced BOLD responses. Using data from the same young participants as their study in 2009 combined with new data from an older group, they found decreased task-induced activity in the older compared to the younger carriers of the *APOE* ε4-allele. Using almost twice the sample size of Westlye et al. (In press), we investigate whether we can replicate findings of increased synchronicity in the MTL for carriers compared to non-carriers of the ε4-allele.

Analysis of functional connectivity data: ICA and dual-regression

Analyses of RSFC have mainly been done in two different ways: one which is hypothesis driven and looks for patterns of connectivity between manually, anatomically, or functionally defined seed regions (regions or voxels; Greicius et al., 2003), and another multivariate data-driven approach (Independent Component Analysis; Cole et al., 2010). Independent Component Analysis (ICA) has rapidly become the tool of choice for resting-state fMRI analyses. This is due to its ability to extract reliable functional RSNs based purely on the covariance structure present in the data, and also to its relative independence of *a priori* assumptions of the localisation of functionally homologous regions (Beckmann, DeLuca, Devlin, & Smith, 2005; Calhoun, Adali, Pearlson, & Pekar, 2001). This data-driven method follows a trend in the neuroscience community towards discovery science approaches, allowing novel, data-driven, and efficient explorations of the functional significance of RNSs (Biswal et al., 2010).

The dual-regression analysis method has recently become more progressively employed. In conjunction with ICA, this method utilises temporal concatenation on a group level with a back reconstruction to the individual participants' component activation (Beckmann et al., 2005; Calhoun et al., 2001; Zuo et al., 2010). This method extracts components with more reliable force than running ICA on a single subject basis, but still allows for cross-subject statistical analyses on the extracted RSNs. In this study we utilise the dual-regression approach to explore the resting-state fMRI dataset.

Aims of this study

Associating RSNs with genetic variability might help explain the mechanisms controlling the RSNs in the aging brain. No one has, to our knowledge, looked at *APOE* and age interactions on resting-state data throughout the adult lifespan. Here, we first test whether there is an effect of age on resting-state connectivity in a wide-spanning age group (20-78), and secondly whether these effects are modulated by *APOE* allelic variation. We have to hypotheses: The first is based on the presented theories of cognitive aging and previous studies where we predict an increase in DMN functional connectivity with age. This would be predicted by the compensation and dedifferentiation theories of aging, and in accordance with the frontal shift, the largest increases in connectivity should be in the frontal cortical regions of the brain. Secondly we predict replicating the elevated DMN RSFC in the hippocampus for carriers of the ε4-allele previously found by others.

Materials and Methods

Participants

All participants read an information sheet and signed a statement of informed consent approved by the Regional Committee for Medical Health Research Ethics (South-East Norway; Project ID: S-03116). The Norwegian Department of Health gave permission to obtain and store blood samples for genotyping, as well as cognitive and MRI data, in a bio bank, and to establish a registry with relevant information for a 10 year time period. The research was carried out in compliance with the Helsinki Declaration.

One hundred and ninety-four individuals (120 females) in the age range 20-78 participated (Mean = 46,80, SD = 16,50). Twelve people were excluded for various reasons: three due to enlarged ventricles (which would severely complicate the registration procedures employed in the fMRI analyses), another three because of technical issues with their fMRI datasets, and four owing to MRI artefact disturbances. The last two participants were excluded due to their *APOE* allele-combination (ε2ε4), as this combination is thought to eliminate the two alleles' unique effects (Mahley & Rall, 2000). This left 182 participants for analysis (113 female, age range 21-78, Mean = 47,44, SD = 15,61). Participants completed several

Table 1. Group Descriptives

	APOE no ε4 (n = 119)	APOE ε4 hetero (n= 54)	APOE ε4 homo (n = 9)	t*	Sig.*
Female/Male	75/44	35/19	6/3		
Age	46.37 (±15.70)	50.15 (±15.46)	51.56 (±15.08)	-1.64	.10
Range	21 - 78	21 - 72	24 - 72		
Education	14.46 (±2.02)	14.93 (±2.58)	14.56 (±3.13)	-1.17	.24
Range	9 - 18	9 - 20	11 - 19		
IQ	119.78 (±10.48)	119.65 (±10.44)	115.56 (±10.30)	.44	.66
Range	88 - 141	88 - 138	94 - 126		
MMSE**	29.22 (±0.84)	29.21 (±0.80)	29.14 (±0.90)	-.036	.97
Range	27 - 30	27 - 30	28 - 30		
BDI	4.01 (±4.16)	4.75 (±4.53)	4.00 (±4.06)	-.95	.34
Range	0 - 22	0 - 19	0 - 13		
CVLT Learning	58.90 (±10.60)	56.50 (±9.81)	59.00 (±6.48)	1.28	.20
Range	24 - 78	24 - 76	50 - 69		
CVLT Delay	13.80 (±2.40)	13.26 (±2.67)	14.11 (±2.15)	1.08	.28
Range	5 - 16	5 - 16	9 - 16		

Table for participants without the ε4-allele (no ε4), with one allele (ε4 hetero) and with two alleles (ε4 homo), for education measured in years, full scale IQ measured with WASI Scale, MMSE (max score 30), BDI (max score 30), and CVLT. Measures are reported in means (SD).
*Two-tailed independent samples comparisons between carriers (ε4 hetero and ε4 homo) and non-carriers (no ε4) of the ε4-allele (p<0.05).
** Only on participants above 40 years (n = 124).

neuropsychological tests, including the California Verbal Learning Test (CVLT), Wechsler Abbreviated Scale of Intelligence (WASI; vocabulary and matrix reasoning) and Beck Depression Inventory (BDI). Individuals above 40 years of age were additionally tested with the Mini-Mental State Examination (MMSE). All participants performed within normal range (see Table 1), and there were no significant differences between the carriers and non-carriers of the ε4-allele on any of the demographic or neuropsychological variables.

Genotyping

Genotyping was done on the LightCycler™ system (Rouche Diagnostics, Mannheim, Germany) by real-time PCR with allele-specific fluorescence energy transfer probes and melting curve analyses. DNA was extracted from 300 μL whole blood using MagNA Pure LC DNA Isolation Kit – Large Volume on the MagNA Pure LC (Roche), eluted and diluted to 1 mL, of which 5 μL was applied in each assay. Section for Genetic Analyses, Department of Medical Biochemistry, Oslo University Hospital, Norway performed the genotyping. The analyses were done in batches of 17-30 samples. The call rate was 100%. No genotyping errors were identified on control repeat analyses or DNA sequencing. Genotype frequencies after exclusions were: ε2 7,4%, ε3 72,8%, and ε4 19,8%.

MR acquisition and analyses

MR data was collected with a 1.5 Tesla Siemens Avanto scanner (Siemens Medical Solutions, Erlangen, Germany), using a 12-channel head coil. Acquisition parameters for the Siemens Avanto: Two 3D magnetization prepared rapid gradient-echo (MP-RAGE) T1-weighted sequences. TR/TE/TI/FA=2400 ms/3.61 ms/1000 ms/8°. Matrix: 192 x 192. Scan time: 7 min and 42 s per volume. Each volume consisted of 160 sagittal slices (1.20 x 1.20 x 1.25 mm). These were used to co-register the fMRI data to standard space coordinates.

During the whole-brain functional resting-state procurement the participants were instructed to have their eyes closed, stay awake and not think of anything in particular during the 10 minute sequence (which was verbally confirmed after its completion). T2* weighted echo-planar images were collected with these specifications: TR/TE/TI/FA= 3000 ms/70 ms/90º. The resting state scan comprised of 200 volumes of 28 slices (3,475 × 3,485 × 4 mm). The first three volumes were deleted during processing in order to account for T1 equilibrium effects.

Pre-processing of structural data was done using FreeSurfer 5,0 (http://surfer.nmr.mgh.harvard.edu/), including averaging of the two MPRAGEs, motion correction and removal of non-brain tissue. Functional data was pre-processed with FSL's Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC; Beckmann et al., 2005) with motion correction, brain extraction, spatial smoothed with a 3D kernel of FWHM = 5 mm and filtered using a 120s high-pass temporal filter. FMRIB's Nonlinear Image Registration tool (FNIRT) was used to register the participant's fMRI volumes to standard space

using the structural scan as an intermediate. Additionally, an in-house FSL/FMRIB version of boundary based registration (BBR; Greve & Fischl, 2009) was performed to further improve the FNIRT registrations. Improvement was confirmed with visual comparisons between FNIRT registration and BBR.

Dual-regression

      The dual regression method is composed of three stages. First, multi-session temporal concatenation (group-ICA) was performed using 94 of the 182 participants. A representative group was chosen in order to avoid biases in the estimation of the gICA –maps, e.g. induced by including less old than young subjects etc. The group subjects were selected to best represent the sample in terms of age, sex and genetic variation. MELODIC was set to automatically estimate the number of independent components using a Laplacian approximation. Figure 2 displays the 17 components that were not visually identified as scanner of physiological noise or white matter activation. With visual inspection, three components were identified as
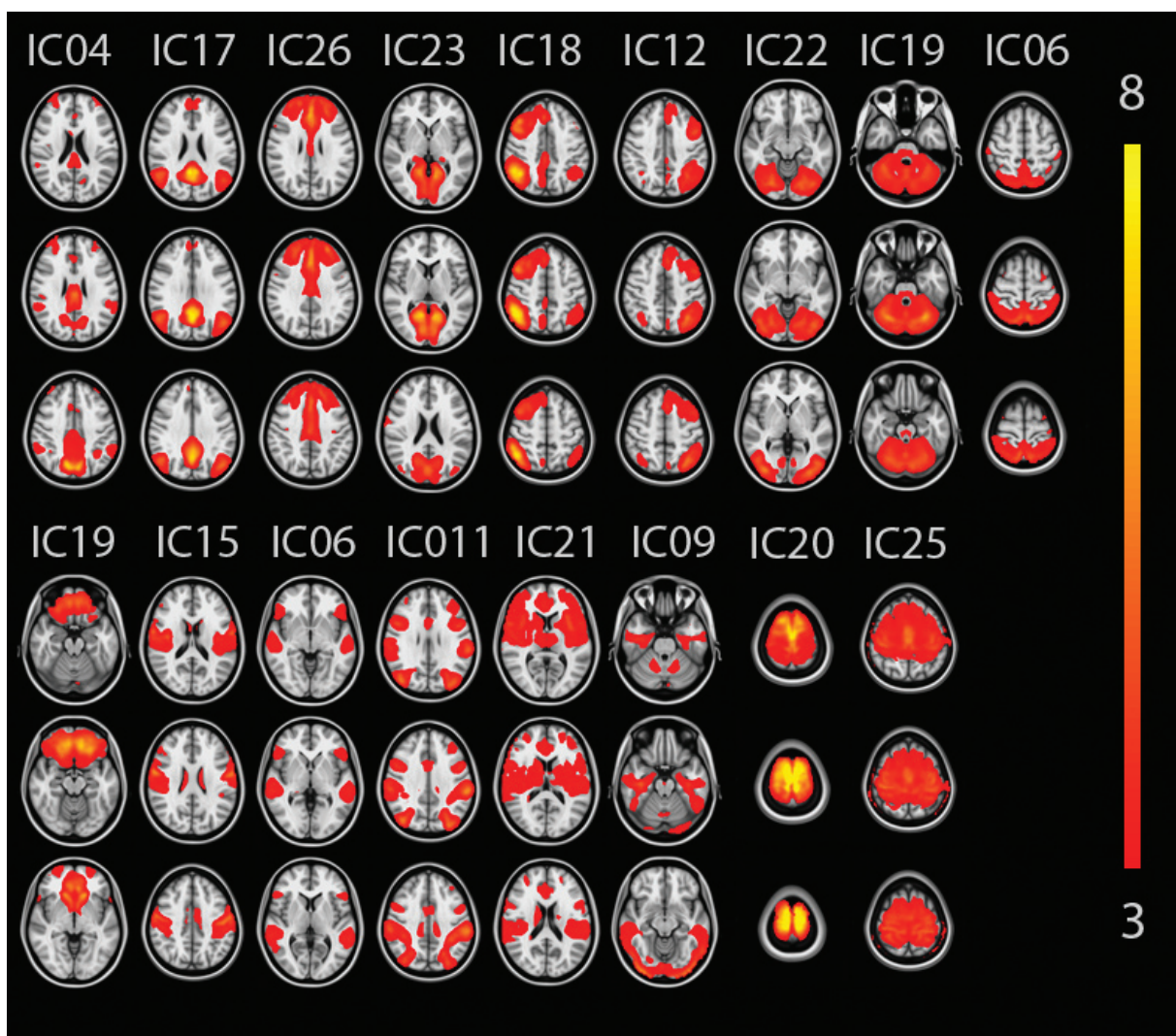

Figure 2. Representation of the independent components that were not associated with noise, here grouped together after cortical areas in proximity to each other.

belonging to the DMN. These three components in addition to a visual control component were selected for further analyses (see Figure 3). Next, we performed the dual-regressions on the full number of components. This creates subject-specific time courses and spatial maps by 1) doing a linear-model fit (spatial regression) from the group component spatial maps to the subject-specific data sets which creates matrices of time courses for each component and subject, 2) these matrices are then used for temporal regression (linear model fit) alongside the associated fMRI data which identifies subject-specific spatial maps, and lastly 3) a single 4D file with the fourth dimension being the subject identification are collected across subjects for statistical analysis (Filippini et al., 2009).

Collectively, the steps in the dual-regression approach employ several multiple linear regressions on each of the group ICs, effectively regressing out the shared variance with all other includes components. This approach thus yields subject-specific maps of each IC, not contaminated by indirect correlations between components or other artefact-related variance (including motion, blood-flow etc., which is effectively regressed out during the dual-regressions). Also, correlations with ICs displaying spatial correspondence are accounted for.

Statistical analyses

Cross-subject voxel-wise statistical analyses were performed on the three DMN components and the medial visual control component using non-parametric permutation based methods as employed in randomise, part of FSL (Smith et al., 2004). For each contrast, 5000 permutations were run. The permutations result in spatial maps describing the voxel-wise between-subject differences (Filippini et al., 2009). In order to avoid randomly having to set cluster size thresholds and smoothing levels, Threshold-Free Cluster Enhancement (TFCE) was used (Smith & Nichols, 2009), and significance threshold was set to $p < 0,05$ corrected for multiple comparisons across space (Westlye et al., In press).

Sociodemographic variables, neuropsychological test scores, and effect sizes were calculated with PASW statistics 18.0 (SPSS). Effect sizes were estimated by running an ANCOVA using mean RSFC in areas showing significant association with the corresponding contrast as dependent variable, sex and *APOE* as fixed factors and age as covariate. Furthermore, for effects of *APOE* the difference between the carrier mean was subtracted from the non-carrier mean, divided by the non-carriers mean and multiplied by 100, giving a percentage of the difference between groups. We also calculated a corresponding Cohen's d (Cohen, 1992) as a supplemental measure of effect size in order to ease comparison with previous studies. Note that the statistical testing was performed voxel-wise using randomise, and these additional analyses were only performed in order to estimate common measures of effect sizes, and should therefore not be regarded as a violation of circular inference (Kriegeskorte, Simmons, Bellgowan, & Baker, 2009).
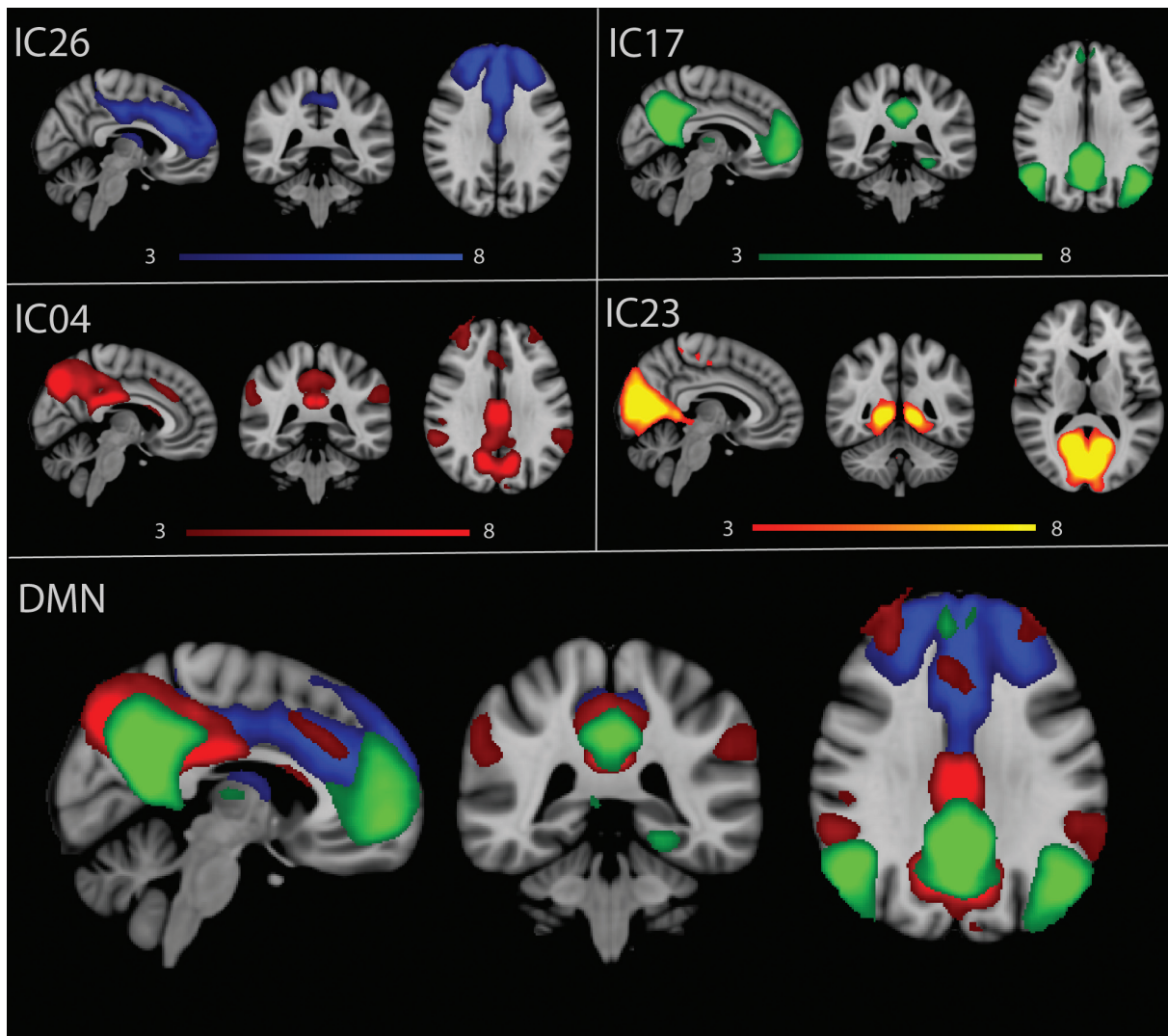
Figure 3. Independent components chosen for analysis. IC04, IC17 and IC26 make up the DMN and are shown in relation to each other at the bottom. IC23 is the visual control component.

Results

Independent component analysis

A total of 27 components were automatically extracted with MELODIC from the group ICA analyses, which characterize temporally correlated BOLD signals of group-averaged networks. Ten of these networks were visually identified as noise. The first four components from the top left in Figure 2 are the components chosen for further analyses: three components making up the DMN (IC04, IC17, and IC26) and a visual control component (IC23). The three DMN components encompass the cingulate, lateral parietal and inferior/middle temporal gyri, prefrontal cortex, the thalami, and the hippocampi (Damoiseaux et al., 2006; Fox et al., 2005; Greicius et al., 2003; Raichle et al., 2001), an amalgamation of these are displayed in the bottom part of Figure 3. The visual control component covers the primary visual cortex. The components used in this study are highly similar to the ones used in Westlye et al. (In press).
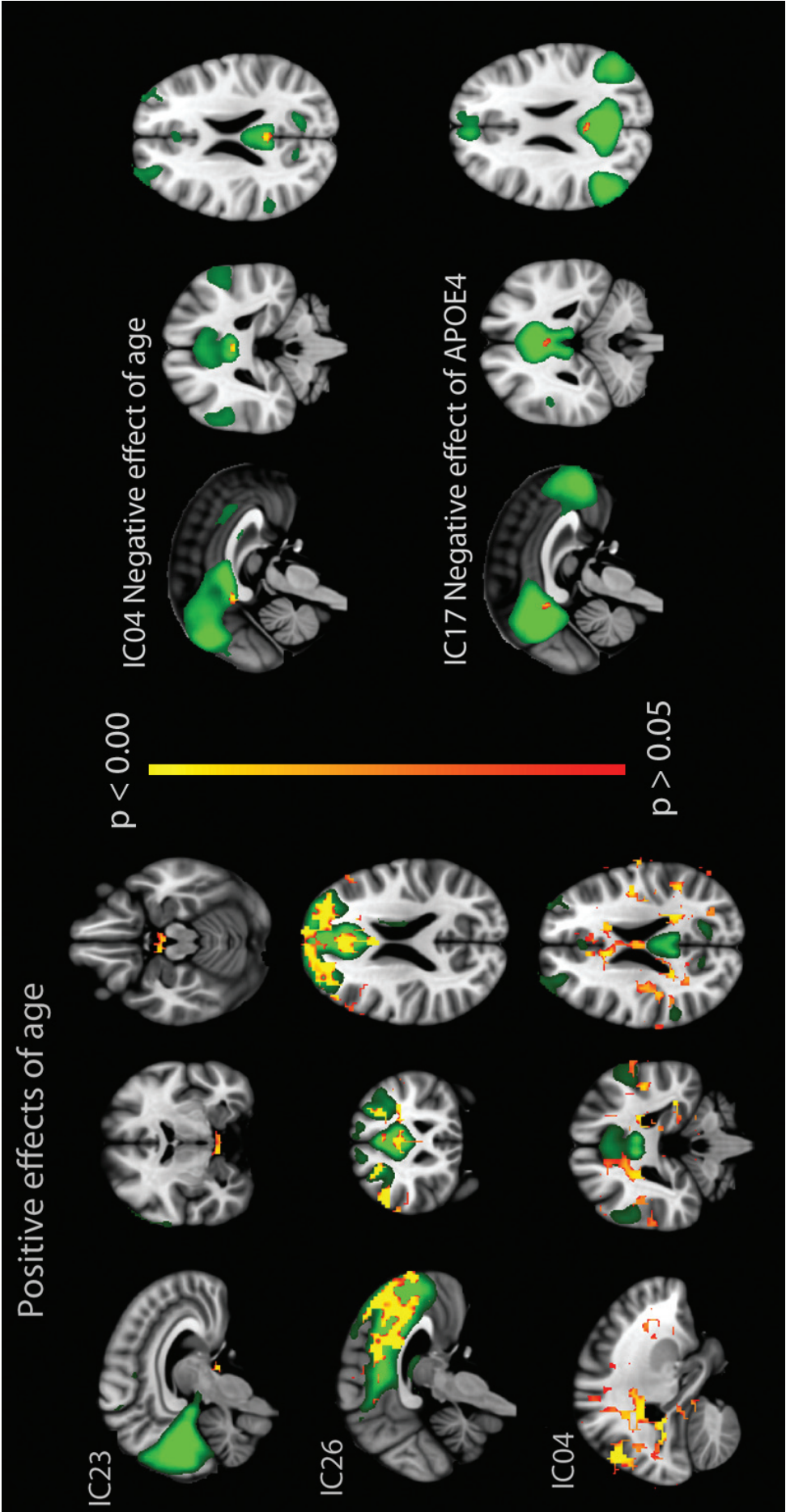
Figure 4. The significant effects after permutations that surpassed the significance threshold of 0.05. These effects are seen in red and yellow, while the corresponding components are displayed in green. The positive effects og age and APOE are shown in B.

DMN RSFC

Figure 4 shows the results from the voxel-wise permutations based testing in the various ICs. Permutation testing yielded significant positive correlations between age and functional connectivity in frontal and temporal areas for DMN components IC04 and IC26. IC04 covers part of the medial and lateral parietal cortex and the PCC, and also small areas of the frontal lobule. IC26 encompasses parts of the medial and orbitofrontal cortex and most of the cingulate cortex. The significant effects in IC26 are centered within core areas of the component including the frontal lobe and the anterior cingulate cortex (ACC). IC04 shows effects primarily outside the core areas of the component, and the effects are scattered around various frontal and parietal areas, including parietal areas close to the sensory cortex and the ACC. Furthermore, there is a positive effect of age on the functional connectivity of the visual control component (IC23). However, the significant cluster is situated outside the brain (bafflingly close to the chiasm), suggesting that this is explained by age-related differences in the cardiovascular system, e.g. the anterior bacillary artery. For IC04, there was additionally age-related increase in RSFC in the posterior parts of the brain outside the DMN. IC04 was the only component that showed a negative effect of age and this was within the posterior cingulate (PCC) in the core parts of the DMN.

Negative effects of *APOE* ε4 in comparison to *APOE* ε3 reached significant levels in a small part of PCC for IC17. This component covers both the anterior (orbitofrontal cortex) and the posterior (PCC and medial/lateral parietal cortex). There were no areas where
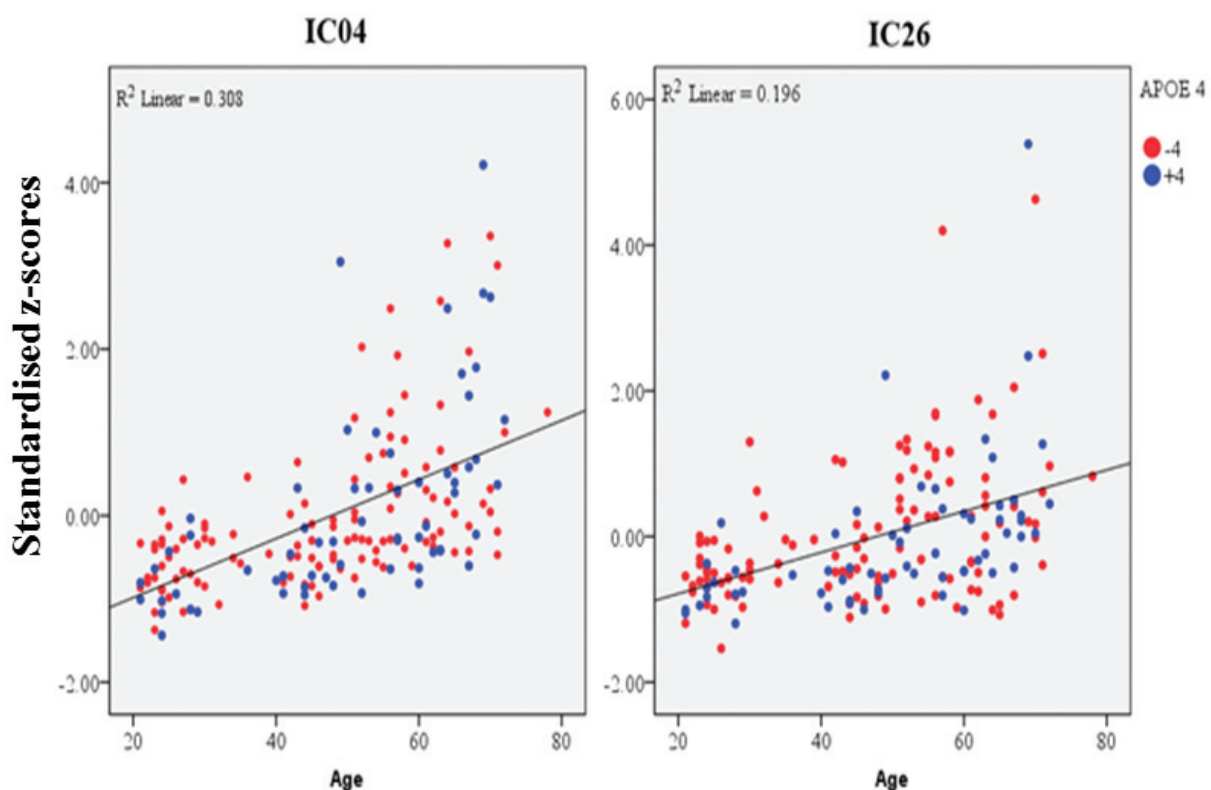

Figure 5. Scatter plots showing the spread of the significant t-values after permutation testing that have been converted to standardised z-scores.

ε4-carriers showed significantly increased co-activation in comparison to non-carriers. Also, we found no evidence of age*APOE interaction on the functional connectivity in any of the tested components.

Figure 5 shows a scatter plot with the RSFC from voxels showing a significant positive effect of age in IC04 and IC26 plotted as functions of age. Tables 2 and 3 summarize the results and effect sizes from the different significant regions. IC04 revealed significant main effect of age (t = 6.41; p < .00) and sex (t = 2.90; p < 0.04), indicating a positive effect of age and a higher RSFC in female compared to male participants. This same trend for age (t = 4.50; p< .00) and sex (t = -2.13; p < 0.34) was found in IC26. The only negative effect of age is evident in IC04 (t = -4.18; p < .00). IC17 showed a significant negative effect of the *APOE* ε4-allele (t=5.10; p < .00) and a significant age*APOE interaction (t = -4.52; p < .00), demonstrating decreased RSFC in *APOE* ε4-carriers. Note that the sex-effects in IC04 and IC26, and the age*APOE interaction in IC17 should be interpreted with great caution as these are run on data already known to show significant effects of age (Kriegeskorte et al., 2009), and no voxels reached significance in the main voxel-wise permutation-based analyses.

For the purpose of interpretation, mean RSFC in ε4 carriers in IC17 (mean = 41.72; SD = 12.91) was subtracted from mean RSFC for non-carriers (mean = 47.73; SD = 14.81),

Table 2. Positive effects

		Positive effects of age			
		B	Std. Error	t	p
IC04					
	Age	0.21	0.03	6.41	.000*
	APOE	3.25	2.07	1.57	.119
	Sex	1.78	0.61	2.90	.040*
	APOE*Age	-0.06	0.04	-1.51	.133
IC23					
	Age	0.94	0.21	4.52	.000*
	APOE	-4.09	13.21	-.314	.754
	Sex	-2.36	3.85	-.613	.541
	APOE*Age	-0.002	0.25	-.010	.992
IC26					
	Age	0.34	0.75	4.50	.000*
	APOE	5.70	4.72	1.19	.309
	Sex	2.97	1.39	2.13	.034*
	APOE*Age	-0.07	0.09	-755.00	.451

Effect sizes of positive effects of age (IC04, IC23, IC26) calculated with univariate ANCOVA. Showing significant effects of age (p < .000) in all three components. IC04 and IC26 additionally show a significant sex effect, though this effect has not been contrasted in the initial analyses run with FSL.
*Significant (p < 0.05).

Table 3. Negative effects

	Negative effect of age					Negative effect of *APOE* ε4			
	B	Std.Error	t	p		B	Std.Error	t	p
IC04					IC17				
Age	-.56	.13	-4.18	.000*	Age	.29	.11	2.63	.090
APOE	-4.76	8.47	-.61	.575	*APOE*	35.68	7.00	5.10	.000*
Sex	-1.59	2.50	-.63	.527	Sex	2.31	2.07	1.12	.266
APOE*Age	0.14	.16	.86	.393	*APOE**Age	-.62	.14	-4.52	.000*

Effect sizes of negative effects of age (IC04) and APOE (IC17) calculated with univariate ANOVA. Showing significant effects of age (p < .000) in IC04 and APOE (p < .000) on IC17. IC17 additionally shows a significant interaction between age and APOE, though this should be interpreted with caution as this is computed from data that have already survived significance testing (p < 0.05).
*Significant (p < 0.05).

divided by the non-carrier mean, and multiplied by 100. This gives a representation of the percentage of difference between carriers and non-carriers, with the non-carriers as the baseline. The effect size was shown to be 12.6% indicating a decrease in DMN RSFC in the PCC for *APOE* ε4-carriers in IC17, with a corresponding Cohen's d at 0.416. According to Cohen (1992) this is a "moderate" effect.

Discussion

Using a combination of ICA and dual-regression, we have reported novel findings of elevated DMN RSFC with increasing age in healthy participants aged 20-78 years. There was augmented synchronicity for two of the DMN components (IC04 and IC26): the frontal DMN (IC26) showed effects within the core areas of the component, and the posterior DMN (IC04) displayed scattered parietal effects in addition to the amplified frontal synchronisation. We also found evidence of decreased PCC RSFC in carriers of the *APOE* ε4-allele compared to non-carriers in the second posterior DMN component (IC17), and a negative correlation with age in another posterior component of the DMN (IC04). The implications of these findings are discussed in detail below.

Effects of Age on DMN RSFC

The results from the present study support both the dedifferentiation and compensation theory in that there is elevated synchronicity with increased age. There are several areas, both within and outside, of the DMN components that show higher co-activation with increased age, indicating that higher number of neurons are firing at the same time with similar temporal patterns. The dedifferentiation theory posits that old age is followed by difficulties in recruiting specialised circuits (Cabeza, 2001) which ultimately would lead to using more general neurons to do the same job that few specialised neurons did earlier. This is possibly what the present results reflect: increases in amounts of unspecialised neurons firing as a result of inadequate recruitment of fewer specialised circuits.

The use of different methods makes it difficult to compare the results between studies (methodological considerations are discussed later), but some speculation is possible. Andrews-Hanna et al. (2007) found decreased temporal correlations between an anterior and a posterior part of the DMN with age. Decreases in correlations between seeds of the DMN can arguably be different measures of the same phenomenon, where the decreases in correlations might reflect the reductions of specialised circuits, rather than the utilisation of several other more general neurons. Similarly, at the other end of the developmental trajectory, Dosenbach et al. (2010) demonstrated that measures of functional connectivity could be used as a predictor of age in a developing sample (ages 8-30), where weakened RSFC was an indicator of neural differentiation associated with development in this age range.

Our findings of increased DMN functional connectivity in frontal areas contrast some previous studies. Koch et al. (2010) found lower co-activation of the anterior cingulate cortex (ACC) in the DMN in an older group compared to younger participants. There was lower co-activation over-all for the DMN in the older group, but only decrease in the ACC surpassed the significance threshold. Damoiseaux et al. (2008) additionally found negative effects of age in the anterior DMN. These apparent discrepancies may be related to different methodologies applied in the respective studies (see discussion below).

It is possible that the age-related increased DMN RSFC observed in the present study, along with higher co-activation between the DMN and other cortical areas as indicated by previous studies, indicate a relative inability to attenuate activity of the brain during rest (Westlye et al., In press). If the hallmark of a healthy brain is its ability to switch between rest-activity and task-activity (Grady et al., 2006), an elevated rest-activity could be negative. If located outside the primary DMN areas, amplified activity could indicate increased coupling between the RSN and other cortical areas during rest. In line with this hypothesis, Sambataro et al. (2010) reported a decreased ability in their older participants to deactivate the DMN when performing a working memory task and that the individuals with lower task performance also showed decreased attenuation of the DMN. Similar patterns have been found by several other researchers (Grady et al., 2006; Lustig et al., 2003; Persson, Lustig, Nelson, & Reuter-Lorenz, 2007). These studies buttress the possible link between attenuation problems of the DMN during task-execution and poorer task performance.

We believe our findings are in correspondence with the default-mode interference hypothesis (Sonuga-Barke & Castellanos, 2007), as it is reasonable to assume that elevated RSFC for the DMN will make it harder to suppress DMN activation when this is needed. Our results indicate a constant higher affinity of the DMN in old age, which would, according to the default-mode interference hypothesis, result in the need for larger cognitive effort to successfully execute an action.

Relations to theories of cognitive aging

The findings reported above might be consistent with both theories of cognitive aging discussed previously, and is in line with a large body of structural and functional studies suggesting increased vulnerability of anterior brain areas in aging. The increases of DMN synchronicity observed bilaterally in the PFC are in line with the compensatory theory, positing that the brain compensates for e.g. structural losses by increasing the functional co-activation.

However, an intriguing question remains: what is being compensated for? The resting brain is by no means inactive. As discussed above, Raichle (2006) notes that only about 1% of the brains' energy consumption is tied to responses to external stimuli, but the function of the idle brains' high activity is still largely unknown. Fransson (2006) discusses the possibility that default mode activity might reflect future planning and imagination, and the areas linked to future planning show similarities to the default mode network (for review see: Buckner & Vincent, 2007). Fransson (2005) also notes the possibility that the DMN is associated with self-referential thoughts, where there is a higher attentiveness to the internal state (e.g. hunger, pain sensations, proprioception etc.). The stability of the DMN through sleep (Horovitz et al., 2009; Horovitz et al., 2008) and anaesthesia (Greicius et al., 2008) and the long-held assumption that sleep is needed for some types of memory consolidation (Stickgold, 2005), further supports the possibility that the DMN is associated with memory consolidation. This also supported by recent findings of a correlation between DMN RSFC and memory performance (Westlye et al., In press).

Buckner (2010) argues for the crucial role of memories for future planning, and these two concepts seem to coincide. Disputably, if the DMN is associated with e.g. level of daydreaming (Mason et al., 2007), DMN activity might be modulated by various states of consciousness (Greicius et al., 2008), like sleep and anaesthesia. The neurocognitive function of the DMN must arguably be some sort of cognitive process that is sustained through different conscious states. Although speculative, it is possible that the aged brain engages in higher amounts of future planning and memory consolidation in a waking state than does the younger brain, consequently resulting in higher DMN co-activation. However, this hypothesis remains to be tested.

The compensation theory in general and HAROLD in particular, do not fully predict our findings, since the effects of age were mainly bilateral as opposed to unilateral as predicted from this theory alone. That is, young participants displayed bilateral synchronisation, and this bilateral co-activation was further increased with age. The dedifferentiation theory might thus better account for our results, (Cabeza, 2001). Based on this theory one would expect an age-related increase in the spatial distribution of the DMN which would manifest as increased DMN RSFC in some areas. This is in line with our results. However, it does not necessarily explain why this dedifferentiation occurs. The higher synchronisation of the DMN in frontal areas, both for the anterior and posterior components of the DMN, might indicate a higher reliance on these frontal areas in old age during rest. Though not in direct opposition to

the compensation theory, it is the primary rise in co-activation, rather than reliance on novel regions, that buttresses the dedifferentiation theory.

Also in support of this theory, previous studies of the effects of aging on the brain have reported non-linear correlations that indicate little to no dedifferentiation in young individuals with an exponential effect after a pivotal age (de Frias et al., 2007), which is also in line with when the age-related white matter differences accelerates (Westlye et al., 2010). Figure 5 indicate such trends, but a non-linear effect has not been tested for here. It is possible that a DMN RSFC has a non-linear trajectory, but this is difficult to estimate with a sample that does not encompass the whole life-span (see limitations for a discussion of this).

Even though previous studies have found decreased connectivity with age instead of augmented connectivity (Andrews-Hanna et al., 2007; Persson et al., 2007), these changes have not been observed markedly before the age of 58 (Bluhm et al., 2008). However, like here, the changes that have been observed seem to occur after the approximate age of 50 (Andrews-Hanna et al., 2007). Grady et al. (2006) propose that a shift in the balance between default mode activity and task-activity may result in behavioural symptoms of cognitive aging. This shift in balance might, as previously mentioned, manifest as a failure to properly attenuate the DMN when this is needed, due to an increase of the DMN RSFC. This supports the dedifferentiation theory in a cognitive aspect. The problem in switching between the task-negative and the task-positive state might indicate a dedifferentiation of these two states.

The rise in DMN RSFC might be related to decreases in grey matter, which reportedly has a decreasing trajectory with age (Raz, 2005). Kalpouzos, Persson and Nyberg (2011) tested whether loss of grey matter volume (GMv) was related to increases or decreases in BOLD signals associated with memory tasks. At encoding, GMv loss was correlated to reductions in signal strength, while in the prefrontal and lateral parietal cortex GMv loss was associated with increases in BOLD activation. It is possible that the amplification of DMN synchronicity with age in frontoparietal areas is the result of decreased GMv in older age (Andrews-Hanna, Reidler, Sepulcre, Poulin, & Buckner, 2010). Older individuals might need to augment activation in certain areas to compensate for neural loss in regions that would normally execute a certain function, being resting-state or task-related. This elevation of DMN RSFC in specific areas might result in older people having problems in deactivating this system when needed, in effect causing problems in solving cognitive tasks (Sonuga-Barke & Castellanos, 2007). Based on this, one could argue that dedifferentiation is a compensatory mechanism itself.

Thorough inspection of Figure 5 indicates that there is a group of individuals that show quite high DMN RSFC, and there is possibly something that segregates this group of people from the rest of the sample. Elucidating what distinguishes the individuals that show such high DMN RSFC in old age from the individuals who do not, might aid the understanding of the individual variability in the aging process. However, further studies delineating the cognitive correlates of DMN RSFC are needed in order to make stronger interpretations of the consequences of elevated DMN connectivity with increased age.

Effects of APOE on DMN RSFC

We observed a significant negative main effect of the ε4-allele in the PCC of the posterior DMN (IC17), demonstrating lower RSFC in carriers compared to non-carriers. This is an interesting finding, and not in line with our hypothesis and previous findings. Recent studies have found increased posterior DMN RSFC activity in medial temporal areas, including the hippocampi, for *APOE* ε4 carriers (Filippini et al., 2011; Filippini et al., 2009; Fleisher et al., 2009; Westlye et al., In press). Fleisher et al. (2009) found decrease in the DMN in a variety of areas in relation to *APOE* ε4 (which is in line with our results), but did not use a specific resting-state paradigm. It is therefore hard to compare their results with ours. Koch, Teipel, Mueller and Benninghoff (2010) used ICA on a sample of 21 healthy individuals and found no effect of *APOE* between carriers and non-carriers in in the DMN, but hypothesise that a correlation would be found if they had more statistical power.

The discrepancy between our *APOE* results and those of Filippini et al. (2009) and of Westlye et al. (In press) cannot be attributed to the use of different methods, as both studies utilised ICA in combination with dual-regression. Vitally, the two previous studies reported effects in areas not spatially overlapping the core areas of the posterior DMN, namely in the MTL. It is likely that previous findings of increased hippocampal DMN RSFC reflect decreased DMN functional decoupling of the hippocampus. This might elevate the total hippocampal metabolic burden in carriers, which again might raise the risk of memory decline, which is one of the cognitive hallmarks of MCI and AD (Lustig et al., 2003). Importantly, the present finding of decreased RSFC in areas within the core regions of the IC does probably not reflect decreased *decoupling* in carriers, but rather decreased *activity* in the posterior DMN during rest. If DMN activity partly reflects the adaptive functional patterns of the idle brain, the present findings could be taken to indicate a relative failure to engage the posterior DMN during resting periods in carriers. Although speculative, this hypothesis might explain the conflicting findings, and further emphasises the importance of careful considerations of the spatial localisations of effects in relation to the relevant network.

The lack of significant age by *APOE* interaction for the two components showing significant positive effects of age can be quite informative for additional reasons. We found no difference between carriers and non-carriers for either of the DMN components with significant age effects, which reveals that *APOE* allelic variation does not help predict which individuals will show higher RSFC with age. Our study cannot elucidate what is the cause of this spread in the data. It does, however, imply that *APOE* allelic variation is not a significant contributor to this development.

Methodological considerations

The discrepancies between studies are likely partly explained by methodological variability. Essentially, dual regression employs several multiple linear regressions on each of the group ICs, effectively regressing out the shared variance with all other included components.

This approach thus yields subject-specific maps of each IC, not contaminated by indirect correlations between components or other artefact-related variance, including motion, blood-flow etc. Also, correlations with ICs displaying spatial correspondence are accounted for. The combination of ICA and dual-regression is a purely data-driven approach that does not impose any assumptions on how the data should act (Beckmann, Mackay, Filippini, & Smith, 2009).

Importantly, using manually, anatomically or functionally (i.e. using functional loca-lisers) defined regions of interest (ROIs) to assess functional connectivity has several limita-tions. Defining ROIs is rooted in *a priori* ideas of which processes are associated with which regions of the brain. These ROIs are then used as seed-loci for seed-based analyses of correla-tion between the ROIs chosen (Cole et al., 2010). These areas will vary between subjects due to e.g. brain size and small organisational differences, and deciding how much each ROI will encompass can yield different results. Smith et al. (2011) clearly demonstrated how inappro-priately defined ROIs damaged network estimations, and strongly argued for caution in inter-preting results from such studies. Utilizing ICA and dual-regression minimises the negative impact of ill-defined ROIs as these are estimated *from* the data, as opposed to *imposed on* the data. As discussed above, this multivariate approach also regresses out the common temporal variance from all included components, including RSN's with spatial correspondence.

The "true" number of functional components in the brain is not known, and an ap-proximation would necessarily depend on the level of analysis (e.g. single-cell versus "cogni-tive" maps), and there is currently no consensus regarding how many components is optimal (Kiviniemi, Kantola, Jauhiainen, Hyvärinen, & Tervonen, 2003). Forcing the ICA algorithm to extract a high number of components (as opposed to letting it compute an automatic num-ber of components based on the data) will result in splitting components into several subcom-ponents (Kiviniemi et al., 2009). Koch et al. (2010) investigated whether analysis method and choices would have an effect on the outcomes. Their study showed that the number and exact spatial patterns of the components extracted by ICA with an automatic configuration might vary between each separate run. This is not surprising given the randomness in some ini-tialisation and optimisation parameters of the ICA algorithms. In order to test the stability of the components chosen for analyses, we ran another gICA with a model order of 80, forcing ICA to extract 80 components. Vitally, the four components selected for the present analysis remained relatively unchanged, while there was a clear split of other components into several smaller and well-defined subcomponents. The stability of the DMN components is in line with a recent study demonstrating excellent reliability of the DMN subcomponents (Franco, Pritchard, Calhoun, & Mayer, 2009).

Limitations and future research

As discussed above, even with a large sample, our participants might not properly represent the population. There are more females than males (2/3rds female), which could skew the results. There were no significant differences between men and women on the socio-

demographics or the neuropsychological test scores, and any effects of sex would have been regressed out of the results due to the setup of the general linear model (GLM). However, we did not test for any possible voxel-wise sex differences on RSFC, as this was outside the scope of the present study.

There should ideally have been a higher percentage of ε4-carriers (especially homozygotes of which we only have 9) to, disputably, estimate *APOE*s effect properly. A notable problem with *APOE* is its association with Alzheimer's disease (AD). Due to this relation, carriers of the ε4-allele are in a high-risk group for developing AD symptoms, resulting in fewer healthy, older participants who carry the ε4-allele. Farrer et al. (1997) report that the correlation between *APOE* ε4 and dementia decreases with increased age, and after the age of 80 the effects of *APOE* on cognitive measures seem to disappear (Negash et al., 2009). This possibly indicates a vulnerability to a selection bias that might lead to cohort effects in both cross-sectional and longitudinal designs (Espeseth et al., 2010). The functional effects on the brain of a single gene (especially if connected to pathologies) might thus be small when studying a life-span sample, which might be a source to this study's results.

There is also a problem in inferring developmental trajectories of a phenomenon when the whole age-scope is not represented. We present the possibility that there is a non-linear effect of age on DMN RSFC, but that we have not tested for this effect. Fjell et al. (2010) demonstrate how a quadratic regression model is affected by the age-range of the study. Their report shows that the estimated decline between individuals aged 60-80 years is affected by how young the youngest participants are, the estimated slopes are different if the age-range starts at 8, 20, or 30 years. Future studies seeking to elucidate life-span changes of DMN RSFC should also include children and adolescents to avoid this problem.

Differences in morphology or volumetric variances might be partially correlated to our effects, and have not been controlled for in this study. There are known changes in volume and morphology with age (Fjell, Walhovd, et al., 2009; Raz, 2005; Walhovd et al., 2005), and these differences could explain some of the variance we have found here. Future studies might benefit from controlling for such factors. However, there are no simple and generally agreed-upon methods to incorporate structural information in functional analysis, and previous studies have found APOE effects to be robust when co-varying for grey matter density in a voxel-wise manner (Westlye et al., In press)

Conclusion

In line with our first hypothesis, we have reported novel findings of amplified DMN resting-state activity mainly in the frontal areas of the brain with increased age. This amplification of synchronicity of the DMN with age might result in problems attenuating the system for goal-directed activity, which is purported to have negative effects on cognitive measures (Grady et al., 2006). Our results are in line with the general assumption of a relative vulnerability of anterior brain areas during the course of aging. Activity in the DMN has previously

been related to memory consolidation and future planning (Fransson, 2006; Greicius et al., 2008), and the results presented here might reflect that these cognitive processes manifest differently in old age. Further studies are needed to investigate the cognitive correlations of increased DMN RSFC in aging. The second hypothesis by *APOE* modulations of DMN RSFC in the aging brain was not supported, partly contrasting previous findings of elevated connectivity in the hippocampus (Filippini et al., 2009; Westlye et al., In press). Various factors might explain the discrepancies between the present and previous findings, and more research is needed to assess the effect *APOE* on DMN RSFC.

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