FUNCTIONAL CONNECTIVITY OF THE AGEING BRAIN

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ABSTRACT

One of the major challenges facing a society with an ageing population is to understand exactly how the ageing process affects cognitive function, and how factors, such as sleep quality, may contribute to such age-related cognitive decline. This thesis investigated the impact of advancing age on modifying the functional connectivity (FC) of both typical cortical resting-state networks and subcortical structures in the human brain. Furthermore, it explored how any differences in FC may be associated with changes in sleep quality, also thought to be affected by age, and how such interactions may contribute to typical cognitive disruption associated with older age.

The results suggest that older age is associated with the heterogeneous, spatially specific re-organisation of resting-state networks (RSNs), as well as indicating gender-specific spatial re-organisation. Investigation of thalamic FC revealed that older adults exhibited greater thalamo-sensory and thalamo-hippocampal FC, which was related to cognitive performance on RT and memory tasks, respectively. Dynamic evaluation of both cortical and thalamic FC revealed very similar patterns compared to the static measures, suggesting that differences in FC between age groups were not driven by temporal variations in FC strength.

Investigation into participant's sleep patterns provided evidence that sleep quality was more variable amongst the older participants. Furthermore, older adults that slept the longest each night were found to exhibit patterns of thalamic FC which were associated with better cognitive performance, than seen in older shorter sleepers. These results provide preliminary evidence that sleep may be associated with more 'preferable' patterns of FC in older adults which may be beneficial for cognitive function.

In summary, the results presented in this thesis suggest that the integration of measures of sleep, brain function and cognition may well provide a clearer understanding of the intricacies of the ageing process. Further research which establishes links between brain connectivity and cognition and how potentially modifiable behaviours, i.e. sleep, moderate such interactions may allow the development of behavioural interventions which could potentially slow the cognitive ageing process.

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COMMONLY USED ACRONYMS

ACC	Anterior cingulate cortex	SWA	Slow wave activity
BOLD	Blood oxygen level dependent	SWS	Slow wave sleep
CBF	Cerebral blood flow	TE	Echo time
CBV	Cerebral blood volume	TR	Repetition time
CSF	Cerebrospinal fluid	TST	Total sleep time
CVR	Cerebrovascular reactivity	TT	Temporal thalamic sub-region
DAN	Dorsal attention network	V1	Primary visual cortex
DMN	Default mode network	WASO	Wake after sleep onset
EEG	Electroencephalography	WM	White matter
FC	Functional connectivity		
fMRI	Functional MRI		
FT	Frontal thalamic sub-region		
gICA	group ICA		
GM	Grey matter		
GS	Global signal		
GSR	Global signal regression		
HRF	Haemodynamic response function		
ICA	Independent component analysis		
IPL	Inferior parietal lobe		
IPS	Intraparietal sulcus		
M1	Primary motor cortex		
MT	Motor thalamic sub-region		
mPFC	Medial PFC		
MTL	Medial temporal lobe		
NS	Non-significant		
OFC	Orbitofrontal cortex		
OT	Occipital temporal sub-region		
PAL	Paired associates learning task		
Pre-MT	Pre-motor thalamic sub-region		
PCC	Posterior cingulate cortex		
PFC	Prefrontal cortex		
PT	Posterior parietal cortex thalamic sub-region		
REM	Rapid eye movement		
ROI	Region of interest		
RSN	Resting state network		
RT	Reaction time		
SE	Sleep efficiency		
SMA	Supplementary motor area		
SN	Saliency network		
SRT	Simple RT task		
ST	Somatosensory thalamic sub-region		
STG	Superior temporal gyrus		

CHAPTER 1. INTRODUCTION

1.1 THE COGNITIVE NEUROSCIENCE OF AGEING

Advancements in modern medicine over the past century have resulted in large increases in life expectancy across Western Europe. One third of those born in 2013 in the United Kingdom (UK) are expected to celebrate their 100th birthday and in 2007 the number of people in the UK aged over 65 outnumbered the number of people under 16 for the first time (Office for National Statistics, 2014). The result of such changes, combined with reduced birth rates, creates an ageing population which poses a number of societal challenges, including gaps in the job market and putting additional pressure on healthcare, public finances and social services. However, arguably the most troubling effect of ageing for the individual is the gradual decline of cognitive function, which is observed even for those considered 'healthy agers'.

The purpose of this introduction is to provide an overview of: 1) the effects of ageing on a range of cognitive abilities; and 2) the current evidence which suggests changes to brain structure and function may be largely responsible for such cognitive change. Furthermore, it discusses recent research which highlights the importance of investigating the brain as a network if we are to develop unified models of cognitive brain ageing. Understanding how age affects the brain is vital for furthering our scientific knowledge of the brain and the ageing process. While currently the possibility for direct intervention to improve cognitive function in older adults, as a response to the scientific literature, is limited, the first step towards such a goal is to understand how advancing age affects the brain. The second part of this introduction will introduce evidence from the field of sleep research, which suggests that changes to sleep quality with age may be associated with the typical differences in

cognitive abilities and brain connectivity patterns seen between young and older adults. Mapping how patterns of connectivity support cognitive function and how such patterns can be affected by sleep, may allow the development of implementable behavioural (i.e. sleep) interventions which may prevent some of the typical age-related cognitive decline by means of improving sleep patterns.

1.1.1 Ageing and cognition

Ageing is associated with decline in a wide range of cognitive processes, including; processing speed, attention, episodic memory, visual memory, and learning (Hedden & Gabrieli, 2004; Kausler, 1994; Nilsson, 2003; Salthouse, Atkinson, & Berish, 2003; Simpson et al., 2005; Sliwinski & Buschke, 1999b; West & Bell, 1997). Executive function deficits are also commonly associated with advancing age (Buckner, 2004; De Luca et al., 2003; Turner & Spreng, 2012) and it has been reported that the neuropsychological profiles of young and older adults are best distinguished by executive function scores (Whelihan & Lesher, 1985). In addition, older adults have been shown to exhibit slower and more variable responses on inhibition tasks, in comparison to younger adults (McAuley, Yap, Christ, & White, 2006), while Robbins and colleagues (1998; 1994) found that the oldest groups of participants had scores on attention shifting tasks that were similar to patients with frontal lobe lesions. Furthermore, studies have shown that for both humans (Rabbitt & Lowe, 2000; Robbins et al., 1998; Robbins et al., 1994) and rhesus monkeys (Nagahara, Bernot, & Tuszynski, 2010), increasing age results in a 25-35% reduction in working memory performance, compared to younger adults.

This widespread decline in cognitive ability has led some authors to propose that a more general aspect of cognition is affected by age, which has a detrimental impact on a wide range of specific cognitive processes (Salthouse, 2001). In light of the extremely robust

finding that processing speed is detrimentally affected by age (see Eckert (2011) for a review), Salthouse (1996) proposed that a global slowing of processing speed is responsible for the majority of age-related deficits reported in more specific cognitive domains. Indeed, the phenomenon of "cognitive slowing" is one of the most commonly documented aspects of cognitive ageing (Fozard, Vercruyssen, Reynolds, Hancock, & Quilter, 1994; Schaie, 1989) and Salthouses' theory has received some supporting evidence (Birren, 1995). However, others have shown that age-related deficits in cognitive abilities persist even after controlling for processing speed (Bugg, Zook, DeLosh, Davalos, & Davis, 2006; Joy, Kaplan, & Fein, 2004; Keys & White, 2000). In addition, Sliwinski and Buschke (1999b) reported that although controlling for processing speed differences greatly reduced the effect of age on cognition in cross-sectional designs they did not attenuate the longitudinal age-effect. These findings suggest that this theory of cognitive slowing may be oversimplified and that other factors, alongside the slowing of processing speed, drive cognitive decline with age.

Similar to Salthouse's theory, is the suggestion that age-related decline in executive function is due to changes in a global factor, such as a 'general intelligence factor' (g_r) (Spearman, 1927), This suggestion is largely a result of the finding that performance on executive function tasks is predicted by general intelligence test scores (Duncan, Burgess, & Emslie, 1995). Rabbitt (1993) reported that a number of cognitive abilities (not solely executive function) for participants aged 50-90 were almost entirely predicted by intelligence test scores. Similarly, even supposedly 'simple' cognitive processes, such as reaction time, have been associated with intelligence (Rabbitt & Goward, 1994). However, a number of other studies have reported significant cognitive deficits in older, compared to younger adults, which are not fully accountable by measures of intelligence (Hart & Bean, 2011; Mohn, Sundet, & Rund, 2014; Rabbitt & Lowe, 2000). Rabbitt and Lowe (2000) found that although general intelligence best predicted performance on "frontal lobe" tasks, for 60-

80 year olds, memory tests were most affected by age, even when intelligence was controlled for. These more recent results suggest that although executive function performance is often best predicted by general intelligence score, this may be due to their general nature, i.e. they depend on a range of cognitive processes, rather than providing direct evidence that a decline in general intelligence causes decline in executive function with age.

A review by Hedden and Gabrieli (2004) suggested that cognitive abilities are differentially affected by age and that the onset of decline differs across cognitive domains. This casts doubt on theories which propose that a global change with age is responsible for all aspects of age-related cognitive decline. They report that, based on cross sectional data from the Seattle Longitudinal study (Schaie, 1996), which included 22-70 year olds, linear age-related decreases in performance were identified for: spatial orientation, inductive reasoning, episodic memory and processing speed while verbal and numerical ability were spared the detrimental effect of advancing age. Longitudinal data from the same study revealed that most cognitive abilities showed a decline after the age of fifty five, with the exception of processing speed, which started to decline after the age of 25. Semantic knowledge and vocabulary were found to be relatively stable across the lifespan, until later life (~80 years), when they began to decline (Park et al., 2002; Schaie, 1996). Similarly, data from a study by Gregoire and Van Der Linden (1997) identified that short-term memory is affected by a "late life decline." This is characterised by small declines in memory that occur throughout the adult lifespan, until around seventy years old when a sharper decline occurs. It is worth stating that these discrepancies between longitudinal and cross-sectional results are fairly typical in the field of cognitive ageing. That is, that results from longitudinal studies (i.e. more than one data point from the same participant, across time) suggest more stable cognitive performance across middle-age, followed by sharper declines in later life,

while results from cross-sectional studies typically suggest that cognitive decline occurs in a linear fashion (Hedden & Gabrieli, 2004; Knopf & Neidhardt, 1995; Ronnlund, Lovden, & Nilsson, 2008). However, evidence also suggests that discrepencies in cognitive performance between the two methods may, largely, be due to practice effects (Salthouse, 2010). Nonetheless, although the time-scale and degree of decline tend to differ, both methods corroborate on the types of cognitive domains which are negatively affected by age. The pros and cons of longitudinal and cross-sectional study designs is discussed in more detail in the discussion chapter. Throughout this thesis, cross-sectional and longitudinal studies are considered together and results that are the outcome of longitudinal studies are highlighted.

Despite widespread changes in cognitive ability, certain cognitive functions seem largely unaffected by age. A number of forms of memory, including: autobiographical (Fromholt et al., 2003), implicit (La Voie & Light, 1994), semantic, (Lacombe, Jolicoeur, Grimault, Pineault, & Joubert, 2015; Nilsson, 2003), procedural (Churchill, Stanis, Press, Kushelev, & Greenough, 2003), and, more generally, some long-term memory processes which depend on temporal-hippocampal systems (Hanninen et al., 1997; Kausler, 1994) show little decline with ageing. In addition, performance that depends on automatic processes, such as recognition, shows very little disruption with age (Jacoby, 1999; Spencer & Raz, 1995). More recently, studies have provided evidence that only certain components of executive functioning are affected by age (Dorbath, Hasselhorn, & Titz, 2011). For example, Verhaeghen and Hoyer (2007) reported that although older adults exhibited a reduced ability to maintain representations outside their focus of attention, their ability to switch spatial attention was maintained, when compared to younger adults.

Furthermore, research suggests that a large degree of individual variability in cognitive performance exists within groups of older participants (Bielak, Cherbuin, Bunce, & Anstey,

2014; Buckner, 2004; Das et al., 2014; Mella, de Ribaupierre, Eagleson, & de Ribaupierre, 2013; Wilson et al., 2002). Using a longitudinal design, Bielak and colleagues (2014) identified that increased intra-individual variability of cognitive speed is a fundamental consequence of the ageing process. For simple tasks, increased variability across the eight-year period of testing existed only for the oldest group of participants, while variability increased for complex tasks from the age of forty. These results clearly highlight the variability of the ageing process on cognitive function, suggesting that chronological age alone does not drive cognitive dysfunction.

In summary, advancing age is associated with disruption to a wide range of cognitive domains. However, recent evidence has highlighted that specific aspects of cognitive abilities are variably affected by age. Therefore, it seems likely that the effect of age on cognitive function is both domain specific and variable between individuals. One possible explanation for such variability, and the differing ages of onset for different cognitive domains, is that cognitive decline is driven by changes to brain structure and function which may not be global and homogeneous or consistent across all participants. The next section discusses the link between brain structure, function and cognition and how brain changes may be responsible for cognitive disruption, and preservation, in older age.

1.1.2 Linking cognitive dysfunction to brain changes with age

Advancing age is associated with a number of changes to brain structure and function (Raz & Rodrigue, 2006) and much research has attempted to link such changes with cognition. However, it is worth considering that the general neuroscientific goal of linking cognition to brain function is a complex task, even before the additional factor of ageing is considered. Until recently, studies investigating how brain differences due to older age may drive differences in cognitive function have largely employed task-fMRI. These studies

attempt to isolate individual regions which exhibit differential BOLD responses to specific stimuli in comparison to younger adults (see Eyler, Sherzai, and Jeste (2011) for a review). A more recent development, which is the approach that will be followed in this thesis, is to examine the intrinsic activity of the brain 'at rest'.

One theory that attempts to explain changes in cognition with age is the "frontal ageing hypothesis." This theory proposes that cognitive abilities which depend on the frontal lobe (e.g. executive functions) would exhibit the greatest declines with ageing because the frontal lobe suffers the greatest age-related deterioration (Raz et al., 1997; Salat et al., 2004; Salat, Kaye, & Janowsky, 2001; Salat et al., 2005). Similarly, others have suggested that anterior brain regions suffer greater disruption compared to posterior regions (Sowell et al., 2003; Sowell, Thompson, & Toga, 2004). Studies have also suggested that cognitive abilities which depend on anterior regions are most vulnerable to age-related decline (Band, 2000; Greenwood, 2000; West, 1996, 2000). However, Greenwood (2000) evaluated the "frontal ageing hypothesis" and concluded that since many non-prefrontal brain areas undergo similar declines in volume, blood flow and metabolism, there is only weak evidence to suggest that the frontal lobes are preferentially affected by ageing.

The common finding that older adults typically exhibit greater frontal task-fMRI activity, compared to younger adults, may correspond with Greenwood (2000) and provide evidence that frontal brain regions are not more specifically compromised than others (see Spreng, Wojtowicz, and Grady (2010) for a meta-analysis review). However, this theory works on the assumption that greater fMRI activity is beneficial for performance, but it is overly simplistic to directly associate the size of fMRI activations with the quality of behaviour. A number of theories of cognitive ageing have attempted to explain the common finding that older adults typically over-recruit frontal brain regions in task-fMRI studies. The Posterior-Anterior Shift in Ageing (PASA) model of cognitive ageing suggests that the posterior-

anterior shift in the recruitment of brain regions in older adults is necessary to maintain younger adult like performance (Davis, Dennis, Daselaar, Fleck, & Cabeza, 2008). For example, studies have found that visual tasks, which require posterior visual regions, come to be associated with greater frontal fMRI signal in older, compared to younger, adults (Cabeza et al., 2004; Madden, Spaniol, Bucur, & Whiting, 2007). This suggests a compensatory role of frontal activity in older adults, rather than widespread declines in the functioning of anterior brain regions. Another such theory which posits that older brains are capable of exhibiting 'compensatory' activity to maintain task performance is the Hemispheric Asymmetry Reduction in OLDer adults (HAROLD) (Cabeza, 2002; Dolcos, Rice, & Cabeza, 2002). This theory suggests that older adults, whose task performance is of a similar quality to that of younger adults, tend to exhibit less-lateralised brain activity and more extensive recruitment of the pre-frontal cortex (PFC). However, despite numerous studies reporting that older adults may exhibit this additional, compensatory-type brain activity (Cabeza, Anderson, Locantore, & McIntosh, 2002; Cabeza et al., 2004; Daselaar, Fleck, Dobbins, Madden, & Cabeza, 2006; Daselaar, Veltman, Rombouts, Raaijmakers, & Jonker, 2003; Park et al., 2002; Park & Reuter-Lorenz, 2009; Reuter-Lorenz & Cappell, 2008), a number of other researchers have reported only reductions in task-fMRI responses in older adults (Dennis & Cabeza, 2008; Schneider-Garces et al., 2010), or that the recruitment of additional brain regions in older adults is associated with worse, rather than better, task performance (de Chastelaine, Wang, Minton, Muftuler, & Rugg, 2011; Garrett, Kovacevic, McIntosh, & Grady, 2011; Morcom, Li, & Rugg, 2007). The relationship between fMRI task responses and behaviour in older adults is therefore complicated, and the idea that additional brain regions are recruited to act as 'compensatory' mechanisms is likely an over-simplification. Furthermore, the majority of task-fMRI studies are confounded by differences in performance between age groups. For example, if task-fMRI differences exist

between age groups, but the groups differ significantly on the performance of the task (as is typically the case for memory, processing speed and executive function tasks) it is difficult to establish whether fMRI differences are related to age-related changes in brain function or a consequence of the differing task performance, or a combination of both. Similar to theories of compensation is the theory of 'dedifferentiation' (Goh, 2011). Dedifferentiation posits that the brain regions, or networks, that are recruited in response to a task in older adults are less specific, or differentiated, compared to younger adults. A study by St-Laurent, Abdi, Burianova, and Grady (2011) revealed differentiation between the brain regions involved in episodic, semantic and autobiographical memory in younger adults (i.e. they identified three distinct memory networks). However, such differentiation was not present in older adults (i.e. there was much more overlap between the brain regions involved in the three memory systems), although there was no difference in memory performance between the two age groups for any of the three memory types. As above, dedifferentiated recruitment of brain regions could reflect a compensatory type method, where the involvement of additional brain regions results in greater neural resources and thus better performance. Alternatively, it could reflect greater neural 'noise' in the older brain, where cortical regions are less distinctly represented, which could have an interference effect on cognitive performance (Li, Lindenberger, & Sikstrom, 2001).

The Compensation-Related Utilization of Neural Circuits Hypothesis (CRUNCH) (Reuter-Lorenz & Cappell, 2008) is one theory that attempts to explain both performance gains and deficits associated with the recruitment of additional brain resources in older adults. Although this theory also suggests that recruitment of additional brain regions in older adults is compensatory in nature, it suggests that there are limitations to compensatory gains. This theory states that older adults are required to recruit additional neural rescources at lower loads (i.e. when task difficulty is low) than younger adults. This is

thought to result in a ceiling effect; where there are no neural resources left to recruit as task demands increase, which results in impaired performance compared to younger adults who are able to recruit additional resources at higher loads because resources were spared at lower loads (Schneider-Garces et al., 2010). The idea of 'resources' in this theory provides a theoretical framework for how factors, such as sleep deprivation or exercise, may alter cognitive performance in older adults. For example, sleep deprivation may reduce available resources, while exercise or cognitive training may increase available resources, thus lowering or raising the threshold for the 'resource ceiling' effect, respectively. However, the evidence to support this theory is currently lacking.

The results from these studies lead to a somewhat fragmented account of changes to task-related brain responses with age meaning that, to date, a unified, accepted theory of cognitive brain ageing does not exist. This is likely due to the fact that disentangling the mutual relationships between different cognitive processes is not simple (Albinet, Boucard, Bouquet, & Audiffren, 2012). Similarly, as the field of neuroscience has recently highlighted the importance of evaluating the brain as a network, or series of networks, (see Sporns (2014) for a review), modular approaches to cognitive ageing are perhaps less useful than theories that adopt network-based methodologies. Using such network-based methodologies offers the potential to unify some of the age-related cognitive findings. For example, it is possible that age-related disruption to a particular network may account for disruption to a number of cognitive functions which are supported by that network but not dependent upon a single brain region. Understanding how brain networks may be re-organised with advancing age, has the potential to allow us to establish a greater insight into the changes occurring in the functional architecture of the older brain and how such changes may explain differences in a range of cognitive abilities. A number of researchers have used network-based approaches to investigate cognitive ageing and attempt to unify some of the

theories discussed above (Salami et al., 2010; Salami, Eriksson, & Nyberg, 2012; Salami, Pudas, & Nyberg, 2014; Vallesi, McIntosh, & Stuss, 2011). The next section summarises the results from studies of both structural and functional network connectivity in the older brain and provides evidence for the role of network reorganisation in cognitive disruption.

1.1.2.1 Ageing and structural connectivity

Early research into structural brain changes with age provided the first preliminary evidence that brain connectivity may be disrupted with age. It is well established that total brain volume declines with age (Courchesne et al., 2000). More specifically, it has been established that after the age of 60 there is an annual tissue loss of approximately 1.6% (Resnick, Pham, Kraut, Zonderman, & Davatzikos, 2003), 2003), which increases to approximately 2.1% between the ages of 70-80 (Tang, Whitman, Lopez, & Baloh, 2001). These losses in brain volume, which includes both grey and white matter, were initially attributed to neuronal loss. However, more recent research has shown that the number of neurons remains relatively stable across the lifespan, and that volume loss could instead be attributed to synaptic density loss and neuronal shrinkage (Haug & Eggers, 1991). Therefore, it is possible that age-related changes in brain volume could be a result of synaptic, and thus connectivity, degradation rather than a reduction of total available neurons. Peters (2002) reported that in humans over the age of 50, there is a 46% reduction in the number of neuronal spines and synapses, compared to younger adults, which co-occurs with the degeneration of myelinated axons. Furthermore, as well as affecting the integrity of connections, the degeneration of myelin sheaths may also affect the timing of network communication, via disruption of conductance rates (Peters, 2009). Synaptic loss and myelin degeneration have been associated with white matter atrophy in both monkeys and humans (Hof & Morrison, 2004; Peters, 2002) and white matter volume loss is

commonly associated with older age (Bartzokis et al., 2003; Good et al., 2001; Raz et al., 2005; Resnick et al., 2000). Therefore, it could be suggested that older adults experience reduced or disrupted connectivity between brain regions, which could be responsible for ineffective communication and cognitive disruption (Bartzokis, 2004).

Gunning-Dixon and Raz (2000, 2003) and Van Petten and colleagues (2004) have shown that white matter pathological markers of vascular disease are associated with cognitive decline, while Bartzokis (2004) reported that white matter decline is associated with a decline in the performance of both attentional control and executive function tasks; likely due to the disruption of inter-hemispheric connections. This finding corresponded with previous work by Peters (2002), who found that disrupted axonal conduction was associated with age-related cognitive deficits. Colcombe, Kramer, Erickson, and Scalf (2005) also supported these findings, identifying that white matter tracts differed as a function of performance. They proposed that hemispheric connectivity may influence performance via different patterns of recruitment of brain regions.

A number of recent studies have revealed the detrimental effect of age on measures of structural connectivity (Salat et al., 2005), rather than just regional white matter effects, and have also identified associations with cognitive performance (see Bennett and Madden (2014) for a review). Typically, diffusion tensor imaging techniques have identified decreased white matter density with age and associations with disrupted cognitive performance on a range of memory, RT and executive function tasks (Brickman et al., 2006; Gunning-Dixon, Brickman, Cheng, & Alexopoulos, 2009; Sullivan & Pfefferbaum, 2006; Sullivan, Rohlfing, & Pfefferbaum, 2010; Voineskos et al., 2012; Zahr, Rohlfing, Pfefferbaum, & Sullivan, 2009). Studies have also suggested that cognitive abilities that depend on anterior brain regions, which are most vulnerable to white matter changes, suffer the greatest age-related decline (Band, Ridderinkhof, & Segalowitz, 2002; Greenwood, 2000;

Sullivan et al., 2010; West, 2000). Similarly, Brickman (2006) found that frontal and temporal white matter volume showed significant decline with age and that this decline explained a significant proportion of between-subject variance in a range of cognitive tasks. They also reported that the steepest decline of white matter loss occurred after approximately 50 years of age. This late life decline could implicate white matter atrophy in the disruption of cognitive abilities which is commonly observed in older age (Hedden & Gabrieli, 2004), as a consequence of large-scale degradation of brain connectivity (O'Sullivan et al., 2001). Furthermore, a study by Bucur and colleagues (2008) suggested that differences in the white matter integrity of the corpus callosum and frontal regions explain inter-individual differences in perceptual speed and episodic memory function in older adults. More recently, these changes to the large-scale structural brain architecture with age have been identified to be heterogeneous across the brain, highlighting the intricate, complex nature of the effect of ageing on brain connectivity (Zhao et al., 2015). Taken together, these findings suggest that pronounced changes in structural brain connectivity and/or white matter integrity with age may partly explain age-related cognitive decline.

1.1.2.2 Functional networks

Mapping of large scale brain networks, i.e. the interconnecting pathways between brain regions, can also be investigated with functional imaging techniques and a large body of work has linked functional network integrity with cognition (see Bressler and Menon (2010) for a review). In this section I will first provide an overview of functional networks before discussing the impact of ageing upon such networks. A functional network consists of a number of 'nodes', which can be defined based on anatomical brain regions, or by parcellating the brain into individual voxels and then performing some sort of clustering analysis to identify 'nodes' based on some pre-defined characteristics (Sporns, 2013).

Traditionally, nodes are considered to belong to the same 'network' if their fMRI time-courses are temporally similar, which can be assessed by correlating the derived time-courses with each other. Therefore, a network can be defined as a subset of voxels (nodes) whose time-courses significantly correlate with a reference, or 'seed' time-course (Raichle & Snyder, 2007; Vincent et al., 2006). The most central, or influential nodes of a network are referred to as 'hub' regions, although there is no standard way to define a 'hub,' which means that their definition depends on the method used to identify them (e.g. graph theory metrics, known functional role, or structural connectivity). Graph theory measures, such as path length, degree, segregation and integration, can also be used to identify networks. However, these methods are not discussed in detail in this thesis, namely because they are a novel area of research and pose a number of complex methodological questions when attempting to compare groups, as in ageing research.

1.1.2.2.1 Resting-state networks

As well as investigating the BOLD response to tasks, assessing spontaneous fluctuations in the BOLD time-course provides another informative way of probing brain function (see Fox and Raichle (2007) for a review). In contrast to task-fMRI methods, resting-state approaches investigate the brain at 'rest', i.e. in the absence of a task. Participants are typically asked to simply keep their eyes open or focus on a fixation cross and to think of nothing in particular. Spontaneous low frequency BOLD oscillations (~0.01-0.1Hz) are then obtained and analysed (see below). Therefore, resting-state fMRI (rfMRI) approaches provide a way of assessing brain function between groups of participants which could otherwise be confounded by differences in task performance (e.g. younger vs. older, adults vs. children, healthy vs. patient groups) and may afford new clinical applications (Fox & Greicius, 2010). However, the nature of rfMRI also means that the data is very

unconstrained and thus potentially less reliable than task fMRI, although compliance and sustained attention is not necessarily guaranteed for task fMRI, particularly for patient groups, older participants and children. A number of studies have provided evidence that despite the unconstrained nature of rfMRI, rfMRI network measures (at both voxel and region analysis level) are reliable within and across sessions, both within and between participants (Choe et al., 2015; Chou, Panych, Dickey, Petrella, & Chen, 2012; Damoiseaux et al., 2006; Franco, Mannell, Calhoun, & Mayer, 2013; Guo et al., 2012; Shehzad et al., 2009). Furthermore, rfMRI may afford up to three times the signal to noise ratio (SNR) compared to task-fMRI (Fox & Greicius, 2010), which has relatively low SNR as the signal being measured (task-related modulation) accounts for only ~20% of the BOLD signal variance (Fox, Snyder, Vincent, & Raichle, 2007; Fox, Snyder, Zacks, & Raichle, 2006). This gain in SNR when adopting rfMRI methods has the advantage of facilitating the detection of group differences and patient abnormalities, further advocating the use of rfMRI for investigating age-related functional brain changes.

By adopting rfMRI methods, it has been identified that spatially distinct regions are found to exhibit temporally similar BOLD time-series, suggesting that a common function is shared between the regions. These temporally coherent regions are thus referred to collectively as a resting-state network (RSN). The integrity of RSNs, or how the connectivity within and between RSNs may differ between groups (see van den Heuvel and Hulshoff Pol (2010) for a review) is assessed by functional connectivity (FC). The term 'functional connectivity' can broadly be defined as the statistical association or dependency between anatomically distinct brain regions (Friston, 2011; Friston, Frith, Fletcher, Liddle, & Frackowiak, 1996; Horwitz, 2003), which is typically assessed using correlation coefficients. The first study to identify the existence of coherent spontaneous BOLD activity across brain regions in this way was conducted by Biswal, Yetkin, Haughton, and Hyde (1995). The

authors noted that spontaneous BOLD fluctuations measured in the left somatomotor cortex were correlated with spontaneous fluctuations in the right somatomotor cortex and with medial motor areas in the absence of overt motor behaviour. This is a finding that has been replicated by numerous studies, and has resulted in the identification of the functional motor resting-state network (RSN). Similar results were identified for visual and auditory regions (Cordes et al., 2000; Lowe, Mock, & Sorenson, 1998), confirming the existence of equivalent sensory RSNs for these modalities. These findings, which identify that brain regions with similar functions, and which are typically anatomically connected, suggest that FC can be interpreted as a measure of coherent neuronal activity between regions which form a functional network during rest. Although previously some have suggested that FC may be artefactual and potentially induced by physiological processes (such as cardiac or respiratory oscillations) (Birn, Diamond, Smith, & Bandettini, 2006; Birn, Smith, Jones, & Bandettini, 2008; Chang, Cunningham, & Glover, 2009; Shmueli et al., 2007; Wise, Ide, Poulin, & Tracey, 2004), spontaneous BOLD signals are largely dominated by low frequencies (<0.1Hz) with a relatively small contribution from higher frequency physiological confounds (>0.3Hz) (Cordes et al., 2001; Cordes et al., 2000). This suggests that FC is not simply an artefactual measurement induced by physiological factors, although it may still be confounded by non-neuronal factors (discussed in more detail in Chapter 3). Similarly, others have reported likely associations between spontaneous neuronal firing and gamma local field potentials (LFPs) and the amplitude of FC (Nir et al., 2008), associations between spontaneous BOLD fluctuations and: spontaneous neuronal spiking (Shmuel & Leopold, 2008; Shmuel et al., 2002), slow EEG oscillations (He, Snyder, Zempel, Smyth, & Raichle, 2008; Lu et al., 2007; Pan, Thompson, Magnuson, Jaeger, & Keilholz, 2013) and low frequency local field potentials (in rat cortex) (Lu et al., 2016). Recently, Hiltunen and colleagues (2014) identified associations between infra-slow (0.01-0.1 Hz) EEG and BOLD

fluctuations in regions which correspond to typical RSNs. Similarly, other researchers have identified associations between BOLD signal fluctuations and concurrent EEG oscillations of RSNs (Sadaghiani et al., 2010). Furthermore, a study by Mantini, Perrucci, Del Gratta, Romani, and Corbetta (2007) identified a robust relationship between BOLD signal RSNs and EEG power. The authors report that although RSNs were generally associated with more than one EEG frequency band, RSNs could be separated by their EEG power profile. For example, visual network BOLD time courses were more associated with delta and theta rhythms, while DAN BOLD time courses were more associated with alpha and beta rhythms. However, this distinction was attenuated during visual tasks when the networks are, presumably, both engaged on the task. Overall, there is a growing consensus that FC measures likely represent, at least in part, patterns of slow neuronal fluctuations, reflecting functional communication between regions (Shmuel & Leopold, 2008; van den Heuvel & Hulshoff Pol, 2010).

More recently, independent component analysis (ICA), which decomposes the entire resting-state fMRI data set into maximally spatially independent, individual components, has also revealed the existence of a number of non-sensory RSNs (Di & Biswal, 2013; Fox et al., 2005). These include the default mode (DMN), dorsal attention (DAN) and saliency (SN) networks, all of which are thought to contribute to cognitive functioning (Stevens & Spreng, 2014). RSNs are robustly identified across the lifespan (Betzel et al., 2014; Bo et al., 2014; Cao et al., 2014; Wang, Su, Shen, & Hu, 2012) and varying states of consciousness (Horovitz et al., 2008; Larson-Prior et al., 2009; Spoormaker et al., 2010; Wilson et al., 2015) and are highly replicable within (Choe et al., 2015; Chou et al., 2012; Guo et al., 2012) and between (Damoiseaux et al., 2006; Franco et al., 2013) participants. One of the most commonly studied RSNs is the DMN, which is most active when the participant is at rest and there is no task or action to engage in, hence the name “default mode” (Raichle et al., 2001). The DAN,

thought to be important for attentional processes (Fox, Corbetta, Snyder, Vincent, & Raichle, 2006; Ptak, 2012; Szczepanski, Pinsk, Douglas, Kastner, & Saalmann, 2013) is commonly thought of as an 'anti-correlated' network to the DMN (Fox et al., 2005; Fransson, 2005; Gao & Lin, 2012; Greicius, Krasnow, Reiss, & Menon, 2003). For example, when a participant engages attentional resources to complete a task (i.e. engages the DAN, increase in fMRI signal) the DMN becomes 'disengaged' (decrease in fMRI signal) until the participant returns to their resting-state. Although initial studies focussed on investigating how the activity of nodes within a network is associated, studies have also investigated how these RSNs may interact with each other. Researchers have identified that the right insula, a main node of the SN, may be important for facilitating the network switching between the DMN and DAN, for example, when 'disengaging' the DMN and 'engaging' the DAN in response to a task/stimuli and vice versa (Menon & Uddin, 2010; Sridharan, Levitin, & Menon, 2008). This suggests that connectivity between networks may be as important, if not more, than intra-network connectivity for efficient brain processing.

1.1.2.2.2 Static vs. dynamic functional connectivity

FC analysis has predominantly been calculated as a 'static' measure (i.e. correlation between BOLD time-courses across the entire scan duration of several minutes, resulting in one FC measure per pair of regions). However, recent research has begun to investigate changes in dynamic FC (i.e. calculating FC strengths at multiple time-points throughout a scan, resulting in multiple FC measures per pair of nodes) (see Calhoun, Miller, Pearlson, and Adali (2014) for an in-depth overview). Investigating dynamic FC in terms of ageing may be particularly important, as a) measures of static FC may mask fluctuating age-related differences across the time-course that are not well represented by the average time-course, b) some evidence suggests that older brains are inherently less variable (Grady & Garrett,

2014) which could, again, result in age-related FC differences which are better identified when using more sensitive, dynamic measures of FC. However, although dynamic FC results in greater temporal information regarding how FC may fluctuate over the length of a scan, the sheer number of measures make summarising differences between groups problematic. This issue, and an investigation of dynamic FC for younger and older adults, is presented in Chapter Seven. The effect of ageing on FC in the following section refers to measures of, typical, static FC.

1.1.2.2.3 The relationship between functional connectivity and cognition

A large body of work has linked functional network integrity with cognition (see van den Heuvel and Hulshoff Pol (2010) for a review) and further studies have identified that white matter integrity and patterns of FC are strongly associated (Andrews-Hanna et al., 2007; Chen, Chou, Song, & Madden, 2009; Damoiseaux & Greicius, 2009), suggesting a relationship between the two. However, a definitive one-to-one mapping is not always identified (Greicius, Supekar, Menon, & Dougherty, 2009; Honey et al., 2009; Vincent et al., 2007). While FC is often identified between regions without strong structural connectivity (Honey et al 2009), regions with structural connectivity almost always have strong corresponding FC.

One of the main advantages of using FC to investigate age-related changes in the older brain is that fMRI BOLD data is acquired at rest. Therefore, the confounding effect of performance differences between age groups (which often exist in task-related fMRI studies of age) is removed, allowing the investigation of differences in the intrinsic functional architecture of the young and old brain. However, differences in a number of physiological factors which can influence the BOLD signal (as discussed briefly above and in more detail in Chapter 3) still exist between age-groups. Therefore, it is imperative to control for these

physiological differences as much as possible in order to reliably assess the extent to which RSNs undergo re-organisation with advancing age.

1.1.2.2.4 Effect of ageing upon functional networks

Recently, research has begun to investigate changes in FC in the older brain (see Dennis and Thompson (2014); Ferreira and Busatto (2013) and Sala-Llonch, Bartres-Faz, and Junque (2015) for comprehensive reviews). Increasing evidence has found that age has a significant impact on inter-individual variability of the spatial extent and strength of resting-state networks (Allen et al., 2011; Biswal et al., 2010). Similarly, graph metrics, such as topological efficiency, which can be used to summarise functional networks, are also seen to be detrimentally affected by advancing age (Achard & Bullmore, 2007). Wang, Li, Metzak, He, and Woodward (2010) reported that advancing age results in disrupted long range functional connectivity, identified between: fronto-temporal, fronto-occipital, fronto-parietal and temporal-parietal regions, as well as impaired short-range connectivity in frontal circuits. Similarly, other researchers have suggested that long-range connections are particularly affected by age (Meier et al., 2012; Meunier, Achard, Morcom, & Bullmore, 2009; Sala-Llonch et al., 2014; Tomasi & Volkow, 2012a; Toussaint et al., 2011). The DMN, which comprises the posterior cingulate cortex (PCC), medial prefrontal cortex (mPFC), bilateral inferior parietal lobes (IPL) and the medial temporal lobes (MTL) (Andrews-Hanna, Reidler, Sepulcre, Poulin, & Buckner, 2010; Buckner, Andrews-Hanna, & Schacter, 2008), has been the most commonly studied RSN in relation to age. Numerous studies have reported decreased FC within the DMN in older compared to younger adults (Andrews-Hanna et al., 2007; Tomasi & Volkow, 2012a; Wu et al., 2011). In addition, Damoiseaux and others (2008) reported that out of a number of RSNs, only the DMN showed decreases in FC with age, suggesting that this network is particularly susceptible to ageing effects. However, more

recently, others have reported age-related FC decreases in the SN (Onoda, Ishihara, & Yamaguchi, 2012), motor network (Wu et al., 2007) and visual network (Yan, Zhuo, Wang, & Wang, 2011). Furthermore, Mevel and others (2013) reported that there is a linear effect of age on the disconnectivity between medial frontal and parietal regions from ages 19-80 years.

Although the majority of resting state studies have found decreases in intra-network FC with advancing age, there have been several instances where increases in FC have been identified. Mowinckel, Espeseth and Westyle (2012) reported that alongside decreases in DMN FC, older adults also exhibited increased FC, compared to young adults, in five RSNs, which included frontal and association networks. Touissant and colleagues (2011) also identified, using graph theoretic techniques, that older adults had increased intra-network integration of frontal and parietal sub-regions of the DMN. Similarly, Tomasai & Volkow (2012a) reported increased FC within somatosensory and motor cortices, as well as the cerebellum and brain stem while Meier (2012) also found increased FC in sensorimotor cortices in older age. While decreases in FC with age are often interpreted as indicators of reduced processing ability of the networks involved, the interpretation of increases in FC with age is currently lacking. Increases could be related to neurotransmitter changes with age, or even non-neural factors, such as changes in vasculature with age (Peters, 2006; Riddle, Sonntag, & Lichtenwalner, 2003a). Alternatively, increased FC with ageing could indicate compensatory network reorganisation in response to a decline in the FC of critical networks, such as the DMN, analogously to the compensatory task-related activations often seen in older age as discussed above. However, it is important to consider that greater FC may not always be beneficial. Although greater FC may represent a stronger communication exchange or a more efficient network in a variety of situations, segregation of the activity of

particular brain regions is likely to be just as important for efficient cognitive performance (Antonenko & Floel, 2014; Bullmore & Sporns, 2012a).

Indeed, a number of researchers have suggested that such age-related de-differentiation (i.e. increased inter-network FC which results in a loss of network modulation and functional specialisation) is responsible for the disruption of cognitive function with older age (for a review see Grady (2012)). Clapp and colleagues (2011) identified age-related deficits in re-establishing FC between the parahippocampal place area (PPA) and PFC during the post stimulus period of a switching task which was associated with poorer performance on a working memory task in older adults. They suggest that older adults are less efficient at modulating network connectivity and that differences in FC compared to younger adults may reflect the inability to inhibit the previous task and successfully switch to the next, as indexed by their behavioural performance. Furthermore, this deficit in the modulation of connectivity may result in inefficient resource allocation to a task, resulting in poorer performance commonly seen in advancing age on a range of tasks. Similarly, Ystad, Eichele, Lundervold, and Lundervold (2010) provided evidence for a negative correlation between FC and performance. They found that reduced FC between subcortical regions (thalamus and basal ganglia) correlated with better verbal memory scores in older adults. Furthermore, age-related increases in hippocampal FC have been associated with both impaired cross-sectional episodic memory performance and longitudinal memory performance over 20 years (Salami et al., 2014). This study also provided evidence to suggest that greater hippocampal FC at rest was associated with reduced hippocampal recruitment and hippocampal-cortical FC during a memory task. Taken together, these findings support the suggestion that in some cases, segregation of brain regions is also important for efficient recruitment of a network to ensure optimal performance on a task. Similarly, greater FC within a region (e.g. left and right hippocampi)

may reduce the capacity for that region to interact with other brain regions, resulting in inefficient network activation which may impact cognitive performance, as suggested by Salami et al. (2014). Further support for this theory is reviewed by Antonenko and Floel (2014) who summarise that selective connectivity (i.e. maintaining specificity between RSNs) is important for 'successful' cognitive ageing.

An increasing number of studies have now provided evidence that reductions in the brain's intrinsic FC with age are associated with cognitive disruption, suggesting that less connected brains are less cognitively able (Hampson, Driesen, Skudlarski, Gore, & Constable, 2006; Koyama et al., 2011; Mevel et al., 2013; Onoda et al., 2012). Similarly, many other studies have provided evidence that intrinsic DMN FC is associated with cognitive performance on a wide range of tasks for older adults (Andrews-Hanna et al., 2007; Clapp et al., 2011; Damoiseaux et al., 2008; Persson et al., 2006; Wang et al., 2010). More specifically, Buckner (2004) identified that age-related reductions in fronto-striatal functional connectivity were associated with memory deficits, while Dennis and colleagues (2008) and Daselarr and others (2006) reported similar findings for temporal-parietal FC. Similarly, reduced fronto-parietal FC has been associated with greater distractibility during an implicit memory task, likely due to impaired inhibition which would typically prevent distraction (Campbell, Grady, Ng, & Hasher, 2012). Furthermore, reductions in FC between frontal regions in older adults, compared to younger adults, have been shown to correlate with processing speed (Chen et al., 2009) while reduced long-range FC observed in older adults has been associated with poorer memory performance (Sala-Llonch et al., 2014; Wang et al., 2010). Such findings have led to theories such as O'Sullivan's 'disconnection hypothesis' (2001), which proposes that decline in cognitive ability with age is due to disconnection of brain regions. O'Sullivan suggests that "changes in functional integration between systems of brain areas" results in the deficits identified in numerous cognitive domains. However,

studies investigating measures of inter-network FC suggest that advancing age is not simply associated with reductions in FC and that these initial theories of brain connectivity in ageing are perhaps over-simplified.

Instead, it is now apparent that the inclusion of inter-network FC analysis may also enhance our understanding of how FC alters with age (Betzel et al., 2014). By investigating how nodes of a particular network may become more or less connected to nodes of a separate network we may be able to gain a greater insight into the network re-organisation that potentially occurs with age. To date, few studies have focussed their attention on inter-network FC. However, those that have report altered inter-network FC with age (Onoda et al., 2012; Tomasi & Volkow, 2012a). He and colleagues (2013) focussed on the right insula, a main node of the SN, which is thought to be important for network switching between the DMN and task-positive networks (Menon & Uddin, 2010; Sridharan et al., 2008). They identified that inter-network FC between the right insula and nodes of the DMN and DAN were significantly reduced with age. In contrast, others have recently suggested that inter-network FC is often increased in older age, suggesting a more complex picture of connectivity alterations in the ageing brain. Meier and colleagues (2012) investigated the FC of 100 seed regions and reported that the majority of paired connections that exhibited greater FC for older adults were inter-network connections, particularly between nodes of sensorimotor, SN, DAN and default mode networks. Betzel and colleagues (2014) recently reported that while intra-network FC was reduced with advancing age in a number of RSNs, inter-network FC increased between nodes of the DAN and SN/ventral attention/somatomotor networks. Similarly, Geerligs, Renken, Saliasi, Maurits, and Lorist (2014b) reported reduced intra-network FC of higher cognitive networks (DAN, DMN, SN) and increased inter-network FC between visual, DAN and sensorimotor networks with advancing age. Similar findings have been reported during task-based FC analysis as both

Geerligs, Maurits, Renken, and Lorist (2014a) and Voss and others (2010) identified reductions in intra-network and increases in inter-network FC in comparison to younger participants during task fMRI. Taken together these recent findings seem to suggest that older age may be associated with reduced specificity of RSNs, which become less modular and distinct and more inter-connected and diffusely distributed across the brain as we age.

A review by Stern (2009) suggests that such reorganisation of brain networks in older adults can be beneficial and is associated with 'cognitive reserve.' Broadly speaking, this is the suggestion that altered patterns of brain connectivity may be responsible for maintained cognitive function despite age-related changes to brain structure and function which may be expected to hinder cognitive performance (Davis, Kragel, Madden, & Cabeza, 2012; Meunier, Stamatakis, & Tyler, 2014; Voss et al., 2013). Vallesi, McIntosh, and Stuss (2011) reported that over-recruitment of the DAN (i.e. greater task-fMRI response) in older adults, compared to younger, was associated with better performance on tasks requiring response inhibition. Furthermore, the greatest increases in DAN response were identified for the hardest conditions, suggesting that greater network response was behaviourally necessary for older adults. Similarly, Oh and Jagust (2013) reported that increased fronto-temporal FC at rest was associated with larger task-fMRI responses to a memory task and greater memory performance. Therefore, a considerable amount of evidence suggests that the reorganisation of functional brain networks, typically in the form of increased inter-network FC, maintains cognitive function in older adults and thus could be thought of as a compensatory mechanism to counter the effects of the typical ageing process.

In summary, it is well established that a wide range of cognitive abilities are detrimentally affected by advancing age and that particular cognitive abilities are more affected than others. Recent evidence suggests that employing neuroimaging methods that

allow us to investigate widespread network changes with age may provide greater insight into exactly how brain changes with age drive changes in cognition.

1.2 THE POTENTIAL INFLUENCE OF SLEEP ON THE AGEING PROCESS

This section summarises the impact of prolonged wakefulness on cognitive ability and presents evidence which suggests that older age is associated with disrupted sleep quality. Based on this evidence, I will discuss the implication that sleep may be a mediating factor in the cognitive ageing process. This is a finding which is often overlooked within the field of brain ageing research but may be vital for the development of behavioural interventions which could improve cognitive performance and/or minimise cognitive decline.

1.2.1 Sleep and cognition

Studies which systematically induce sleep deprivation in younger adults have firmly established a link between sleep and cognitive function (see Banks and Dinges (2007) & Killgore (2010) for reviews). Typically, depriving someone of sleep overnight results in a reduced ability to sustain attention, which results in lapses in concentration and slowed reaction times (RTs) (Binks, Waters, & Hurry, 1999; Durmer & Dinges, 2005; Gujar, Yoo, Hu, & Walker, 2010; Lim & Dinges, 2008). Higher cognitive functions are particularly susceptible to the effects of sleep loss (Durmer & Dinges, 2005). Specifically, researchers have identified impaired inhibition (Chuah, Venkatraman, Dinges, & Chee, 2006; Drummond, Meloy, Yanagi, Orff, & Brown, 2005b), as well as impaired decision making and increased risk taking (Killgore, Balkin, & Wesensten, 2006; McKenna, Dickinson, Orff, & Drummond, 2007) following a lack of sleep. Furthermore, a range of memory processes are also impaired following sleep deprivation (Drummond et al., 2000; Harrison & Horne, 2000; Lim & Dinges, 2010; Walker & Stickgold, 2006). A number of studies have identified that the cognitive

impairments resulting from sleep deprivation are similar to those seen during elevated levels of blood alcohol concentration (BAC) (Falleti, Maruff, Collie, Darby, & McStephen, 2003). Falleti and colleagues (2003) found similarities between 24 hours of wakefulness and 0.05% BAC, which is the legal maximum BAC limit for driving in most European countries (0.08% in UK). They reported that both fatigue and alcohol affected performance on a range of tasks, with fatigue resulting in a greater impairment on the speed of simple RT tasks. These results were similar to those of Dawson and Reid (1997) who had participants perform visual-motor tasks during 28 hours of sleep deprivation and at different alcohol concentrations (conducted on a different day). They reported that after 10 hours of wakefulness, every additional hour resulted in performance deficits equivalent to a 0.004% BAC increase. After 24 hours awake they found that visual-motor performance was equivalent to a BAC of 0.10% (exceeding most legal maximum BAC limits for driving).

Fewer studies have investigated the effect of chronically partially restricted sleep in younger adults (i.e. reducing sleep to less than seven hours per night over four or more nights rather than depriving sleep for an entire night), despite the fact that this type of sleep deprivation is arguably more comparable to the type of sleep loss that occurs naturally in response to work/lifestyle demands, compared to total sleep deprivation, which is rarer in a real-life environment. However, the few existing studies provide evidence that chronic partial sleep restriction has a cumulative effect which results in behavioural deficits similar to total sleep deprivation (Banks & Dinges, 2007; Belenky et al., 2003; Dinges et al., 1997; Drake et al., 2001; Van Dongen, Maislin, Mullington, & Dinges, 2003) and provide further evidence for the detrimental effects of sleep loss on cognitive function, even when sleep is only partially restricted.

1.2.2 Sleep and age

1.2.2.1 Sleep deprivation

Given the firm association between sleep loss and cognitive performance in younger adults, it seems imperative to explore how sleep quality and quantity may change across the lifespan and the outcome such changes may have on brain function and cognition. Perhaps surprisingly, a number of studies have provided evidence that younger adults are more negatively affected by the process of total sleep deprivation than older adults (Adam, Retey, Khatami, & Landolt, 2006; Blatter et al., 2006; Duffy, Willson, Wang, & Czeisler, 2009; Landolt, Retey, & Adam, 2012; Philip, Taillard, Quera-Salva, Bioulac, & Akerstedt, 1999; Philip et al., 2004; Silva, Wang, Ronda, Wyatt, & Duffy, 2010; Stenuit & Kerkhofs, 2005). However, the reduced impact of sleep deprivation in older adults may be due to the fact that the vast majority of these studies remove a large degree of age-related variance by excluding comorbidities which are more common in older adults as well as explicitly excluding those older adults with poor sleep (i.e. less than 85% sleep efficiency at baseline). Alternatively, it could be argued that older adults are less affected by sleep deprivation because of a flooring effect. For example, the age-related disruption to cognitive ability and PFC function, thought to be common to both ageing and sleep deprivation (Harrison, Horne, & Rothwell, 2000), may be of greater magnitude than that caused by the effects of acute sleep deprivation. Therefore, one could suggest that sleep depriving older adults does not result in equivalent performance deficits to those seen in younger adults because the largest changes to cognitive ability and PFC function have already occurred in the older brain and thus any additional impact of sleep deprivation is relatively unimportant. Currently, the research into sleep deprivation in older adults cannot provide a conclusive answer for this effect. Future research using large, representative samples of older participants may reveal whether this result is due to methodology (i.e. only selecting older adults with high sleep efficiencies, who

may be less susceptible to sleep deprivation) and whether there is a differential effect of sleep deprivation depending on baseline cognitive performance of PFC function. Furthermore, this field of research ultimately asks an interesting but separate question regarding how sleep deprivation may have a further impact on any existing sleep deficits rather than how endogenous changes to nocturnal sleep may affect cognition in older age. Currently, research investigating the links between habitual sleep patterns (and their changes with ageing) and waking cognition is lacking.

1.2.2.2 Nocturnal sleep and age

Nocturnal sleep undergoes fundamental changes with age (Bruce & Aloia, 2006; Edwards et al., 2010; Roepke & Ancoli-Israel, 2010; Schmidt, Peigneux, & Cajochen, 2012; Wolkove, Elkholy, Baltzan, & Palayew, 2007). It is estimated that approximately 40-50% of older adults (over 65 years old) have insomnia symptoms and they report more sleep complaints than any other age group (Foley et al., 1995). However, these sleep problems are frequently undiagnosed and untreated (Ancoli-Israel, Poceta, Stepnowsky, Martin, & Gehrman, 1997). A number of studies which have compared sleep architecture, i.e. the temporal structure and pattern of sleep, between young and older adults have shown that increasing age causes a decline in overall sleep duration and sleep efficiency (total time sleeping in relation to total time in bed) (Stanley, 2005). Increased intra-night wakefulness and difficulty initiating and returning to sleep are also common features of advancing age (Hoch et al., 1997; Roepke & Ancoli-Israel, 2010). In addition, older adults are more likely to wake during non-REM periods of sleep, rather than transitioning from non-REM to REM sleep, compared to younger adults (Klerman et al., 2013). Sleep electroencephalography (EEG) microstructure changes with age have also been identified. These typically concern particular patterns of electrical activity known as spindles and K-complexes, which are

discharged from thalamo-cortical neuronal circuits and are a marker of sleep. Feinberg (1967) reported a reduction in number of spindles and spindle frequency power in older adults, similarly Guazzelli and colleagues (1986) identified a reduction in spindle quantity, amplitude and duration in older participants, while Principle and Smith (1982) identified an increase in spindle frequency with age. Others have noted significant decreases in both spindles and k-complexes with age (Crowley, Trinder, Kim, Carrington, & Colrain, 2002; Wauquier, 1993) and reported the difference between wake and sleep EEG profiles becomes smaller with increasing age (Munch et al., 2005). However, the most commonly reported change in sleep architecture seen with age is the dramatic decrease in slow wave activity (SWA) (synchronised oscillatory activity at 0.5 to 4.0Hz) (Backhaus et al., 2007; Cajochen, Munch, Knoblauch, Blatter, & Wirz-Justice, 2006; Edwards et al., 2010; Mander et al., 2013), which is most evident in the deepest stage of non-REM sleep (i.e. slow wave sleep (SWS)). In addition to a general decrease in SWA in older adults, there is also a loss of the frontal predominance of this activity which is observed in younger adults and is commonly associated with 'restorative function' (Cajochen et al., 2006). However, these studies are largely observational and have not linked these changes to worsening sleep complaints, or waking cognitive function, in their older cohorts.

Despite these commonly identified sleep changes with age, others have suggested that sleep complaints in older adults are closely associated with co-morbid factors, such as illness, pain or depression, rather than age per-se (Ancoli-Israel, 2009; Bliwise, King, Harris, & Haskell, 1992; Duffy, 2005; Foley et al., 2007; Zimmerman, Bigal, Katz, Derby, & Lipton, 2013) and that healthy older adults are capable of maintaining satisfactory sleep quality (Driscoll et al., 2008). A recent study by Chien and Chen (2015) reported that subjective measures of poor sleep were associated with disability, independent of other co-morbid factors, in a large sample of older adults. A large study conducted by Foley, Ancoli-Israel,

Britz, and Walsh (2004) found that depression, heart disease, pain and memory problems were associated with more prevalent symptoms of insomnia, while obesity, arthritis, diabetes, lung disease, osteoporosis and stroke were associated with sleep related problems. They argue that these results support those reported by epidemiological studies, which found that sleep disruption in older adults was a secondary factor to physical and mental health issues, as these factors better predicted sleep disruption, compared to chronological age (Ancoli-Israel et al., 1991; Foley, Monjan, Simonsick, Wallace, & Blazer, 1999; Foley et al., 1995; Maggi et al., 1998; Vitiello, 1997). A study by Ohayon, Carskadon, Guilleminault, and Vitiello (2004) reported that when controlling for factors commonly associated with older age (e.g. disease, drug use, mental health problems) the majority of the age-related decline in sleep quality occurred by the age of 60. Between the ages of 60 and 102, changes in sleep quality were relatively small, suggesting that the oldest age is not necessarily associated with worsening sleep.

In summary, although the disruption of sleep quality and quantity in older age is not doubted, the exact mechanisms of such decline remain unclear. In addition to the theory that such changes are largely driven by illness it is also possible that biological changes which result in disrupted circadian rhythms or other external factors (e.g. reduced physical activity) may contribute to changes in sleep in older age. The next section will present the research that has investigated such possibilities.

1.2.2.3 Potential influences on sleep quality with age

Although older age does not necessarily guarantee sleep disruption, it can still be associated with shorter sleep duration, independent of physical or mental illness, potentially due to changes in circadian rhythms, sleep hygiene and physical activity (Roepke & Ancoli-Israel, 2010; Van Someren, 2000; Vitiello, 2006, 2009).

1.2.2.3.1 Circadian rhythms

Circadian rhythms, first identified in 18th century studies of plants, are controlled by biological oscillators ('circadian clocks') which are principally entrained by light and synchronised to the 24-hour solar day (See Czeisler and Gooley (2007) for a review). Synchronisation with the rising and setting of the sun is presumably to ensure physiological and behavioural rhythms are appropriately timed with daily environmental changes. In mammals, the generation and synchronisation of circadian rhythms is controlled by the central neural pacemaker, which exists within the suprachiasmatic nucleus (SCN) of the hypothalamus (Rosenwasser & Turek, 2015). Circadian rhythms are assessed by phase and amplitude, which are thought to be determined by period (PER) and clock (CLOCK) genes, among others (see Goel (2011) and Sehgal and Mignot (2011) for reviews). Phase is defined with respect to an established reference point in the rhythm, such as the body temperature nadir, while amplitude refers to the half-distance between the minimum and maximum points of the rhythm.

The regulation of the sleep-wake cycle depends on an interaction between circadian processes and a homeostatic sleep drive process, which increases pressure for sleep with each hour awake and is only dissipated once sleep has occurred (Achermann, 2004; Schmidt, Cajochen, & Chellappa, 2014). The homeostatic drive for sleep accumulates throughout the day, and is opposed by the circadian pacemaker which provides an increasingly strong drive for wakefulness. Before bedtime, melatonin is released into the bloodstream, which binds to receptors in the SCN and suppresses SCN neuronal firing, thus quietening the signal for wakefulness (Dijk, Shanahan, Duffy, Ronda, & Czeisler, 1997). At this point, when the homeostatic and circadian drives for sleep coincide, the drive for sleep is the strongest. Given that the interaction between these processes drives the sleep-wake cycle and also the role of circadian rhythms in facilitating sleep and daily variations in

sleepiness, any changes to circadian rhythms with age could potentially affect sleep quantity and quality (Nakamura et al., 2015).

The majority of studies investigating how circadian processes change with age report an advance in circadian phase and a decline in the amplitude of circadian markers (such as body temperature, melatonin and cortisol) (Czeisler et al., 1992; Edwards et al., 2010; van Coevorden et al., 1991; Van Someren, 2000; Van Someren, Raymann, Scherder, Daanen, & Swaab, 2002; Wauquier, 1993; Weitzman, Moline, Czeisler, & Zimmerman, 1982; Wolkove et al., 2007; Youngstedt, Kripke, Elliott, & Klauber, 2001). Numerous studies have provided evidence for an association between circadian amplitude and sleep quality (see Myers and Badia (1995) for a review), thus suggesting that these changes in circadian markers may impact on the sleep quality of older adults. Others have reported a reduction in the amplitude of the sleep-wake cycle itself with age, in animals (see Ingram, London, and Reynolds (1982) and humans (Huang et al., 2002), again suggesting that disruption to the circadian system with age may affect sleep. However, although circadian phase advances have been thought to explain why older adults typically have earlier habitual bed and rise times, these differences in endogenous bed/rise times are not always associated with advances in circadian phase (Duffy & Czeisler, 2002; Yoon et al., 2003). This suggests that circadian changes are not solely responsible for changes in sleep patterns in older age.

Furthermore, Cajochen and colleagues (2006) reported that although melatonin secretion was reduced in older compared to younger adults, (as others have typically found, see Pandi-Perumal, Zisapel, Srinivasan, and Cardinali (2005) for a review), there were no differences between young and older age groups in the phase of the melatonin rhythm or the timing of the sleep-wake cycle. However, they did report that older, compared to younger, adults, had a reduced circadian drive to promote sleep/wake at the 'correct' times of day. For example, older adults exhibited more sleep during periods when the circadian

process was promoting wakefulness and there was less circadian modulation of REM sleep, compared to younger adults. A number of other studies have also reported a disruption in circadian rhythm signal promotion (Buysse, Monk, Carrier, & Begley, 2005; Dijk, Duffy, Riel, Shanahan, & Czeisler, 1999; Haimov & Lavie, 1997; Richardson, Carskadon, Orav, & Dement, 1982; Silva et al., 2010). Further evidence is provided for the reduced influence of circadian rhythms in older adults by the finding that performance during wakefulness follows a linear decline throughout the day in older adults, compared to a circadian trend in younger adults (Dijk, Duffy, & Czeisler, 1992; Dijk et al., 1999).

In summary, evidence strongly supports the argument that older age is associated with disruption to circadian processes, although a review by Monk (2005) suggests that not all of the common assumptions regarding age and circadian rhythms are particularly strongly supported. Nonetheless, the evidence for circadian phase shifting in older age, at least, is firmly established. However, despite the acknowledgement of a number of changes to circadian rhythms with age, how these changes manifest themselves in terms of changes to nocturnal sleep quality remains to be firmly established. Furthermore, as studies have reported changes in sleep parameters independently from circadian phase shifts, changes to circadian rhythms with age are unlikely to solely drive disruption to sleep quality and are instead one of many factors which may result in impaired sleep in older age.

1.2.2.3.2 Sleep hygiene and physical activity

Sleep hygiene is one such additional factor which may interact with circadian changes to disrupt sleep in older age. Sleep hygiene refers to behavioural and environmental practices which facilitate quality nocturnal sleep and ensure daytime alertness (Irish, Kline, Gunn, Buysse, & Hall, 2015) and is commonly assessed with the Sleep Hygiene Index (Mastin, Bryson, & Corwyn, 2006). Examples of practicing good sleep hygiene include: maintaining a

regular sleep-wake cycle, engaging in regular exercise, and refraining from certain behaviours which disrupt sleep, such as daytime napping and consuming stimulants before bed. The positive association between good sleep hygiene and good sleep quality has been well documented (Brick, Seely, & Palermo, 2010; Brown, Buboltz Jr, & Soper, 2002; Chou, Chang, & Chung, 2015; LeBourgeois, Giannotti, Cortesi, Wolfson, & Harsh, 2005; Lee et al., 2015a; Lee, Paek, & Han, 2015b) and a number of studies have reported that behavioural interventions, predominantly aiming to improve sleep hygiene, improved insomnia symptoms in both younger and older adults (McCrae, McGovern, Lukefahr, & Stripling, 2007; Reid et al., 2010). However, improvement is often only assessed using subjective measures of sleep and others have suggested that groups of better/poorer older sleepers (60-96 years old) are not differentiated by sleep hygiene practices (McCrae et al., 2006).

Specifically, physical activity/exercise has been linked to sleep quality (see Chennaoui, Arnal, Sauvet, and Léger (2015), Driver and Taylor (2000) and Kredlow, Capozzoli, Hearon, Calkins, and Otto (2015) for reviews). Typically, greater levels of exercise are associated with shorter REM and stage one periods of sleep, greater SWS, longer total sleep time (TST) and shorter sleep onset latencies (de Aquino-Lemos et al., 2016; Driver & Taylor, 2000). The influence of exercise on such sleep variables is mediated by individual factors, such as age, gender, and fitness level, as revealed by meta-analyses (Kredlow et al., 2015; Kubitz, Landers, Petruzzello, & Han, 1996). However, studies specifically investigating the association between exercise and older age also largely report the general association between levels of exercise and sleep quality (Morgan, 2003; Tsunoda et al., 2015). Edinger and colleagues (1993) reported that physically fit older men had superior sleep quality to physically unfit men within the same age group, as assessed by polysomnography (PSG). This included shorter sleep latencies, less wake after sleep onset and greater SWS for the physically fit, compared to the physically unfit, older men. More recent studies have also

provided evidence that moderate-intensity exercise programs can improve both objective and subjective measures of sleep quality in older adults (Benloucif et al., 2004; King, Oman, Brassington, Bliwise, & Haskell, 1997; King et al., 2008; Naylor et al., 2000; Singh, Clements, & Fiatarone, 1997) suggesting that reduced levels of physical activity in older age may be partly responsible for reductions in sleep quality. Indeed, Morgan (2003) investigated the potential role of physical and social factors on reported sleep problems in a large, nationally representative UK sample (n=1042) of older adults, who were initially interviewed in 1985 and then again in 1989 and 1993. By applying logistic regression models they identified that risk factors for insomnia were: lower physical health, depressed mood and lower physical activity levels. Age alone was unrelated to any insomnia variables; again suggesting that changes to 'secondary' factors, such as exercise and physical health, may be responsible for any disruption to sleep quality in older adults.

1.2.2.4 Nocturnal sleep, age and cognitive performance

Regardless of whether sleep disruption with age is an independent consequence of the ageing process, is co-morbid with disease (or other factors discussed above), or occurs via interaction between multiple such factors, research has suggested that changes in sleep with age have a detrimental effect on cognitive ability (see Altena, Ramautar, Van Der Werf, and Van Someren (2010) for a review). A number of recent studies have reported that performance on a range of cognitive and motor tasks is associated with sleep quality (Blackwell et al., 2006; Bonnet, 2000; Bonnet & Arand, 2003; Nebes, Buysse, Halligan, Houck, & Monk, 2009; Oosterman, van Someren, Vogels, Van Harten, & Scherder, 2009; Pace-Schott & Spencer, 2011). For example, Oosterman and colleagues (2009) found moderate inverse correlations between sleep fragmentation and mental speed, memory and executive function, while others have reported older adults with greater sleep quality

perform better on a number of tasks, including attention, executive functioning, and working memory (Blackwell et al., 2006; Nebes et al., 2009; Pace-Schott & Spencer, 2011). Others have suggested that the increased prevalence of sleep problems in older adults may contribute to age-related decrements of processing speed. Rayman and Van Someren (2007) used a psychomotor vigilance task and found that performance was worst in the elderly participants with poor sleep. Similarly, Crenshaw and Edinger (1999) found that reaction times, measured during wake, correlated with the power of EEG slow wave activity during sleep, for elderly poor sleepers. A study by Jelicic and colleagues (2002) reported that after controlling for a number of factors (gender, disease, baseline cognitive performance) subjective sleep quality was associated with changes in performance on the Mini Mental State Examination (MMSE) over a period of three years. Furthermore, they reported that waking up too early was the complaint that was most strongly associated with poorer MMSE performance, suggesting sleep duration was compromised. A large study conducted by Blackwell and others (2006) reported that reduced sleep continuity caused by frequent awakenings, rather than total sleep time, has the greatest association with cognitive performance. This is a finding that has been reported by others (Bastien et al., 2003; Bonnet, 2000; Bonnet & Arand, 2003; Foley et al., 2004; Nebes et al., 2009; Schmutte et al., 2007) and suggests that amount of uninterrupted sleep is more important for optimal cognitive performance than the total number of minutes asleep. A longitudinal study, which assessed self-reported TST in a large number of middle-aged to older adults at baseline and at a follow up session, on average 5.4 years later, found that both increases and decreases in TST were associated with reduced cognitive performance across a wide range of domains excluding memory (TST increases associated with: impaired reasoning, vocabulary, phonemic and semantic fluency, MMSE, TST decreases associated with: impaired reasoning, vocabulary and MMSE) (Ferrie et al., 2011).

Studies that have focused specifically on SWS and cognition have identified that disruption of (i.e. waking up during) SWS in younger adults is found to commonly result in 'sleep inertia'; performance impairments that occur immediately after waking (Stanley, 2005). In addition, experimental induction of slow oscillation-like field potentials by transcranial current stimulation during early non-REM sleep facilitates SWS and improves memory performance (Marshall, Helgadottir, Molle, & Born, 2006). Similarly, Backhaus and others (2007) found that a decline in SWS in middle-aged adults occurred in parallel with a decline in declarative memory. These findings suggest that the significant disruption of SWS commonly seen in older adults could feasibly affect cognitive ability. Evidence from Anderson and Horne (2003) seems to support this theory. They identified that performance on executive function and verbal fluency tasks was associated with frontal delta (0.5-1Hz) EEG activity, i.e. SWA, during the first non-REM period in older adults. Finally, Mander and colleagues (2013) reported that SWA positively correlated with memory performance for both young and older adults and memory deficits were proportional to declines in SWS for the older adults. In addition, they also detected a decline in medial PFC (mPFC) volume with age. The declines in SWS and mPFC volume were not independently associated with age; instead, they found that the decline in SWS with age was mediated by the decline in mPFC volume.

However, a recent review by Scullin and Bliwise (2015) reported that there is not always a clear relationship between sleep and cognition in older adults. Across the range of epidemiological, experimental, clinical, and neuropsychological fields reviewed, it was apparent that variability in sleep quality in older adults was not often associated with cognitive performance. The authors also highlight that the effect of sleep (typically SWS) on memory consolidation is reduced in older adults and that sleep deprivation has a smaller effect on the cognitive performance of older, compared to younger adults, which is a

common finding (Adam et al., 2006; Blatter et al., 2006; Duffy et al., 2009; Landolt et al., 2012; Philip et al., 1999; Philip et al., 2004). They purport three potential reasons why sleep may not be related to cognition in older age. The first, functional weakening, suggests that age-related differences in brain structure and function (e.g. neural atrophy, reduced connectivity, disrupted neurotransmitter systems) will persist, regardless of sleep quality, and thus, improving sleep quality is unlikely to reverse changes in cognition. For example, even if SWS was improved in older adults, if connectivity between hippocampus and cortex is impaired and this connectivity is required for SWS memory consolidation processes, increasing SWS alone is not enough to improve memory consolidation because the connectivity required to facilitate it remains impaired. The second reason relates to 'sleep need' and suggests that because the amount of SWS is often linked to the amount of daytime learning that has occurred, SWS may be reduced in older adults because the need for SWS in older age is reduced, due to reduction in the amount of day-time learning that occurs in older age (Cirelli, 2012). The third reason suggests that SWS may be reduced in older age because it is simply a phenomenon that is remnant from early life maturation processes (Feinberg, 2000). Similarly, a recent study by Wilckens, Woo, Erickson, and Wheeler (2014) reported that less wake after sleep onset and longer TST was associated with better attentional processes, however, this effect was independent of age; both young and older adults showed a similar effect. Others have reported that, after controlling for general intelligence, a group of older adults suffering from sleep maintenance problems did not differ in working memory performance compared to a group of age-matched controls (Lovato, Lack, Wright, Cant, & Humphreys, 2013). Furthermore, although reduced subjective sleep quality was associated with the expansion rate of ventricles over a two year period, the effect on cognitive performance was much less clear (Lo, Loh, Zheng, Sim, & Chee, 2014).

In summary, it appears that the relationship between sleep, age and cognition remains unclear, as also summarised by Schmidt et al. (2012) who conclude that although older adults typically exhibit attenuated circadian rhythms, slower RT performance and neuronal degeneration to the SCN (responsible for circadian processes), the interaction between these factors remains unclear. A number of studies have highlighted the influence of sleep quality on cognitive performance in older age. However, more recent evidence has suggested that sleep changes with age may have a smaller impact on cognitive performance than was previously suggested. Despite this uncertainty, it remains the case that research into cognitive ageing often overlooks the findings from the field of sleep, which clearly show that sleep quality declines with age. Only with better integration across these fields of research may we develop a clearer, more coherent picture of how sleep, age and changes to the brain may interact to have an impact on cognition in older age.

CHAPTER 2. METHODS

2.1 GENERAL METHODS

This section will provide a description of the participants and methodological procedure which were common to all experimental chapters in this thesis.

2.1.1 Participants

Twenty younger (M= 27, ± 3 years, 10 male) and twenty older (M=74, ± 4 years, 9 male) participants took part. Older participants were screened for cognitive impairment with the Advanced Mini-Mental State Test (3MS) (Teng & Chui, 1987); the group's average score was 97.65 (± 2.6, range: 88-100). No participants scored below the cut-off (79/100) for normal cognitive ability. All participants (excluding two younger participants for whom English was not their native language) also took part in the National Adult Reading Test (NART) as an estimator of IQ (Nelson & Willison, 1991). Younger participants had an average 'full IQ' score of 114 (± 7.56), compared to a score of 119 (± 7.07) for the older participants. IQ scores were not significantly different for the two groups, as assessed by a one-way ANOVA ($F(1,37)=3.811, p=0.059$).

2.1.2 Procedure

Participants gave written informed consent and the study was approved by the Research Ethics Board of the University of Birmingham. On the first visit to the lab, participants were screened for MR compliance and then completed the Insomnia Severity Index (ISI) (Bastien, Vallieres, & Morin, 2001; Morin, Belleville, Belanger, & Ivers, 2011) and Fatigue Severity Scale (FSS) (Kaida et al., 2006). This allowed us to screen for any participants who could be

defined as having a clinical sleep disorder. No participants scored above the cut off scores for either scale (ISI = 15/32, FSS = 36/73). Participants who met the criteria for the study were then given a sleep diary to complete and an actiwatch to wear for the next 14 days. All participants were given detailed instructions on how to use the actiwatch and how to complete the sleep-diary and were given the opportunity to ask questions about the procedure. Participants were also encouraged to contact the researchers with any questions they may have had over the 14 day period. See Chapter 6 for detailed actiwatch and actigraphy methods.

Following this 14 day, sleep quality assessment phase, participants returned to the lab for the MRI scan and cognitive testing session. All participants first completed the MRI session and, approximately 30 minutes later, completed the cognitive session. This consisted of the NART and The Pittsburgh Sleep Quality Index (PSQI)(Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) and Epworth Sleepiness Scale (ESS)(Johns, 1991) as measures of subjective sleep quality. Older participants also completed the 3MS to screen for severe cognitive deficits.

Participants then underwent the MRI session. During the resting-state scan participants were asked to keep their eyes open. This was done for two reasons, 1) to avoid participants falling asleep (see below) and 2) to ensure coherence across participants and age groups as studies have shown that different resting conditions (i.e. eyes open/closed) can result in small, but significant, differences in RSN FC (Patriat et al., 2013). Participants were also asked to try and think of nothing in particular, to avoid engaging in any specific cognitive activity which could alter RSN FC. Immediately after the resting-state scan, participants subjectively rated their sleepiness using the Karolinska Sleepiness Scale (KSS) (Kaida et al., 2006; Shahid, Wilkinson, Marcu, & Shapiro, 2012), and were asked to report if they were aware of falling asleep during the scan. Approximately twenty minutes after the MRI

session, participants completed tests of memory and reaction time (Simple Reaction Time: SRT), from the Cambridge Neuropsychological Test Automated Battery (CANTAB, Cambridge Cognition). All tasks were computed in a quiet testing room, on an 11" Samsung tablet (XE700T1C; Intel 1.7GHz i5 processor, 4GB RAM, 64-bit Windows 7). Upon completion, participants were thanked and debriefed.

2.2 COGNITIVE TASKS

This section will provide a description of the cognitive tasks used to assess memory performance and reaction time (RT) and the outcome measures which were used to investigate the associations between FC and cognition. These tasks were chosen to investigate ageing as memory and RT deficits are key characteristics of older age. Furthermore, as this was a preliminary investigation into how FC may be associated with performance, age and sleep, we chose relatively simple tasks, which have feasible connectivity substrates (e.g. the association between hippocampal-thalamic connectivity in memory is well established) which allowed us to be selective with FC analysis.

Paired Associates Learning

This task is a measure of visual spatial memory. During the task, boxes are displayed on the screen in a radial configuration and opened, one at a time, in a randomised order. One or more of the boxes will contain a pattern. After all boxes have been opened, the patterns shown in the boxes are then displayed in the middle of the screen, one at a time, and the subject must touch the box where the pattern was originally located. For each stage, participants are allowed up to ten attempts in total. If the participant makes a mistake, the patterns are re-presented to remind them of their locations. When the participant gets all of the locations correct, they proceed to the next stage. If a stage is not completed within the

ten attempts, the task is terminated. The task consisted of eight stages. Stages one to seven consisted of six boxes, with either 1 (Stages 1 & 2), 2 (stages 3 & 4), 3 (stages 5 & 6) or 6 (stage 7) patterns to remember. Stage eight consisted of eight boxes and eight patterns to remember. Two initial practice trials, which featured one pattern to remember, familiarised participants with the task.

The outcome measure used to assess memory performance was the number of errors made at stage 7 of the task (where there is a pattern in each of the 6 boxes displayed), thus a lower score indicates better performance. This measure was selected as it is sensitive to memory impairment (Sahakian & Owen, 1992) and capable of characterising patients with Alzheimer's disease and healthy older controls with an accuracy of 98% (Swainson et al., 2001).

Simple Reaction Time

SRT delivers a known stimulus to a known location to elicit a known response. In this task, a stimulus (a white square) was presented on a black background and participants were instructed to respond by pressing a key on a two-button button box whenever they saw the stimulus appear on the screen. The only uncertainty is when the stimulus will appear, as there is a variable interval between the previous trial response and the onset of the stimulus for the next trial. An initial practice block of 24 trials familiarised participants with the task. Following this, participants completed two assessment blocks of 50 trials each.

As it well established that older age is associated with slowing of reaction times (Der & Deary, 2006; Dykiert, Der, Starr, & Deary, 2012; Woods, Wyma, Yund, Herron, & Reed, 2015), the outcome measure used to assess performance on this task was mean reaction time (RT).

2.3 MAGNETIC RESONANCE IMAGING METHODS

This section will provide an overview of the principles of magnetic resonance imaging (MRI) which allows for the non-invasive, in-vivo assessment of the effects of ageing on the human brain. Furthermore, it discusses the neurophysiological underpinnings of the signal obtained by functional MRI (fMRI), known as the blood oxygen level dependent (BOLD), and introduces the caveat that this measure may be confounded by changes in neurovascular coupling with age which is discussed in more detail in Chapter 3.

Furthermore, I will introduce resting-state networks and functional connectivity analyses, which form the basis of this thesis. I also provide an explanation of the pre-processing techniques applied to the data and the methods adopted to define resting-state nodes.

2.3.1 MR physics

The fundamental principles of nuclear magnetic resonance (NMR) imaging are underpinned by the fact that atomic nuclei have two properties; spin and magnetic moment, which can only take discrete values. Nuclear spin can be visualised as a small sphere of distributed electromagnetic charge which rotates at a high speed around its axis. This rotation produces an electric current which results in a small magnetic field, known as the magnetic moment. The magnetic moment allows the atomic nucleus to interact with a magnetic field, almost like a small bar magnet, where it will precess about the direction of the magnetic field. According to quantum mechanics, the spin of an atomic nucleus can only take one of two orientations, therefore, in NMR, because of the externally applied magnetic field, these two orientations correspond to low or high energy states. If a radio frequency (RF) electromagnetic field is applied whose frequency matches the precession frequency of

the atomic nucleus, energy will be absorbed into the nucleus, changing it from the low to the high energy state. This concept is 'magnetic resonance'. The radio frequency required to excite a nucleus to the higher energy spin state is referred to as the Resonant Frequency and is determined by the Larmor Equation. The Larmor Equation posits that the resonance frequency of a nucleus is proportional to the magnetic field it experiences. As atomic nuclei with different magnetic properties resonate at different frequencies, NMR sequences can be developed to image the brain in a number of ways.

As the human body is composed of approximately 70% water, MRI is able to take advantage of the high abundance of hydrogen atoms within water molecules. The nuclei of hydrogen atoms are protons, which exhibit the NMR properties described above. Therefore, by targeting hydrogen protons, it is possible to create images of both brain structure and function using MRI. In order to do this, three main components are required: 1) a large static magnetic field (typically generated by superconducting electromagnets), 2) a radio-frequency coil and 3) a gradient coil. In the absence of a magnetic field, the spin axes of all hydrogen protons are orientated in random directions, meaning that the net magnetisation of the tissue is zero. In response to the strong static magnetic field (B_0), a proportion of the magnetic moments align themselves either in the parallel state (i.e. parallel to the magnetic field) or anti-parallel state. A weaker magnetic field (B_1) is then induced, using a RF pulse delivered by the RF coil, the frequency of which is determined by the Resonant Frequency. Following this, low-energy (i.e. parallel state) hydrogen protons take on a high energy state and flip over, the angle of which is determined by the flip angle parameter. This results in a change to the net magnetisation from the longitudinal into the transverse plane, referred to as excitation. After this RF excitation, the hydrogen protons emit this energy at the same frequency until they return to their preferred low energy state, to re-establish thermal equilibrium, and re-align to the static magnetic field. It is this emitted energy which is

acquired through receiver coils and provides the raw data for an MR image. This measured RF signal decays over time (spin relaxation) with the amount of signal loss depending on the time between excitation and data acquisition; referred to as echo time (TE). The timing of MR signal loss is driven by both T_1 and T_2 relaxation times. T_1 recovery refers to the mean time it takes for the return of net magnetisation to the parallel, low energy state along the longitudinal direction. Anatomical (T_1 weighted images) reflect the relative T_1 values of tissue, therefore, as these T1/T2 values vary with tissue type as they exhibit different relaxation times, we are able to create MR contrasts between grey and white matter. In contrast, BOLD (see below) functional images reflect the T_2* signal decay. T_2* decay is the time constant that describes the decay of the net transverse magnetisation, due to a combination of a reduction in phase coherence between spins (T_2) and local magnetic field inhomogeneities. By applying a third kind of magnetic field, a 3-dimensional spatial gradient (induced by the gradient coil), it is possible to obtain MR signal at specific locations, i.e. slices, throughout the brain. This is possible as the spatial gradient ensures that the process of excitation occurs selectively for protons within a selected slice, but not for those outside of the slice. The typical echo planar imaging (EPI) method that is common in fMRI acquisitions uses rapid gradient switching which allows imaging of the entire brain, in a few seconds, following a single excitation. Each slice is sampled in units of voxels, which are 3 dimensional pixels, the size of which are determined by the sampling matrix (the total number of data points acquired in the phase and frequency directions), the field of view (the spatial encoding area of the image) and the slice thickness. Generally speaking, the smaller the voxel the greater the spatial resolution, although voxels that are too small will lack enough signal to generate a reliable image.

2.3.2 Imaging BOLD

The use of T_2* weighted contrast imaging facilitates the investigation of brain function via functional MRI (fMRI). This was made possible by the discovery that oxygenated and deoxygenated haemoglobin have different magnetic properties meaning that changes in blood oxygenation could be visualised using T_2* weighted imaging. Oxygenated haemoglobin is diamagnetic (i.e. has no magnetic moment) while deoxygenated haemoglobin is paramagnetic (i.e. does have a magnetic moment) meaning that fully oxygenated blood has a magnetic susceptibility that is approximately 20% less than that of fully deoxygenated blood (Huettel, Song, & McCarthy, 2009). This means that spatial and temporal differences in oxygenated blood (assumed to be related to brain function, see below) result in inhomogeneities in the magnetic field. Greater inhomogeneity results in reduced MR signal intensity, because protons at different locations will experience different magnetic field strengths which will cause them to precess at different frequencies, leading to a loss of phase coherence, and thus MR signal, between protons. BOLD fMRI is able to image differences in blood oxygenation, in space and time, because the decay of transverse magnetisation (as assessed by T_2* decay) depends on the inhomogeneities induced by the de/oxygenation of haemoglobin. Therefore, BOLD contrast refers to the signal intensity in T_2* weighted images as a function of blood oxygenation, and reveals stronger MR signal when blood is oxygenated, compared to deoxygenated.

2.3.2.1 Neurophysiological underpinning of the BOLD signal

As detailed above, the BOLD signal is an indirect measure of brain function, related to the different magnetic properties of oxygenated and deoxygenated haemoglobin. Although we know neuronal activity is an energy demanding process, which results in changes to oxygenated haemoglobin levels, the BOLD response cannot be thought of as a simple one-to-

one mapping of neuronal firing. The exact relationship, called the neurovascular coupling, between neuronal activity and the BOLD signal is complicated, particularly because the dynamics of the two processes are so different; the neuronal response to a stimulus occurs on the scale of milliseconds, while the first observable BOLD response occurs seconds after stimulus presentation. Although the contribution of CBF and CBV to the BOLD response have long been known (for reviews see Buxton and colleagues (2004) and Kim and Ogawa (2012), the association between the BOLD signal and specific neuronal activity is yet to be firmly established. Oxygen is used by neurons to fuel a variety of functions, such as neurotransmitter release and clearance from synapses, pre- and post-synaptic action potentials and changes to membrane potentials. Therefore, in order to reliably interpret the BOLD response of brain regions, it is important to investigate whether particular neuronal activity is more associated with the BOLD signal than others. Seminal studies conducted by Logothetis and colleagues (2001; 2012) have investigated the association between neuronal activity and BOLD signal increases. Their work has identified that the neurophysiological basis of the BOLD signal primarily reflects LFPs, i.e. the summed post-synaptic electrical potential within the extracellular space around neurons, rather than neuronal spiking (action potential) activity. Although the neuronal basis of the positive BOLD response is largely accepted within the neuroimaging community, much work is currently being conducted to establish the neurophysiological underpinning of the negative BOLD response (Mayhew, Ostwald, Porcaro, & Bagshaw, 2013b; Mullinger, Mayhew, Bagshaw, Bowtell, & Francis, 2014).

Clearly, the BOLD signal is a complex measure which is determined by several vascular factors (i.e. CBF, CBV, $CMRO_2$) and likely reflects the collective neuronal activity and consequent metabolic demand of neuronal populations. It is important to keep this in mind when comparing the BOLD response across different groups as differences in neurovascular

coupling may exist between them; which may result in differences in the BOLD response which are not related to neuronal function. This is discussed in more detail, with specific relation to ageing, in Chapter 3.

2.3.2.2 The BOLD haemodynamic response function

Initial fMRI studies used simple visual and motor tasks to assess the BOLD response to a simple stimulus, in order to validate the utility of the BOLD signal as a marker of neuronal activity against existing electrophysiological evidence. Studies revealed a replicable BOLD response to a very brief, impulse stimulus, now referred to as the Haemodynamic Response Function (HRF). The HRF is a relatively slow response, lagging behind the onset of neuronal activity by approximately 1-2 seconds and lasting approximately 30 seconds in total. The HRF is typically described as comprising the following components: 1) an 'initial dip' in BOLD signal from baseline prior to the onset of a large BOLD signal increase 2) a large increase in BOLD signal from baseline ~2 seconds after the onset of neuronal activity 3) a peak in the BOLD signal at ~6 seconds (for a stimulus presented with a short (<1s) duration) 4) a decrease in the BOLD signal amplitude below the pre-stimulus, or "baseline" level (known as the post-stimulus undershoot), remaining below baseline for an extended period of time (up to ~30 seconds post-stimulus onset) 5) BOLD signal returns to the baseline level. Although the specific mechanism responsible for the post-stimulus undershoot is a subject of much debate (Mullinger, Mayhew, Bagshaw, Bowtell, & Francis, 2013; van Zijl, Hua, & Lu, 2012), it is well established that interactions between vascular processes such as; cerebral blood flow (CBF), cerebral blood volume (CBV) and the metabolic rate of oxygen ($CMRO_2$) contribute to the HRF (Buxton, Uludag, Dubowitz, & Liu, 2004; Kim & Ogawa, 2012). Typically increases in BOLD signal are caused by increases in CBF and CBV with a proportionately smaller increase in $CMRO_2$.

The work presented in this thesis does not model and explore changes in the HRF in response to stimuli, as task fMRI studies do. Instead it is concerned with the spontaneous fluctuations in the BOLD signal at rest, which are discussed in more detail below.

2.3.3 MRI procedure

For the work in this thesis, a Philips Achieva 3T MR scanner with a 32-channel head coil was used to acquire MRI data. A fifteen minute resting-state scan was acquired (T2*-weighted BOLD fMRI data with whole brain coverage: 3x3x4mm voxels, TR=2000ms, TE = 35 ms, SENSE factor = 2, flip angle = 80°, volumes= 450). In addition, a high-resolution (1 mm isotropic) T_1-weighted anatomical image was also obtained. During the resting-state scan, participant's cardiac and respiratory cycles were measured using pneumatic bellows and a pulse oximeter. Foam padding was positioned around the head to reduce motion artifacts.

2.3.4 MRI data analysis

For clarity, an overview of the successive stages of MRI data analysis are presented in Section 2.2.4.1. These methods are used to calculate FC between the paired nodes of all of the brain's major RSNs and are explained in more detail in the following sections. All brain images presented in this thesis are in radiological format.

2.3.4.1 Analysis summary

Stage 1: Identify RSNs and create individual node definitions for each participant by transforming RSN nodes from standard to functional space.

Stage 2: Segment each participant's T_1 image into CSF, WM and GM partial volume maps. Transform these into functional space.

Stage 3: Create a 5x5x5 voxel ROI for each RSN node (centred on the peak z-statistic for all nodes except thalamus/hippocampus, see Section 2.3.4.5). For each ROI, exclude any voxels which exist within the participant's CSF/WM maps created in Stage 2.

Stage 4: Initial pre-processing of resting-state BOLD data (slice-time correction, high pass filter, spatial smoothing)

Stage 5: Correction for respiratory and cardiac confounds within resting-state BOLD data

Stage 6: Apply a band pass filter to the resting-state BOLD data

Stage 7: Removal of further confounds (WM, CSF, motion, global signal) from resting-state BOLD data by linear regression

Stage 8: Extract the BOLD time-series for each ROI voxel, using the definitions created in Stage 3, and calculate the average time-series across voxels for each RSN node ROI

Stage 9: Calculate FC by correlating these average ROI time-series, for each participant.

2.3.4.2 Definition of network nodes

The spatial locations of each RSN's individual nodes were defined from six-minute resting-state scans (3x3x4mm voxels, TR=2000ms, TE=35ms, flip angle 80°, SENSE factor = 2) acquired from an independent cohort of fifty five subjects (28 male, age 25±4yrs) which was collected as part of a previous study (Przezdzik, Bagshaw, & Mayhew, 2013). Using FSL 4.1.8 (www.fmrib.ox.ac.uk/fsl) data were motion corrected, spatially smoothed (5mm), temporally concatenated across subjects and decomposed into 20 spatially independent components using MELODIC (Beckmann & Smith, 2004). The dorsal attention network (DAN), default mode network (DMN), saliency network (SN), motor, visual and auditory networks were visually identified from individual components, based on their spatial similarity to previous reports (Damoiseaux et al., 2006). Each component was thresholded

at a Z-statistic > 4, based on previous methodology (Khalsa, Mayhew, Chechlacz, Bagary, & Bagshaw, 2013), to ensure that each of the network nodes were spatially distinct (see Figure 1). The individual nodes consisted of: left and right orbitofrontal cortex (OFC), left and right intraparietal sulcus (IPS) [**DAN**], pre-frontal cortex (PFC), posterior cingulate cortex (PCC), left and right inferior parietal lobule (IPL), left and right medial temporal lobe (MTL) [**DMN**], left and right insula and anterior cingulate cortex (ACC) [**SN**], left and right lateral and primary visual regions [**Visual**], left and right superior temporal gyrus (STG) [**Auditory**], left and right M1 and supplementary motor area (SMA) [**Motor**]. We chose to use this low model ICA in order to 1) provide clean differentiation between networks; if we had adopted higher order ICA, it is likely that nodes will split across components and networks will not be confined to one component. Our method allowed us to define robust network ROIs for comparison across groups. 2) reduce the number of nodes for comparison. Given that this was a preliminary investigation and that our sample size was limited, we did not want to compare an even larger number of regions, which would have resulted in a greater multiple comparison problem. Furthermore, as we were also investigating the association between FC, cognition, sleep and age, we wanted to ensure that we could reliably interpret our results, which would have been more difficult with a large number of network nodes resulting from a higher order ICA.

2.3.4.3 Definition of hippocampus and thalamus

In addition to the network ROIs defined from ICA, we also anatomically defined left and right hippocampal and thalamic nodes (see Figure 1c). This was done by thresholding the hippocampal and thalamic probability maps, provided by the Harvard-Oxford subcortical structural atlas included in FSL, to retain the top 75% of voxels. These thresholded masks were then binarised.

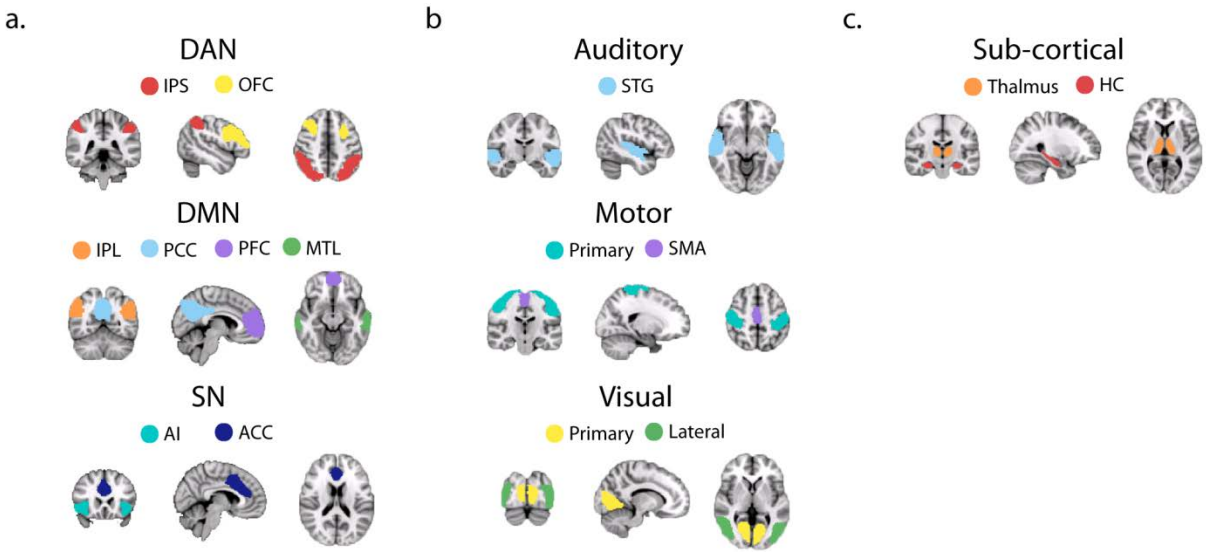

Figure 1a: Illustration of each node of the three main 'cognitive' RSNs: DAN, DMN and SN. DAN comprises: IPS intraparietal sulcus, OFC orbitofrontal cortex, IPL intraparietal lobe. DMN comprises: IPL intraparietal lobe, PFC pre-frontal cortex, MTL medial temporal lobe. SN comprises: AI anterior insula, ACC anterior-cingulate cortex.
Figure 1b: Illustration of each node of the three main sensory RSNs: auditory, motor and visual. Auditory comprises: STG superior-temporal gyrus. Motor comprises: M1 primary motor cortex, SMA supplementary motor area. Visual comprises: primary and lateral visual cortices.
Figure 1c: Illustration of the anatomically defined masks for HC hippocampal complex and thalamus.

2.3.4.4 Segmentation of thalamus

In order to conduct more fine-grained FC analysis of the thalamus, and to compare these more specific results to those from the anatomical thalamic masks, which make no distinction between thalamic sub-regions, we used the Oxford Thalamic Connectivity Atlas, (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases; Behrens et al. (2003)). The probabilistic masks from this atlas, thresholded at a probability of 25% as applied previously (Serra et al., 2014), comprise seven bilateral sub-regions that have been identified to be structurally connected (as assessed by DTI) predominantly to: primary motor cortex (MT), pre-motor cortex (Pre-MT), somatosensory cortex (ST), occipital cortex (OT), frontal cortex (FT), posterior parietal cortex (PT) and temporal cortex (TT) (see Figure 2).

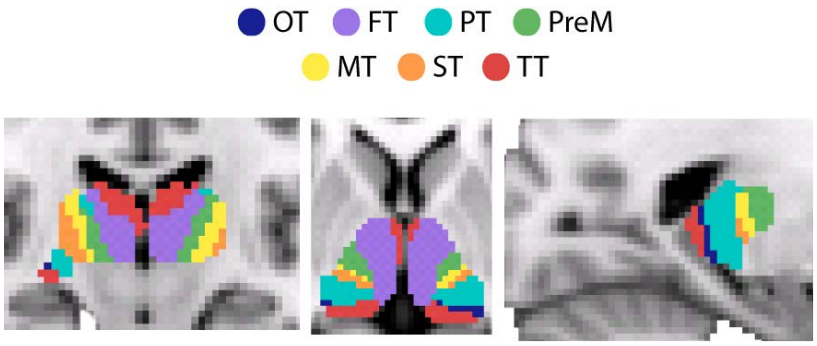

Figure 2: Depiction of each thalamic sub-region from the Oxford Thalamic Connectivity Atlas (Behrens et al., 2003). The descriptions below detail the cortical region that each thalamic-sub-region is thought to be most strongly connected to and the corresponding thalamic nuclei each sub-region is said to contain. OT visual cortex (LGN, inferior pulvinar and some intralaminar nuclei), FT frontal cortex (some of MD, VA, parts of anterior complex), PT posterior parietal cortex (anterior pulvinar), Pre-MT pre-motor cortex (VLa and VA), MT primary motor cortex (VLp), ST somatosensory cortex (LP and VPL), TT temporal cortex (some of MD, parts of anterior complex, medial and inferior pulvinar).

2.3.4.5 Region of interest definition

FSL's Brain Extraction Tool (BET) (Smith, 2002) was first used to remove the skull from each T_1 image. The centre co-ordinates of each brain were entered into the BET command for optimal brain extraction. Each brain extracted image was visually inspected and if the quality of skull removal was poor, centre co-ordinates were adjusted until the best possible brain extraction was achieved.

Following brain extraction, FLIRT (Jenkinson, Bannister, Brady, & Smith, 2002) was then used to transform the node masks into functional space, using the T1 as an intermediate step, for each participant. For each cortical RSN node a 5x5x5 voxel cube ROI, centred on the maximum Z-statistic voxel, was defined (Table 1). This resulted in an ROI of 125 voxels in size, for each RSN node.

For hippocampal and thalamic nodes, the same method could not be applied as the node masks contained binary values rather than z-statistics. In order to create ROIs of the same

size, the subcortical masks were transformed into anatomical space and then voxel values (which could be considered to represent a goodness-of-fit value after registration) were ranked and the 125 voxels with the largest intensity were selected to form the ROI. This ensured that ROIs were centred within the thalamic and hippocampal nodes, and excluded voxels which less accurately represented thalamic/hippocampal nodes, as a consequence of the warping caused by registration into a different space.

Table 1: MNI co-ordinates of the peak voxel for each network node, around which 5x5x5 voxel ROIs were created.

	x	y	z		x	y	z
DAN				**Auditory**			
Left IPS	67	39	60	Left STG	75	54	39
Right IPS	25	37	61	Right STG	17	53	39
Left OFC	71	75	52				
Right OFC	21	83	45	**Motor**			
DMN				Left M1	67	55	67
PCC	45	37	53	Right M1	23	55	67
mPFC	45	89	39	SMA	45	53	65
Left IPL	71	29	55				
Right IPL	19	29	55	**Visual**			
Left MTL	77	58	27	Left lateral	69	26	41
Right MTL	19	64	21	Right lateral	21	29	41
SN				Left primary	49	27	39
ACC	45	76	51	Right primary	39	29	39
Left AI	65	71	37				
Right AI	27	75	37				

2.3.4.5.1 Accounting for ROI grey matter differences

In order to account for differences in the proportion of grey/white matter voxels within each ROI between the two age groups additional analyses were performed. Following brain

extraction, FAST (Zhang, Brady, & Smith, 2001) was used to segment each individual's brain-extracted T_1 image into grey matter, white matter and CSF (Figure 3a). These partial volume maps were then transformed into functional space using FLIRT (Jenkinson et al., 2002; Jenkinson & Smith, 2001), with nearest neighbour interpolation and a threshold of 0.5 to preserve approximately the size of the original partial volume maps (Figure 3b).

These partial volume maps were used to exclude any CSF/WM voxels within the RSN node ROIs to ensure that only GM voxels were included for FC analysis (Figure 4). However, as the grey matter segmentation of the thalamus was not adequate for all participants, we chose instead to exclude only voxels that had been identified as CSF. This allowed us to at least control for potential differences in ventricle size between the two age groups. See Section 2.4.3 for a discussion regarding the impact of differing ROI sizes for the two age groups on FC.

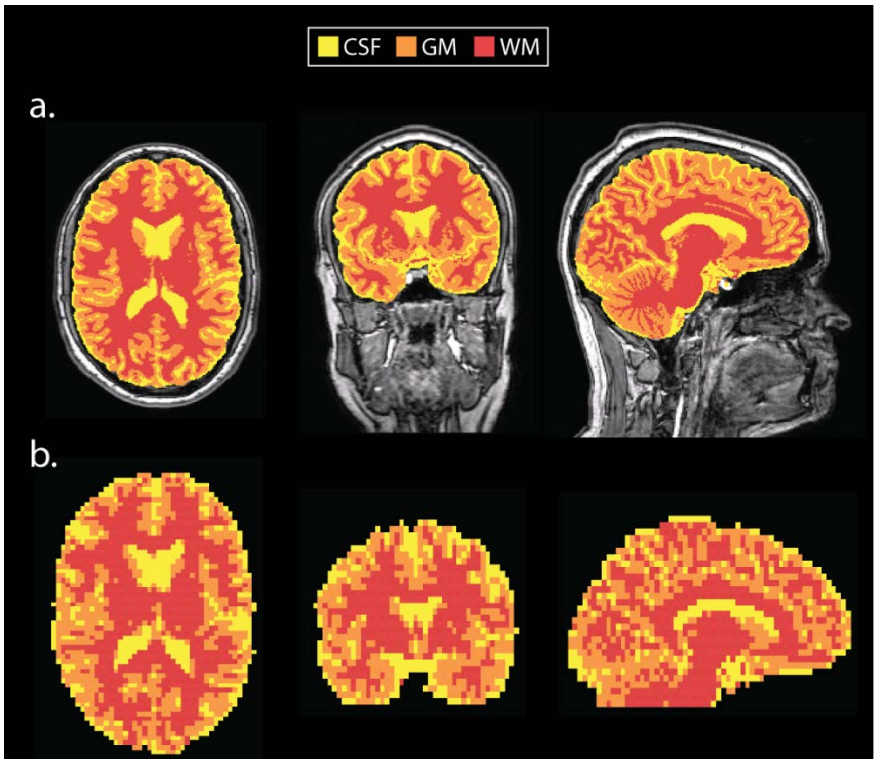

Figure 3: An example of the segmentation results in anatomical (a) and transformed into native functional space (b), shown for a randomly selected younger participant. The three partial volume maps (CSF (yellow), grey matter (orange), white matter (red)) are presented overlaid on the participant's T_1 image and then the corresponding slice is presented in native functional space, for comparison. Participant permission was obtained to produce these images.

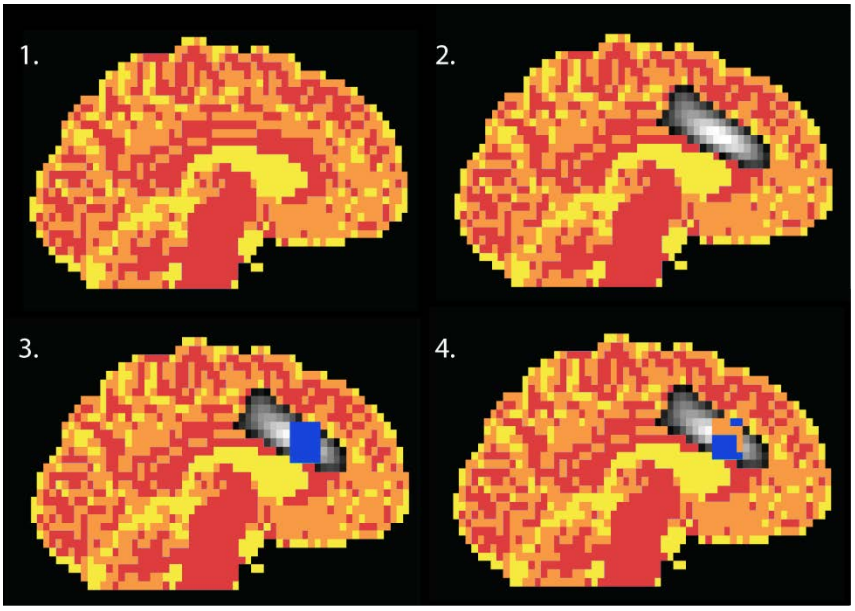

Figure 4: A pictorial representation of the steps for ROI voxel selection. 1) partial volume maps are transformed into functional space 2) RSN mask (ACC is shown as an example in greyscale) is also transformed into functional space 3) A 5x5x5 ROI is created around the peak RSN mask voxel (shown in blue) 4) Exclusion of CSF and WM ROI voxels, i.e. only the voxels which exist in the grey-matter partial volume map are included in the final ROI (remaining GM voxels depicted in orange).

2.3.4.6 Pre-processing

Resting-state BOLD data were pre-processed according to standard methodology prior to FC analysis (Fox et al., 2005). Data were motion and slice-time corrected, spatially smoothed (5mm) and high-pass filtered using FEAT. Following this, data were further filtered by applying a temporal band-pass filter (0.009<Hz<0.08), using custom MATLAB code, in order to investigate the spontaneous, low-frequency fluctuations typically associated with resting-state networks. The effect of respiratory and cardiac confounds (RETROICOR) (Glover, Li, & Ress, 2000) and subsequently variations in breathing depth and heart rate interval (Birn et al., 2006; Chang et al., 2009) were then reduced using custom MATLAB code. Further potential confound signals were removed using multiple linear regression, these included: the six motion parameters of head rotation and translation,

white matter and CSF signals and the global signal, calculated by averaging the BOLD time-series across all brain voxels.

2.3.4.7 Calculating functional connectivity

Following pre-processing, FC strength was calculated for each pair-wise combination of RSN nodes as the correlation coefficient (Pearson's R-value) between the mean ROI BOLD time series of each node pair. Correlation coefficients were then converted to a normal distribution using Fisher's *r*- to-z Transform ($z = 0.5 \text{ Ln } [(1 + r) / (1 - r)]$) (Jenkins & Watts, 1968). These values were converted into z-scores by dividing by the square root of the variance ($1/\sqrt{(n - 3)}$, where n is the degrees of freedom in the measurement, i.e. (number of volumes-2). All negative correlations were replaced with 0 in order to address the fact that negative correlations may have been artificially induced following global signal regression (Murphy, Birn, Handwerker, Jones, & Bandettini, 2009) and that global signal regression has been shown to bias estimates, particularly when comparing between groups (Gotts et al., 2013a; Saad et al., 2012a). For a more detailed discussion regarding global signal regression and its impact on FC, see Chapter 3.

2.4 ANALYSIS OF POTENTIALLY CONFOUNDING METHODOLOGICAL FACTORS

This section will present analyses employed to investigate a number of methodological factors which could have potentially contributed to any group differences in FC.

2.4.1 Head motion

As head motion has been identified as a potential confound when comparing FC between groups (Van Dijk, Sabuncu, & Buckner, 2012), we explicitly compared head motion, defined

from the MCFLIRT parameters, between the two age groups before calculating FC. IBM SPSS Statistics for Windows (Version 20.0) was used to conduct mixed design ANOVAs to assess potential differences in head motion between the two age groups as a whole, and divided by gender. Younger and older adults did not differ significantly in terms of relative or absolute head motion parameters as revealed by no significant main effect of age ($F(1,38)=0.13$, $p=0.73$, $\eta^2=0.003$) and no significant age*motion interaction ($F(1,38)=0.46$, $p=0.50$, $\eta^2=0.01$). Similarly, we found no effect of gender. See Table 2 for the average head motion values for the participant groups and Table 3 for all statistical outcomes.

Table 2: Average (± standard deviation) absolute and relative motion parameters (mm) for the two age groups, as a whole and divided by gender. No significant differences were identified between the participant groups.

		F	p	η^2
Young	Male vs. female	2.476	0.133	0.12
	Motion * gender	2.27	0.149	0.11
Older	Male vs. female	1.241	0.280	0.06
	Motion * gender	0.639	0.435	0.03
Male	Young vs. old	0.046	0.832	0.003
	Motion * age	0.626	0.439	0.03
Female	Young vs. old	0.283	0.601	0.02
	Motion * age	0.013	0.910	0.001

Table 3: Outcome of mixed ANOVAs testing differences in motion (absolute and relative) for participants divided by age and gender. ANOVAs consisted of the main effects motion (absolute and relative) and either gender or age and the interaction terms motion*gender or motion*age group. F= F statistic, p= p-value, η^2 = partial eta squared (effect size).

	Group		Male		Female	
	Young	Older	Young	Older	Young	Older
Absolute	1.43 ± 0.33	1.41 ± 0.36	1.53 ± 0.22	1.49 ± 0.35	1.30 ± 0.42	1.34 ± 0.38
Relative	0.08 ± 0.04	0.13 ± 0.07	0.08 ± 0.03	0.14 ± ±0.05	0.07 ± 0.04	0.13 ± 0.08

2.4.2 Potential sleeping in the scanner

One possible confound with resting-state analyses is the potential for participants to fall asleep in the scanner during the resting-state fMRI acquisition. This has been previously reported and associated with changes in RSN organisation (Tagliazucchi & Laufs, 2014). Although yet to be established, it is also possible that the propensity for sleep inside the scanner differs between younger and older adults. Our study employed an eyes open protocol, in an attempt to ensure participants maintained vigilance, although some evidence has suggested participants can enter light sleep even under these conditions (Tagliazucchi & Laufs, 2014). We found that self-reported daytime sleepiness and sleepiness upon exiting the scanner did not differ significantly between the two age groups as assessed by the Epworth Sleepiness Scale (Younger M = 5.85 ± 3.48, Older M = 4.9 ± 4.52), or post-scan sleepiness, as assessed by the Karolinska Sleepiness Scale (Younger M = 4.7 ± 1.9, Older M = 4.65 ± 1.81). This was revealed by a non-significant (NS) main effect of age ($F(1, 38)=0.48$, $p=0.49$, $\eta^2=0.013$), and a NS age*sleep measure interaction ($F(1, 38)=0.44$, $p=0.51$, $\eta^2=0.011$).

Within the limitation of assessing subjective sleepiness, these results suggest that, at least for this cohort, older adults were not considered more likely to fall asleep during periods of immobility, compared to younger adults. However, the inclusion of objective measures of vigilance fluctuations inside the scanner during future resting-state studies will ensure any differences in FC identified between groups of participants are not associated with a greater propensity to fall asleep during the scan

2.4.3 ROI sizes

The caveat of correcting ROIs for differences in grey matter volume is that, on average, older participant's ROIs contained fewer voxels compared to younger participants. Table 4

displays the average ROI sizes for the two age groups, after including only grey matter voxels. A mixed design ANOVA with the main effects age and node and the interaction term age*node revealed that for all RSN nodes, aside from left IPL, older adults had significantly fewer voxels within each ROI, after excluding white matter/CSF voxels, as indicated by a significant main effect of age ($F(1,38)=107.68$, $p<0.001$, $\eta^2=0.74$) and a significant age*node interaction, following a mixed design ANOVA ($F(11.38, 132.27)=6.92$, $p<0.001$, $\eta^2=0.778$. See Table 4 for the results from pairwise comparisons. Similarly, analyses conducted specifically for the thalamic sub-regions identified that older adults had significantly fewer remaining voxels within thalamic sub-region ROIs, as indicated by a significant main effect of age ($F(1,38)=17.95$, $p<0.001$, $\eta^2=0.321$). A significant age*sub-region interaction ($F(1.42, 53.8)=13.37$, $p<0.001$, $\eta^2=0.26$) revealed that this was the case for all sub-regions ($p<0.005$), excluding Pre-MT ($p=0.18$) and MT($p=0.11$). See Table 5 for ROI sizes for the two age groups, after excluding CSF voxels, and the results from pairwise comparisons.

Table 4: Final ROI size (group mean number of voxels and standard deviation across participants) after transforming ROIs into individual space and selecting only grey matter voxels, are displayed. Significant differences in ROI size between young and older adults are highlighted (***p<0.001, **p<0.01, *p<0.05). Partial eta squared (η^2) is also displayed for each significant comparison.

	Average ROI size ± SE			
	Younger	Older	η^2	Sig.
DAN				
Left IPS	59 ± 8.81	49 ± 6.35	0.32	***
Right IPS	61 ± 5.94	53 ± 6.14	0.32	***
Left OFC	59 ± 6.83	51 ± 8.06	0.21	**
Right OFC	62 ± 5.81	55 ± 9.50	0.19	*
DMN				
PCC	79 ± 7.18	67 ± 8.76	0.33	***
mPFC	85 ± 6.45	66 ± 8.74	0.61	***
Left IPL	53 ± 10.14	49 ± 9.63	0.05	
Right IPL	47 ± 8.37	38 ± 7.17	0.24	**
Left MTL	69 ± 7.04	52 ± 13.69	0.37	***
Right MTL	68 ± 6.57	52 ± 12.68	0.37	***
SN				
ACC	75 ± 6.91	42 ± 12.73	0.57	***
Left AI	83 ± 7.52	61 ± 7.49	0.72	***
Right AI	85 ± 7.51	65 ± 10.06	0.67	***
Auditory				
Left STG	71 ± 8.32	58 ± 5.73	0.43	***
Right STG	72 ± 5.99	61 ± 9.22	0.23	**
Motor				
Left M1	54 ± 5.53	43 ± 6.33	0.26	**
Right M1	46 ± 7.32	38 ± 5.82	0.46	***
SMA	61 ± 9.25	50 ± 9.55	0.31	***
Visual				
Left lateral	67 ± 7.34	59 ± 8.35	0.21	**
Right lateral	71 ± 6.51	62 ± 8.13	0.27	**
Left primary	54 ± 8.23	48 ± 7.51	0.13	*
Right primary	51 ± 5.68	43 ± 6.19	0.32	***

Table 5: Final ROI size (group mean number of voxels and standard deviation across participants) after transforming anatomically defined ROIs into individual space and selecting only grey matter voxels (or excluding CSF voxels for thalamic regions), are displayed. Significant differences in ROI size between young and older adults are highlighted (***p<0.001, **p<0.01, *p<0.05). Partial eta squared (η2) is also displayed for each significant comparison.

	Average ROI size ±SE			
	Younger	Older	η^2	Sig.
Hippocampus				
Left HC	90.35 ± 6.85	73 ± 9.46	0.52	***
Right HC	91.15 ± 5.78	75 ± 7.93	0.59	***
Thalamic sub-regions				
OT	162 ± 15.52	137 ± 16.84	0.40	**
FT	575 ± 55.20	514 ± 61.27	0.22	**
PT	403 ± 14.45	346 ± 38.88	0.36	**
Pre-MT	156 ± 24.96	149 ± 11.08	0.05	
MT	269 ± 15.52	260 ± 17.93	0.07	
ST	154 ± 15.25	140 ± 11.22	0.22	**
TT	553 ± 60.08	456 ± 81.01	0.33	**

2.4.3.1 The effect of ROI size on FC strength

In order to ensure that the number of voxels within an ROI did not drive FC strength, additional analyses were conducted. We selected the ACC node, which we found exhibited some of the greatest FC changes with age (Chapter 3) and investigated its FC with all other nodes, when the target node size was restricted to either 10, 20, 30 or 40 voxels. For each target ROI, GM voxels were ordered by spatial proximity to the peak voxel and then the top 10, 20, 30 or 40 voxels were selected as a target ROI subset. All ACC ROI GM voxels were used to calculate the average seed time-course, as usual, which was then correlated with each of the voxels within each target ROI subset. Correlation coefficients were averaged and converted into z-scores (as described on page 54). This resulted in an average FC strength for each ROI subset size (Figure 5).

Selecting target ROIs with differing voxel numbers, comparable to the differences in ROI sizes seen between younger and older adults, had no effect on ACC FC. From this, we can be confident that any FC differences between the two age groups are not driven by small differences in ROI sizes that result from different proportions of GM between young and older adults. A mixed design ANOVA with the factors node and ROI size revealed that there was no effect of ROI size on FC, as indicated by a NS main effect of ROI size ($F(4,95)=0.01$, $p=1.0$, $\eta^2= 0.001$) and a NS node*ROI size interaction ($F(11.01, 261.52)=0.12$, $p=1.0$, $\eta^2= 0.005$). Removing white matter and CSF voxels from ROIs ensures that the average ROI time-course is not contaminated by these signals of no interest and thus prevents the potential skewing of FC differences between the two age groups. We argue that as the consequence of having differing numbers of ROI voxels does not bias FC results in any way, this is a worthwhile step for comparing FC between younger and older adults.

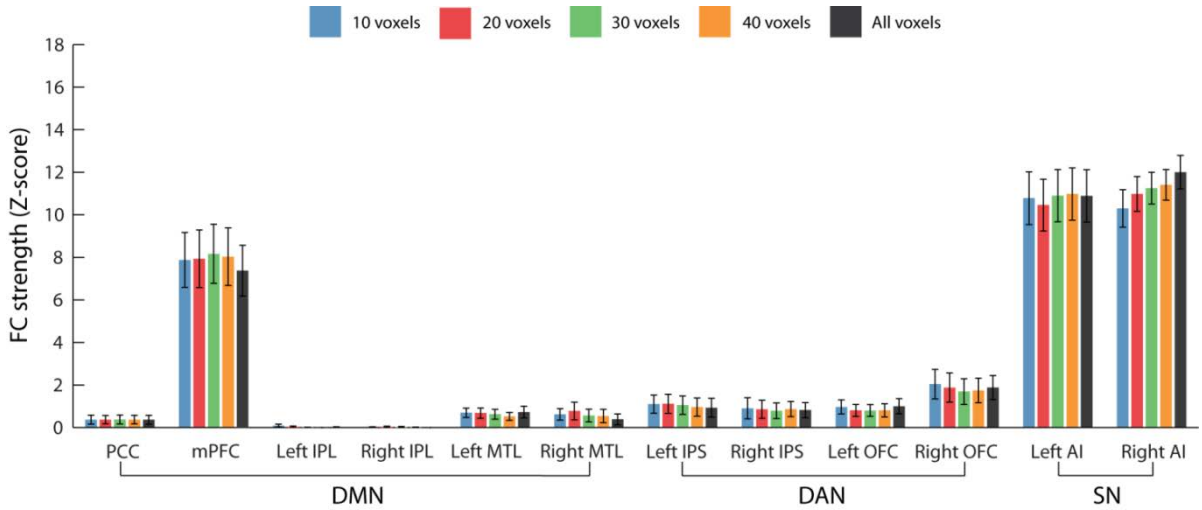

Figure 5: Average ACC- node FC for younger participants for ROIs of differing sizes (10, 20, 30, 40 voxels). ACC node- FC for ROIs that include all GM voxels (as used in our main analysis) are shown in black, for comparison. Error bars are SEM calculated across participants.

2.4.3.2 The effect of ROI size on age-related FC differences

In order to explicitly test whether differences in ROI sizes between groups affected between group FC differences, ACC-RSN FC was calculated for the two age groups using the

two voxel selection methods (i.e. GM only voxels or all voxels). The ACC was selected as it is the main node of the SN and is a main component of later analyses in Chapters 4 and 7. Mixed model ANOVAs revealed no significant differences in FC strength between the two methods for either age group (See Figure 6).

2.4.5 Summary

In summary, the results from analyses investigating the potential impact of a number of methodological factors on FC suggest that the methodological choices made for FC analysis throughout this thesis are unlikely to drive FC differences between the two age groups. Head motion and subjective sleepiness were not found to differ between groups, suggesting that these factors were not responsible for any FC differences identified between groups. Furthermore, considering that ROI size was not found to be associated with FC strength, we chose to adopt the CSF/WM voxel exclusion method detailed above, as a way of attempting to account for GM volume differences between age groups. Failing to do so could result in 'noisier' TCs for older adults, as ROIs would contain more voxels of no interest which would be averaged with GM TCs for FC analysis. The next chapter will investigate in more detail how the typical pre-processing step of global signal regression and underlying BOLD signal properties may affect group FC differences.

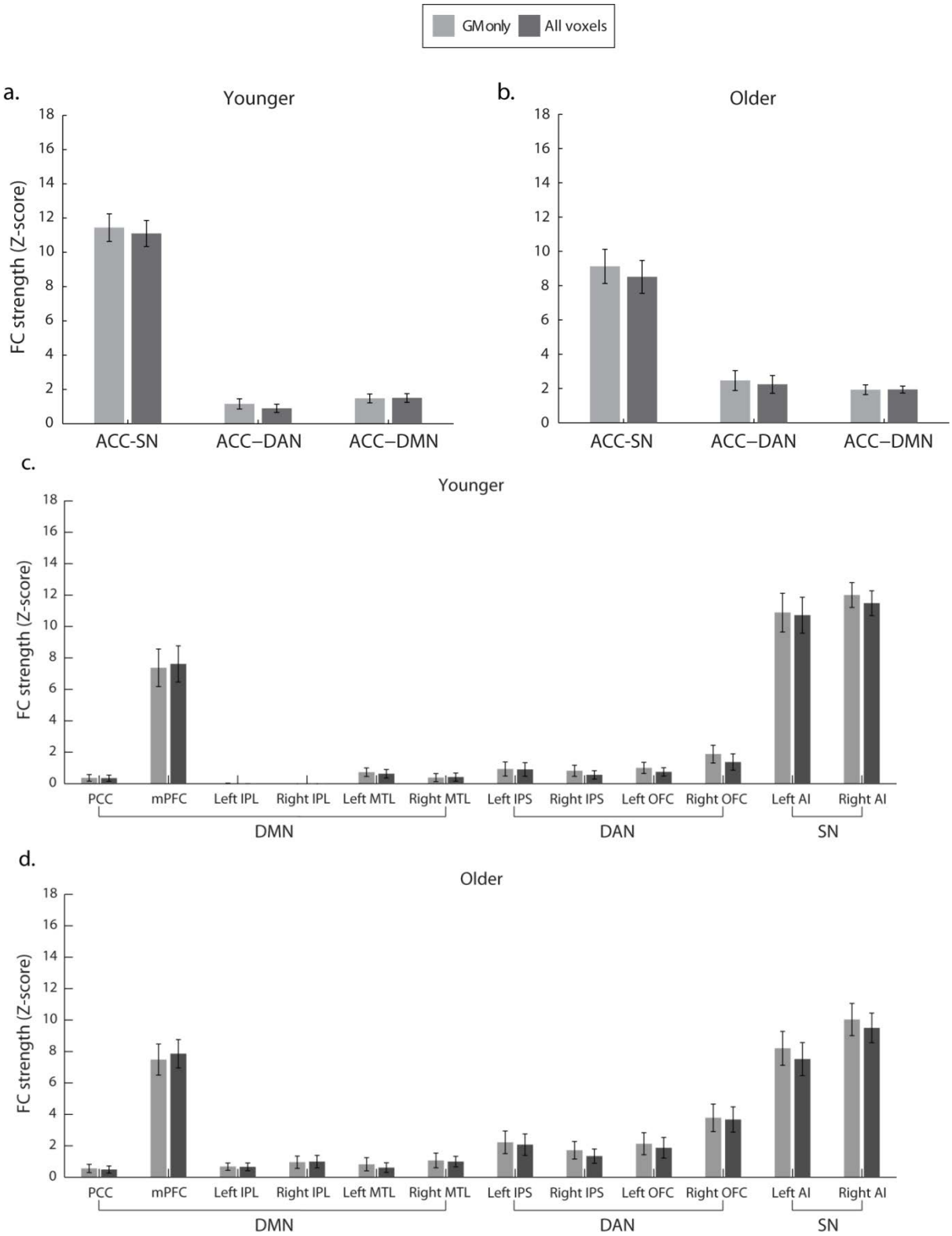

Figure 6: A comparison of the average ACC inter-network FC for younger (**6a**) and older (**6b**) participants calculated by 1) restricting FC analysis to grey matter voxels only (light grey) and 2) including all ROI voxels (dark grey) for analysis. Figures 6a and 6b depict the ACC-node FC for younger and older participants respectively, using the two methods. No significant differences were identified.

CHAPTER 3. THE POTENTIAL EFFECT OF CONFOUNDING GROUP DIFFERENCES ON FUNCTIONAL CONNECTIVITY MEASURES.

ABSTRACT

This chapter investigated whether differences in the underlying signal properties of the BOLD time series and the global signal (GS) existed between age groups. The results found that differences in cardiovascular reactivity, as indexed by resting-state fluctuation amplitude (RSFA), existed between age groups, confirming previous findings. However, RSFA did not relate to FC strength and nor did differences in RSFA correlate with FC differences between the two age groups. Similarly, the global correlation between GS and voxel time-series did not differ between groups. This means that FC results were not differently affected by the removal of GS, which could have been the case if the degrees of GS correlation for particular RSN nodes differed between the two age groups. Furthermore, patterns of FC group differences were largely similar both with and without GS regression.

3. 1 INTRODUCTION

The BOLD signal is influenced by a number of vascular responses, secondary to neural activity, such as changes in cerebral metabolic rate of oxygen consumption ($CMRO_2$), cerebral blood flow and volume (CBF and CBV respectively) (Buxton et al., 2004; Buxton, Wong, & Frank, 1998; Davis, Kwong, Weisskoff, & Rosen, 1998; Hoge et al., 1999; Liau & Liu, 2009; Logothetis & Wandell, 2004). Therefore, as the BOLD signal depends on vasodilation, voxel-wise BOLD signal amplitude depends on cerebrovascular reactivity (CVR) (i.e. the ability of vessels to respond to a vasodilatory stimulus) (Lipp, Murphy, Caseras, & Wise, 2015), which varies both within- and between individuals. If left unmodelled, these differences between individuals can reduce the statistical power of task-fMRI results, and potentially lead to both type I and type II errors when comparing across groups who may differ in terms of CVR, CBF and CBV.

This is a particular concern when comparing brain function, using fMRI, between young and older participants (D'Esposito, Deouell, & Gazzaley, 2003), as older age is associated with an array of changes to physiological factors, such as respiration, cardiovascular function, $CMRO_2$, CBF, CBV, CVR and arterial and vascular integrity (Buijs et al., 1998; Chester & Rudolph, 2011; Kannurpatti, Motes, Rypma, & Biswal, 2010; Leenders et al., 1990; Marchal et al., 1992; Martin, Friston, Colebatch, & Frackowiak, 1991; Nakano, Asada, Matsuda, Uno, & Takasaki, 2000; Riecker et al., 2003; Strandgaard, 1991; Takahashi, Yamaguchi, Kobayashi, & Yamamoto, 2005), as well as reductions in microvascular plasticity (see Riddle, Sonntag, and Lichtenwalner (2003b) for a review). These differences can alter the properties of the BOLD signal compared to young adults and also create variability between older participants which could potentially confound age-related differences in FC. These confounds may be directly associated (e.g. reduced CBF = reduced FC in older adults) or may be indirectly induced via global signal regression (GSR), a typical pre-processing step

for FC analysis which is thought to be associated with a number of physiological sources (Birn et al., 2006). This chapter will discuss 1) how CVR differs with age and investigate how such differences may be associated with measures of cortical and subcortical FC and 2) the effects of GSR on age-related FC differences.

3.1.1 Controlling for differences in CVR

In order to address differences in vascular responses between subjects, calibrated fMRI methods (Davis et al., 1998; Hoge et al., 1999) can be used. These methods typically combine BOLD MRI data with measures of perfusion in an attempt to separate neural from non-neural physiological sources, and thus improving group fMRI comparisons by controlling for voxel-wise differences in CBF/CBV (Ances et al., 2009). However, perfusion images typically suffer from lower SNR, fewer slices and reduced temporal resolution compared to BOLD acquisition (Silva & Kim, 2003) and require alternative imaging sequences (arterial spin labelling, ASL) and analysis. An alternative approach is to induce hypercapnia (increases in cerebral and end-tidal carbon dioxide) via either CO_2 challenges (participants inhale air with ~5% CO_2 concentrations) or breathhold (BH) tasks (participants cycle between breathing normally and holding their breath for ~20 seconds). These methods are adopted because during hypercapnia, CBF increases diffusely resulting in a global increase in oxygenated compared to deoxygenated haemoglobin, thus inducing an increase in BOLD signal, in all grey matter. The resultant BH BOLD response is thought to be linearly related at the voxel level to the amplitude of the BOLD response to a cognitive or sensory task, but independent of changes in neural activity (Bandettini & Wong, 1997; Davis et al., 1998; Hoge et al., 1999; Kannurpatti, Motes, Rypma, & Biswal, 2011a; Riecker et al., 2003). Thus, scaling voxel-wise task-fMRI responses with the BOLD response to the BH normalises an individual's BOLD response and accounts for regional variability in CVR, thus revealing BOLD task responses

that are not driven by underlying vascular properties. A number of studies have provided evidence that controlling for the BH response in every voxel reveals more specific differences in BOLD activation between young and older adults (Hamzei, Knab, Weiller, & Rother, 2003; Handwerker, Gazzaley, Inglis, & D'Esposito, 2007; Thomason, Burrows, Gabrieli, & Glover, 2005; Thomason, Foland, & Glover, 2007). Typically, older adults exhibit reductions in CVR, as assessed by BH tasks. This suggests that reductions in task-BOLD signals with age, prior to BH correction, are partly driven by underlying differences in CVR (Riecker et al., 2003). Therefore, any differences in task-BOLD response between age groups, which persist after BH normalisation, are more likely to arise from differences in underlying neuronal activity.

3.1.2 Resting-state fluctuation amplitude (RSFA)

Although the BH task is more easily implemented and accepted by participants, compared to the CO_2 challenge tasks (Lipp et al., 2015), it may still be a challenge for some participants, particularly older and patient groups with weak cardiovascular fitness. As a result, participants may differ in their compliance with the task and also their depth of inhalation. Furthermore, some have argued that the BOLD response to the BH task may not be entirely non-neuronal in origin and at the very least causes changes in subject's arousal levels (Hall et al., 2011; Zappe, Uludag, Oeltermann, Ugurbil, & Logothetis, 2008). For this reason researchers have searched for a way to account for voxel-wise differences in CVR, using measures obtained from resting-state fMRI data, negating the need for hypercapnia tasks or additional perfusion scans. One such method is quantification of the resting-state fluctuation amplitude (RSFA) which is typically calculated by computing the standard deviation, or the ratio between the standard deviation and the temporal mean (Kannurpatti & Biswal, 2008), of voxelwise BOLD timecourses. Similarly to the known association

between task-BOLD and BH-BOLD response, a voxel-wise linear association between RSFA and task-BOLD response has also been identified, suggesting a common physiological origin (Kannurpatti, Motes, Rypma, & Biswal, 2011b; Kannurpatti, Rypma, & Biswal, 2012). A number of studies have evaluated the suitability of using RSFA to normalise task-BOLD responses. The amplitude and spatial extent of task-BOLD, scaled by RSFA has been shown to be very similar to the results obtained when scaling by both BH- and CO_2 challenge- BOLD (Liu et al., 2013). Furthermore, large correspondences between RSFA and BH measures (>80%) have been identified, both within and between participants (Kannurpatti & Biswal, 2008), suggesting that RSFA accurately assesses CVR across all age ranges (Kannurpatti, Motes, Biswal, & Rypma, 2014). However, there has been no definitive demonstration of the origin and composition of RSFA and some have suggested that RSFA may also reflect other factors such as breathing variations and potentially neural signals (Liu et al., 2013). For these reasons some authors suggest that convolving the end-tidal CO_2 trace (post expiration, measured by nasal cannula) with a standard HRF and including this as a co-variate in GLM analyses is superior to correction by BH, CO_2 challenges and RSFA (Lipp et al., 2015; Murphy, Harris, & Wise, 2011; Scouten & Schwarzbauer, 2008).

However, a number of studies have provided evidence that scaling task-BOLD responses using RSFA is more accurate for identifying differences between young and older adults (Kannurpatti et al., 2011a; Tsvetanov et al., 2015). Recently, Tsvetanov and colleagues (2015) compared RSFA against heart rate and heart rate variability, as well as task-BOLD and resting-state magnetoencephalography (MEG). Using mediation analysis on a large sample of participants, they identified that age effects on RSFA were driven by vascular rather than neuronal factors, providing further support for using RSFA as a measure of CVR. Overall, RSFA has generally been established as a reliable estimator of CVR, without the need for additional apparatus and/or ASL acquisition, which allows for useful scaling of the

BOLD response in order to accurately assess age-related differences. Although the analysis of FC does not require such scaling, as it is not calculated based on BOLD amplitude, it remains to be seen how regional differences in RSFA, between age groups, may be associated with differences in FC. For example, it is possible that the degree of fluctuation may affect the oscillatory properties of the BOLD signal, thus resulting in spurious FC differences between groups with different RSFA properties. This could be complicated further if RSFA differences between groups are region dependent.

3.1.3 Global signal regression (GSR)

The global signal (GS) is a time-series computed by averaging the resting-state BOLD signal time courses of all brain voxels. The rationale for applying global signal regression (GSR) to fMRI data is that the GS time-course is a common signal across all brain regions, not restricted to those sharing functional or anatomical connections. Therefore, it is suggested that the GS provides no spatially specific information regarding neuronal activity and can be removed (Macey, Macey, Kumar, & Harper, 2004). Global effects are commonly thought to obscure more specific, local fMRI effects and are thought to reflect 'background' fluctuations, such as physiological confounds which are not captured by the removal of cardiac and respiratory confounds, as well we motion artifacts and scanner instabilities (Birn et al., 2006; Desjardins, Kiehl, & Liddle, 2001). Specifically, in terms of FC analysis, global signal fluctuations are thought to mask more spatially specific network FC, therefore it has been argued that the application of GSR enhances the strength and reliability of FC results (Fox, Zhang, Snyder, & Raichle, 2009; Keller et al., 2013; Kruschwitz et al., 2015a).

Initial studies investigating the use of GSR for FC analysis reported enhanced detection of FC and improved correspondence between FC and anatomy (Fox et al., 2009) and improved accuracy of identification of RSNs (Birn et al., 2006), presumably via the removal

of residual motion and global physiological artifacts which are not removed by typical confound regression of motion parameters or average CSF and white matter signals (Keller et al., 2013; Satterthwaite et al., 2013; Yan, Craddock, Zuo, Zang, & Milham, 2013). However, it was quickly discovered that GSR skews the distribution of FC correlation coefficients from being positively biased as they are prior to GSR (Fox et al., 2009; Hayasaka, 2013), to negatively biased (with a mean of 0), thus inducing negative correlations, particularly between regions with zero FC pre-GSR (Murphy et al., 2009). This finding cast some doubt over the reliability of the method and the true existence of anti-correlated networks which appeared to arise simply as a consequence of GSR. Although GSR has been seen to improve the specificity and reliability of positive FC (Keller et al., 2013; Kruschwitz et al., 2015b; Weissenbacher et al., 2009), and has been associated with removing physiological artifacts (Birn et al., 2006), some researchers are concerned that the GS contains signals related to neural activity. The GS is known to localise primarily to grey matter, correlating most strongly with sensory, thalamic and midline regions, while being least correlated to white matter and CSF signals (Vincent, Kahn, Snyder, Raichle, & Buckner, 2008), although it could be argued that these patterns most resemble the global patterns of BOLD signal associated with respiratory and cardiac factors (Birn et al., 2006; Chang et al., 2009). More specifically, a study by Scholvinck, Maier, Ye, Duyn, and Leopold (2010) in anaesthetised monkeys reported that the BOLD GS is related to the LFPs recorded from electrodes implanted in visual, parietal and frontal cortex, particularly for bands of upper gamma range frequencies (40-80Hz) and lower frequencies (2-15Hz). This suggests that the BOLD GS may contain a significant amount of neural activity. The origin and functional interpretation of global neuronal signals remains unclear, however this work raises the question of whether by removing the BOLD GS distortions are introduced into the FC of RSNs

Furthermore, the finding that the GS is distributed heterogeneously across the cortex (Fox et al., 2009) has led to concerns that its removal could result in shifting the spatial distribution of positive FC. This effect was reported by Saad and colleagues (2012b) who used simulated data sets, which allows for calculated FC to be evaluated against known patterns of 'true' connectivity unlike in real imaging data where the ground truth is unknown. They reported that GSR resulted in group differences being falsely identified in a number of locations, rather than just at those appropriate locations where differences did exist. They also reported that this GSR distortion was spatially dependent, different regions/ROIs were differentially affected by GSR, the effect of which depended on the 'true' connectivity between regions. A specific investigation into how GSR may alter FC and induce differences between groups was conducted by Gotts and others (2013b). They reported that GSR does not simply rescale or re-centre FC, but instead, warps correlation matrices, which means it alters the ranking of correlations, and that the extent of warping may differ between groups. They also found that GSR had the greatest impact on those pairs of connections which had the largest group differences (i.e. patient vs. normal) when no GSR was applied. This meant that those connections which had previously been most different between groups (without GSR) were the same connections which showed the greatest attenuation of group differences following GSR. They also reported that GSR can alter the direction of group FC comparisons. This is because if a difference in FC between groups exists prior to GSR, the magnitude of re-centring correlations to 0 (as GSR does) will differ for the two groups, i.e. the magnitude will be greater for the group with the strongest FC. This can result in both the attenuation and reversal of differences found when no GSR is applied, thus under GSR they report more instances of patient>normal FC, while without GSR they report greater instances of normal>patient FC. The authors agree that GSR

removes global artifacts, but they argue that it is at the cost of warping FC matrices and thus altering group differences.

Following these observations, Gotts and colleagues (2013b; 2012) argue for the use of other global correction methods, such as GCOR (correction for the average 'global' FC between all possible voxel pairs). However, as there is no way of partitioning this global correlation into noise/meaningful sources, the effect of removing meaningful correlation may still persist with this method. They state that if only a relatively small number of subjects have large amounts of global correlation (GCOR), which could be associated with motion, it increases the variability in FC strength across subjects, which in turn reduces differences when comparing between groups. Thus by applying correction for GCOR the within-group FC strength variability is reduced, revealing 'true' group differences. They state that patterns of FC using this method more closely resemble those using no GSR. However, a recent study by Yan and colleagues (2013) investigated a number of different pre-processing and standardisation techniques and reported that generally, post-hoc correction (i.e. correction for GS after calculation of FC such as GCOR) was more effective than traditional GSR (which is typically conducted prior to FC calculation) for a number of resting-state measures. However, for FC specifically, GSR and post-hoc methods resulted in very similar FC strengths and the authors report no evidence of GSR inducing differences in FC related to gender or age.

Despite the criticisms surrounding GSR, a recent electrocorticography (ECoG) study by Keller and colleagues (2013) reported that both positive and negative BOLD FC (identified both with and without GSR) has neurophysiological correlates and that GSR ensured greater spatial correspondence between BOLD FC and high gamma power ECoG FC (50-150Hz). Similarly, a recent study investigating the reliability of using FC to segment the fusiform gyrus into known sub-regions (Kruschwitz et al., 2015a), reported that GSR resulted in

greater specificity of FC results and more reliable segmentation of the fusiform gyrus, which was replicated across sites, compared to when GSR was not applied. Fox and colleagues (2009) also reported improved thalamo-cortical FC and greater correspondence between FC and structural connectivity across the brain. Therefore, the authors suggest that the GS obscures underlying neuroanatomy and neurophysiology and that GSR affords more sensitive FC results. Similarly, a previous study by Weissenbacher and colleagues (2009) reported that GSR almost doubled the spatial specificity of positive FC and suppressed spurious positive FC. In summary, the enhancement of neuronal-haemodynamic correspondence and improved detection of underlying neuronal fluctuations (Keller et al., 2013) as well as improved specificity of positive FC (Hayasaka, 2013; Kruschwitz et al., 2015a; Weissenbacher et al., 2009), suggest that although GSR can induce artifacts (e.g. some negative correlations) the benefit of improved detection of true neuronal patterns of connectivity may outweigh this cost.

3.1.4 Chapter overview

This chapter explored potential differences in RSFA between age groups for all the RSN nodes used for FC analysis in Chapters 2-5 and whether the amplitude of RSFA within individuals was associated with FC, or whether differences in RSFA between groups was associated with between-group differences in FC. Furthermore, this chapter also investigated the impact of GSR on differences in FC between age groups which will form the basis for Chapters 2-5. This included assessing 1) inter-network FC of two main RSN nodes (PCC and ACC) and the possible interaction with gender 2) thalamo-cortical FC.

3.2 METHODS

3.2.1 RSFA

3.2.1.1 Voxelwise RSFA

For each participant, RSFA was calculated for each brain voxel, by computing the standard deviation of the BOLD time course, following all pre-processing steps described in the methods section. After calculating voxelwise RSFA, whole brain RSFA maps were created for each participant. These maps were transformed into MNI space and averaged across participants in order to assess the global pattern of RSFA between the two groups, as an additional step to comparing RSFA within the specific RSN nodes used for FC analysis.

In order to assess whether voxelwise RSFA was significantly different between the two groups, FSL's Randomise tool was used to perform non-parametric permutation testing to determine significance (Winkler, Ridgway, Webster, Smith, & Nichols, 2014). For this, each participant's RSFA brain map was transformed into MNI space, and then all standardised maps were concatenated across all participants. A two-sample t-test then assessed RSFA differences between age-groups at each voxel, 5000 permutations were computed for each contrast (e.g. young>older) and threshold free cluster enhancement (TFCE) (Smith & Nichols, 2009) was applied to identify 'clusters' of significant voxels, thus preventing the need to set an arbitrary threshold.

3.2.1.2 RSFA by RSN node

RSFA was also assessed for all RSN nodes previously used for the FC analysis. For this, RSFA was calculated for each voxel within the ROI used for FC analysis, for each participant. RSFA was then averaged across voxels, resulting in an average RSFA value for each node, for each participant. A mixed design ANOVA, with main effects of age and node and the interaction term, was then used to assess differences in RSFA between the two age groups.

3.2.1.3 RSFA and FC strength

To assess whether RSFA was associated with FC strength, a number of correlational analyses were performed. For this, first FC between the main nodes of the SN and DMN (ACC and PCC) and all other RSN nodes was calculated. These individual node FC strengths were then averaged within networks (e.g. ACC-rAI, ACC-lAI were averaged to create ACC-SN FC) for each participant. These composite FC strengths were then correlated with the average seed (i.e. ACC or PCC) RSFA. Furthermore, in order to assess whether subcortical RSFA and FC were associated, RSFA for bilateral thalamus (i.e. the whole thalamic mask) was correlated with bilateral thalamic – sensory cortical FC.

Furthermore, it was also investigated whether age-related ACC and PCC FC differences were proportional to the age-related RSFA differences. The difference in average RSFA between the two age groups was calculated for the 22 cortical nodes, as well as the difference in average ACC/PCC-node FC between the two age groups for all 22 nodes. These two difference scores were then correlated in order to assess if the difference in ACC/PCC-node FC between the two groups could be associated with the difference in RSFA for each target node.

3.2.2 Global Signal

3.2.2.1 Voxelwise GS

Similar to the voxelwise RSFA analysis, the correlation between global signal and each voxel's BOLD time course was calculated in order to create a voxelwise map for each participant. These maps were transformed into MNI space and averaged across participants in order to assess the global pattern of RSFA between the two groups, as an additional step to comparing RSFA by the specific RSN nodes used for FC analysis. In order to assess whether voxelwise GS correlation was significantly different between the two groups, we

applied the same methods used for the RSFA voxelwise analysis, using FSL's Randomise tool to perform non-parametric permutation testing to determine significance (Winkler et al., 2014).

3.2.2.2 GSR impact on FC

3.2.2.2.1 Cortical ROIs

To investigate the impact of GSR on FC of cortical ROIs, two main nodes of the SN and DMN were selected (ACC and PCC respectively) as examples. FC was calculated using both methods (GSR and no GSR) and mixed effects ANOVAs with the main effects of age, network (i.e. ACC-DAN, ACC-DMN, ACC-SN and PCC-DAN, PCC-DMN, PCC-SN) and method (i.e. GSR vs. no GSR) and their interaction terms assessed whether the process of applying GSR altered the differences in FC between young and older adults.

3.2.2.2.1.1 Cortical ROIs by gender

As one of the main findings presented in Chapter 2 related to gender, the effect of GSR was also investigated in relation to gender. Specifically, it was identified that for ACC, inter-network FC age differences were gender specific (greater ACC-DAN FC in older, compared to younger, male participants). Therefore, the results of this finding are also presented with and without GSR. For comparison to the gender effects reported for ACC-network FC, the effect of gender and age was also explored for PCC-network FC, for the two GSR methods.

3.2.2.3 Thalamic FC

As Chapters 5 and 6 specifically investigate the potential differences in thalamic FC between the two age groups, the effect of GSR method on thalamic-sensory FC was also explored. For this, average FC was calculated between bilateral thalamic FC and each node of

the sensory RSNs using both GSR methods. These connections were chosen as these thalamic-sensory connections are most well understood in terms of thalamic connectivity and are the main focus of the investigation in Chapters 5 and 6.

3.3 RESULTS

3.3.1 RSFA

3.3.1.1 Voxelwise RSFA

Although the pattern of RSFA strength was similar between the two groups, qualitatively, older adults exhibited reduced RSFA across the cortex, but higher RSFA in sub-cortical regions, compared to younger adults. This is seen most clearly in slice numbers 25 and 29 of Figure 7, which were found to consist of a combination of regions, including: the occipital pole, parahippocampal gyrus, areas surrounding the brainstem and temporal pole (typically areas associated with noisy BOLD signal due to close proximity to tissue boundaries and B_0 inhomogenities) and white matter regions.

Results from the Randomise analysis revealed that, following family-wise error (FWE) correction, older adults did not exhibit significantly greater RSFA, compared to younger adults, in any regions. In contrast, RSFA was significantly greater for younger, compared to older adults, for a number of cortical regions, including: visual cortex, precuneus, pre- and post- central gyrus, insula, supramarginal gyrus and putamen. Significant differences, thresholded at $p<0.05$ are presented in Figure 8.

3.3.1.2 RSFA by RSN node

Young and older adults exhibited significantly different RSFA, for specific nodes only, as indicated by a significant age*RSN node interaction ($F(8.74, 331.98)=5.65$, $p<0.001$,

η^2=0.13) and a NS main effect of age (F(1,38)=2.86, p=0.099, η^2=0.07). Pairwise comparisons revealed that RSFA was significantly greater for younger participants, compared to older, for: left and right IPS, left OFC, PCC, left and right IPL, left and right STG, left and right lateral visual regions and right V1 (Figure 9). However, only left IPS, left OFC and PCC survived Bonferroni correction for multiple comparisons. Results from these pairwise comparisons are displayed in Table 6. The reduction in cortical RSFA seen in older adults was fairly homogenous, affecting all cortical regions similarly (as seen in Figure 10). This suggests that comparisons of FC will not be confounded by nodes that have differing RSFA and cannot directly be accountable for the combination of both increases, and decreases, in FC we see in older adults compared to younger.

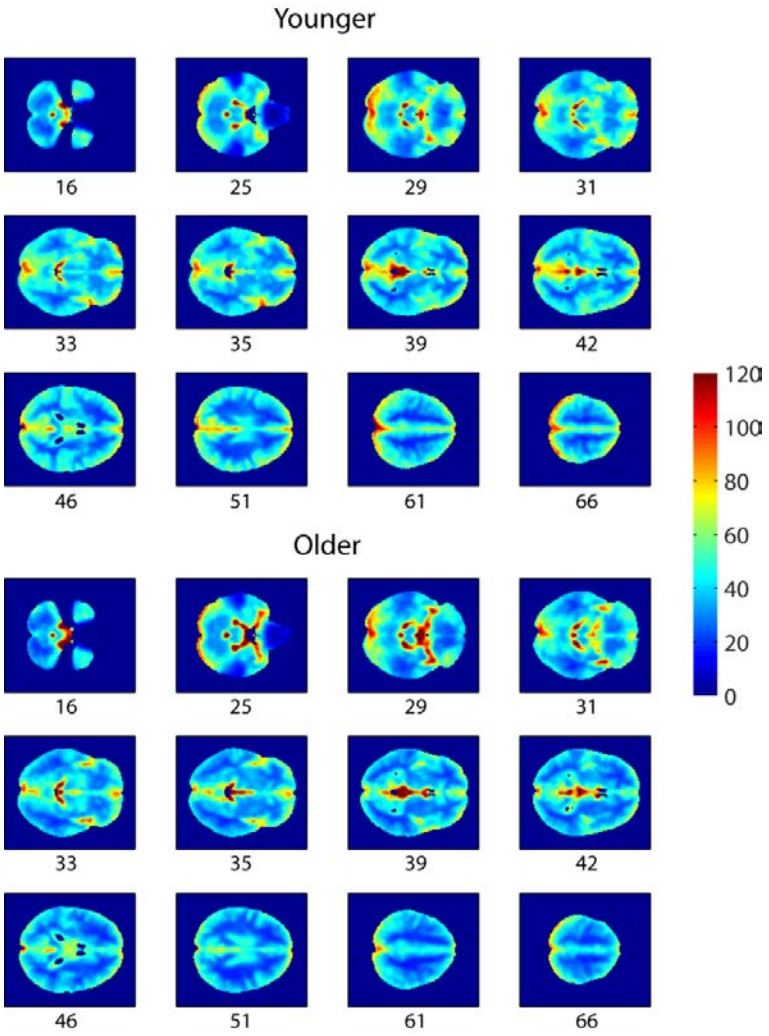

Figure 7: Average RSFA for each brain voxel, displayed for axial slices in MNI space, for the two age groups.

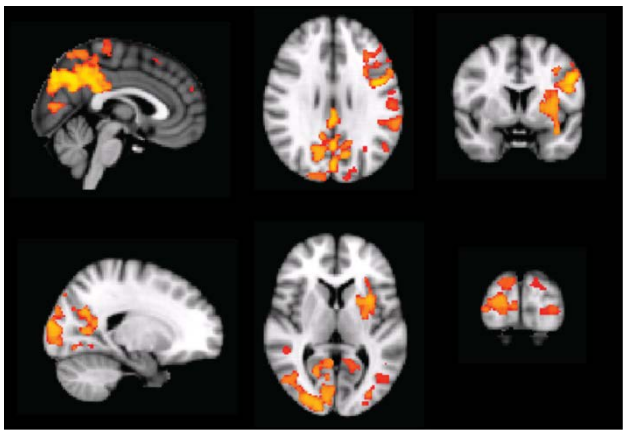

Figure 8: Regions for which younger participants exhibited significantly greater RSFA than older adults, as identified by non-parametric permutation testing. The top row presents significant differences for precuneus (left), pre-cuneus, pre- and post-central and supramarginal gyri (centre) and insula and pre-central gytus (right). The bottom row presents significant differences identified for visual cortex and putamen (centre). Maps are FWE corrected and thresholded at $p<0.05$.

Table 6: Results from the pairwise comparisons comparing RSFA for each RSN node for the two age groups. Differences between the two groups that remain significant following Bonferroni correction are highlighted in blue.

	Mean difference (young – older)	SE	Sig.	η^2
DAN				
Left IPS	8.50	2.62	0.044	0.217
Right IPS	8.75	3.44	0.330	0.145
Left OFC	10.04	3.01	0.044	0.226
Right OFC	5.89	4.37	1.000	0.046
DMN				
PCC	17.74	3.72	<0.001	0.374
mPFC	2.57	5.27	1.000	0.006
Left IPL	10.55	3.74	0.176	0.173
Right IPL	10.33	3.44	0.110	0.192
Left MTL	3.50	4.01	1.000	0.020
Right MTL	5.59	3.67	1.000	0.057
SN				
Left AI	8.91	4.72	1.000	0.086
Right AI	1.61	3.55	1.000	0.005
ACC	1.74	3.23	1.000	0.008
Motor				
Left M1	6.442	4.50	1.000	0.051
Right M1	6.989	4.83	1.000	0.052
SMA	6.134	4.07	1.000	0.056
Auditory				
Left STG	11.09	3.54	0.066	0.205
Right STG	9.28	3.63	0.330	0.146
Visual				
Left lateral visual	8.15	3.13	0.286	0.151
Right lateral visual	10.29	3.90	0.264	0.155
Left V1	11.70	6.28	1.000	0.084
Right V1	17.47	5.44	0.066	0.213
Subcortical				
Left HC	-4.08	4.15	1.000	0.025
Right HC	-1.15	4.04	1.000	0.002
Left thalamus	-3.77	3.44	1.000	0.031
Right thalamus	-3.54	4.05	1.000	0.020
FT	-3.73	3.85	1.000	0.024
OT	-2.78	2.45	1.000	0.033
PT	-1.71	2.54	1.000	0.012
Pre-MT	1.27	2.74	1.000	0.006
MT	0.92	2.14	1.000	0.005
ST	0.96	2.12	1.000	0.005
TT	-5.23	3.81	1.000	0.047

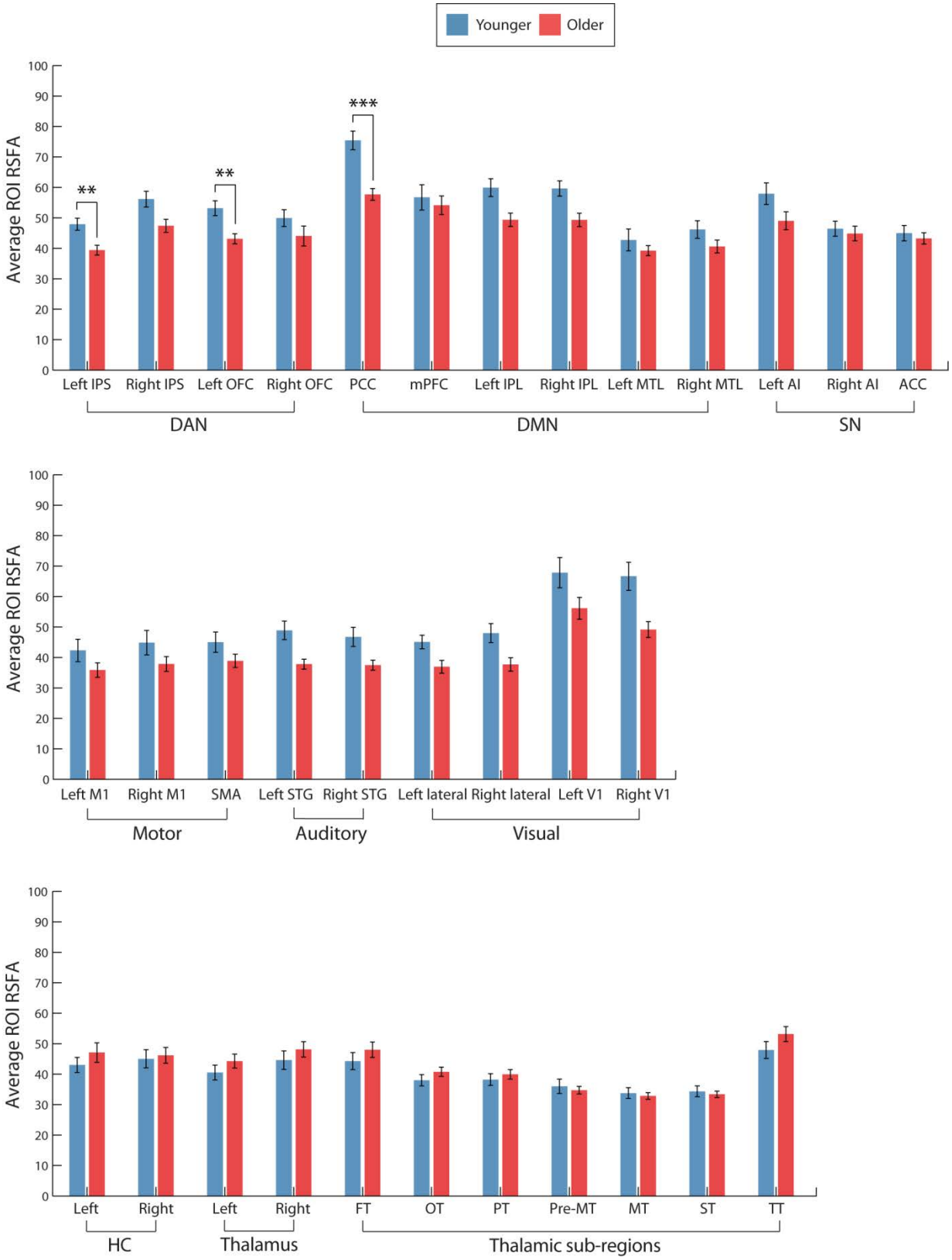

Figure 9: RSFA for each RSN node is displayed for the two age groups, for 9a) 'Cognitive' RSNs, 9b) Sensory RSNs and 9c) Sub-cortical nodes. Error bars are standard error across participants. **p<0.01, ***p<0.001.

3.3.2 RSFA and FC

Although we do not expect differences in RSFA, which seems to be quite globally reduced across cortical regions for older adults, to drive the age-related differences in FC reported in Chapters 3-7, specific investigations were conducted in order to test this possibility. Results from correlating RSFA and FC strength suggested that RSFA was not associated with FC as no significant correlations were identified following Bonferroni correction. Tables 7-10 present the results from these correlation analyses. As PCC RSFA showed the greatest difference with age, PCC RSFA and FC associations were explored for all nodes of the DMN, however, no significant association was identified (Table 9). All results have been Bonferroni corrected for multiple comparisons.

Table 7: Results of correlating ACC RSFA and ACC-network FC for the two age groups.

	Younger			Older		
	DAN	DMN	SN	DAN	DMN	SN
Pearson's r	0.344	0.179	0.257	0.281	0.325	-0.103
p-value	0.411	1.0	0.825	0.690	0.489	1.0

Table 8: Results of correlating PCC RSFA and PCC-network FC for the two age groups.

	Younger			Older		
	DAN	DMN	SN	DAN	DMN	SN
Pearson's r	0.222	0.042	-0.162	0.317	0.406	-0.162
p-value	1.0	1.0	1.0	0.522	0.123	0.164

Table 9: Results of correlating PCC RSFA with PCC-DMN node FC for older adults only.

	mPFC	Left IPL	Right IPL	Left MTL	Right MTL
Pearson's r	0.057	0.235	0.088	0.330	0.448
p-value	1.0	0.957	1.0	0.465	0.144

Table 10: Results of correlating bilateral thalamic RSFA with bilateral thalamic-sensory RSN FC.

	Younger			Older		
	Motor	Auditory	Visual	Motor	Auditory	Visual
Pearson's r	0.025	-0.184	0.164	-0.399	-0.107	-0.089
p-value	1.0	1.0	1.0	0.246	1.0	1.0

Results from the analyses which investigated whether differences in RSFA were proportional to FC differences between the two age groups suggested no associations between ACC and PCC RSFA and FC differences. This was revealed by NS correlations (Pearson's r= -0.06, p=0.80 & Pearson's r= -0.27, p=0.24) suggesting that the difference in RSFA between the two age groups was not associated with age-related differences in FC between the main nodes of two of the cortical RSNs (ACC, PCC) and each of the other cortical nodes (see Figure 10).

These results suggest that any differences in RSFA between the two age groups are unlikely to be responsible for any differences in FC.

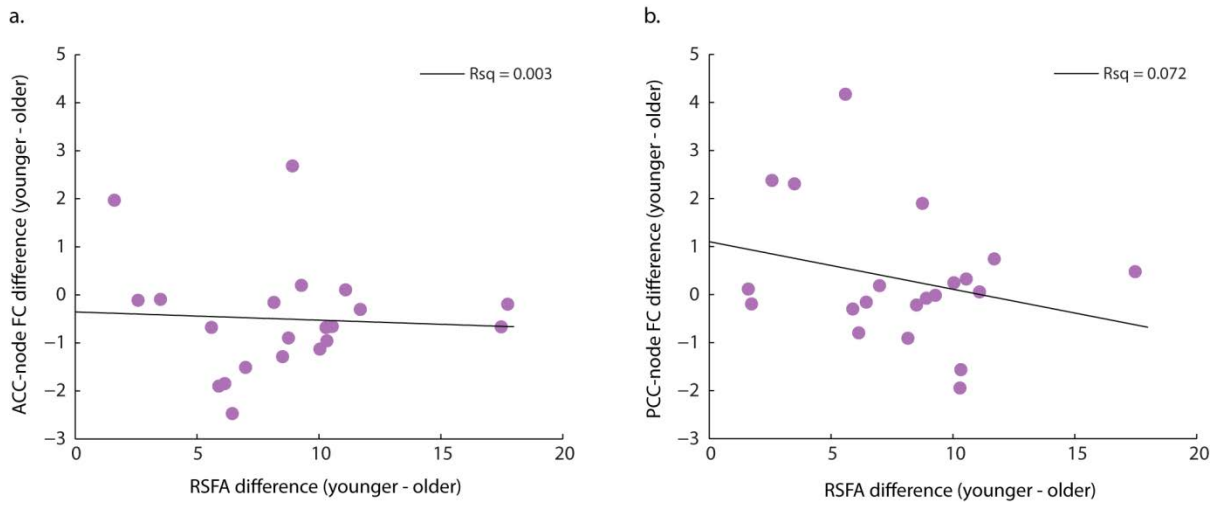

Figure 10: Average RSFA difference between the two age groups is plotted against the difference in node-FC between the two age groups for a) ACC and b) PCC. No significant correlation was identified between these two difference measures for either node.

3.3.3 Global signal

3.3.3.1 Voxelwise GS

The extent to which global signal correlated with BOLD time courses across the brain was very similar for the two age groups (Figure 11). Results from the Randomise analysis revealed that, following family-wise error (FWE) correction, older, compared to younger,

adults did not exhibit significantly greater correlation between GS and voxel time-series at any voxels. Younger adults exhibited one significant cluster around the temporal pole, which extended to the amygdala and included some orbitofrontal cortex (OFC). The temporal pole and nearby OFC commonly suffer signal loss in older adults, which may explain why only this one region was identified to differ significantly between the two age groups. Significant differences, thresholded at $p<0.05$ are presented in Figure 12.

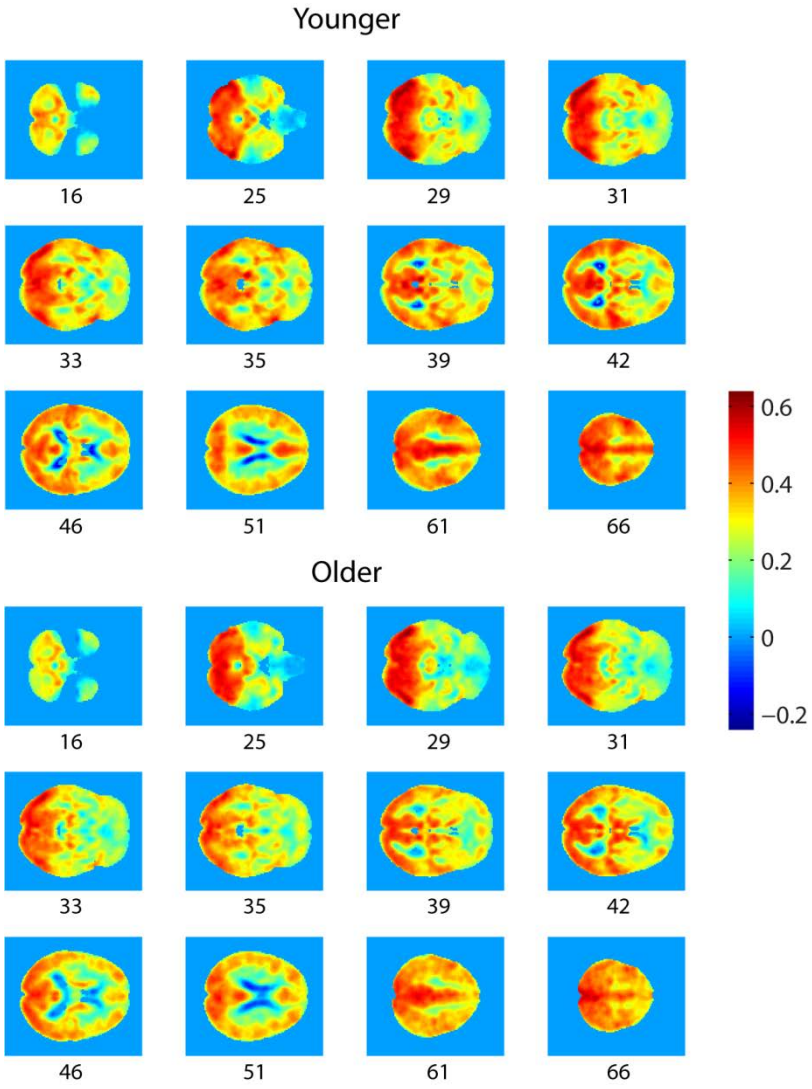

Figure 11: The average correlation between global signal and BOLD time course for each brain voxel, displayed for axial slices in MNI space, for the two age groups.

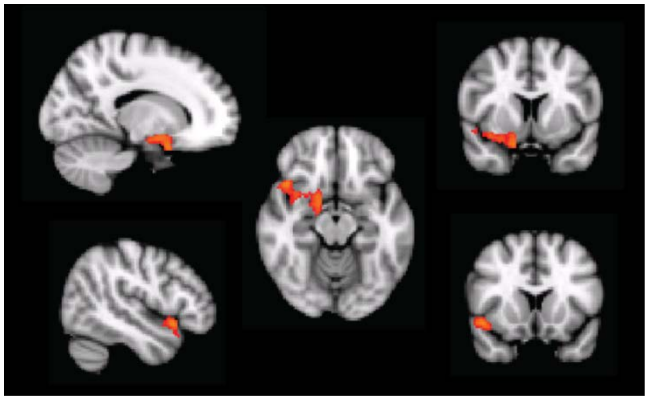

Figure 12: Regions for which younger participants exhibited significantly stronger correlation between GS and voxel time-series, as identified by non-parametric permutation testing. The centre figure presents the extent of the cluster which extends from right OFC (top left and right) to the right temporal pole (bottom left and right) and also includes the amygdala (see centre image). Maps are FWE corrected and thresholded at p<0.05.

3.3.3.2 GSR impact on FC

3.3.3.2.1 Cortical ROIs

For both of the main nodes (ACC and PCC), GSR was found to lower FC strength, independent of network and age, compared to not applying GS regression, as indicated by a main effect of method ($F(1,38)=142.80$, $p<0.001$, $\eta^2=0.79$ & $F(1, 38)=108.90$, $p<0.001$, $\eta^2=0.71$, for ACC and PCC respectively). Similarly, all node-RSNs were affected by GSR, that is; they all exhibited greater average FC when GS was not applied, as revealed by a significant method*network interaction ($F(1.91, 72.54)=11.27$, $p<0.001$, $\eta^2= 0.23$ & $F(1.84,69.95)=15.05$, $p<0.001$, $\eta^2=0.28$, for ACC and PCC respectively) and subsequent pairwise comparisons ($p<0.001$ for GS vs. no GS for all three RSNs, for both nodes). However, NS method*age ($F(1, 38)=3.16$, $p=0.08$, $\eta^2=0.08$ & $F(1, 38)=0.02$, $p=0.89$, $\eta^2=0.001$, for ACC and PCC respectively) and network*method*age interactions ($F(1.91, 72.54)=2.61$, $p=0.08$ & $F(1.84, 69.95)=3.184$, $p=0.05$, $\eta^2=0.08$, for ACC and PCC respectively) suggested that the effect of GSR on ACC/PCC-RSN FC was not differentially affected by age. As potential trends were identified for the network*method*age interactions, pairwise comparisons were explored. However, no significant differences between the age groups were identified

for any networks, for either GS method, confirming the lack of influence of GS method on age-related differences in ACC/PCC-network FC (See Figures 13 and 14 for ACC- and PCC-network FC).

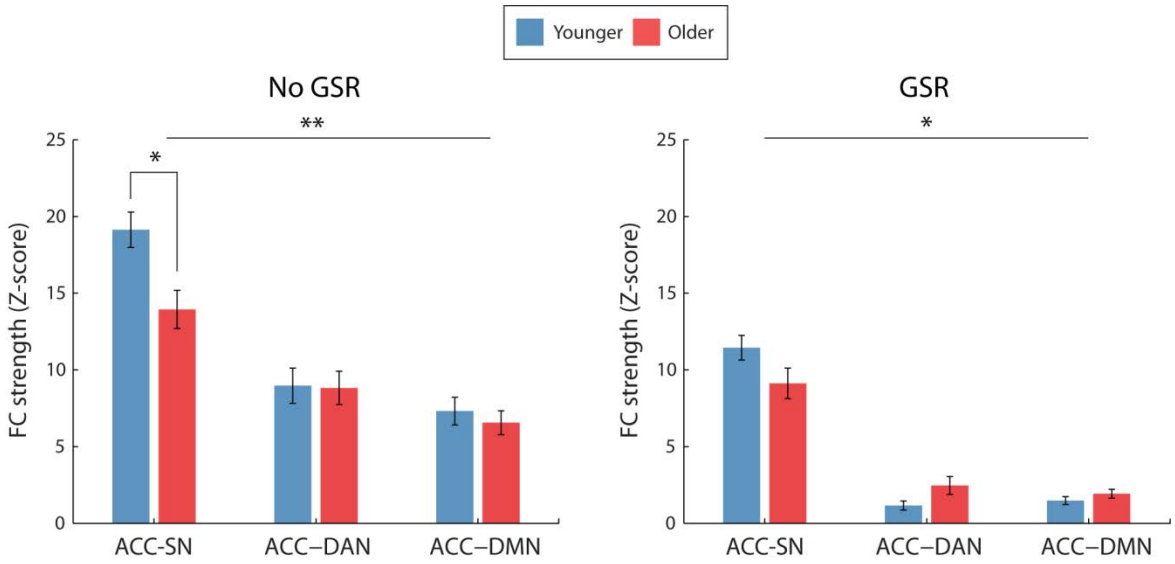

Figure 13: A comparison of ACC- RSN FC between the two age groups, calculated without (left) and with GSR (right). Error bars are standard error across participants. Asterisks depict significant age*network interactions (above horizontal lines) and pairwise comparisons, to compare how GSR affects group differences. *p<0.05, **p<0.01.

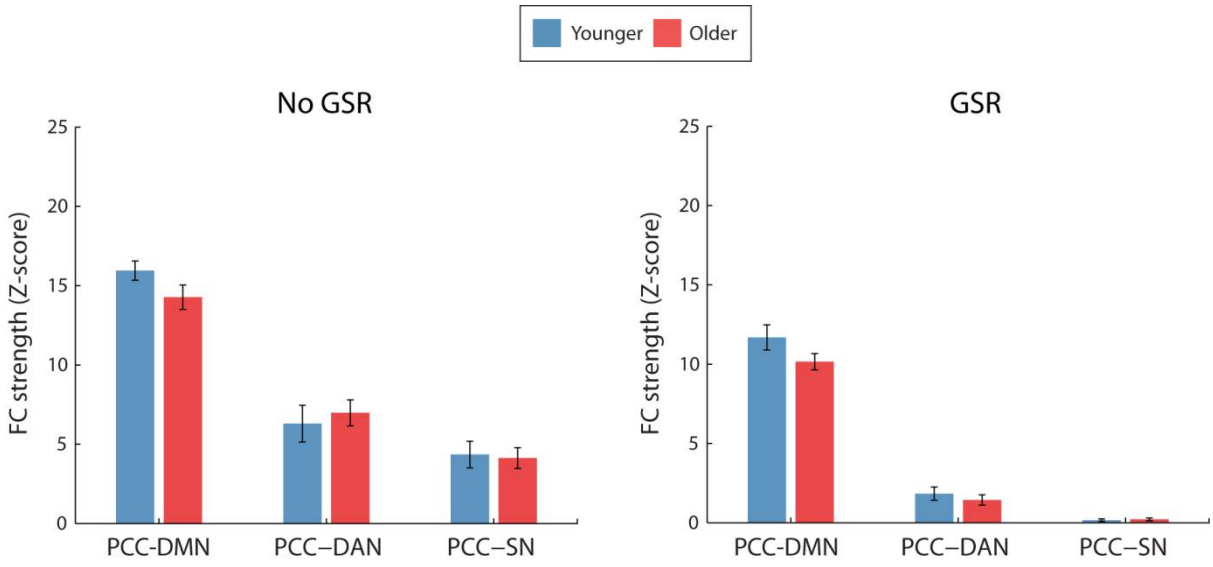

Figure 14: A comparison of PCC- RSN FC between the two age groups, calculated without (left) and with GSR (right). Error bars are standard error across participants.

3.3.3.2.1.1 Cortical ROIs by gender

Male participants

Results from the ANOVA for male participants only echoed the results from the ACC-network ANOVA for the groups as a whole. GSR resulted in significantly reduced FC compared to no GSR, as indicated by a significant main effect of method ($F(1,18)=105.12$, $p<0.001$, $\eta^2=0.85$). Pairwise comparisons following a significant network*method interaction ($F(1.89, 34.02)=4.37$, $p=0.02$, $\eta^2= 0.20$) revealed that all three ACC-RSNs were affected in this way ($p<0.001$). Again, NS method*age and network*method*age interactions ($F(1, 18)=0.19$, $p=0.66$, $\eta^2=0.01$ & $F(1.89, 34.02)=1.04$, $p=0.36$, $\eta^2=0.05$, respectively) suggested that the effect of GSR on ACC-RSN FC was not differentially affected by age. Therefore, although the previously identified ACC-DAN gender difference failed to reach significance without GSR, the general trend was the same (Figure 15).

Similarly, results from the PCC ANOVA for male participants revealed that GSR resulted in significantly reduced FC compared to no GSR, as indicated by a significant main effect of method ($F(1,18)=70.85$, $p<0.001$, $\eta^2=0.80$). Pairwise comparisons following a significant network*method interaction ($F(2,36)=$, $p=0.01$, $\eta^2= 0.22$) revealed that all three PCC-RSNs were affected in this way ($p<0.001$). However, NS method*age and network*method*age interactions ($F(1, 18)=0.66$, $p=0.43$, $\eta^2=0.04$ & $F(2, 36)=2.06$, $p=0.16$, $\eta^2=0.10$, respectively) suggested that the effect of GSR on ACC-RSN FC was not differentially affected by age for male participants (Figure 16).

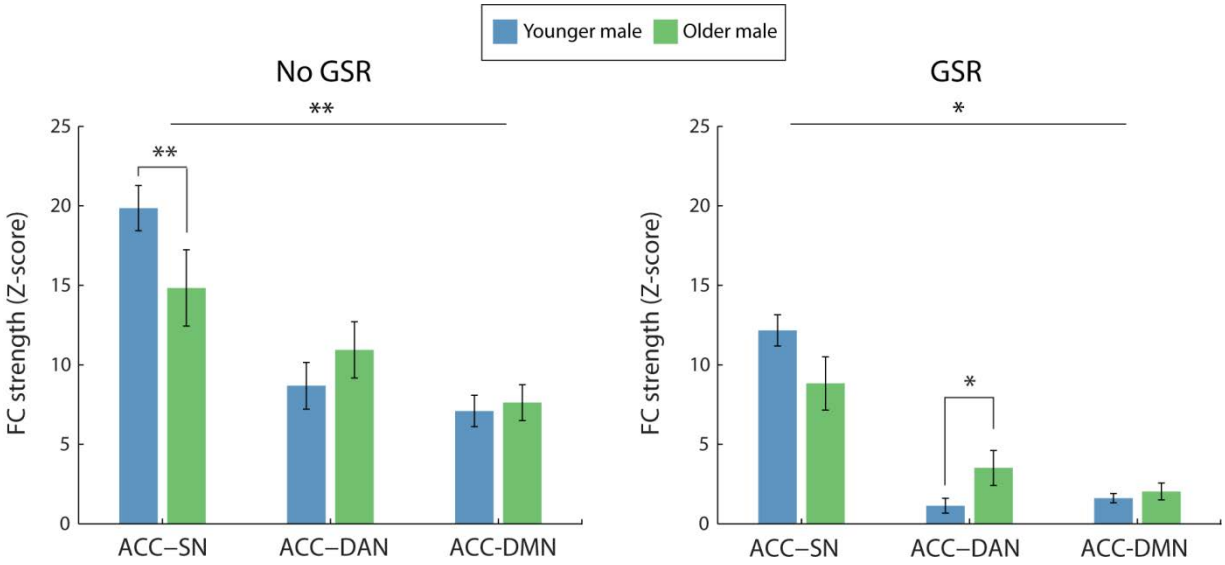

Figure 15: A comparison of ACC- RSN FC between the two age groups for male participants only, calculated without (left) and with GSR (right). Error bars are standard error across participants. Asterisks depict significant age*network interactions (above horizontal lines) and pairwise comparisons, to compare how GSR affects group differences. *p<0.05, **p<0.01.

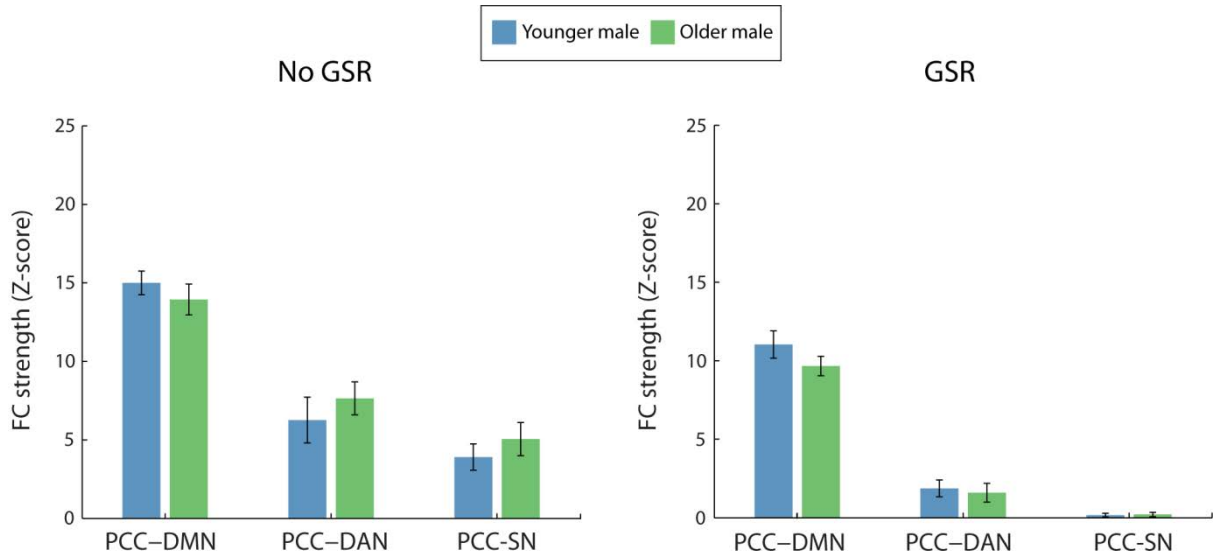

Figure 16: A comparison of PCC- RSN FC between the two age groups for male participants only, calculated without (left) and with GSR (right). Error bars are standard error across participants.

Female participants

Results from the ACC ANOVA for female participants revealed that GSR resulted in significantly reduced FC compared to no GSR, as indicated by a significant main effect of method ($F(1,18)=51.42$, $p<0.001$, $\eta^2=0.74$). Pairwise comparisons following a significant

network*method interaction (F(1.88, 34.02)=8.11, p=0.02, η^2= 0.31) revealed that all three ACC-RSNs were affected in this way (p<0.001). A trend suggested that the effect of GSR may have been greater dependent on age group (F(1, 18)=3.7, p=0.09, η^2=0.15), however, a NS network*method*age interaction (F(1.88, 3.75)=1.5, p=0.24, η^2=0.08) suggested that this effect was similar across networks. See Figure 17 for a comparison between the two GSR methods for female participants.

Similarly, results from the PCC ANOVA for female participants revealed a similar pattern. GSR resulted in significantly reduced FC compared to no GSR, as indicated by a significant main effect of method (F(1,18)=41.89, p<0.001, η^2=0.70). Pairwise comparisons following a significant network*method interaction (F(2,36)=2.45, p=0.10, η^2= 0.12) revealed that all three PCC-RSNs were affected in this way (p<0.001). However, NS method*age and network*method*age interactions (F(1, 18)=0.20, p=0.66, η^2=0.01 & F(2,36)=2.45, p=0.10, η^2=0.12, respectively) suggested that the effect of GSR on ACC-RSN FC was not differentially affected by age for female participants (Figure 18).

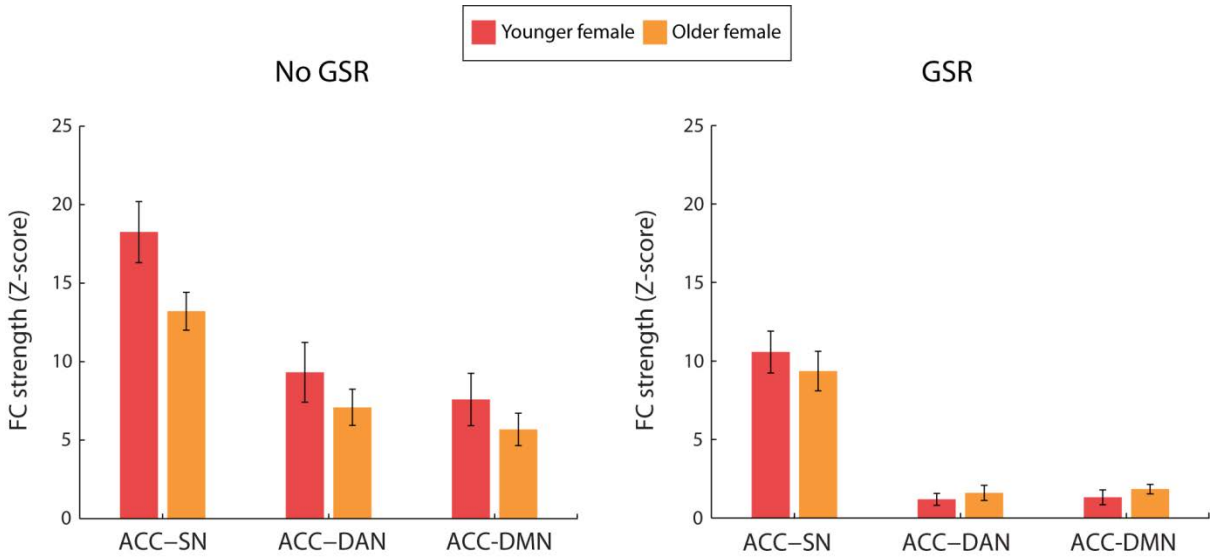

Figure 17: A comparison of ACC- RSN FC between the two age groups for female participants only, calculated without (left) and with GSR (right). Error bars are standard error across participants.

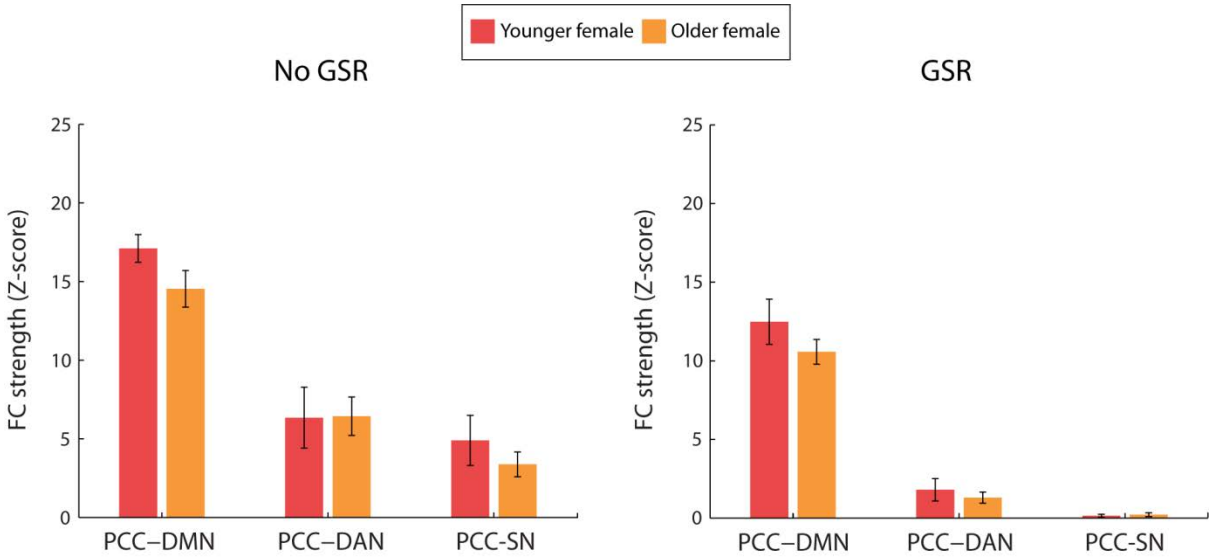

Figure 18: A comparison of PCC- RSN FC between the two age groups for female participants only, calculated without (left) and with GSR (right). Error bars are standard error across participants.

3.3.3.3 Thalamic FC

Finally, an ANOVA was applied to FC calculated between bilateral thalamus- auditory, motor and visual RSNs and again, revealed similar findings to those found for PCC and ACC. Applying GSR resulted in significantly reduced FC compared to not applying, as indicated by a significant main effect of method ($F(1,38)=165.52$, $p<0.001$, $\eta^2=0.81$). Pairwise comparisons following a significant network*method interaction way ($F(1.99, 75.51)=3.22$, $p<0.046$, $\eta^2= 0.08$) revealed that all three thalamic-sensory RSNs were affected in this way ($p<0.001$). Again, NS method*age and network*method*age interactions ($F(1, 38)=0.28$, $p=0.60$, $\eta^2=0.007$ & $F(1.99, 75.51)=0.22$, $p=0.81$, $\eta^2=0.006$, respectively) suggested that GSR does not differentially affect the FC between sub-cortical and cortical sensory regions for the two age groups (Figure 19). Differences between bilateral thalamus and each sensory RSN node are presented in Figure 20 to further highlight that specific differences in thalamic-sensory FC for the two age groups between primary/lateral regions persist, regardless of whether GSR is applied or not. For example, when GSR is applied, significant differences in

thalamo-sensory FC between the two age groups exist for left/right lateral visual regions, but not primary visual regions. Although less distinct, this same pattern exists when GSR is not applied. A similar distinction between primary motor and SMA is also seen.

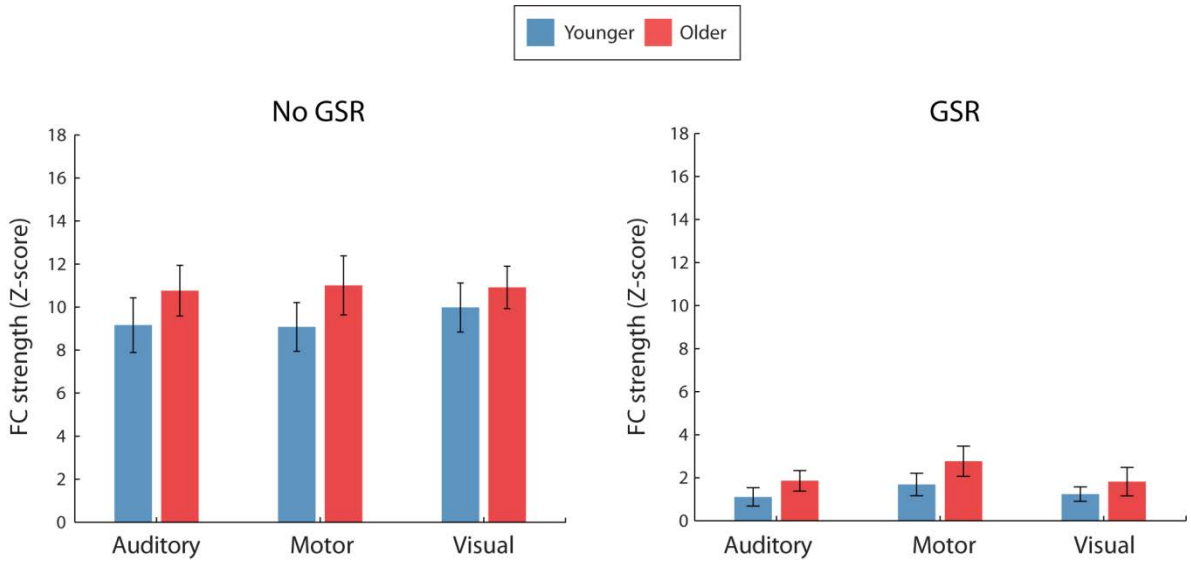

Figure 19: A comparison of thalamic-sensory RSN FC between the two age groups, calculated without (left) and with GSR (right). Error bars are standard error across participants.

3.4 Discussion

This chapter investigated differences between young and older subjects in two fundamental parameters for BOLD images, RSFA and GS, and their potential influence on FC.

3.4.1 RSFA

Our results provided evidence that older adults exhibited significantly reduced RSFA, and therefore likely reduced cerebrovascular reactivity (CVR), across nearly all cortical network nodes. This is in line with a number of previous studies which have reported reduced CVR in older adults as assessed by both PET and fMRI (Buijs et al., 1998; Kannurpatti et al., 2010; Leenders et al., 1990; Marchal et al., 1992; Martin et al., 1991; Nakano et al., 2000; Riddle et al., 2003b; Riecker et al., 2003; Strandgaard, 1991; Takahashi

et al., 2005). Controlling for differences in vasculature with age has been shown to reveal more reliable and specific task-BOLD differences between younger and older adults (Ances et al., 2009; Hamzei et al., 2003; Handwerker et al., 2007; Kannurpatti et al., 2014; Kannurpatti et al., 2011a; Thomason et al., 2005; Thomason et al., 2007; Tsvetanov et al., 2015), and has provided evidence that not controlling for differences in CVR may result in reductions in older adult's task-BOLD responses being falsely attributed to disrupted neural activity, when they are actually a result of underlying CVR differences (Riecker et al., 2003; Tsvetanov et al., 2015).

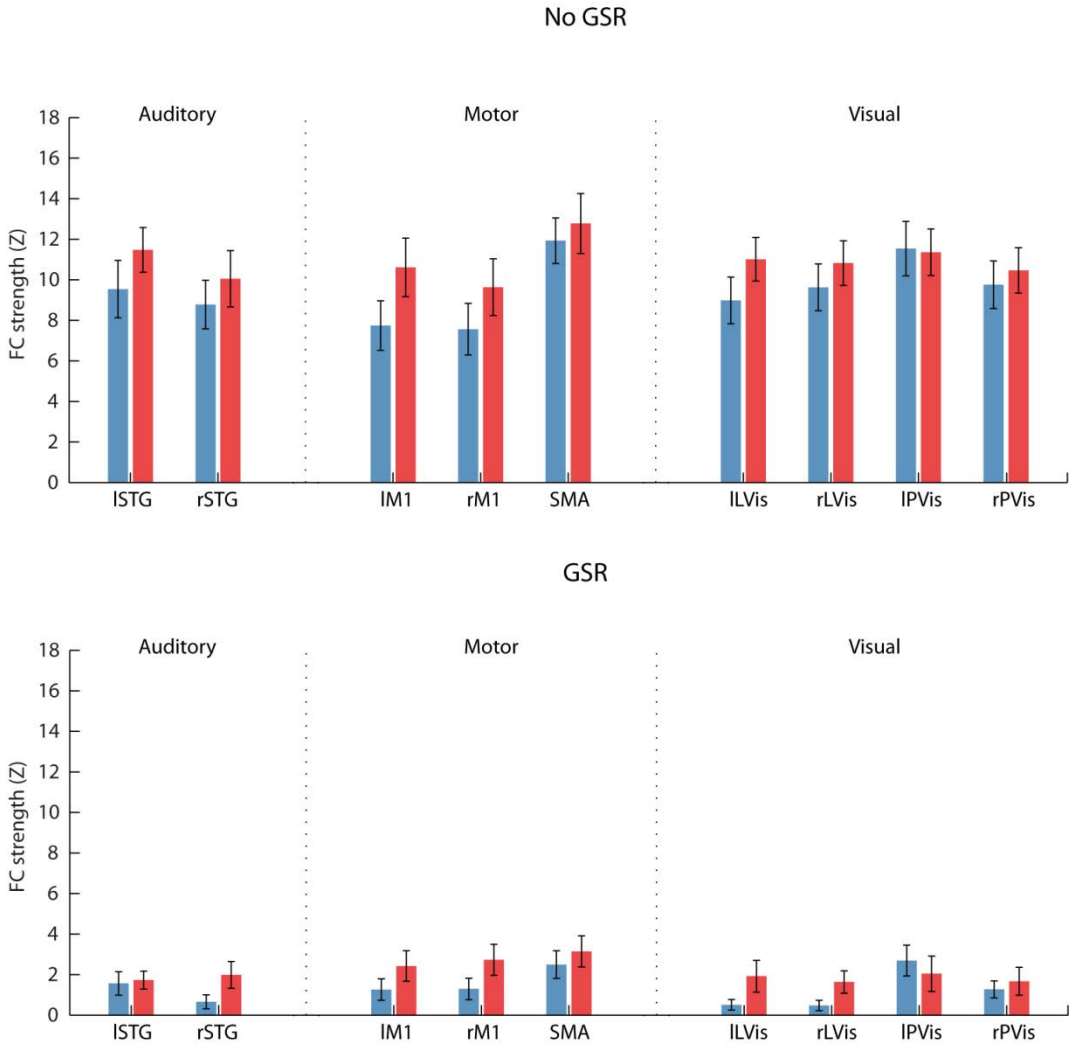

Figure 20: Thalamic-sensory RSN node FC is illustrates that the node-specific age-related differences in thalamo-sensory FC, persist for the two approaches. Error bars are SE.

Despite it being unnecessary to scale resting-state BOLD when calculating FC, to date, no studies have investigated whether age-related differences in CVR of RSN nodes are associated with age-related FC differences of those nodes. The results presented here provide evidence that for both young and older adults, node RSFA is not associated with node FC, as assessed by correlating main RSN node RSFA (e.g. PCC) with node FC (e.g. PCC-DAN). Furthermore, differences in RSFA between age groups for a given node were not associated with age differences in the FC of that node. These results suggest that despite RSFA differences between the two age groups for a number of the cortical nodes, these differences do not seem related to FC differences identified with age.

Furthermore, these results also highlight an interesting finding that despite differences in RSFA across the cortex, RSFA of sub-cortical regions did not differ between the two age groups. This could be a consequence of the close proximity of spatial regions to ventricles, which could result in noisier BOLD time-series for the two age groups, thus reducing the specificity of RSFA measure in detecting CVR differences in these regions. However, there are a number of reasons which suggest this may not be the case: 1) average CSF signals are regressed from the resting-state data before RSFA is calculated, thus reducing their impact on grey matter voxel time-series 2) older adults have considerably larger ventricles in comparison to younger adults, so if RSFA of these regions were linked to noise from ventricles, one might expect there to be a significant difference between the two groups 3) average RSFA of subcortical regions is not significantly different to that of cortical regions, which again, one might expect not to be the case if subcortical time-series contained substantially more noise compared to cortical. Future research should look to investigate the potential differences in CVR of cortical/subcortical regions between age groups using potentially additional measures of CVR, such as perfusion or BH data. Furthermore, if it is the case that RSFA differences between age groups are regionally specific in this way, it

suggests against the use of global measures of CVR and instead the importance of scaling fMRI data on a region or voxel level. Although the evidence specifically investigating whether there are regional differences in the way age affects cerebrovasculature is currently lacking, studies suggest that the effect of ageing is heterogeneous across the arterial tree (Lee & Oh, 2010). Future work establishing that particular cerebral vessels and arteries are affected by age would support the argument for regional/voxel based CVR correction between age groups.

Here we provide evidence that although older adults exhibited significantly reduced CVR across the majority of cortical regions, compared to younger adults, these differences do not seem associated with age-related FC differences identified for these regions. For this reason we do not consider differences in RSFA further in terms of FC results, but it is important to keep in mind that these differences do exist and may affect FC between groups in a way that is not easy to assess given the current data set consisting of BOLD time series. Nonetheless, differences in CVR with age are often overlooked when comparing BOLD responses between age groups and warrant further investigation in terms of both task-fMRI and FC measures. Although the vast majority of studies have provided evidence for coupling between RSFA and CVR, some authors have suggested that RSFA measures may not be as reliable as direct measurement of end-tidal CO2 levels (Lipp et al., 2015; Murphy et al., 2011; Scouten & Schwarzbauer, 2008). However, additional equipment, analyses and demand on the participant (wearing a nasal cannula) means this approach is often unfeasible. As the field of ASL and multi-band imaging advance (Chen et al., 2015; Francis & Panchuelo, 2014), these methodologies will allow us to more easily assess the differences between CVR in different populations, using more direct measures of blood flow without the need for monitoring CO2 levels, and the contribution of these factors to age-related differences identified for both measures of FC and task fMRI.

3.4.2 GSR

The results presented here suggest that applying GSR shifts FC strength downwards in a global manner. Although the extent of this shift may differ between networks (e.g. the reduction in PCC-DMN is smaller compared to the reduction for PCC-DAN FC, see Figure 14), it is not systematically different for the two age groups. To a large extent, the greatest age-related FC differences identified when applying GSR show similar patterns when GSR was not applied, suggesting that the overall distribution of FC strengths is not warped by GSR in this dataset, contrary to the suggestion presented by Gotts and others (2013b). Likewise, even FC differences identified between genders for the two age groups, where sample sizes were smaller, showed similar patterns using the two GSR methods. However, significant age*gender differences were only identified after applying GSR, which might indicate the additional specificity that is considered the primary reason to perform it (Fox et al., 2009; Kruschwitz et al., 2015a).

Currently, a definitive conclusion regarding whether or not to apply GSR for studies of FC is still lacking. Even after physiological correction (e.g. methods such as RETROICOR) and confound regression of motion parameters, white matter and CSF signals, a large amount of GS correlation across the brain still persists. GS clearly consists of multiple sources, some of which are artifacts (physiological noise, motion, scanner instabilities) and some of which are likely to be of neuronal origin and may even represent global network activity of interest. When investigating FC it is imperative that any global noise which may confound FC analysis of RSNs is removed. This is particularly important when comparing between groups where global confounds may differ for the two groups and thus induce false FC differences if not controlled for. However, currently there is no clear method which will allow for the clean removal of global artifacts which do not contain meaningful signal. The number of studies which have provided evidence for GSR improving the sensitivity and reliability of FC,

especially compared to anatomical and MEG findings, suggests that it is a necessary pre-processing step, particularly when investigating sub-cortical structures.

However, it is important to be mindful of the limitations associated with GSR. There is evidence that GSR does skew FC distributions negatively (Murphy et al., 2009; Weissenbacher et al., 2009), however, Keller and colleagues (2013) provided evidence that negative FC (identified with and without GSR) does have neuronal origins. Furthermore, Weissenbacher and colleagues (2009) reported that when simulated data contained anti-correlations, both GSR and no GSR methods identified them, suggesting that comparison of negative FC before and after GSR allows identification of 'true' anti-correlated networks, which are not purely a function of GSR. Given these results, it seems sensible to ignore negative FC if applying GSR, or only focussing on those negative connections identified with both methods. Similarly, understanding that GSR may enhance group differences is also worth keeping in mind (Gotts et al., 2013b; Gotts et al., 2012; Saad et al., 2012b). For this reason, the process of comparing results both with and without GSR may be informative when comparing groups. However, with no ground truth it remains difficult to know which patterns of FC differences are 'true' and which may be a function of pre-processing strategies for the majority of studies.

3.4.3 Conclusion

In conclusion, this chapter provides evidence that although age-related differences in CVR, as assessed by RSFA, exist these differences are not associated with age-related FC differences. Therefore, although it is important to be mindful of such CVR differences and consider that they may affect FC in ways that are undetected here, it is unclear how else to investigate the impact of such differences, or even how to control for them based upon the currently available data. For these reasons, differences in RSFA between age groups are not

investigated further in the following chapters. Furthermore, GSR was not found to differentially affect FC between the two age groups and the spatial extent of GSR correlation and voxel time-courses did not differ between age groups, suggesting a common GS for the two groups. For these reasons, we took the decision to apply GSR for this dataset, ignoring all negative correlations, in an attempt to reveal more specific age-related FC differences.

CHAPTER 4. GENDER SPECIFIC RE-ORGANISATION OF RESTING-STATE NETWORKS WITH AGE.

ABSTRACT

Recently, the suggestion that alterations in brain connectivity may drive disruption in cognitive abilities with age has been investigated. However, the interaction between the effects of age and gender on the reorganisation of RSNs is not fully understood. This study sought to investigate the effect of both age and gender on intra- and inter-network FC and the extent to which RSN node definition may alter with older age. The FC of three main cortical networks: DAN, DMN and SN, was assessed. Older adults exhibited reduced DMN intra-network FC and increased ACC inter-network FC in comparison to younger participants, which was driven largely by male participants. However, further analyses suggested that the spatial location of ACC, bilateral anterior insula and orbitofrontal cortex RSN nodes changed with older age and that age-related gender differences in FC may reflect spatial re-organisation rather than increases or decreases in FC strength alone. These differences in both the FC and spatial distribution of RSNs between younger and older adults provide evidence of reorganisation of fundamental brain networks with age, which is modulated by gender. These results highlight the need to further investigate changes in both intra- and inter- network FC with age, whilst also exploring the modifying effect of gender. They also emphasise the difficulties in directly comparing the FC of RSN nodes between groups and suggest that caution should be taken when using the same RSN node definitions

for different age or patient groups to investigate FC. This chapter contains material that is now published (Goldstone et al., 2016).

4.1 INTRODUCTION

In addition to the influence which advancing age has on the brain, it is well established that gender differences in brain structure, chemistry and function exist (Cosgrove, Mazure, & Staley, 2007; Ingalhalikar et al., 2014; Luders, Gaser, Narr, & Toga, 2009; Ruigrok et al., 2014). A study by Sowell and colleagues (2007) reported that reductions in cortical thickness with age, within dorsal frontal and temporal regions, were modulated by gender. Others have reported similar significant age*gender interactions for grey (Gur, Gunning-Dixon, Turetsky, Bilker, & Gur, 2002; Nunnemann et al., 2009; Raz et al., 1997) and white (Riello et al., 2005) matter volume. However, studies have also failed to find such interactions (Greenberg et al., 2008; Lemaitre et al., 2005; Salat et al., 2004) and a recent study reported that controlling for brain size resulted in a substantial decrease in the effect of gender (and gender*age interactions) on brain volume (Jäncke, Mérillat, Liem, & Hänggi, 2015). Given these differences in brain structure associated with gender, it is likely that gender also influences brain function. A number of studies have indeed identified such modulations (Allen et al., 2011; Biswal et al., 2010; Filippi et al., 2013; Smith et al., 2014). During task fMRI studies, it is commonly reported that males show increased recruitment of parietal networks, while females typically recruit more frontal networks (Bell, Willson, Wilman, Dave, & Silverstone, 2006; Christakou et al., 2009; Hill, Laird, & Robinson, 2014; Rubia et al., 2013). In addition, Hong and colleagues (2014) reported that males had greater anterior insula (AI) intra-SN and inter-SN (AI – MPFC) FC. Similarly, a number of studies have reported gender differences in the lateralisation of RSNs, a typical finding being that the function of male brains was more strongly lateralised in comparison to female brains (Agcaoglu, Miller, Mayer, Hugdahl, & Calhoun, 2015; Liu, Stufflebeam, Sepulcre, Hedden, &

Buckner, 2009). However, others have reported that gender has a relatively small effect on RSNs (Bluhm et al., 2008; Lopez-Larson, Anderson, Ferguson, & Yurgelun-Todd, 2011) while some studies have found a lack of significant gender differences in RSNs (Nielsen, Zielinski, Ferguson, Lainhart, & Anderson, 2013; Weissman-Fogel, Moayedi, Taylor, Pope, & Davis, 2010). Studies that have investigated the effects of both advancing age and gender on FC have largely been limited to investigation of lateralisation of RSNs. Recently, Agcaoglu and colleagues (2015) assessed voxel-wise laterality of RSNs of more than 600 participants and identified that age differentially affected lateralisation of RSNs for the two sexes. Similarly, Zuo and others (2010) identified a general decrease in homotopic FC (FC between any pair of symmetric interhemispheric voxels) with age in higher order cognitive RSNs and an increase in sensory/motor RSNs. However, they also identified differences in homotopic FC that were dependent on gender.

It is clear that both age and gender modulate patterns of resting-state FC; however few studies have included gender when investigating how age may modulate intra- and inter-network FC. Furthermore, to date, no studies have specifically investigated whether the spatial location of RSN nodes differs with older age. Considering that age-related differences in grey matter are not homogeneous across the brain (Raz et al., 1997; Sowell et al., 2003; Tisserand et al., 2004), it is plausible that the spatial extent of particular RSN nodes, or indeed their location, may alter with age. It thus remains to be seen whether age-related FC differences are driven by changes to the connections between nodes or changes to RSN definitions, or both.

4.1.1 Chapter objectives

Our study aimed to comprehensively investigate the interactions between age and gender on both intra- and inter- network FC of the DMN, DAN and SN. For this, we

investigated FC using 1) the same node definitions for the two age groups (defined using data from an independent sample of participants) and 2) nodes defined separately for the two groups. We focussed our analysis on these RSNs as advancing age has been associated with disrupted FC of both the DAN and DMN (Andrews-Hanna et al., 2007; Zhang et al., 2014a), while the SN is thought to be responsible for switching between the DMN and task-positive networks (Sridharan et al., 2008) and has also been shown to be affected by age (He et al., 2013; Onoda et al., 2012). While this approach may not capture all possible changes in functional connectivity associated with age and gender, we were motivated in our analysis by the benefits in terms of interpretability and comparability with previous studies. We concentrated on these 'cognitive' RSNs as the primary behavioural changes that occur with age tend to be in higher order cognitive functions, rather than basic sensory processing. However, we also present an overview of the results of the sensory RSNs, for completeness.

4.2 METHODS

4.2.1 Functional connectivity measures

In order to reduce the number of pairwise comparisons in our FC measures, we calculated composite intra- and inter-network FC for each participant. Intra-network measures consisted of averaging the FC strengths of each pair of nodes within a network (i.e. for DMN we averaged: PCC-all other DMN nodes, mPFC-all other DMN nodes, left and right IPL-all other DMN nodes, left and right MTL-all other DMN nodes). Inter-network FC strengths (ACC-DAN, ACC-DMN, right insula-DAN, right insula-DMN, PCC-DAN, PCC-SN) were calculated by averaging the FC strengths between the main node of each network, as identified previously (Buckner et al., 2008; Buckner et al., 2009; Leech, Braga, & Sharp, 2012; Seeley et al., 2007), and all nodes of the other network. For example, ACC-DAN FC was calculated by averaging FC strengths across all ACC-DAN node pairs. We chose to limit our

FC analysis in this way for brevity and specificity. Seeding from each node of the DMN separately would have resulted in six measures for both DMN-SN and DMN-DAN inter-network FC, making it harder to statistically compare and interpret differences between the two age groups. The main nodes we chose to focus our analysis on have all been consistently previously identified as main nodes of their corresponding networks. Figures 29a and 29b show FC between each of these seed regions (ACC and PCC respectively) and all other individual nodes of each network. These figures illustrate that the average inter-network FC measures (Figures 25 and 27) are representative of the patterns of age-group differences in FC that are seen at the individual seed node level for each RSN. For example, the average inter-network FC from the ACC increases with age with the DAN (Figure 25), and the same pattern is seen for the individual nodes that comprise the DAN (Figure 29a). We chose to calculate inter-network FC in this manner as our initial analyses suggested that inter-network FC differed depending on the main node chosen for analysis. E.g. ACC-node inter-network FC differed to right AI-node inter-network FC (Figures 29a and 29c), even though they were nodes of the same network (SN) and their intra-network FC was similar. If we had calculated inter-network FC in the same way as intra-network FC (e.g. for SN inter-network FC: averaged ACC-all nodes, right AI-all nodes, left-AI-all nodes) we would have missed the specific age-related ACC inter-network FC effect.

4.2.2 Assessing changes in spatial location of ROIs in older adults

We also assessed whether any observed differences in the strength of FC between age groups arose as a consequence of changes in the centre point of the RSN nodes with older age. For this, RSN nodes were redefined for older participants using the spatial components from a separate group ICA (gICA) of only the older participant's data. While this gICA includes fewer subjects than that used for the original node definition, it has the advantage

of allowing a direct examination of the spatial location of RSN nodes in this specific group of older subjects. For node definition, the same methods were applied as for the independent cohort, except data were decomposed into 15 (rather than 20) spatially independent components with MELODIC to identify the RSNs most comparable to the initial analysis. Restricting this data set to 20 components resulted in the RSNs splitting into multiple components, presumably because of the smaller sample size compared to the independent cohort of younger participants used in the original analysis. As with the definition of the younger RSN nodes, the components corresponding to the DAN, DMN and SN were visually identified and thresholded at a Z-statistic > 4, based on previous methodology (Khalsa et al., 2013), to ensure that each of the RSN nodes were spatially distinct. Cubic ROIs were then centred on the peak voxel (as was done for the younger ROI node definition) for each of the nodes defined from this separate gICA (*Table 11*). Table 11 also presents the spatial differences in peak voxel location for the two methods of RSN node definition (i.e. independent cohort vs. defined specifically within the older cohort). Table 12 reports average final ROI sizes for the two ROI definition methods. Using these alternative ROIs we re-assessed 1) intra-network 2) ACC inter-network and 3) PCC inter-network FC in the older subjects and compared it with FC measures obtained using the young ROIs. This allowed us to investigate whether any age-related FC differences were a result of changes in FC strength or a spatial re-organisation of RSN nodes in older adults.

4.2.3 Statistical analysis

IBM SPSS Statistics for Windows (Version 20.0) was used to conduct mixed design ANOVAs with the factors age and network and the interaction term age*network to assess differences in intra- and inter-network FC between the two age groups. In addition, we also used this ANOVA configuration to assess whether age modulated FC differentially for the

two genders. Finally, mixed design ANOVAs with the factors gender and network and the interaction term gender*network were uses to assess sex differences in FC within the two age groups.

4.2.3.1 Potential group confounds

Initial analyses were run to establish whether there were any significant differences in age between the female and male sub-groups for the two age groups, which could have confounded any effect of gender on FC. Splitting the younger group into female and male participants resulted in sub-group sizes of n=9 and n=11 and average ages of 27 (± 3.11) and 26 (+/- 3.95) years, respectively. Splitting the older group into female and male participants resulted in sub-group sizes of n=11 and n=9 and average ages of 73 (± 5.02) and 74 (± 4.87) years, respectively. Results of one-way ANOVAs revealed that there were no significant differences in ages between female and male participants for both younger ($F(1, 18)=0.63$, $p=0.44$) and older ($F(1,18)=0.13$, $p=0.73$) participants.

Table 11: MNI co-ordinates of the peak voxel for each RSN node, around which 5x5x5 voxel ROIs were created, defined using: 1) an independent cohort of 55 young participants (aged 25±4yrs); and 2) only the older participant's data. The final column reports the difference between the two sets of co-ordinates, for each direction.

	Independent cohort			Older gICA			Difference		
	x	y	z	x	y	z	x	y	z
DAN									
Left IPS	67	39	60	64	36	60	-3	-3	0
Right IPS	25	37	61	29	37	58	4	0	-3
Left OFC	71	75	52	67	84	48	-4	9	-4
Right OFC	21	83	45	22	70	52	1	-13	7
DMN									
PCC	45	37	53	45	35	52	0	-2	-1
mPFC	45	89	39	45	92	43	0	3	4
Left IPL	71	29	55	70	30	51	-1	1	-4
Right IPL	19	29	55	21	30	53	2	1	-2
Left MTL	77	58	27	75	62	28	-2	4	1
Right MTL	19	64	21	14	63	25	-5	-1	-4
SN									
ACC	45	76	51	43	70	59	-2	6	8
Left AI	65	71	37	73	66	39	8	-5	2
Right AI	27	75	37	26	68	38	-1	-7	1

Table 12: Final ROI size (group mean number of voxels and standard deviation across participants) after transforming ROIs into individual space and selecting only grey matter voxels, are displayed. Average ROI sizes are reported for the nodes defined using an independent cohort of 55 young participants (columns two and three) and only the older participant's data (column four). Significant differences in ROI size between young and older adults are highlighted (***p<0.001, **p<0.01). Partial eta squared (η^2) is also displayed for each significant comparison. Spatial location of ROI peak voxels for young and older definitions are plotted in Figure 21.

	Average ROI size			
	Younger	Older	Older (re-defined)	η^2
DAN				
Left IPS	59 ± 8.81	49 ± 6.35 ***	48 ± 6.95 ***	0.33
Right IPS	61 ± 5.94	53 ± 6.14 **	42 ± 7.55 ***	0.58
Left OFC	59 ± 6.83	51 ± 8.06 **	54 ± 8.21	0.15
Right OFC	62 ± 5.81	55 ± 9.50 **	53 ± 6.20 **	0.23
DMN				
PCC	79 ± 7.18	67 ± 8.76 ***	70 ± 7.67 **	0.28
mPFC	85 ± 6.45	66 ± 8.74 ***	55 ± 9.55 ***	0.68
Left IPL	53 ± 10.14	49 ± 9.63	59 ± 8.34	0.18
Right IPL	47 ± 8.37	38 ± 7.17 **	58 ± 6.91 ***	0.53
Left MTL	69 ± 7.04	52 ± 13.69 ***	53 ± 11.90 ***	0.31
Right MTL	68 ± 6.57	52 ± 12.68 ***	43 ± 12.79 ***	0.46
SN				
ACC	75 ± 6.91	42 ± 12.73 ***	52 ± 10.29 ***	0.70
Left AI	83 ± 7.52	61 ± 7.49 ***	57 ± 7.04 ***	0.68
Right AI	85 ± 7.51	65 ± 10.06 ***	62 ± 9.51 **	0.65

4.3 RESULTS

4.3.1 Spatial reorganisation of RSN nodes in older adults

Qualitative comparison of the locations of the RSN nodes' peak Z-statistic voxels between the two age groups suggests that the centre of a number of the RSN nodes may shift with age. This was particularly apparent for left and right OFC, AI, and ACC (see Figure 21). Using the ROIs defined specifically for the older adults, we re-evaluated RSN intra-network FC and inter-network FC of two main RSN nodes (ACC, PCC) (see below). We identified that by calculating older FC using the age-group specific ROIS, the differential effect of age on the two genders was attenuated. This suggests that the spatial reorganisation of RSN nodes may be gender specific, in addition to the gender specific effects on FC strength, as seen above for the original node definitions. Specific results are presented below.

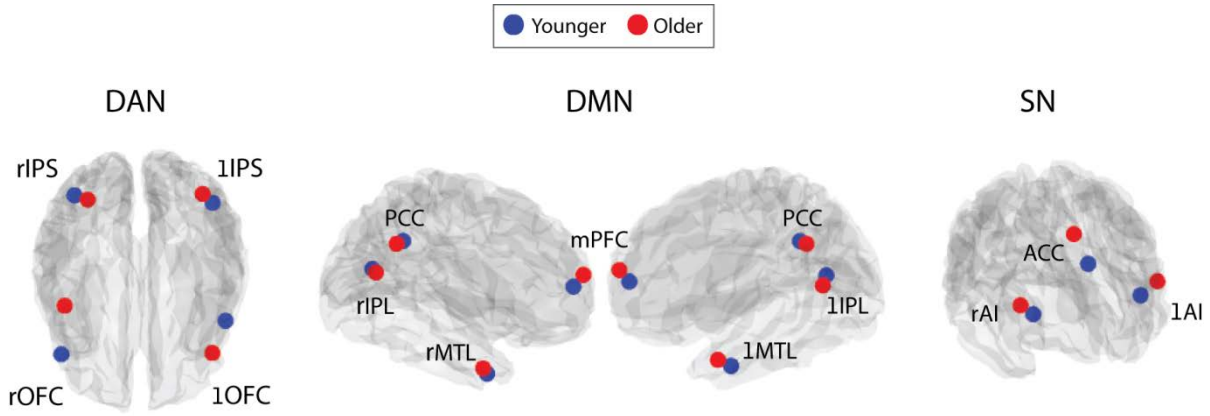

Figure 21: Depiction of the peak z-statistic voxel location, around which 5x5x5 voxel ROIs were made, for each RSN node. These were defined from gICAs performed on: an independent cohort of fifty-five younger participants (blue); and twenty older participants (red). L and R prefixed before node names indicates the left and right hemispheres. MNI co-ordinates for these voxels are presented in Table 12.

To compare the spatial location of nodes defined in young and old subjects we thresholded the DMN, DN and SANIC maps with a Z-score of 4 (as with the original node masks) and isolated each of the individual nodes and present overlap between young and old definitions for all nodes in Figure 22.

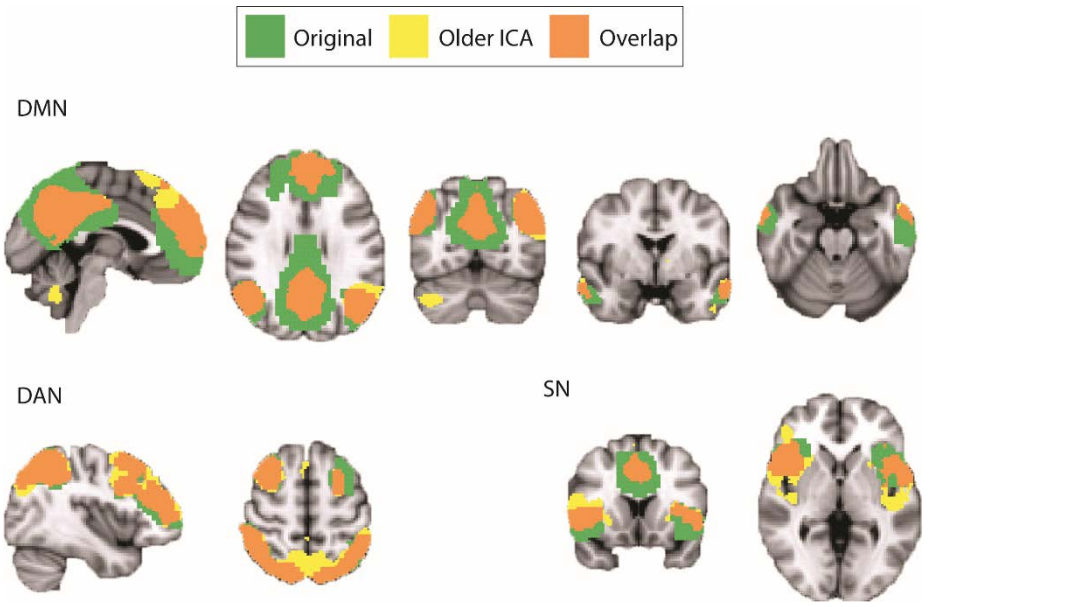

Figure 22: Depiction of the original nodes used in our analyses (defined in an independent cohort of 55 participants) are depicted in green and nodes generated from the group ICA (conducted on the 20 older participants in our sample) are depicted in yellow. Finally, voxels that overlap between the two methods are shown in yellow. All masks are displayed in MNI space.

4.3.2 Functional connectivity

Results are presented first for the original node definitions produced from the independent younger cohort, followed by those obtained from the older participants. All significant age and gender effects on FC can be found in Table 13.

Table 13: Significant statistical effects of age (Younger Y vs Older O) and gender on intra- and inter- network FC measures, following FC analysis using 1node definitions defined from the younger, independent cohort,, and 2nodes defined separately for the older adults.

		F	P	η^2
Intra-network FC (group)				
Main effect: Age[1]	Y>O	10.606	0.002	0.218
Intra-network FC (male)				
Main effect: age[1]	Y>O	8.18	0.01	0.312
ACC inter-network FC (group)				
Interaction: age*network[1]		4.48	0.026	0.106
Interaction: age*network[2]		7.33	0.004	0.16
	ACC-DAN (O>Y)		<0.001	0.46
	ACC-DMN (Y>O)		0.006	0.18
ACC inter-network FC (male)				
Interaction: age*network[1]		4.55	0.028	0.202
	ACC-DAN (O>Y)		0.047	0.20
Interaction: age*network[2]		4.76	0.024	0.21
	ACC-DAN (O>Y)		0.008	0.33
ACC inter-network FC(female)				
Interaction: age*network[2]		4.17	0.042	0.19
	ACC –DAN (O>Y)		<0.001	0.60
	ACC-DMN (Y>O)		0.03	0.24

4.3.2.1 Intra-network FC

4.3.2.1.1 Young ROI definitions

Older participants were found to have reduced intra-network FC compared to younger adults, as revealed by a significant main effect of age (F(1,38)=10.606, p=0.002, η^2=0.218). No significant interaction between age and network was found (F(2,76)=0.214,p=0.808, η^2=0.006), suggesting that this reduction in intra-network FC with age was not specific to any one RSN.

4.3.2.1.2 Older ROI definitions

Following the re-definition of the older RSN nodes, we found no significant intra-network FC differences between age groups, as indicated by a NS main effect of age and a NS age*network interaction (F(1,38)=1.73, p=0.20, η^2=0.04 & (F(2,76)=1.03, p=0.36, η^2=0.026, respectively). These results diverged from previous findings (see Figure 23) that older adults exhibited significantly weaker intra-network FC, obtained when using the same ROIs for both age groups.

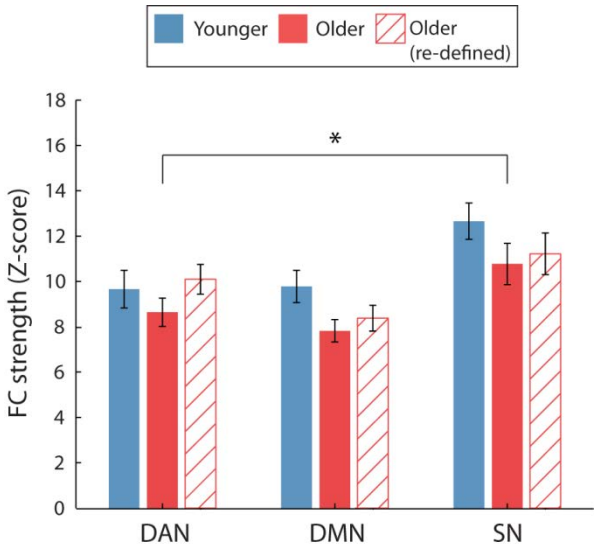

Figure 23: Average intra-network FC for the two age groups. For FC calculated using the young ROIs only (solid bars), a significant main effect of age identified that intra-network FC was weaker for older, compared to younger participants (depicted by a horizontal line, *p<0.05). No significant age-group difference was identified when FC was calculated using age-group specific ROIs for the older participants (hatched bars). Error bars are SEM calculated across participants.

4.3.2.2 Intra-network FC: sex differences

4.3.2.2.1 Young ROI definitions

Female vs. male: by age

We first investigated whether there were any sex differences in intra-network FC within the two age groups. For both age groups, there was no evidence to suggest that intra-network FC was significantly different between genders. This was evident by the lack of a significant main effect of gender (F(1,18)=0.350,p=0.562, η^2=0.019 &

F(1,18)=0.054,p=0.819, η^2=0.003) and gender*network interactions (F(2,36)=1.739,p=0.190, η^2=0.088 & F(2,36)=0.440,p=0.648, η^2=0.024).

Younger vs. older: by gender

We then sought to identify whether age differentially modulated intra-network FC differences for the two sexes. For the females, age did not modulate intra-network FC and nor was there a significant difference in intra-network FC strength across the three networks, as revealed by the lack of a significant main effect of age (F(1,18)=2.863,p=0.108, η^2=0.137) or a significant interaction of age*network (F(2,36)=1.66,p=0.205, η^2=0.084).

For males, intra-network FC was significantly reduced in older, compared to younger, participants, as indicated by a main effect of age (F(1,18)=8.18,p=0.01, η^2=0.312). No significant age*network interaction was identified (F(2,36)=0.209,p=0.812, η^2=0.011), indicating that in male participants age affected intra-network FC in a global manner , rather than on a network-specific level. See Figures 24a and 24b for intra-network FC for the two sexes, divided by age group.

4.3.2.2.2 Older ROI definitions

The previous finding which suggested that the reduction in intra-network FC was specific to male participants was not fully replicated (see Figure 24). Although a similar trend was identified for male participants, intra-network FC did not differ significantly between the age groups for either female or male participants. This was indicated by a NS main effect of age (F(1,18)=0.20, p=0.88, η^2=0.001 & F(1,18)=4.04, p=0.06, η^2=0.18) and age*network interactions (F(2,36)=2.57, p=0.09, η^2=0.13 & F(2,36)=0.66, p=0.52, η^2=0.04), for female and male participants respectively.

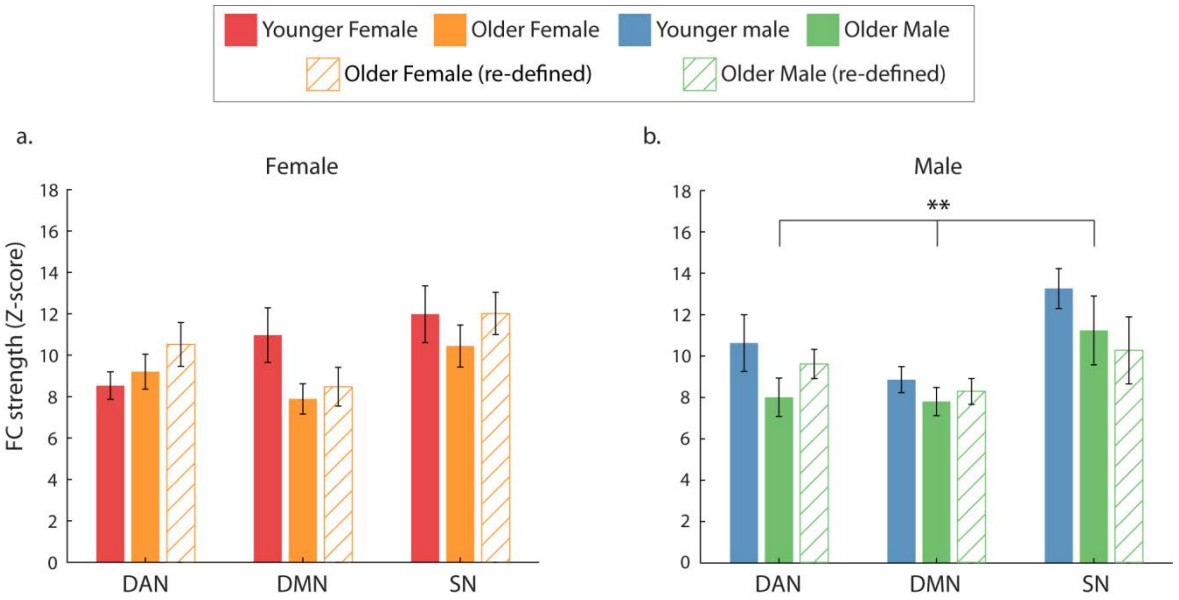

Figure 24: Average intra-network FC for the two age groups, split by gender. Figures 24a and 24b depict the effect of age on intra-network FC separately for female (24a) and male (24b) participants. When comparing FC using the young ROIs (solid bars), a significant main effect of age was identified for male participants only (**p=0.01, indicated by the horizontal line). However, no significant differences between the age groups were identified when FC was calculated using age-group specific ROIs for the older participants (hatched bars).

4.3.2.3 ACC inter-network FC

4.3.2.3.1 Young ROI definitions

Overall, inter-network FC was not significantly affected by age, as shown by a NS main effect of age ($F(1,38)=0.18, p=0.676, \eta^2=0.005$). A significant interaction effect showed that ACC FC was differentially affected by age, depending on the network ($F(2,76)=4.48, p=0.026, \eta^2=0.106$), with a trend for reduced ACC-SN FC and increased ACC-DAN FC in older adults (Figure 25). However, tests of simple effects revealed that taken independently, ACC-SN, ACC-DAN and ACC-DMN FC were not significantly different for the two age groups, ($p=0.078, \eta^2=0.08$ & $p=0.055, \eta^2=0.093$ & $p=0.250, \eta^2=0.035$ respectively), although the largest effect size was identified for ACC-SN, suggesting this inter-network FC differed the most with age.

4.3.2.3.2 Older ROI definitions

Following the re-definition of the older RSN nodes, we report greater ACC-DAN FC in older, compared to younger, adults as previously identified (see Figure 25). This was confirmed by a significant age*network interaction ($F(1.43, 54.3)=7.33$, $p=0.004$, $\eta^2=0.16$) and a significant pairwise comparison for ACC-DAN ($p<0.001$, $d=1.79$), we also identified weaker ACC-DMN ($p=0.006$, $d= -0.93$) FC for older, compared to younger adults. ACC-SN FC did not differ significantly between the two age groups ($p=0.8$, $d= -0.08$).

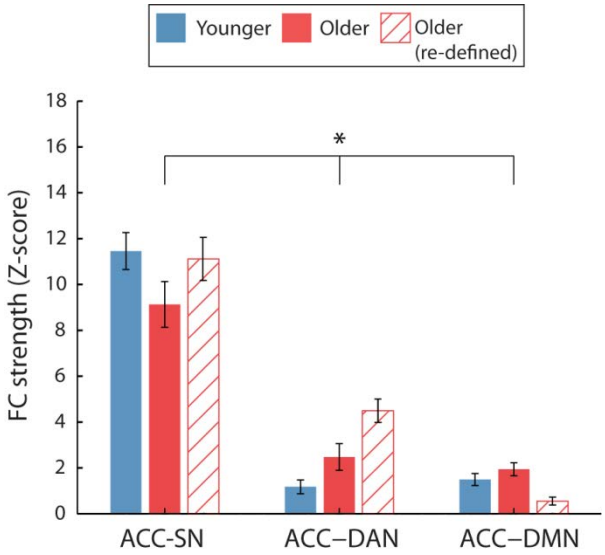

Figure 25: Average ACC inter-network FC for the two age groups. Inter-network FC is calculated by averaging FC between ACC and each node of the target network. For FC calculated using the young ROIs only (solid bars), a significant age*network interaction was identified (depicted by the horizontal line, *p<0.05). No significant age-group difference was identified when FC was calculated using age-group specific ROIs for the older participants (hatched bars). Error bars are SEM calculated across participants.

4.3.2.4 ACC inter-network FC: sex differences

4.3.2.4.1 Young ROI definitions

Female vs. male: by age

Within both age groups, female and male participants did not exhibit significantly different ACC-inter network FC. This was shown by the lack of significant main effects of gender ($F(1,18)=1.273$,$p=0.274$, $\eta^2=0.066$) & $F(1,18)=0.501$,$p=0.488$, $\eta^2=0.027$; for younger

and older participants respectively) and gender*network interactions (F(2,36)=0.620,p=0.475, η^2=0.033 & F(2,36)=0.755,p=0.445, η^2=0.04; for younger and older participants respectively).

Younger vs. older: by gender

For female participants, ACC inter-network FC was not significantly different between younger and older participants (Figure 26a). This was shown by a NS main effect of age (F(1,18)=0.129,p=0.885, η^2=0.001) and age*network interaction (F(2,36)=4.67,p=0.526, η^2=0.035).

For male participants, ACC inter-network FC was modulated by age. We identified no general effect of age (F(1,18)=0.07,p=0.794, η^2=0.004). However, we found that ACC inter-network FC was differentially affected by age, as shown by a significant age*network interaction (F(2,36)=4.55, p=0.028, η^2=0.202). Analysis of simple effects revealed that older men had significantly greater ACC-DAN FC compared to younger men (p=0.047, η^2=0.202 Figure 26b). No significant differences in FC with age were identified for ACC-SN or ACC-DMN (p= 0.09, η^2=0.151 & p= 0.469, η^2=0.02 respectively).

4.3.2.4.2 Older ROI definitions

Greater ACC-DAN FC was no longer specific to male participants as was previously identified (see Figure 26). Significant age*network interactions were identified for both female and male participants (F(1.33, 23.91)=4.17, η^2= 0.19 & (F(1.52, 27.41)=4.76, p=0.024, η^2=0.21, respectively). Pairwise comparisons revealed that both female and male older participants exhibited greater ACC-DAN FC, compared to their younger counterparts (p<0.001, η^2= 0.60 & p=0.008, η^2=0.33, respectively). Older female participants exhibited significantly weaker ACC-DMN FC compared to younger female participants (p=0.03,

η^2=0.24), while both younger and older male participants exhibited similar levels of ACC-DMN FC (p=0.134, η^2=0.12). ACC-SN FC did not differ significantly between the two age groups for both female (p=0.32, η^2=0.05) and male (p=0.20, η^2= 0.09) participants.

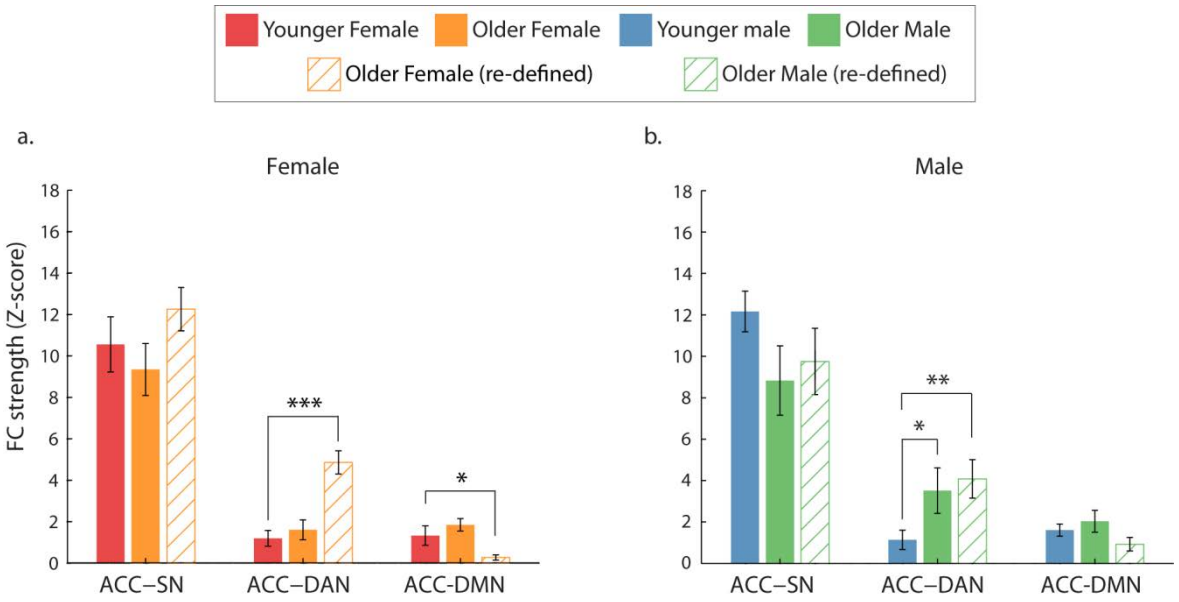

Figure 26: Average ACC inter-network FC for the two age groups, split by gender. Figures 26a and 26b depict the effect of age on ACC-network FC for female (26a) and male (26b) participants. When comparing FC using the young ROIs (solid bars), greater ACC-DAN FC was identified for older, compared to younger, male participants only. However, when FC was calculated using age-group specific ROIs (hatched bars), greater ACC-DAN FC was identified for older, compared to younger participants, for both males and females. In addition, weaker ACC-DMN FC was found for older, compared to younger, females using this method. An asterisk over a horizontal line depicts a significant pairwise comparison, *p<0.05, **p<0.01, ***p<0.001. Error bars are SEM calculated across participants.

4.3.2.5 PCC inter-network FC

4.3.2.5.1 Young ROI definitions

PCC inter-network FC was not significantly different between the two age groups, as indicated by a NS main effect of age (F(1,38)=3.468,p=0.07) and age*network interaction (F(2,76)=1.731,p=0.191, η^2=0.044). See Figure 27 for PCC-network FC.

4.3.2.5.2 Older ROI definitions

As was previously identified, PCC inter-network FC was not significantly different between the two age groups (See Figure 27), as indicated by a NS main effect of age ($F(1,38)=2,58, p=0.116, \eta^2=0.06$) and age*network interaction ($F(1.25, 47.46)=0.30, p=0.64, \eta^2=0.008$).

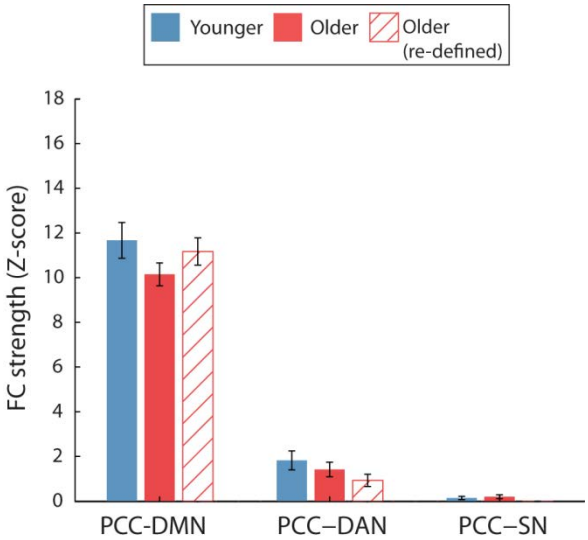

Figure 27: Average PCC inter-network FC for the two age groups, calculated using the young ROIs only (solid bars) and age-group specific ROIs for the older participants (hatched bars). No significant differences were identified using either method to define RSN nodes. Error bars are SEM calculated across participants.

4.3.2.6 PCC inter-network FC: sex differences

4.3.2.6.1 Young ROI definitions

PCC inter-network FC was not significantly different for female and male participants within age groups and neither did age differentially affect PCC inter-network FC for the two sexes (Figures 28a and 28b). This was identified by the NS effect of gender ($p=0.476, \eta^2=0.029$ & $p=0.563, \eta^2=0.019$ for younger and older participants respectively) and age ($p=0.200$, 0.089 $\eta^2=$ & $p=0.166, \eta^2=0.104$ for female and male participants respectively), as well as NS gender*network ($p=0.382, \eta^2=0.052$ and $p=0.684, \eta^2=0.021$ for younger and older

participants respectively) and age*network (p=0.251, η^2=0.074 & p=0.566, η^2=0.031 for female and male participants respectively) interactions.

4.3.2.6.2 Older ROI definitions

Similarly, in correspondence with the previous results, age was not found to differentially affect PCC inter-network FC for the two sexes (See Figure 28). This was identified by NS main effects of age (F(1,18)=1.55, p=0.23, η^2=0.08 & F(1,18)=1.32, p=0.27, η^2=0.07 and NS age*network interactions (F(1.20, 21.66)= 0.16, p=0.74, η^2=0.009 & F(1.35, 24.74)=0.16, p=0.77, η^2=0.009) for both female and male participants, respectively.

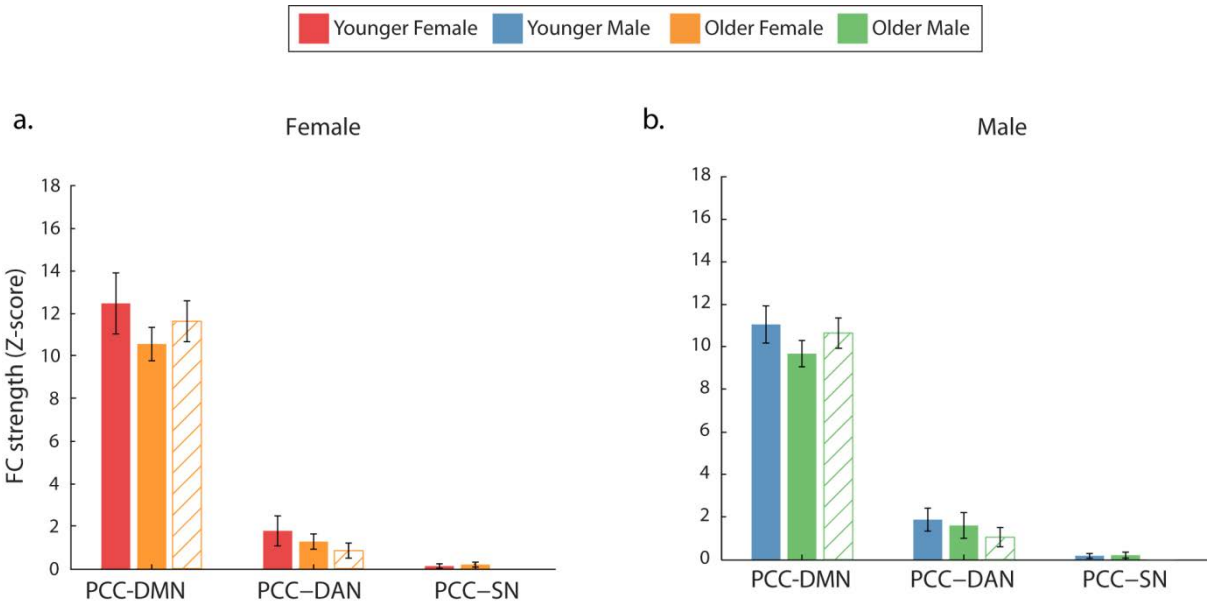

Figure 28: Figures 28a and 28b depict the effect of age on PCC-FC for female (28a) and male (28b) participants. No significant differences were identified using either ROIs defined from a young cohort (solid bars) or age-group specific ROIs for the older participants (hatched bars). Error bars are SEM calculated across participants.

4.3.2.7 FC by individual node

Here we present the FC between the main nodes used for inter-network FC analysis, and all other RSN nodes. FC strengths were averaged across the individual nodes within a network, to create the composite scores presented in the manuscript. Figures 29a and 29b

show FC between each of the seed regions (ACC and PCC respectively) and all other individual nodes of each network. These figures illustrate that the average inter-network FC measures (Figures 25 and 27) are representative of the patterns of age-group differences in FC that are seen at the individual node level for each RSN. For example, the average inter-network FC from the ACC increases with age with the DAN (Figure 25), and the same pattern is seen for the individual nodes that comprise the DAN (Figure 29a). FC between right AI (another main node of the SN) is presented for comparison to ACC FC, the results from this figure (and subsequent analysis not presented here for brevity) suggest that the age/gender differences in SN inter-network FC were specific to the ACC (Figure 29c).

4.4 DISCUSSION

We investigated the influence of both age and gender on intra- and inter- network FC. We identified that, when using the same RSN node definitions for the two age groups, older adults were found to have reduced intra-network FC, particularly in the DMN, and increased ACC-DAN inter-network FC in comparison to younger participants. Upon further investigation of FC differences by gender and age, we identified that the increased inter-network FC in older age was driven specifically by the male participants in our sample. Additional evaluation of these FC differences, using RSN node definitions that were specific to the older cohort, suggested that there was a gender-specific spatial reorganisation of some RSN nodes, which were predominantly frontal in location. While greater ACC-DAN FC was still identified in older, compared to younger, participants the difference was exhibited by both genders rather than being specific to male participants. This suggests that the node definitions provided by the independent younger cohort remain appropriate for older males, but not for older females.

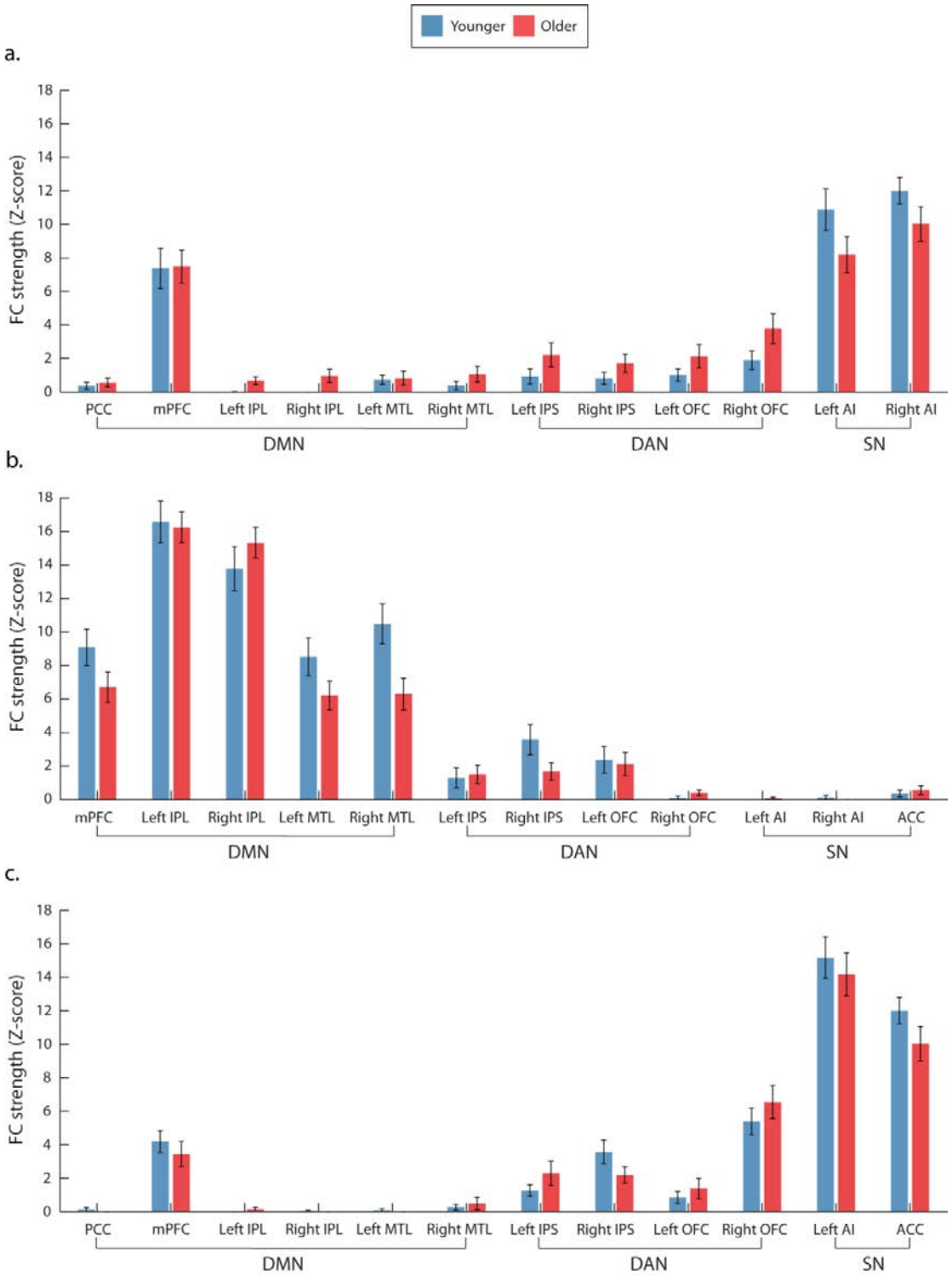

Figure 29: FC between ACC (29a), PCC (29b), right AI (29c) and all other nodes of DMN, DAN and SN. Error bars are SEM calculated across participants.

Our findings are largely in agreement with previous studies which have identified that advancing age is associated with reductions in the modularity of RSNs, meaning that while intra-network FC is reduced in older participants, inter-network FC is increased in comparison to younger adults (Betzel et al., 2014; Geerligs et al., 2014a; Geerligs et al., 2014b; Voss et al., 2010). Geerligs, Saliasi, Renken, Maurits, and Lorist (2014d) identified that older adults had increased inter-network FC in four of twenty-five independent components identified by ICA, including an ACC component. While younger brains are typically highly modular, with limited inter-network FC (Achard & Bullmore, 2007; Bullmore & Sporns, 2012b), older brains are seen to have greatly reduced intra-network FC, particularly of higher cognitive networks, such as the DAN (Andrews-Hanna et al., 2007), DMN (Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Tomasi & Volkow, 2012a; Wu et al., 2011) and SN (Onoda et al., 2012). However, it remains to be determined whether 1) such additional brain connectivity is a compensatory mechanism which is beneficial for brain function in response to reduction of intra-network FC which is commonly found in the ageing brain; or 2) such a reduction in RSN specificity results in interference between network activity, which is not conducive to efficient task performance (Baltes & Lindenberger, 1997). Geerligs and colleagues (2014a) reported that reductions in intra-network FC were associated with poorer performance on cognitive tasks but did not find evidence to suggest that additional inter-network FC was associated with better performance. However, Geerligs, Saliasi, Maurits, Renken, and Lorist (2014c) , reported that increased inter-network connectivity during selective attention was compensatory in nature, as it was associated with greater performance.

Increased inter-network FC associated with older age is often compared to a typical finding from task-based fMRI studies, which have provided evidence for reduced lateralisation with age (Buckner, 2004; Cabeza, 2002; Dennis et al., 2008; Park & Reuter-

Lorenz, 2009). That is, during a task, older adults are typically shown to recruit additional frontal regions as well as greater bilateral activity, compared to younger adults who tend to show left/right lateralisation of the same regions (Reuter-Lorenz et al., 2000). This 'over-recruitment' in ageing is commonly thought of as a compensatory or beneficial mechanism to maintain or improve function (Buckner, 2004; Cabeza et al., 2002; Park & Reuter-Lorenz, 2009). However, as stated above, it is possible that additional brain activity in older adults increases interference and results in disruption of performance. A number of studies have now provided evidence that over-recruitment in older adults is associated with poorer performance on a number of cognitive tasks (Morcom et al., 2007; Stevens, Hasher, Chiew, & Grady, 2008). Recently, Geerligs and colleagues (2014d) reported that although older adults were capable of flexibly modulating inter-network FC to meet task demands by recruiting additional resources in comparison to younger adults, there was a limit to the compensatory nature of this additional FC. Older adults reached a 'resource ceiling' which resulted in age-related deficits in performance in more difficult conditions. It is apparent that more research is required to fully understand the phenomenon of increased BOLD response during tasks or increased inter-network FC in older adults and to establish whether particular inter-network FC patterns are more or less beneficial than others. The relationship between increased task responses and changes to FC also remains to be clarified, both in younger and older subjects (Mennes et al., 2010; 2011).

Relatively few studies have investigated the interaction between age and gender on intra- and inter-network FC changes with age for multiple networks. One study by Biswal and colleagues (2010) revealed that both age and gender were significant determinants of FC while Fillipi and others (2013) reported that males had stronger FC of visual, sensorimotor (including insula) and parietal RSNs, while women were found to have stronger FC of frontal, temporal and cerebellar RSNs. They conclude that males have

stronger FC between cognitive and sensory networks, while females exhibit greater FC between frontal/working memory networks. Similarly, Smith and others (2014) identified reliable gender differences in fronto-parietal, visual and auditory RSNs, in a large sample of younger adults. Similar to our initial findings, Hong and colleagues (2014) identified, for a group of younger participants, that males exhibited greater intra-SN FC (dorsal AI to dorsal posterior insula) and greater SN inter-network FC (AI-mPFC). These studies combined have led to a suggestion that males tend to exhibit increased inter-network FC between sensory and cognitive RSNs, while females tend to exhibit increased FC to working memory/attention networks.

Studies that have focussed on the lateralisation of RSNs have provided evidence that male brains tend to be more lateralised than female brains (Agcaoglu et al., 2015; Liu et al., 2009; Tomasi & Volkow, 2012b; Wang et al., 2010; Zuo et al., 2010), particularly for short-range connections (Tomasi & Volkow, 2012b). However, some studies have reported no effect of gender on measures of RSN connectivity (Nielsen et al., 2013; Weissman-Fogel et al., 2010) while others have argued that the effect of gender on RSN connectivity is relatively small. For example, Bluhm and colleagues (2008) reported that, for a group of participants aged between 17-58 years, gender and age had little effect on DMN connectivity. In addition, Lopez-Larson and colleagues (2011) found that only a middle temporal region exhibited a gender effect for local brain connectivity for younger (11-35 years) participants. They reported that women exhibited greater local connectivity (right-lateralised) within the hippocampus and amygdala. However, this study used an anatomical parcellation method (AAL) and a voxelwise 'regional homogeneity' measure to assess FC, which makes it hard to compare to the results of the current study.

Studies that have investigated the effects of both age and gender on RSN FC have shown that age can differentially affect RSN lateralisation for the two sexes. Agcaoglu et al. (2015)

identified that younger males were found to have lateralised visual networks whilst older males exhibited a right lateralised attentional network and older females had left lateralised attention and frontal RSNs. Similarly, Zuo and others (2010) identified linear, quadratic and cubic changes to FC with age, dependent on the RSN, with a decrease in homotopic FC (FC between any pair of symmetric interhemispheric voxels) with age in higher order cognitive RSNs and an increase in sensory/motor regions. In addition, they identified homotopic FC differences, which were dependent on gender. A recent study by Scheinost and colleagues (2014) also provided evidence to suggest that men and women exhibit differential patterns of FC change with age. In a sample of participants aged between 18-65, older male participants were found to have a sharper decline of FC in nodes of the DMN but increased FC of lateral parietal and frontal nodes, compared to females, who exhibited reduced visual cortex and frontoparietal network FC and increased subcortical (hippocampus, parahippocampal gyrus, thalamus and insula) FC with age. Furthermore, Allen and colleagues (2011) compared intra- and inter- network FC for a large sample of 12-71 year olds and found that although gender had less of an effect on FC than age, women were seen to have stronger intra-network FC whilst men had greater inter-network FC.

The results from the current study provide evidence that age-related RSN re-organisation may be modulated by gender. Although our initial results suggested that changes to FC strength with age were associated with gender, further investigation suggested that the spatial organisation of RSN nodes may be differentially affected by age for the two sexes. For example, the fact that greater ACC-DAN FC for older, compared to younger, male participants was identified using both RSN node definition methods, but only when using age group-specific RSN nodes for female participants, suggests that the spatial extent and peak location of certain RSN nodes may differ between male and female older participants. Although age related ACC-DAN FC strength was no longer modulated by gender

after applying age-group specific RSN node definitions, this result instead reflects gender specific differences in the spatial definition of RSN nodes. Future work should look to investigate the interaction between age and gender on the re-organisation of RSNs. With larger sample sizes, it would be interesting to investigate FC from gender specific RSN nodes defined separately for each age group. One caveat of the secondary analysis presented here is that the gICA used to create specific older adult RSN nodes only contained 20 participants compared to the independent cohort of 55 participants, which was used to define the RSN nodes originally. Therefore, it is possible that the differences in FC between the two node definition methods is due to the reduced reliability of RSN definition due to the smaller sample size of the older gICA. This may be particularly true for RSN nodes where older adults typically exhibit greater signal loss in comparison to younger participants. Future work should also look to validate the node definition we present here to confirm the finding that RSN nodes may be spatially reorganised with older age.

Furthermore, we specifically identified that increased SN-DAN FC in older adults, was being driven by the ACC, rather than the right AI node, of the SN. When we explored the increased inter SN-DAN FC, we found that although right insula showed increased FC to some DAN nodes in older participants, the pattern was less consistent compared to ACC-DAN FC (Figures 29a and 29c). This suggests that ACC-DAN FC is specifically disrupted with older age, rather than a whole network reorganisation of the SN. This finding provides evidence that although it is often useful to combine nodes of the same network to provide more summarised measures of network FC, we cannot assume that all nodes of the same network are affected by age (or gender) in the same way. This is particularly true when investigating inter-network FC. If we had looked only at inter-network FC of the SN as a whole, we would have failed to identity the increase in SN-DAN inter-network FC. Similarly, Hoffstadter and others (2014) investigated FC using two seeds within the motor network,

and found that age was associated with reductions in FC of the motor initiation seed but increases in FC of the motor execution seed, providing evidence that nodes of the same network can be differentially affected by age. This should be considered carefully when combining measures of FC and when investigating inter-network FC. The underlying reasons behind age-related changes in the FC of some nodes, while others are unaffected, remains unclear. In this study, we identified that for both age groups, left-right insula FC were the most connected nodes of the SN, meaning that the least functionally connected node (ACC) of a network was found to be modulated by age. Further work could look to identify whether this effect also occurs in other RSNs and to what extent it is behaviourally relevant.

The fact that the increase in ACC FC was found to be specific to nodes of the DAN, rather than a global increase of ACC FC to all nodes of the RSNs investigated, suggests that this shift in ACC FC may serve to support a function. Recent research has implicated nodes of the SN, namely the AI and ACC, as being responsible for cognitive control (Dosenbach et al., 2007; Gehring, Goss, Coles, Meyer, & Donchin, 1993; Ham, Leff, de Boissezon, Joffe, & Sharp, 2013). Evidence suggests that while the right AI plays a greater role in detecting salient stimuli and facilitating attention by switching between DMN and DAN in response to task/rest demand (Allman et al., 2010; Ham et al., 2013; Menon & Uddin, 2010; Sridharan et al., 2008), the ACC modulates responses in sensory/motor/association cortices (Crottaz-Herbette & Menon, 2006; Menon & Uddin, 2010; Sridharan et al., 2008). This modulation of such a wide range of brain areas is possibly due to the dense and diverse connectivity of the ACC (Margulies et al., 2007; Seeley et al., 2007; Shackman et al., 2011; Yu et al., 2011). A sub-region of the ACC, the anterior mid-cingulate cortex (aMCC) which is analogous to our ACC ROI, is known to have connections to subcortical, sensorimotor, cognitive, salience, pain and affective networks (Dosenbach et al., 2007; Margulies et al., 2007; Vogt, 2005; Yu et al., 2011) and is strongly

implicated in a wide array of cognitive processes (Koski & Paus, 2000; Shackman et al., 2011; Stevens, Hurley, & Taber, 2011). The aMCC region also contains the rostral cingulate zone (RCZ), which is known to project directly to the spinal cord (Morecraft & Tanji, 2009), allowing the top-down control of motor action. In addition, specialised von Economo neurons (VENs) (Allman, Tetreault, Hakeem, & Park, 2011; Nimchinsky et al., 1999) are found exclusively within the ACC and AI (Watson, Jones, & Allman, 2006), are known to have large axons which facilitate the rapid relay of signals to other cortical regions (Allman et al., 2010) and have been proposed as the mechanism by which rapid switching between the DMN and DAN is facilitated (Sridharan et al., 2008). Taken together, the ACC's dense connectivity across the brain, including the spinal cord, presence of VENs, and role in a range of cognitive processes, suggest that the ACC is a hub that is well situated to integrate information from multiple areas and regulate action/behaviour (Shackman et al., 2011). The same reasons also suggest that the ACC may be well positioned to facilitate compensatory connectivity in response to disruption of FC with age. However, although it seems intuitive to hypothesise that this ACC inter-network FC increase is a compensatory mechanism in response to reduced intra-network FC with age, we must also consider the possibility that such network reorganisation is detrimental to brain function, as previously discussed. Future work should look to establish whether increased ACC-DAN FC is associated with better cognitive performance, or whether additional ACC inter-network FC is associated with poorer cognitive functioning.

This study explicitly attempted to address potential grey matter loss with age, which could have resulted in different proportions of grey/white matter and CSF within the ROIs for the two age groups. By segmenting each participant's anatomical scan into the three tissue classes, and transforming partial volume maps into functional space, we were able to include only grey matter voxels in our FC analysis. This meant that for most ROIs, excluding

left IPL and left OFC, older adults had, on average, significantly fewer voxels retained compared to younger adults, after correcting for multiple comparisons. The difference in number of grey matter voxels justifies our decision to include only grey matter voxels in our analysis, as ROIs for the older adults would have contained significantly more white matter/CSF voxels of no interest compared to younger adults, which could potentially skew FC differences between groups. Such differences in grey matter volume between age groups poses a difficult problem for researchers investigating changes in FC with age. Not controlling for these differences results in potentially noisier data for older participants i.e. due to the inclusion of CSF voxels, while controlling for them can result in unequal node/ROI sizes between groups. In the Methods Chapter I present the ACC-network FC results following an analysis which included all ROI voxels for both age groups (i.e. did not exclude CSF and white matter voxels) and report that the FC differences identified remain the same (See Chapter 2, Figure 6). Therefore, any differences in ROI size between age groups are unlikely to be driving any of the FC differences we have identified. Nonetheless, this is a difficult problem that requires consideration when investigating age-related differences in FC.

By exploring FC differences in this way we have identified that, in addition to intra-network FC, there is further information to be gleaned from studying the inter-network FC and from considering the FC of individual network nodes. Individual network nodes appear to differ in their inter-network connectivity, as well as how they are affected by age and gender, which is something that is not well understood. Only by studying more RSNs in this fashion, and incorporating measures of cognition, may we be able to build a more coherent picture of what happens to brain connectivity as a result of the ageing process. Finally, we also provide preliminary evidence that in addition to changes in the connectivity between RSN nodes, the spatial location of certain RSN nodes may change with advancing age, the

extent of which may be differentially affected by gender. These results highlight the problematic nature of comparing RSN FC changes between groups. For example, if the spatial locations of RSN nodes alter with age or disease, applying the same RSN node definition to all participants may result in spurious differences in FC strength between groups. We found that spatial re-organisation was RSN node specific, highlighting the importance of investigating individual RSN nodes to identify which are most prone to spatial re-organisation with advancing age or neurological disorder. Future work should further investigate the spatial re-organisation of RSN nodes with age and utilise larger data sets to create reliable age or patient group specific RSN node definitions which can be made publically available for researchers investigating FC.

CHAPTER 5. THALAMIC FUNCTIONAL CONNECTIVITY AND ITS ASSOCIATION WITH COGNITIVE PERFORMANCE IN OLDER AGE

ABSTRACT

Despite the thalamus' dense connectivity with both cortical and subcortical structures, few studies have specifically investigated how thalamic connectivity may change with age and how such changes may be associated with changes in cognitive function. This study investigated the effect of age on thalamo-cortical and thalamo-hippocampal FC and the association between thalamic FC and memory and reaction time (RT) performance in older adults. A seed-based approach assessed the FC between the thalamus and: 1) sensory resting-state networks; 2) the hippocampus. Older adults exhibited a loss of specificity in the FC between sensory thalamic sub-regions and corresponding sensory cortex. Increased thalamo-motor FC in older adults was associated with faster RTs. Furthermore, older adults exhibited increased thalamo-hippocampal FC, which was greatest for those with the poorest memory performance. These results highlight the importance of including the thalamus in studies of cognitive ageing in order to fully understand how brain changes with age may be associated with cognitive function.

5.1 INTRODUCTION

5.1.1 The role of the thalamus in the human brain connectome

The thalamus has long been thought of as a sensory integrative centre whose primary function is to integrate incoming sensory information and project it to the relevant cortical regions (Walker, 1938). This view is largely due to the long established presence of first order nuclei, which relay messages to the cortex from primary sensory pathways or subcortical centres (Jones, 1985; Walker, 1938). However, it is now well established that the majority of the main driver inputs to the thalamus come from the cortex, rather than the sensory periphery (Sherman & Guillery, 2013). Higher order nuclei receive dense input from both layers five and six of the cortex, resulting in cortico-thalamo-cortical pathways which create trans-thalamic indirect connections between cortical areas, (Saalmann, 2014; Sherman & Guillery, 2013). Every dorsal thalamic nucleus receives fibres back from the cortical region it projects to, resulting in large scale cortical reciprocal connectivity (Jones, 1985; Sherman & Guillery, 2013). The first evidence for thalamo-cortical reciprocity came from evidence that damage to cortical regions also resulted in impairment of thalamic nuclei. For example, frontal cortex lesions have been associated with medial dorsal (MD) nucleus damage (Schwartz, Dekker, & Goldman-Rakic, 1991), while damage to visual cortex is known to be reciprocated in the pulvinar (Mathers, 1972; Ogren & Hendrickson, 1977). However, despite our knowledge of the existence of such diffuse thalamo-cortical connectivity, its function currently remains poorly understood.

Recent studies have provided evidence that higher order thalamic nuclei have a strong influence over cortical activity (Purushothaman, Marion, Li, & Casagrande, 2012; Saalmann, Pinsk, Wang, Li, & Kastner, 2012; Theyel, Llano, & Sherman, 2010) and even first order nuclei have been shown to alter sensory information before it projects it to the cortex (McAlonan, Cavanaugh, & Wurtz, 2008; O'Connor, Fukui, Pinsk, & Kastner, 2002). Some have

argued that higher order nuclei may function to modulate the synchrony between different cortical regions and networks, to increase the efficiency of information transfer (see Saalmann (2014) for a review). Furthermore, 90% of thalamic input is provided by modulatory axons, which do not carry a message to be relayed, unlike driver axons which provide input to first and higher order nuclei for relay. Instead, these axons play a role in modulating the way incoming messages are relayed to the cortex, or whether they are relayed at all; a process referred to as thalamic gating (Sherman & Guillery, 2013). These recent studies provide convincing evidence for the role of higher order thalamic nuclei in modulating cortical activity, but again the precise mechanisms for such modulation remain unclear.

5.1.2 Segmenting the thalamus

In humans, brain imaging and particularly fMRI are the best tools available for understanding the function of the thalamus. As discussed above, the thalamus is not a homogenous structure, and its nuclei are known to possess differential connectivity and function. For this reason, recent studies have looked at identifying ways to segment the thalamus to identify thalamic sub-regions. Diffusion tensor imaging (DTI) (Behrens et al., 2003; Duan, Heckenberg, Xi, & Hao, 2006; Jang & Yeo, 2014; Kumar, Mang, & Grodd, 2014; Ye, Bogovic, Ying, & Prince, 2013) and FC analysis of fMRI data (Hale et al., 2015; Kim, Park, & Park, 2013a; Zhang et al., 2008) have been successfully used to parcellate the thalamus into sub-regions which show preferential connectivity to certain cortical regions. These sub-regions generally correspond with the overall picture from histological and anatomical studies, although the evidence provided by invasive and non-invasive modalities is not always easy to compare. Hale and colleagues (2015) provided quantitative evidence that thalamic segmentation using both FC and ICA largely correspond with a histological atlas,

although neither functional method provided the same level of specificity as the atlas. Similarly, although results from FC and ICA corresponded with a structural atlas, similar to other studies who report correspondence between structure and function (Jeon, Anwander, & Friederici, 2014; Zhang, Snyder, Shimony, Fox, & Raichle, 2010), neither functional segmentation were directly comparable to the structural parcellation. However, the relationship between structure and function is not straightforward and others have shown structure and function do not always result in one to one mapping (Greicius et al., 2009; Honey et al., 2009; Vincent et al., 2007). Finally, Hale and colleagues (2015) also compared thalamic segmentation results provided by the FC and ICA methods and reported that although both methods are capable of revealing sub-regions of the thalamus, comparable to previous studies (Behrens et al., 2003; Zhang et al., 2008; Zhang et al., 2010), segmentation using ICA may provide additional specificity and was found to correspond more highly to histology compared to segmentation via FC.

5.1.3 The thalamus and cognition

The re-appraisal of the thalamus' role in cortical information processing has led researchers to investigate the role of the thalamus in cognition. Cole, Pathak, and Schneider (2010) assessed which brain regions were most globally connected, using resting-state functional connectivity, and reported that as well as nodes of the DAN and DMN, medial dorsal and lateral thalamic regions were most strongly connected to regions throughout the brain. They argue that this connectivity may allow the integration and modulation of information streams, thought to be a key role of thalamic function. More recent studies have highlighted the role of the thalamus, particularly higher order thalamic nuclei, in cognition. A review by Mitchell (2015) highlights the specific role of the mediodorsal (MD) thalamus in learning and decision making. The nucleus, which is known to have extensive cortico-

thalamo-cortical connections, particularly with the PFC (Klein et al., 2010; Ray & Price, 1992, 1993) and temporal lobes (Mitchell & Chakraborty, 2013) has been shown to be particularly important during rapid trial-by-trial associative learning and decision making which require multiple cognitive processes. Similarly, Parnudeau and colleagues (2015) also highlight the importance of MD in goal-directed behaviour and report that hypofunction of this nucleus results in reduced cognitive flexibility. Piras, Caltagirone, and Spalletta (2010) presented results from a DTI study on 181 healthy participants and reported that out of a number of subcortical regions (thalamus, caudate nucleus, putamen, hippocampus, amygdala and pallidum) only the micro-structure (mean diffusivity) of the thalamus was associated with working memory performance. This finding was particularly pronounced for nuclei projecting to pre-frontal and posterior parietal regions, highlighting the role of thalamo-cortical connectivity in cognitive function.

Furthermore, studies that have focussed on patient groups have also highlighted the importance of thalamic connectivity for cognitive performance. Patients with cognitive impairment associated with multiple sclerosis were found to have greater atrophy of anterior thalamic nuclei as well as abnormal DTI indices of all cortico-thalamic tracts studied (Bisecco et al., 2015). In addition, executive function deficits, which are commonly identified in Huntington's disease, have been associated with thalamic degeneration (Kassubek, Juengling, Ecker, & Landwehrmeyer, 2005). Similarly, Serra and colleagues (2014) reported that a patient with a thalamic infarct who suffered from memory and executive functioning deficits exhibited disrupted structural connectivity for a thalamic region most strongly connected to ACC, dlPFC and motor areas, compared to a patient with memory problems alone who only exhibited thalamic-PFC disruption. In addition, others have highlighted thalamic connectivity differences in traumatic brain injury (Nathan et al.,

2012; Tang et al., 2011), schizophrenia (Rose et al., 2006) and bipolar (Teng et al., 2014) patients, mainly affecting connectivity between thalamic and frontal brain networks.

5.1.4 The thalamus' potential role in cognitive ageing

One argument that has been poised to explain the observed cognitive declines with advanced age points to the importance of disrupted cortical connectivity (O'Sullivan et al., 2001). There is evidence that the structural connectivity of frontal circuits degrades with age, while posterior connectivity is largely maintained (O'Sullivan et al., 2001; Pfefferbaum, Adalsteinsson, & Sullivan, 2005). Studies of functional networks reach similar conclusions that brain connectivity, particularly of networks including frontal regions, decreases with age (Andrews-Hanna et al., 2007). Others have also reported increased inter-network connectivity with age (Geerligs et al., 2014a; Geerligs et al., 2014b), although the consequence of this increased connectivity has yet to be fully understood. See Chapter 1, Section 1.1.2 for a more detailed discussion.

Despite the evidence for dense thalamo-cortical connectivity and the importance of the thalamus in a range of cognitive abilities, few studies have focussed on changes in thalamic connectivity with age and how such changes may impact on cognition. Ystad et al. (2010) identified, using resting-state fMRI, that functional connectivity strength between the dorsomedial nucleus of the thalamus and parts of the striatum was negatively associated with episodic memory functioning in a sample of 49-80 year olds. Similarly, Ystad et al. (2011) combined measures from DTI and resting-state fMRI and identified clear thalamo-cortical (as well as thalamo-cerebellar) connections. They also reported that measures of executive function and processing speed were associated with the fiber integrity between subcortical regions (thalamus and putamen) and frontal cortical regions. A number of recent studies that have used measures of structural connectivity have suggested that the integrity

of thalamic nuclei and their projections to cortical regions decline with age (Hasan et al., 2011; Hughes et al., 2012; Ota et al., 2007) and that these changes have implications for attention, processing speed working and episodic memory (see Fama and Sullivan (2015) for a review).

Decline in memory performance is perhaps the most commonly associated cognitive deficit with advancing age (see (Craik & Rose, 2012) and (Khan, Martin-Montanez, Navarro-Lobato, & Muly, 2014) for reviews). The medial temporal lobe (Halgren, Wilson, & Stapleton, 1985) and, more specifically, the hippocampus has long been implicated as a vital structure for explicit memory (Riedel et al., 1999; Schacter, Alpert, Savage, Rauch, & Albert, 1996; Squire, 1992). However, researchers have highlighted the importance of connectivity of the hippocampus to other brain regions (Izquierdo & Medina, 1997) prompting the suggestion that the hippocampus is integral to memory via a number of memory circuits, rather than as an isolated region (Aggleton, 2014). The importance of hippocampus – anterior thalamic connectivity in memory has long been discussed (Aggleton & Brown, 1999; Aggleton et al., 2010; Child & Benarroch, 2013; Jankowski et al., 2013) and a number of studies have now highlighted the importance of hippocampal-pre-frontal cortex connectivity for memory function (See Preston and Eichenbaum (2013) for a review) and the wider interactions between thalamic, cortical and sub-cortical regions (Aggleton, 2014; Mitchell & Dalrymple-Alford, 2006; Nishio et al., 2014). Evidence from patients with thalamic infarcts also provide evidence that disrupted thalamo-cortical structural connectivity is associated with memory problems (Serra et al., 2014). Furthermore, FC strength between the dorsomedial nucleus of the thalamus and parts of the striatum has been associated with episodic memory functioning in a sample of 49-80 year olds (Ystad et al., 2010).

As well as memory deficits, slowing of processing speed is also commonly associated with older age (Albinet et al., 2012; Nilsson, Thomas, O'Brien, & Gallagher, 2014; Papp et al.,

2014; Salthouse, 2009; Sliwinski & Buschke, 1999a). A large study by Der and Deary (2006) which included 7130 adults revealed that reaction times (RTs) on simple RT tasks slows after age 50, while RT on more complex, choice RT tasks slows throughout the adult age range. As well as increases in mean RT with age, increased individual variability in RT is also associated with advancing age, particularly for choice RT tasks (Dykiert et al., 2012). A number of studies have now highlighted the association between thalamic connectivity and processing speed in both young (Tuch et al., 2005) and older (Ystad et al., 2011) adults. Sasson and colleagues (2012) identified that in a sample of 25-82 year olds, processing speed was associated with white matter integrity of brain regions including the parietal cortex and medial thalamus, while executive function and memory were associated mainly with measures in frontal and temporal (for memory only) white matter.

In addition to studies of thalamic connectivity, a number of studies have also reported changes in thalamic shape and volume with advancing age (Goodro, Sameti, Patenaude, & Fein, 2012; Long et al., 2012). Serbruyns and colleagues (2015) highlighted both global and specific volume declines of thalamic nuclei with age in a group of 20-79 year olds. Furthermore, volume loss in thalamic nuclei connected to pre- and primary- motor cortex, as well as somatosensory regions, was associated with sensorimotor performance deficits with older age. Sullivan, Rosenbloom, Serventi, and Pfefferbaum (2004) reported that thalamic volume decreased linearly with age with no significant differences in decline for the two sexes. However, an earlier study by Good and colleagues (2002) which performed voxel-based morphometry on 465 brains, identified that thalamic grey matter volume was preserved in older age, unlike other structures (including anterior cingulate, temporal gyri, left frontal gyrus and insula). Finally, Mather and Nga (2013) reported that older adults exhibited greater amplitude of thalamic low-frequency (0.01-0.10 Hz) fMRI oscillations, compared to younger adults who showed greater amplitude for frequencies in the range of

0.198-0.25Hz. The authors argue that these shifts in low-frequency oscillatory activity within the thalamus with advancing age may alter the thalamus' influence over cortical and subcortical regions, which could impact on cognition.

5.1.5 Chapter objectives

Taken together, previous research provides strong evidence for the thalamus' role in cognition as well as its potential role in mediating cognitive decline with age, via disrupted connectivity and changes in structural and functional integrity. Here we sought to compare thalamo-cortical functional connectivity between younger and older adults to investigate the association between thalamic connectivity and disruption of memory and processing speed with age. This is, to our knowledge, the first study to investigate the FC of thalamic sub-regions in older age. We focussed this study on first-order nuclei and sensory cortex, as these connections are better understood and perhaps more intuitive to understand than the connectivity between higher-order nuclei and higher cortical regions. We also investigated hippocampal-thalamic FC, across all sub-regions, as this subcortical region has also been studied much more extensively in terms of connectivity to individual thalamic nuclei.

5.2 METHODS

5.2.1 Functional connectivity analyses

5.2.1.1 Intra-thalamic FC

To assess whether the FC between each of the thalamic sub-regions differed as a function of age we also calculated the intra-thalamic FC for the two age groups. This was done by correlating the time-course of each thalamic sub-region with all other thalamic sub-regions, defined as detailed in section 2.3.4.4.

5.2.1.2 Thalamic sub-regions to sensory RSNs

We explored the differences between thalamic-sensory FC for the two age groups by assessing FC between sensory thalamic sub-regions and sensory RSNs. FC was calculated by seeding from each of the primary sensory (primary motor, occipital) and the temporal thalamic sub-regions to each of the nodes of the sensory RSNs. These consisted of: left and right M1, SMA (motor), left and right STG (auditory) and left and right lateral and primary visual regions (visual). Below, the primary motor thalamic sub-region will be referred to as MT, occipital sub-region as OT and temporal sub-region as TT.

5.2.1.3 Thalamic sub-regions to hippocampus

FC was also calculated between each of the thalamic sub-regions and left and right hippocampi.

5.2.2 Cognitive measures

Paired Associates Learning (PAL) task and Simple Reaction Time (SRT) task from the CANTAB battery were employed as measures of memory (visual spatial) and reaction time, both of which are affected by advancing age (Der & Deary, 2006; Dykiert et al., 2012; Petersen, Smith, Kokmen, Ivnik, & Tangalos, 1992; Skolimowska, Wesierska, Lewandowska, Szymaszek, & Szelag, 2011; Sliwinski & Buschke, 1999a). Computer expertise was not required to complete these tasks as responses were recorded via a touchscreen (PAL) and a button box (SRT). This ensured that any differences in computer familiarity between the two age groups did not confound performance.

5.2.3 Statistical analysis

5.2.3.1 Good vs. poor performers

To address the question of whether any age-related differences in FC are beneficial or detrimental, we assessed whether older "good" performers had thalamic FC that was more similar to younger participants than "poor" performers in their own age group. For this, older adults were split into good and poor performers based on a median split of the group's memory or reaction time performance. This meant that for PAL, participants with <6 errors on stage 7 of the task were classified as "good performers" whilst those with >6 were classed as "poor performers." FC of good and poor performers was then compared to younger performers (2 of whom were excluded from this analysis for having errors >6, which was the criterion for an older, "poor" performer on this task). Final sample sizes using this categorisation were: 18 younger, 9 older good and 11 older poor performers. No significant differences in age existed between the two older performance groups ($F(1,18)=0.58$, $p=0.46$), older good performers had an average age of 74 (± 3.09) years while older poor performers had an average age of 73 (± 5.35) years. Similarly, there was a fairly even gender distribution for the two performance groups. Within the older good performance group there were 4 males and 5 females and within the older poor performance group there were 5 males and 6 females. Therefore, it is unlikely that any confounding effect of unequal gender distribution, or differences in average age between the two performance groups, accounted for any FC differences that were identified.

For SRT, participants with RTs shorter than the median value of 295ms were classified as "good" performers, whilst those with RTs above 295ms were classified as "poor". FC of good and poor performers was then compared to younger performers (3 of whom were excluded from this analysis for RTs >295ms, which was the criterion for an older, "poor" performer on this task). Final sample sizes using this categorisation were: 17 younger, 10

older good and 10 older poor performers. Again, no significant differences in age existed between the two older performance groups ($F(1,18)= 1.78$, $p=0.20$), older good (faster) performers had an average age of 72 (± 4.42) years while older poor (slower) performers had an average age of 75 (± 4.28) years. Similarly, there was a fairly even gender distribution for the two performance groups. Within the older good performance group there were 4 males and 6 females and within the older poor performance group there were 5 males and 5 females. Therefore, it is unlikely that any confounding effect of unequal gender distribution, or differences in average age between the two performance groups, accounted for any FC differences that were identified.

To link FC with task performance, we focussed on paired connections which we hypothesised would be most relevant to the two tasks. The hippocampus is a vital structure for memory formation and retrieval (for reviews see Bird & Burgess, 2008 & Squire, 1992) and, specifically, spatial memory (Burgess, Jeffrey, & O'Keefe, 1999; Cohen et al., 1999; Eichenbaum, Dudchenko, Wood, Shapiro, & Tanila, 1999). In addition, the role of the hippocampal-anterior thalamic axis is also implicated in memory processes (Aggleton & Brown, 1999; Jankowski et al., 2013; Warburton, Baird, Morgan, Muir, & Aggleton, 2001). We therefore sought to investigate whether differences in thalamo-hippocampal FC with age were associated with memory performance on the PAL task. For SRT performance, we examined thalamic-motor FC.

5.2.3.2 Specific Analyses

IBM SPSS Statistics for Windows (Version 20.0) was used for all statistical analyses. All results presented were corrected for multiple comparisons with Bonferroni correction with an error rate of 0.05. All p-values presented in the text are corrected. For ANOVAs where the

principle of sphericity was violated, Greenhouse-Geisser correction was applied to degrees of freedom.

We assessed whether FC between thalamic sub-regions and RSNs differed with age by using mixed design ANOVAs with three factors: age, RSN node and thalamic sub-region, and their interaction terms. Finally, mixed design ANOVAs with three factors: performance group (i.e. young, old good performers & old poor performers), RSN node and thalamic sub-region, and their interaction terms, were used to assess whether older "good" performers had thalamic FC that was more similar to younger participants than "poor" performers in their own age group.

5.3 Results

5.3.1 Cognitive results

Average performance on the two cognitive tasks is shown in Figure 30. Older adults made significantly more errors on stage seven of the PAL task ($F(1, 38)=9.01$, $p=0.005$) and had significantly slower RTs ($F(1,38)=5.83$, $p=0.021$) compared to younger participants.

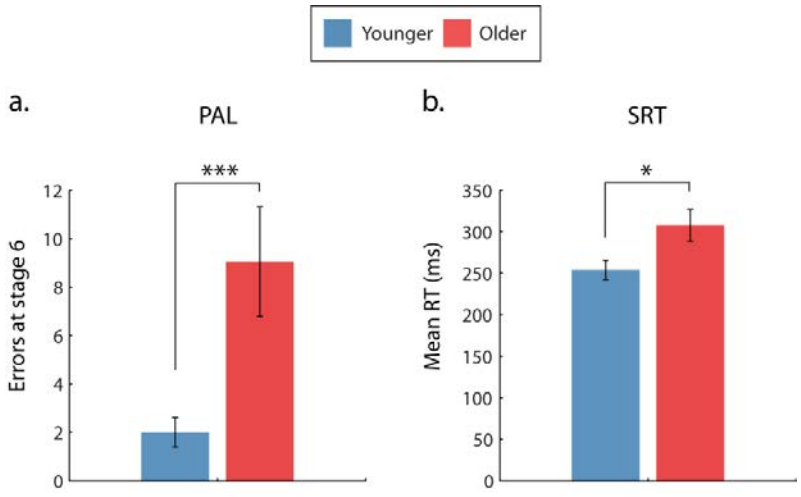

Figure 30: The average number of errors made on stage 7 (6 boxes all containing a pattern) of the PAL task for young and older adults (**30a**). The average reaction times on the SRT task for young and older adults (**30b**). ***p=0.005, *p<0.05. Error bars represent standard error, calculated across participants.

5.3.2 Thalamic FC

5.3.2.1 Intra-thalamic FC

Although younger adults exhibited stronger intra-thalamic FC, compared to older adults, the general pattern of FC across the thalamic sub-regions did not differ. See Figure 31.

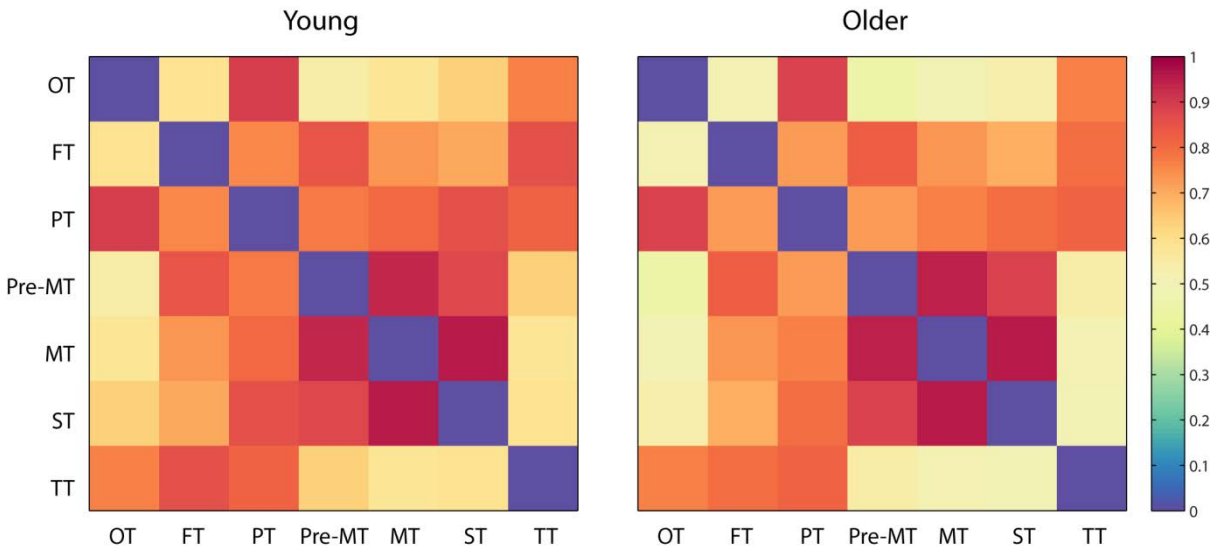

Figure 31: Correlation matrices illustrating the intra-thalamic FC of the two age groups. Young adults exhibited stronger FC between some thalamic sub-regions, compared to older adults (e.g. OT-ST), however the general pattern of FC between the sub-regions remained the same.

5.3.2.2 Thalamo-hippocampal FC

Older adults exhibited significantly greater thalamo-left hippocampal FC, averaged across all sub-regions of the thalamus, as indicated by a significant main effect of age ($F(1,38)=6.17, p=0.018, \eta^2=0.140$) and a NS age*sub-region interaction ($F(2.04, 77.47)=2.88$, $p=0.063, \eta^2=0.071$) (see Figure 32a). However, young and older adults did not differ in terms of thalamo-right hippocampal FC, as revealed by a NS main effect of age ($F(1,38)=2.68$, $p=0.11, \eta^2=0.066$) and a NS age*sub-region interaction ($F(1.96,74.50)=1.42$, $p=0.247, \eta^2=0.036$) (see Figure 32b). For both hippocampi, independent of age, FC was found to vary with thalamic sub-regions as indicated by a significant main effect of sub-region.

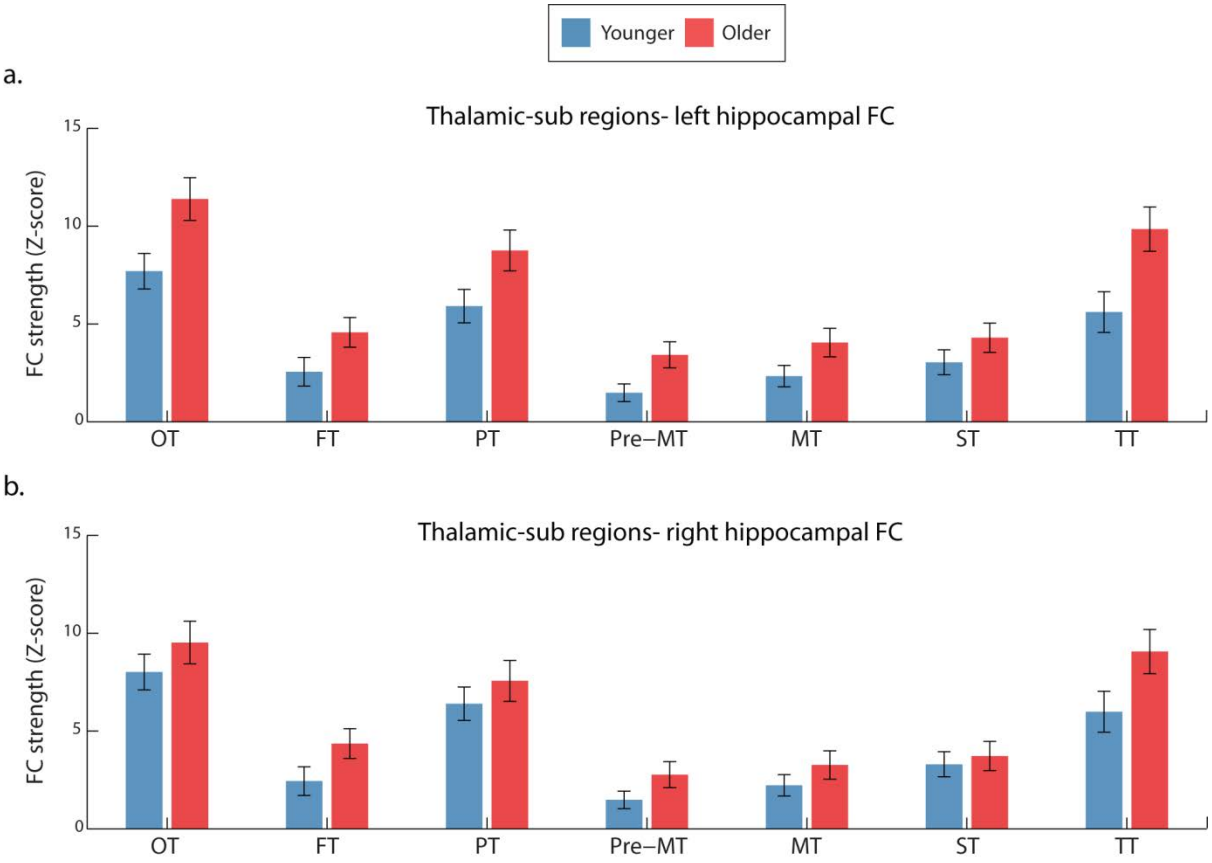

Figure 32: The average thalamo-left hippocampal (**32a**) and thalamo-right hippocampal (**32b**) FC for the two age groups. For thalamo-left hippocampal FC, older adults exhibited significantly greater thalamo-hippocampal FC, compared to young (independent of sub-region). No significant difference in FC strength was identified between the two groups for thalamo-right hippocampal FC. Error bars represent standard error, calculated across participants.

5.3.2.3 Thalamic – sensory cortex FC

5.3.2.3.1 Auditory RSN

The two age groups did not differ in average thalamic-auditory RSN FC, across thalamic sub-regions ($F(1.67, 63.60)=1.1$, $p=0.052$, $\eta^2=0.08$), or RSN nodes ($F(1,38)=0.185$, $p=0.669$, $\eta^2=0.01$). Similarly, there was no significant interaction between age*thalamic sub-region*RSN node ($F(1.6, 60.81)=0.015$, $p=0.985$, $\eta^2=0$). See Figure 33.

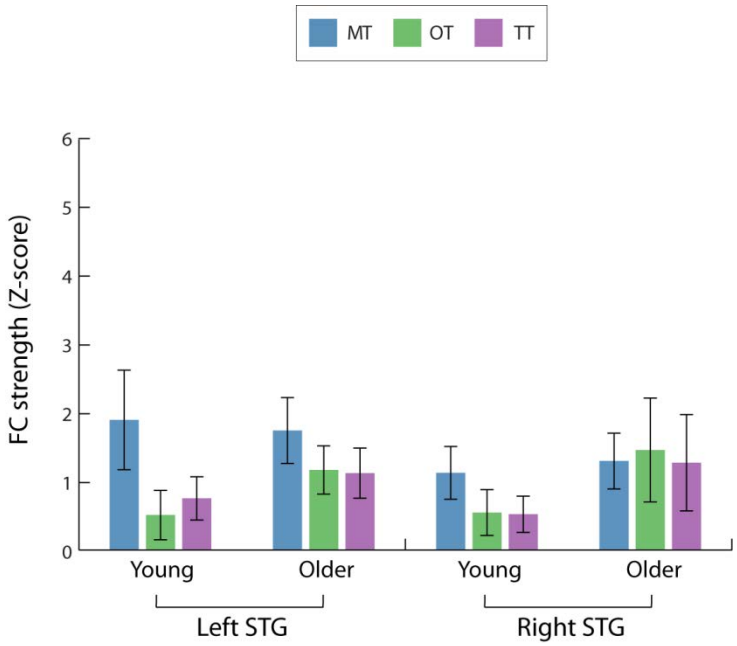

Figure 33: The average FC between each thalamic sub-region and each auditory RSN node, for the two age groups. Blue bars depict MT, green depicts OT and purple depicts TT. No significant difference in thalamus-auditory RSN FC was identified between the two age groups. Error bars are standard error calculated across participants

5.3.2.3.2 Motor RSN

Thalamic-motor FC differed significantly between the two age groups, dependent on sub-region, as indicated by a significant RSN node*thalamic region*age group interaction ($F(4,152)=4.056$, $p=0.01$, $\eta^2=0.1$). Pairwise comparisons revealed that older adults exhibited greater TT –left M1 FC ($p=0.03$, $\eta^2=0.12$) compared to younger adults. Similarly, older adults showed greater TT –right M1 FC ($p=0.003$, $\eta^2=0.22$) and OT-right M1 FC ($p=0.007$, $\eta^2=0.18$) compared to younger adults. Thalamic region-SMA FC did not differ for the two age groups (MT-SMA: $p=0.355$, $\eta^2=0.02$, OT-SMA: $p=0.153$, $\eta^2=0.05$, TT-SMA: $p=0.415$, $\eta^2=0.018$).

Thus, although older and younger adults did not differ in FC between MT and motor cortex, older adults had significantly greater OT–motor cortex and TT –motor cortex FC compared to younger adults. This suggests that older adults lose the FC specificity between MT and motor cortex, which is present in younger adults. See Figure 34 for comparisons across thalamic sub-regions and age groups.

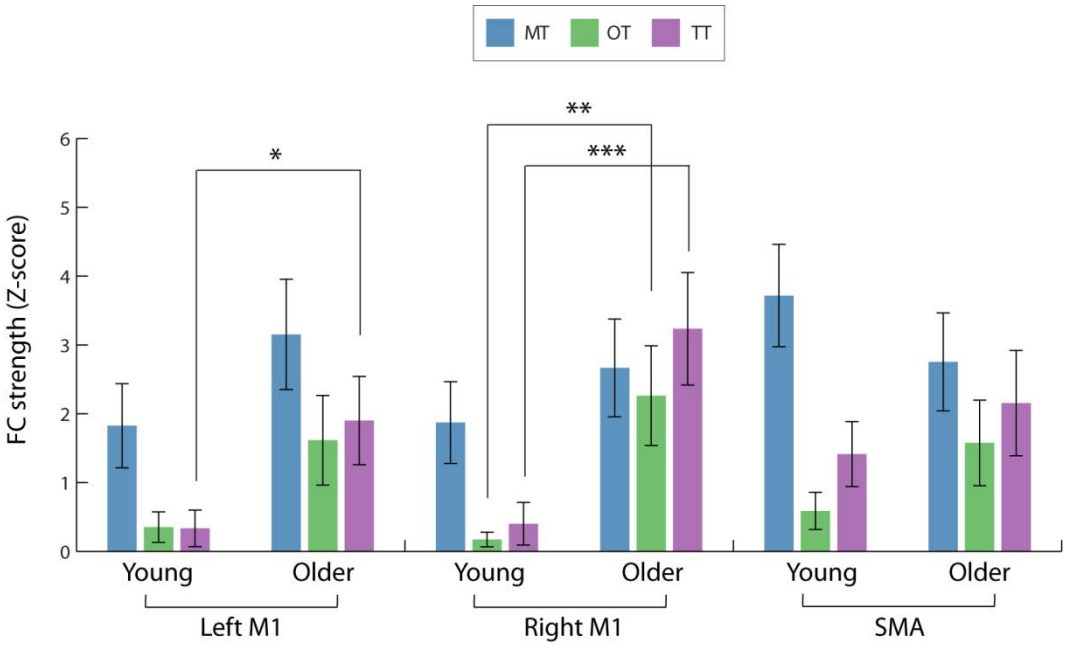

Figure 34: The average FC between each thalamic sub-regions and each motor RSN node, for the two age groups. Blue bars depict MT, green OT and purple depicts TT. Older adults exhibit significantly greater OT – and TT- primary motor FC compared to younger adults. An asterisk over a horizontal line depicts a significant pairwise comparison, *p<0.05, **p<0.01, ***p<0.001. Error bars are standard error calculated across participants

5.3.2.3.3 Visual RSN

The two age groups did not differ in average thalamic-visual RSN FC, across thalamic sub-regions ($F(2,76)=0.57$, $p=0.57$, $\eta^2=0.02$), or RSN nodes ($F(2.18, 82.70)=1.67$, $p=0.176$, $\eta^2=0.04$). Similarly, there was no significant interaction between age*thalamic sub-region*RSN node ($F(3.55, 134.81)=0.50$, $p=0.716$, $\eta^2=0.01$). See Figure 35.

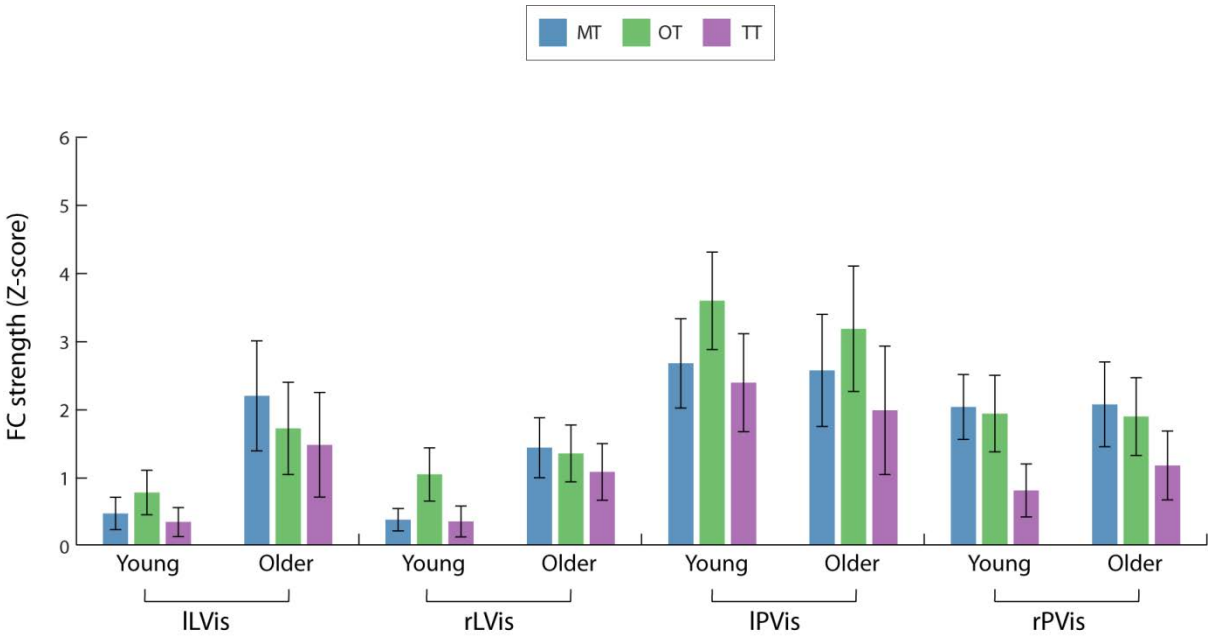

Figure 35: The average FC between each thalamic sub-region and each visual RSN node, for the two age groups. Blue bars depict MT, green depicts OT and purple depicts TT. No significant difference in thalamus-visual RSN FC was identified between the two age groups. Error bars are standard error calculated across participants

5.3.3 Thalamic FC and behavioural performance

5.3.3.1 Thalamo-hippocampal FC and memory performance

We identified that older poor PAL performers had significantly greater thalamo- left hippocampal FC, independent of thalamic sub-region, compared to younger participants only (participants ($p=0.007$), as indicated by a main effect of performance group ($F(2,35)=5.42$, $p=0.009$, $\eta^2=0.24$). The fact that there was no significant difference between older poor and older good PAL performers ($p=0.38$) suggests that thalamo-left hippocampal FC differences were predominantly driven by age, rather than performance. A significant performance group*thalamic sub-region interaction ($F(4.23, 74.1)=3.01$, $p=0.02$, $\eta^2=0.021$) suggested that this effect was driven by particular sub-regions (see Figure 36a).

Analysis of thalamo-right hippocampal FC revealed similar patterns of thalamo-hippocampal FC. However, older poor PAL performers were found to exhibit significantly greater thalamo- right hippocampal FC, independent of thalamic sub-region, compared to

both older good PAL performers (p=0.03) and younger adults (p=0.008), as revealed by a significant main effect of performance group (F(2,35)=5.97, p=0.006, η^2=0.25). A marginally significant performance group*thalamic sub-region interaction (F(4.1, 71.71)=1.823, p=0.046, η^2=0.094) suggested that these differences may be driven by the thalamo-hippocampal FC of the FT, Pre-MT and TT sub-regions (See Figure 36b). The largest effect sizes were identified for the TT sub-region, for both left and right hippocampi. The effect sizes of pairwise comparisons for each thalamic sub-region, for left and right hippocampi, are reported in Table 14.

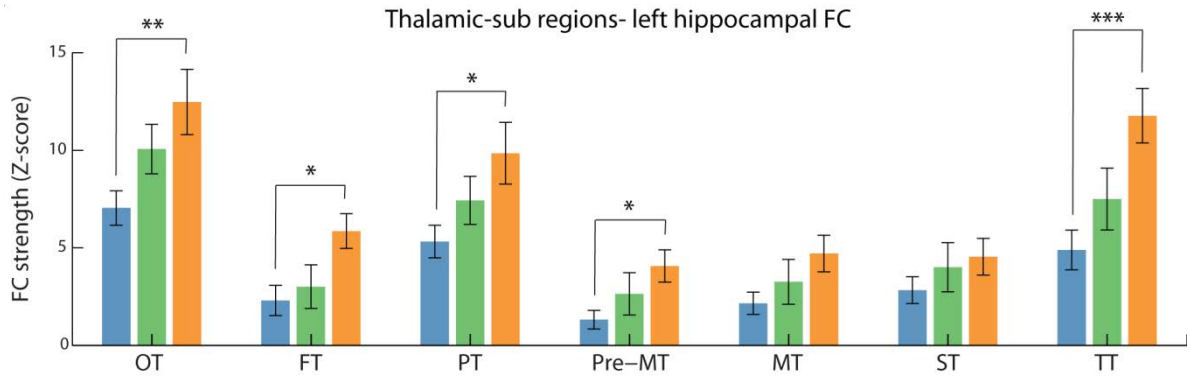

Figure 36a: The average FC between each thalamic sub-region and the left hippocampus for younger participants, good older PAL performers and poor older PAL performers. Poor older PAL performers exhibited significantly greater thalamic- average hippocampal FC compared to younger adults only, for OT-, FT-, PT-, pre-MT- and TT- hippocampal FC. An asterisk over a horizontal line depicts a significant pairwise comparison, *p<0.05, **p<0.01, ***p<0.005. Error bars are standard error calculated across participants.

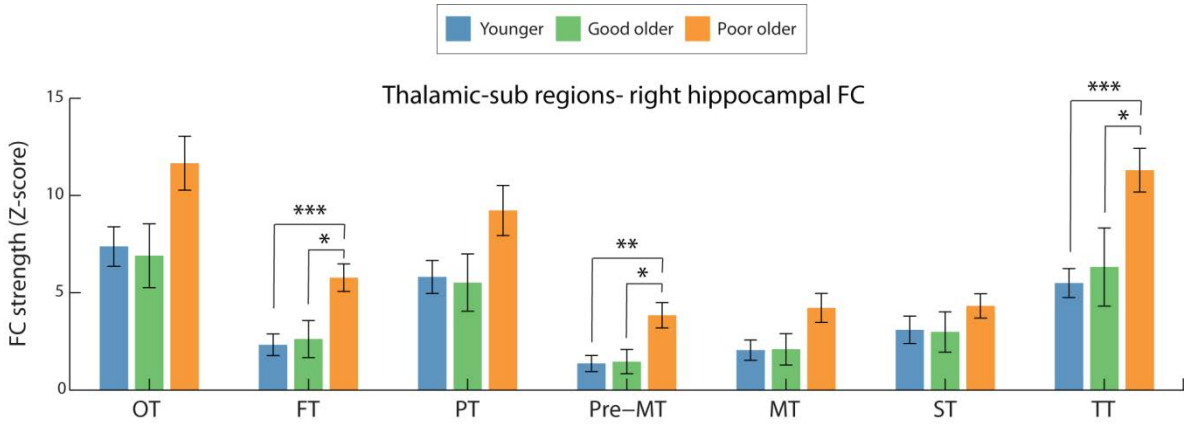

Figure 36b: The average FC between each thalamic sub-region and the right hippocampus for younger participants, good older PAL performers and poor older PAL performers. Poor older PAL performers exhibited significantly greater thalamic- average hippocampal FC compared to good older PAL performers and younger adults, for FT-, pre-MT- and TT- hippocampal FC. An

asterisk over a horizontal line depicts a significant pairwise comparison, *p<0.05, **p<0.01, ***p<0.005. Error bars are standard error calculated across participants.

Table 14: Effect sizes (Cohen's d) for the significant pairwise comparisons following the significant performance group*thalamic sub-region interactions for thalamo- left and right hippocampi. HC= hippocampus. Significant differences are highlighted in blue, *** p<0.005, **p<0.01, *p<0.05.

			Left HC	Right HC
	Group a	Group b	Effect size (d) (Group a – b)	Effect size (d) (Group a – b)
OT	Old Poor	Young	1.21 **	0.97
		Old good	0.49	1.00
FT	Old Poor	Young	1.12 *	1.46***
		Old good	0.91	1.22 *
PT	Old Poor	Young	1.06 *	0.88
		Old good	0.52	0.85
Pre-MT	Old Poor	Young	1.19 *	1.29**
		Old good	0.48	1.17 *
MT	Old Poor	Young	0.94	0.94
		Old good	0.44	0.87
ST	Old Poor	Young	0.57	0.46
		Old good	0.16	0.52
TT	Old Poor	Young	1.55 ***	1.72 ***
		Old good	0.91	1.02 *

5.3.3.2 Thalamic-motor FC and SRT performance

Older, good (faster) SRT performers exhibited significantly greater thalamic-motor cortex FC compared to younger participants (p=0.037, d=0.81) whereas older poor (slower) SRT performers did not (p=0.39, d=0.53) differ significantly compared to older, faster performers, as shown by a significant main effect of performance group (F(1,34)=3.5, p=0.042, η^2=0.17). A significant interaction between thalamic nuclei*RSN node*performance group (F(6.89,117.19)=2.17, p=0.037, η^2=0.12) revealed that, for older good SRT performers, FC was specifically increased between TT-left M1 (p=0.018, d=1.09), TT-right M1 (p=0.003, d=1.63), OT-right M1 (p=0.027, d=1.32), compared with younger adults, while older poor SRT performers did not differ in FC compared to younger participants or older

good SRT performers. This suggests that increased thalamus-motor cortex FC may not be entirely restricted to faster performers. See Figure 37 for comparison of thalamic-motor cortex FC between young participants and good/poor performing older participants.

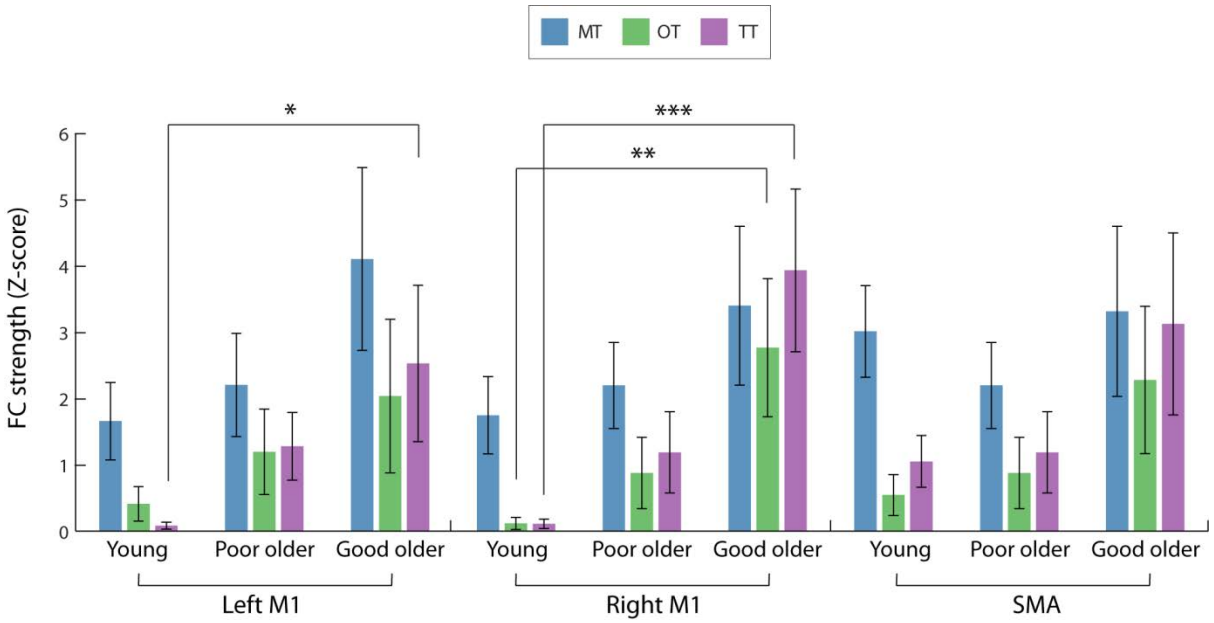

Figure 37: The average FC between each thalamic sub-region and each motor RSN node, for younger participants, poor (slower) older SRT performers and good (faster) older SRT performers. Blue bars depict MT, green depicts OT and purple depicts TT. Older good SRT performers exhibit significantly greater OT – and TT- primary motor FC compared to younger adults. An asterisk over a horizontal line depicts a significant pairwise comparison, *p<0.05, **p<0.01, ***p<0.005. Error bars are standard error calculated across participants.

5.4 Discussion

We investigated age-related differences in thalamo-cortical and thalamo-hippocampal FC and their association with age-related changes in cognitive performance on a memory and a SRT task. Our results highlight that advanced age was associated with poorer spatial memory performance, as has been shown previously (Hayat et al., 2014; Lee, Archer, Wong, Chen, & Qiu, 2013; Rabbitt & Lowe, 2000). In addition, we provide evidence that memory performance was inversely associated with thalamo-right hippocampal FC, as older poor memory performers exhibited significantly greater thalamo-right hippocampal FC

compared to both younger adults and older good memory performers. Interestingly, this is in contrast to the results of comparing the two groups as a whole, which did not differ significantly in terms of thalamo-right hippocampal FC strength. This suggests that thalamo-hippocampal FC increases as a function of age, but that increases in thalamo-right hippocampal FC may be more detrimental to memory performance compared to increases in thalamo-left hippocampal FC. Comparison of effect sizes revealed the greatest differences in thalamo-hippocampal FC existed between young and older poor performers for the temporal thalamic sub-region (TT). These results highlight the behavioural importance of thalamic FC, as well as specifically demonstrating that increases in FC are not necessarily advantageous. Similar findings were reported by Ystad and colleagues (2010) who found that dorsomedial thalamus-striatum FC was negatively related to verbal episodic memory functioning in a sample of 49-80 year olds. Our findings provide further evidence for the hippocampus' well established role in memory processes (Bird & Burgess, 2008; Eichenbaum et al., 1999; Henry, Petrides, St-Laurent, & Sziklas, 2004; Riedel et al., 1999; Schacter, Alpert, Savage, Rauch, & Albert, 1996; Squire, 1992). However, they also highlight the important role of the thalamus in memory processes, particularly during aging. A number of previous studies have provided evidence of the thalamus' role in memory, for example, Aggleton (2014) proposes that there are three parallel, yet distinct, 'information streams' within the anterior thalamic nucleus (ATN) which integrate and work together to support episodic memory, while Nishio and colleagues (2014) demonstrated that the disruption of multiple thalamo-cortical circuits can lead to pre-frontal cortex dysfunction and memory deficits. Furthermore, numerous studies have highlighted the importance of thalamic-PFC connectivity for memory and cognition (Cross, Brown, Aggleton, & Warburton, 2012; Funahashi, 2013; Gaffan, Murray, & Fabre-Thorpe, 1993; Watanabe & Funahashi, 2012). Further research is required to fully understand how age may impact on these

hippocampal-thalamic-cortical networks, and how their potential reorganisation with age may impact on memory performance. Our results provide a starting point for this research, by indicating the feasibility and benefit of parcellating the thalamus in terms of identifying age-related alterations to FC but also in distinguishing between good and poor memory performers. Future work should use such parcellations to further investigate the potential deterioration of thalamic-temporal systems during ageing, and the impact on memory performance, by specifically targeting the 'temporal sub-region' of the thalamus for thalamic-temporal FC analysis.

In addition to a reduction in memory performance with age, we observed slowing of information processing in older adults, as assessed by the SRT task, and demonstrated that older faster SRT performers exhibited significantly greater thalamic-motor cortex FC compared to younger adults, suggesting that faster SRT performance was associated with greater thalamic-motor cortex FC. However, the increase in FC in older fast performers was not significantly different to older slow performers, suggesting that increased thalamus-motor cortex FC may not be entirely restricted to faster performers or the sole cause of their improved performance. This lack of differentiation could be due to statistical power, as by splitting our older group into good and poor performers we reduced the group sizes to 10 participants instead of 20. Alternatively, a full explanation of these differences may require a more holistic investigation of the motor system, of which the thalamo-cortical interactions we have examined are only a part.

The role of thalamic-motor RSN connectivity on SRT performance and the potential modulation with age is a finding that warrants further investigation. To date, functional links between the thalamus and motor cortex have been identified using DTI and fMRI (Guye et al., 2003; Hale et al., 2015; Lehéricy et al., 2006; Zhang et al., 2008) and anatomical evidence has shown that the thalamus is substantially connected to subcortical motor

regions (Sherman & Guillery, 2013, pg. 169). Despite this known thalamic-motor connectivity, few studies have investigated their role in measures of RT. Those that have identified associations between RT and thalamic white matter connectivity, diffusivity and gamma oscillations (Brucke et al., 2013; Fall, Querne, Le Moing, & Berquin, 2015; Tuch et al., 2005). Taken together, this evidence suggests that the thalamus is well situated to contribute to individual differences in RT as well as age-related slowing, via re-organisation of thalamic connectivity or structural and/or functional thalamic changes with age. Future research should look to probe thalamic-motor cortex connectivity more specifically using segmentations of the thalamus to investigate the connectivity between individual thalamic sub-regions and motor cortex.

In older adults, increased activity (Buckner, 2004; Cabeza et al., 2002; Reuter-Lorenz et al., 2000) or connectivity (Campbell et al., 2012; Davis et al., 2008; Geerligs et al., 2014c) is often considered to be compensatory in nature. The recruitment of additional brain regions or increased connectivity between brain regions has been suggested to support the maintenance of cognitive function which would otherwise be disrupted due to age-related brain changes, such as loss of grey matter or reductions in within-network connectivity. However, other evidence has shown that increased FC does not always equate to better performance (Geerligs et al., 2014a; Grady et al., 2010; Salami, Eriksson, & Nyberg, 2012). One explanation of this finding could be that older age is associated with reduced specificity of brain networks, which results in less efficient processing, potentially by increasing interference between network activity (Baltes & Lindenberger, 1997) and thus causing deficits in cognition (Antonenko & Floel, 2014). Our results provide evidence for both scenarios and suggest that the relationship between changes in brain networks and behavioural performance with age may be quite specific to individual behavioural domains. We found that faster RT in older adults was associated with increased thalamic-motor

cortex FC, compared to younger adults, while increased thalamo-hippocampal FC (particularly from the thalamic sub-region that connects mainly to the temporal lobe) in older adults was associated with poorer memory performance. Further research is required to investigate the differential effects of increased or decreased thalamo-cortical connectivity with age on cognition, their domain specificity, as well as the relationship between changes in task-related activations and changes in FC.

By performing FC analysis using thalamic sub-regions we were able to present more specific results of the effect of age on thalamic FC, compared to results using thalamic masks which treat the thalamus as a homogeneous structure, which, in our study, showed a less clear effect of age. Many studies have now provided evidence that it is possible to segment the thalamus using non-invasive DTI and fMRI data into sub-regions which largely correspond with known sub-divisions identified from anatomical and histological evidence (Hale et al., 2015; Jang & Yeo, 2014; Kumar et al., 2014; Zhang et al., 2008; Zhang et al., 2010). However, Hale and colleagues (2015) highlight the differences between analysis methods even within a single imaging modality. Although their results suggest that ICA may provide a more specific definition of thalamic sub-regions, we chose to use a structural atlas to segment the thalamus for the following reasons 1) it is less intuitive to interpret ICA results for defining thalamic sub-regions, particularly when comparing between age groups 2) Hale and colleagues reported there was largely a correspondence between the results from the structural and ICA definitions, suggesting that the added specificity provided by ICA may not warrant the additional interpretation complexity for this preliminary study.

One potential limitation to the current study is the presence of non-neuronal confounds in fMRI connectivity measurements, which may artificially induce, or exaggerate, differences between age groups, as highlighted by a recent study by Balsters and colleagues (2013). In order to account for differences in breathing and heart rate across age groups, and

individuals, we collected both respiratory and cardiac pulse data for all participants and regressed these from participant's functional data. Nonetheless, the possibility of age-related differences in other non-neuronal factors, such as vasculature and cerebral blood flow (CBF) (Beason-Held et al., 2012; Peters, 2006; Riddle et al., 2003a), may have had an impact. However, a recent study revealed that although older adults did exhibit reduced CBF in comparison to younger adults, the uptake of oxygen, lactate and glucose did not differ between the two age groups, suggesting that reduced CBF in older adults does not affect the brain's ability to uptake nutrients (Fisher et al., 2013). Nonetheless, these issues require further investigation. The use of EEG-fMRI or arterial spin labelling, which provides a more direct and quantifiable measure of cerebral haemodynamics, may go some way to addressing such potential differences between age groups.

An additional caveat of investigating differences in brain function with advancing age is differences in grey-matter volumes between age groups and the variability of such age-related differences between individuals. Recent studies using grey-matter volume as voxel-based regressors have provided evidence that some functional differences between age groups can be a consequence of grey-matter atrophy (Kalpouzos, Persson, & Nyberg, 2012), while others persist after correction for grey matter volume (Salami et al., 2012). In this study, we addressed differences in grey-matter volumes within cortical ROIs (and the hippocampus) using partial volume maps following segmentation to exclude any voxels not classified as grey-matter from any analyses. However, segmentation of subcortical structures, such as the thalamus, can be less reliable and grey-matter is often misclassified as white. For this reason, we chose to exclude any CSF voxels, to go some way to addressing differences in thalamic volume between the two age-groups, but, currently, this remains a methodological issue for researchers investigating thalamic connectivity differences in older age.

Our understanding of thalamo-cortical connectivity has undoubtedly increased since Jones (1985) stated that we "have to confess to almost total ignorance regarding cortico-thalamic connectivity''. This is due to a combination of detailed electrophysiological and histological work (Steriade & Deschenes, 1984), as well as the studies that have been discussed above combining neuroimaging with behavioural measures. It is now apparent that the thalamus plays an important role in not only integrating and transmitting sensory information, but in also regulating cortical regions and both directly and indirectly supporting cortico-cortical connectivity. Understanding the connectivity between brain regions is imperative to understanding brain function. A systematic review of the functional neuroanatomy of the thalamus by Power and Looi (2015) highlighted that although the precise role of the thalamus remains unclear, its importance in the functional connectome is beyond doubt. Some have argued that a significant factor in determining the functions that any cortical region is capable of is its connectivity to the thalamus (Sherman & Guillery, 2013). However, there are still a number of questions to be answered regarding: 1) how these connections support cognitive function 2) how changes with age or disease disrupt thalamic connectivity to both cortical and subcortical brain regions and 3) the impact of such connectivity changes on cognition.

Our work has provided new evidence of the importance of thalamo-cortical and thalamo-hippocampal connectivity in supporting reaction times and memory in ageing. As evidence mounts, it seems unlikely that a single thalamic nucleus is responsible for a specific cognitive ability or memory function and a distributed system appears more probable where the integration of information and connectivity across thalamic, subcortical and cortical regions is involved in a range of cognitive abilities (Mitchell & Chakraborty, 2013; Mitchell & Dalrymple-Alford, 2006). The role of the thalamus in terms of ageing, disease and

cognition cannot be denied and future research should look to integrate measures of the thalamus alongside cortical networks which are often the focus of studies of cognition.

CHAPTER 6. THE ASSOCIATION BETWEEN HABITUAL SLEEP AND FUNCTIONAL CONNECTIVITY.

ABSTRACT

It is well established that the thalamus plays a pivotal role in sleep/wake mechanisms and much research has suggested that sleep quality may alter with advancing age. However, to date, the interactions between sleep quality, brain connectivity and cognition remain unclear. This chapter builds on the results of Chapter 5 which identified that patterns of thalamo-hippocampal and thalamo-cortical FC are associated with cognitive performance. Here, these connections are re-examined in terms of sleep quality. By dividing older participants into long and short sleepers, two main findings were identified: 1) older, shorter sleepers exhibited the poorest memory and RT performance and 2) older, longer sleepers were more likely to exhibit patterns of FC which were previously associated with better cognitive performance. These results suggest an interaction between sleep and patterns of FC which may be important for maintaining relatively good cognitive function in older age.

6.1 INTRODUCTION

6.1.1 Sleep and brain functional connectivity

One way of addressing the inconsistencies identified in the literature relating to sleep, ageing and cognition is to attempt to understand how sleep may affect brain efficiency; namely by using measures of brain connectivity. Considering the brain as a network, brain efficiency refers to how optimally organised the brain is. It is well established that the brain follows certain organisational properties, such as 'small world' properties: which means a number of highly connected nodes with short path lengths between nodes and few long-range connections allow high information processing efficiency at low cost (Bullmore & Sporns, 2012a). Recent EEG studies by Verweij and colleagues (2013; 2014) have highlighted that sleep deprivation disrupts the synchronisation and topological organisation of the global brain network (Koenis et al., 2013) and that PFC connectivity is specifically disrupted (Verweij et al., 2014) . One previous study used self-report measures of sleep time and identified that greater sleep durations were associated with greater waking-rest DMN FC (mPFC-PCC) and greater anti-correlation with parietal, occipital and lateral PFC regions (Killgore, Schwab, & Weiner, 2012). Similarly, a recent study by Khalsa and colleagues (2015) identified an association between cumulative total sleep time, as assessed by actigraphy, and waking DMN intra-network FC as well as inter-network FC between the DMN and SN. Furthermore, studies have reported reduced intra-DMN FC and reduced anti-correlations between DMN and task-positive networks following sleep deprivation (De Havas, Parimal, Soon, & Chee, 2012; Gujar et al., 2010; Sämann et al., 2010). Similarly, Ward and colleagues (2013) reported that decreased waking DMN FC was associated with increased daytime sleepiness, in both young and older adults. In summary, a number of studies have identified that less sleep, either naturally occurring or induced by sleep

deprivation, is associated with disrupted brain network connectivity, and more specifically, weaker intra- and inter-network FC.

However, in addition to reductions in FC following sleep deprivation, some studies have also reported increased FC. Currently, it is still unclear whether such increases are compensatory, i.e. to maintain function which would otherwise be compromised following sleep deprivation, or interfering, i.e. a result of inefficient brain network organisation, due to sleep deprivation, which disrupts cognitive function. For example, Liu, Li, Wang, and Lei (2014) reported that small worldness properties were increased following sleep deprivation and that this alteration was compensatory in nature. Similarly, Zhu and colleagues (2015) identified greater inter-hemispheric FC for a number of brain regions (including the thalamus and SMA) following sleep deprivation. They suggest this may be a compensatory response which maintains cognitive ability that would otherwise be impaired by sleep deprivation. Studies that have focussed on changes in BOLD signal rather than FC also report similar increases following sleep-deprivation, compared to rested wakefulness. Drummond (2001; 2000) highlighted increases in task-related BOLD signal in pre-frontal and parietal regions following sleep deprivation. They suggest that these increases reflect compensatory brain responses to sleep deprivation, as the greatest increases in BOLD signal, following sleep deprivation, were identified in the hardest task conditions. Similarly, Chuah et al. (2006) reported that maintained inhibition following sleep deprivation was associated with increased activity in right ventral PFC. One possible mechanism which may account for increases in BOLD signal following sleep deprivation is altered cortical responsiveness. A previous study by Huber and colleagues (2013) reported that increased frontal excitability was associated with time spent awake, from morning to night and following one full night of sleep deprivation but not related to performance on an attentional task. This suggests that increased brain function following sleep deprivation is

not necessarily compensatory and may instead reflect neurophysiological processes associated with a 'build-up' of sleep pressure, such as changes to neurotransmitter levels or synaptic plasticity, which are restored following sleep.

Furthermore, results from a number of other sleep deprivation studies have suggested that increased brain function following sleep deprivation is not always beneficial in terms of cognitive performance. Although maintained cognitive performance following sleep deprivation has been associated with increased BOLD signal in PFC/parietal regions, increased BOLD signal in right inferior frontal gyrus has been negatively associated with memory recall (Drummond et al., 2005b), while increased task-related DMN activity following sleep deprivation has been linked to impaired cognitive function (Gujar et al., 2010). Chee and Choo (2004) suggest that some increases in BOLD signal following sleep deprivation may actually reflect reduced de-activation (or smaller amplitude negative BOLD response) (e.g. reduced capacity to de-activate nodes of DMN during cognitive tasks). Indeed, a study by Drummond and colleagues (2005a) reported that, following sleep deprivation, participants with poorest performance on a psychomotor vigilance task exhibited greater BOLD signal within regions commonly associated with the DMN, compared to those who performed best. These results suggest that not all increased brain activity following sleep deprivation, compared to rested wakefulness, is compensatory; it appears that it can also be interfering.

6.1.2 The role of the thalamus in sleep

Much of the work investigating the effects of sleep deprivation, or sleep in ageing, and brain connectivity has focussed on cortical brain regions and networks. However, the thalamus has long been known to play an important role in sleep (Brown, Basheer, McKenna, Strecker, & McCarley, 2012; Coulon, Budde, & Pape, 2012) and its diffuse

connectivity with the cortex places it in a good position for manipulating conscious awareness (Evans, 2003; Halassa, 2011). Steriade (2003) reported that prolonged hyperpolarisation of thalamocortical neurons prevented input from the outside world reaching the cortex, while sleep spindles, generated by reticular thalamic nuclei neurons, induced rhythmic inhibitory activity in thalamocortical neurons. This results in a state of cortical synchronisation, until arousal occurs, by inhibition of reticular neurons which stop sleep spindle generation. A number of studies have provided evidence for the role of the thalamo-cortical loops in sleep spindle generation (Bartho et al., 2014; Bonjean et al., 2011; Jan, Reiter, Wasdell, & Bax, 2009; Lustenberger, Maric, Durr, Achermann, & Huber, 2012; Tsai et al., 2010), while damage to thalamic nuclei has been shown to result in the loss of sleep patterns (Lugaresi, 1992). REM sleep has been associated with increases in cerebral blood flow and glucose metabolism in the thalamus, as well as increased thalamic EEG activity and BOLD signal, while NREM sleep has also been associated with decreases in CBF of the thalamus (Dang-Vu et al., 2010). Although SWS is typically thought to be cortically generated (Brown et al., 2012; Sanchez-Vives & McCormick, 2000; Timofeev, Grenier, Bazhenov, Sejnowski, & Steriade, 2000), David and colleagues (2013) found that blocking thalamic output to the cortex, in rats, reduced the frequency of SWS by 50%, suggesting that although thalamo-cortical activity is not the only mechanism required for SWS, it still plays a vital role in its generation, or expression.

While thalamo-cortical connections are thought to be responsible for the activation and deactivation of the cortex for sleep/wake regulation, and specifically the control of sleep spindles, the hypothalamus is known to be responsible for circadian timing and sleep/wake promotion (Saper, 2013). A recent review by Colavito, Tesoriero, Wirtu, Grassi-Zucconi, and Bentivoglio (2015) highlights the potential role of the paraventricular nucleus of the thalamus (PT) in integrating the sleep/wake mechanisms of these two systems, for

transmission to the limbic system. They highlight that the PT's role in sleep is firmly established, as it controls arousal and circadian timing, receives direct input from the retina and is connected with the circadian pacemaker (hypothalamic suprachiasmatic nucleus) and is also supplied by orexinergic neurons that are implicated in arousal. However, further investigation is required to firmly establish the PT as the site for the integration of sleep/wake mechanisms.

Despite the strong evidence for the role of the thalamus in sleep/wake regulation, few studies have looked to investigate its FC specifically in sleep, particularly in relation to age. A study by Shao and colleagues (2013) identified reduced FC between the thalamus and middle temporal gyri, medial and superior frontal gyri during rest, following sleep deprivation. Similarly, Tomasi and colleagues (2009) reported reduced FC of the ventral lateral thalamic nucleus and left pre-central and middle frontal gyri following one night of sleep deprivation. Others have reported reductions in thalamic BOLD signal during working memory tasks, following sleep deprivation (Chee & Choo, 2004; Chee et al., 2006; Chee & Chuah, 2007). Similarly, PET studies have shown that reduced glucose metabolism within the thalamus is associated with lorazepam-induced sleepiness (Volkow et al., 1995), decreases in thalamic glucose metabolism following sleep deprivation (Thomas et al., 2000) and reduced thalamic CBF during loss of consciousness following propofol (Fiset et al., 1999). However, it is debatable whether drug-induced losses of consciousness and natural sleep are similar enough to generalise results across the two fields.

6.1.3 Chapter objectives

It is clear that the thalamus plays a pivotal role in sleep/wake mechanisms, however what remains less clear is the extent to which its connectivity is altered in response to inadequate sleep, either as a result of sleep deprivation in younger adults or sleep changes

associated with older age. Furthermore, it remains to be clarified whether advancing age, independent of physical or mental health conditions, is more strongly associated with poorer sleep quality than is seen in younger adults, and if so, what impact this may have on cognition. Finally, the majority of studies investigating sleep quality have used self-report measures of sleep, which are known to often be unreliable estimators of objective sleep quality (Auger, Varghese, Silber, & Slocumb, 2013), particularly in older adults (Landry, Best, & Liu-Ambrose, 2015). In this chapter I sought to investigate the objective sleep quality of healthy young and older adults and whether variability in sleep quality was associated with thalamo-cortical and cortico-cortical FC.

6.2 METHODS

6.2.1 Functional connectivity analysis

Functional connectivity analysis follows the same procedure as Chapter 5. However, as well as calculating FC strength between the mean BOLD time-series from the thalamus (by thalamic sub-region) and the mean BOLD time series of each RSN node, we also calculated FC strength between the mean BOLD time-series from the PCC and all other nodes of the DMN (mPFC, left and right MTL, left and right IPL).

6.2.1.1 Thalamo-cortical connectivity

In order to explore thalamo-cortical FC we calculated FC between thalamic sub-regions and nodes of the cortex. As in Chapter 5, we chose to focus our analysis on first-order thalamic sub-regions (regions of the thalamus that project sensory information from sub-cortical brain regions and sensory afferents to the cortex) and sensory cortex, as these are perhaps the most well understood, and intuitive thalamo-cortical connections. In order to further explore thalamus-frontal cortex FC, we also calculated FC between the PFC thalamic

sub-region and mPFC, and compared with sensory thalamic sub-regions (motor (MT), occipital (OT), temporal (TT)) - mPFC FC.

6.2.1.2 Thalamo-subcortical connectivity

In Chapter 5 it was identified that older adults exhibited significantly greater thalamic-hippocampal FC compared to younger adults. In older to investigate whether sleep quality interacts with this age-effect, we also chose to include the hippocampus in our analysis. FC was calculated between the whole thalamus and whole hippocampus, as well as between each thalamic sub-region and the hippocampus.

6.2.1.3 PCC-DMN connectivity

As the PCC has been associated with consciousness, and sleep (Hannawi, Lindquist, Caffo, Sair, & Stevens, 2015; Herbet et al., 2014; Vogt & Laureys, 2005), we also investigated whether PCC intra-network FC was associated with sleep quality and whether any association interacted with age. For this, FC between PCC and all nodes of the DMN (mPFC, left and right MTL, left and right IPL) was calculated for all participants.

6.2.2 Cognitive measures

As in Chapter 5, cognitive tasks Paired Associates Learning (PAL) and Simple Reaction Time (SRT) were included for this analysis. See Sections 2.2 and 5.2.2.

6.2.3 Sleep/activity monitoring

6.2.3.1 Actiwatch

Sleep quality was assessed via ambulatory actigraphy using the Actiwatch 2® device (Respironics, Philips). The Actiwatch was worn on the non-dominant wrist and provided

continuous monitoring of the participant's activity levels for approximately 15 days (14 nights of sleep). 14 nights of actigraphy assessment has been suggested to provide a reliable assessment of sleep quality, as there can be considerable intra-subject variability in sleep quality (Van Someren, 2007). Movement is detected by an accelerometer, with a sensitivity of 0.025 G (a 2 count level), encased in the device. Each time movement is detected an activity count is generated. Counts were stored on the device in 1-minute epochs. An ambient white light detector also tracks how much light the participant is exposed to, recorded in units of lux.

6.2.3.2 Actigraphy analysis

Data were extracted using Philip's Respironics Actiware software (version 6.0.4). Data recorded by participants in their sleep diary were used to validate the analysis of the actigraphy data. Any periods highlighted by the participant as time when they had removed the watch were excluded from analysis. Participants were instructed to press the button on the Actiwatch to indicate when they were turning the lights off to try and go to sleep and when they were getting out of bed to start their day. This inserts markers onto the actigraph when it is imported into the Actiware software, to allow further validation, or adjustment, of the automatic detection of sleep/wake times. Figure 38 provides an example of Actigraphy data after any adjustments using information provided by sleep diaries.

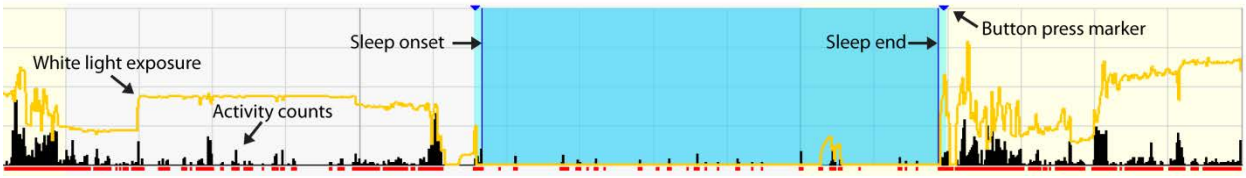

Figure 38a: An example of a portion (24 hour period) of an actigraph from a representative younger participant from this dataset. The activity count for each epoch is indicated by the height of a black vertical bar. Epochs classified as wake (see below) are marked as red along the bottom of the actigraph. The level of white light exposure is shown with the yellow trace. Markers inserted by a participant's pressing of the actiwatch button to indicate the point at which they attempt to go to sleep and get out of bed are shown at the top of the actigraph. Dark blue vertical lines at the beginning and end of the sleep period indicate sleep onset and end. Cyan indicates periods of sleep and the lighter blue portion of the actigraph before and after sleep periods indicate automatically defined periods of rest (i.e. prolonged periods of low activity) that are not classified as sleep.

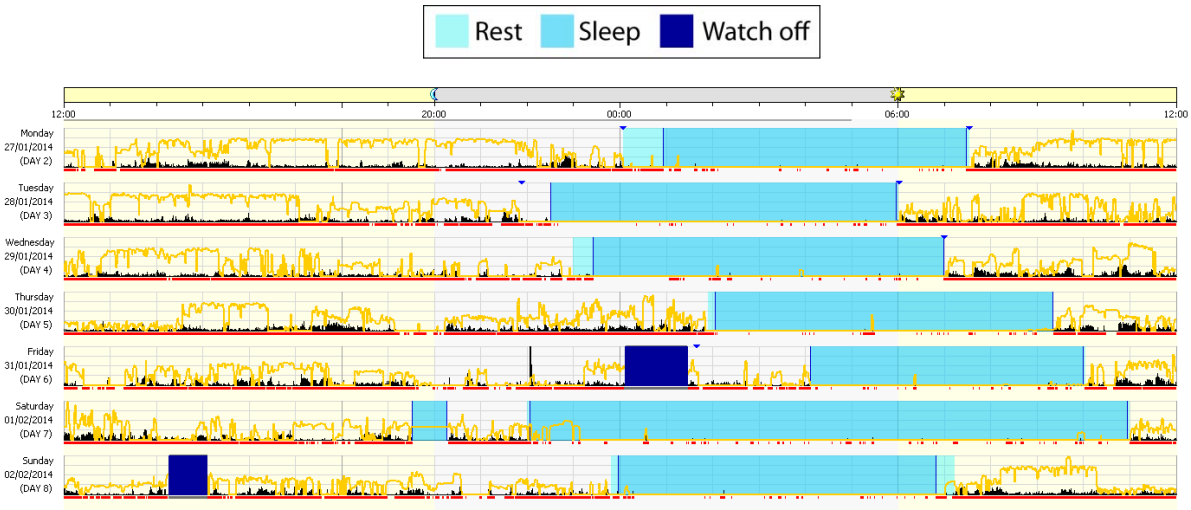

Figure 38b: An example of a portion of a two-week actigraph. Each row indicates a 24 hour period, beginning and ending at 12pm. Dark blue periods depict time when the participant indicated they took the watch off for prolonged periods (i.e. exercise, bathing, forgetting to put the watch back on after removing it).

6.2.3.2.1 Sleep/wake threshold

A pre-determined analysis threshold of 'low' (i.e. high sensitivity, ACT20) was selected, as this has been shown to have the best specificity and overall accuracy out of the three threshold options (high, medium, low) provided by the software (Cellini, Buman, McDevitt, Ricker, & Mednick, 2013; Kushida et al., 2001; Taibi, Landis, & Vitiello, 2013) and provides the highest correlations with polysomnography (PSG) measured wake after sleep onset

(Chae et al., 2009) and total sleep time in healthy older adults (Colling et al., 2000). Others have highlighted that medium (ACT40) and high (ACT80) thresholds overestimate the amount of wake, in comparison to PSG (Belanger, Bernier, Paquet, Simard, & Carrier, 2013). Using this threshold, the Actiware software classifies each one-minute epoch by comparing the epoch in question and those immediately surrounding it, to the threshold value of 20 activity counts per minute. Figure 39 displays how the activity counts of the epochs surrounding the 'epoch of interest' are multiplied to create a total activity count.

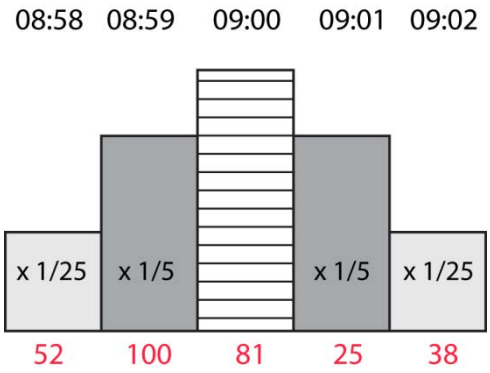

Figure 39: A graphical depiction of the weighted windowing function used for classifying an epoch as wake or sleep. Here, bar height represents the respective weighting of that epochs activity contribute to the calculation of total activity count. The epoch in the centre of the figure is the epoch to be determined. Example activity counts for each epoch are shown below in red. The activity counts of the surrounding epochs are multiplied by 1/25 or 1/5 and then summed together with the epoch to be defined. If the total exceeds the pre-determined wake threshold (low: 20 in our case) the epoch is defined as wake, if the total is less than, or equal to, the total, the epoch is defined as sleep.

So, for the activity counts displayed above, the epoch activity count for time 09:00 would be: Total activity count = 52*(1/25) + 100*(1/5) + 81 + 25*(1/5) + 38*(1/25)= 109.6

If the total activity count exceeds 20 it would be classed as a wake epoch, if it was equal to or less than 20, it would be classed as a sleep epoch.

6.2.3.2.2 Sleep onset

We used a sleep onset threshold of 5 minutes, as this has previously been shown to give the most accurate 'wake after sleep onset' durations when compared to PSG (Chae et al., 2009). Sleep onset is determined by identifying the first group of five epochs within a 'major rest' period (periods of low activity that are more than three hours in duration) for which at least four epochs are scored as sleep. Sleep onset is then set to the first epoch of the period satisfying these requirements.

6.2.3.3 Objective sleep quality measures

Sleep measures used for analysis were: *total sleep time (TST)*; total time scored as asleep during the night, *cumulative total sleep time (cTST)*; participant's total sleep time summed across the 14 nights, *wake after sleep onset (WASO)*; time scored as awake during the participant's night of sleep, *sleep onset latency (SOL)*; the time between the start of a given rest interval and the sleep interval start time, controlled by the sleep onset threshold, and *sleep efficiency (SE)*; the number of minutes asleep divided by the duration of the sleep interval.

6.2.3.3.1 Shorter/longer sleepers

In order to assess how thalamic/DMN FC may differ between young and older adults depending on habitual sleep patterns, we divided young and older participants separately into shorter/longer sleepers. Within each age group, a median split was performed on cTST to divide participants into shorter and longer sleepers. cTST was chosen as it is well known that actigraphy more accurately scores sleep than wake and thus it is more reliable at estimating global sleep measures (e.g. TST) than variables that include wakefulness (i.e. number of awakenings) (McCrae et al., 2005).

6.2.3.4 Subjective sleep quality measures

In addition to the objective measures of sleep, subjective measures of sleep quality were also included for analysis. These included:

1) Epworth Sleepiness Scale (ESS) (Johns, 1991): a subjective measure of a participant's propensity to fall asleep under different conditions. Participants rate how likely they are to fall asleep under certain conditions (e.g. "sitting inactive in a public place") on a scale of 0 (would never doze) to 3 (high chance of dozing). Scores are summed across the scenarios to create a 'daytime sleepiness' score where a greater value equals greater sleepiness.

2) Fatigue Severity Scale (FSS) (Krupp, LaRocca, Muir-Nash, & Steinberg, 1989): a subjective measure which summarises the severity of a participant's 'fatigue symptoms' by rating their agreement with nine statements related to fatigue, e.g. "fatigue interferes with my general functioning". Participants respond on a 7-point Likert scale and answers are summed across the questions, a greater score indicates greater fatigue severity.

3) Insomnia Severity Index (ISI) (Morin et al., 2011): this is a clinical screening tool for insomnia. Participants respond on a 4-point Likert scale (where 4 equals the most negative response to each question) to questions such as "To what extent do you consider your sleep problems to INTERFERE with your daily functioning?" Again, scores are summed across questions so that a greater score indicates greater likelihood of insomnia. Participants were screened with this questionnaire before taking part in our study, no participants scored over the cut-off score for risk of clinical insomnia (15/32).

4) Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989): participants respond to a number of questions designed to assess the participant's usual sleep habits during the past month. Participants are asked "During the past month, how often have you had trouble sleeping because you…" (e.g. felt too hot, have pain, need the toilet etc) and score 0-3 (where 0 equals 'not during the past month' and 3 equals 'several times a week'). Scores are

summed across the answers to give a 'sleep quality index.' Again, a greater score is associated with poorer sleep quality.

5) We also included the average subjective nightly sleep quality rating provided by the sleep diaries. For this question, participants were asked to rate each night of sleep as either poor, satisfactory, good or excellent.

6.2.4 Statistical analysis

Participants were divided into shorter/longer sleepers, as described above; as there was little variability in sleep quality between younger long/short sleepers (further explained in results) we compared older long/short sleepers to the younger age group as a whole. IBM SPSS Statistics for Windows (Version 20.0) was used to conduct mixed design ANOVAs with two factors: sleep status (i.e. young, long or short older sleepers) and network, and the interaction term sleep status*network to assess whether FC between 1) thalamic sub-regions and RSNs, 2) thalamic sub-regions and hippocampus and 3) thalamic sub-regions and PFC differed between young participants and older shorter/longer sleepers by using mixed design ANOVAs with three factors: sleep status, RSN node and thalamic sub-region, and their interaction terms. All results presented were corrected for multiple comparisons with Bonferroni correction. For ANOVAs where the principle of sphericity was violated, Greenhouse-Geisser correction was applied to degrees of freedom.

6.3 RESULTS

6.3.1 Sleep quality

6.3.1.1 Younger vs. older

6.3.1.1.1 Objective sleep measures

On average, older participants had a bed time of 23:09 and a rising time of 07:34. They spent an average of 8 hours and 42 minutes in bed, had an average TST of 7 hours and 2 minutes and an average cTST of 101.7 hours. In addition, they had a sleep onset latency of 8 minutes, 66 minutes of wake after sleep onset and an average SE of 82%. In comparison, younger participants had a bed time of 00:13 and a rising time of 8:00. They spent an average of 7 hours and 58 minutes in bed, had an average TST of 6 hours and 19 minutes and an average cTST of 102.2 hours. Their average sleep onset latency was 6 minutes; they had 78 minutes of wake after sleep onset and a SE of 80%. A mixed design ANOVA with the factors objective sleep quality and age group and their interaction terms was used to assess differences between TST, WASO and sleep onset latency. A significant sleep quality*age interaction ($F(1.17,44.59)=8.1$, $p=0.005$, $\eta^2=0.176$) indicated that sleep quality measures were differentially affected by age. Pairwise comparisons revealed that TST was significantly greater for older, compared to younger adults ($p=0.006$), while WASO and sleep onset latency did not differ between the two age groups ($p=0.08$, $p=0.14$ respectively). Similarly, cTST and SE were not significantly different for young and older adults, as assessed by one way ANOVAs ($F(1,38)=0.115$, $p=0.736$, & $F(1,38)=0.987$, $p=0.327$ respectively).

6.3.1.1.2 Subjective sleep measures

Older and younger adults did not differ on any of the subjective measures of sleep quality, as assessed by one way ANOVAs: Epworth sleepiness scale (ESS) (p=0.461), FSS (p=0.430), ISI (p=0.337), Pittsburgh Sleep Quality Index (PSQI) (p=0.073) and the average subjective rating stated nightly in the sleep diaries (p=0.269). Figure 40 displays objective (40a) and subjective (40b) sleep quality measures for the two age groups.

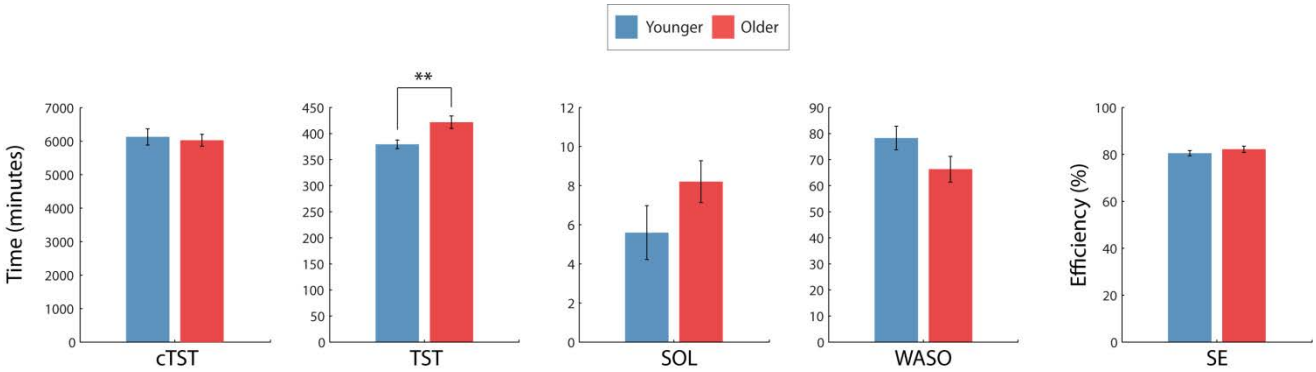

Figure 40a: Objective sleep measures compared between young (blue) and older (red) age groups. Error bars represent standard error across participants. An asterisk over a horizontal line depicts a significant pairwise comparison, **p<0.01.

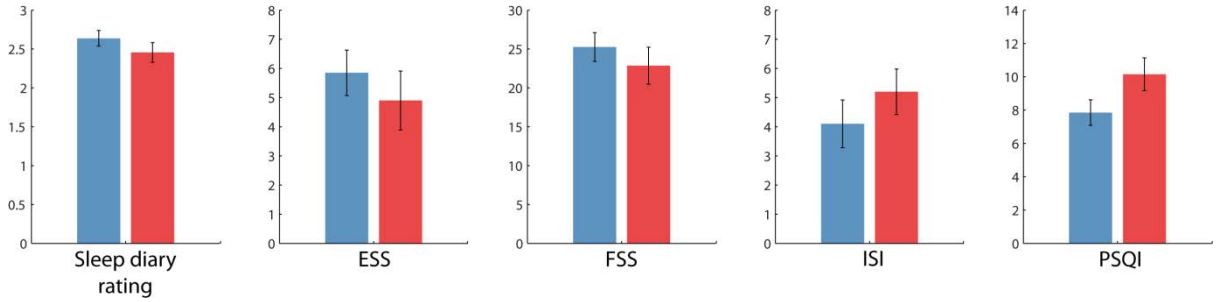

Figure 40b: Subjective sleep measures compared between the two age groups. Error bars represent standard error across participants.

6.3.2 Longer vs. shorter sleepers

6.3.2.1 Younger participants

For younger participants, a mixed-design ANOVA with the main effects: objective sleep quality and sleep performance (i.e. longer or shorter) and their interaction terms assessed the differences in WASO and sleep onset latency for the two sleep performance groups. A NS

main effect of sleep performance ($F(1,18)=0.581$, $p=0.456$, $\eta^2=0.03$) and a NS sleep quality*sleep performance interaction ($F(1,18)=1.98$, $p=0.177$, $\eta^2=0.099$) revealed that there was no significant difference in these sleep measures between the two sleep performance groups. Nor was a significant difference in SE or TST identified for the two sleep performance groups ($F(1,18)=1.689$, $p=0.210$ & $F(1,18)=1.85$, $p=0.19$). No significant differences were identified for any of the subjective sleep quality measures between the two sleep performance groups.

6.3.2.2 Older participants

For older participants, longer and shorter sleepers did not differ in terms of WASO ($p=0.504$) or sleep onset latency ($p=0.24$) as indicated by a NS main effect of performance group ($F(1,18)=0.792$, $p=0.385$, $\eta^2=0.042$) and a NS sleep measure*performance group interaction ($F(1,18)=0.185$, $p=0.672$, $\eta^2=0.01$). However, longer sleepers were found to have significantly greater SE and TST in comparison to shorter sleepers ($F(1, 18)=4.36$, $p=0.04$ & $F(1,18)=29.35$, $p<0.001$). No significant differences were identified for any of the subjective sleep quality measures between the two sleep performance groups. Table 15 presents descriptive statistics for actigraphy assessed sleep measures for both performance groups and both age groups.

Table 15: Descriptive statistics assessed by actigraphy for longer and shorter young sleepers and longer and shorter older sleepers, standard deviation is displayed in brackets.

	Younger Longer	Younger shorter	Older Longer	Older shorter
cTST	96.52 hours *(5.77)*	82.22 (4.10)	110.92 (5.45)	90.04 (9.86)
TST	06:44:33 *(00:23:46)*	05:48:20 *(00:25:17)*	07:42:45 *(0:29:09)*	06:20:39 *(0:38:02)*
Sleep onset latency	5.64 *(3.47)*	5.54 *(8.68)*	6.92 *(2.07)*	9.49 *(6.35)*
WASO	79.01 *(17.80)*	77.32 *(23.94)*	62.90 *(22.63)*	69.72 *(22.05)*
SE	81.46 *(3.44)*	79.29 *(6.26)*	84.69 *(5.09)*	79.66 *(5.68)*
Bed time	23:33:06 *(01:00:30)*	01:01:45 *(00:40:31)*	22:51:00 *(00:50:38)*	23:28:23 *(00:47:34)*
Rising time	07:46:58 *(00:44:54)*	08:16:56 *(00:52:13)*	07:53:31 *(00:42:48)*	07:13:45 *(00:26:03)*

6.3.3 Younger vs. older longer and older shorter sleepers

As there was no significant difference in sleep measures between the 'longer' and 'shorter' younger participants, we compared the FC of older longer (OLS) and older shorter sleepers (OSS) to the younger age group as a whole. Figures 41 and 42 display objective and subjective sleep quality measures for younger vs OLS and OSS.

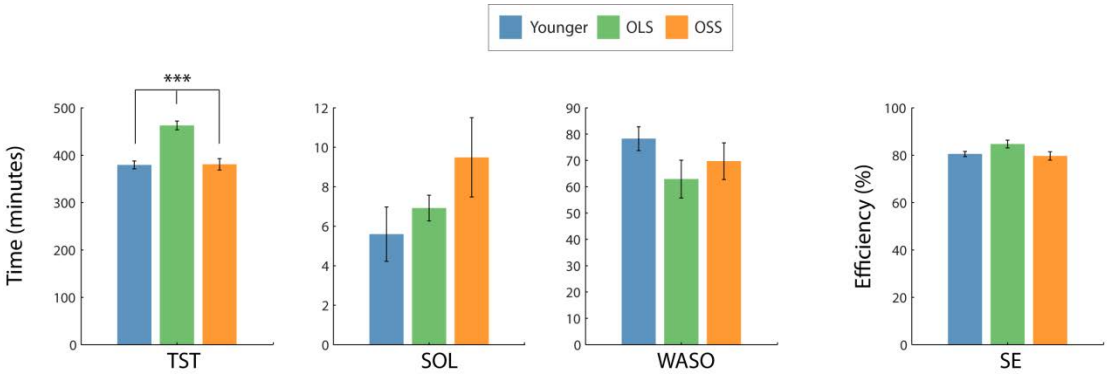

Figure 41: Comparison of objective sleep quality measures between younger participants, OLS and OSS. OLS had significantly greater TST compared to OSS and younger participants as depicted by the asterisk over the horizontal line, depicting the significant pairwise comparisons, ***p<0.001. Error bars represent standard error across participants.

A mixed-design ANOVA with the main effects objective sleep measure and participant group (i.e. younger, OLS, OSS) and their interaction terms assessed the differences in TST, WASO and sleep onset latency (SOL) for younger, OLS and OSS. A significant main effect of participant group ($F(2,37)=14.69$, $p<0.001$, $\eta^2=0.44$) and a significant sleep quality*participant group interaction ($F(2.49, 46.04)=15.40$, $p<0.001$, $\eta^2=0.45$) indicated that average sleep measures differed across participant groups and that there was a specific effect of participant group on sleep. Pairwise comparisons assessing the significant interaction between participant group and sleep measure revealed that these differences between participant groups were only significant for TST. OLS had significantly greater TST compared to OSS ($p<0.001$, $d=2.42$) and younger participants ($p<0.001$, 2.39), while OSS and younger participants did not differ significantly in their average TSTs ($p=0.9$, $d=0.04$). For WASO and sleep onset latency, the three groups did not differ significantly. However, for SE, a trend was identified ($F(2, 37)=2.94$, $p=0.066$), which suggested that OLS had greater SE in comparison to OSS ($d= 0.93$) and younger participants ($d= 0.85$). See Figure 41.

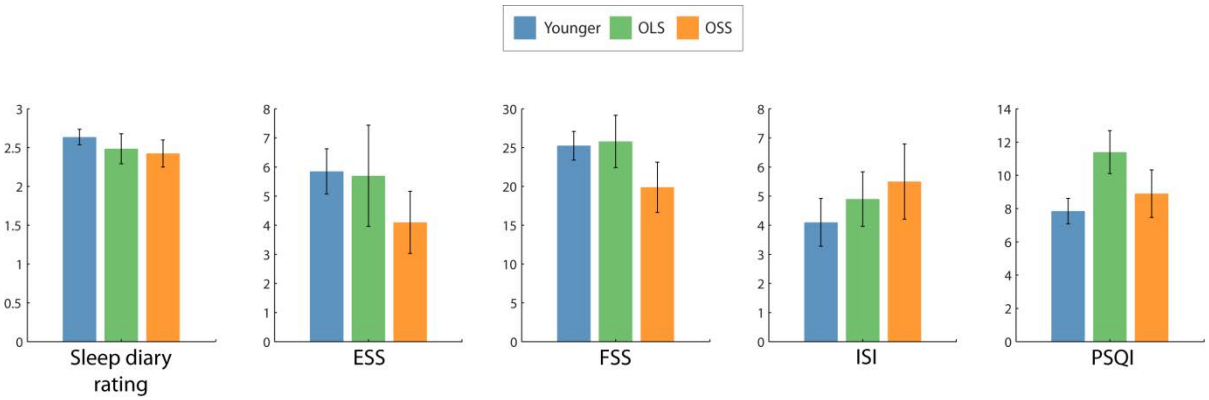

Figure 42: Comparison of subjective sleep quality measures between younger participants, OLS and OSS. Error bars represent standard error across participants.

The three groups were not found to differ significantly on the subjective sleep measures: ESS ($F(2,37)=0.667$, $p=0.519$), FSS ($F(2,37)=1.313$, $p=0.281$), ISI ($F(2,37)=0.530$, $p=0.593$) and the average subjective rating recorded nightly in the sleep diaries ($F(2,37)=0.649$,

p=0.528). However, a trend suggested that PSQI ratings differed between the three groups, with greatest scores reported for OLS (F(2,37)=2.781, p=0.075). See Figure 42.

6.3.3.1 Cognitive results

A significant one-way ANOVA (F(2,37)=6.72, p=0.003) revealed that OSS exhibited significantly slower reaction times compared to younger (p=0.001) and OLS (p=0.014). Younger adults and OLS did not differ significantly in their average reaction times (p=0.540).

Similarly, a significant one-way ANOVA (F(2,37)=4.85, p=0.013) revealed that OSS made significantly more errors on stage 6 of the PAL task compared to younger (p=0.006) adults. A trend suggested that OLS also made more errors on the PAL task compared to younger adults (p=0.06), however, OLS were not found to make a significantly different number of errors compared to OSS (0.390). See Figure 43.

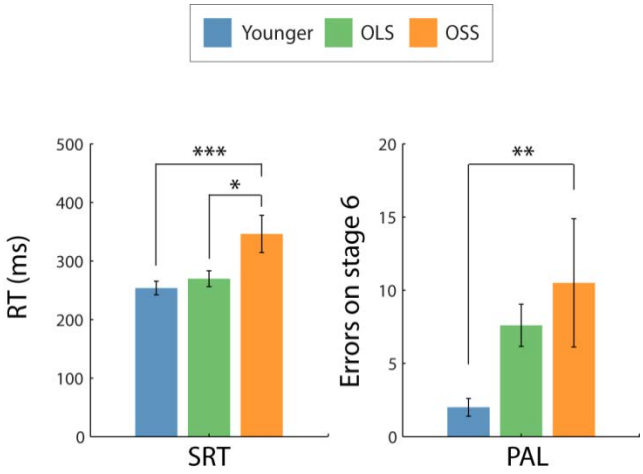

Figure 43: Comparison of cognitive performance on a simple reaction time task (SRT) and paired associates learning (PAL) test of memory, between younger participants, OLS and OSS. An asterisk over a horizontal line depicts a significant pairwise comparison, **p<0.01, ***p<0.001, *p<0.05. Error bars represent standard error across participants.

6.3.3.2 FC results

6.3.3.2.1. Sensory thalamic regions – sensory RSNs

Auditory RSN

Average thalamic-auditory RSN FC did not differ significantly between younger participants, OLS and OSS, independent of thalamic sub-region and RSN node, as shown by a NS main effect of participant group ($F(2,37)=0.538$, $p=0.588$, $\eta^2=0.028$). Nor did FC differ between thalamic sub-region dependent on participant group or RSN node, as shown by NS sub-region*participant group and sub-region*RSN node* participant group interactions ($F(3.36, 61.2)=0.75$, $p=0.54$, $\eta^2=0.039$ & $F(3.3, 61.2)=1.79$, $p=0.15$, $\eta^2=0.088$ respectively). See Figure 44.

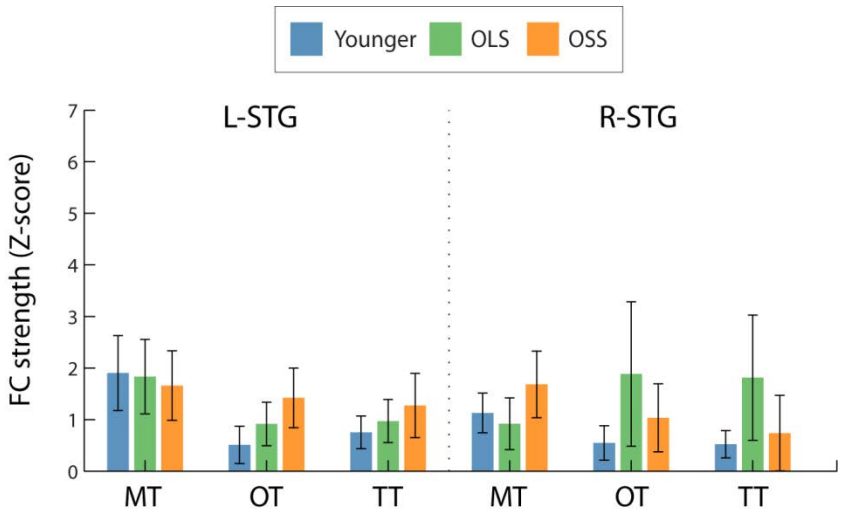

Figure 44: FC between first-order thalamic sub-regions (MT, OT, and TT) and nodes of the auditory RSN, for the three participant groups.

Motor RSN

Average thalamic-motor RSN FC did not differ significantly between younger participants, OLS and OSS, independent of thalamic sub-region and RSN node, as shown by a NS main effect of participant group ($F(2,37)=1.88$, $p=0.167$, $\eta^2=0.092$). Similarly, average thalamic sub-region FC did not differ significantly between the three participant groups, as

revealed by a NS sub-region*participant group interaction (F(2.6, 105.58)=1.18, p=0.325, η^2=0.06). However, thalamic-motor RSN FC differed across sub-regions for young/shorter and longer older sleepers, depending on RSN node; as indicated by a significant sub-region*participant group*RSN node interaction (F(5.70, 105.48)= 2.56, p=0.026, η^2=0.12). Pairwise comparisons revealed that longer older sleepers had significantly greater OT-left M1 FC compared to younger participants (p=0.02), and OSS (p=0.04). Furthermore, both older shorter and OLS had significantly greater TT-right M1 FC compared to younger participants (p=0.03 & p=0.048 respectively), but older shorter and longer sleepers did not differ significantly in TT-right M1 FC (p=1.0). For SMA, no significant differences were identified between thalamic sub-region and participant groups. See Figure 45.

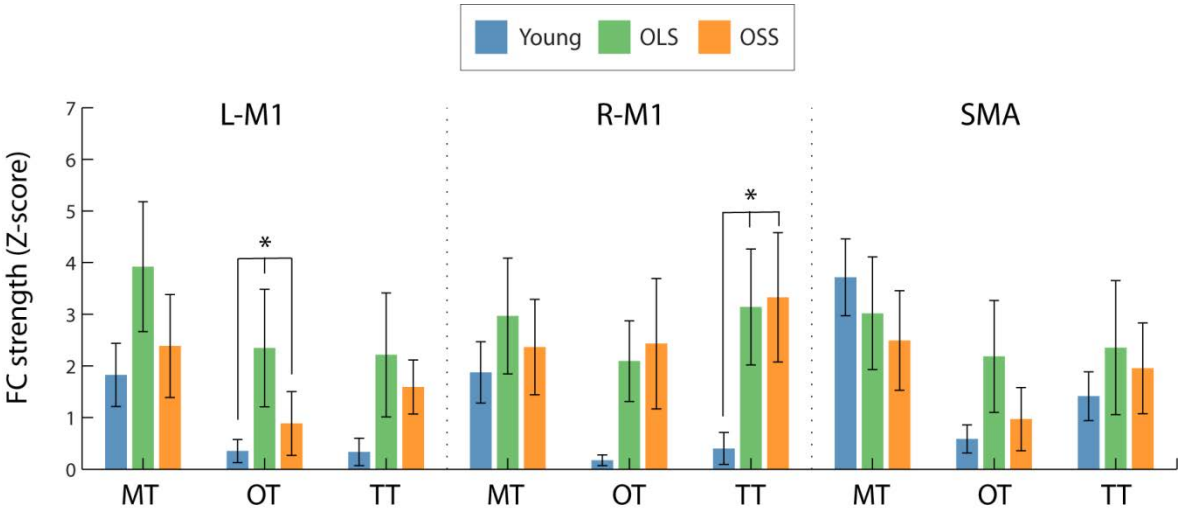

Figure 45: FC between first-order thalamic sub-regions (MT, OT, and TT) and nodes of the motor RSN, for the three participant groups. Asterisks over horizontal lines depicts the significant pairwise comparisons, *p<0.05.

Visual RSN

Average thalamic-visual RSN FC did not differ significantly between younger participants, OLS and OSS, independent of thalamic sub-region and RSN node, as shown by a NS main effect of participant group (F(2,37)=0.288, p=0.751, η^2=0.015). Similarly, average

thalamic sub-region FC did not differ significantly between the three participant groups, as revealed by a NS sub-region*participant group interaction ($F(3.67, 130.31)=0.42$, $p=0.793$, $\eta^2=0.022$). Nor did thalamic-visual RSN FC differ across sub-regions for younger/shorter and longer older sleepers, depending on RSN node; as indicated by a NS sub-region*participant group*RSN node interaction ($F(7.04, 130.31)= 1.14$, $p=0.34$, $\eta^2=0.058$). Despite the lack of significant interaction, there is some suggestion that for lateral visual regions, similar to the result found for motor cortex, OLS show reduced specificity in FC between thalamus and visual cortex, i.e. they show increased FC between the motor thalamic region and visual cortex. See Figure 46.

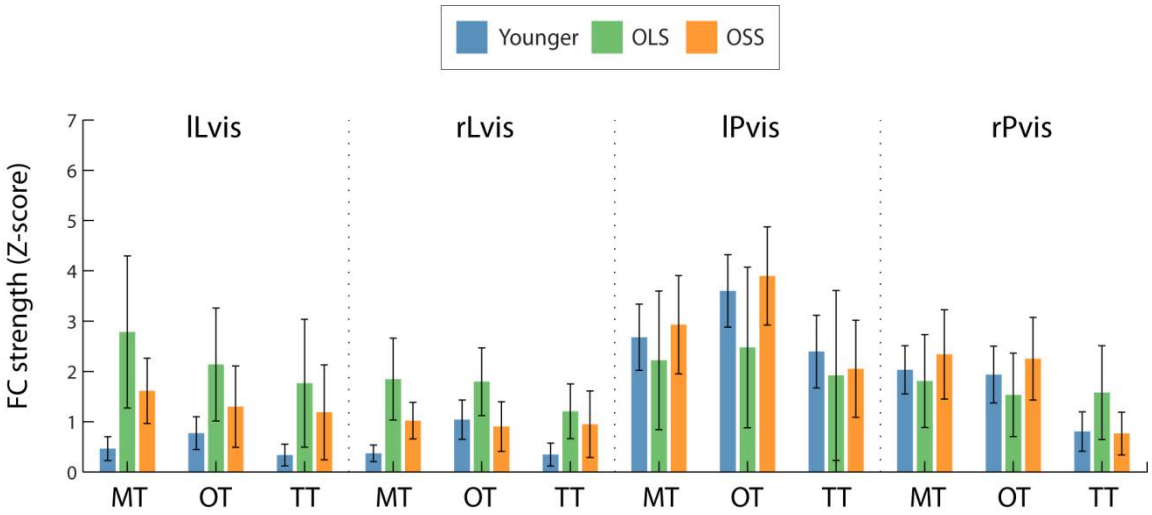

Figure 46: FC between first-order thalamic sub-regions (MT, OT, and TT) and nodes of the visual RSN, for the three participant groups.

6.3.3.2.2 Thalamic sub-regions – mPFC FC

Average thalamic-mPFC FC did not differ significantly between younger participants, OLS and OSS, independent of thalamic sub-region, as shown by a NS main effect of participant group ($F(2,37)=0.995$, $p=0.379$, $\eta^2=0.051$). Furthermore, younger, OLS and shorter sleepers did not differ in terms of thalamic sub-region-mPFC FC as indicated by a NS

sub-region*participant group interaction (F(3.33, 61.56)=0.652, p=0.6, η^2=0.034). See Figure 47.

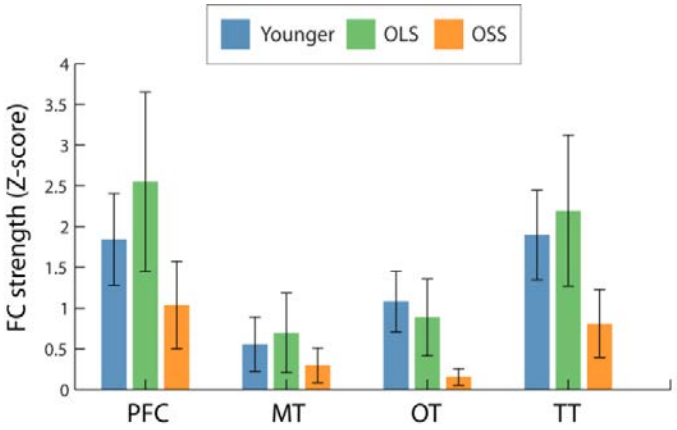

Figure 47: FC between higher order thalamic sub-region PFC and first-order thalamic sub-regions (MT, OT, and TT) and mPFC, for the three participant groups.

6.3.3.2.3 Thalamic sub-regions – hippocampal FC

Average thalamic-hippocampal FC did not differ significantly between younger participants, OLS and OSS, independent of thalamic sub-region, as shown by a NS main effect of participant group (F(2,37)=2.49, p=0.096, η^2=0.119). Furthermore, younger, OLS and shorter sleepers did not differ in terms of thalamic sub-region-hippocampal FC as indicated by a NS sub-region*participant group interaction (F(4.14, 76.58)=1.38, p=0.248, η^2=0.069). Despite the lack of significant interaction identified by this ANOVA we identify a trend where, across the majority of thalamic sub-regions, OLS exhibit greater thalamo-hippocampal FC compared to younger adults, and OSS exhibit greater thalamo-hippocampal FC compared to both groups, See Figure 48.

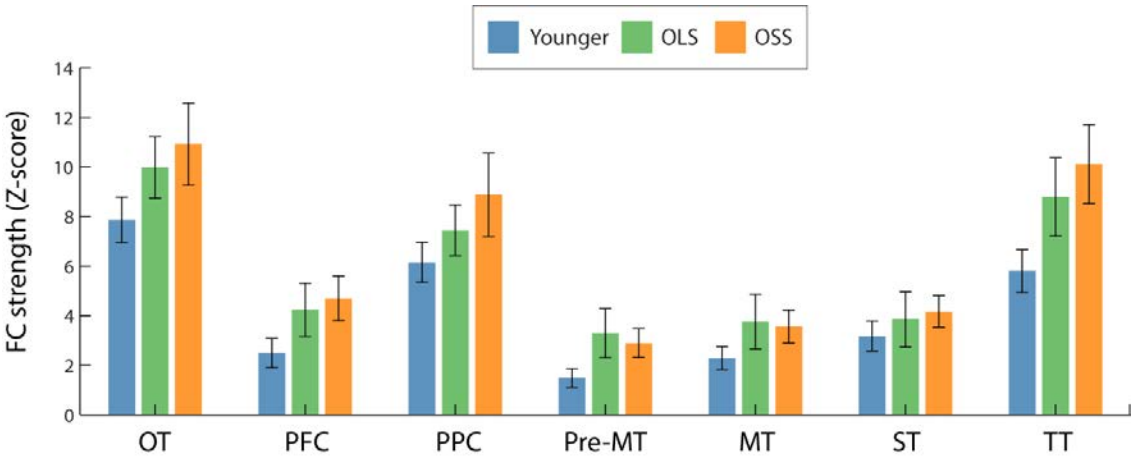

Figure 48: FC between all thalamic sub-regions and whole hippocampus, for the three participant groups.

6.3.3.2.2 DMN FC

Intra-network FC

Younger, OLS and shorter sleepers did not differ in DMN intra-network FC strength, as indicated by a NS one-way ANOVA ($F(2, 37)= 5.23$, $p=0.093$). See Figure 49.

PCC-DMN node FC

Average PCC-DMN FC did not differ significantly between younger participants, OLS and OSS, independent of DMN node, as shown by a NS main effect of participant group ($F(2,37)=1.58$, $p=0.219$, $\eta^2=0.079$). Furthermore, younger, OLS and shorter sleepers did not differ in terms of PCC-DMN node FC as indicated by a NS DMN node*participant group interaction ($F(8,148)=1.76$, $p=0.089$, $\eta^2=0.087$). Although this interaction is not statistically significant, it identifies a trend that suggests OSS exhibit reduced PCC-DMN node FC, particularly for mPFC, left IPL and left MTL, compared to both younger adults and OLS. OLS on the other hand, show PCC-IPL FC that is more similar to the FC strength seen in the younger adults. See Figure 49.

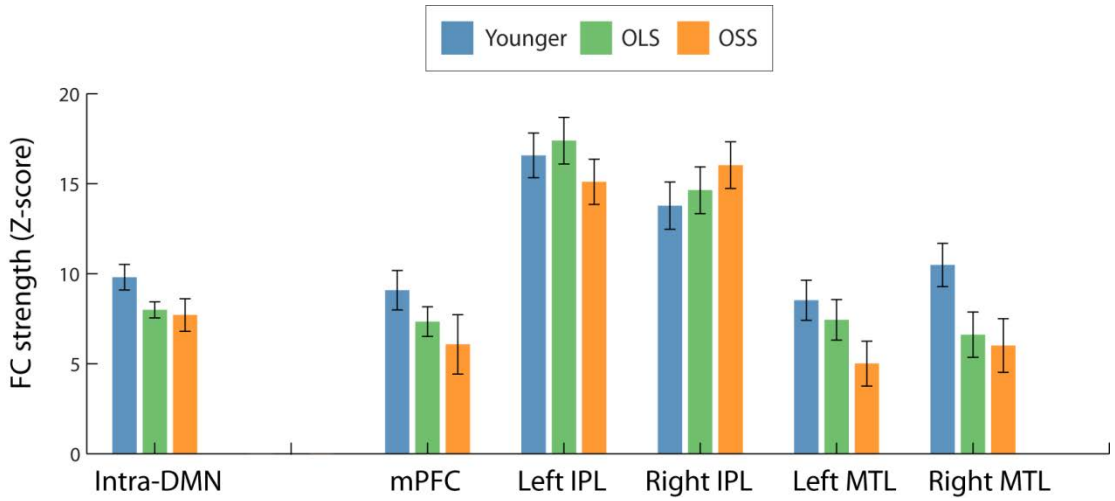

Figure 49: Average intra-network DMN FC and average PCC-DMN node FC for the three participant groups.

6.5 Discussion

This study investigated whether advancing age was associated with reduced sleep quality and whether thalamo-cortical and thalamo-hippocampal functional connectivity (FC) in older age differed in comparison to younger participants, depending on sleep quality. Older adults were found to have significantly greater total sleep time (TST) compared to younger adults, as assessed by wrist actigraphy. However, the two age groups did not differ on any other objective or subjective measures of sleep quality. By dividing the older group into longer and shorter sleepers, we found that half of the older participants had significantly greater TST compared to young participants. OSS were found to exhibit significantly slower reaction times to both younger and OLS, while younger participants and longer older sleepers exhibited similar mean reaction times. In addition, OSS were found to make significantly more errors on a memory task, compared to younger adults. Following the identification of cognitive differences between the three participant groups, we then sought to investigate how differences in functional connectivity may support the inter-group variability in cognitive performance.

Patterns of thalamo-cortical and thalamo-hippocampal FC were largely similar between the three groups. Although both groups of older participants showed increased thalamus-motor cortex FC (between visual and temporal thalamic sub-regions and M1) there was some evidence that longer sleep in older adults may enhance this effect. However, more research is required to investigate how variability in sleep quality may modulate increases (or decreases) in FC with age. Similarly, we identified a trend ($p=0.05$) for OSS to have greater thalamo-hippocampal FC compared to both OLS and younger participants. What is interesting to observe is that previously (Chapter 2) we identified within older adults that higher thalamus-motor cortex FC was associated with faster reaction time on the same simple reaction time task while higher thalamo-hippocampal FC (across all thalamic sub-regions) was associated with poorer memory performance. It is possible that greater sleep quality in older participants may be associated with patterns of FC which support preserved cognitive ability, or superior cognitive ability compared to those with shorter sleep. Alternatively, these FC difference may just represent a marker of preserved ability rather than specifically supporting improved/preserved cognitive ability. This is something that requires further investigation.

A number of recent studies have provided evidence that additional/increased FC in older adults is associated with both compensatory (Geerligs et al., 2014c) and interfering (Stevens & Spreng, 2014) effects, although to our knowledge, no studies to date have investigated how sleep may be associated with such changes in functional brain connectivity. A recent study by Wilckens and colleagues (2014) suggested that although better sleep quality was associated with better attentional performance, this effect was not mediated by age. However, the study did not consider good/poor sleepers within the older group. It remains to be seen whether older adults with the greatest sleep quality or longest sleep times, exhibit superior attentional performance to their poorer/shorter sleeping counterparts.

As well as exploring thalamic FC and sleep/age differences we also investigated DMN FC but again, identified no clear effect of sleep length in older age on PCC-DMN node FC. Results revealed a trend that suggests that shorter sleep durations may be associated with reduced PCC-DMN node FC for certain nodes of the DMN, (mPFC, left IPL, left MTL), compared to younger adults and OLS. Although this is a finding that requires further investigation. A number of studies have provided evidence for the disruption of the DMN with advancing age (Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Tomasi & Volkow, 2012a; Wu et al., 2011), including our own previous work (Chapter 2). The lack of considerable differences in sleep quality with age in our sample may explain why we fail to find any significant relationship between sleep length and DMN FC. Although it remains to be formally investigated, it may be the case that the DMN is fairly resilient to changes in sleep quality, and that differences are only noticeable once there has been a large disruption, for example following sleep deprivation. Alternatively, it could be that the additional effects of poor sleep quality are relatively small in comparison to age-related disruption of the DMN, particularly to frontal regions, which make up a large portion of the DMN.

A number of previous studies have suggested that poorer sleep quality in older adults is associated with impaired cognitive performance (Blackwell et al., 2006; Bonnet, 2000; Bonnet & Arand, 2003; Ferrie et al., 2011; Nebes et al., 2009; Oosterman et al., 2009; Pace-Schott & Spencer, 2011). While we also provide evidence for this finding, since the poorest older sleepers had significantly slower reaction times compared to young adults, we also provide preliminary evidence that OLS had reaction times comparable to younger participants. This suggests that for the healthy older adults tested, maintaining particularly good sleep quality, i.e. sleep quality superior to younger adults, was associated with superior cognitive performance to participants of similar chronological age but with inferior sleep quality. We also identified a similar finding with memory performance but to a lesser

degree. Although only OSS had significantly impaired memory in comparison to younger adults, after correction for multiple comparisons, memory performance between the two older groups was not significantly different. Further research is required to investigate differences in sleep quality within groups of older adults and how these differences may relate to cognitive performance. If future work were to replicate the findings presented here we will provide strong evidence that sleep quality in older adults should not be ignored when exploring cognitive disruption in age. By including all older participants in one homogeneous group (as in Chapter 2) we identified that older adults exhibited significantly slower reaction times and significantly shorter memory performance compared to younger adults. However, by segregating the older group based on sleep quality we have provided preliminary evidence to suggest that age alone may not result in cognitive impairment (e.g. slowing of reaction times) but that age-related disruption to certain cognitive abilities may be further impacted by poor sleep. As a group, the older participants tested here did not show significantly impaired sleep quality on any measures compared to the younger adults, showing no indication of inadequate or impaired sleep. Therefore, it seems quite promising to have identified such an effect within a group of relatively good, older sleepers. It would be interesting to replicate this study on a much larger group of both young and older adults, with much greater variability in sleep quality measures to identify whether the effect of this finding is greater when poorer, older sleepers are also included.

Some have argued that sleep disruption occurs up to around 60 years of age and then plateaus (Ohayon et al., 2004). However, others have identified that longitudinal declines in sleep quality were greater over a period of three years for very old adults (75-87 years) compared to a younger group of 65-71 year olds (Hoch et al., 1997). Furthermore, declines in sleep quality in this older group were not associated with changes in medical condition. Similarly, a recent study by Leigh, Hudson, and Byles (2015) identified sleep disruption in a

large sample (10, 721) of older women aged 70-75 and very old adults (85-90). They also suggested that older adults can be separated into several categories of sleep disruption and that a simple dichotomous distinction between 'disrupted sleep' and 'non-disrupted' sleep is too simplistic. This could mean that our distinction of 'longer' and 'shorter' sleepers based on TST did not adequately address the different ways and combinations of factors which may affect classification of longer or shorter sleepers (e.g. early waking, trouble initiating sleep, disrupted sleep). However, due to the relatively small sample size here, and the fact that sleep quality was obtained via actigraphy, we chose to focus this preliminary study on one global sleep measure that is relatively accurately measured via actigraphy methods (McCrae et al., 2005).

This study provided limited evidence for disrupted sleep in older adults; in fact, we identified increased TST with older age and a group of particularly good, older sleepers. Despite a considerable amount of research which has associated older age with reduced sleep quality (Bruce & Aloia, 2006; Hoch et al., 1997; Klerman et al., 2013; Roepke & Ancoli-Israel, 2010; Stanley, 2005; Van Someren, 2000), a number of studies have suggested that age-related sleep changes occur as a secondary consequence to other factors, such as impaired physical and mental health, medications, changes to sleep hygiene and reduced physical activity (Ancoli-Israel et al., 1991; Foley et al., 2004; Foley et al., 1999; Foley et al., 1995; Maggi et al., 1998; Vitiello, 1997). Therefore, it is possible that our results reflect a typical finding; that healthy older adults show little sleep disruption. It is also possible that older adults who chose to volunteer for this study may not be representative of the general population; they may be more active and social members of the community and may have a specific interest in cognitive research. Additionally, by screening for MRI suitability, we also risk recruiting the healthiest participants of the population group, as we exclude participants who have had heart surgery, knee and hip replacements and any physical

impairment that would prevent lying still for an hour. The participants included in this study were particularly healthy, with no chronic health conditions that required medication and had been screened for any clinically diagnosed sleep disorders.

Furthermore, although actigraphy has largely been successfully validated against the gold standard for sleep quality measurement (PSG) (Acebo & LeBourgeois, 2006; Ancoli-Israel et al., 2003; Blackwell et al., 2008; Morgenthaler et al., 2007), and provides a convenient way of assessing long periods of sleep quality, it does have limitations. Actigraphy has been shown to overestimate TST and sleep efficiency (Blackwell et al., 2008; Kanady, Drummond, & Mednick, 2011), and be relatively poor at detecting wakefulness during sleep (McCrae et al., 2005; Paquet, Kawinska, & Carrier, 2007; Sivertsen et al., 2006). However, it is worth keeping in mind that these previous studies have used less sensitive settings compared to the current study. Additionally, there is currently a lack of standardisation across devices and algorithms used for sleep/wake detection (Ancoli-Israel et al., 2003). Relatively ew studies have looked to validate the reliability and validity of different actigraph settings (Belanger et al., 2013; Cellini et al., 2013; Colling et al., 2000; Kim et al., 2013b; Kushida et al., 2001), especially to specifically compare measure between different populations. For this reason, we chose to use the same wake threshold detection for both age groups, however it is possible that the use of 'low' (high sensitivity) was reliable in older adults but may have underestimated sleep quality in younger adults. Alternatively, it may have been the case that actigraphy, or the thresholds/settings applied, was not sensitive enough to discriminate between healthy older vs younger sleep. Future studies should look to use simultaneous PSG and actigraphy to determine if there are more suitable detection thresholds and settings for different populations Finally, it is also possible that older adults exhibited greater periods of motionless wake, due to reduced mobility, which is impossible to detect via actigraphy (Morgenthaler et al., 2007). This could have resulted in

overestimated sleep quality in older adults who were actually awake but immobile. However, as the older participants in our study were particularly healthy, this is unlikely to be a concern. However, future work using simultaneous PSG and actigraphy could look to establish if this is a common phenomenon in older adults. Despite its limitations, actigraphy provides a means for monitoring habitual sleep patterns in the participant's natural home environment over prolonged periods of time, which would be otherwise very costly, time consuming and intrusive if using the "gold standard" for sleep assessment; PSG.

In summary, although this study did not provide evidence for an average decline in sleep quality with advancing age it highlighted some potential FC differences between longer and shorter older sleepers which could be related to cognitive function. Further research is required to fully understand how longer/shorter sleep interacts with advancing age and how such changes may impact on the re-organisation of functional brain networks with age. It remains unclear how greater brain activity and connectivity in older age impact on cognition, with evidence for both compensatory and interfering mechanisms. Perhaps by combining sleep measures with measures of brain connectivity and cognitive performance, this distinction may become clearer. However, the field of sleep research must continue to thoroughly validate measures of actigraphy against PSG in older adults, particularly comparing settings and thresholds between participant groups with simultaneous PSG-actigraphy. Without a thorough investigation and validation, we run the risk of applying inadequate methodology to an already complicated problem.

CHAPTER 7. DYNAMIC FUNCTIONAL CONNECTIVITY IN OLDER AGE.

ABSTRACT

The FC analyses presented until now have used measures of 'static' FC, i.e. a single measure calculated across an entire time series. However, a number of recent studies have provided evidence that RSN FC fluctuates over time in younger adults. To date, studies investigating how such RSN fluctuations may change with age are lacking. This chapter uses dynamic analysis to re-evaluate measures of intra- and inter- network FC where age-related differences were previously identified using static measures. This allowed us to assess whether measures of dynamic FC provide any additional information to that obtained from measures of static FC which could allow us to more specifically interpret age-related differences in RSN connectivity. Similarly, PCC inter-network FC, which did not differ significantly in static measures between age groups, was explored with dynamic FC to identify whether any age-related differences in FC were lost by using static measures. We identified that the spatial pattern and strength of dynamic FC was largely similar to that identified using static measures. Furthermore, age-groups did not differ in the degree of dynamic FC variability (i.e. how much FC fluctuated across a time series). This suggests that age-related differences in FC strength identified by static measures are not driven by changes to FC variability between age groups. The importance of developing informative summary measures for comparing dynamic FC between groups is discussed.

7.1 INTRODUCTION

The brain's activity is inherently variable, at all spatial scales (see Faisal Faisal, Selen, and Wolpert (2008) and Stein Stein, Gossen, and Jones (2005) for reviews) and the existence of dynamic, spontaneous neuronal signals has long been documented by both EEG and single cell recordings (Arieli, Sterkin, Grinvald, & Aertsen, 1996; Makeig, Debener, Onton, & Delorme, 2004; Mayhew, Hylands-White, Porcaro, Derbyshire, & Bagshaw, 2013a; Mayhew et al., 2013b; Onton, Westerfield, Townsend, & Makeig, 2006). A number of researchers have provided evidence that this temporal variability in activity has a functional role, and results in a greater dynamic range which allows for more adaptive and flexible responses to stimuli or cognitive demand (See Garrett and colleagues (2013b) for a review). However, the majority of FC studies to date have used measures of 'static' FC, i.e. a single correlation co-efficient calculated across the entire time-course of a scan of several minutes, thus assuming that FC between nodes, and networks, is temporally stable. However, considering our understanding of brain variability, it seems plausible to suggest that FC between resting-state networks (RSNs) and nodes may also be a dynamic, transient process, just as behaviour, attention and arousal states are transient. Recent studies have begun to investigate measures of dynamic FC, which typically uses a 'sliding window' method to divide time-courses into short, often over-lapping, temporal periods and calculates FC for each window, rather than over the whole time-course. Such studies have now provided evidence that FC between RSN nodes fluctuates across time and is perhaps not as stable as first thought (Allen et al., 2014; Chang & Glover, 2010; Handwerker, Roopchansingh, Gonzalez-Castillo, & Bandettini, 2012).

In a large study (n=405), Allen and colleagues (2014) identified transient 'states' of FC. That is, short periods of time where nodes typically thought of as 'belonging' to one RSN

temporarily form new networks, and then return to the typical pattern of FC. This suggests that the topology of RSNs may not be as stable and distinct as originally suggested by studies that have employed measures of static FC. Specifically, they identified that the 'typical' DMN actually subdivides into smaller RSNs over time, which have complex interactions with other networks, a similar finding to Smith and colleagues (2012) and Karahanoglu and Van De Ville (2015). Similarly, Chang and Glover (2010) identified that the PCC was dynamically functionally connected to nodes of the attention and salience networks, a finding which is typically not identified with measures of static FC. They also reported that even the relationship between the DMN and DAN, which is thought to be relatively well established, fluctuates over time. These findings highlight the potential over-simplification of our understanding of even the most well studied RSNs (i.e. the DMN) afforded by typical, static measures of FC.

Researchers attempting to understand what may drive such fluctuations in FC have investigated whether spontaneous BOLD events may be responsible. One commonly used method is point-process analyses (PPA), which typically identifies certain 'points' of a time-course to be included for FC analysis. One such method of point definition is to standardise the BOLD time-course and select all points that are 1SD above the mean (Tagliazucchi, Balenzuela, Fraiman, & Chialvo, 2012a). Studies which have employed PPA type methods have suggested that the FC of RSNs is driven by BOLD fluctuations at a few critical time points, rather than continuous, stable synchronisation across the entire time-course (Liu & Duyn, 2013; Tagliazucchi et al., 2012a; Wu et al., 2013). Specifically, Tagliazucchi and colleagues (2012a) reported that extracting data at only these time points resulted in a 94% dimensionality reduction yet typical RSNs were still identifiable, even from only 6% of the original fMRI data. This suggests that the typical patterns of RSN FC are driven by a relatively small number of large, spontaneous BOLD events. Tagliazucchi and colleagues

(2012a) also report that at the time of these spontaneous ”events”, the BOLD signal assumes the shape of the haemodynamic response function (HRF), which typically occurs as a result of an external stimulus , despite the fact the participants were simply ‘at rest’. Therefore, as spontaneous events follow the same properties of the BOLD response, which is used to map neuronal activity, it can be argued that by extension spontaneous events also represent neuronal activity. This provides further evidence that these spontaneous events contain meaningful information and are not simply a consequence of noise. Furthermore, Di and Biswal (2015) identified that the intrinsic activity of SN, DMN and motor RSNs was associated with both local and global FC measures. By dividing average ROI time-courses into ‘high’ and ‘low’ time points (categorised based on a median split of the BOLD signal amplitude across all time points) they identified that timepoints with greatest motor RSN signal were associated with greatest intra-motor FC and greatest brain modularity, which they suggest may serve to facilitate local information processing by supressing other RSNs.

However, one caveat of this type of analysis is that the thresholds used to define the ‘points’ to include in these types of analyses are somewhat arbitrary. More recent work by Allan and others (2015) used paradigm free mapping (PVM) to provide evidence that short, spontaneous BOLD events drive measures of FC. PVM allows the identification of spontaneous events simply by assuming that each spontaneous BOLD event assumes the form of an HRF and is less susceptible to confounds compared to PPA. By creating ‘activation time series’ for each voxel, which represent the timing and amplitude of detected events, it is possible to calculate FC before and after removal of such events to investigate their influence on measures of FC. In the study conducted by Allan and colleagues (2015) this was done by subtracting an identified event’s time-series (convolved with the HRF) from each brain voxel and re-calculating FC. The authors identified that when all spontaneous events were removed, FC dropped by 5-15%, thus indicating the involvement of spontaneous BOLD

events in measures of FC. However, only 29% of that reduction was attributed to 'co-ordinated network events' (i.e. when all nodes of a network simultaneously demonstrated a spontaneous event), suggesting that FC is driven by spontaneous events across distributed nodes, rather than co-ordinated events within networks. This study also provided evidence for the fluctuation of FC between RSN nodes, resulting in transiently connected sub-networks, atypical to the traditional RSN definitions. The results from this study support earlier findings which also reported that RSN intra-network FC fluctuated over time and peaked at the time of spontaneous events and that by regressing out these events FC strength was reduced (Petridou, Gaudes, Dryden, Francis, & Gowland, 2013). Taken together, these studies seem to confirm the contribution of transient BOLD fluctuations to FC measures, a view that is opposed to the sole importance of low frequency oscillations as first assumed.

Investigations into the sources of dynamic connectivity have found evidence that a significant amount of temporal fluctuations in FC have a neurophysiological origin (Brookes et al., 2011; Jerbi et al., 2010; Miller, Weaver, & Ojemann, 2009; Tagliazucchi, Von Wegner, Morzelewski, Brodbeck, & Laufs, 2012b; Thompson et al., 2013) and potentially reflect changes in vigilance, arousal or mind-wandering (Chang, Liu, Chen, Liu, & Duyn, 2013). This would presumably be particularly true for resting-state scans in which FC is typically assessed, as there are no tasks to restrict mind-wandering or random shifts in cognitive state, meaning that such fluctuations may be particularly frequent (Tagliazucchi & Laufs, 2014). However, a recent study by Elton and Gao (2015) found that although dynamic FC variability was reduced during a task, a substantial amount of variability still persisted. This suggests that fluctuations in FC are functional and not driven specifically by mind-wandering or shifts in cognitive state. However, it is possible that mind-wandering and loss of vigilance still occur whilst a participant is taking part in a task. Future work investigating

the modulation of FC depending on task difficulty/cognitive demand is required to shed light on cognitive factors which may drive fluctuations in FC at 'rest'.

As research into dynamic FC expands, it seems a natural progression to investigate the impact of age on dynamic FC measures. Despite a number of studies providing evidence that BOLD signal variability is generally reduced with older age (Garrett et al., 2011; Garrett, Kovacevic, McIntosh, & Grady, 2013a; Grady & Garrett, 2014; Grady et al., 2010), to date, no studies have directly assessed whether FC fluctuates to a greater or lesser extent with advancing age. One study by Madhyastha and Grabowski (2014) used factor analysis (computed on correlation coefficients across nodes and epochs) to investigate the dynamic FC of a group of 56-89 year olds. They identified a number of sub-networks for each main RSN investigated (DMN, DAN, fronto-parietal) and identified negative correlations between age and intra-network FC for specific sub-regions, rather than global effects on the FC of the entire RSN. However, it was also reported that this pattern of results was similar to those found from assessing FC 'statically' and the study did not report whether variability of FC across time windows was associated with age. Thus, it remains unclear exactly how dynamic FC may be affected by age and how such dynamic FC differences are related to age-related 'static' FC differences.

7.1.1 Chapter objectives

The current chapter addressed the question of whether investigating measures of dynamic FC provides any additional information regarding differences in FC between age groups, compared to those identified using 'static' FC (as in Chapters 4-5).

7.2 METHODS

7.2.1 Node choice

Dynamic FC was investigated for the nodes/networks where age differences have already been identified using 'static' FC (Chapters 4 and 5). These analyses included; 1) intra-network 2) ACC inter-network 3) thalamus-sensory cortex 4) thalamus-hippocampus. As it was identified (Chapter 4) that age differences in ACC-DAN 'static' FC were driven by gender, we also assessed the effect of gender on dynamic ACC inter-network FC. Furthermore, we assessed PCC inter-network dynamic FC (by age and gender), which did not show age differences at the static level. This allowed us to assess whether dynamic FC could reveal differences between groups that were not apparent when FC is calculated in a 'static' manner

7.2.2 Calculation of dynamic FC

The average resting-state time-course of each RSN node was segmented into 32-second epochs, with a 50% overlap, resulting in 55 epochs per time-course. This window length was chosen because a number have studies have highlighted that this is an adequate time window to conduct reliable dynamic FC analyses, as it replicates similar spatial patterns of FC calculated at longer windows but with improved temporal information (Leonardi & Van De Ville, 2015) (Chang & Glover, 2010; Elton & Gao, 2015; Hutchison, Womelsdorf, Gati, Everling, & Menon, 2013; Jones et al., 2012; Wilson et al., 2015). The FC between seed-target pairs was then calculated for each of these epochs, resulting in 55 correlation coefficients per seed-target pair (See Figure 50). In order to summarise and compare the dynamic FC of the two age groups, two measures were used: 1) dFCp: the percentage of epochs 'strongly' functionally connected (i.e. with a correlation co-efficient > 0.2), and 2) dynamic FC variability (dFCv): the standard deviation of FC strengths (correlation coefficients) across

epochs. We chose to ignore negative FC as it is well established that global signal regression can induce spurious negative correlations (Murphy et al., 2009; Saad et al., 2012a),

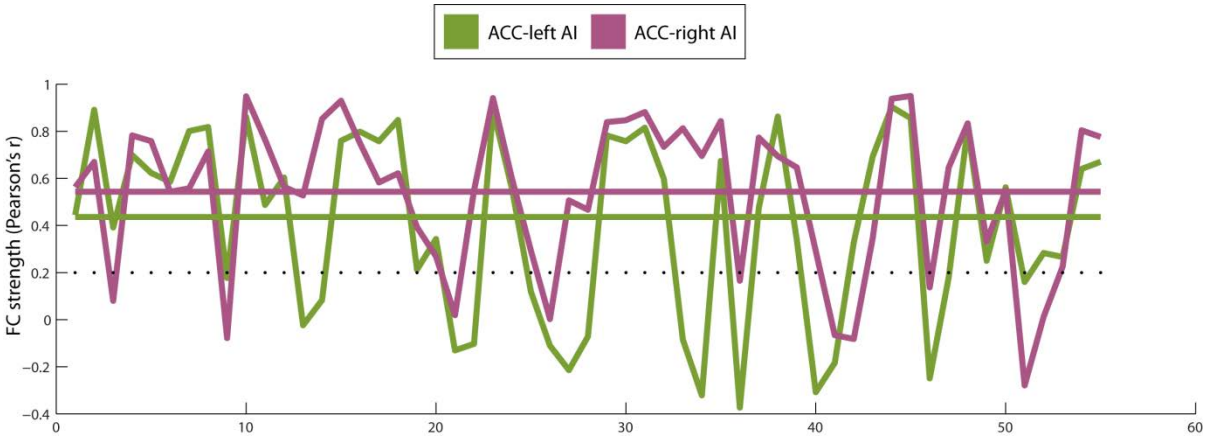

Figure 50: An example of ACC- left AI and ACC-right AI FC across the 55 epochs for one representative younger participant from this sample. Solid lines depict the average 'static' FC value (calculated across the whole time-courses rather than at each epoch) for this participant for comparison. All dynamic FC points above the black dotted line are included in the dFCp measure (i.e. >0.2).

7.2.2.1 Composite FC measures

In line with the analyses conducted in Chapters 2-5, composite network measures for dFCp and dFCv were separately calculated by taking the mean score across all paired connections to create an average measure for each network. This method was used for both measures of intra- and inter- network dynamic FC. For ACC and PCC inter-network dynamic FC figures also depict the individual ACC/PCC-node dFCp strengths that are averaged within a network to create the composite scores depicted in the composite figures.

7.2.3 Statistical analysis

IBM SPSS Statistics for Windows (Version 20.0) was used to conduct mixed design ANOVAs with between-subject factors of age group or gender to assess differences in either dFCv or dFCp between the two groups. Within subject factors consisted of either RSN for intra- and inter-network analyses or thalamic sub-region for dynamic FC analyses of the

thalamus. This allowed us to assess differential effects of age/gender dependent on RSN or thalamic sub-region. Individual ANOVAs were run for dFCv and dFCp and all results presented were corrected for multiple comparisons with Bonferroni correction. For ANOVAs where the principle of sphericity was violated, Greenhouse-Geisser correction was applied to the degrees of freedom.

7.4 RESULTS

7.4.1 Dynamic investigation of RSNs/nodes where age differences were previously identified by static FC measures

7.4.1.1 Intra-network dFC: cognitive RSNs

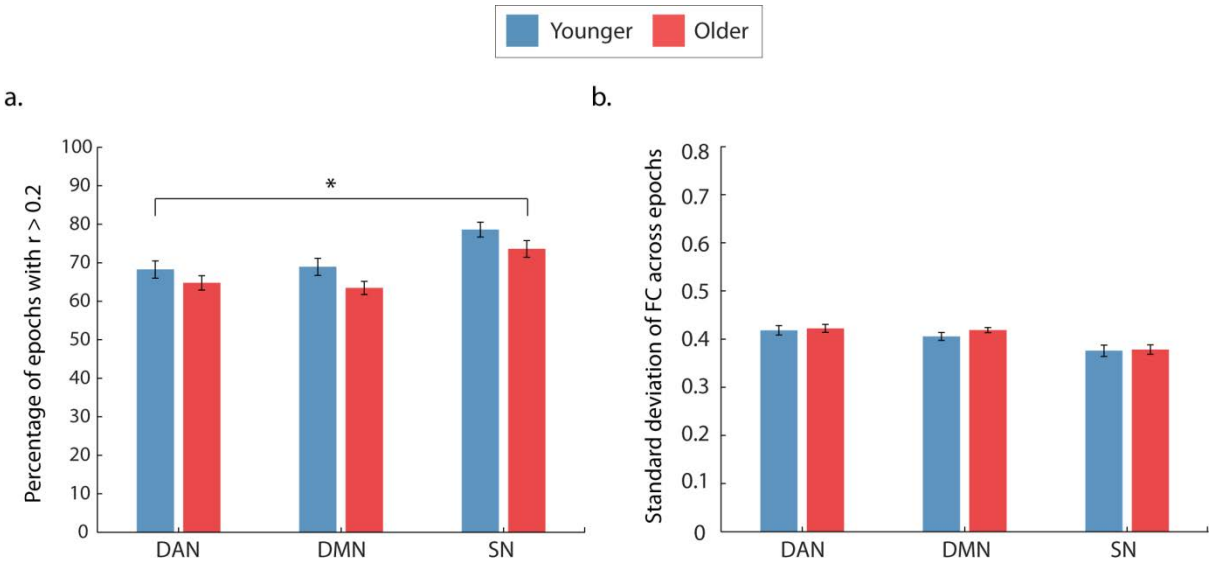

Figure 51: average intra-network dFCp (**51a**) and dFCv (**51b**) for the three main cognitive RSNs. These composite network measures are created from the average scores across all pairs of nodes. An asterisk over a horizontal line depicts a significant main effect of age, *p<0.05. Error bars represent standard error across participants.

7.4.1.1.1 dFCp

Younger adults were found to exhibit significantly greater intra-network dFCp for each of the three cognitive networks, as highlighted by a significant main effect of age

(F(1,38)=5.42, p=0.025, η^2=0.125). This age effect was independent of RSN, as indicated by a NS age*network interaction (F(2,76)=0.27, p=0.767, η^2=0.007). See Figure 51a.

7.4.1.1.2 dFCv

Younger and older adults did not differ in terms of intra-network dFCv for the three cognitive networks: DAN, DMN and SN, as highlighted by a NS main effect of age (F(1,38)=2.91, p=0.096, η^2=0.071) and a NS age*network interaction (F(2,76)=0.15, p=0.858, η^2=0.004), thus suggesting that dFCv does not drive the age–related differences in dFCp presented in Figure 51a. See Figure 51b.

7.4.1.2 ACC-network dFC

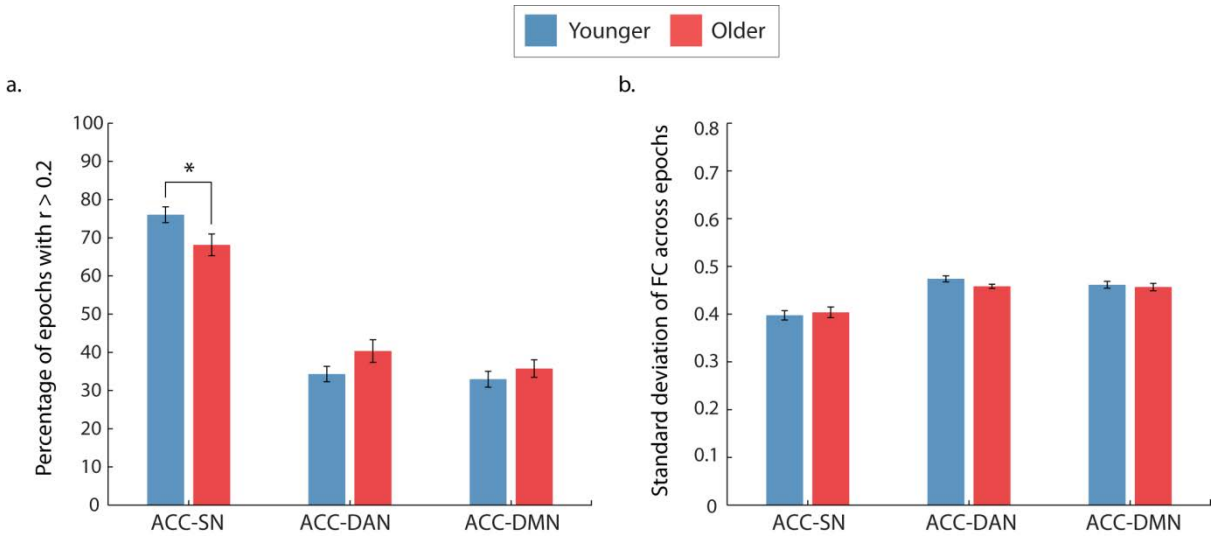

Figure 52: average ACC-network dFCp (Figure **52a**) and dFCv (Figure **52b**) for the three main cognitive RSNs. An asterisk over a horizontal line depicts a significant pairwise comparison, *p<0.05. Error bars represent standard error across participants.

7.4.1.2.1 dFCp

Age had a network-specific effect on ACC-network dFCp, as evidenced by a significant age*network interaction (F(2, 76)=3.95, p=0.023, η^2=0.094) and a NS main effect of age (F(1,38)=0.038, p=0.847, η^2=0.001). Pairwise comparisons revealed that older adults had

significantly weaker ACC-SN dFCp (p=0.031), but NS differences in ACC-DAN (p=0.1) or ACC-DMN (p=0.374) dFCp, in comparison to younger adults (see Figure 52a).

7.4.1.2.2 dFCv

Younger and older adults did not differ in terms of ACC-network dFCv, as highlighted by a NS main effect of age ($F(1,38)=0.37$, $p=0.548$, $\eta^2=0.01$) and a NS age*network interaction ($F(1.68, 63.92)=1.15$, $p=0.315$, $\eta^2=0.029$). See Figure 52b.

7.4.1.2.3 dFCp by gender

Age did not differentially affect ACC-network dFCp for female participants (Figure 53a) nor was ACC-network dFCp significantly different between female and male participants within age groups. This was identified by the NS effect of age (p=0.566, η^2= 0.019 for female participants) and gender (p=0.905, η^2=0.001 & p=0.234, η^2=0.076 for younger and older participants respectively), as well as NS age*network (p=0.556, η^2=0.027 for female participants) and gender*network (p=0.667, η^2=0.022 & p=0.549, η^2=0.032 for younger and older participants respectively) interactions

However, ACC-network dFCp was differentially affected by age for male participants, as indicated by a significant age*network interaction ($F(2, 36)=4.34$, $p=0.021$, $\eta^2= 0.194$). Pairwise comparisons revealed that older male adults had significantly weaker ACC-SN dFCp compared to younger male participants (p=0.04), however ACC-DAN and ACC-DMN dFCp did not differ significantly between the two age groups (p=0.119 & p=0.12 respectively). See Figure 53b.

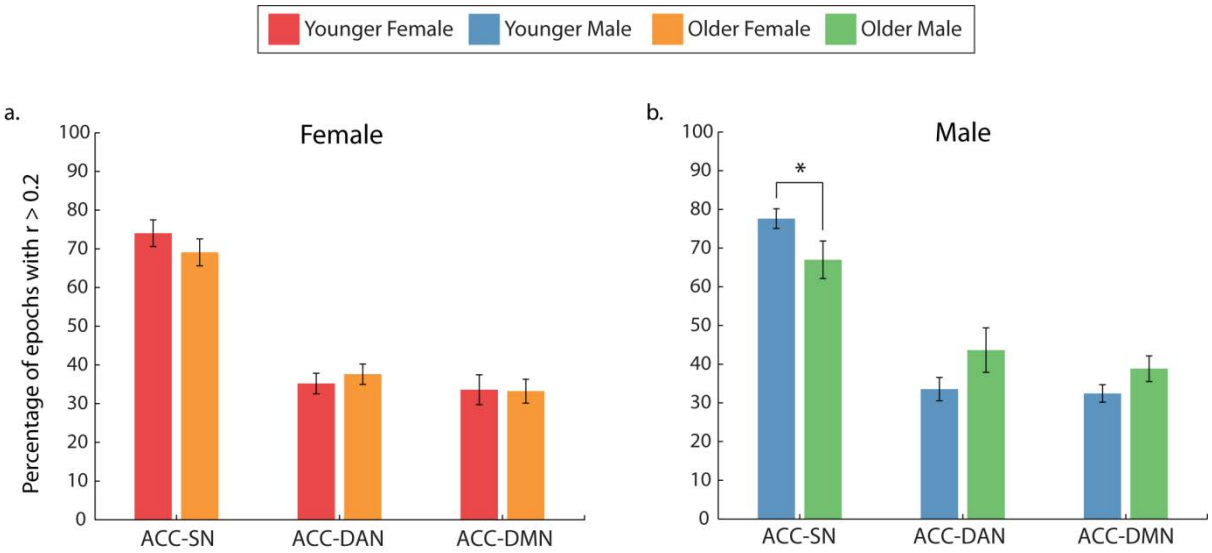

Figure 53: ACC –network dFCp for younger and older participants divided by gender (**53a**: female and **53b**: male). An asterisk over a horizontal line depicts a significant pairwise comparison, *p<0.05. Error bars represent standard error across participants.

7.4.1.3 Thalamus-sensory cortex dFCp: by RSN node

As the results from Chapter 5 revealed age-related FC differences between specific sensory thalamic sub-regions and sensory RSN nodes, we also investigated dFC in this way. dFCp was calculated (as this measure showed greater differences between age group for the composite thalamus-sensory RSN dFC, presented above, compared to dFCv) between each thalamic sensory sub-region (MT, OT, TT) and each sensory RSN node. This allowed us to assess paired thalamocortical dynamic connections that previously showed age-related differences (e.g. OT-left M1) and those that didn't (e.g. OT-left STG), using static FC measures.

7.4.1.3.1 Auditory RSN

Thalamic-auditory RSN dFCp did not differ as a function of individual RSN node, for the two age groups, as indicated by a NS RSN node*age interaction ($F(1,36)=0.379$, $p=0.542$,

η^2=0.01). Furthermore, age did not modulate patterns of dFCp between individual RSN nodes and thalamic sub-regions, as evidenced by a NS RSN node*thalamic sub-region*age interaction (F(2,76)=0.384, p=0.682, η^2= 0.01). See Figure 54a.

7.4.1.3.2 Motor RSN

Thalamic-motor RSN dFCp did not differ as a function of individual RSN node, for the two age groups, as indicated by a NS RSN node*age interaction (F(2, 76)=1.68, p=0.194, η^2=0.042). However, a RSN node*thalamic sub-region*age interaction trend (F(4, 152)=2.33, p=0.058, η^2=0.06) suggested that patterns of thalamic-motor RSN node dFCp differed for the two age groups. Pairwise comparisons revealed that older adults exhibited greater TT-left M1 dFCp compared to younger adults (p=0.021), OT-left M1 and MT-left M1 dFCp did not differ significantly between the two age groups (p=0.078, p=0.184 respectively). Similarly, older participants were found to exhibit greater MT-right M1 and TT-right M1 dFCp compared to younger participants (p=0.017, p=0.036 respectively). OT-right M1 did not differ significantly between the two groups (p=0.574). No significant dFCp differences were identified between thalamic sub-regions and SMA for the two age groups. See Figure 54b.

7.4.1.3.3 Visual RSN

Thalamic-visual RSN dFCp did not differ as a function of individual RSN node, for the two age groups, as indicated by a NS RSN node*age interaction (F(2.15, 81.64)=1.11, p=0.37, η^2=0.028). Furthermore, age did not modulate patterns of dFCp between individual RSN nodes and thalamic sub-regions, as evidenced by a NS RSN node*thalamic sub-region*age interaction (F(4.41, 167.65)=0.43, p=0.808, η^2= 0.011). See Figure 54c.

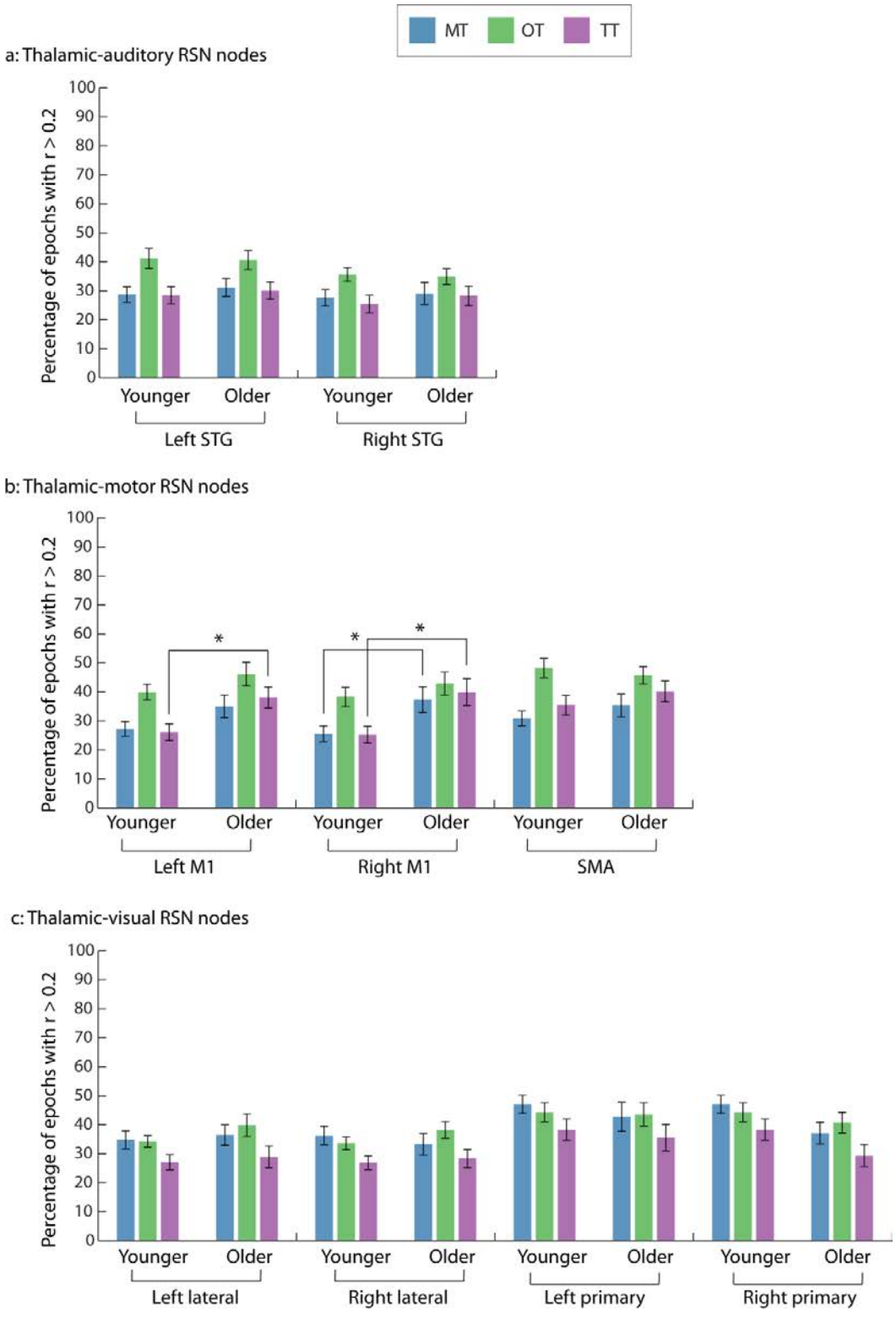

Figure 54: Thalamic – sensory RSN dFCp for each thalamic sub-region -individual sensory RSN node, for the two age groups: Auditory RSN (**54a**), Motor RSN (**54b**) and Visual RSN (**54c**). An asterisk over a horizontal line depicts a significant pairwise comparison, *p<0.05. Error bars represent standard error across participants.

7.4.1.4 Thalamic-hippocampal dFC

7.4.1.4.1 dFCv

In comparison to younger adults, older participants were found to exhibit reduced thalamo-hippocampal dFCv, independent of thalamic sub-region, as indicated by a significant main effect of age ($F(1, 38)=7.11$, $p=0.011$, $\eta^2=0.158$) and a NS age*sub-region interaction ($F(2.406, 91.43)=1.01$, $p=0.379$, $\eta^2=0.026$). See Figure 55a.

7.4.1.4.2 dFCp

Increased thalamo-hippocampal dFCp was identified in older, compared to younger, participants as indicated by an age effect trend ($F(1,38)=3.89$, $p=0.056$, $\eta^2=0.093$). A NS age*sub-region interaction indicated that this was a global effect, independent of thalamic sub-region, ($F(1.974, 75.03)=1.52$, $p=0.227$, $\eta^2=0.038$). See Figure 55b.

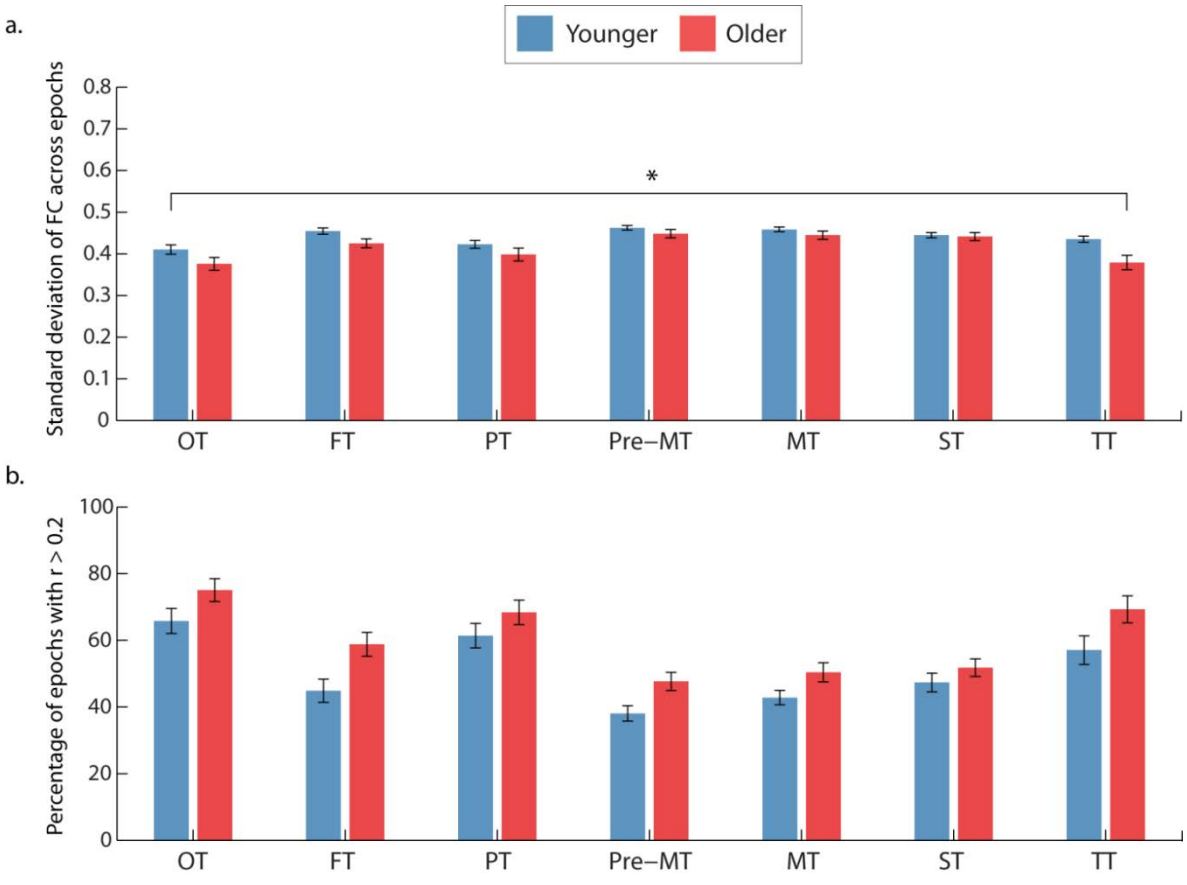

Figure 55: Average thalamic-hippocampal dFCv (**55a**) and dFCp (**55b**) between each thalamic sub-region and the whole hippocampus, for the two age groups. An asterisk over a horizontal line depicts a significant depicts a significant main effect of age at p<0.05. Error bars represent standard error across participants.

7.4.2 Dynamic investigation of RSNs/nodes where age differences have not previously been identified by static FC measures

7.4.2.1 PCC-network dFC

7.4.2.1.1 dFCp

Younger and older adults did not differ in terms of PCC-network dFCp, as indicated by a NS main effect of age ($F(1, 38)=0.13$, $p=0.718$, $\eta^2=0.003$) and a NS age*RSN interaction ($F(2,76)= 0.55$, $p=0.582$, $\eta^2= 0.014$). See Figure 56a.

7.4.2.1.2 dFCv

Similarly, younger and older adults did not differ in terms of PCC-network dFCv, as indicated by a NS main effect of age ($F(1, 38)=0.32$, $p=0.578$, $\eta^2=0.008$) and a NS age*RSN interaction ($F(2,76)= 1.38$, $p=0.257$, $\eta^2= 0.035$). See Figure 56b.

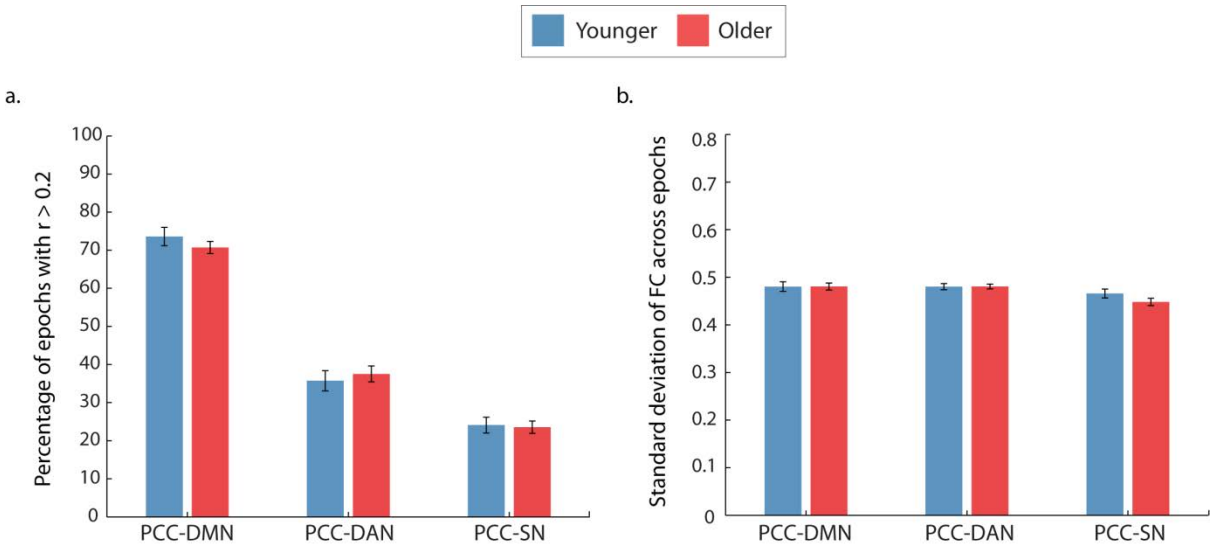

Figure 56: Average dFCp (56) and dFCv (56) between the PCC and each of the three main cognitive RSNs. Error bars represent standard error across participants.

7.4.2.1.3 dFCp by gender

PCC-network dFCp was not differentially affected by age, for the two genders (Figures 57a and 57b), nor was PCC-network dFCp significantly different between male and female participants within age groups. This was identified by the NS effect of age (p=0.365, η^2=0.046 & p=0.663, η^2=0.011 for female and male participants respectively) and gender (p=0.641, η^2= 0.012 & p=0.348, η^2= 0.049 for younger and older participants respectively), as well as NS age*network (p=0.399, η^2=0.05 & p=0.745, η^2=0.016 for female and male participants respectively) and gender*network interactions (p=0.534, η^2=0.034 & p=0.505, η^2=0.037 for younger and older participants respectively).

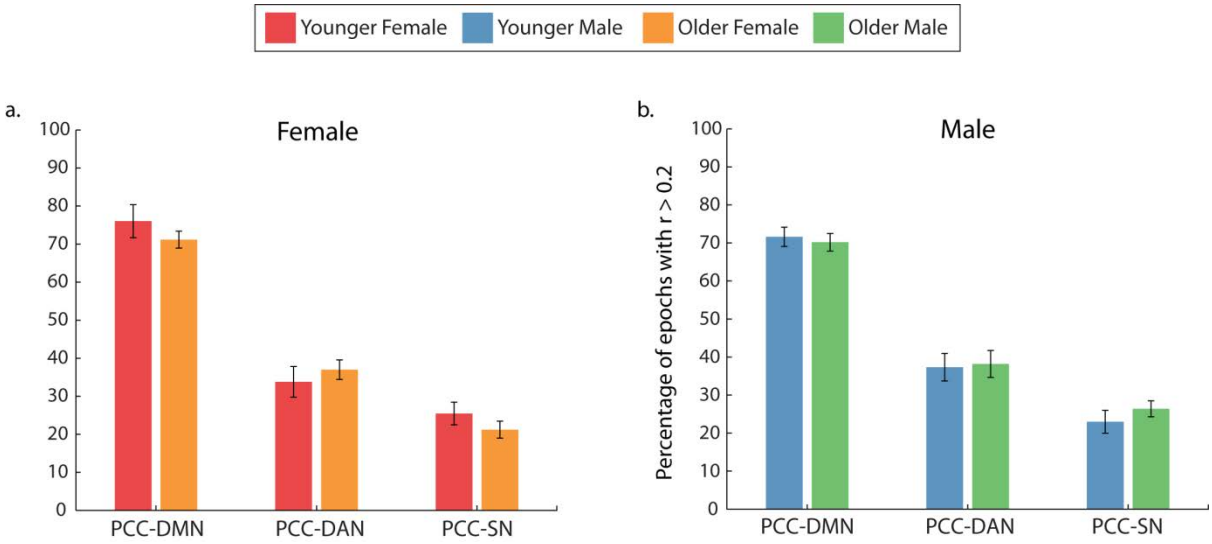

Figure 57: PCC –network dFCp for younger and older participants divided by gender (57a: female and 57b: male). Error bars represent standard error across participants.

7.5 Discussion

In this chapter we investigated whether measures of dynamic FC would provide greater information concerning differences in FC between younger and older adults, compared to the results we have already identified using 'static' FC measures. We identified that, at least by using the summary measures dFCv and dFCp, results from dynamic FC analysis were

largely similar to those we have already reported with 'static' FC. The two age groups did not differ in measures of dynamic FC variability (dFCv), other than for thalamo- visual cortex FC and thalamo-hippocampal FC, for which younger participants exhibited greater FC variability compared to older. All of the other main findings from our original static FC analyses were also identified using the dFCp measure. For example, younger, compared to older, adults exhibited greater intra-network dFCp for cognitive (DAN, DMN, SN) RSNs as well as greater ACC-SN dFCp, which was only identified for male participants when we divided the groups by gender. Similarly, older adults exhibited greater thalamo-hippocampal and thalamo-motor cortex dFCp, compared to younger participants. These results suggest that, at least for this data set and set of comparisons, measures of dynamic FC do not provide any more specific age-related differences compared to static FC. However, the fact that the standard deviation of dynamic FC (dFCv) largely did not differ between the two age groups provides us with some reassurance that the underlying signal properties of the BOLD time courses are not different between the two age groups, suggesting that the FC differences we have identified are not a consequence of differing physiology or signal property characteristics.

Despite the evidence provided by a number of researchers for fluctuating, dynamic FC between pairs of nodes and RSNs (Allan et al., 2015; Allen et al., 2014; Chang & Glover, 2010; Chang et al., 2013; Di & Biswal, 2015; Handwerker et al., 2012; Hutchison et al., 2013; Karahanoglu & Van De Ville, 2015; Liu & Duyn, 2013; Petridou et al., 2013; Smith et al., 2012; Tagliazucchi et al., 2012a; Tagliazucchi & Laufs, 2015; Tagliazucchi et al., 2012b; Wilson et al., 2015; Wu et al., 2013; Zalesky, Fornito, Cocchi, Gollo, & Breakspear, 2014), to what extent these fluctuations are neuronal in origin is still a question that is currently being investigated. A number of studies have suggested that these fluctuations have a neurophysiological underpinning (Brookes et al., 2011; Jerbi et al., 2010; Miller et al., 2009;

Tagliazucchi et al., 2012b; Thompson et al., 2013) and studies have reported correlations between dynamic FC and EEG; typically positive correlations between dynamic FC and the gamma band (30-80Hz) and negative correlations with the alpha band (8-12Hz) (See Tagliazucchi and Laufs (2015) for a discussion). However, to date, no studies have assessed dynamic FC with simultaneous EEG-fMRI in humans, which may shed more light on the origin of FC fluctuations, at least for cortical regions. A recent study by Thompson, Pan, and Keilholz (2015), simultaneously recorded fMRI and electrophysiology in rats and divided both time-series into epochs of matching length to correlate neural activity and dynamic FC at each time window. The authors demonstrated that sliding window FC measures were significantly correlated with high frequency neural activity across time, thus suggesting a neural origin to dynamic FC. Although these results cannot be directly applied to humans, they provide some evidence that the process of sliding window correlation may not induce spurious correlations (see discussion below). Similarly, although some have reported that dynamic FC and head motion/cardiac artefacts/respiration are not associated (Tagliazucchi et al., 2012a) one study has provided some evidence that spontaneous events in motor cortex were related to small physical movements (e.g. hand movement), although the relationship was not always consistent (Petridou et al., 2013). Further research is required in order to firmly establish the lack of influence of non-neuronal confounds on dynamic FC and spontaneous BOLD events and the true extent to which dynamic FC reflects fluctuations in neural networks.

The use of measures of cognitive ability may also help to shed some light on the cognitive consequences, and neurophysiological origin, of dynamic FC. A recent study by Elton and Gao (2015) identified that the brain was globally more efficient during rest compared to task periods, despite the fact that dynamic FC was most variable during rest. This confirms results from a previous study by Zalesky et al. (2014) which suggested that

frequent state changes during rest may result in greater global brain efficiency. They argue that at rest the brain is free to spontaneously fluctuate, naturally exhibiting properties that make it globally efficient; however, when a task begins some global efficiency is compromised in order to sustain task-relevant connectivity/networks. Similarly, others have argued the functional importance of brain variability, suggesting that the brain functions "at the edge of criticality" (Deco, Jirsa, & McIntosh, 2011; Ghosh, Rho, McIntosh, Kotter, & Jirsa, 2008; McIntosh, Kovacevic, & Itier, 2008)(Deco, 2011; Ghosh 2008; McIntosh 2008).This suggests that the brain maintains an optimal balance between numerous possible functional states, thus affording the greatest dynamic range and the most adaptable and efficient processing (See Garrett 2013b for a review). Furthermore, research explicitly investigating age-related differences in BOLD variability have largely identified decreases in variability in older age (See Grady and Garrett (2014) for a review). Furthermore, (Garrett, Kovacevic, McIntosh, & Grady, 2010) report that the age-predictive power of BOLD variability is five times greater than that of mean BOLD signal, suggesting that variability measures may provide a greater insight into functional brain changes with age compared to mean amplitude differences. Similarly, studies investigating BOLD variability and cognitive function have reported that greater BOLD variability is associated with faster and more stable performance (Garrett et al., 2010, 2011, 2013a). Specifically, Garrett and colleagues (2013a) identified that younger and older fast performers typically exhibited the greatest variability in task conditions, while older slower performers exhibited reduced variability within task conditions and less differentiation in variability between fixation and task. Despite the typical finding of reduced cortical BOLD variability with age, some studies have also provided evidence for increased subcortical BOLD variability in older adults (Garrett et al., 2010, 2011, 2013a; Samanez-Larkin, Kuhnen, Yoo, & Knutson, 2010). Recently, a study by Guitart-Masip (2015) also identified increased sub-cortical and decreased BOLD

variability in older, compared to younger adults, during a working memory task, both of which were associated with poorer performance. Interestingly, they provided evidence that reduced dopamine receptor density (assessed by PET imaging) was associated with the increased BOLD variability in subcortical regions. These studies provide evidence that increased variability of subcortical regions in older adults is associated with slower performance. Our own results have identified that younger and older adults differ in terms of dynamic FC for a number of thalamo-cortical connections which we have previously associated with differences in cognitive performance (Chapter 3). However, despite identifying greater subcortical dFCp in older, compared to younger adults, dFCv was largely similar for the two age groups and in some cases (thalamo-hippocampal, thalamo-visual) lower for older adults. Although based on Garret and colleagues' findings one may expect increased subcortical dFCv, the results in Chapter 1 clearly highlight that RSFA (i.e. BOLD SD) is not linearly associated with FC. Future work is required to develop more sophisticated summary measures of dynamic FC variability and to establish how variability in BOLD may be associated with dynamic FC, for both rest and task states. By assessing how patterns of dynamic FC change with age (or disease) and associating such changes with cognitive deficits, or improvements, we may better understand both the consequences of brain changes on cognitive function, as well as how the dynamic nature of brain RSNs may support cognitive function in the first place.

Despite the potential for dynamic FC analyses to enrich our understanding of the brain's functional connectivity, the method does currently have some limitations. For example, due to the poor temporal resolution of fMRI, one of the caveats of dynamic FC analysis is how to select the length of the sliding window, as it is difficult to naturally divide the BOLD time-course into meaningful windows. Research has provided evidence that sliding window analysis can induce spurious correlations (Robinson, de la Peña, & Kushnir, 2008). Similarly,

a recent study by Leonardi and Van De Ville (2015) highlighted that the window length chosen for dynamic FC analysis requires careful consideration, because combined with other factors (e.g. high pass filtering) it can result in spurious correlations. To address this concern, a recent study by Handwerker and colleagues (2012) used simulated time-series, which contained real fMRI data, but with randomised phase (i.e. the timing relationships between voxels were randomised). They revealed that FC fluctuations still appeared, even when the phase was randomised to remove temporal correlations between voxels, and were in fact based on the frequency content of the BOLD signals, as opposed to their amplitude fluctuations. They also reported that often the magnitude of changes in structured noise was larger than the magnitude of FC changes. These results cast some doubt over whether fluctuations in FC are always meaningful or whether they are affected by the methodology commonly employed in measures of dynamic FC. Similarly, when it comes to investigating how age may interact with dynamic FC, it remains to be seen whether age effects may be greater for certain temporal window durations. One recent study by Madhyastha and Grabowski (2014), which assessed the dynamic FC of a group of 56-89 year olds, reported no age* window length*FC interactions, suggesting that for this age group at least, the relationship between FC and window length did not significantly alter with age. However, future research will need to investigate whether this relationship is consistent when directly comparing groups of older and younger adults, or similarly, healthy vs. patient groups. Clearly, while the evidence for the fluctuation of FC over time is not disputed, further research is required to establish how best to segment resting-state time courses to ensure that measures of dynamic FC are meaningful, in that they are aligned with neuronal activity or behaviour. At this point, until the development of more robust analysis methods, such as data driven techniques used to detect time points (Cribben, Haraldsdottir, Atlas, Wager, &

Lindquist, 2012), current evidence suggests that measures of dynamic FC are determined by a combination of both neurophysiological and methodological factors.

Another caveat of dynamic FC analysis is the vast number of measurements generated. For example, in this study each seed-node pair resulted in 55 correlation coefficients, compared to just one when calculating 'static' FC. This poses a problem when it comes to comparing groups as it is unclear how best to summarise measures for comparisons across groups. In order to do this, we simply calculated the standard deviation of correlation coefficients across all epochs and compared these between the two groups. However, this is a fairly simplistic method which may simply reduce the richness of the data provided by calculating dynamic FC, so that the end result is not much more informative compared to calculating static FC. Similarly, we also calculated the percentage of epochs that exhibited FC greater than a certain threshold (r=0.2), Which, although arbitrary, can be considered moderate network adherence, particularly when GSR has been applied (Wilson et al., 2015). However, this measure is limited and may not provide much more information compared to static FC measures. For example, if the FC is large at the average (static) level, it makes sense that it would also be large when summarising in this way. However, this may not necessarily be the case if static FC is driven by relatively few, isolated peaks in BOLD signal as suggested by some recent studies (Allan et al., 2015; Liu & Duyn, 2013; Tagliazucchi et al., 2012a; Wu et al., 2013). However, this method provides a crude way of assessing whether average FC is high because of a relatively small number of spikes in dynamic FC, or whether FC is fairly consistent across time. By comparing this result between the two age groups we at least get an idea of whether there are gross differences in the static and dynamic FC relationship between the two ages. However, it is important to keep in mind that the lack of differences in dynamic FC between the two age groups identified here may be a consequence of the summary measures that we have employed. A recent review by Calhoun et al. (2014)

reported that the accuracy of between-group FC differences was vastly improved when measures of both static and dynamic FC were included in a classifier to identify different FC 'states'. They suggest that often, differences in FC between groups occur within specific 'states', which may not be detected by more simple summary dynamic FC measures (as used here). Future work should look to further validate such classifier and clustering methods, as well as establishing more informative summary measures, an example of which may be to compare the distribution of dynamic FC measures. Generally, these should allow the reduction of dimensionality for comparison, whilst retaining the richness of the data afforded by dynamic, compared to static, FC analysis.

However, despite these caveats, dynamic FC analysis is an interesting area of research which, with more refined methodology and analysis, will allow for a more thorough understanding of RSNs, and more generally, brain function. Recent dynamic FC findings pose some interesting questions regarding the ageing brain and may provide some working theories based on changes to FC may result in cognitive decline. For example, one could posit that older adults with more variable dynamic FC may exhibit superior cognitive performance to their peers with less variable FC, or that those with poorest cognitive abilities show less distinction, in terms of RSN configuration, between task and resting-states. Future work will need to focus on assessing dynamic FC between age groups, in order to firmly establish whether dynamic FC does alter with advancing age, and if so, what the cognitive consequences may be.

THESIS SUMMARY AND CONCLUDING REMARKS

This chapter provides a brief summary of the main findings presented in the experimental chapters. I will also discuss some potential limitations to this work and suggestions for future research directions.

8.1 SUMMARY OF FINDINGS

This thesis investigated the impact of advancing age on the FC of both typical cortical resting-state networks and subcortical structures. Furthermore, it explored how such differences may be driven by changes in sleep quality, also thought to be affected by age, and how such interactions may contribute to typical cognitive disruption associated with older age.

The results suggest that older age is associated with the re-organisation of resting-state networks, with older adults typically exhibiting weaker intra- and greater inter-network FC compared to younger adults. These changes in FC were not found to be homogenous across the brain and were instead specific to individual RSN nodes. Furthermore, re-organisation of cortical RSNs with older age was found to be driven by gender-specific spatial re-organisation of RSN nodes, as well as simple changes to FC strength. Further investigation of thalamic FC revealed that older adults also exhibited greater thalamo-sensory and thalamo-hippocampal FC, which was related to cognitive performance on RT and memory tasks, respectively. Dynamic evaluation of these functional connections revealed very similar patterns of FC compared to the static measures, suggesting that differences in FC between age groups were not driven by how variably connected nodes were and instead age-related FC differences were consistent across the length of the scan.

Investigation into participant's sleep patterns suggested fairly similar sleep profiles between the two age groups, however sleep quality was more variable amongst the older participants. After dividing the older group into long and short sleepers we identified that the two sub-groups differed in terms of cognitive performance; i.e. older longer sleepers had significantly better cognitive performance compared to older shorter sleepers. In addition, older longer sleepers seemed to exhibit patterns of thalamic FC which had previously been associated with better cognitive performance (i.e. similar levels of thalamo-hippocampal but stronger thalamo-motor FC compared to younger participants). These results provide preliminary evidence that sleep may be associated with more 'preferable' patterns of FC in older adults, which are beneficial for cognitive function.

These results highlight the importance of assessing FC across the whole brain, including subcortical structures such as the thalamus, when investigating cognitive functioning in older adults. To date, the majority of studies have focussed on identifying how the well documented age-related differences within the cortex, particularly the PFC, may be associated with cognitive disruption. However, the thalamus is a structure that is densely and reciprocally connected to the cortex which could feasibly alter cortical functioning if it is detrimentally affected by advancing age. Therefore, the thalamus' role in cognitive ageing clearly warrants further investigation. Similarly, the results from Chapter Six highlight the potential role of sleep in the cognitive ageing process. Even in a group of healthy older participants we identified an association between sleep length and cognitive performance as well as a potential link between thalamic FC and cognition, related to sleep. Investigating this finding in a larger sample with greater variability in sleep quality will allow us to establish exactly how sleep and patterns of FC may interact within the ageing brain.

8.1.1 Potential impact of findings on the field of ageing research

The results presented in this thesis highlight a number of factors that are currently commonly overlooked in imaging studies but which require consideration when using fMRI to investigate the ageing brain. Chapter 3 presented clear evidence that differences in the basic BOLD signal properties exist between younger and older adults. It is possible that these differences could drive, or at least contribute to, group differences in task and resting fMRI measures between age groups thus causing incorrect conclusions to be drawn regarding the effect of ageing on brain function. Furthermore, Chapter 4 provided evidence that the effect of ageing on FC strength and spatial reorganisation of RSN nodes may be differentially affected by gender. Typically studies of brain ageing consider male and female brains as synonymous, however, if it is the case that interactions between ageing and gender exist, studies which do not explicitly consider gender may result in skewed conclusions. Finally, Chapter 6 provides preliminary evidence that total sleep time prior to scanning may be associated with patterns of FC. Sleep quality is commonly overlooked within the field of cognitive ageing, but is a factor that could feasibly have a large impact on both cognitive performance and patterns of brain FC. For example, if a study happened to recruit a sample of particularly poor older sleepers, the FC differences between young and old may be considerably different to a study who recruited a sample of particularly good older sleepers. Similarly, recruiting younger participants who are particularly sleep deprived (or not) may also distort the conclusions drawn between age groups relating to brain function and cognitive performance.

Combining measures of sleep quality and gender and controlling for differences in the underlying BOLD signal properties as best we can may allow us to establish a more coherent, reliable understanding of cognitive ageing.

8.2 POTENTIAL LIMITATIONS AND FUTURE DIRECTIONS

8.2.1 Numerous physiological processes change with age

The process of ageing is associated with changes to a number of biological (circadian rhythms, melatonin production) and external (physical mobility impairments) factors, combined with structural degradation of the cortex and thalamus. A number of these factors can be associated with both reduced sleep quality and cognitive impairment, making it difficult to identify which, if any, are most responsible for typical patterns of cognitive disruption with age. It could be argued that the relationship between poor sleep and brain function (and thus potentially cognitive ability) is coincidental. The combination of these changes with age may lead to both sleep disruption and cognitive decline, but the relationship may not necessarily be a causal one. In all likelihood, the relationship between the numerous physiological changes with age is intricate and not straightforward. For example, production of melatonin which plays an important role in the initiation of and maintenance of sleep, declines after middle age and may be responsible for some of the typical insomnia symptoms commonly reported in older adults. However, melatonin is also an endogenous anti-oxidant which may play an important role in protecting neurons from free radicals, which induce oxidative damage (Ortiz, Benítez-King, Rosales-Corral, Pacheco-Moisés, & Velázquez-Brizuela, 2008; Parmar, Limson, Nyokong, & Daya, 2002; Rahman, 2007). Therefore, reductions in melatonin may result in independent changes to sleep behaviour and brain integrity, rather than one causing the other. Adopting a multi-faceted approach is required if we are to understand the complex interactions between the numerous biological changes with older age. Establishing a link between sleep, brain network integrity and cognition may provide us with the opportunity to develop implementable interventions in middle age to prevent, or slow the rate of, cognitive decline.

However, developing effective interventions is only possible if we fully understand the mechanisms which are responsible for sleep changes with age.

In addition to the biological changes potentially responsible for sleep the results from Chapter Three suggested that differences in neurophysiological factors, such as CVR, also differ between age groups. Although these differences were not correlated with FC strength or differences in FC strength between age groups, they do confirm that modifications to BOLD signal properties exist between younger and older adults. Furthermore, it is possible that such differences affected measures of FC in a way that we cannot easily identify with the methods that are currently widely used. Therefore, these results highlight the need for multimodal imaging (e.g. BOLD and ASL combined, MEG) in order to reveal reliable age-related FC results, not confounded by differences in physiological sources, such as CBF and CVR.

8.2.2 Functional connectivity analysis measures

Recently, there has been a shift within the field of neuroimaging away from identifying age-related functional differences for isolated brain regions towards understanding how the brain functions as a network, or series of networks. However, there are currently a number of different ways that this type of analysis can be conducted and until a standard methodology is developed it remains difficult to compare and combine across studies (Cole, Smith, & Beckmann, 2010). This is a particular problem when comparing across groups as the number of methodological decisions to be made increases even further (for example, how to deal with potential differences in grey matter volume and motion between groups). One such decision is how to define RSNs and RSN nodes for calculating FC and comparing between groups. Since we were primarily interested in whether there are age-related differences in connectivity, within specific RSNs, we focussed on comparing connectivity

strength between groups within the same spatial locations and took the approach of using an independent definition of the nodes and applying them equally to both young and old. However, this does not allow us to disambiguate changes in the spatial extent and location of connectivity with age, which is a subject for more detailed investigation in future work. We made the decision to use RSN nodes that were previously defined in a younger cohort of 55 participants, as this gave a robust, independent definition of typical RSN nodes which were also comparable to nodes from a larger, functionally defined node atlas (Shirer, Ryali, Rykhlevskaia, Menon, & Greicius, 2012). However, as the results from Chapter Four highlight, there is the potential for the centre of some RSN nodes to shift with age. These results emphasise the difficulties in directly comparing the FC of RSN nodes between groups and suggest that caution should be taken when using the same RSN node definitions for different age or patient groups to investigate FC. However, the alternative approach, to apply individual node definitions for each participant group, has its own limitations. By applying different node definitions, it is then difficult to know whether you are truly assessing FC of the same nodes in different groups.

A number of studies have advocated for calculating FC on a voxel-wise level and thus avoiding the need for RSN node definitions at all. Similarly, results from Allan and colleagues (2015) suggest that valuable information about spontaneous network dynamics can be lost when averaging across voxels within an ROI. However, voxel-wise FC analyses typically requires some sort of clustering (Lee et al., 2012; van den Heuvel, Mandl, & Hulshoff Pol, 2008; Wang & Li, 2013) to be applied to identify between group FC differences, or for the use of global network measures calculated by graph theoretical approaches (Friston, Kahan, Razi, Stephan, & Sporns, 2014; Micheloyannis et al., 2009). Furthermore, both of these methods still requires a number of methodological decisions to be made (i.e. levels at which to threshold graphs and determine clusters), for which there are no standard definitions.

Furthermore, it becomes more difficult to interpret differences between groups at the voxel-wise level. Although applying RSN node definitions and calculating FC between such nodes is more limited, in spatial resolution, than voxelwise measures, the results are more interpretable in the context of the literature as RSN nodes have been associated with particular functions or networks with defined functions. By increasing the number of measures between groups, i.e. by adopting large parcellation schemes or conducting voxel-wise analysis, it also introduces a multiple comparison problem. That means for moderate sample sizes, such as in this thesis, it is often necessary to collapse FC measures across networks in order to reduce the number of comparisons. This may also be a limited approach as we have identified that not all nodes of the same network are affected by ageing in a similar way (Chapter Four). The use of generalised linear mixed models (GLMMS) may be more useful when comparing numerous measures from the same subjects, as they allow for more specific modelling of the within subject variability for 'nested' data. However, sample size still remains a problem when comparing hundreds, or thousands of measures, thus highlighting the need for larger scale studies with greater statistical power.

Similarly, results from a number of studies (Grady & Garrett, 2014; Liu & Duyn, 2013; Tagliazucchi & Laufs, 2015; Tagliazucchi et al., 2012b; Wu et al., 2013), suggest that investigating the effect of ageing on brain variability may also be important for understanding exactly how the ageing process disrupts cognition. However, the numerous outcome measures of dynamic FC further increases the multiple comparison problem, as each spatial node has multiple FC measures. For example, assuming a full matrix correlation of a voxel-wise parcellation with 100 nodes and 55 FC measures per node (using 32 second epochs with 50% overlap for a typical 15minute scan), results in ((100*100-1)/2)*55 = 272,250 possible measures (selecting only the top half of the matrix). Again, this problem requires researchers to decide between applying potentially less interpretable clustering

type methods (Zhang et al., 2014b) or by summarising methods relatively crudely (as in Chapter Seven) in order to compare between groups. The development of adequate, summary measures which still capture the richness of the dynamic data will allow us to develop a greater understanding of how the dynamics of functional networks may differ with age.

8.2.3 The concept of compensation in the ageing brain

Studies linking patterns of network functional connectivity and cognition in older age have provided evidence for compensatory-type increases in FC within the older brain, typically for inter-network connections. The recruitment of additional brain regions in this way is thought to be a response to the fact that canonical RSNs function sub-optimally in older age (Stern, 2002; Stern, 2009). Furthermore, a number of researchers have also highlighted the importance of segregated networks for efficient brain processing, rather than distributed whole brain networks. This finding suggests that increased inter-network FC in older adults is not necessarily compensatory and in fact results in interference between networks and thus disrupted cognition, which has been reported by a number of studies. The results presented here provide evidence for both scenarios. For older adults, greater thalamo-hippocampal FC was associated with the poorest memory scores, while greater thalamo-motor FC was associated with the fastest reaction times. This suggests that segregation of certain networks or connections is important for some tasks and that greater thalamo-hippocampal FC may result in interference which disrupts memory ability. In contrast, greater thalamo-motor FC may facilitate faster RTs in response to disrupted or reduced connectivity of other brain regions. Therefore, there may be a limit to the usefulness of additional inter-network FC in the older brain before it starts to become an interference for cognitive performance. This finding is one that requires more thorough

investigation, in order to establish which connections facilitate compensatory responses and which are more associated with interference. The FC presented in this thesis was calculated from data obtained during rest. This suggests that the patterns of thalamic FC which are associated with cognitive performance are more of a global index of potential network function, which are present even when the participant is not engaged in a task. We assume that the FC differences observed at rest are also likely to be reflected during a task, however, some have reported that there are explicit differences in the patterns of FC during rest and task (Arbabshirani & Calhoun, 2011; DeSalvo, Douw, Takaya, Liu, & Stufflebeam, 2014; Finn et al., 2015). Future research should look to compare such differences in older FC during both states in order to assess whether patterns of FC at rest associated with task performance predict FC during completion of the associated task. As differences in task-fMRI measures between age groups are confounded by differences in performance level, comparing age-group differences at rest and then comparing rest and task FC within participants may be another way of investigating how differences in FC of brain networks with age may be responsible for cognitive deficits.

8.2.4. Longitudinal vs. cross-sectional studies

As briefly discussed in Section 1.1.1 another confounding factor when investigating any aspect of the ageing process, is whether the methodology, or analysis, is longitudinal or cross-sectional in nature. A number of studies have provided evidence that the conclusions drawn by the two methods can be considerably different (Hedden & Gabrieli, 2004; Knopf & Neidhardt, 1995; Ronnlund et al., 2008). A recent study by Pfefferbaum and Sullivan (2015) reported that cross-sectional and longitudinal analyses of the same hippocampal volume data resulted in vastly different results. While outcomes from cross-sectional analyses suggested a 'bowed' trajectory of hippocampal volume (i.e. increases in younger age

followed by decline in older age), longitudinal analyses did not identify the same trend and instead suggested a highly significant effect of age across the lifespan and only a moderate effect of accelerated hippocampal volume in older age. Given the richness of longitudinal data, one could argue that outcomes from a longitudinal study reveal 'true' effects compared to a cross-sectional design. However, as also highlighted by Pfefferbaum and Sullivan (2015) there are also a number of pitfalls to consider. First, for most longitudinal studies (or longitudinal analyses using data from a cross-sectional design) the baseline data-point is collected from participants of a variety of ages, which means that age-trajectories are confounded by cohort effects; for example, the ageing process may differ for people born in different eras. In addition, the intervals between data-points are often heterogenous across subjects, meaning that different ages are sampled at different rates. Furthermore, a number of practical considerations limit the feasibility of longitudinal studies, particularly for investigating the entire life-span. Considering these limitations, the use of 'individual trajectories,' which typically occur on shorter time-scales, rather than more demanding longitudinal studies, may be a reliable alternative for investigating cognitive ageing. Researchers should continue to develop analytical tools and statistical methods in order to ensure that such novel, longitudinal approaches can become well-established practices. However, any repeated study design is considerably impacted by practice effects (Salthouse, 2010), making it more difficult to establish the 'true' effect of ageing. More advanced methods for statistically modelling such practice effects are required if longitudinal analyses are to become more typical.

Despite the discrepancies identified between cross-sectional and longitudinal methods, a number of previous studies have reported a considerable amount of overlap between the two. While both methods will be necessary to continue to inform the field of cognitive

ageing, it is important to consider the limitations of both when assimilating results across studies to build theories of ageing.

8.3 CONCLUSION

The integration of measures of sleep, brain function and cognition will likely provide a clearer understanding of the intricacies of the ageing process which is required for the development of successful cognitive interventions. However, this is not a simple task, as a multifaceted approach is required to investigate the mechanisms most responsible for changing sleep and exactly how such changes manifest themselves as brain and/or cognitive dysfunction. A recent move within the neuroimaging field to investigate the brain as a network provides a more holistic approach for understanding the effect of the ageing process on brain structure, function and associated cognitive performance. However, currently, the lack of standard methodologies, particularly for comparing across groups, also poses a challenge for such research. The work presented here provides evidence that associations can be established between sleep, brain functional connectivity and cognition even in a sample of particularly healthy older adults. These results highlight the importance of investigating this trivariate relationship in the future. By building larger theories of cognitive ageing we may become closer to developing interventions which could potentially lessen the impact of one of the most distressing factors of ageing, cognitive decline.

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