NEUROIMAGING INVESTIGATIONS OF THE FUNCTIONAL AND STRUCTURAL CHANGES OF INTRINSICALLY CONNECTED BRAIN NETWORKS IN RELATION TO HABITUAL SLEEP STATUS

by

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ABSTRACT

The work presented in this thesis uses functional magnetic imaging fMRI and diffusion tensor imaging (DTI) neuroimaging modalities to investigate the relationship between chronic habitual sleep status in waking normal control subjects and functional and structural changes in higher order intrinsically connected brain networks (ICNs). The first study investigates the methodologies and compares the use of deterministic and probabilistic tractography approaches in combination with functional imaging to characterise structural connectivity with respect to functional connectivity in a single ICN. The following chapter examines whether inter-individual differences in habitual sleep patterns are reflected in waking measurements of intra- and inter- network functional connectivity (FC) between major nodes of three ICNs. Subsequent work investigates group differences in fractional anisotropy (FA) and Mean diffusivity (MD) structural connectivity metrics with respect to habitual sleep duration, as well as whole brain changes in white matter architecture in relation to subjective habitual sleep quality using Tract based spatial statistics (TBSS). The final experimental chapter builds on the work from previous chapters by examining a wider range of sleep features and examining overall network FC as opposed to regional specific changes. The results presented in this thesis provide evidence of functional and structural brain connectivity changes, which are modulated by chronic habitual sleep durations and in some cases by sleep quality. This may help to elucidate the link between sleep, waking sleep status, cognition and explain individual differences in susceptibility to sleep deprivation, as well as potentially the networks and systems responsible for variations in sleep patterns themselves.

Dedication:

I would like to dedicate this thesis to the memory of my brother Rick Khalsa; you have been a motivating factor for so many things in my life, 'thanks Rick!'

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The work presented here would not have been possible without the help of a number of people to whom I am greatly indebted. First, I would like to thank Dr.Andrew P Bagshaw my supervisor for transforming me in to something resembling a scientist, without his valued knowledge, experience, guidance and continued support and encouragement this thesis would never have been written. Secondly, I would like to thank Dr.Manny Bagary from the department of neuropsychiatry at the Barberry national centre for mental health for having enough faith and confidence in my abilities to take me on as his PhD research fellow and provide research funding. I would like to thank my colleagues in the neurophysiology department at the Barberry for giving me the research time needed to complete my PhD. I would also like to thank Dr.Stephen Mayhew for all his encouragement, ideas and discussions in the early days of my research and for his continued support throughout. I would like to express my gratitude to Dr.Joanne Hale for her advice and contributions during the latter part of my research and to all my colleagues in the research corridor who helped with data collection and were always happy to discuss, advise or listen. I would like to say thanks to my mother for giving me her ‘can do!’ attitude to life. Finally I want to thank Kully, your support and understanding during this journey has been invaluable.

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List of Abbreviations

ACC	Anterior cingulate cortex
ANOVA	Analysis of variance
BOLD	Blood oxygenation level dependent
CEN	Central executive network
CNS	Central nervous system
CFS	Cerebro spinal fluid
rCBF	Regional cerebral blood flow
dLPFC	Dorsal lateral prefrontal cortex
DAN	Dorsal attention network
DMN	Default mode network
DTI	Diffusion tensor imaging
DWI	Diffusion weighted imaging
EEG	Electroencephalography
EMG	Electromyography
EOG	Electrooculography
FC	Functional Connectivity
FDG	Flourodeoxyglucose
FLIRT	FMRIBs registration tool
fMRI	Functional magnetic resonance imaging
FSL	FMRIB software library
GABA	*Gamma*-Aminobutyric acid
GSR	Global signal regression
ICA	Independent component analysis

GICA	Group independent component analysis
ICN	Intrinsically connected network
KSS	Karolinsk sleep scale
lHC	Left hippocampus
lAI	Left insula
lIPL	Left inferior parietal lobule
lIPC	Left inferior parietal cortex
lMTL	Left medial temporal lobe
MELODIC	Multivariate exploratory linear optimised decomposition into independent components
MNI	Montreal neurological institute
MR	Magnetic resonance
MEG	Magneto-electroencephalography
NREM	Non-rapid eye movement sleep
PLM	Periodic limb movements
PSG	Polysomnography
PSQI	Pittsburgh sleep quality index
PVT	Psycho-motor vigilance task
PET	Positron emission tomography
PCC	Posterior cingulate cortex
REM	Rapid eye movement sleep
rHC	Right hippocampus
rAI	Right insula
RF	Radio frequency
rIPC	Right inferior parietal cortex
rIPL	Right inferior parietal lobule
rMTL	Right medial temporal lobe

ROI	Region of interest
SD	Sleep deprivation
SN	Saliency network
SPECT	Spin positron emission computer tomography
STD	Standard deviation
SWS	Slow wave sleep
TE	Echo time
TR	Repetition time
TST	Total sleep time
cTST	Cumulative total sleep time
WASO	Wake after sleep onset

STATEMENT OF AUTHORSHIP

This thesis contains work that has been published[1,2,3] or has been submitted for publication[4] and the authorship on each paper or chapter indicates collaborative work. Two sections of chapter 1 (Resting state fMRI, and Functional connectivity) of this thesis are based on a published book chapter which I co-authored with my supervisor Dr.Bagshaw. The work from the published chapter[1] included in chapter 1 of this thesis is solely my contribution and has been modified to fit in with the rest of the work in chapter 1 of this thesis. I collected all the data for chapter 3[2] of this thesis. For chapter 4[3] and 5[4], as well as myself, Dr.Hale, Dr. Wilson and Dr.Goldstone contributed to data collection. Miss Przezdzik produced the regions of interest (ROI) representing the nodes of the DMN, CEN and the SN from data from a separate cohort of 55 subjects for chapter 4. I carried out all preprocessing and performed the data analysis for all chapters, I wrote all the manuscripts and I am the primary author of all the work within this thesis. Dr.Mayhew contributed to chapters 3 and 4 by developing the Matlab code used to perform the correlational analysis and provided advice on the design of the study for chapter 5[4]. Dr.Hale also helped develop the Matlab code for chapter 4. Dr.Chechlacz provided me with the basic information on how to process MRI data for diffusion weighted imaging and streamline deterministic tractography for chapter 3. Dr.Bagary is the head of our clinical sleep and epilepsy service in the Neuropsychiatry department at the Barberry Centre for Mental Health and provided me with clinical guidance on the work within this thesis. Dr.Bagshaw, my supervisor, is named as a co-author on chapter 3 and 4 and 5 as he helped to develop my original ideas and provided valuable feedback on all of my work.

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* publications or contributions not included in this thesis.

CHAPTER 1

GENERAL INTRODUCTION

‘Sleep that knits up the ravelled sleave of care
The death of each day's life, sore labour's bath
Balm of hurt minds, great nature's second course,Chief nourisher in life's feast.’
~William Shakespeare, Macbeth

INTRODUCTION

The work presented in this thesis will investigate the relationship between chronic habitual sleep status and functional and structural changes in specific higher order brain networks. Habitual sleep patterns are important markers of sleep status, but are less widely investigated than short term experimental manipulations of sleep. Habitually short sleep durations in particular may result in cumulative chronic sleep debt, which may have individual consequences for higher order cortical functioning. Disruption may be caused to higher brain networks, which are responsible for attention, salience, memory, introspective thought and executive functions. Global or region specific functional or structural changes to these networks may account for subtle cognitive impairments associated with habitual sleep status. This chapter introduces the reader to the literature, basic definitions, principles and general methodologies, which are directly or indirectly related to the research presented in this thesis. The sections are structured to allow the reader to gain a progressive understanding of the research area.

SLEEP

It has become increasingly evident that sleep is a necessity and not a luxury, as evidence of sleep behavior is seen across species (Cirelli and Tononi, 2008, Mignot, 2008). For example, cockroaches, honeybees, fish, birds, mice, as well as mammals including humans all fit the behavioural definition of sleep (Cirelli and Tononi, 2008, Mignot, 2008). This definition of sleep behaviour consists of: sleep being rapidly reversible (unlike hibernation or coma for example), the preference of species to sleep in a specific position and in specific places, a heightened arousal threshold (a reduction in responsiveness to external sensory stimuli), the need for an organism to recover from reduced sleep opportunity such as partial or total sleep deprivation (homeostatic regulation), and for most species circadian regulation.

Understanding the function of sleep is something humans have been attempting for millennia. Many ancient cultures attempted to explain or understand sleep; Kirsch (2011) illustrates historical examples such as the Chester Beatty papyrus, which focuses on dream interpretation and the importance of dreams in the Egyptian culture. Another example is the opus written by Aristotle in ancient Greece around 300 bc which translates as '*on sleep and sleepiness*', and is devoted to the processes of sleep and waking. In China, *Huangdi Neijing,* translated as, 'Canon of Medicine', may have been written as early as 2900 BC, and introduced the theory of yin and yang. This is a symbol that has been used to represent the sleep and wake state (Kirsch, 2011).

Over time, a number of theoretical positions emerged as to why organisms sleep (Mignot, 2008). By the 1970's-80's the main theories that had developed were based on five

theoretical perspectives (Webb, 1981). Restorative theories (Hartmann 1973, Moruzzi 1972, Oswald 1970) hold that sleep is a period of maximal restoration of functional states, which have been depleted during wakefulness. For example, Morzzuri hypothesized a primary recovery process during rapid eye movement (REM) sleep to nerve synapses and glial cells associated with waking neural plasticity, and in essence memory and learning (Moruzzi, 1972). The second perspective, protective theories (based on Pavlov's theory of sleep, Pavlov 1929), proposed sleep to be an inhibitory process which protects the brain from continuous and excessive stimulation. The key concept of Pavlov's theory of sleep was a spread of cortical inhibition to prevent the central nervous system (CNS) from excessive and conflicting stimulation. Energy-conservation (Zepelin and Rechtschaffen 1974) was a third perspective, which emerged from the strong empirical data on metabolic rates and total sleep time. The main premise was that sleep enforces rest and as a consequence reduces metabolic requirements. This theory is closely linked with the evolutionary relationships between temperature regulation systems and the emergence of slow wave sleep (Zepelin and Rechtschaffen 1974). The Instinctive theories (Moruzzi 1972) regard sleep as a species-specific pattern of behaviour. Sleep is seen as an instinctive built-in stereotyped behaviour, which is elicited by inducing stimuli. The fifth theoretical perspective was the ethologic theories (Meddis 1977, Webb,1974), which suggest that sleep evolved as a system of behavior to ensure survival of a species within a particular ecological niche. By the 1980's it was proposed that sleep was regulated by biological rhythms (Webb 1981, also see appendix for the neurobiology of sleep status) and in 1982 the two process homeostatic model was published (Borbély,1982). According to this model, two processes regulate sleep. These are the

homeostatic process (S) which is determined by sleep status (i.e. the longer an individual remains awake, the greater the sleep pressure and the greater the need for sleep), and the circadian process (C) which determines the thresholds for sleep onset and offset. Process C is controlled by an endogenous circadian pacemaker (the suprachiasmatic nucleus). The interaction of S and C characterises the sleep/wake cycle (figure 1.1) and can explain fluctuations in alertness and vigilance and increases in sleep pressure due to resisting the need to sleep which may consequently lead to a state of sleep deprivation (Alhola and Polo-Kantola, 2007). For example, in modern society the drive to take advantage of the full 24 hours of the day is increasing (Dinges 1995). The demand for wakefulness at all hours of the day has steadily increased. (Alhola and Polo-Kantola, 2007). Unfortunately, biological rhythms such as S and the C cannot adapt readily to such situations. There is an imperative need for sleep, which is defined by process S. While it is possible to postpone S, it eventually needs to be fulfilled. We also need to consider process C that drives wakefulness during the day, but not at night (fig 1.1). In essence, living in a 24-hour culture and with extended periods of sleep deprivation can lead to neurobehavioural deficits in response to increasing homeostatic sleep pressure combined with circadian-modulated withdrawal effects on waking drive (Van Dongen, and Dinges, 2001). A model-based understanding of sleep deprivation can be helpful to explain neurobehavioural deficits, which may manifest due to prolonged wakefulness whether that be due to total sleep deprivation or habitual chronic sleep restriction. Research studies investigating sleep deprivation use the two process classical model in order to measure cortical and behavioral changes as a consequence of sleep deprivation. The work in this thesis is based on the two process model of sleep regulation

(fig 1.1). In this thesis we investigate cortical changes in relation to chronic habitual sleep status in subjects with long or short habitual sleep durations. As mentioned above the two process model consists of a S and C, which interact to determine the timing of sleep onset and offset, as well as the stability of waking neurocognitive functions (Van Dongen and Dinges 2003).

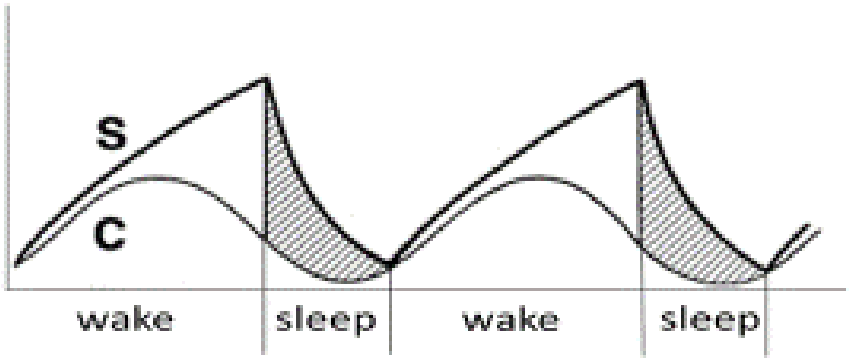

Fig 1.1 Two process model of sleep regulation. Homeostatic process S=sleep need, Circadian rhythmicity C=sleep timing. (Modified from Borbély 1982).

SLEEP STATUS

Sleep deprivation:

Sleep deprivation may have a marked impact on an individual's quality of life (Alhola and Polo-Kantola 2007). This can be due to multiple factors (Colten and Altevogt 2006). One particularly important, but underestimated, factor which explains why individuals may become sleep deprived is sleep loss due to work and life style (Ohida et al 2001, Swanson et al 2011, Van Dongen and Kerkhof 2011). Long working hours, nightshift working, the type of job an individual does (e.g. healthcare professionals, truck drivers and other

transport workers, security guards) as well as extended leisure hours can all result in sleep loss (Härmä et al 1998). Total sleep deprivation (SD) which is defined as at least 24 hours without sleep, is known to have a significant effect on performance (Pilcher and Huffcutt 1996, Williamson and Feyer, 2000), for example slowing of response speed (Williamson and Feyer, 2000), and reduced alertness, attention and vigilance (Lim and Dinges, 2008). Most research studies investigate sleep deprivation from this extreme view point.

The effects of partial sleep deprivation should also not be taken lightly (Durmer and Dinges 2005, Van Dongen, et al 2003, Williamson and Feyer 2000). Studies have shown that chronic partial sleep restriction can lead to similar, deficits as seen post total SD (Banks and Dinges, 2007). Individuals tend to overestimate their ability to function to an appropriate and safe standard after night time sleep has been compromised (Alhola and Polo-Kantola 2007). The cumulative effect of poor sleep patterns is subsequent chronic SD. Studies have shown that the cumulative effect of partial sleep deprivation appears to be rate sensitive (Drake et al 2001). The impairment of alertness and performance due to 8 hours of total sleep deprivation has been found to be more severe when experienced acutely in comparison to cumulative sleep deprivation of 8 hrs over several nights, suggesting the presence of a compensatory mechanism operating in conjunction with the accumulation of a moderate sleep debt (Drake et al 2001). Although Memory, salience and attention are known to still be affected by partial as well as total SD and can result in numerous negative effects including impairments to cognitive performance, Killgore et al 2006, Harrison and Horne 2000).

Theoretical perspective of SD:

A variety of theoretical perspectives have been proposed to explain the detrimental effect on cognitive processes of SD or extended periods of wakefulness. An example is the lapse hypothesis (Dorrian et al 2005, Kjellberg 1977,Williams et al 1959). The lapse hypothesis proposes that sleep deprivation can have general effects on alertness and attention. According to this hypotheses sleep deprivation results in lapses in attention and alertness and slow responses, which are a result of cognitive changes and reduced cognitive performance due to lack of sleep resulting in wake-state instability. Neurophysiological sleep-like changes as demonstrated by EEG (electroencephalography, see PSG section below) recordings illustrate microsleeps which are thought to account for these lapses (Priest et al 2001). Originally, it was proposed that cognition remained unaffected between lapses, but studies have shown cognitive slowing independent of these lapses (Dorrain 2005, Kjellberg 1977). Based on the initial observations of the lapse hypothesis, the study by Doran et al 2001 proposed the wake state instability hypothesis. Doran et al found that increases in performance variability in relation to psycho-motor vigilance testing (PVT) with increasing sleep loss in sleep deprived subjects was due to the internal influence of sleep initiating mechanisms attempting to maintain attention and alertness subsequently leading to an unstable state that fluctuates in seconds and cannot be characterised as fully awake or asleep. Such theories as above indicate cognitive disturbances in a sleep deprived individual to be most obvious during the performance of long simple tasks such as reaction time tasks or vigilance tasks (Doran et al 2001, Lisper and Kjellberg 1972, Lorenzo et al 1995).

Another school of thought suggests that the effects of sleep deprivation cause disruption to the functioning of specific cortical regions and consequently impairs cognitive function. This is known as the sleep-based neuropsychological perspective or the selective impact school of thought (Babkoff et al 2005). The prefrontal vulnerability hypothesis proposed by Horne 1993 is an example of one of the more influential theories from this school. Horne's theory suggests that impairment to cognitive functioning in the prefrontal cortex including higher order functions, for example executive functions, saliency and attention, are most affected as a result of sleep deprivation. Functional magnetic imaging (fMRI) and positron emission tomography (PET) have been used to investigate specific regions of cortex thought to be affected by total or partial sleep deprivation (Durmer and Dinges 2005, Jones and Harrison 2001). Durmer and Dinges report findings from functional metabolic and neurophysiological studies, which show that neural systems involved in executive function such as the prefrontal cortex, were more vulnerable to sleep deprivation in some individuals compared to others. Sleep deprivation has also been shown to result in a significant decrease in relative metabolism of the frontal cortex in a PET study by Wu et al 2006. Therefore suggesting that sleep may be especially important for maintenance of appropriate functioning of the frontal cortex, but no imaging studies have investigated the effect of short cumulative habitual sleep on these regions to date.

Sleep debt:

Taken in the context of chronic sleep restriction, sleep debt can be defined as the accumulation of the total hours of sleep lost with respect to the individual's specific daily need for sleep. Sleep debt is a major problem in industrial western adult populations, but sleep patterns in pre-industrial communities have also been found to be similar to

industrial societies (Yetish et al 2015). Therefore the sleep debt acquired in western industrial populations may be determined by inter-individual susceptibility to sleep pressure and not purely on chronic habitual sleep behaviour. Sleep debt is not easily estimated as each individual's daily need for sleep is different and the majority of studies use measures of sleep duration to objectively assess the effects of restricted sleep times and subsequent sleep debt. Epidemiological studies in adults and children report a statistically significant clinical risk in adults with habitually short sleep of approximately 5 hours, which presumably results in habitual sleep debt accumulated over numerous years (Bonnet and Arand 1995). Sleep studies have reported that sleep periods reduced by as little as 1.3 to 1.5 hours for one night result in the reduction of daytime alertness by as much as 32% (Bonnet and Arand 1995). In view of the above, it is not surprising to find that poor sleep is a factor in 57% of road traffic accidents leading to fatalities in truck drives and in 10% of fatal car accidents in the United States costing billions dollars per year (Bonnet and Arand 1995).

NORMAL VARIABILITY IN SLEEP PATTERNS

Sleep patterns can vary between individuals and one key determinant is the subject's tolerance to sleep pressure (Aeschbach et al 1996).

Habitual Short sleepers:

Short sleepers can be defined as individuals who attain less sleep than average sleep and sleep for < 7 hours (Taub 1978, Geol et al 2009).

Short sleepers have been shown to demonstrate more consolidated sleep (slow wave sleep, see PSG section below), but less REM and light (N1 and N2, see PSG section

below) sleep in comparison to longer sleepers. There is evidence to suggest that sleep homeostatic mechanisms and circadian rhythms are different in short sleepers (Aeschbach et al 1996). Aeschbach et al found short sleepers compared to long sleepers demonstrated differences in recovery from sleep deprivation, which indicates differences in homeostatic sleep mechanisms. Short sleepers were found to have shorter sleep onset latencies, and higher sleep efficiency compared to longer sleepers. Short sleepers also demonstrated increased slow wave sleep. These differences demonstrate increased homeostatic sleep pressure in short sleepers compared to longer sleepers. Long sleepers subjected to sleep restriction demonstrated greater EEG changes in sleep architecture than short sleepers (Aeschbach et al 1996). These findings support the premise that short sleepers live under greater homeostatic sleep pressure than longer sleepers and that short sleepers may be more tolerant to homeostatic sleep pressure than longer sleepers. Higher homeostatic pressure load is a possible explanation of performance deficits seen in SD (Aeschbach et al 2001).

It has also been reported that individuals given unlimited sleep opportunity under laboratory conditions sleep longer than their habitual sleep patterns indicate (Klerman and Dijk 2005, Webb and Agnew 1975). Individuals with shorter habitual bed rest duration have been shown to sleep more than those with longer habitual bed rest duration when given unlimited sleep opportunity under laboratory conditions (Klerman and Dijk 2005). This suggests that habitual sleep habits may be prone to significant amounts of cumulative sleep debt even in self-reported short sleepers.

Habitual Long sleepers:

Long sleepers by definition are individuals who sleep longer than average, but are able to function without any impairment and feel well in themselves. It is important to be able to make distinctions between long sleep and idiopathic hypersomnolence which is characterised by individuals sleeping long hours but still not feeling refreshed (Thorpy 2012). Adult long sleepers typically sleep around 9 hours (or more, Grandner and Drummond 2007) when not previously sleep deprived. Studies have shown individuals classified as long sleepers based on long habitual sleep times show no significant differences in their sleep in comparison to individuals reporting normal sleep times with the exception of an increased amount of time spent in bed and asleep (Patel et al 2012). This suggests that any links between self-reported long sleep durations and adverse health effects are not likely to be due to poor sleep quality, sleep disorders or circadian phase abnormalities, in agreement with the above definition of long sleepers. Further research focusing on understanding the causes for an increased time asleep in bed are needed to get a better overall understanding of long sleepers. Others have shown depression, worry and being introverted show more prevalence in long sleepers compared to normal sleepers, although these differences have been found to be very small (Patel et al 2006, Patel et al 2012).

Long sleepers have been reported to have a greater incidence of sleep related problems, for example: difficulty initiating sleep, awakening more frequently during the night and waking too early and not feeling refreshed and subsequently being more sleepy in the daytime compared to normal sleepers (Grandner and Drummond 2007). It is possible in these cases, the definition of long sleep is interpreted as time spent in bed, but not

sleeping, as multiple awakenings and early waking suggest shorter actual sleep durations, and possibly greater time spent in bed attempting to sleep. Therefore, these individuals do not truly fit the above definition for long sleepers and early bed times may be a result of cumulative sleep deprivation resulting in hypersomnolence and increased sleep pressure which may be secondary to a primary sleep disorder such as psychophysiological insomnia.

SUBJECTIVE SLEEP MEASURES

Questionnaires:

In a clinical setting subjective reports of sleep quality are a useful screening tool for the initial assessment of sleep complaints (Akerstedt et al 2002, Suzuki et al 2004, Vitiello et al 2004). There are several assessment questionnaires available to clinicians, and these sleep rating measures focus on subjective estimates of sleep duration, sleep fragmentation (e.g. awakenings during the night) and other factors which can determine sleep quality and sleep time, and other issues for example in relation to medication or co-morbidity. The Pittsburgh sleep quality index (PSQI) is one of the most widely used sleep questionnaires (Buysse et al 1989). The PSQI is a useful tool for overall assessment of sleep quality in general terms, but was not designed for the assessment of sleep quality for any particular night. In a study which used the PSQI in a cohort of young and old subjects it was found older subjects demonstrated worse subjective sleep quality component scores than younger subjects, although the overall global score for the majority of older subjects was within the good sleeper range (Buysse et al 1991). When comparison between subjective PSQI and objective PSG data was made (which included the comparison of sleep

efficiency, sleep latency, time asleep, sleep maintenance and % delta against global PSQI scores) no significant correlations between the subjective and objective measures was found (Buysse et al 1991). This lack of agreement between subjective and objective assessments of sleep seems to be quite general. Studies comparing the Karolinska sleep scale (KSS), which is a sleep questionnaire developed to assess subjective sleep quality (Akerstedt et al 1994) with objective data from PSG showed that overall subjective sleep quality was related more to sleep efficiency and continuity, but not to individual sleep stages, and that sleep efficiency in the young adults studied had to be >87% to be subjectively rated as good (Akerstedt et al. 1994). In addition, the authors reported that the ease of awakening was related to poor objective sleep quality.

While subjective questionnaires described above have clinical relevance, their primary purpose is to assess large changes in sleep quality attributed to sleep disorders or other medical conditions, as opposed to assessing normal sleep patterns. Also it is important to reiterate that sleep questionnaires such as PSQI and KSS are designed to rate overall sleep quality not sleep quality for a single night. As sleep can vary from night to night, obtaining chronic habitual sleep data allows the assessment of sleep patterns and average sleep durations which can be important to determine if a subject has poor cumulative sleep quality or a poor habitual sleep status.

Sleep diaries:

Sleep diaries are assessment tools which allow subjects to record their sleep behaviors on a daily basis. These include sleep patterns, sleep quality, daytime sleepiness and stimulant use. Similar to actigraphy sleep diaries can be used to assess night to night

variability allowing a more representative sample of an individual's sleep than two nights of PSG or a single time-point questionnaire. Sleep diaries have been shown to correspond well to actigraphy and PSG, although studies have found subjects to overestimate sleep latency and awake time after sleep onset on sleep diaries (Coates et al 1982, Monk et al 1994, Vallieres and Morin 2003).

OBJECTIVE SLEEP MEASURES

PSG:

To examine sleep, laboratory-based polysomnography (PSG) is regarded as the gold standard as an objective measure (Ancoli-Israel et al 2003, Blackwell et al 2008, Kushida et al 2001). The PSG is the standard method for sleep measurement in clinical practice. The minimum physiological variables measured with PSG are EEG, elctrooculogram (EOG), electromyogram (EMG).

The EEG measures electrophysiological cortical sleep changes, the EOG measures eye movements which can help to identify REM sleep together with EMG which measures muscle tone usually form the mentalis or sub-mentalis muscle.

The EEG component of the PSG is invaluable in terms of characterizing sleep and EEG defined sleep stages can be represented in a hypnogram (Figure 1.2). The EEG can be used in conjunction with the other PSG variables to characterise the sleep cycle electrographically into 4 sleep stages N1, N2, N3 (Non-REM sleep or NREM) and R (REM sleep, Iber et al 2007). Each sleep stage is characterised by more than 50% of an epoch demonstrating the charateristics of that sleep stage (an epoch being defined as 30-seconds) which consists of continuous EEG activity demonstrating the following: N1

defines the onset of sleep, and consists of slow rolling lateral eye movements, low amplitude mixed frequency activity on the EEG, predominantly 4-7Hz, and presence of vertex sharp waves. Vertex sharp waves (or vertex sharp transients of sleep) are benign paroxysmal discharges which characterise well established N1 sleep. They are seen over the central (vertex) regions of the cortex. N2 requires the presence of K-complexes and sleep spindles. K-complexes resemble vertex sharp transients of sleep with sleep spindles (sigma spindles) attached and are evident over the fronto-central regions of the cortex. Sigma spindles can also be seen independently over the fronto-central regions of the cortex and have a frequency of 12-16Hz. These paroxysmal discharges are benign and may be associated with arousal responses due to noise. They have also been associated to a range of cognitive functions and memory (Schabus et al 2006, Walker, 2009. Plihal and Born,1997) and it has been suggested they play a protective role in sleep (Wauquier et al 1995). N3 (Slow wave sleep, SWS), demonstrates large amplitude slow waves (0.5-3Hz) and is thought to play a role in memory processing and consolidation (Walker 2009). In the R phase, there are rapid eye movements, low amplitude mixed frequency (wake like) EEG, and low chin EMG tone.

Additional measures such as airflow, respiration and oxygen saturation levels can identify airway obstructions which may cause arousals and awakenings from sleep for example due to sleep apnea. Clinical applications of PSG for assisting in the identification of sleep pathology are many, including; insomnia, narcolepsy, periodic limb movement disorder (PLM), apnea (obstructive or central), non-REM sleep parasomnias, REM parasomnias, differential diagnosis between parasomnias and nocturnal frontal lobe epilepsy (Kushida, et al 2005).

Many PSG laboratory based studies have investigated SD (Goulart et al 2014, Tarokh et al 2015, Van Dongen et al 2003), and chronic sleep restriction (Van Dongen et al 2003).

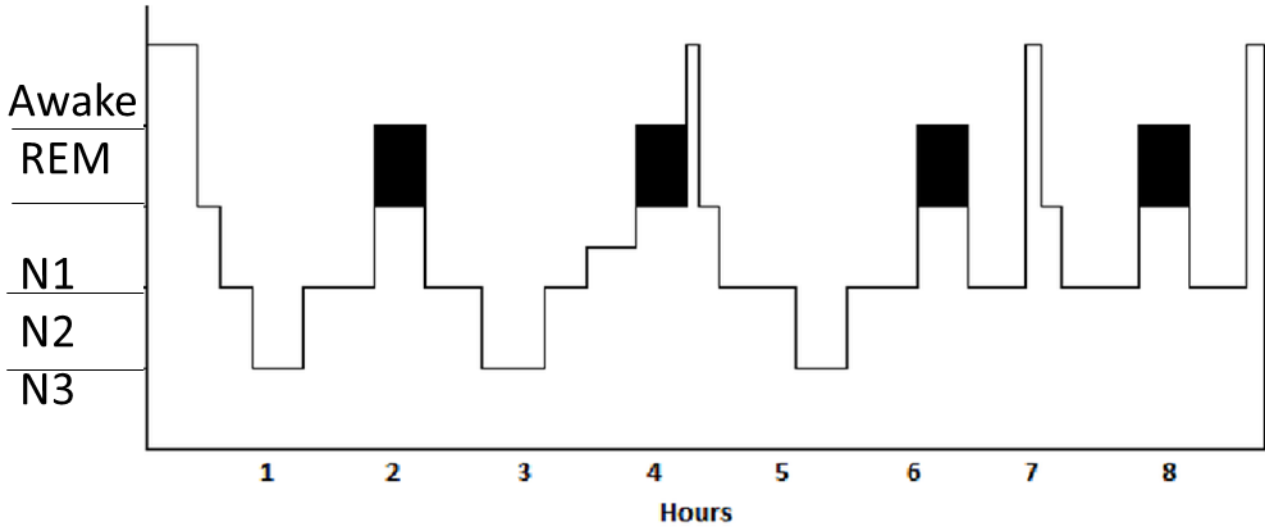

Fig 1.2 above, adult hypnogram showing normal sleep cycling and sleep stages based on EEG (adapted from Miller et al 2014).

Although PSG is without question the investigative tool of choice in the clinical setting when investigating sleep disorders such as insomnia, parasomnia, apnea, periodic limb movement disorders etc. (Kushida et al 2005), when attempting to charaterise specific regional changes in cortical activity, EEG is known to have poor spatial localisation (Burle et al 2015, Niedermeyer and da Silva, 2005). This makes PSG impractical to assess very specific regional cortical changes. PSG studies are also limited in their efficacy in determining chronic habitual sleep durations. PSG studies are one or two nights in duration and therefore are limited in terms of providing long term habitual sleep data which is required to charaterise habitually short or long sleep in a participant's normal

environment. Actigraphy and sleep diaries are a much more effective tool for characterising habitual sleep patterns and sleep times (Ancoli-Israel et al 2003).

Actigraphy:

Actigraphs are watch like devices, which can be worn on the wrist or less commonly on the ankle to measure and record movements. As frequency and level of movement change when subjects settle for sleep as opposed to being awake and active, activity can be used to approximate the sleep-wake cycle. This characteristic can be used to determine sleep patterns and sleep times over days or weeks in conjunction with sleep diary records (figure1.3). The collected data is downloaded on to a computer and analysed for activity verses inactivity and further analysis performed to estimate wakefulness and sleep (Ancoli-Israel et al 2003). The earliest actigraphs were developed in the 1970's . Kripke et al 1978, published some of the first studies to demonstrate the reliability of the use of actigraphy for sleep assessment. Modern digital actigraphy devices are available which can analyse light changes as well as movement using accelerometers in order to assess sleep. They are equipped with enough memory to record for several weeks and parameters such as total sleep time (TST), percent of time spent asleep, total wake time and awakenings after sleep onset (WASO) or number of awakenings can all be easily calculated. Actigraphy is highly correlated with PSG for differentiating sleep from wake (Blood et al 1997, Jean-Louis et al 1996) with TST correlations of 0.97 and 91-93% overall agreement for marked epochs of sleep and awake in adults (age 20-30 years, Ancoli-Israel et al 2003). In healthy adult subjects, actigraphy is a valid method for the assessment of sleep durations and sleep/wake activity, although it is less reliable for more specific measures such as sleep offset or sleep efficiency (Ancoli-Israel et al 2003).

Actigraphy is useful for recording multiple nights of sleep, is easy to use, can be used in the subject's normal environment and has minimal effect on the subject's natural sleep behavior and data can be represented in a graphical format (figure 1.3).

Actigram:

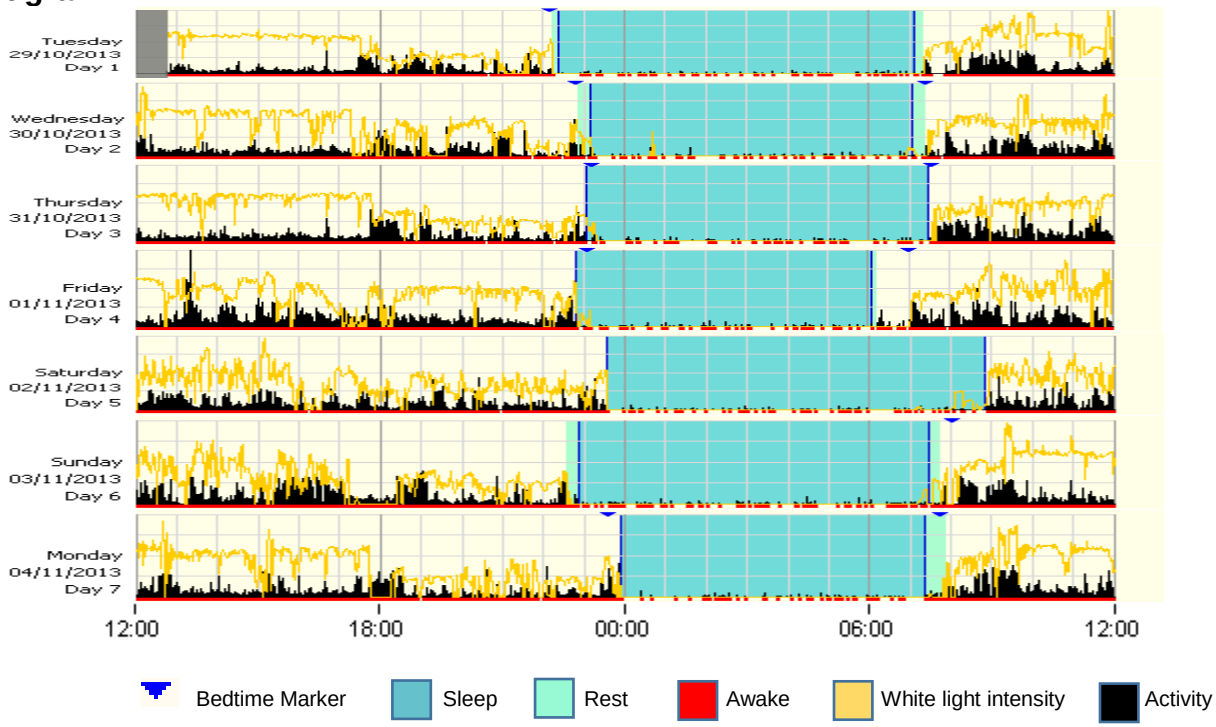

Fig 1.3 Actigraphy data for 7 days form an adult subject with consistent normal habitual sleep durations.

FUNCTIONAL NEUROIMAGING

The organ showing the clearest changes during sleep compared with relaxed wakefulness is the brain. These changes are evident in fMRI functional imaging studies (Horovitz et al 2008, Horovitz et al 2009, Larson-Prior et al 2009, Sämann et al 2011). Focussing on the brain using these techniques is appropriate, as not only does it contain numerous control mechanisms for sleep (Geiger-Brown et al 2012), but of all the body's

organs, it is the brain (especially the cerebral cortex) for which sleep seems to be the most vital in terms of actual recovery from deficits associated with sleep deprivation (Alhola and Polo-Kantola 2007, Babkoff et al 2005, Chee et al 2006, Horne 1993). Although the human adult cortex comprises about 2% of total body weight, it is highly metabolically active during wakefulness, requiring 20% of total resting oxygen intake even in the awake resting state (Clarke and Sokoloff 1999, Kety 1957, Rolfe and Brown 1997,). Resting with eyes closed and the mind cleared of all thoughts is not sufficient for brain rest and recovery. Only sleep can achieve these goals.

As mentioned above, EEG which is a key component of PSG investigations, provides very good temporal resolution, but poor spatial resolution due to the effects of volume conduction (Burle et al 2015). In addition, it is not possible to infer specific structural changes in ICNs (see below) using superficial electrophysiological potentials. On the other hand, functional imaging modalities such as fMRI and PET have very good spatial resolution, but poor temporal resolution. These imaging modalities allow characterisation of ICNs and comparisons of any changes in network functional connectivity (see below) from specific regions of cortex. Structural neuroimaging modalities such as DTI also allow structural comparisons, which provide a complementary measure of the impact of sleep on the brain.

In the work presented in this thesis, fMRI and DTI were used. The focus of the work was to determine functional and structural changes within specific regions of ICNs in relation to habitual sleep status. Therefore, spatial information was paramount to determine any specific or subtle changes in network connectivity whether structurally or functionally. Before discussing the specific imaging modalities used to charaterise changes associated

with chronic habitual sleep status in this thesis, I briefly discuss work involving other commonly used functional imaging methods with respect to sleep status.

PET and SPECT studies with respect to sleep status:

PET and SPECT (Spin positron emission computed tomography) are functional imaging techniques in nuclear medicine, which are used to observe metabolic processes using a positron-emitting radionuclide tracer. At present, there are no nuclear imaging studies that have investigated functional cortical changes in the context of chronic habitual sleep status to our knowledge. Therefore the studies discussed here will be based on SD, related to cognitive performance or to insomnia, a sleep disorder characterised by poor sleep or the inability to initiate adequate amounts of sleep. The most common PET tracer is fluorodeoxyglucose (FDG) which is an analogue of glucose. Therefore regional glucose metabolic uptake can be measured to characterise metabolic activity within tissues. PET studies have shown reduced behavioral performance post SD, associated with a reduction in global levels of glucose metabolism, together with reduced local activation in attention and arousal-related brain areas, such as the thalamus (Thomas et al 2000, Wu et al 1991). Reduced glucose metabolism post SD has also been found to be positively correlated between the thalamus and prefrontal cortex, suggesting sleep deprivation impacts these areas together as a functional network (Thomas et al 2003). Frontal and temporal lobes have been shown to demonstrate a significant decrease in absolute metabolic rate in response to SD where subjects were sleep deprived from 7am until 1-5pm the next day (Wu et al 1991). A PET study by Nofzinger et al 2004 suggests daytime fatigue in insomniacs may reflect decreased activity in the prefrontal cortex resulting from inefficient nocturnal sleep. The serial addition/subtraction task used in Thomas et al

(2000) required arithmetic working memory in addition to attentional demands and demonstrated decreased activation in regions associated with such tasks such as prefrontal cortex, inferior parietal lobe, and anterior cingulate gyrus. Molecular radiotracers (C-11 raclopride and C-11 cocaine radiotracers) tracers have been used in a PET study to investigate the effects of sleep deprivation on dopamine neurotransmission in humans. Volkow et al (2008) proposed increases in dopamine post SD are possibly responsible for maintaining levels of arousal under increasing homeostatic sleep pressure, but do not exert enough influence to prevent behavioral and cognitive impairment.

SPECT is a nuclear imaging technique similar to PET, but has poorer temporal resolution (several minutes for SPECT apposed to 45 seconds to a minute or so for PET) and uses only blood flow analogues opposed to PET which generally uses glucose or water/blood flow analogues. Smith et al 2002, found reduced regional cerebral blood flow from a SPECT study, in the basal ganglia in insomniacs. Another preliminary study by Smith et al 2005, which compared 5 insomniacs with 4 normal sleepers using SPECT, found no significant regional increase during NREM sleep but reduced regional cerebral blood flow (rCBF) in frontal medial, occipital, and parietal cortices, as well as in the basal ganglia. This suggests these brain areas are possibly most affected by chronic inadequate sleep (as chronic inadequate sleep is the main problem for insomniacs) during wakefulness.

The above PET and SPECT studies are consistent with the behavioural observation that SD affects cognitive domains such as the temporal and parietal regions and in particular the pre-frontal cortex. While PET and SPECT can be very specific in terms of

characterising cerebral metabolism with the use of radioisotopes, it must be stated that the temporal resolution for PET and SPECT is slower in comparison to fMRI studies (in the order of minutes) due to the slow tracer and blood flow kinetics (Maquet 2000).

Nuclear Magnetic Resonance Imaging (Basic principles of MRI):

The main imaging modality used in this thesis was MRI. Here, the basic knowledge of MRI and fMRI physics is presented, with several publications available for additional details (e.g., Pooley 2005, Deichmann 2010). Nuclear Magnetic Resonance (NMR) is a physical effect used in medical imaging since the 1970's. Nuclei are composed of protons and neutrons. The proton number determines the element and its position in the periodic table while the neutron number can provide variations in mass between nuclei of atoms of the same element resulting in their isotopes. The neutrons and protons have a property of angular momentum known as spin. The motion of electrically charged particles such as protons results in a magnetic force orthogonal to the direction of motion. Therefore, nuclei of isotopes with an equal number of protons and neutrons will not spin as the contra-rotation of their component particles will cancel out. Isotopes with an odd number of nucleons will display net spin. These nuclei behave like minute bar magnets as they are rotating charges. Materials made of such isotopes will be undistinguishable from other materials under 'normal' conditions, as atoms tend to be orientated at random. In essence, they will show no evidence of magnetism. If an external magnetic field of significant strength is applied, some alignment of magnetic moment (the magnetic effect of a particle with spin) will occur and it is under these conditions that certain isotopes can show NMR properties. Examples of the nuclei amenable to resonance are hydrogen,

phosphorus, fluorine and carbon. Of these hydrogen is found in the greatest quantity in living tissue because of the large amount of water present, and generally forms the basis for magnetic resonance imaging described below.

Magnetic fields are described in terms of x, y and z-axes, with the z-axis being that of the applied magnetic field (M). When this is the main magnetic field it is known as B0. When a body is exposed to a strong magnetic field, a proportion of the protons will attempt to align parallel with this field. Some protons will align in the up spin position and others in the down spin position. The spin up position requires less energy, therefore it is the preferred direction. In other words there are always more protons in the up spin position. It is this excess which creates the weak net magnetisation in the field direction. The protons are then in an equilibrium state with the external field, and are proposed to be precessing around the z-axis, similar to the analogy of a spinning top which precesses around the vertical field of gravitation.

The number of times a nucleus precesses in one second is called the precessional or Larmor frequency ($\omega 0$). This is an important distinguishing characteristic of a nucleus. For instance, in a magnetic field of a given strength, all of the hydrogen nuclei will precess at the same frequency. Other types of nuclei will precess at their own characteristic frequencies. Therefore the Larmor frequency depends on the type of nucleus and the strength of the applied magnetic field.

Magnetic field strength is measured in Tesla (T), which is the SI unit of magnetic flux density. Fields used in human NMR can range from 0.02-7.0 T, and as the magnetic field strength increases an increasing proportion of the nuclei will align, leading to a stronger

signal which can result in improvements in image contrast and spatial resolution. Compared to the Earth's magnetic field (5x10 -5T) these are very strong.

Nuclei do not initially precess in-phase with each other in the magnetic field. Phase coherence can be achieved by introducing energy in the form of Radio Frequency electro-magnetic radiation (RF) and the RF magnetic field is known as B1. To be useful the RF pulse must have the same frequency as the resonance (Larmor) frequency of the nuclei of interest, which are generally protons. This is known as the spin precession of the RF field. As soon as the RF pulse is applied, the effect is that of a rotating magnet which 'pulls' the magnetisation along.

For the duration of the RF pulse, the magnetisation precesses about a new time-dependent axis created by the field lines along the z-axis and the rotating magnetic field in the x-y plane. The stronger the energy of the stimulating RF pulse the greater the angle of deflection, or flip angle. Common flip angles include 90 degrees which flips the magnetisation directly into the x-y plane, while 180 degrees inverts the magnetisation and flips it exactly into the opposite direction.

The magnetisation can be separated into two vector components located perpendicular to one another. Longitudinal magnetisation (Mz) is the portion of the vector in the direction of the z-axis and so aligned with the external magnetic field (B0, see figure 1.4 below). Transverse magnetisation (Mxy) is the component of the vector located in the x-y plane perpendicular to the external magnetic field (see figure 1.4).

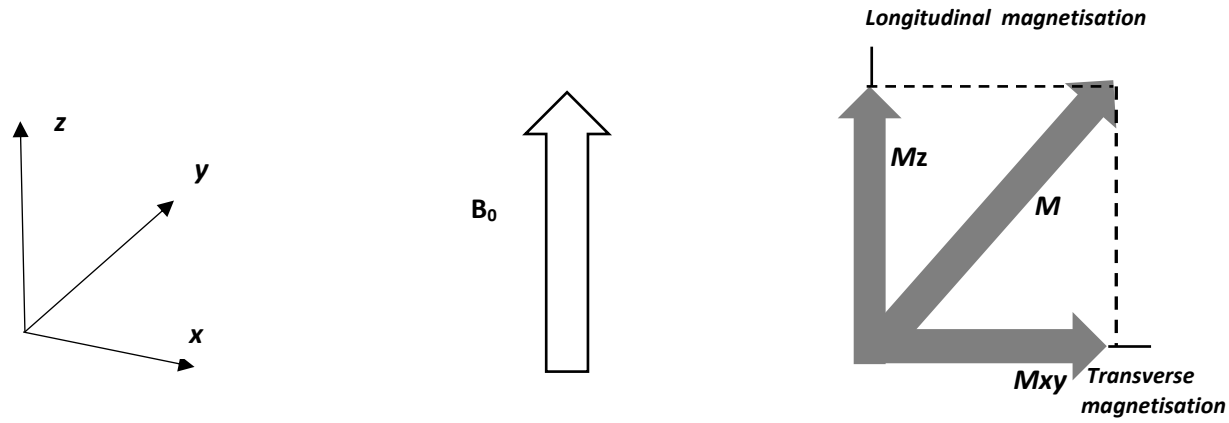

Figure 1.4 Diagram of the magnetisation M separated into two vector components located perpendicular to one another (Mz and Mxy), longitudinal magnetisation and transverse magnetisation (B_0 is the direction of the external magnetic field and the longitudinal magnetisation is aligned with B_0).

The Magnetic Resonance (MR) signal is generated as the flipped spins return to their original state. This causes the induction of an electrical voltage in the receiver coils of the scanner, because of emission of RF energy from the protons. After the RF pulse is switched off Mxy decays quickly, as the spins lose their phase coherence again. This is known as free induction decay. Mz recovers fully after a brief period as the excess energy is absorbed into the atomic and molecular environment surrounding the proton.

The restoration of the Mz magnetisation is the relaxation time. The time required to restore the equilibrium between the two energy states of the spin is a function of tissue as well as field strength. T1 (longitudinal relaxation time), which is approximately 900ms for grey matter and 600ms for white matter (Huettel et al 2004), which describes the recovery time of the longitudinal magnetisation Mz (but there can be variations in times due to field

dependence effects which are determined by the types of tissue being imaged, Korb and Bryant 2002). As each T1 time passes the magnetisation increases to approximately 63% of the remaining differential value. After a total time of approximately 5 times T1, the process is close to completion. Therefore T1 is a tissue specific time constant which tells us how quickly the spins of each tissue will emit their absorbed RF energy. It is dependent on the size of the tissue molecule and its type of surroundings. A small water molecule will move quickly and randomly through its molecular environment. It will have little opportunity per unit time to emit energy by interacting with its neighbouring molecules. Pure water and cerebral spinal fluid (CSF) therefore have very long T1 constants. A large slow moving fat molecule in a dense atomic lattice has a very short T1. This is key to the sharp image contrast obtained in MRI.

As Mz is restored, so Mxy decays. The decaying process for Mxy is much faster than the recovery process of Mz. After a 90 degree RF pulse has been applied the vector of the Mxy magnetisation consists of a large number of spins which precess in phase in x-y plane. Their individual contributions to the magnetisation are summative. The spins are phase coherent and they behave as one large magnet which rotates in the x-y plane. Due to their unavoidable interactions with neighbouring molecules the precessing spins lose their phase coherence. The rotating Mxy magnetization dephases or fans out into individual spins and begins to decay. T2 (transverse relaxation time) corresponds to the time taken for 37% of the Mxy magnetisation to decay.

Mxy magnetisation usually decays before Mz has recovered because the interaction occurring between spins is stronger than the spin-lattice interaction. T2 is also tissue

specific. In grey and white matter which contain a more rigid atomic lattice than fluids the nuclear spins are constantly exposed to fluctuating local magnetic fields. T2 is therefore very short in the order of 100ms for grey matter and 80ms for white matter (Huettel et al 2004). However in fluids the nuclear spins move in random molecular motion which minimises field fluctuations. For this reason, T2 is longer in fluids (around 400ms in CSF). There is a second type of transverse relaxation time which is known as T2* which is similar to T2 but includes overall decay from transverse magnetization static field inhomogeneity over a macroscopic region (mm) as opposed to T2 which is intrinsic decay of transverse magnetization over a microscopic region (5-10 microns). T2* decays more quickly than T2 (by a factor of about 2). T2 and T2* time constants have useful properties which are used in fMRI imaging which is discussed later on in this section.

Pulse sequences such as the combination of 90 degree and 180 degree RF pulses are used to measure T1 and T2 times. The time from the 90 degree RF excitation pulse to the peak of the signal induced in the receiver coil is known as the echo time (TE). TE controls the amount of T2 contrast in the image. The time from one 90 degree RF pulse to the next one is the repetition time (TR). TR controls the amount of T1 contrast in the image. T1 weighting requires a short TR and a short TE, while a T2 weighting requires a long TR and a long TE. Other sequences and contrasts can be developed using different combinations, with for example Proton Density weighting requiring a long TR and a short TE.

As mentioned the Larmor frequency of the nuclei is directly proportional to the strength of the magnetic field. The nuclei are therefore stimulated by the RF pulse of the same

frequency and so no spatial information is obtained as all the protons have the same resonance frequency and emit an unidentifiable signal. To overcome this and obtain a spatially specific response the spatial structure of the magnetic field needs to be changed. A magnetic field gradient generated using a pair of gradient coils is superimposed on the homogenous magnetic field. The current within the poles is the same but of opposite polarity. One coil increases the magnetic field by a specific amount while the other coil decreases it by a specific amount. Most MR scanners are equipped with three sets of linear field gradients (G_x, G_y, G_z). Application of these gradients leads to linear modulation of the z-component of the B0 field along either the x, y, or z spatial axis. Gradients are also used for slice selection. RF pulses have a finite bandwidth that can be mapped to a spatial band by the use of a gradient pulse during RF excitation. Conventional MRI yields images of anatomy. A block diagram of a conventional MRI scanner is shown below (figure 1.5).

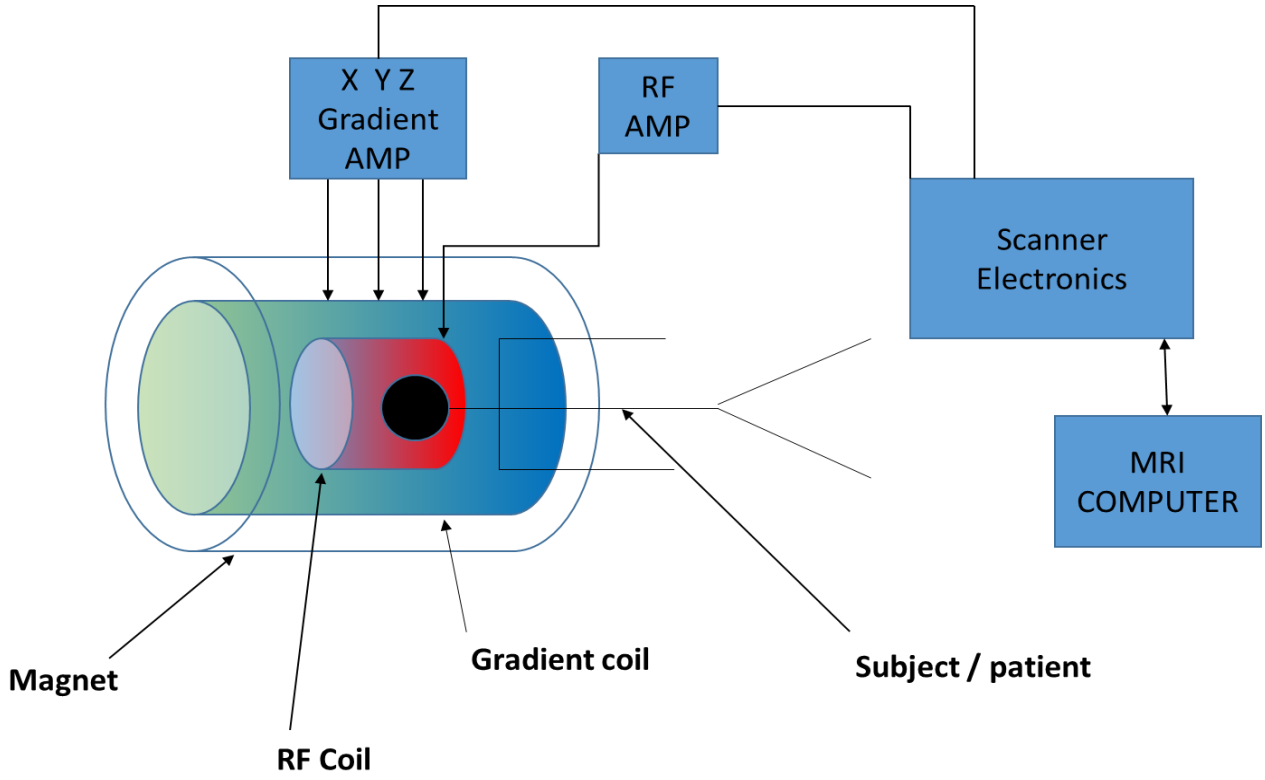

Figure 1.5 Basic block diagram of an MRI scanner

fMRI basic principles:

Most fMRI measures the blood-oxygen-level dependent (BOLD, Logothetis and Wandell, 2004) contrast, first reported in 1990 by Seiji Ogawa and his lab (Ogawa et al 1991). The first successful fMRI study in humans was reported by Belliveau et al 1991. Below I describe the basic principle of fMRI.

The insertion of a person in to a B0 field will cause the B magnetic field to become non-uniform. This effect is caused by susceptibility (χ) which is the production of magnetic fields in materials that are immersed in an external magnetic field. For large scale inhomogeneities of >10cm, the MRI scanner magnetic field can be adjusted to reduce the

field inhomogeneities in the B0. This is called shimming. Susceptibility artefacts can also occur close to junctions of air and tissue, such as the sinuses and ear canals. In addition, red blood cells are responsible for changes in susceptibility during activation (which is the BOLD response due to neural activity). This results in local increases in blood flow together with some increase in oxygen consumption resulting in a rise in oxyhaemaglobin levels and a reduction in deoxyhaemoglobin. Oxyhaemoglobin in the blood becomes deoxygenated to produce deoxyhaemoglobin which is paramagnetic. Therefore susceptibility differences between venous vasculature and the surrounding tissues cause magnetic field shifts This results in an increased T2* which results in increased fMRI signal intensity (Deichmann et al 2010).

The spatial resolution of fMRI is better than PET and far greater than EEG (spatial resolution in centimetres, Rodic and Zhao 2015), allowing it to distinguish between smaller regions of activity. This may seem insignificant, but such differences in resolution are substantial if we take in to consideration that a few millimetres of cortical grey matter are made up of millions of neurons which subsequently constitutes billions of synaptic connections (Rodic and Zhao 2015). fMRI also detects activity inside the brain in three dimensions, while superficial EEG generally reflects global radial dipoles of neurophysiological activity on the cortical surface (Rodic and Zhao 2015). In comparison to PET, fMRI imaging negates the need for radioactive contrast agents or metabolites and provides higher temporal resolution.

Activation fMRI in relation to sleep:

Historically, most functional neuroimaging studies have employed tasks to identify brain regions whose activity responds to a certain category of stimuli, or is different for two different categories (Bagshaw and Khalsa 2013). Activation fMRI studies looking at the effects of habitual sleep status in adults have not been performed as far as we know, but activation studies investigating the effects of SD on performance have been performed. For example (Lim et al 2007), assessed the reproducibility of fMRI activation and performance on a working memory task before and after 24 hours of sleep deprivation. They found that the modulation of the parietal regions were possibly good markers of vulnerability to SD, with a drop in left parietal activation correlating with SD. Drummond et al (2001) found SD was associated with increased activation in the bilateral prefrontal cortex and parietal lobes for a verbal learning and attention task. They also found an arithmetical task led to significantly reduced activation in the bilateral prefrontal cortex and parietal lobes. They suggest these findings demonstrate an adaptive cerebral response during cognitive performance due to SD and specific patterns of adaptation depending on the cognitive process being performed. Chee et al (2008) found SD related lapses in attention differed from lapses of equivalent duration after a normal night's sleep. They found a reduced activation of fronto-parietal control regions in response to attentional lapses, as well as dramatically reduced visual sensory cortex activation and reduced thalamic activation during lapses in SD individuals. Chee et al (2008) illustrate some neural consequences of the interaction between efforts to maintain wakefulness and processes which initiate involuntary sleep in SD subjects. fMRI studies investigating sleep deprivation have demonstrated attention to be particularly sensitive. Portas et al

1998 found that different levels of arousal (sleep deprived low levels or caffeine stimulated heightened levels for example) modulated thalamic activation. With sleep deprivation demonstrating greater thalamic activation. Many other fMRI activation studies have investigated the effects of SD on memory as well as SD effects on attentional functioning (Drummond et al 1999, Bell-McGinty et al 2004, Chee and Choo 2004, Habeck et al 2004, Caldwell et al 2005, Mu et al 2005, Mander et al 2008, Sterpenich et al 2007).

Resting state fMRI:

There is another way of analysing brain imaging data, and of conceptualising brain function, which is to move away from a sole emphasis on stimulus responses towards an examination of the brain's intrinsic functional properties (Raichle 2010). Resting state activity can be detected with advances in imaging techniques which allow the use of fMRI to reveal fluctuations in BOLD signal intensity from each voxel of the brain (Biswal et al 1995). These fluctuations are of low frequency (<0.08Hz), and their time courses are highly correlated temporally (Biswal et al 1995). It has been suggested they reflect the neuronal baseline activity of the human brain (Damoiseaux et al 2006), and represent the resting neuronal activity in the absence of goal directed tasks or external input. Whether these low frequency oscillations of the BOLD fMRI signal are a result of neuronal activity of the cortex or low-frequency artefacts due to other physiological processes causing variations in cerebral blood flow has been considered, such as movement, breathing and heart rate (Maldjian et al 2001). The overwhelming body of research now suggests the fluctuations are a direct result of neuronal activity (Biswal et a 1995, Damoiseaux et al 2006, Greicius et al 2003, Lowe et al 1998, Raichle et al 2001, Shulman et al 1997). Non-neuronal ‘noise’ signals are

a component of the BOLD signal which may occur from head motion (Van Dijk, Sabuncu and Buckner, 2012), fluctuation in heart rate (Change et al 2009) and from respiratory artefacts . Several preprocessing steps can be performed prior to seed-based FC analysis to reduce artifacts and noise from the BLOD data. Including: regressing motion parameters of the white matter and ventricular time series (Weissenbacher et al., 2009), regressing breathing and cardiac artefact (Khalili-Mahani et al 2013). Global signal regression (GSR, regressing the whole global mean of the bold signal) is also commonly used to remove signals between two regions and has been known to improve positive correlations and give greater spatial specificity (Fox et al 2005, Weissenbacher et al 2009).

Functional Connectivity:

The degree of correlation of resting state activity within the brain is a measure of the FC of those regions, and the fMRI signal is now widely used to study the functional relationships between brain areas. Functional connectivity is generally defined by examining correlations between regions in the low-frequency (<0.1Hz) part of the BOLD signal (Fox and Raichle 2007). Analytically, this can be done in two main ways. On the one hand, an initial 'seed' region can be defined based on some previous anatomical or functional prior information, and its activity correlated with all other brain regions (fig 1.6). For example, resting state functional connectivity of ICN neural networks can be examined in control subjects (figure 1.6, and expanded upon in the next section). Alternatively, multivariate techniques such as independent component analysis (ICA, Beckmann and Smith 2004) can be used. ICA decomposes the data into multiple components based purely on its statistical properties with no prior functional or anatomical

constraints (figure 1.7). This has proved particularly powerful for identifying brain networks for which no previous hypotheses exist. For example, Damoiseaux et al 2006 found 10 consistent resting patterns with relatively large coherent fluctuations in the BOLD signal in their study of control subjects using ICA. The results showed very plausible and consistent brain networks that were in line with findings in previous ICA research (Biswal et al 1995, Beckmann 2005, De Luca et al 2006, Fox et al 2005, Grecius 2003)

The identification of how regions coordinate their activity and interact has become an increasingly important approach for characterising ICNs and understanding the neural underpinnings of sensation, salience and cognition (Bressler and Menon 2010, Fox and Raichle 2007, Raichle 2010). Crucially, the brain's functional architecture can be characterised in the absence of any specific external input (i.e., while the subject is at rest). Raichle's 2010 paper (the two views of brain function) describes brain functions as mainly intrinsic, involving information processing for interpreting, responding to and predicting environmental demands. This idea corresponds well with metabolic studies which report the adult cerebral cortex to be highly metabolically active during the awake resting state requiring 20% of the total resting oxygen intake despite the human adult cortex comprising about 2% of total body weight (Clarke and Sokoloff 1999, Rolfe and Brown 1997). Prior to Raichle's work on resting state (intrinsic) brain network activity (Raichle et al 2001) most studies of brain function focused on activation (task evoked, see above) responses. Although important, these studies by design encourage a reflexive view of brain function.

FC can be studied with any of the available non-invasive methods that probe human brain function (i.e. PET and SPECT see above), electrophysiological methods (EEG and magnetoencephalography (MEG)), or fMRI. In the research setting fMRI is the most widely used tool for studying human brain function and has some advantages over the other techniques either in terms of spatial and temporal resolution (compared to PET and SPECT), or sensitivity to cortical and subcortical structures (compared to EEG/MEG). It also has the potential to be widely available clinically, since clinical MRI scanners can be used in principle allowing developments in understanding of the brain's functional architecture and their modifications to impact on clinical management.

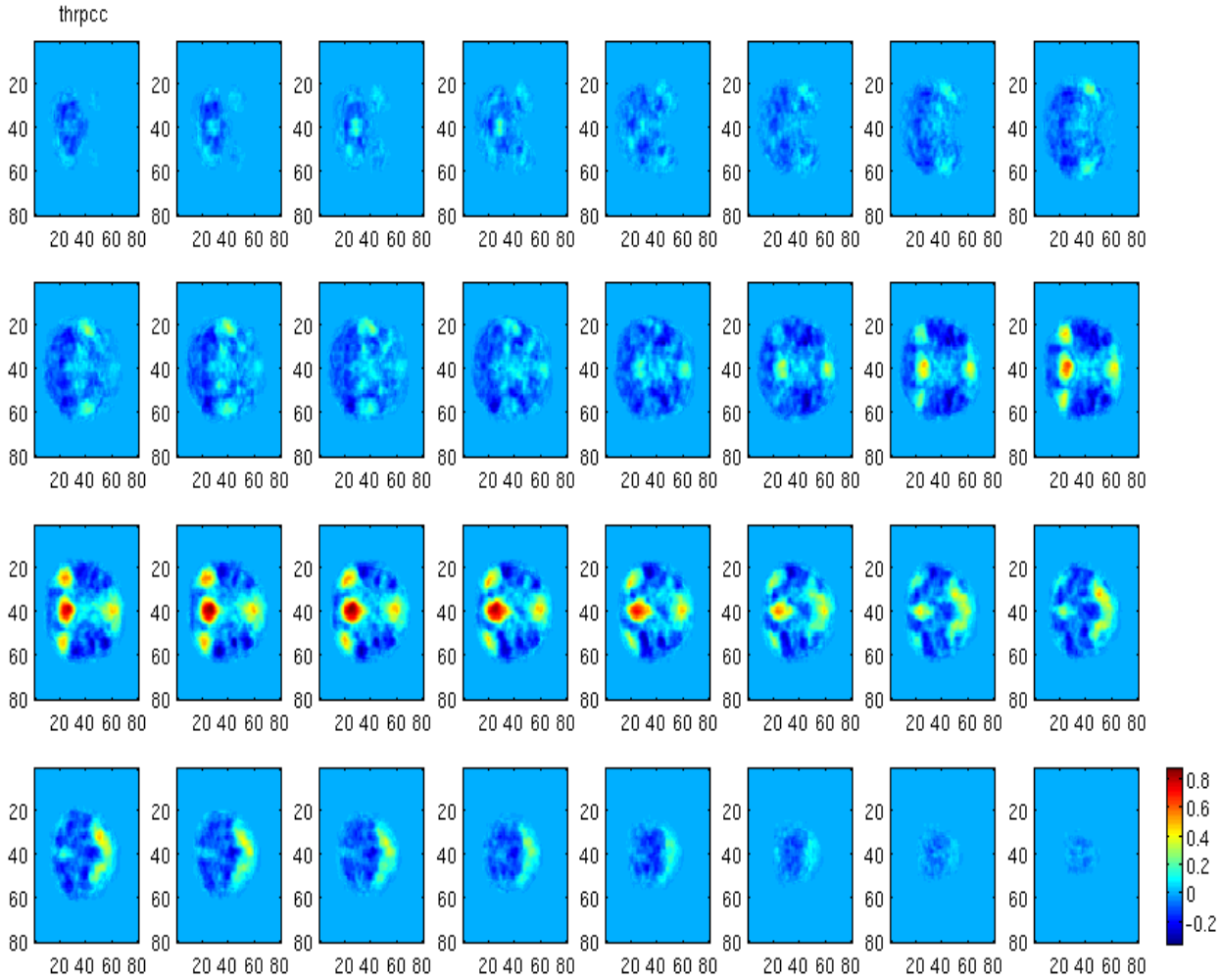

Figure 1.6 group correlational FC maps in axial view of the DMN with the PCC as the seed region produced from fMRI data (X and Y axis represent arbitrary units for comparison of ROIs between maps. The colour bar scale represents degree of correlation for the seed region to the other ROIs).

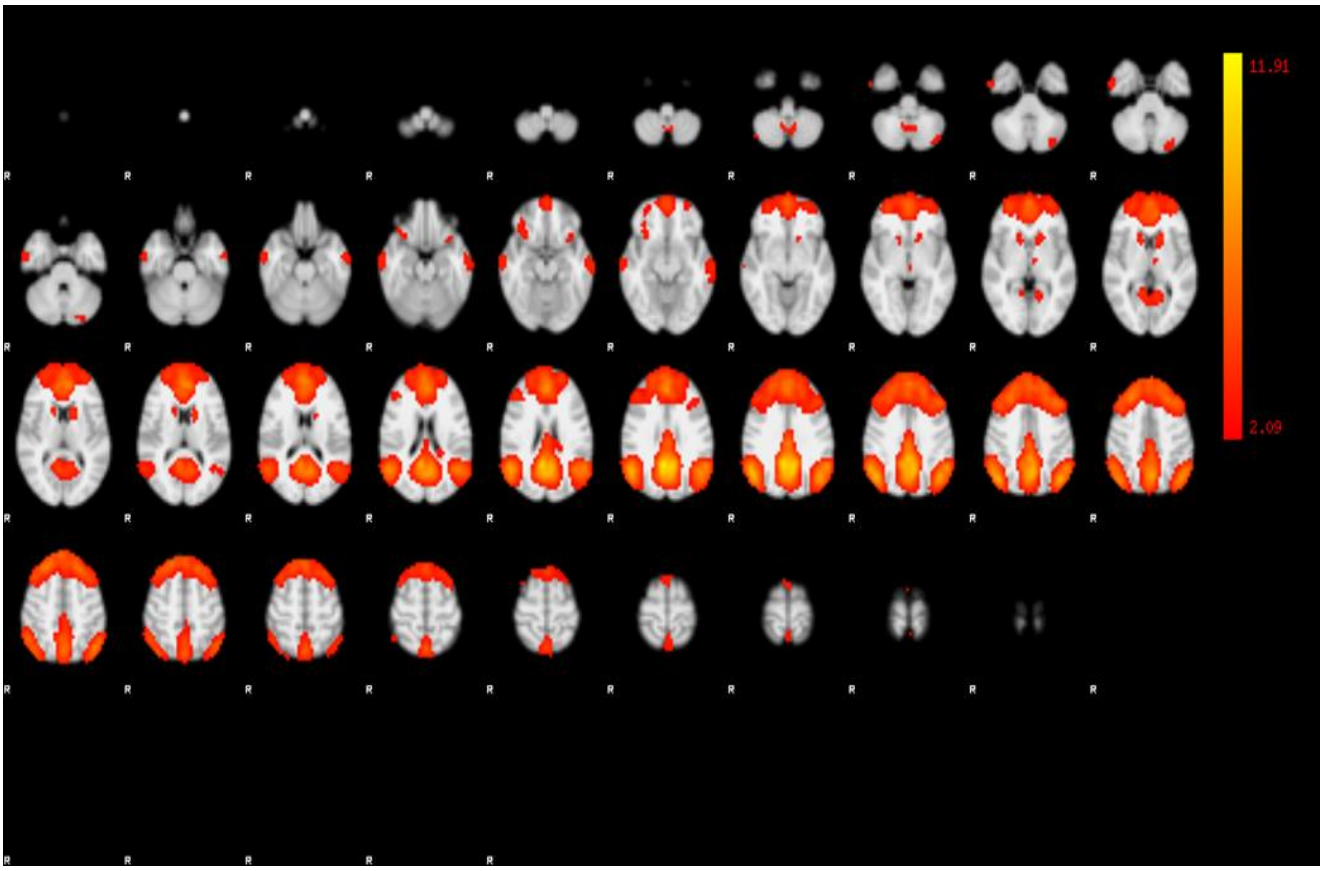

Figure 1.7 above resting state group FC correlation maps in axial view of the DMN using independent component analysis processed from fMRI data (scale represents the z-score which represents the degree of correlation of the voxel time course with the independent component time course).

FUNCTIONAL NEUROIMAGING AND ICNs

Intrinsically connected networks:

The brain is organised into a series of correlated functional networks characterised by FC, even in the absence of stimulation (Biswal 1995, Lowe, et al.1998, Raichle et al. 2001). Correlated networks can be defined as brain regions which are preferentially functionally (or structurally) connected, so the regions within the network are more connected than they are with regions outside the network. Using fMRI, approximately ten resting state networks (RSNs) or ICNs (to avoid the assumption that they are only seen

in data acquired at rest) can be identified. These range from regions known to be involved in motor function (the sensory-motor network) and primary sensory processing (auditory phonological and visual processing networks), to those involved with higher level cognitive functions such as executive functioning (Central executive network CEN), attention, salience (Salience network SN) and memory, awareness, and conscious self-perception (Default mode network DMN). While in some cases the precise functional purpose of ICNs is not absolutely clear, their activity has been shown to have functional significance. ICN activity is modified in a multitude of neurological and neuropsychiatric conditions (Broyd et al 2009, Greicius et al 2008, Monk et al 2009, Rombouts et al 2005, Zhang et al 2011), as well as in the different stages of sleep (De Havas et al 2012, Horovitz et al 2009, Larson-Prior et al 2009, Samann et al 2011) and following sleep deprivation (Bosch et al 2013, De Havas et al 2012, Gujar et al 2010, Tomasi et al 2009, Sämann et al 2011, Verweij et al 2014). This opens up the possibility that they can provide clinically-relevant information that might be of use not only in patients with various neurological disorders (Bozzali et al 2002, Gattellaro et al 2009, Kinnunen et al 2011, Zhang et al 2011), but also in control subjects in relation to subtle changes to the waking state in relation to habitual sleep status (Chapter 4, Khalsa et al 2016).

Default Mode Network:

One of the most studied ICNs is the DMN (Greicius et al 2003, Horovitz et al. 2009, Lowe et al 1998, Raichle et al 2001, Shulman et al 1997). The DMN consists of the posterior cingulate/precuneus cortex (PCC), the left and right inferior parietal/angular gyrus (l/rIPC), medial prefrontal/anterior cingulate cortex (mPFC), the left and right mesial temporal regions (l/rMTL) and left and right hippocampi (l/rHC). The DMN is thought to be

instrumental in promoting awareness and conscious self-perception in the human brain at rest and is seen to deactivate during task performance (Gusnard et al 2001). Many studies have been conducted using fMRI (Greicius et al 2003,Horovitz et al 2009, Lowe et al 1998, Raichle et al 2001) which report a similarity between the patterns of FC identified in the resting brain across individuals. Numerous studies have indicated the high rate of glucose metabolism of the PCC compared to other brain regions (Pfefferbaum et al, 2011, Raichle et al 2001) suggesting this region plays an important role in the regulation of resting state activity, self referential intrinsic thoughts and cognition (Binder et al 1999, Leech et al 2012, Mitchell et al 2003, Pearson et al 2011). Subsequently the DMN nodes have been identified as components of the consciousness system (Blumenfeld 2012, Danielson et al 2011).

In addition to the proposed role of the overall network, individual nodes have been linked with specific functions for example, it has been proposed that when an individual is awake, but not actively engaged in cognitive task performance, the PCC promotes information gathering and representation of oneself and the world around us (Binder et al 1999, Leech et al 2012, Pearson et al 2011,). When an individual focuses on a goal directed task this disrupts the resting state processes (Gusnard et al 2001,Raichle et al 2001). This in turn is a reflection of the disruption of cortical neuronal activity devoted to self-referential and general information gathering processes (Cavanna et al 2006, Leech et al, 2011). In a PET study investigating the neural correlates of the hypnotic state (Rainville et al 1999) the PCC and rIPC demonstrated a decrease in rCBF compared with other regions of cortex. In addition, fMRI studies investigating FC in the DMN of individuals in the descent to sleep demonstrated a reduction in FC of the PCC to the mPFC (Horovitz et al 2009,

Samaan et al 2010). Since impaired consciousness is a key feature of sleep onset and is manifest in the sleep deprived state (Bosch et al 2013, De Havas et al 2012, Gujar et al 2010, Horovitz et al 2009, Larson-Prior et al 2009,Samann et al 2011, Tomasi et al 2009, Verweij et al 2014) these observations provide further evidence of the involvement of default mode regions in higher order processes of conscious behaviour. Furthermore Fiset et al 1999 investigating the effect of Propofol (an anaesthetic) induced unconsciousness demonstrated marked reductions in rCBF to the PCC and mPFC regions of cortex. The progressive decrease in PCC and mPFC activity correlated directly with increasing anaesthesia and inversely with the restoration of consciousness. These studies lend weight to the notion that DMN activity is directly involved in the maintenance of the resting alert conscious state.

Central Executive Network:

The CEN is frequently seen to activate during tasks involving executive function such as vigilance and alertness during fMRI studies (Collette and Van der Linden 2002, Fan et al 2005). Seeley et al 2007 found the CEN to consist of the dorsolateral prefrontal cortex (DLPFC) and posterior lateral inferior parietal lobule (IPL), particularly in the intraparietal sulcus, and reported that activity from the CEN correlated with performance on executive control tasks. There is evidence to suggest the intra-network FC strength of the CEN (also known as the fronto-parietal control network Vincent et al 2008, or the executive control network Lie et al 2015) is associated with elevated levels of I.Q (a measure of intelligence) in children, adolescents and adults (Langeslag et al 2013). It has also been shown that the CEN is anti-correlated with DMN activity in healthy adults (Fox et al 2005,

Mennon and Uddin 2010, Sridharan et al 2008). There is a possibility that it may even inhibit DMN activity under certain conditions (Chen et al 2013).

Salience network:

The SN is comprised of the anterior cingulate cortex (ACC), the left and right anterior insula (lAI, rAI). The sub-cortical amygdala and substantia nigra (ventral tegmental region) and thalamus are also associated regions. The SN was identified by Seeley et al 2007 using region of interest (ROI) and ICA of resting state fMRI data. The SN detects the most relevant stimuli from internal and extrapersonal stimuli in order to guide behaviour (Seeley et al 2007). There is evidence from several brain imaging studies to suggest that the SN responds to varying degrees of subjective salience which can be cognitive, emotional or homeostatic (Craig 2002, Craig 2009). The AI has been identified as an integral node of the SN which is tightly coupled to this network. It has been found that the ACC and AI are co-activated during a wide range of cognitive tasks.

Sridharan et al (2008) demonstrated that the rAI plays a critical role in switching between the DMN and CEN, two networks known to demonstrate competitive interactions during cognitive information processing (Fox et al 2005, Greicius et al 2003). The rAI was shown to play a major role in the activation of the CEN and the deactivation of the DMN (Sridharan et al 2008), suggesting that the rAI acts as a control switch between brain networks across task paradigms and stimulus modalities. The AI functions to detect transient salient stimuli and initiate attentional control signals which are sustained via the ACC and ventrolateral and dorsolateral prefrontal cortex (Mennon and Uddin 2010, Seeley et al 2007). Therefore the core function of the SN is to identify stimuli from a plethora of sensory stimuli that are imposed on the senses. Upon detection of such stimuli

the AI instigates task-related information processing via appropriate transient control signals initiating brain regions concerned with attentional, working memory, and higher order cognitive processes while deactivating the DMN. These switching mechanisms direct attention to external stimuli which results in such stimuli taking on added significance or saliency.

Higher ICNs and sleep status:

Sleep is crucial for maintaining normal waking cognitive functioning (Alhola and Polo-Kantola 2007, Babkoff et al 2005, Belenky et al 2003, Horne 1993, Dinges et al 1997, Harrison et al 2000,Van Dogen et al 2003). It has been suggested that the cognitive processes of the human brain are regulated via ICNs (Bonnelle et al 2012, Mennon and Uddin 2010). One of the ICNs that has been consistently implicated in alterations to consciousness is the default mode network (DMN, for a review see the chapter by Bagshaw and Khalsa 2013). As mentioned above impaired consciousness is a key feature of sleep onset and is most evident in the sleep deprived state (Bosch et al 2013, De Havas et al 2012, Gujar et al 2010, Horovitz et al 2009, Larson-Prior et al 2009,Samann et al 2011, Tomasi et al 2009, Verweij et al 2014). A number of studies have investigated DMN functional connectivity during the descent into sleep, with one of the more consistent observations being a reduction in connectivity between the PCC and mPFC in stage N2 and beyond (Horovitz et al 2009, Samaan et al 2011). For example Horovitz et al investigated changes in DMN FC connectivity in relation to the natural sleep induced reductions in consciousness and reported changes in FC between nodes of the DMN. They found the most noticeable reductions in FC between the mPFC and the other nodes of the DMN and they also reported an increase in FC between the PCC and the

IPC nodes during sleep. While during rested wakefulness PCC-mPFC FC was found to be strong. More widespread ICN changes in connectivity have also been observed (Andrade et al 2011, Spoormaker et al 2012). As well as these changes in ICN functional connectivity during sleep itself, alterations have also been noted during wakefulness following sleep deprivation (Gujar et al 2009, Lei et al 2015) or partial sleep deprivation (Samaan et al 2010) and in relation to self-reported sleep duration on the night prior to a waking scan (Kilgore et al 2012). For example Yoo et la 2007 reported a single night of sleep deprivation produced a significant deficit in FC activity of the hippocampus during episodic memory encoding, subsequently causing worse memory retention of new experiences. They also report hippocampal FC impairment characterises a different pattern of FC in alertness networks of the brain stem and thalamus in subjects sleep deprived for a single night compared to control subjects. In addition, Yoo et al report FC of the prefrontal regions is predictive of the success of memory encoding for sleep-deprived individuals in comparison to 'normal' sleepers. Their overall findings show that lack or absence of prior sleep substantially compromises the capacity for committing new experiences to memory. The researchers express concerns from their findings in relation to ever decreasing sleep time in today's developed industrial societies and the possible impact this may have on memory consolidation of new experiences. Also Lei et al 2015 investigated the FC of the DMN, SN and executive control network (CEN) in relation to subjective sleepiness scores, sleep pressure index (a measure of the degree of homeostatic sleep pressure) and cognitive tasks (working memory, reaction time) in SD subjects. They found significant increases in the FC between the DMN and SN with respect to all the above measurers after 36 hours of SD. Lei et al 2015 suggest these

findings represent an increased instability of the waking state with the brain favouring enhanced DMN-SN connectivity opposed to DMN-CEN connectivity. This suggests an up regulation of salience in relation to internal mental events (for example as might be required to counteract the increased biological pressure to sleep). Kilgore et al examined the relationship between sleep duration and resting state connectivity among healthy volunteers who slept at home according to their own schedules. Thirty nine subjects filled in questionnaires about their recent sleep habits and entries in sleep diaries for the previous night. They underwent a resting state fMRI scan at T3. Kilgore's group found that longer self-reported overnight sleep duration had an association with significantly enhanced functional connectivity between the medial prefrontal cortex and posterior cingulate. Overall findings from Kilgore's study suggest that even normal variations in sleep duration over one night measured by self-report are related to FC strength within select nodes of the DMN.

Other studies suggest that an appropriate level of AI activity is required to sustain an alert signal to initiate brain responses to salient stimuli (Menon and Uddin 2010). Bell-McGinty et al 2004 report increased FC activation in the AI together with the claustrum and right putamen in sleep deprived subjects during a non-verbal recognition memory task in comparison to responses in the same subjects post normal sleep. The AI of the SN is thought to behave as an integral hub in mediating dynamic interactions between other ICNs, which are involved in externally oriented attention or internally oriented, or introspective cognition (Menon and Udin 2010). From such observations it is not unreasonable to surmise that changes in inter-network modulation may occur albeit to a lesser degree in response to changes in chronic sleep status, as a consequence of partial

sleep deprivation or as a result of short habitual sleep durations for example. It has been shown that sleep deprivation effects FC of the DMN and other ICNs (Gujar et al 2009, De Havas et al 2012, Lei et al 2015, Samaan et al 2010, Yoo et al 2007) cognition and task performance (Belenky et al 2003, Dinges et al 1997, Lei et al 2015 Van Dongen et al 2003, Bell-McGinty et al 2004) and the prefrontal cortex (Harrison et al 2000, Horne 1993, Naghavi and Nyberg 2005). Therefore if sleep itself and variations in sleep pressure affect the FC between the nodes of the DMN and it has been shown that the SN is involved with the modulation of degrees of consciousness in conjunction with the DMN and the CEN (Menon 2010). It is reasonable to propose that variations in habitual sleep durations may also cause network changes in FC between the nodes of the SN, CEN as well as the DMN in the waking state and subsequent ICN FC changes may be an indicator of prior sleep status.

These studies indicate that the integrity of ICN FC is a sensitive marker of prior sleep history, which may therefore help to shed light on the link between sleep and cognition and conscious behaviour, the neurobiological underpinnings of individual differences in susceptibility to sleep deprivation, as well as the mechanisms behind sleep disorders.

STRUCUTURAL NEUROIMAGING

Structural neuroimaging methodologies have been used in the work presented in this thesis. The structural connectivity (SC) that ultimately provides the anatomical substrate for functional interactions, and the relationship between FC and SC, is less well understood than FC (Damoiseaux and Greicius, 2009). SC can most readily be defined

non-invasively using diffusion-tensor imaging (DTI) approaches allied with tractography analysis.

Diffusion weighted imaging:

Diffusion weighted imaging (DWI) is a non-invasive MRI technique which allows a greater understanding of the brain's white matter architecture and neuroanatomy in normal and pathological conditions with detail which was not previously possible with non-invasive techniques. DWI is therefore important in allowing greater understanding of brain structure, and also structural and functional relationships in the brain. MRI is sensitive to water in tissues and the passive random thermal motion of water molecules in bulk at ambient temperatures, also known as Brownian motion, which describes diffusion properties. Diffusion properties can be measured with a diffusion coefficient (also known as diffusivity, S.I unit m^2/s) which is the measure of the degree of free random particle movement within a liquid or gas and is dependent on both temperature and pressure. The higher the diffusivity of one substance with respect to the other the faster they diffuse in to one another. The diffusion of water is measured by an Apparent Diffusion Coefficient (ADC) as opposed to a diffusion coefficient because as the water molecules move within tissue they encounter restrictions and hindrances such as macromolecules and cell membranes. Therefore, we do not observe truly free random movement of water molecules. This restrictive effect on water molecules forms the basic principle for DWI. Subtle variations in the amount of restriction to the diffusion of water molecules are seen as changes in the diffusion weighted image signal. Conventional MRI sequences are made sensitive to molecular diffusion by adding two extra gradients (the work in this thesis is based on diffusion data acquired with two gradients using 61 diffusion gradient

directions) to a standard ultrafast MRI sequence (which is usually a T2- weighted echo planar sequence).The diffusion gradients are symmetrically centered around a 180 degree refocusing radiofrequency pulse and equal in magnitude. The first gradient causes molecules to acquire phase shifts and the second cancels these phase shifts in non-moving stationary spins (rephasing). Moving spins however acquire an effective phase shift as their motion limits rephasing by the second gradient. The degree of diffusion (random movement of water molecules) determines the degree of MRI signal loss. Therefore the higher the rate diffusion (for example in CSF) the greater the loss of signal. Inversely the lower the diffusion rate (for example in grey or white matter) the lower the MRI signal loss. MRI signal loss can be enhanced by increasing the strength, directions, and duration of diffusion gradients. The gradients are characterised by their b value (s/mm^2). This value is determined by the Stejskal Tanner equation: b factor = $\gamma^2 G^2 \delta^2(\Delta - \delta/3)$ where γ is the gyrometric ratio, G is the strength of diffusion gradient pulses, δ is the duration of the diffusion gradient pulse and Δ is the time between diffusion gradient RF pulses (Huisman 2010).Therefore the diffusion weighted image signal is determined by ADC (Basser et al 1994) together with a weighting factor b often called the b-factor which is expressed in mm-2 and the acquisition parameters and pulse sequence.

Movement of water molecules within white matter is further restricted and tends to be along axons and white matter tracts rather than across them (similar to diffusion within a cylinder). The diffusion of water along a particular axis (i.e. directional diffusion) as opposed to non-directional diffusion (i.e., diffusion in all directions) is called anisotropic diffusion (Henkelman et al 1994, Moseley et al 1990, Moseley et al 1991). In an anisotropic environment, for example white matter, where measured diffusivity depends

on the orientation of the tissue, a single scalar quantity such as the ADC is not appropriate to use as a measure of water mobility (Henkelman et al 1994, Moseley et al 1990, Moseley et al 1991). The next most complex model which describes anisotropic diffusion replaces the scalar ADC with an Apparent Diffusion Tensor (D) (Crank, 1975).

The diffusion tensor model:

Within the diffusion tensor model, anisotropic diffusion is represented by a 3D ellipsoid (the diffusion ellipsoid, figure 1.8). The ellipsoid is the space representing the distance that a molecule will diffuse to with equal probability from the origin. The diffusion tensor represents the 3D probability of the displacement of water molecules. The reference frame within a diffusion tensor is the Eigensystem. Three eigenvalues (λ1, λ2, λ3) represent the ADCs measured along the principal axes giving the strength of diffusion. Eigenvectors (e1, e2, e3) represent the orientation of the principal axes and therefore are related to the direction of fibres. The eigenvector corresponding to the largest eigenvalue is the Principal Diffusion Direction (PDD) (figure 1.8).

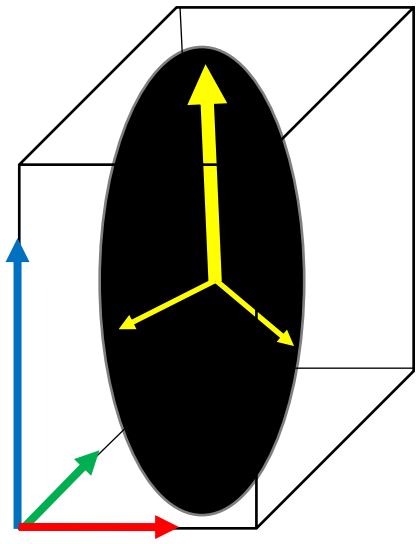

Figure 1.8.The diffusion tensor model. The thick yellow arrow represents the principle eigenvalue $\lambda 1$ (which corresponds to e1 = PDD). e_1(blue arrow), e_2 (red arrow), e_3 (green arrow) represent the direction (eigenvector) of diffusion (Based on *Basser, et al 1994)*.

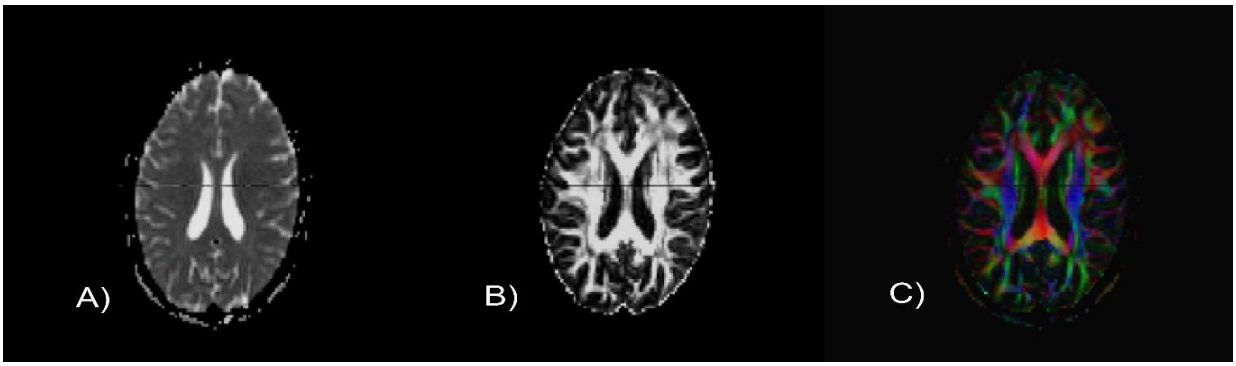

Figure 1.9. Above image A) is a diffusion weighted image as constructed using ADC, image B) is a diffusion weighted FA image as constructed using the diffusion tensor model, image C) is a colour coded diffusion weighted FA image constructed using the diffusion tensor model. Colour coded directional information about diffusivity: The green represents tracks running in an anterior – posterior direction, red represents tracks running left – right and blue represents tracks running in a superior-inferior direction. All slices are axial and all images are from the same subject.

Fractional anisotropy:

Fractional anisotropy (FA) is the indicator of how strong the directional diffusion is and therefore, is a good marker for white matter integrity. FA is a measure of delimiting free water diffusion. In white matter this is mainly due to axon and myelination features. Therefore, compromises to axon integrity and demyelination result in an increase in free water diffusion, a consequent decrease in directional diffusion and a subsequent reduction in FA anisotropic values.

FA values are low in grey matter (~0.2), variable in deep grey matter (0.2-0.4) and higher in white matter from ~0.45 in the subcortical white matter in the gyri to ~0.8 in the corpus callosum of the healthy human brain (Johansen-Berg, Behrens, 2009) This property allows us to effectively use DWI to compare SC between individuals by comparison of the FA values acquired from their DWI data.

Mean diffusivity:

The mean diffusivity (MD) is used in many published studies and is simply the sum of the eigenvalues divided by three (MD = $(\lambda 1+\lambda 2+\lambda 3)/3$), which is equivalent to the average of the eigenvalues. MD increases in regions where changes in white matter cytoachitecture cause reductions in directional diffusion, which is measured as mean FA (figure 1.8 illustrates a diffusion tensor showing anisotropic diffusion). As directional diffusion is compromised, an increase in mean diffusion is evident (figure 1.10 is a diffusion tensor illustrating MD). Therefore, MD can be used as a marker of axonal structural integrity in conjunction with FA. In healthy axons high FA values would be expected with low MD

values, but in compromised white matter an increase in MD would be seen together with reduced FA. Therefore MD can be used as an additional measure to assess axonal microstructural integrity.

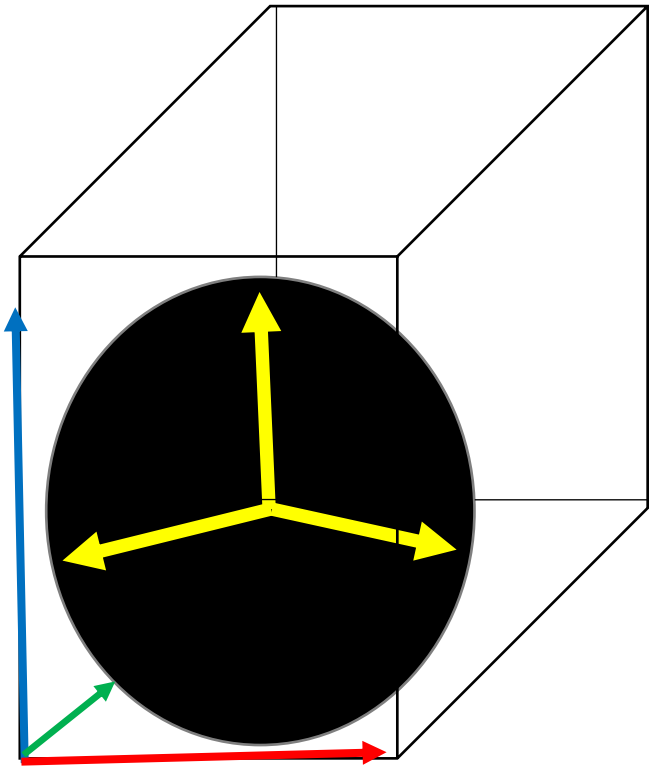

Figure 1.10 diffusion tensor model illustrating MD. The Eigenvalues and Eigen vectors are of approximately equal magnitude in all three directions. This therefore represents mean diffusivity (compare with the diffusion tensor in Fig 1.8 which illustrates anisotropic diffusion).

TRACTOGRAPHY

Streamline deterministic tractography:

Voxelwise estimation of directional anisotropy of water diffusion as characterised by diffusion tenors provides unique opportunities for modelling of white matter architecture in the human brain in vivo. The algorithms used to map white matter tract trajectories are referred to as tractography. Tractography can be achieved in a variety of ways (Conturo et al 1999,Tuch al 2004,). Deterministic tractography methods are primarily based upon streamline algorithms where the local tract direction is defined by the major eigenvector of the diffusion tensor. A streamline is any line through a vector field whose tangent is always parallel to the vector field. The streamline follows the orientations of least resistance to diffusion (in other words the principle axis of diffusion tensors, PDD), thus producing a track (Johansen-berg, Behrens 2009). Streamlines are used in deterministic streamline tractography. A limitation of streamline deterministic tractography is that it can only work in regions of high FA where confidence in PDD is also high. In real terms this means that streamline deterministic methods use a threshold on FA (usually 0.2), below which pathways are terminated. In other words while it is possible to track major white matter pathways in white matter, but it is not possible to continue to track these pathways to their grey matter destinations or through regions of fiber complexity or crossing.

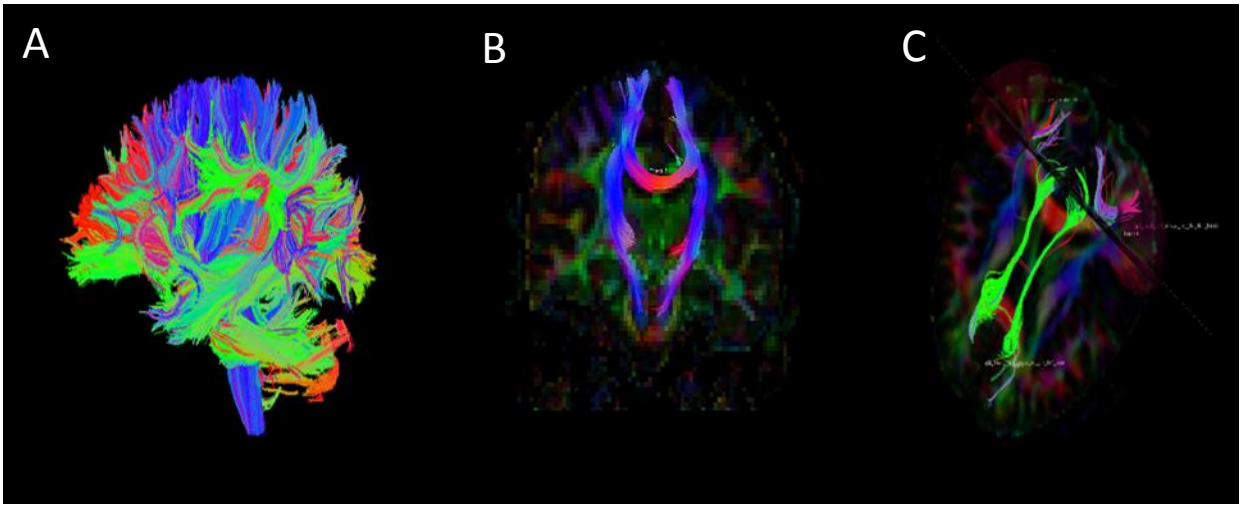

Figure 1.11 A) above whole brain streamline deterministic tractrography of a single subject. Fig B) above corpus callosum and the internal capsule white matter tracts superimposed on a colour coded diffusion tensor image map. Fig C) cingulum bundle tracts and left and right angular gyrus white matter connections to the lateral parietal regions.

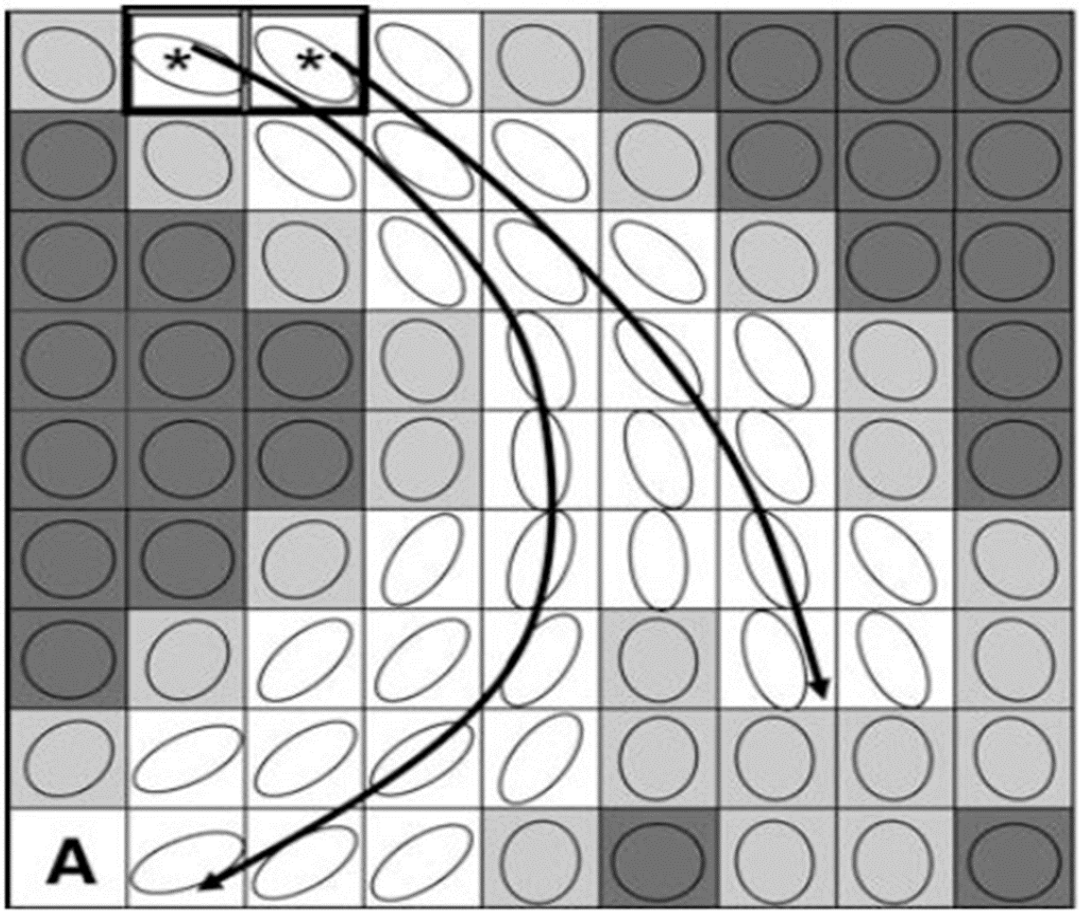

Figure 1.12 The diagram above illustrates deterministic streamline tractography. The grid represents individual voxels. Each voxel containing diffusion tensor ellipsoids of various shapes. Those demonstrating the greatest anisotropy (most elliptical) are shown in white and the least anisotropic are dark grey. The dark outlined boxes with * represent the seed regions; the black curved arrows represent the streamline path of the PDD through each of the voxels producing a tract. (From Johansen-Berg et al. Ann Rev. Neurosci 32:75-94 ,2009).

Probabilistic tractography:

Probabilistic tractography was developed a few years after streamline deterministic tractography (Behrens et al 2003, Hagmann et al 2003, Lazar and Alexander 2005, Parker et al 2003). These probabilistic methods account for the uncertainty in estimates

of PDD, therefore allowing tracking without the need for thresholds on FA. Instead of a single orientation estimate, a distribution of orientations in each voxel are inferred. Probabilistic tractography is used to build up thousands of streamlines and subsequently produce a probability distribution of different pathways from a given seed point (figure 1.13). Such a methodology has allowed detailed research into patterns of cortico-cortical connectivity (Broser et al 2012, Huang et al 2009, Parker and Alexander, 2005, Zarei et al 2006). Probabilistic methods now extend beyond the estimation of one diffusion direction per voxel (Behrens et al 2007, Hosey et al 2005, Parker and Alexander 2005).

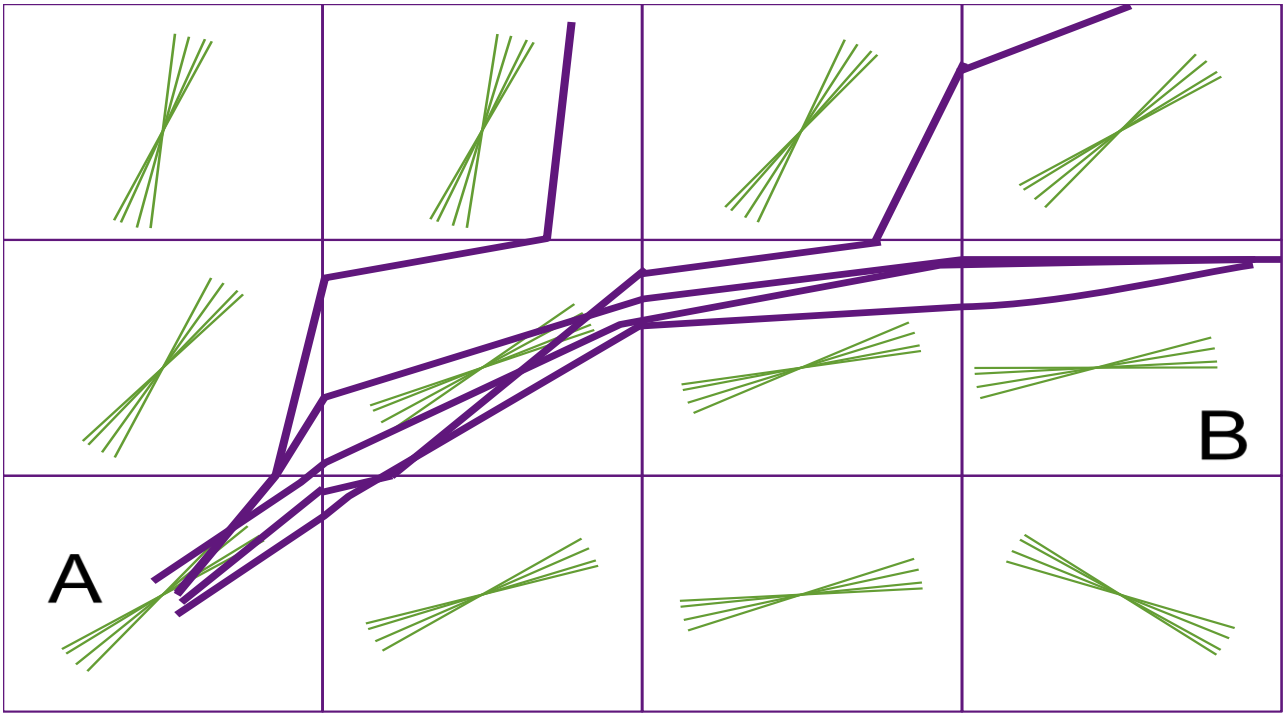

Figure 1.13. propagation of a probability distribution. (adapted from Bherens et al 2003 and parker et al 2003).

Figure 1.13 above shows a grid of voxels with a number of streamlines (green) propagated from a seed at each voxel. Thus Building a spatial distribution of curves

(purple) that simulates overlapped results from multiple deterministic streamline tracking on multiple scans. The probability of a curve starting at A and going through B (P_{AB}, figure 1.13 above) is expressed as $P_{AB}=M/N$ (where M is the number of streamlines that go through B and N is the total number of streamlines generated from A. (adapted from Bherens et al 2003 and parker et al 2003)

Voxel Based Analysis

Group statistical analysis can be performed on whole brain DTI data. Most researchers are interested in group comparisons of DTI metrics and the methods to extract these measures differ mainly in the way the alignment across subjects is achieved. Analyses on a voxel-by-voxel basis is popular in DTI research due to the fact that such analysis is automated, requires minimum intervention and is not influenced by the researcher carrying out the analysis. Voxel Based Analysis (VBA) is one such method which involves registration of diffusion maps containing (FA and MD metrics) into a standard space (known as normalisation) to achieve coherence across subjects and voxels and subsequently their anatomical structures . This allows the comparison of DTI metrics such as FA and MD between groups and correlations with covariates of interest, such as total sleep time for example. This type of analysis allows spatially specific and unbiased analysis of DTI metrics and does not require prior assignment of ROIs. The main issue with standard VBA analysis of diffusion tensor data is the degree of accuracy of the registration algorithms using tensor datasets. (Mukherjee et al., 2008b; Abe et al., 2010; Astrakas and Argyropoulou, 2010; Jones and Cercignani, 2010; Van Hecke et al., 2010).

Tract based spatial statistics

A methodology which is designed to overcome the problems associated with VBA analysis with respect to registration algorithms and arbitrariness of spatial smoothing is tract based spatial statistics (TBSS). TBSS is used to compare two DTI image groups. The first step in TBSS is preprocessing the images for possible artifacts such as eddy currents in the same way as for all other DTI analysis (see chapter 2). The next TBSS uses non-linear alinement of images, by applying affine transformation. It also creates a mean FA skeleton and this skeleton is used as the framework for the comparison of the two DTI data sets. TBSS uses a skeletonisation process which involves applying non-maximal suppression perpendicular to the local tract structure (known as thinning). In this way, a skeleton of average FA values of all subjects is produced (figure 1.14). This is achieved by projecting each subjects FA data onto the skeleton . The skeleton is filled with FA values from the nearest track center via the generation of a distance map (figure 1.15). Finally voxelwise statistics is preformed across all subjects to identify possible differences between the two groups.

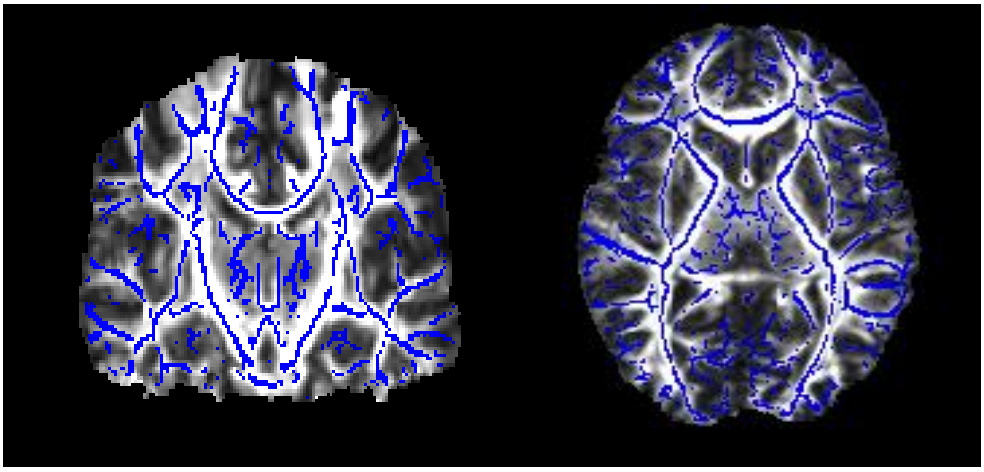

Figure 1.14) mean FA skeleton projected on to the mean FA image of all subjects (coronal and axial view).

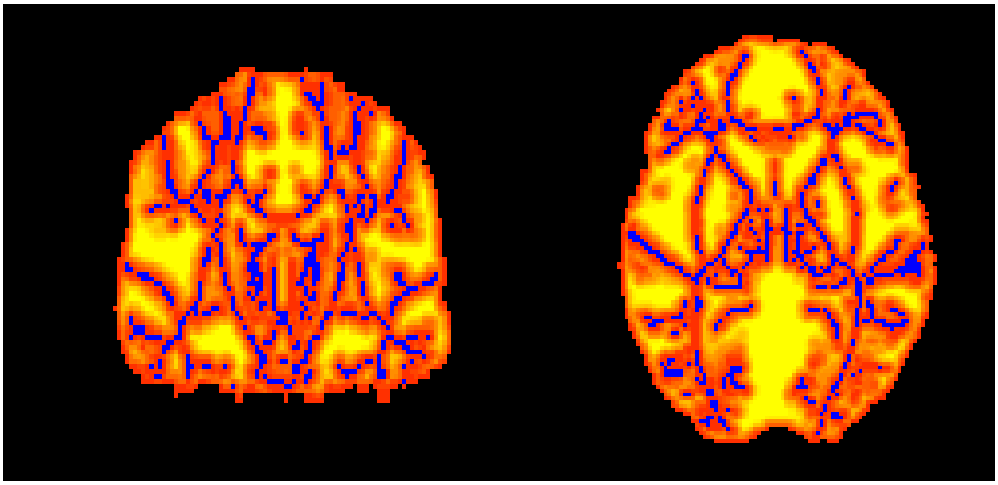

Figure 1.15), distance map showing distance of each voxel from mean FA skeleton (Blue), bright orange regions indicate the highest FA values close to the mean FA skeleton.

TBSS is similar to VBA in many ways for example it is an automated method for detecting group voxel-wise changes in DTI metrics in the whole brain. But it has important differences too, due to TBSS analysis being based on the skeletonisation (see chapter 4 for more details) of group registered FA maps. Also importantly in comparison to VBA TBSS removes the need to perform spatial smoothing, and increases statistical power due to reducing the total number of voxels being tested.

Non -tensor tractography models:

Alternative approaches to the tensor model include q-ball imaging (Tuch 2004), or diffusion spectrum imaging (Weedon et al 2005). These approaches take a direct approach to estimate the orientation distribution function without having to impose a particular distribution (e.g Gaussian) on the data. Such methods can resolve multiple fibre orientations within a voxel and can characterise fibre orientation structure at high spatial resolution and angular resolution. Such models can be used to perform tractography

(Wedeen et al 2008) and have been used to characterise high resolution cerebella circuits (Granziera et al 2009). These approaches are also time-consuming due to the high number of diffusion directions required during each scan and can lead to subjects becoming restless due to the long scan times. They also require longer analysis processing time due to extra computation demand as a consequence of the high volumes of data acquired.

Structural connectivity:

Due to the invasive nature of traditional tract tracing methods used to assess SC in the brain, they are not appropriate for use in vivo. Therefore, tract tracing studies in humans relating structural features to brain dynamics or behavior are impossible, although tract tracing is extremely useful and important in studying anatomical connections in animal models. Tract tracing is vital for validating tractography derived from diffusion MRI data (as discussed above). Such validation has been performed and has demonstrated similarities between projections identified by tractography and classical anatomy (Lawes et al 2008, Seehaus,et al 2013).

Structural connectivity describes anatomical white matter connections between cortical and/or sub-cortical regions. Structural connectivity is relatively stable on shorter time scales (seconds to minutes) but may be subject to neuroplasticity effects which are determined by experience dependent changes at longer time scales (days, months or years, May et al 2007, Mackey et al 2012, Dayan and Cohen, 2011). Diffusion MRI SC is usually measured as a set of undirected connections, since the directionality of projections at this present time cannot be discerned. Structural connectivity can be assessed qualitatively by visual inspection of tracts reconstructed from DTI data via

tractography and comparing these to known white matter pathways using atlases (Lawes et al 2008, Oishi et al 2010). These tracts can be quantitatively measured by measuring the strength of FA , MD or other DTI metrics(Johansen-Berg and Behrens 2009,Wahl et al 2010) or by calculating the number of activated voxels within a tract probability distribution between pairwise connections (Johansen-Berg and Behrens 2009).

STRUCTURAL NEUROIMAGING OF ICNs IN RELATION TO SLEEP

Understanding the relationship between functional and structural connectivity is an active area of research (Damoiseaux and Greicius 2009, Guye et al 2008,). The goal of the majority of research is to understand how underlying brain structure, and the modifications to structure brought about by disease processes, affect functional networks and behaviour. Little work has been done in terms of examining FC and SC in functional neural networks in relation to sleep deprivation and even less when considering chronic changes due to habitual sleep status in control subjects.

Structural connectivity and sleep status:

Differences in distributed white matter pathways reflect, and may contribute to, a person's ability to function effectively when sleep deprived. Rocklage et al 2009, in a TBSS study found lower FA values significantly positively correlated to the level of performance in a spatial visual motor task in SD subjects compared to non-SD participants. The SD subjects demonstrated reduced FA in multiple white matter regions including the superior corona radiata , cortico-spinal tracts and in the cingulum bundle. A study by Rosenberg et al 2014 while not considering direct sleep deprivation, investigated late, early and intermediate sleep chronotypes in relation to white matter architecture using TBSS.

Rosenberg's group found that late sleepers (those subjects who remain awake until the early hours and have difficulty getting up in the mornings) demonstrated significant white matter differences in the way of reduced structural connectivity metrics in subjects who stayed up until the early hours compared to control groups with 8 hours sleep. These differences were found in the temporal lobes, cingulate gyrus and corpus callosum. The researchers discuss these findings in terms of a chronic form of jet lag and sleep deprivation. The widespread nature of these differences supports the view that SD has a global effect on brain functioning (Rocklage et al 2009). Studies involving patients with chronic insomnia have demonstrated that grey matter in the frontal lobe may be altered with respect to normal sleepers (Altena et al 2010), and acute sleep deprivation has been shown to reduce thalamic volume (Liu et al 2014), suggesting that there is a link between sleep duration and brain structure. However, the white matter SC that ultimately provides the anatomical substrate for functional interactions is less well understood and very few studies have investigated white matter changes in relation to sleep (Elvsashagen et al 2014, Piantoni et al 2013, Rocklage et al 2009). These observations indicate that the structural correlates of sleep phenomena and even short term alterations to sleep patterns can be investigated with DTI. In combination with the changes to functional connectivity discussed above, they may also suggest that white matter connectivity and organisation moderates the cognitive effects of sleep deprivation and may affect a person's ability to function effectively when sleep deprived.

AIMS AND OBJECTIVES:

The aim of the work presented in this thesis was to investigate the relationship between chronic habitual sleep status and changes in functional connectivity and structural

connectivity metrics in higher order ICNs using fMRI and DTI imaging modalities in normal awake adult control subjects. The main aims were: 1) To use fMRI and DTI neuroimaging modalities to advance our understanding of habitual sleep status and its associations with ICN functional and structural changes in the human brain. 2) To investigate the sensitivity of fmri and DTI metrics in order to determine the degree of association between ICN FC and SC and to investigate the effect of habitual sleep status on these measures. 3) To determine whether whole ICN FC is associated with a broad range of quantitative and subjective sleep metrics.

The hypothesis for this work is based on the premise that the integrity of ICNs, in terms of both their functional and structural connectivity, may be a sensitive marker of prior chronic habitual sleep history and that ICN network FC and SC changes have covariance with habitual sleep status metrics and habitual sleep time in particular.

I report the findings of 4 experiments which examined the structural and functional properties of higher intrinsically connected brain networks to determine if functional and structural changes are associated to chronic habitual sleep status.

At present there have been no studies that have investigated the functional or structural connectivity of ICNs in relation to habitual sleep status. The hypothesis for the work presented in this thesis is based on the premise that the integrity of higher intrinsically connected brain networks, in terms of both their functional and structural connectivity, may be a sensitive marker of prior habitual sleep history.

This thesis is composed of 7 chapters; the research chapters (3-6) differ to varying degrees in the methods used to charaterise FC and SC. These methods were chosen in

order to investigate the specific questions being asked in each study. This combination of measures of FC and SC allows this work to build up a strong argument based on experimental evidence to support the hypothesis of this thesis. Chapters 3-6 are self-contained experimental chapters which are presented as manuscripts including relevant literature review, detailed description of specific methods used and the justification of methods and analyses as well as discussion and concluding remarks. The chapters have been published or are intended for publication, therefore some overlap in content is unavoidable, but this has been kept to a minimum and the published work has been modified and integrated into this thesis.

Chapter 3 investigates and compares the use of deterministic and probabilistic tractography approaches in combination with functional imaging to characterise structural connectivity with respect to functional connectivity in a single ICN, the DMN. The overall aim of the work in chapter 3 was to review and make direct comparisons of tractography algorithms and their ability to charaterise SC and relate this to FC within an ICN. This is a necessary step in order to understand the structural basis of functional connectivity in normal individuals. A better understanding of how structural connections relate to functional connectivity in ICNs is imperative in order to enhance our understanding of changes in SC and FC that may occur as a consequence of chronic habitual sleep status within the general population. The work from chapter 3 has been published in Neuroimage.

Chapter 4 aimed to examine whether inter-individual differences in habitual sleep patterns were reflected in waking measurements of intra- and inter- network FC between major nodes of three ICNs: DMN, SN, CEN. The study improves our understanding of the

relationship between intra- and inter-network FC of ICNs in relation to habitual sleep quality and duration, which may underlie the link between sleep status and cognitive performance. This work has been published in Sleep.

Chapter 5 investigated group differences in FA and MD, structural connectivity metrics, with respect to habitual sleep duration using TBSS. The study also investigated whole brain changes in white matter architecture in relation to subjective habitual sleep quality. The findings from this study support for the first time the notion that reduced habitual cTST, as well as being associated with FC changes within networks which may affect cognition (chapter 4), is also involved in the modulation of the micro structural integrity of specific white matter regions which form the structural backbone of important higher cognitive networks. This work is currently under review at Neurobiology of Sleep and Circadian Rhythms.

Chapter 6 builds on the work from chapter 4 by examining a wider range of sleep features and examining overall network FC as opposed to regionally specific changes. We used habitual sleep time and sleep quality metrics to gain a greater understanding of the importance of these sleep measures in relation to FC network changes.

Chapter 7 the closing chapter of my thesis (General discussion) provides a summary of the overall findings and discusses the potential impact of the findings in the field of neuroimaging sleep research and clinical applications. Subsequently the chapter goes on to discuss the limitations and future directions of this work and ends with the thesis conclusion.

CHAPTER 2

COMMON METHODOLOGIES

This chapter introduces the common methodology, which is used within the experimental chapters of this thesis. The chapter explains participant recruitment, experimental procedures, data acquisition and analysis protocols. Justification for the use of methods is also given where appropriate. Additional methods specific to each experiment are incorporated in to the methods section of that particular chapter.

Subjects:

Data were acquired from healthy adults using a 3 Tesla Philips Achieva MRI scanner at Birmingham University Imaging Centre (BUIC), University of Birmingham. Participants had no history of neurophysiological, neuropsychological or neurological illness. Written informed consent was obtained from all participants, and the studies were approved by the University of Birmingham Research Ethics Committee.

For chapter 3, DTI and fMRI data were acquired from fifteen healthy adults (right handed, 10 female, age 23-29 years, mean age=24.6 years).

For chapter 4 and 6, data were acquired from 37 healthy adults (right handed, 17 female, age 20-59 years, mean age (+/-SD)=35.0+/-11.7 years).

For chapter 5, DTI and fMRI data were acquired from 38 healthy adults (right handed, 10 female, age 20-34 years, mean age=25.4 years). From the original 38 subjects, 5 were subsequently excluded due to actigraphy and diary data demonstrating erratic sleep patterns, leading to a final cohort of 33 participants

Image acquisition and preprocessing:

fMRI:

Subjects underwent a single resting-state fMRI session in the early afternoon during which they were instructed to lie still in the scanner and relax with eyes open. All participants confirmed that they remained awake and alert through the scanning session. Each subject underwent one resting-state fMRI scan of 12 minutes duration (this was 5 minutes for chapter 3), with the following parameters: repetition time (TR) = 2000 ms, echo time (TE) = 35 ms, flip angle = 80 degrees, voxel size 3x3x4 mm, 32 slices giving whole brain coverage. A standard T1-weighted anatomical scan (1mm isotropic voxels) was acquired to facilitate image co-registration.

Pre-processing of the fMRI data was performed using the FMRIB Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl, Smith et al 2004). The following procedures were applied: motion correction using MCFLIRT (Jenkinson et al 2002) slice timing correction, spatial smoothing using a Gaussian kernel (FWHM = 6mm) and a high-pass filter cut off at 100 secs (f>0.01Hz).

DTI:

For chapters 3 and 5 which used structural imaging , DTI scan:13 minute echo planer DTI scan: TR = 5191msec, TE =77 ms, field of view (FOV) = 224x150x224 mm, angulation = 0 degrees, voxel size 2mm isotropic. A total of 75 slices were acquired for b values of b = 0 and b = 1500 mm^2/sec obtained by applying gradients along 61 different diffusion directions. Additionally, a high-resolution (1 mm isotropic) T1-weighted anatomical image was acquired in each subject.

DTI scans were pre-processed using the FSL Brain Extraction Tool (BET, Behrens et al 2003) for skull stripping and the FSL Diffusion Toolkit (Smith et al 2002) to minimise eddy current distortion effects and for registration of the diffusion volumes.

Defining Regions of Interest:

Regions of interest (ROI) representing the nodes of the DMN, CEN and the SN for chapters 4 and 6 were created from data from a separate cohort of 55 subjects from a previous study (Przezdzik et al 2013) 28 male, age 25±4yrs. This allowed an objective identification of the canonical DMN, CEN and SN that was independent from the subjects investigated in chapters 4 and 6. These subjects underwent a 6-minute waking resting state fMRI scan with identical imaging parameters, also at BUIC. Using FSL 4.1.8 data were motion corrected, spatially smoothed (5mm), registered to MNI standard space, temporally concatenated across subjects and decomposed into 20 spatially-independent components with MELODIC (Beckmann et al 2005). This low dimensionality was used to facilitate identification of the ICNs in single components and to avoid individual ICNs being split into their constituent nodes, which would have made unambiguous detection more difficult. For each of the DMN, CEN and SN in turn a single independent component was identified by visual inspection based on spatial similarity to previous reports (Damiseaux et al 2006). The group-level Z-statistical maps were then thresholded at Z=4, and individual ROIs were defined for the following ICN nodes: DMN (PCC, mPFC, left and right IPC, left and right MTL; CEN (left and right DLPFC, left and right IPL); and SN (left and right AI and the ACC, see figure 2.1). The left and right hippocampal regions (HP) were identified independently from the FSL atlas as these regions were included later on as part of the DMN . These group-space ROIs were then registered to individual

subject's fMRI data. We focused on these ROIs as they have been consistently reported as constituting robust regions of the DMN (Horovitz et al 2009,Uddin et al 2009) CEN (Damoiseaux et al 2006,Menon and Uddin 2010,Samann et al 2010) and the SN (Menon and Uddin 2010, Seeley et al 2007).

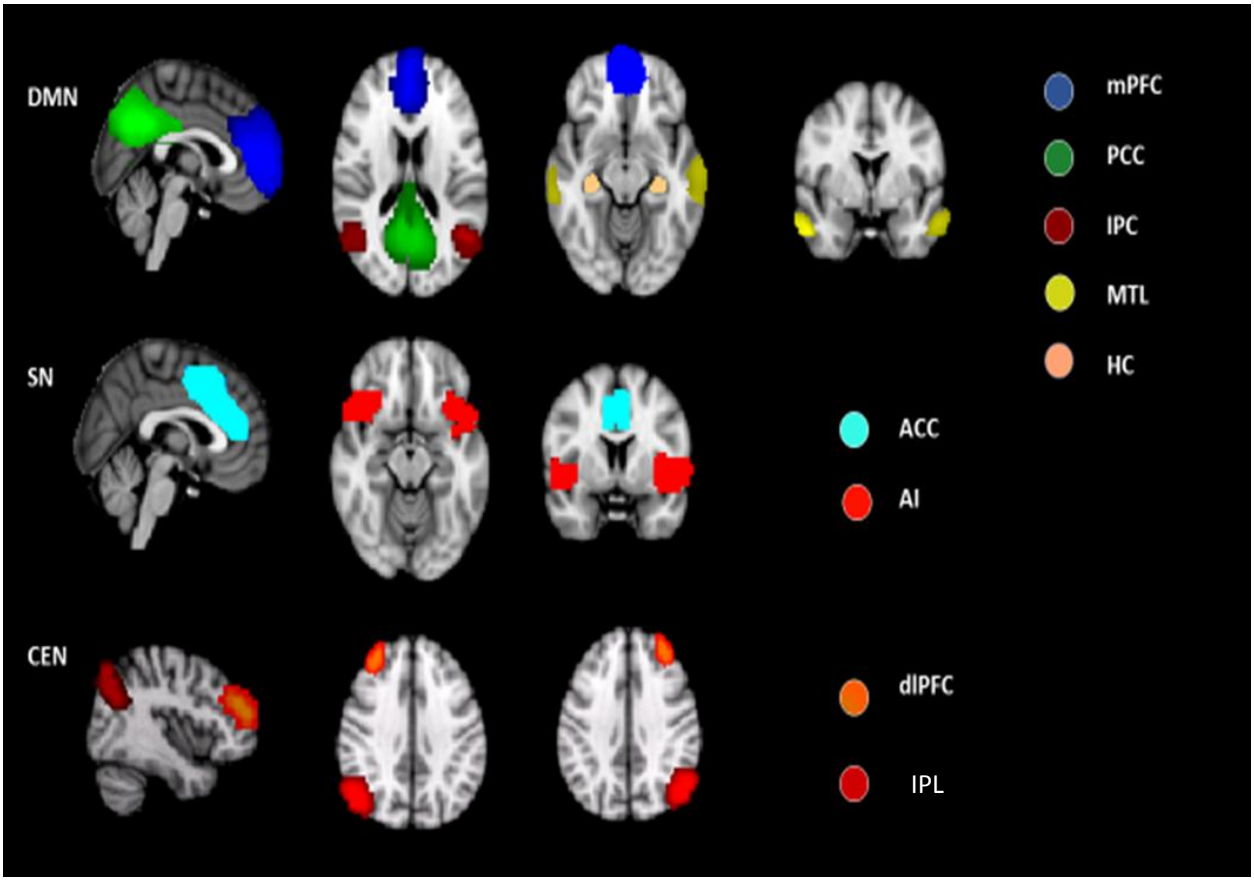

Figure 2.1 ICN ROIs produced form ICA analysis of the DMN, SN and CEN (refer to list of abreviations for full network lables)

Measuring Network FC:

we used seed-based FC analysis performed according to standard methods (Fox et al 2005) using in-house MATLAB code (Mathworks, USA). Using FSL, the pre-processed functional data were further filtered (0.009<f<0.08Hz) and single voxel co-ordinates taken from each subject's individual functional scan to extract signal time courses from white matter and ventricles. The white matter and ventricular signals, the global brain signal and the motion parameters were then removed from the voxel-wise data using linear regression. ROIs were defined from nodes of group ICA and the ROI/node maps were

transformed from MNI space to individual space using FSL. Individual subject ROIs (cubes) were created as 3x3x3 voxel cubes centred on the maximum Z-statistic voxel for each group ROI. The mean fMRI timeseries within each cube was then correlated with the fMRI timeseries of all other brain voxels. This produced a whole-brain map of Pearson correlation coefficients which allowed FC between cube regions of the ICNs (DMN, SN and CEN) to be assessed and quantified. FC was defined by averaging the voxel-wise correlation coefficients within each target cube.

Sleep Patterns and Questionnaires:

Subjects were asked to maintain their normal sleep patterns for the duration of the study. Habitual sleep patterns were assessed for a 14 day period using sleep diaries and wrist actigraphy (Actiwatch 2, Philips Respironics Ltd). The actiwatch measures the amplitude of the movement as part of the sampling process with the minimum and maximum measures being +/- 128. These values are referred to as counts. The number of counts is proportional to the intensity of movement. The highest count value for each sampling period (which consists of 1/32 of a second) was taken for each 1 second interval and the sum of the captured counts form the individual 1 second intervals making up the 1 minute epoch gave us the total count score. Actigraphs were set at a medium sensitivity of one minute epochs, and a total count score of 40 or more was used to signify that the subject was awake. Use of actigraphy in sleep disordered patients (Kushida et al 2001) has shown that medium or high sampling rate sensitivities provide data for total sleep time (TST) per night in close agreement with polysomnography (PSG). Subjects were asked to press a button on the actiwatch when they settled for bed and again on awakening to start their day. These times were defined as a sleep opportunity, and were used to carry

out the actigraph analysis using Philips Respironics Actiwatch2 software. Participants also completed the following questionnaires: Pittsburgh Sleep Quality Index (PSQI, Buysse et al 1989), Epworth Sleepiness Scale (ESS, Johns 1991) Depression, Anxiety and Stress Scale-21(DASS, Lovibond and Lovibond 1995), Karolinska Sleepiness Scale (KSS, Akerstedt and Gillberg 1990). These were administered immediately prior to or following the scanning session, with the exception of the KSS which was administered verbally immediately upon exiting the scanner to assess the level of alertness directly after the scan. Each of the questionnaires resulted in a single score per subject, while TST was determined from the actigraphy and defined as the sleep time for each sleep opportunity and compared with sleep diary data for consistency (Kushida et al 2001, Morgenthaler et al 2007). Habitual TST was calculated as cumulative TST (cTST, sum of TST over the entire two week period). Wake after sleep on set (WASO) was also extracted from the actigraphy data and was the summed value over the entire two week period.

CHAPTER 3

The structural and functional connectivity of the posterior cingulate cortex: Comparison between deterministic and probabilistic tractography for the investigation of structure-function relationships[1]

[1]This chapter is published in Neuroimage: Khalsa, S., Mayhew, S.D., Chechlacz, M., Bagary, M. and Bagshaw, A.P., 2014. The structural and functional connectivity of the posterior cingulate cortex: Comparison between deterministic and probabilistic tractography for the investigation of structure–function relationships. *Neuroimage*, *102*, pp.118-127.

ABSTRACT

The DMN is one of the most studied resting-state networks, and is thought to be involved in the maintenance of consciousness within the alert human brain. Although many studies have examined the FC of the DMN, few have investigated its underlying SC, or the relationship between the two. We investigated this question in fifteen healthy subjects, concentrating on connections to the PCC, commonly considered as the central node of the DMN. We used group independent component analysis (GICA) and seed-based correlation analysis of fMRI data to quantify FC, and streamline and probabilistic tractography to identify structural tracts from DTI data. We first assessed the presence of structural connections between the DMN regions identified with GICA. Of the 15 subjects, when using the probabilistic approach 15(15) demonstrated connections between the PCC and mPFC, 11(15) showed connections from the PCC to the rIPC and 8(15) to the left IPC. Next, we assessed the strength of FC (magnitude of temporal correlation) and SC (mean fractional anisotropy of reconstructed tracts (streamline), number of super-threshold voxels within the mask region (probabilistic). The lIPC had significantly reduced FC to the PCC compared to the mPFC and rIPC. No difference in SC strength between connections was found using the streamline approach. For the probabilistic approach, mPFC had significantly lower SC than both IPCs. The two measures of SC strength were significantly correlated, but not for all paired connections. Finally, we observed a significant correlation between SC and FC for both tractography approaches when data were pooled across PCC-lIPL, PCC-rIPL and PCC-mPFC connections, and for some individual paired connections. Our results suggest that the streamline approach is advantageous for characterising the connectivity of long white matter tracts (PCC-mPFC), while the probabilistic approach was more reliable at identifying PCC-IPC connections.

The direct comparison of FC and SC indicated that pairs of nodes with stronger structural connections also had stronger functional connectivity, and that this was maintained with both tractography approaches. While the definition of SC strength remains controversial, our results could be considered to provide some degree of validation for the measures of SC strength that we have used. Direct comparisons of SC and FC are necessary in order to understand the structural basis of functional connectivity, and to characterise and quantify changes in the brain's functional architecture that occur as a result of normal physiology or pathology.

INTRODUCTION

The human brain is organised into a series of functional networks that exhibit correlations in activity between individual regions even in the absence of stimulation (Biswal et al 1995, Gusnard et al 2001, Raichle et al 2001, Shulman et al 1997,). This resting-state activity can be measured from low frequency fluctuations in the blood oxygen level dependent (BOLD) fMRI signal (Biswal et al 1995). One of the most studied RSNs is the DMN (Greicius et al 2003, Horovitz et al 2009, Raichle et al 2001, Shulman et al 1997) consisting of the PCC, the lIPC, the rIPC and mPFC. Many studies have investigated the FC of the DMN (Damoiseaux et al 2009, Greicius et al 2003, Gusnard et al 2001, Raichle et al 2001, Shulman et al 1997). However, the SC that ultimately provides the anatomical substrate for functional interactions, and the relationship between FC and SC, is less well understood.

SC can most readily be defined non-invasively using DTI approaches allied with tractography analysis. DTI allows the tracking of white-matter pathways by measuring the

FA of water molecules along neuronal axon fibres (Basser et al 1992, 1994, Hagmann et al 2003, Mori et al 2006). Whilst a number of different tractography algorithms of varying complexity exist, the two main distinguishing factors relate to how white matter tracts are modelled within a voxel (i.e. a single or multiple fiber orientations) and how the tracts are reconstructed (i.e. interpolated streamlines or probabilistic global connectivity estimations). These choices can have a profound effect on the estimated white matter fibre tracts, and hence on the judgement of whether two brain regions are structurally connected (Yo et al 2009). The question of how SC underpins and constrains FC, and the extent to which the underlying SC is responsible for the maintenance and regulation of FC, remains unclear. The examination of structure-function relationships in human neuroimaging data is a burgeoning field (Damoiseaux et al 2009, Guye et al 2008,), not least because it may provide a way of understanding the modifications in FC that have been observed in many neurological and psychiatric disorders (Broyd et al 2009). The added value of investigating the relationship between SC and FC has recently been highlighted in patients with idiopathic generalised epilepsy (Zhang et al 2011) and traumatic brain injury (Kinnunen et al 2011).

Several studies have investigated structure-function relationships in a variety of brain networks, and taken together this work displays general agreement that functionally connected regions are also structurally connected (Greicius et al 2009, Hagmann et al 2007,Honey et al 2009, Johansen-Berg et al 2004, Mars et al 2010, Mars et al 2011, Skudlarski et al 2008, van den Heuvel et al 2009, Zhang et al 2010). However, the related question of whether regions that are more strongly functionally connected (i.e., a higher correlation coefficient between paired functional time series) are also more strongly

structurally connected has received less attention (but see Skudlarski et al 2008). One of the reasons for this is that while a higher correlation between fMRI time series, after removal of physiological, scanner and movement confounds, provides a measurement of stronger FC, inferring the 'strength' of SC from metrics that can be extracted from existing diffusion weighted scans, such as fractional anisotropy (FA) or a probabilistic connectivity score, is considerably more difficult. As discussed in detail by Jones et al (2013) diffusion weighted imaging (DWI) provides information about the directionality of water diffusion within the macroscopic volumes that are sampled in typical voxels. With certain assumptions (e.g., the fitting of voxel-wise single tensor models in the simplest case), preferred diffusion directions can be identified, and tractography algorithms can subsequently be used to estimate the likelihood of the existence of connections between two regions. However, there remains considerable controversy over the extent to which variation in structural connectivity metrics can be interpreted as indexing variations in the strength of those structural connections (i.e., is a higher FA indicative of an increased strength of SC?), since there are contributions from several methodological and physiological factors which are not related to the underlying connectivity (Jbabdi and Johansen-Berg 2011, Jones et al 2013). Indeed, at the macroscopic level assessed by DWI, and in the absence of precise and validated markers of specific aspects of the underlying physiology (e.g., myelination), there is ambiguity about the very definition of 'strength' of structural connectivity from DWI data. While it is clear that there are several potential contributions to variation in DWI metrics, it seems a plausible hypothesis that at least part of that variance can be attributed to underlying differences in SC strength, with 'strength' defined in the broadest sense and recognised as a concept that requires further

physiological clarification. One way of testing this hypothesis is by direct comparison with the strength of FC. Considering the potential sources of variability in DWI metrics, a correlation between FC and a particular DWI metric would only be expected if the metric coded for variations in underlying SC.

In the current study we focused on the DMN as one of the most reliably detected RSNs, whose spatiotemporal pattern of activity has been observed to be altered in a range of neurological and psychiatric disorders (Broyd et al 2009), as well as during altered states of consciousness such as sleep (Horovitz et al 2009, Samann et al 2010), coma (Norton et al 2012) and anaesthesia (Fiset et al 1999). Few studies have investigated the SC of the DMN (Greicius et al 2009, Hagmann et al 2008, Skudlarski et al 2008, Van den Huevel et al 2010), and some uncertainty remains concerning the existence of connections between the PCC and inferior parietal cortices (Greicius et al 2009), potentially because of problems related to crossing fibres and the choice of tractography approaches (Yo et al 2009).

We used GICA to spatially identify the DMN from resting-state fMRI data. We subsequently defined the PCC, the core node of the DMN (Hagmann et al 2008, Leech et al 2011, Leech et al 2012), as the seed region from which to assess SC and FC to the three other principle nodes of the DMN (mPFC and bilateral IPC). SC was defined using two tractography algorithms, interpolated streamline (Conturo et al 1999) and probabilistic global connectivity estimation (Behrens et al 2003, Hagmann et al 2003) to investigate whether different analysis methods can lead to different conclusions regarding the relationship between SC and FC.

The study tested four hypothesis: i) The PCC is structurally connected to the other nodes of the DMN, ii) For a given pair of DMN nodes the strength of FC between those nodes is mirrored by the strength of SC, iii) FC and SC are correlated across the nodes of the DMN, iv) The FC-SC relationships identified are not affected by the definition of SC.

METHODS AND MATERIALS

Subjects:

DTI and fMRI data were acquired from fifteen healthy adults (right handed, 10 female, age 23-29 years, mean age=24.6 years). Also see chapter 2.

Image acquisition and preprocessing:

See chapter 2 for further details on fMRI and DTI image acquisition.

Defining Regions of Interest (ROI) from functional scans:

All fMRI data were registered to MNI standard space and temporally concatenated across subjects. To identify the DMN, GICA was then performed using MELODIC (Beckmann et al 2005). The number of output components was set to 10, in accordance with a recent study (Schopf et al 2010) and in the absence of a clear consensus as to the optimum number of components. A low dimensionality reduction ensures that the DMN will be decomposed into a single component, which makes its identification more straightforward. A single independent component representing the DMN was identified by visual inspection from its characteristic spatial map. The DMN Z-statistical map was then thresholded at Z=4 and manually divided into four group-space ROIs: PCC, mPFC and left and right IPC. We focused on these four ROIs as they have been consistently reported

as constituting robust regions of the DMN (Damoiseaux et al 2009, Greicius et al 2003, Horovitz et al. 2009, Raichle et al. 2001). Other brain regions (e.g., hippocampus, parahippocampal gyrus) have been observed, but are less consistently reported.

Measuring DMN FC:

See chapter 2 for details.

Measuring DMN SC:

In order to investigate SC of the DMN, each group-space ROI was co-registered to each individual's native DTI data space using FLIRT (Jenkinson et al 2002). DMN ROIs were then binarised and registered to the b0 volume of each subject. This allowed tractography to be performed to determine whether these functionally connected regions were also structurally connected.

Interpolated streamline tractography:

The interpolated streamline algorithm (Basser et al 1992, Conturo et al 1999,) was used to estimate fibre tracts between ROIs. Using FMRIB's diffusion toolbox (FDT v2.0, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT), DTIFIT was used to fit a single tensor model at each voxel of the preprocessed eddy current corrected diffusion weighted data. Tractography was carried out using the Diffusion Toolkit (DTK) and tracts reconstructed using Trackvis (http://www.trackvis.org/). Tracts were considered as connecting ROIs if any part of them passed through the ROI en route to other cortical regions. Path tracing was permitted to continue until the FA fell below 0.2 or until the maximum angle between path segments was larger than 35 degrees (Johansen-Berg and Behrens 2004). As well as using visual confirmation of the existence of SC, reconstructed tracts were identified

using a white matter atlas (Mori et al 2011) and mean FA values of structural connections were used as an indicator of the strength of structural connections between nodes (Ben-Shachar et al 2007,Bozzali et al 2005,).

Probabilistic tractography:

Probabilistic tractography was performed using FMRIB's diffusion toolbox (FDT v2.0, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT). BEDPOSTX was used to model 5000 iterations within each voxel with a curvature threshold of 0.2, a step length of 0.5 and a maximum number of 2000 steps (Behrens et al 2007). Target masks were used (mPFC, lIPC and rIPC) and a distribution of fibre orientations was calculated between pairs of masks using the PCC as a seed mask (i.e. PCC-rIPC, PCC-lIPC, PCC-mPFC). The connection probability was given by the number of tracts that reached a target voxel (in the target mask) from a given seed voxel (from the seed mask). This is an estimate of the most likely location and strength of a pathway between the two areas (Behrens et al 2007). Thresholding of probabilistic tractography remains an unsolved statistical issue (Morris et al 2008). We used FSLstats to identify the voxel with the maximum connectivity value within the connectivity distribution map of each participant and used thresholds to 50%, 25% and 15% of the maximum connectivity value to determine the optimum threshold value (i.e. keeping all other voxels which had values more than 15%, 25% or 50% of the maximum connectivity value, Bennett et al, 2010, 2011,2014).

Comparison of structure and function:

We assessed the nature of the link between the two measures of SC and between structural and functional connectivity in three ways: 1) Structural pairwise connections were identified between the PCC and each node of the DMN as being either present or absent, by visual inspection of connections between nodes, 2) The strength of connection to the PCC from each of the other three nodes of the DMN was determined separately for FC and the two methods of quantifying SC. Those subjects who did not demonstrate pairwise structural connections for a given pair of nodes were excluded from this analysis, 3) The degree of SC FC coupling was determined by linear correlation analysis for each pairwise connection between nodes, and for the DMN as a whole (i.e., including all pairs together).

Statistical Analysis:

For FC and SC separately (i.e., comparison 2 above) we compared the FC and SC between the three pairs of ROIs (PCC-mPFC, PCC-lIPC, PCC-rIPC) using repeated measures oneway ANOVA (SSPS for windows version 20.0 Inc, Chicago USA). Secondly, bivariate correlation analysis (SPSS for windows version 20.0 Inc, Chicago USA) was carried out to determine the relationship between deterministic and probabilistic tractography, and between SC and FC (comparison 3 above), using mean FA streamline data, mean probability distribution connectivity data and mean FC correlation coefficients.

RESULTS

Functional Connectivity:

A single component containing the major nodes of the DMN was identified visually from the GICA decomposition (Figure 3.1). Seed-based FC was then used to measure the strength of FC for each pairwise connection between the PCC seed and the mPFC, lIPC, rIPC. Repeated measures one-way ANOVA was performed to compare three FC means form the same group of subjects to determine if there was a significant difference in FC between the PCC and each of the other three regions of the DMN i.e. the mean FC of the PCC-mPFC, PCC-llPC, PCC-rIPC for the 15 subjects in this study. The ANOVA indicated a significant main effect of region upon FC, demonstrating differences in FC between the three pairwise connections of the DMN (i.e., PCC-mPFC, PCC-lIPC and PCC-rIPC, $F(2,42)=3.880$, $p=0.033$). Post-hoc T-tests were performed to allow us to discover which specific means differed. The T-tests indicated a significant difference in FC between PCC-mPFC and PCC-lIPC ($p=0.023$), as well as between PCC-rIPC and PCC-lIPC ($p=0.037$). In contrast, the FC between PCC-mPFC was not significantly different to that between PCC-rIPC ($p=0.980$). These group data are plotted in Figure 3.4 a. Mean correlation coefficients (i.e. magnitude of FC) were consistent between subjects for a particular paired connection, as indicated by the relatively small standard errors. The strongest FC was measured between the PCC-rIPC (mean R = 0.1556) and the weakest between the lIPC-PCC (mean R = 0.0998), with the mPFC-PCC intermediate (mean R =0.1362).

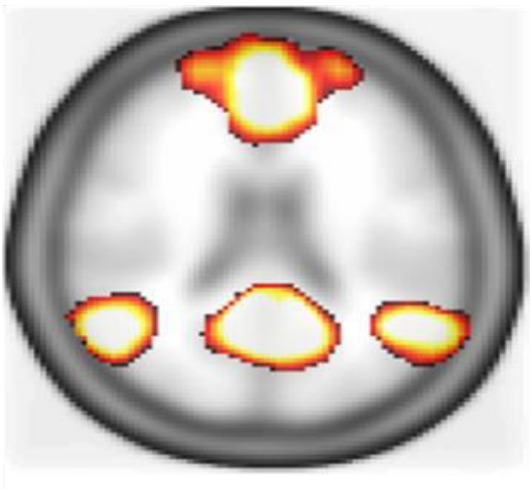

Figure 3.1. The GICA map representing the DMN which was used to define ROIs for all FC and SC analysis.

Structural Connectivity: Streamline Tractography

All of the subjects (15/15) demonstrated clear cingulate tracts connecting the PCC to the mPFC. White matter tracts were observed to link the PCC to the rIPC in 11/15 subjects and to the lIPC in 8/15 subjects. In total 34/45 connections were detected from 15 subjects (see data in Figure 3.2 and examples in Figure 3.3 a-f).

The strength of white matter connections was assessed by measuring the mean FA along reconstructed tracts. Although SC assessed by streamline tractography showed a similar pattern to the FC in terms of the relative strengths between the different nodes (Figure 3.4 b), no significant main effect of region upon SC was detected ($F_{2,31}=0.752$, $P=0.414$).

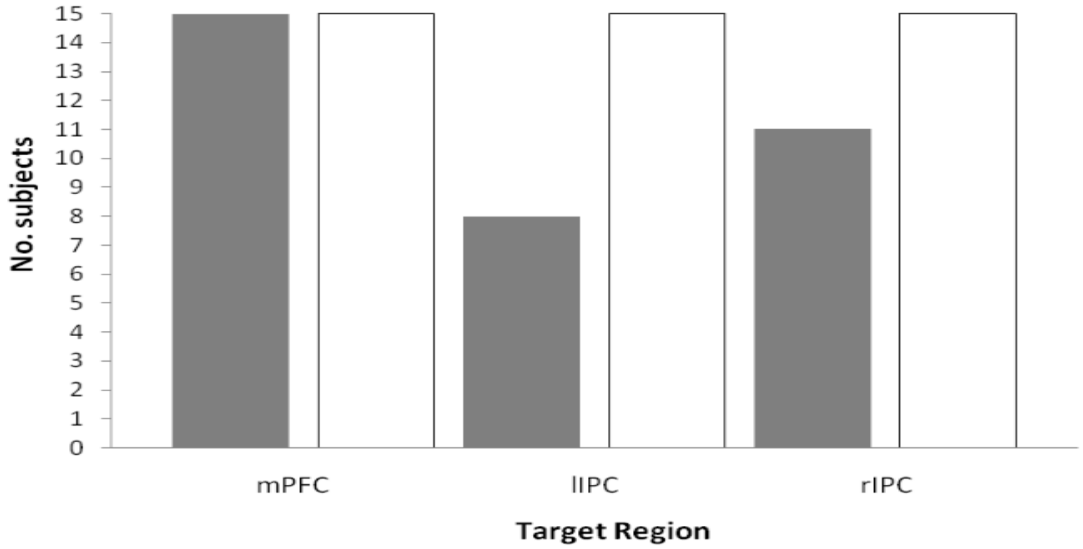

Figure 3.2. The total number of subjects demonstrating SC with the PCC for each target region using deterministic streamline tractography (grey bars) and probabilistic tractography (white bars).

Structural Connectivity: Probabilistic Tractography

For the probabilistic data, SC was found between the PCC and the other three nodes in all subjects (15/15 for all three pairs of ROIs leading to a total of 45 connections, see examples in Figure 3.3g-i). One of the issues related to the use of probabilistic tractography is that at present there is no standard methodology for thresholding maps across subjects (Morris et al 2008). We compared the effect of using minimum thresholds of 15%, 25% and 50% of activated voxels within the connection probability distribution. The 15% threshold (i.e. 85% of voxels above threshold) was chosen as the highest threshold as this was the threshold at which structural tracts could be discerned most

clearly upon visual inspection. When considering the strength of SC (number of voxels above threshold), similar to the deterministic approach the rIPC had greater connectivity than the lIPC, but in contrast the mPFC demonstrated less connectivity than both rIPC and lIPC. The pattern of SC between the pairs of nodes was comparable across all thresholds. The mean SC of the PCC to the different nodes were found to be significantly different at the 15% threshold (one-way ANOVA, $F(2,42)=5.00$, $p=0.029$). Post-hoc T-tests revealed differences in SC between PCC-mPFC and PCC-lIPC ($p=0.015$) connections and also between PCC-rIPC and PCC-mPFC ($p=0.014$) connections. The strength of SC between PCC-lIPC and PCC-rIPC was not significantly different ($p=0.189$, Figure 12 c). At the 25% and 50% thresholds the mean SC between the PCC and the different nodes were not significantly different ($F(2,42)=3.10$, $p=0.90$ and $F(2,42)=0.22$, $p=0.74$). However, at both of these thresholds the pattern of connectivity looked qualitatively similar for the IPC regions to that at a threshold of 15% (Figure 3.4c).

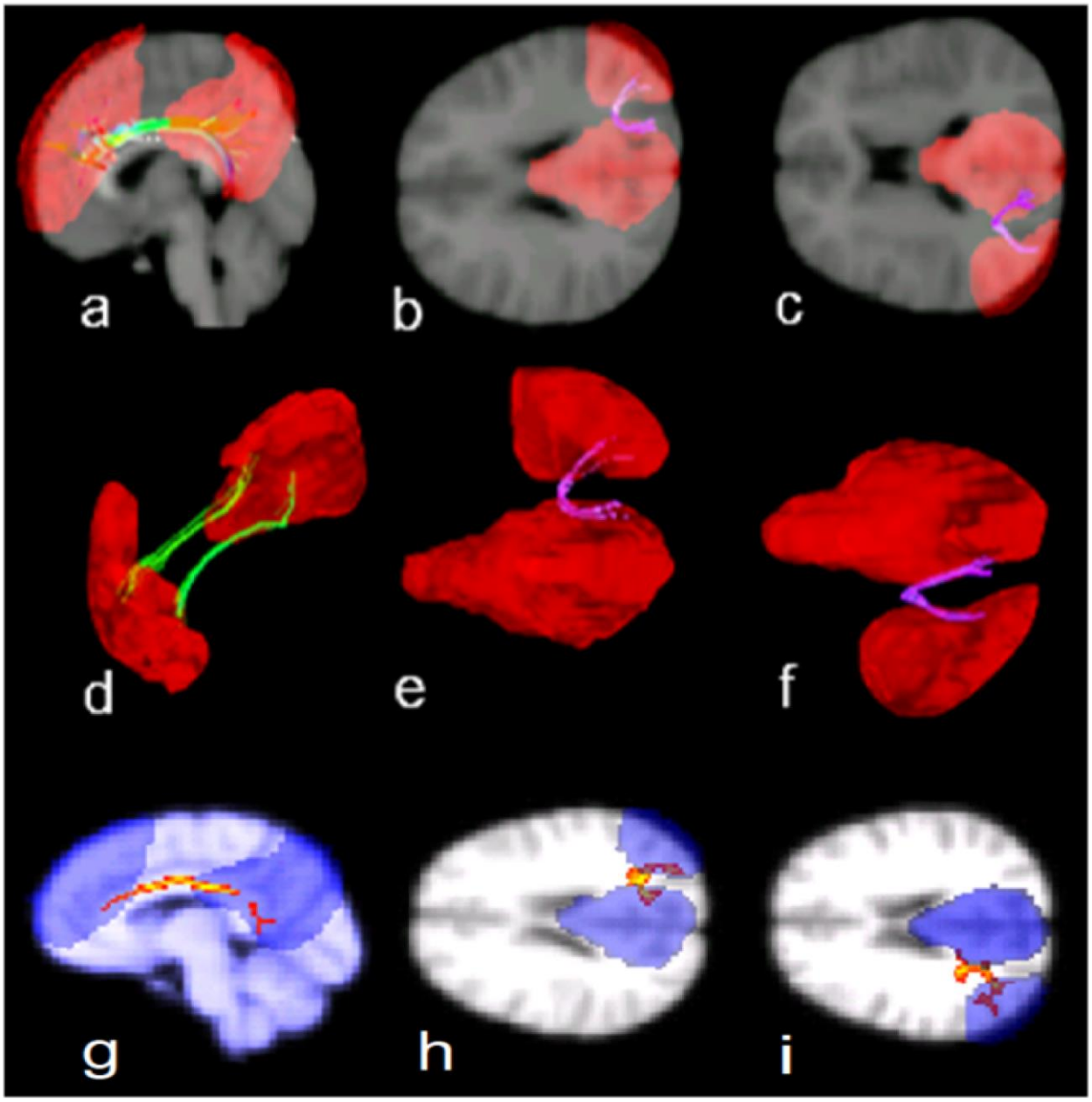

Figure 3.3. An example of structural connections reconstructed using streamline tractography.The functional nodes of the DMN are shown in red. a) and d) show the cingulate tracts reconstructed between the mPFC and the PCC; b) and e) show the right angular/lateral parietal lobule white matter and precuneus/posterior cingulate white matter tracts connecting the PCC to the rIPC; c) and f) show the left angular white matter/lateral parietal lobule and precuneus/posterior cingulate white matter tracts connecting the PCC to the lIPC. In g-i the same tracts are shown reconstructed with probabilistic tractography (the functional nodes of the DMN are shown in blue and tract connection probability distribution in red/orange, the more orange/yellow the colour the greater the probability of a connection).

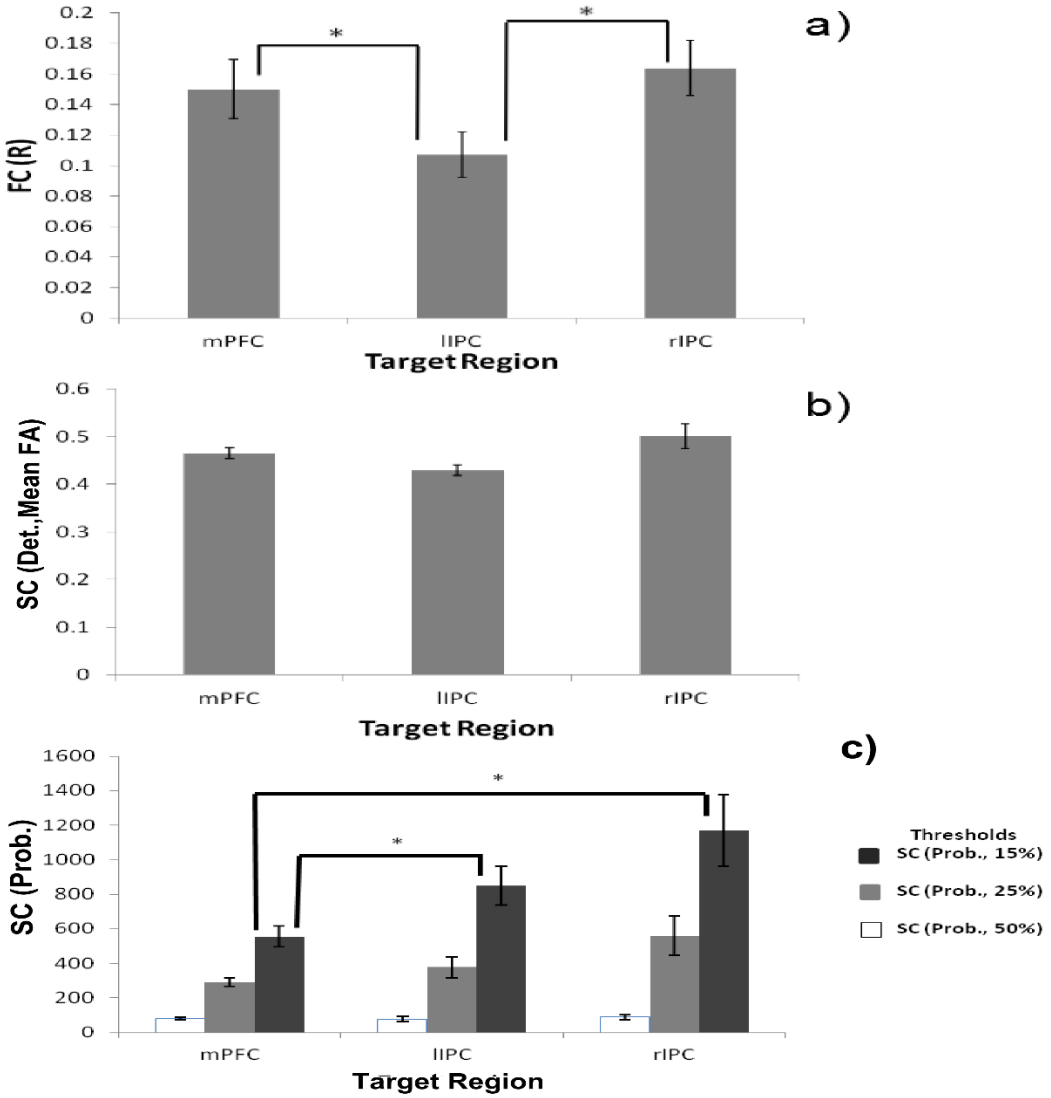

Figure 3.4. Overview of SC and FC for each pairwise connection between the PCC seed and the mPFC, lIPC, rIPC. a) Functional Connectivity: Group mean Pearsons correlation coefficients. b) Structural Connectivity: streamline tractography. Mean FA values as a measure of structural connectivity. c) Structural Connectivity: probabilistic tractography. The total number of activated voxels within the probability connectivity distribution. The three bars for each target region represent the number of activated voxels at each of the thresholds tested (15%, 25%, 50%). In all cases, error bars represent standard error. (*denotes significant difference, $p<0.05$).

Relationship between Deterministic and Probabilistic Tractography:

Figure 3.5 shows the relationship between deterministic and probabilistic tractography strengths (i.e., mean FA along reconstructed tracts vs number of suprathreshold voxels). Only subjects who showed both deterministic and probabilistic connections (PCC-rIPC 11/15, PCC-lIPC 8/15, and 15/15 PCC-mPFC making a total of 34/45) are included. Data are plotted for a probabilistic threshold of 25%, but the results were comparable for 15% and 50%. In figure 3.5a, all paired connections to the PCC across all subjects are plotted together, while in figures 3.5b-d the data for each of the three paired connections are plotted separately (PCC-mPFC in 3.5b, PCC-lIPC in 3.5c and PCC-rIPC in 3.5d). Only the posterior-anterior connections linking the PCC with the mPFC demonstrated a significant correlation ($R=0.76$, $p<0.002$). This positive correlation exists despite the fact that the probability of PCC-mPFC connections was low with probabilistic tractography, as indicated by the relatively small number of voxels above threshold.

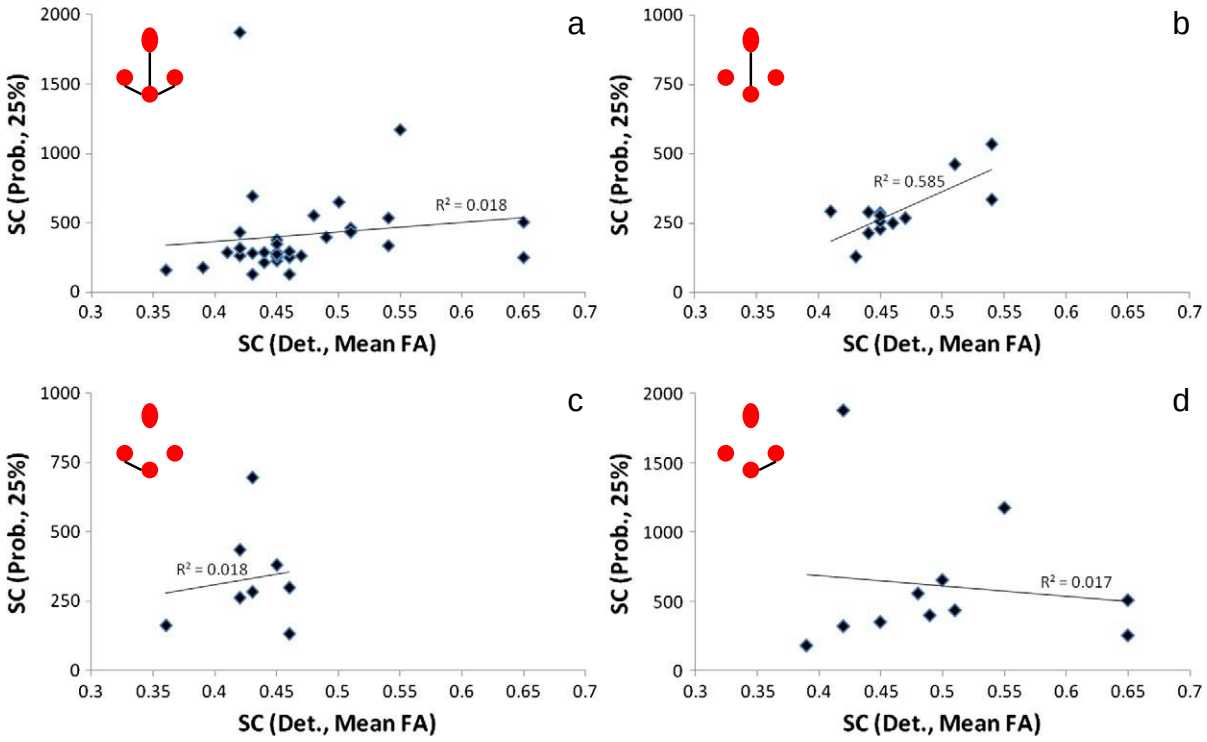

Figure 3.5. Comparison between measures of SC derived from deterministic and probabilistic tractography. a) mean FA from streamline tractography versus number of suprathreshold voxels (25% threshold) from probabilistic tractography for all paired connections pooled together. The same comparison is shown for the individual paired connections in panels b-d (i.e., b) PCC-mPFC, c) PCC-lIPC and d) PCC-rIPC). Subjects who did not show a deterministic structural connection are omitted.

Relationship between Structure and Function:

Figure 3.6 shows the relationship between measures of FC and SC strength for all paired connections together (subplots a, e and i) and separately (subplots b-d, f-h, j-l). The results for deterministic tractography are shown in the top row. The middle row (e-h) shows the results for probabilistic tractography at a threshold of 25%, restricted to those subjects who had deterministic connections to allow a direct comparison with the top row. The bottom row (i-l) again shows the probabilistic results, but with all subjects included since all subjects demonstrated some degree of connectivity with probabilistic

tractography (Figure 3.2). A significant positive correlation was found between FC and SC measured by streamline tractography when pooling across all pairs of connections and all subjects (Figure 3.6a, R=0.48, p=0.005). Considering each of the paired connections to the PCC individually, streamline tractography did not demonstrate any significant correlations between SC and FC (Figure 3.6 b-d, minimum p value 0.098), although there was something of a general positive trend. A similar analysis for probabilistic tractography demonstrated a significant correlation between SC and FC when all paired connections were considered, both when only those subjects who showed deterministic connections were included (Figure 3.6e, R=0.37, p=0.039) and when all subjects were included (Figure 3.6i, R=0.33, p=0.027). Of the paired connections to the PCC considered independently, only PCC-lIPC demonstrated a significant correlation (Figure 3.6g, R=0.72, p=0.045, Figure 14k R=0.52, p=0.048), although again there was some evidence of a general positive trend. Similar results were seen whether all subjects or only those who had deterministic connections were included. At the 15% and 50% thresholds the SC-FC correlation was found not to be significant (R=0.027, p=0.85 and R=0.031, p=0.83).

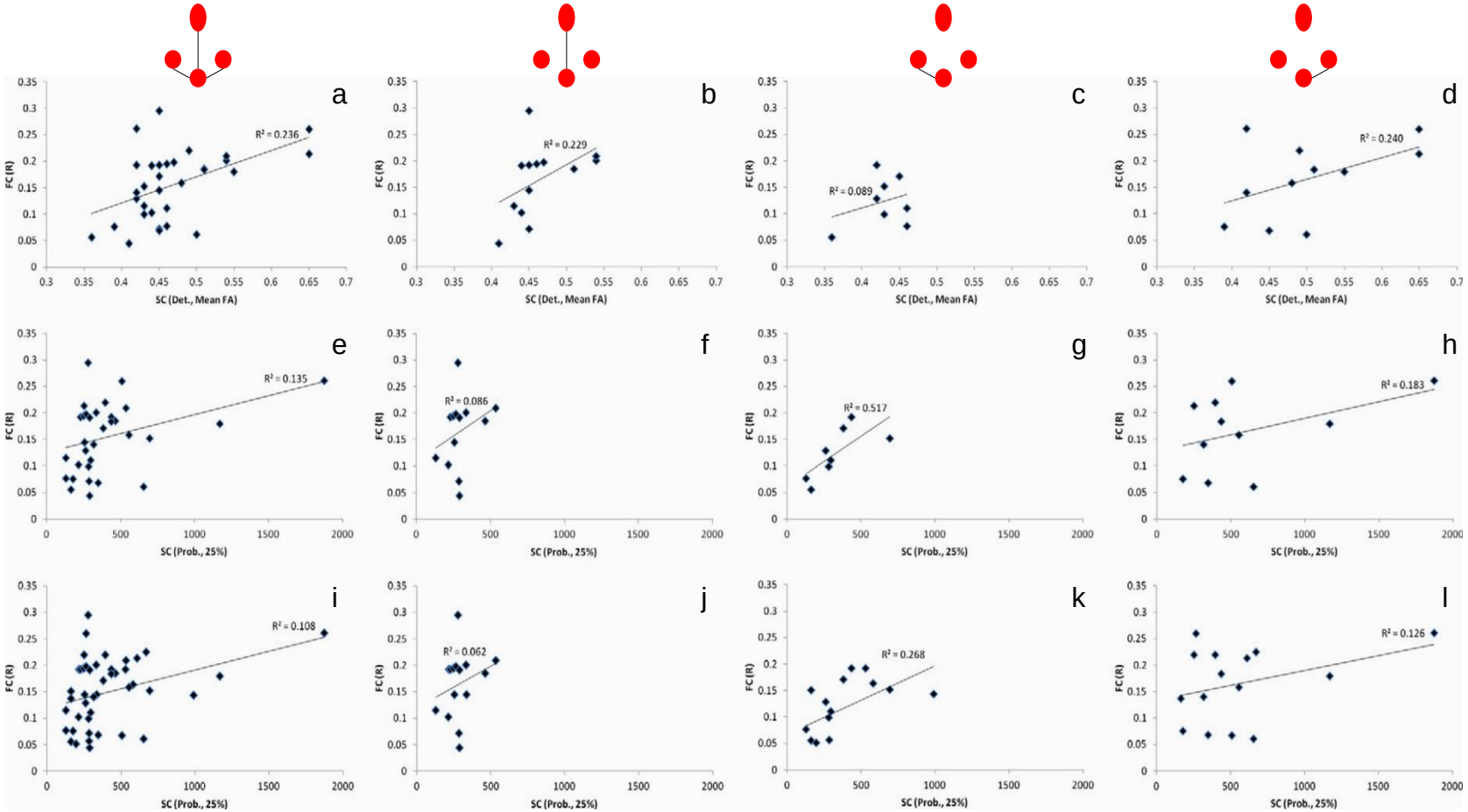

Figure 3.6. Comparison between FC and SC for deterministic tractography (panels a-d) and SC for probabilistic tractography score for 25% threshold (panels e-l). Data are shown pooled across all paired connections (panels a,e,i) and for each of the paired connections separately. a-d) Functional correlation coefficient vs. mean FA along reconstructed tracts. Subjects who did not show a deterministic structural connection were omitted. e-h) FC is plotted against probabilistic SC for the same subjects shown in panels a-d. i-l) FC plotted against probabilistic SC for all subjects.

DISCUSSION

Analysis of intrinsic functional brain activity is becoming an increasingly important and ubiquitous component of brain imaging studies. While RSNs are consistently and robustly identified (Anderson et al 2011, Damoiseaux et al 2009, Smith et al 2009), considerable inter-individual variability exists in the strength of these RSNs. The reasons for this remain unclear, as do the causes of alterations in RSNs that have been observed in various patient populations compared to healthy controls (Andrews-Hanna et al 2007, Rombouts et al 2005). One factor that is likely to contribute is individual variations in the nature and strength of the structural connectivity underlying RSNs. This aspect has received much less attention than characterising functional connectivity itself, and the current study aimed to compare two common approaches for quantifying SC and their relationship with conventional seed-based FC. We concentrated on the default mode network (DMN), and in particular on connections to the posterior cingulate/precuneus cortex (PCC), as previous work suggests it represents the core DMN node (Hagmann et al 2008, Leech et al 2011, 2012) with particular relevance for the maintenance of the conscious state (Cavanna and Trimble 2006, Cavanna 2007). A better understanding of PCC functional and structural connectivity could thus help in understanding physiological and pathological alterations of consciousness, such as in sleep, epilepsy, non-epileptic attacks etc (Bagshaw and Cavanna 2013).

This study improves our understanding of the relationship between structural and functional connectivity in several ways. Perhaps most importantly, it demonstrates a direct correlation between the strength of SC and the strength of FC, as well as between the two measures of SC strength defined from deterministic and probabilistic tractography

(Figures 3.5 and 3.6). This indicates that as well as the previously reported observation that regions that are functionally connected tend to be structurally connected (Hagmann et al 2008, Honey et al 2009, Margulies et al 2009), there is a specific and graded relationship whereby regions which have stronger structural connections also have stronger functional signal coherence. This issue has received much less attention, partly because of the difficulty in defining the strength of SC. Skudlarski et al (2008) noted an approximately linear relationship between resting state functional connectivity and anatomical connectivity determined from a deterministic tractography algorithm (Fiber Assignment by Continuous Tracking (FACT), Mori et al 1999), but the definition of SC strength remains a controversial issue. Metrics that are commonly used to infer the presence of white matter connections (e.g., fractional anistropy, probabilistic score) are not necessarily good indicators of the strength of SC, since their magnitudes may be influenced by a number of methodological and physiological factors which are not related to the underlying connectivity (Jbabdi and Johansen-Berg 2011, Jones et al 2013). This clear need for caution and the ambiguity surrounding the interpretability of measures of SC strength requires theoretical, methodological and empirical investigation and validation.

The results presented here lead to a number of observations which are relevant to this issue. Firstly, Figure 3.4 demonstrates an asymmetry between functional connections from the PCC to the left and right IPC, with higher connectivity for the right compared to the left. This functional asymmetry is mirrored in the two measures of connectivity strength derived from deterministic (mean FA along reconstructed tracts) and probabilistic (number of voxels above threshold) tractography. Secondly, the two measures of SC

strength were significantly correlated (Figure 3.5), but not for all paired connections. This presumably relates to the differential ability of the algorithms to cope with crossing fibres, as discussed in more detail below. Thirdly, when pooling across all paired connections, and in some cases when considering individual paired connections, strength of SC was significantly correlated with strength of FC (Figure 3.6). Given the different pulse sequences used to acquire the data from which FC and SC strength were measured, and that the definition of FC strength is much less ambiguous than for SC, these observations could be considered to provide some degree of validation for these measures of SC strength. If nothing else, the results indicate that there is some shared variance between FC and SC strength which requires further investigation.

The first hypothesis of this study was that the PCC had direct structural connections to the other nodes of the DMN. We observe that, in general, the DMN has reasonably clear and consistent SC, and this conclusion is reached using either tractography algorithm (Figures 3.2 and 3.3). Structural connections via cingulate tracts were found between the mPFC and PCC in all subjects for both the streamline and probabilistic analyses. This is consistent with previous studies that also report robust SC between the mPFC and PCC using streamline tractography (Grecius et al 2009, Hagmann et al 2008, Van den Heuvel et al 2009).

Examination of SC between the PCC and the bilateral IPC is complicated by the crossing fibre tracts of the anterior to posterior superior longitudinal fasciculus and the superior to inferior corona radiata tracts that separate them (Dougherty et al 2005). This is likely to be a particular problem for single tensor streamline tractography (Mori and Zhang et al 2006). Greicius et al (2009) did not examine these connections, as their preliminary data

showed PCC-IPC SC in only 4/23 subjects. They therefore felt that the investigation of the SC between these regions was severely restricted due to their tractography algorithm being unable to resolve the problem of crossing fibres (Grecius et al 2009). Hagmann et al did find evidence of structural connectivity between the PCC and the IPC regions, although this was less consistent than the PCC-mPFC (Hagmann et al 2008). Van den Heuvel et al used the mPFC as the seed region for their study, and therefore did not examine the connections from the PCC to the IPC (Van den Heuvel et al 2009). Margulies et al (2009) compared the functional connectivity of both human and macaque monkey brains against classical and recent anatomical studies. They identified a central zone of the PCC which demonstrated strong functional connectivity with the posterior part of the inferior parietal lobule and adjacent superior temporal sulcus in the Macaque monkey (this corresponds in human brain to the morphology of the angular gyrus/IPC). Margulies et al (2009) found the functional connectivity patterns were remarkably consistent between species and with predictions from previous tract tracing anatomical studies in the macaque.

In the current study, structural connections between the PCC and the bilateral IPC were seen for both tractography approaches, although they were more consistent when using probabilistic tractography. The probabilistic tractography identified connections between the PCC and all other nodes of the DMN (mPFC, lIPC, rIPC) in all subjects. This finding suggests the probabilistic approach is more effective at determining the underlying connections between regions where considerable fibre cross over is apparent (Behrens et al 2007, Hagmann et al 2007,2008, Honey et al 2009, Yo et al 2009). However, a marked reduction in the PCC-mPFC SC was found when using probabilistic tractography

(Figure 3.4c). This finding suggests that the probabilistic approach is not as effective in reconstructing long pathways as streamline tractography (Figure 3.4c). A degree of uncertainty in fibre orientation is apparent when pathways are reconstructed, and as the probability connectivity distribution is propagated, this uncertainty results in a decrease in the connection probability with increasing tract length. Consequently, long range connections are more difficult to characterise and have lower probability values. This effect has been previously reported, with probabilistic tractography demonstrating weaker SC with increasing tract length (Morris et al 2008). For example, long tracts have been found to be weaker using probabilistic algorithms compared to streamline methods (Yo et al 2009). This finding is consistent with our results (cf Figure 3.4b and c).

The second hypothesis of this study was that variations in FC of the ROIs are mirrored by between ROIs variations in SC. The rIPC demonstrated the strongest FC to the PCC, while the weakest FC was demonstrated by the lIPC (Figure 3.4a). These findings show similarities with those of Horovitz et al 2009, who found the strongest FC for the rIPC and weakest for the lIPC when using a PCC seed. A recent magneto-electroencephalography (MEG) study (de Pasquale et al 2012) recorded neuromagnetic signals from the DMN and several other RSNs and found that the lIPC demonstrated marked cross correlation with the dorsal attention network (DAN). This may possibly account for the reduced correlation between the lIPC and PCC in our study, by suggesting that the lIPC is a less consistent member of the DMN.

Given this asymmetry in the FC of the IPCs, and the evidence for non-DMN connectivity of the lIPC, we would expect an asymmetry in the SC of the IPCs. For each tractography algorithm there were two measures of SC, the strength of connections (mean FA for

streamline data or number of activated voxels for probabilistic data) and the number of subjects who demonstrated a particular connection. We found a similar pattern for SC to that found for FC for both tractography algorithms and both measures of SC that came from them when considering the IPC regions. Our findings are consistent with previous work that suggested that regions exhibiting strong SC also exhibit strong FC, and lends weight to the idea that FC is constrained by SC (Hagmann et al 2008, Honey et al 2009).

The third hypothesis of this study was that SC and FC are correlated across the nodes of the DMN. This is a more specific, though more controversial as discussed above, test of the extent to which SC and FC are linked. The correlation analysis demonstrated a significant relationship between functional connectivity and structural connectivity defined using either streamline or probabilistic tractography approaches (Figure 3.6). One of the issues related to the use of probabilistic tractography is that at present there is no standard methodology for thresholding maps across subjects (Morris et al 2008). We compared the effect of using minimum thresholds of 15%, 25% and 50% of activated voxels within the connection probability distribution. The 15%, 25% and 50% thresholds were successful in including only connections consistently observed within the DMN (Figure 3.3g-i) across subjects and allowed specific tracts between ROIs to be identified within all subjects. A significant FC-SC correlation was found only with the 25% threshold (Figure 3.6e,i), but not with 15% and 50% thresholds, although the overall pattern of connectivity was seen to be preserved for the IPC regions at all thresholds (Figure 3.4c). The lack of a significant FC-SC correlation as a whole at the 15% threshold may have been due to the significant difference in the strength of SC of the PCC-mPFC pairwise connections compared to the PCC-IPC connections.

The final hypothesis of this study was that the SC-FC relationships identified would not be dependent on the type of tractography used to define SC. As detailed above, in the majority of cases we were able to show similar relationships between FC and SC calculated using either streamline or probabilistic tractography. However, there were differences between the two tractography approaches both in terms of their definition of SC strength (Figure 3.5) and their relationship with FC (Figure 3.6). These differences might be related to known issues with tract reconstruction such as crossing fibres and dispersion with distance (Morris et al 2008). It is important to consider some advantages and disadvantages of the methods. In favour of the probabilistic approach was its improved ability to cope with regions of crossing fibres, and hence to identify PCC-IPC connections more reliably than the streamline approach. However, for the anterior-posterior connections between the PCC and mPFC deterministic tractography appeared to be more related to FC than probabilistic tractography (Figure 3.6). This was despite a high level of shared variance for this pair of nodes for the two structural approaches themselves (Figure 3.4b). Yo et al (2009) have found probabilistic approaches on average produce more connected regions, but lower individual connectivity values, than streamline approaches. Accordingly, we have found probabilistic structural connections are weaker than streamline SC when considering the mPFC-PCC pairwise connections. This suggests streamline and probabilistic approaches may complement each other with probabilistic tractography detecting more individual connections (especially where crossover is a problem) and streamline tractography demonstrating more consistent individual strength of connectivity. In terms of understanding structure-function relationships this obviously adds a level of complexity and deserves further attention.

This study has several limitations, one of which is likely to be encountered by other studies which seek to compare explicitly the strength of SC and FC, and relates to the relationship between the regions where these quantities are calculated. Firstly, FC was quantified from a relatively small region based on the peak voxel from GICA, and would therefore primarily be in grey matter, while quantification of SC is obviously restricted to white matter. Secondly, the tracts we reconstructed connected the larger ROIs that represented the entire DMN nodes. The spatial group ROIs produced from the GICA analysis, while comparable with those from previous studies (Greicius et al 2009, van den Heuvel et al 2009), were much larger than the peak regions used in the FC correlation analysis, and were therefore composed of various areas of cortex. For example, the PCC ROI consisted of the precuneus, posterior cingulate and retrosplenial cortex, while the IPC consisted of the angular gyrus, lateral inferior parietal lobule and parts of the lateral parietal sulcus. The tracts reconstructed using the two tractography approaches demonstrated connections between the overall GICA ROIs, which included the peak FC correlation voxels. However, the tracts did not pass directly through the peak FC voxel. We therefore cannot assume SC-FC connectivity at the cytoachitectural level and studies accounting for cytoachitectural compartmentalisation may be more suited to the detailed analysis of the functional and structural characterisation of each particular region of cortex (Margulies et al 2009). We have only addressed SC-FC at a macroscopic level, but this spatial discrepancy would be expected to reduce the observed correlation between SC and FC, meaning that the figures we have reported likely represent lower bounds on the true relationship. This study's sample size was comparable to much of the related literature, however investigating structure-function relationships in large samples such as those

provided in data repositories would increase the breadth of conclusions that could be drawn. However, one of the motivations for our methodological choices was that they are readily applicable to standard clinical scanners and can therefore lay the groundwork for ongoing and future work in clinical populations and sleeping subjects. Finally, we did not investigate the effect of different preprocessing strategies on the definition of FC. Foremost amongst these is the widely utilised but much debated application of global signal regression, which can have quite a profound effect on FC, particularly in terms of inducing negative FC between networks (Murphy et al 2009, Fox et al 2009). However as we have focussed on measuring only positive FC within a single network, the effect of global signal removal in our data is simply a reduction in FC consistent for all pairwise comparisons for a given individual. Additionally several other factors such as controlling for residual movement effects have been shown to be important to improve FC measurements in future work (Van Dijk et al 2010, 2012). This highlights the difficulty of studying SC-FC relationships, since methodological uncertainty exists at every stage.

Our findings have demonstrated structural connections between functionally connected regions of the DMN and a significant relationship between DMN FC and SC using both deterministic and probabilistic tractography. A better understanding of how structural connections relate to functional connectivity is imperative in order to enhance our understanding of changes in SC and FC that occur as a consequence of neurological (Bozzali et al 2002, Gattellaro et al 2009, Kinnunen et al 2011, Nierenberg et al 2005, Rigman et al 2007, Zhang et al 2011), psychiatric (Broyd et al 2009, Hubl et al 2004, Li et al 2008,) and sleep (Altena et al 2010, Nofzinger et al 20015) disorders and whether any of these changes are reversible with therapeutic interventions.

This study has demonstrated a degree of co-variance between SC and FC within an ICN therefore providing evidence for a degree of association between the two measures. The study also highlights the difficulty of studying SC-FC relationships. As mentioned above, methodological uncertainty exists at every stage. Future studies investigating chronic habitual sleep status in relation to SC and FC (chapters 4, 5 and 6) will investigate SC and FC individually in order to minimize the effects of such methodological uncertainties.

CHAPTER 4

Variability in cumulative habitual sleep duration predicts waking functional connectivity[2]

[2]This chapter is published in SLEEP: Khalsa, S., Mayhew, S.D., Przezdzik, I., Wilson, R., Hale, J., Goldstone, A., Bagary, M. and Bagshaw, A.P., 2016. Variability in Cumulative Habitual Sleep Duration Predicts Waking Functional Connectivity. *Sleep*. 39(1):87-95.

ABSTRACT

We examined whether inter-individual differences in habitual sleep patterns, quantified as the cumulative habitual total sleep time (cTST) over a two week period, were reflected in waking measurements of intra and inter- network FC between major nodes of three ICNs: DMN, SN, CEN. This was a resting state fMRI study using seed-based FC analysis combined with14 day wrist actigraphy, sleep diaries and subjective questionnaires. Data were statistically analysed using multiple linear regression. The methods included fourteen consecutive days of wrist actigraphy in participant's home environment and fMRI scanning on day 14 at the Birmingham University Imaging Centre. The experimental cohort consisted of 33 healthy adults, mean age 34.3, SD= +/- 11.6 years.Seed-based FC analysis on ICNs from resting-state fMRI data and multiple linear regression analysis performed for each ICN seed and target. cTST was used to predict FC. cTST was specific predictor of intra-network FC when the mPFC region of the DMN was used as a seed for FC**,** with a positive correlation between FC and cTST observed. No significant relationship between FC and cTST was seen for any pair of nodes not including the mPFC. Inter-network FC between the DMN (m PFC) and SN (right anterior insula) was also predicted by cTST, with a negative correlation observed between FC and cTST. In conclusion, this study improves our understanding of the relationship between intra and inter-network FC of ICNs in relation to habitual sleep quality and duration. The cumulative amount of sleep that participants achieved over a 14 day period was significantly predictive of intra- and inter-network FC of ICNs, an observation that may underlie the link between sleep status and cognitive performance.

INTRODUCTION

Sleep is crucial for maintaining normal cognitive performance (Alhola and Polo-Kantola 2007, Babckoff et al 2005, Banks and Dinges 2007, Belenky et al 2003, Dinges et al 1997, Harrison and Horne 2000, Horne et al 1993,Van Dongen et al 2003,) but the precise mechanisms by which the processes that occur during sleep affect waking function remain to be clarified. It is increasingly recognised that FC of ICNs is crucial for the maintenance of proper function in healthy individuals (Bressler and Menon 2010, Damoiseaux and Greicius 2009, Menon and Uddin 2010) and that specific disruptions to intra- and internetwork FC are widespread in neurological and neuropsychiatric disorders (Menon 2011, Zhang and Raichle 2010). Modification of the activity and FC of ICNs has also consistently been observed during the descent into sleep (Horovitz et al 2008, Horovitz et al 2009, Koike et al 2011, Larson-Prior et al 2009, Samann et al 2011) and following sleep deprivation (Bosch et al 2013, De Havas et al 2012, Gujar et al 2010, Samann et al 2011, Tomasi et al 2009, Verweij et al 2014) with the main emphasis having been placed on the default mode network (DMN). The DMN is likely to be particularly important in understanding the link between sleep and waking brain function not only because of its general link with maintenance of consciousness (for review see Bagshaw and Khalsa 2013), but also its importance in a range of cognitive domains which are known to be affected by prolonged wakefulness, including memory (Buckner and Carroll 2007, Drummond et al 2013, Spreng and Grady 2010,),attention (Gumenyuk et al 2011) and emotion processing (Buckner and Carroll 2007).

In parallel with these investigations of FC, studies utilising chronic partial sleep deprivation, which more closely resembles everyday life situations than total sleep deprivation, have reported dose-dependent deficits in cognitive performance (Belenky et al 2003, Dinges et al 1997, Van Dongen et al 2003).The common finding is that the less sleep subjects obtain due to sleep restriction (e.g. subjects restricted to 3, 5 or 7 hours of time in bed compared to controls who spent 8 hours in bed for up to 7 days) the more cognitive performance is impaired (Belenky et al 2003, Dinges et al 1997, Van Dongen et al 2003). Given that ICNs underpin waking function and are affected by prolonged wakefulness (De Havas et al 2012, Gujar et al 2010, Tomasi et al 2009, Verweij et al 2014), one possibility is that sleep is needed to maintain the brain's intrinsic functional architecture, normalising the FC of ICNs to sustain the high level of regionally-appropriate FC that is necessary for waking function. This would suggest that shorter habitual sleep over a prolonged period could have a cumulative effect on FC, which may subsequently result in subtle deficits in higher cognition. However, to date there has been no investigation of whether habitual sleep patterns measured over a prolonged period relate to waking FC. This is important because even a small amount of sleep restriction over a prolonged period can have measureable negative consequences on waking behavioural performance (Bonnet and Arand 1995) and self-imposed short sleep durations are becoming increasingly common and represent a considerable public health burden (Altevogt and Colten 2006,Geol et al 2009, Hillman and Lack 2013). Understanding whether differences in habitual sleep patterns relate to FC thus has considerable practical implications. We examined this issue by comparing cTST, assessed over a two week period with wrist actigraphy and sleep

diaries, with waking FC of three of the most important ICNs for higher level cognitive function the DMN, SN and CEN.

The DMN encompasses the PCC, mPFC and bilateral IPC cortices, with the MTL and the hippocampal regions also sometimes included, although less consistently (Ward et al 2013). Originally identified as a set of regions which are consistently deactivated when attention is directed externally (Shulman et al 1997, Raichle et al 2001) its general importance has subsequently been underscored by its relationship with a wide range of cognitive tasks (Anticevic et al 2012, Shulman et al 1997, Raichle et al 2001, Raichle et al 2007,). Further investigations have also revealed specific roles of the anterior and posterior portions of the DMN (Cavanna and Trimble 2006, Euston et al 2012, Utevsky et al 2014), indicating that while it is certainly a coherent network the individual nodes can have differentiated functions, as well as a specific relationship to task-positive regions (Bressler and Menon 2010, Uddin et al 2009).

A number of studies have investigated ICN FC during sleep (Andrade et al 2011,Horovitz et al 2009, Larson-Prior et al 2009, Samann et al 2011, Spoormaker et al 2010), and alterations have been noted during wakefulness, following full or partial sleep deprivation (De Havas et al 2012, Gujar et al 2010, Samann et al 2010) and in relation to self-reported sleep duration on the night prior to a waking scan (Killgore et al 2012).These studies indicate that integrity of the DMN is a sensitive marker of sleep status and prior sleep history.

While the importance of DMN functional integrity for the maintenance of normal brain function is clear, it is only one of many ICNs ranging from those encompassing

primary sensory regions (e.g., visual, auditory, somatomotor) to higher level networks such as the CEN and the SN. Given previous behavioural observations (Banks and Dinges 2007) it would be expected that, in addition to the DMN, the higher-level CEN and SN would be most affected by sleep, rather than the sensory networks.

The human brain switches from intrinsic thoughts and self-referential activity involving regulation by the DMN, to task positive cognitive activity involving regulation by the CEN (Hasenkamp et al 2012, Manoliu et al 2013). This switching between networks is thought to be regulated by the right AI of the SN, which acts as a control hub between the DMN and CEN and regulates states of consciousness in response to salient events (Sridharan 2008). These three ICNs therefore act in concert to maintain a normal level of brain function.

In the current awake, resting-state fMRI study, we aimed to investigate whether the strength of intra-network FC of the DMN, SN and CEN covaried with the cumulative effect of normal habitual sleep time. Secondly, since the SN is involved in the regulation of activity between the DMN and CEN, we also aimed to investigate how between-subject FC variability in inter-network connectivity of the SN, CEN and the DMN was related to subject's habitual sleep time. The motivation for examining these networks is that they are closely linked with the higher cognitive functions which are mainly affected by sleep deprivation (Alhola and Polo-Kantola 2007' Babkoff et al 2005, Belenky et al 2003, Dinges et al 1997, Harrison and Horne 2000, Horne 1993, Van Dongen et al 2003). A better understanding of how sleep affects ICN FC may help to shed light on the link between sleep and the functions these networks support, in particular cognition and conscious behaviour, as well as the neurobiological

underpinnings of individual differences in susceptibility to sleep deprivation. While the link between individual variability in behavioural performance and sleep history has been extensively studied (Kloss et al 2002), an explicit understanding of susceptibility to sleep loss requires a detailed knowledge of individual differences in the resilience of the brain networks that are responsible for waking function. In addition, as a marker of sleep deprivation, FC of ICNs is particularly attractive as it is not under conscious control and may provide an unbiased measure of sleep history.

We had two hypotheses: i) Longer habitual cumulative total sleep times will be reflected by increases in the intra-network FC between the major nodes of the DMN, SN, CEN measured during wakefulness, ii) Longer habitual cumulative total sleep times will be reflected by network specific increases and decreases in inter-network FC between the DMN, SN and CEN.

METHODS AND MATERIALS

Subjects:

Data were acquired from 37 healthy adults (right handed, 17 female, age 20-59 years, mean age (+/-SD)=35.0+/-11.7 years). See chapter 2 for further details.

The data from 4 subjects were subsequently excluded (corrupted data for one subject, erratic sleep patterns for the second, illness around the time of scanning for the third and fourth), meaning that the final data set that was analysed consisted of 33 participants (right handed, 17 female, age 20-59 years, mean age (+/-SD)= 34.2+/-11.6 years).

Sleep Patterns and Questionnaires:

Subjects were asked to maintain their normal sleep patterns for the duration of the study. Habitual sleep patterns were assessed for a 14 day period using sleep diaries and wrist actigraphy (Actiwatch 2, Philips Respironics Ltd). See chapter 2 for further details.

Demographics (n=33)	**Mean**	**SD**
Age	**34.2**	11.62
Questionnaires		
Epworth	3.94	.79
Karolinska	1.16	.41
Fatigue	12.36	.98
PSQI	2.31	1.65
Depression	1.58	2.74
Anxiety	1.35	.92
Stress	3.61	2.73
Actigraphy		
Mean TST (h)	7.65	1.85
cTST (h)	97.57	13.52

Table 4.1 above summarises the demographic, habitual sleep and questionnaire data for the participants. All subjects were within normal limits and no evidence of depression, anxiety, excessive daytime sleepiness or fatigue was found (Table 4.1). Mean cTST was also within normal limits (7.65+/-1.85 hours).

Image acquisition and preprocessing:

Subjects underwent a single resting-state fMRI session in the early afternoon during which they were instructed to lie still in the scanner and relax with eyes open. All participants confirmed that they remained awake and alert through the scanning session. See chapter 2 for further details.

Defining Regions of Interest:

Regions of interest (ROI) representing the nodes of the DMN, CEN and the SN were created from data from a separate cohort of 55 subjects from a previous study (Przezdzik et al 2013) 28 male, age 25±4yrs. (see chapter 2 for details).

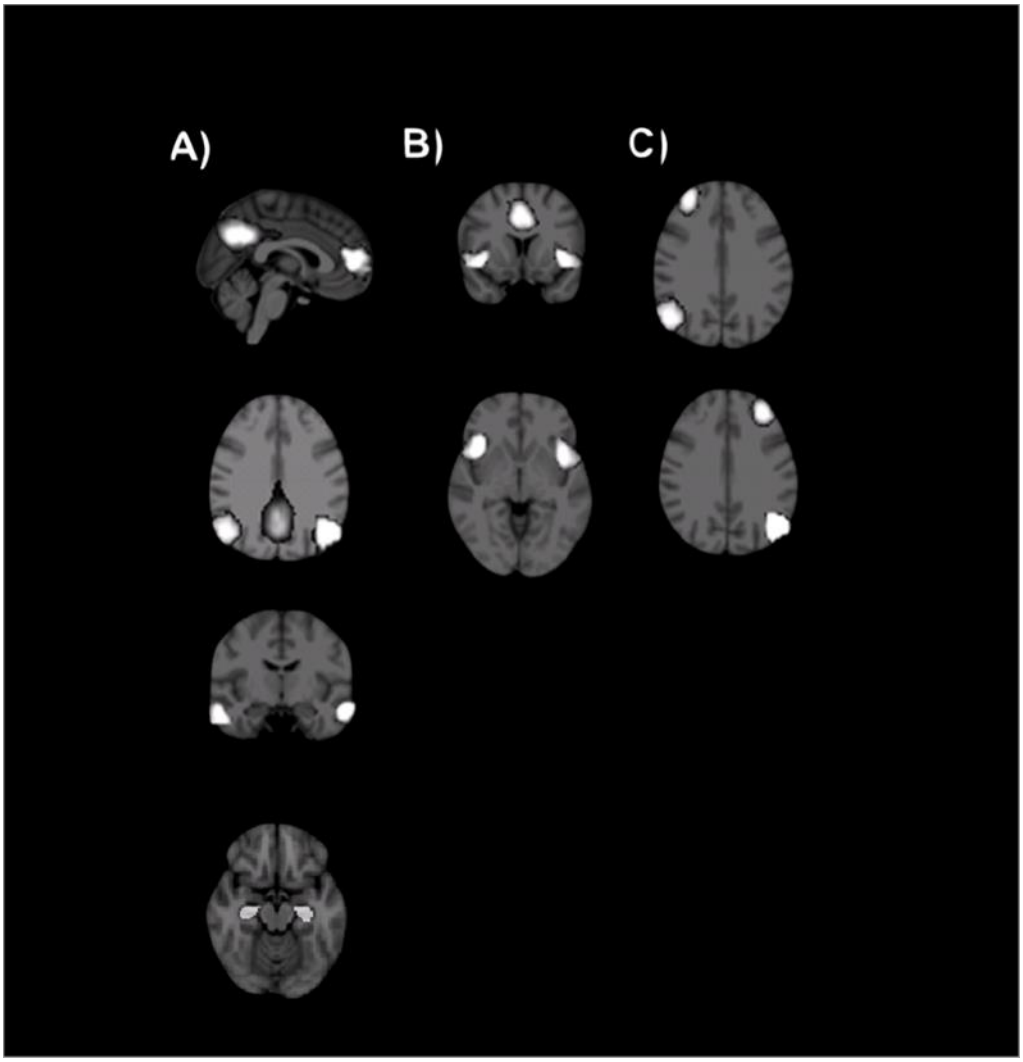

Figure 4.1 ROIs produced form ICA analysis of the DMN A), SN B) and CEN C).

Regions of interest/nodes for all networks	MNI co-ordinates(mm)		
	X (centre)	Y (centre)	Z (centre)
Posterior cingulate cortex (PCC)	0	-52	34
Mesial prefrontal cortex (mPFC)	0	52	6
Left inferior parietal cortex (lIPC)	-52	-68	38
Right inferior parietal cortex (rIPC)	52	-68	38
Left mesial temporal lobe (lMTL)	-64	-10	-18
Right mesial temporal lobe (rMTL)	52	2	-30
Left hippocampus (lHC)	-28	-18	-14
Right hippocampus (rHC)	26	-18	-14
Right Insula (rAI)	36	24	2
Left Insula (lAI)	-40	16	2
Anterior cingulate cortex (ACC)	0	26	30
Left dorsal lateral prefrontal cortex (ldlPFC)	-42	34	24
Right dorsal lateral prefrontal cortex (rdlPFC)	42	44	24
Left inferior parietal lobule (lIPL)	-54	-64	24
Right inferior parietal lobule (rIPL)	56	-66	26

Table 4.2, MNI co-ordinates for the centre voxel for each node/region of interest (ROI) for the three networks (DMN, SN, and CEN) from the ICA .

Measuring DMN, CEN and SN FC:

Following previous methodology (chapter 3,Khalsa et al 2014) we used seed-based FC analysis performed according to standard methods (Fox et al 2005) using in-house MATLAB code (Mathworks, USA). (See chapter 2 for further details).

The fifteen ROIs described above were used in turn as the seed to measure the strength of FC to all other DMN, SN and CEN ROIs for the intra-network and internetwork analysis.

Statistical Analysis:

We investigated the relationship between individual sleep variables and both intra- and inter-network FC. Multiple linear regression analysis (SPSS Inc, Chicago USA) was performed for each DMN, SN and CEN seed and target ROI, with cTST as the criterion variable and including FC as predictor variables. We controlled for false discovery rates (FDR) due to multiple measures by using the Benjamini Hochberg procedure (Benjamini and Hochberg 1995) as used in previous studies (Sridharan et al 2008). The FDR p value adjustment method involved ranking the p values in order with the smallest p value being assigned rank 1, the second rank 2 and the largest rank N. Then each p value was multiplied by N and divided by its assigned rank to give the adjusted p. In order to restrict the FDR to 0.05 significance, all adjusted p values of less than or equal to 0.05 were regarded as significant (Benjamini and Hochberg 1995). All p values reported in the Results section are FDR corrected.

RESULTS

Intra-network FC analysis:

cTST and intra-network FC of the DMN:

Table 4.3 shows the significant regression analysis results for the relationship between cTST and intra-network DMN FC using the mPFC as seed ROI. This analysis indicated that cTST only predicted DMN FC when the mPFC was used as the seed ROI. No significant relationship between FC and cTST was seen for any pair of nodes not including the mPFC (see additional supplementary material for all non-significant results, and Figure S1 for average group FC between the mPFC and other nodes of the DMN).

mPFC (seed)

Model	B	Std. Error	β	t	p	corrected p	Zero-order R
(Constant)	103.334	5.635		18.339	<.001		
lIPC	-41.833	35.807	-.272	-1.168	.254	.444	.118
lMTL	27.679	34.593	.176	.800	.431	.603	.377
lHP	-51.204	22.102	-.699	-2.317	.020	.070	.308
PCC	61.445	24.646	.730	2.493	.020	**.046***	.469
rIPC	13.214	25.319	.133	.522	.606	.707	.480
rMTL	82.223	28.689	.568	2.866	.008	**.056**	.541
rHP	19.448	41.335	.113	.470	.642	.642	.260

Model significance R^2=0.576 F=4.251 P=0.002 (*significant FDR corrected p≤0.05)

Table 4.3 Results of the regression analysis between habitual cTST (dependent variable) and DMN (mPFC seed) intra-network connectivity (table 4.3 above).

cTST demonstrated a significant regression model when the mPFC was used as the seed region for mPFC-DMN intra-network FC and a Model significance R^2=0.576 F=4.251 P=0.002 was found. For all pairs of ROIs that had a significant corrected p value or demonstrated a positive correlational trend (p=0.046, p=0.056 FDR

corrected) in partial correlations to the seed region, the strength of FC between the DMN seed regions and the mPFC increased with cTST.

cTST and intra-network FC of the SN and CEN:

cTST was not a significant predictor of intra-network FC for the SN or the CEN ($p>0.61$, see supplementary material for non-significant results).

Inter-network FC analysis:

cTST and inter-network FC of the DMN and SN:

cTST was a significant predictor of the DMN-SN inter-network FC using the mPFC as the seed region. Specifically, FC between the mPFC and rAI was significantly predicted by cTST. A significant negative correlation was found (Table 4.4). cTST demonstrated a significant regression model when the rAI was used as the seed region for SN-DMN inter-network FC and an uncorrected p value of 0.019 was found, but this did not survive FDR correction (Table 4.5). (In the appendix supplementary material Figures S2 and S3 demonstrate the average group inter-network FC between the DMN and SN).

mPFC (seed)

Model	B	Std. Error	β	t	p	corrected p	Zero-order R
(Constant)	100.696	7.387		13.631	.000		
ACC	-21.558	26.365	-.123	-.818	.420	.560	-.121
lAI	36.623	22.521	.254	1.626	.115	.172	.110
rAI	-57.774	15.544	-.595	-3.717	.001	**.003***	-.510

Model significance R^2= 0.585 F=3.768 P=0.014 (*significant FDR corrected p≤0.05)

Table 4.4. Significant results of the regression analysis between habitual cTST (dependent variable) and DMN (mPFC seed) inter-network connectivity with the SN.

rAI (seed)

Model	B	Std. Error	β	t	p	corrected p	Zero-order R
(Constant)	114.390	14.032		8.152	.000		
lIPC	14.033	32.574	.079	.431	.671	.671	-.093
lMTL	27.629	42.041	.183	.657	.519	.593	.507
LHP	-95.299	81.343	-.258	-1.172	.255	.680	.083
mPFC	-15.415	6.025	-.611	-2.558	.019+	.152	-.473
PCC	30.550	35.605	.294	.858	.401	.641	-.252
rIPC	20.846	24.882	.270	.838	.412	.549	.300
rMTL	51.270	44.162	.311	1.161	.259	.518	.305
RHP	122.529	78.168	.362	1.568	.133	.532	.099

Model significance R^2=0.499 F=2.256 P=0.054 (+significant uncorrected p≤0.05)

Table 4.5. Significant regression analysis model between habitual cTST (dependent variable) and SN (rAI seed) inter-network connectivity with the DMN. On FDR correction of the p values in the model the rAI-mPFC FC association with cTST were found to be non-significant.

cTST and inter-network FC of the CEN:

cTST was not a significant predictor of either DMN-CEN or SN-CEN inter-network FC (see supplementary material in the appendix).

DISCUSSION

This study examined the effect of habitual sleep patterns on the awake, resting-state FC of intrinsically connected networks. We focused on the DMN, SN and CEN as these networks are most closely linked with the higher cognitive functions that have been shown to be most affected by sleep deprivation (Alhola and Polo-Kantola 2007, Babckoff et al 2005, Belenky et al 2003, Dinges et al 1997, Harrison and Horne 2000, Horne et al 1993, Van Dongen et al 2003,). Our main finding was that the cumulative amount of sleep that participants achieved over the 14 day period preceding fMRI scanning was significantly predictive of intra- and inter-network FC of the DMN and SN, but not the CEN. .

The study had two hypotheses. The first suggested that individual differences in sleep patterns, quantified as the cumulative total sleep time over 14 days (cTST), would be reflected in intra-network FC strength between the major nodes of the DMN, SN and CEN measured during wakefulness. Multiple linear regression demonstrated that this was at least partially the case. In terms of the DMN, FC of the mPFC was significantly predicted with cTST model significance R^2=0.576 F=4.251 P=0.002. This result was specific to the mPFC, with only pairwise connections involving the mPFC as the seed showing a relationship between DMN FC and cTST (see Table 4.3). No association between SN or CEN intra-network FC and sleep was found.

The specificity of the relationship between mPFC FC and sleep status is consistent with previous imaging and behavioural investigations. For example, it has been demonstrated that sleep deprivation causes reduced intra-DMN FC strength of the mPFC (Killgore et al 2012, Verweij et al 2014) to the PCC and posterior nodes of the

DMN, while self-reported sleep duration on the night prior to scanning has also been linked with mPFC FC (Killgore et la 2012). Behaviourally, a similar specificity has been observed, with sleep deprivation preferentially impairing cognitive performance on tasks involving the prefrontal cortex (Horne 1993, Harrison and Horne 2000). Although we did not test cognitive performance, it is reasonable to postulate that experimentally-induced sleep deprivation leads to deficits in higher cognitions via its effect on intra- and inter-network FC of ICNs. The implication from our results is that these observations are generalisable to habitual sleep patterns in healthy individuals, and by quantifying FC of the mPFC we provide a mechanism by which habitual sleep status and cognition are linked. The fact that cTST is specifically linked to mPFC-DMN FC, but not FC within the SN or CEN, is a novel observation. The SN and CEN have been linked with salience and attentional processes, which might be expected to be related to cTST, but our results suggest the importance of inter-network FC in mediating the effects of cTST on these processes, as discussed in more detail below.

Our second hypothesis was that inter-network connectivity of the DMN, SN and CEN would be altered in relation to habitual sleep status. This issue has not been previously examined, and the basis of this hypothesis is that for optimal brain performance it is not only crucial that ICNs are internally connected, but they must be able to interact with each other in a consistent and coherent manner. This hypothesis was again partially confirmed, with connectivity between the DMN and SN dependent on cTST. Specifically, FC between the mPFC of the DMN and the rAI of the SN demonstrated a significant negative correlation with cTST, (Table 4.4). It has been shown that when responding to an unexpected event in the environment the internally

focused mode of operation supported by the DMN needs to be inhibited, and that this is achieved by an increase in rAI activity which in turn allows the brain to quickly switch to a controlled mode of operation which is tightly coupled to external events (Menon and Uddin 2010, Sridharan et al 2008, Uddin et al 2009,). We have shown for the first time that a reduction in cTST is associated with an increase in the FC between rAI (SN) and the mPFC of the DMN (Table 4.4). It is possible that this represents an attempt to maintain the appropriate level of rAI activity needed to sustain alertness and ensure the effectiveness in network switching from intrinsic thoughts to external executive functioning. It is thought that the rAI is involved in the regulation of dynamic changes between the DMN and CEN (Manoliu, Menon and Uddin 2010) networks known to have competitive interactions (Sridharan et al 2008). Our results suggest that short habitual sleep durations disrupt right AI connectivity to the DMN and hence the ability to switch between internal and external modes, which may have an effect on widespread cognitive and behavioural domains. Future work will need to address this question with neuropsychological testing, but existing behavioural literature would support the association between working memory and attention and sleep status, albeit generally from the more extreme case of sleep deprivation or restriction (Basner et al 2013).

One factor which complicates the interpretation of this observation is that the DMN and SN are anti-correlated. A negative correlation with cTST therefore suggests that longer habitual sleep durations are related to more negative DMN-SN FC. It has been demonstrated that the use of global signal regression (GSR) as we have done negatively biases correlation measures (Murphy et al 2009). At best this can manifest

as a shifting of all correlations to lower values, including negative values. However, at worst it can result in a distortion of the underlying connectivity which can fundamentally alter interregional correlations within a group, as recently demonstrated (Gotts et al 2013, Saad et al 2012). This makes it difficult to draw detailed conclusions regarding the relationship between negative inter ICN FC (i.e. DMN-SN) and behavioural metrics, and future studies may benefit from more advanced investigations of the impact of GSR (Scholvinck et al 2010) as well as better assessments of the physiological nuisance variables that GSR is intended to mitigate.

A recent study has suggested that a substantial proportion of waking resting-state fMRI scans may be confounded by participants entering early stages of sleep in even relatively short waking scan (Wong et al 2013). While the impact of this observation on the field generally remains to be clarified, it could be argued that in our study participants with shorter habitual sleep times might be more likely to fall asleep during the scanning session. Our cohort consisted of healthy control subjects adhering to their normal sleep routine, verbally indicated that they had not slept during the session, and our questionnaire data demonstrated no evidence of abnormal levels of daytime sleepiness (Epworth score 4.93±1.07, mean±SD). In addition, their responses to the Karolinska Sleepiness Scale indicated a good level of alertness immediately upon exiting the scanner (2.13±0.21, mean±SD, indicating a self-assessment of ‘very alert’, compared to a value of 6 indicating ‘some level of sleepiness’). While subjective ratings cannot be taken as completely reliable, the available evidence is therefore supportive of our resting state data being composed at least predominantly of wakefulness, and as we have pointed out, the changes to

FC that we have observed are consistent with those seen in response to explicit sleep deprivation. However, future studies would need to record EEG data concurrently with the fMRI to allow unambiguous sleep staging, and thereby address this issue.

Our approach of investigating multiple ICNs and the interactions between them in relation to habitual variation in sleeping patterns has the potential to provide a more detailed mechanistic explanation for why some cognitive functions are affected by sleep status, while others are not, as well as for the individual differences that are seen in the effects of sleep deprivation. It would also be interesting to address the issue of how differences in cumulative TST link with sleep debt. In this study, we did not record information about participants' preferred amount of sleep, so we are not able to distinguish between those who achieved that amount versus those who did not. Future studies might examine whether the changes to FC in subjects who are not achieving their preferred amount of sleep are different to those who are, independently of how much sleep that represents.

Overall, this study is the first to address the question of how interactions within and between the major ICNs are related to variations in habitual sleep durations. These effects are not global, but specific to certain connections between certain pairs of nodes. In particular, the mPFC node of the DMN has FC that is related to cTST, while connections between the DMN and SN are also associated with cTST. Future work will need to address the behavioural implications of these observations to determine whether they underlie the known cognitive and behavioural effects associated with short sleep durations (Basner et al 2013).

CHAPTER 5

Habitual cumulative total sleep time and subjective sleep quality are associated with structural white matter differences in the human brain[3]

[3]Khalsa, S., Hale J., Goldstone, A., Wilson, R., Mayhew, S.D., Bagary, M. and Bagshaw, A.P., 2016. Habitual sleep durations and subjective sleep quality predicts structural white matter differences in the human brain. This chapter is submitted as a manuscript for review in Neurobiology of Sleep and Circadian Rhythms.

ABSTRACT

Self-imposed short sleep durations are increasingly commonplace in society, and have considerable health and performance implications for individuals. Reduced sleep duration over multiple nights has similar behavioural effects to those observed following acute total sleep deprivation, suggesting that lack of sleep affects brain function cumulatively. A link between habitual sleep patterns and functional connectivity has previously been observed (chapter 4), and the effect of sleep duration on the brain's intrinsic functional architecture may provide a link between sleep status and cognition. However, it is currently not known whether differences in habitual sleep patterns across individuals are related to changes in brain structure. In the present study we use diffusion–weighted imaging and tract based spatial statistics (TBSS) to investigate bivariate correlational white matter changes in relation to sleep duration and quality, hypothesising structural connectivity as quantified by white matter metrics would demonstrate reduced FA and MD values in association with the long term effects of poor sleep and short habitual sleep patterns. Our findings suggest that reduced cumulative total sleep time (cTST) and poor subjective sleep quality result in subtle white matter micro-architectural changes. The regions we identified as being related to habitual sleep patterns were restricted to the frontal and temporal lobes, and the functions they support are consistent with those which have previously been demonstrated as being affected by short sleep durations. Examining how brain structure and function are related to inter-individual differences in habitual sleep patterns could help to shed light on individual susceptibility to short sleep durations, as well as potentially the networks and systems responsible for variations in sleep patterns themselves.

INTRODUCTION

Sleep patterns have been investigated in relation to behaviour and functional connectivity (FC, De Havas et al 2012, Gujar et al 2010, Khalsa et al 2016), but no studies to date have considered habitual sleep status in relation to white matter structural connectivity. Studies involving patients with chronic insomnia have demonstrated that grey matter in the frontal lobe may be altered with respect to normal sleepers (Altena et al 2010), and acute sleep deprivation has been shown to reduce thalamic volume (Liu et al 2014), suggesting that there is a link between sleep duration and brain structure. However, the white matter structural connectivity (SC) that ultimately provides the anatomical substrate for functional interactions is less well understood, and very few studies have investigated white matter changes in relation to sleep. SC can be characterised using diffusion tensor imaging (DTI), with fractional anisotropy (FA) and mean diffusivity (MD), two commonly used metrics to quantify white matter tracts (Beaulieu et al 2002, Le Bihan et al 2003). MD is dependent on the amount of water molecule movement and independent of direction, while FA assesses the directionality of such movement (Le Bihan et al 2003). Therefore with reductions in FA a corresponding increase in MD values may be seen (see above, chapter 1 for more details). These measures have been used extensively as markers of white matter microstructural changes in a variety of situations (Alexander et al 2007), and may be altered by a variety of changes to the underlying white matter (Jones et al 2013).

In terms of sleep, Rocklage et al 2009 examined cognitive vulnerability to total sleep deprivation in relation to white matter differences. They found differences in the genu, ascending and longitudinal white matter pathways, with significantly higher FA values in subjects with reduced susceptibility to total sleep deprivation. Elvsashagen et al 2014 found that a night of total sleep deprivation was associated with widespread FA decreases mainly explained by reductions in axial diffusivity. Piantoni et al 2013 investigated EEG sleep oscillations and DTI metrics and found that individuals with greater spindle power (a phenomena of N2 and N3 sleep which has been associated with cognitive performance Schabus et al 2006) demonstrated higher DTI metrics in the corpus callosum and temporal lobe. These observations indicate that the structural correlates of sleep phenomena and even short term alterations to sleep patterns can be investigated with DTI. In combination with the changes to functional connectivity mentioned above, they may also suggest that white matter connectivity and organisation moderates the cognitive effects of sleep deprivation and may affect a person's ability to function effectively when sleep deprived.

In the present study, we use tract based spatial statistics (TBSS, with FDT FLS tool box, Smith et al 2004) to investigate white matter changes in relation to habitual cumulative sleep time and sleep quality. We chose to measure habitual sleep patterns as these are more representative of a subject's day to day sleep behaviour than total sleep deprivation. Our overall aim was to investigate the notion that SC is affected by the long term effects of habitual sleep status and habitual sleep debt. We expected group differences in FA and MD with respect to habitual sleep duration, as measured in a group of habitually short sleepers compared to a group of longer sleepers. We also investigated whether

subjective habitual sleep quality as measured using the Pittsburgh Sleep Quality Index (PSQI, Buysse et al 1989) would be related to differences in FA and MD metrics. Furthermore, given the evidence of previous behavioural and functional imaging literature (Belenky et al 2003, De Havas et al 2012, Dinges et al 1997, Gujar et al 2010, Horne 1993, Khalsa et al 2016, Van Dongen et al 2003), we expected these effects to be most prominent in frontal brain regions.

METHODS AND MATERIALS

Subjects:

DTI and fMRI data were acquired from 38 healthy adults (right handed, 10 female, age 20-34 years, mean age=25.4 years). From the original 38 subjects, 5 were subsequently excluded due to actigraphy and diary data demonstrating erratic sleep patterns, leading to a final cohort of 33 participants. (For further details, see chapter 2).

Sleep Patterns and Questionnaires:

Sleep patterns were assessed for a 14 day period using sleep diaries and wrist actigraphs (Actiwatch2, Philips Respironics Ltd, Cambridge, UK). (See chapter 2 for further details).

Demographics (n=33)	Mean	SD
Age	**25.4**	6.27
Questionnaires		
Epworth	3.94	.79
Karolinska	1.16	.41
Fatigue	12.36	.98
PSQI	4.5	1.84
Depression	1.42	2.63
Anxiety	1.21	.84
Stress	3.10	2.43
Actigraphy		
Mean TST (h)	7.38	1.45
cTST (h)	97.21	8.79

Table 5.1. Summary data: demographics, questionnaires, the mean total habitual sleep time (TST), and cumulative habitual total sleep time (cTST) summed over 14 days.

Image acquisition:

Subjects underwent a 13 minute echo planer DTI scan. Each subject also underwent one resting-state fMRI scan of 12 minutes duration, during which they were instructed to lie still and relax with eyes open. All participants confirmed that they remained awake and alert through the scanning session. (See chapter 2 for more details).

Definition of short and long sleepers (cTST):

The short and long sleeper groups were defined by a median split of the 33 subjects based on the cTST. The 17 subjects with the shortest cTST comprised the short sleepers group (mean 88.33, SE 2.19), and the 16 subjects with the longest cTST made up the long sleepers group (mean 105.57, S.E. 1.55).

Definition of poor and good sleepers (PSQI):

PSQI global scores for the assessment of sleep quality were used to define subjectively poor or good sleepers. From the subject group of 33, one subject was excluded due to not filling in the PSQI questionnaire appropriately (responses were vague descriptive words where a tick was required for a specific set of questions). The remaining 32 subjects were split into two groups. The 16 subjects with the lowest PSQI global scores comprised the good sleepers group (mean 2.68, S.E. 0.25), and the 16 subjects with the highest PSQI global scores represented the poor sleepers group (mean 6.31, S.E. 0.37) By definition the lower the global PSQI score the better the subjective sleep quality for each subject. A global PSQI score of > 5 clinically indicates poor sleep quality.

Tract based spatial statistics (TBSS) analysis:

We performed a voxelwise, between group comparison of FA and MD using TBSS (Smith et al 2006) focusing on a cohort of 33 subjects split into two groups for cTST and 32 subjects split into two groups for subjective sleep quality (PSQI) as described above.

A single FA image from each subject was created using tools in the FDT FSL toolbox (Smith et al 2004). The original data were corrected for head movement effects and eddy currents using eddy current correction (Jenkinson et al 2002). A brain mask was created using brain extraction tool (BET, Jenkinson 2002) on the non-diffusion weighted image. The diffusion tensor model was fitted using DTIFIT (Part of FSL Tool Box). We then ran the TBSS script for nonlinear registration, aligning all FA images to 1x1x1mm standard space. The target image used in the registrations was chosen automatically as the most representative of all subjects in the study. This target image was then affine-aligned into 1x1x1mm MNI152 space (1x1x1mm resolution was used as the skeletonisation and projection steps are known to work well at 1x1x1mm resolution, Smith et al 2006). The FA image for each subject had the nonlinear transform to the target and then the affine

transform to MNI152 space applied. Next, the mean of all FA images was created, and this was used to construct the mean FA skeleton. The last TBSS script was used to threshold the mean FA skeleton at the chosen threshold of 0.2 (Smith et al 2006) to exclude voxels consisting of grey matter or cerebral spinal fluid. Distance estimation analysis was also carried out before voxelwise cross-subject statistics was performed using the randomise tool in FSL which carries out permutation testing (5000 permutations, Nichols and Holmes 2002). Thresholding was carried out using threshold-free cluster enhancement (TFCE, Smith and Nichols 2009). The TFCE p-value images produced were fully corrected for multiple comparisons across space to give a significance of $p<0.05$ to determine which FA voxels were statistically significant between the two groups of subjects.

For the MD analysis a diffusion tensor model was fitted at each voxel using the same methodology used for FA voxels (to produce FA maps), to produce the MD maps (Kim et al 2012, Alves et al 2012). All subject's MD warped data were merged in to a 4D file, which was projected onto the mean MD skeleton using the original projection vectors to project the MD data onto the skeleton (which we called the mean MD skeletonised data). Using randomise as above voxelwise statistical analysis was carried out for our two group comparison of MD skeletonised data metrics.

RESULTS

TBSS analysis using cTST:

We found statistically significant decreases in mean FA values in short sleepers compared to long sleepers in three brain regions: the left orbito-frontal region, the right

inferior longitudinal fasciculus and the right superior corona radiata (tracts identified using Mori et al 2011,see Figure 5.1, MNI co-ordinates in table 5.2, figure 5.2 shows the significant correlation plots).

We also found statistically significant increases in MD values in two brain regions when comparing subjects with short cTST against long cTST: the right orbito-frontal white matter and the right inferior longitudinal fasciculus (temporal pole region, Mori et al 2011) as shown in Figure 5.3, correlational plots figure 5.4 and MNI co-ordinates table 5.3.

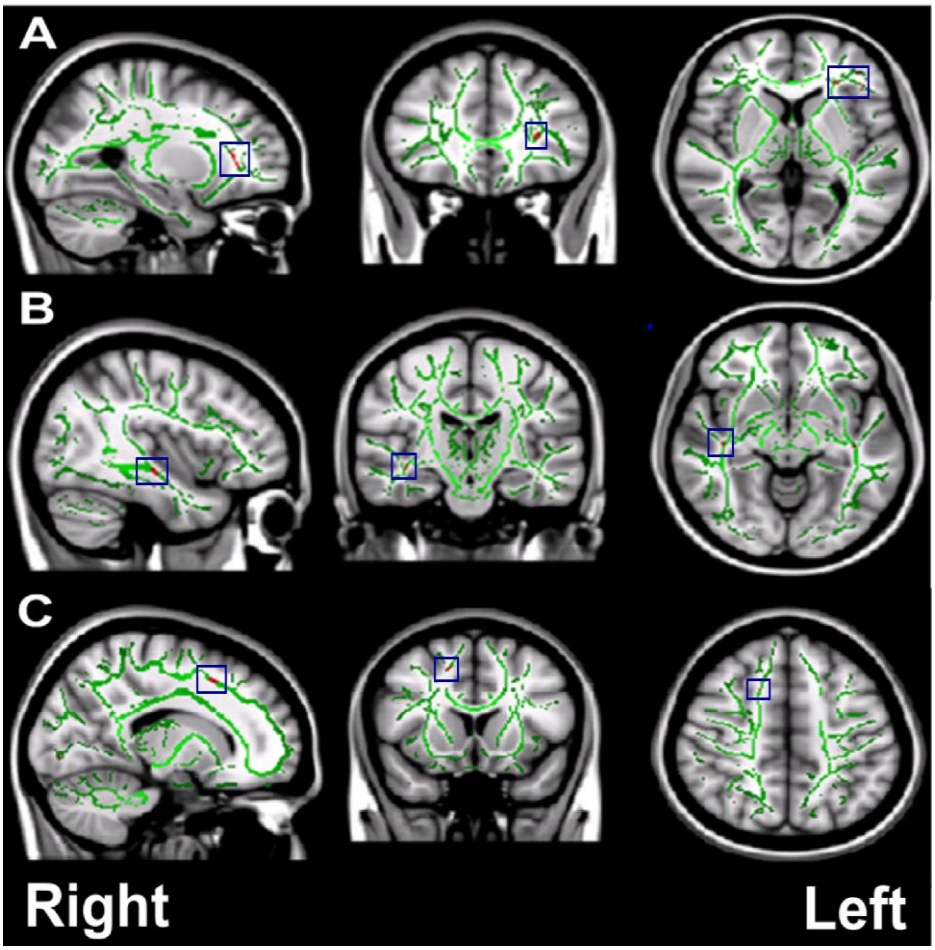

Figure 5.1. Differences in FA between long and short sleepers. The mean all sleepers FA skeleton (green) is projected onto the standardised T1 MNI 1mm brain image. The red regions (highlighted in blue boxes) show statistically significant ($p < 0.05$) reductions in the mean FA of short sleepers compared to long sleepers. The significant reductions correspond to (A) the left orbito-frontal region (B) the right inferior fasciculus and (C) the right superior corona radiata (tracts identified using Mori et al 2011).

Region of white matter with significant mean FA reductions	MNI Co-ordinates		
	X	Y	Z
left frontal orbital/insula region	-27	32	3
right inferior fasciculus	42	-18	-10
right superior corona radiata	16	16	48

Table 5.2. Shows the MNI co-ordinates for the significant group differences at p=<0.05 in mean FA between short and long sleepers shown in figure 5.1 above, from the TBSS analysis. (supplementary material, shows all the DTI metrics for the regions demonstrating significant differences).

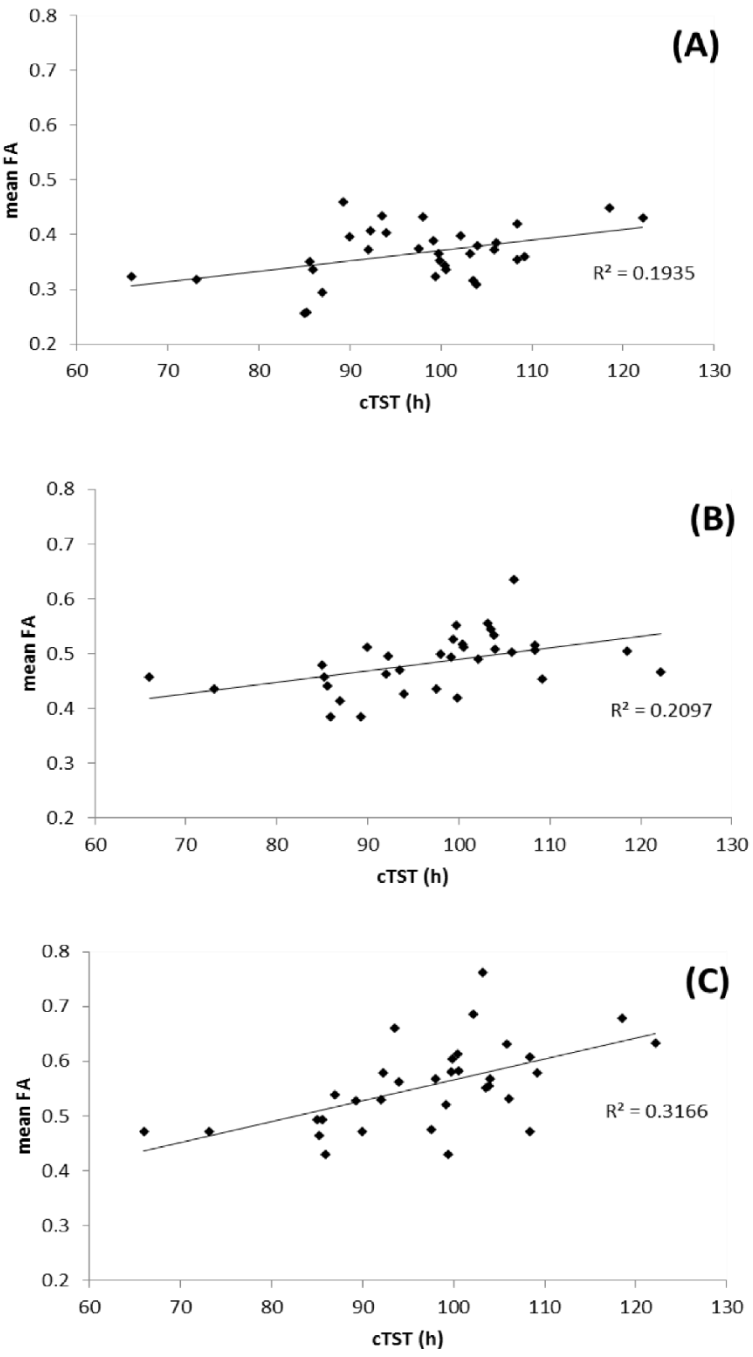

Figure 5.2. Correlational plots of the significant differences in mean FA between long and short sleepers. For A) left orbito-frontal region (p= <0.05), B) the right inferior fasciculus (p= <0.01) and C) the right superior corona radiata (p= <0.01).

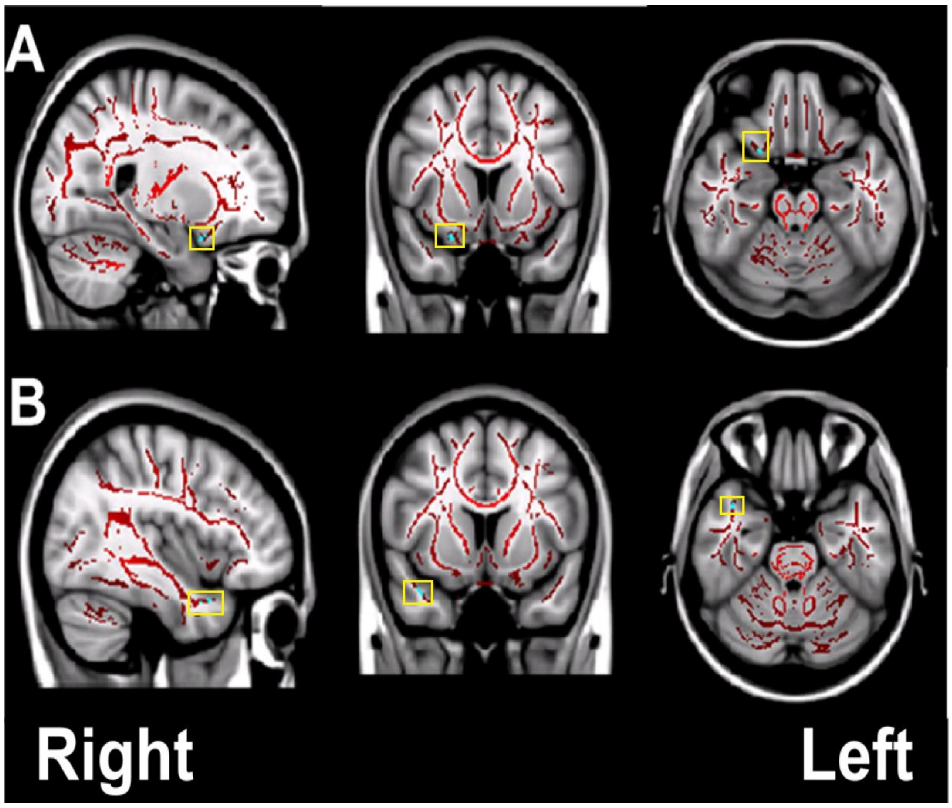

Figure 5.3. Differences in MD between long and short sleepers. The mean all sleepers MD skeleton (red) is projected onto the standardised T1 MNI 1mm brain image. The light blue regions (highlighted in yellow boxes) show statistically significant ($p < 0.05$) increases in the mean MD of short sleepers compared to long sleepers. The significant increases correspond to (A) the right orbito-frontal white matter tracts and (B) the right inferior longitudinal fasciculus (temporal pole region, tracts identified using Mori et al 2011).

Regions of white matter with significant increases in mean MD	MNI Co-ordinates X	Y	Z
right orbito-frontal WM	19	19	-17
right inferior longitudinal fasciculus (temporal pole)	27	-12	-31

Table 5.3 shows the MNI co-ordinates for the significant group differences at p=<0.05 in mean MD between short and long sleepers shown in figure 5.3 above, from the TBSS analysis.

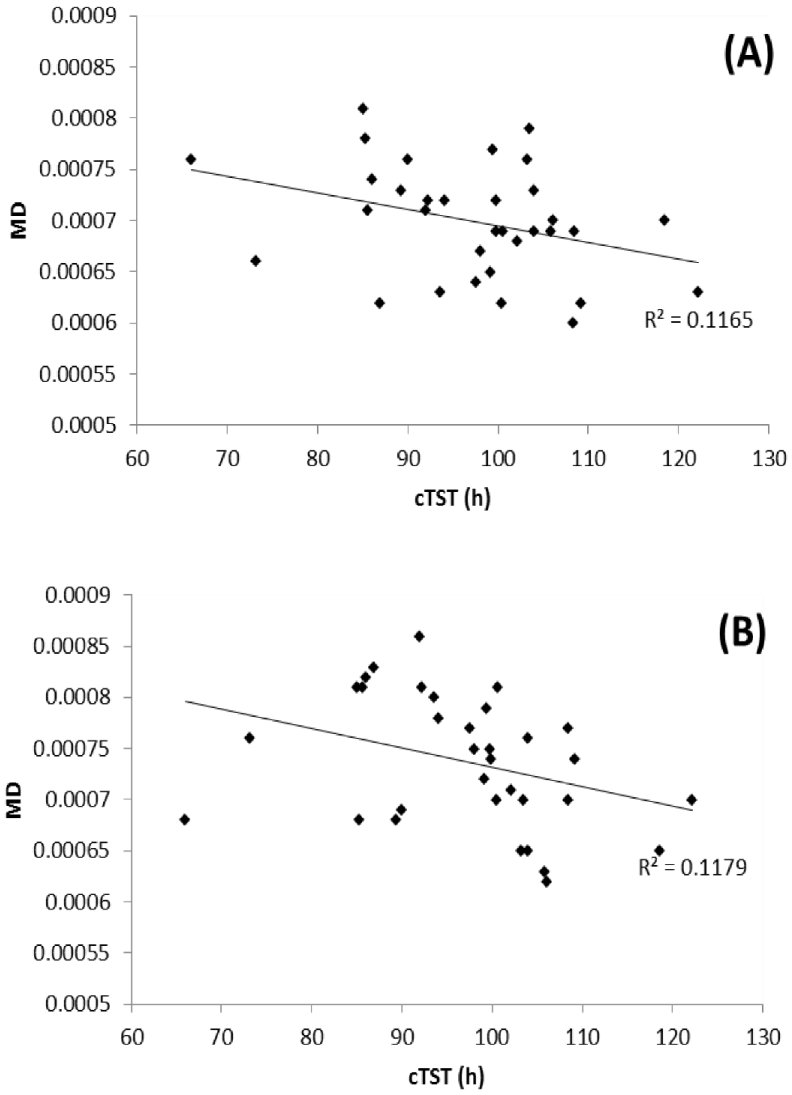

Figure 5.4. Correlational plots of the significant differences in mean FA between long and short sleepers. For A) Right orbito-frontal region (p= <0.05), B) the right inferior fasciculus (temporal pole region, p= <0.05)

TBSS analysis using subject global PSQI scores:

We found statistically significant differences in mean FA and MD values when comparing DTI metrics of subjects with poor subjective sleep quality (high PSQI global scores) against subjects with good subjective sleep quality (low PSQI global scores). Significant decreases in mean FA were found in four white matter brain regions for the subjects with

poor subjective sleep quality relative to those with good sleep quality: white matter tracts to the head of the left caudate nucleus, white matter tracts to the left orbito-frontal region, the left anterior cingulum bundle and the white matter tracts associated with the right operculum and insula (Figure 5.5, figure 5.6 shows the correlational polts for all subjects, table 5.4 shows MNI co-ordinates). Significantly higher mean MD values were found for the left orbito-frontal white matter and the left anterior cingulum bundle (Figure 5.7, figure 5.8 shows correlational plots for all subjects, table 5.5 shows MNI co-ordinates).

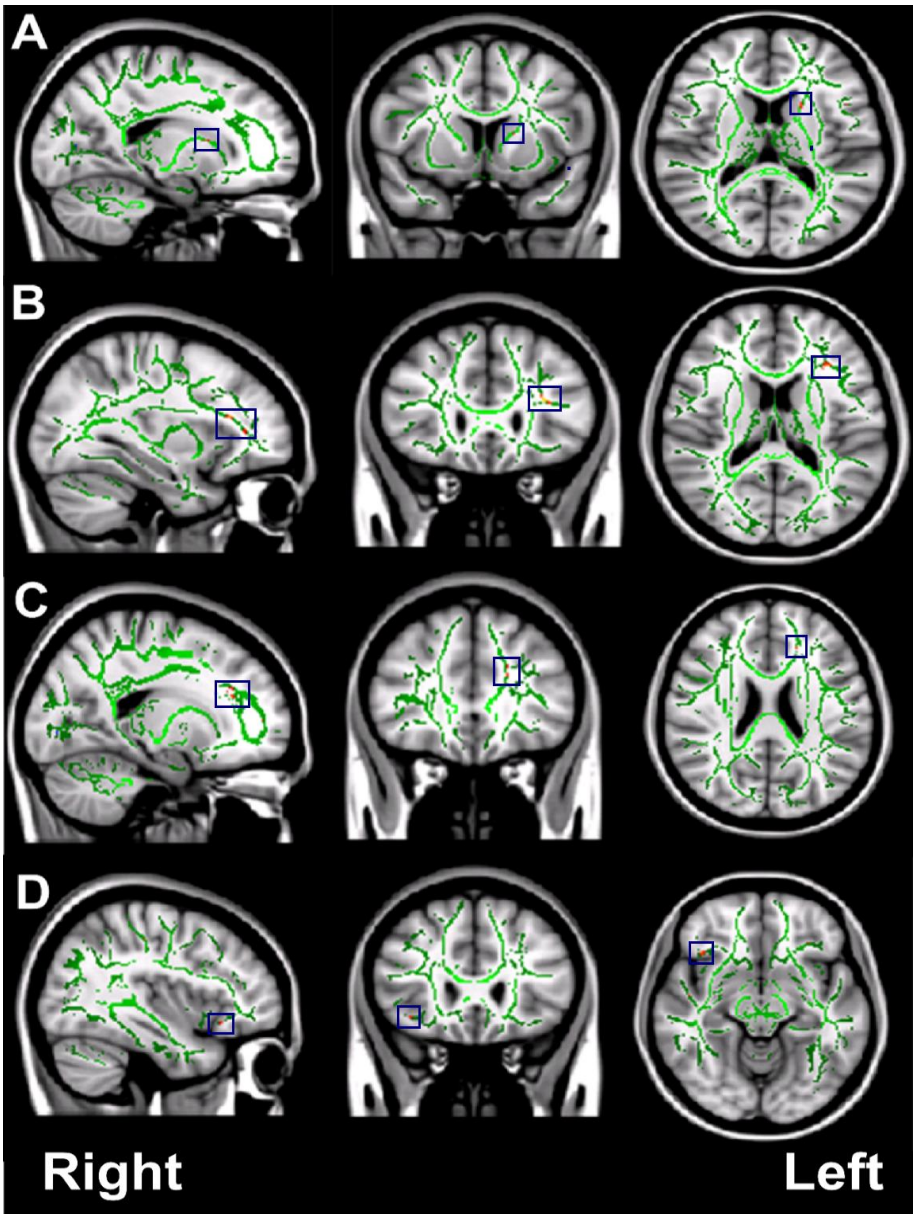

Figure 5.5. Differences in FA between poor and good sleepers. The mean all sleepers FA skeleton (green) is projected onto a T1 MNI 1mm standardised brain image. The red regions (highlighted in blue boxes) show statistically significant ($p < 0.05$) decreases in the mean FA of poor sleepers compared to good sleepers. The significant decreases correspond to (A) the white matter tracts associated with the head of the left caudate nucleus, (B) the white matter tracts associated with the left corona radiata, (C) the left anterior cingulum bundle and (D) the white matter tracts associated with the right operculum and right insula. (tracts identified using Mori et al 2011).

Region of white matter with significant mean FA reductions	MNI Co-ordinates		
	X	Y	Z
head of left caudate WM	-19	18	11
left corona radiata	-34	29	13
left anterior cingulum	-17	23	24
right insula/operculum	39	24	-12

Table 5.4 shows the MNI co-ordinates for the significant group differences at p=<0.05 in mean FA between poor and good sleepers shown in figure 5.5 above, from the TBSS analysis. (Supplementary material shows all the DTI metrics for the regions demonstrating significant differences).

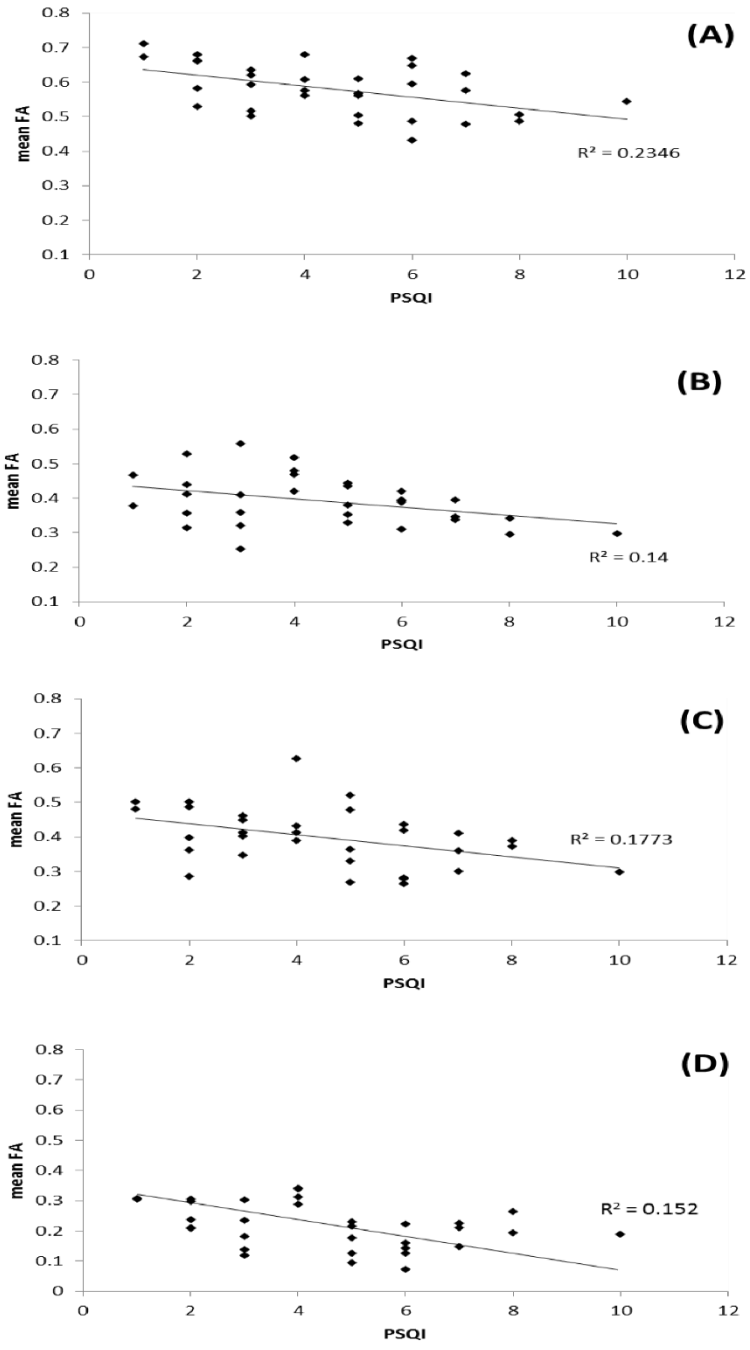

Figure 5.6. Correlational plots of the significant differences in mean FA between long and short sleepers. For A) head of the left caudate nucleus (p= <0.01), B) the left corona radiata (p= <0.05), C) the left anterior cingulum bundle (cingulate, p < 0.05) and D) right insula region (p= < 0.05).

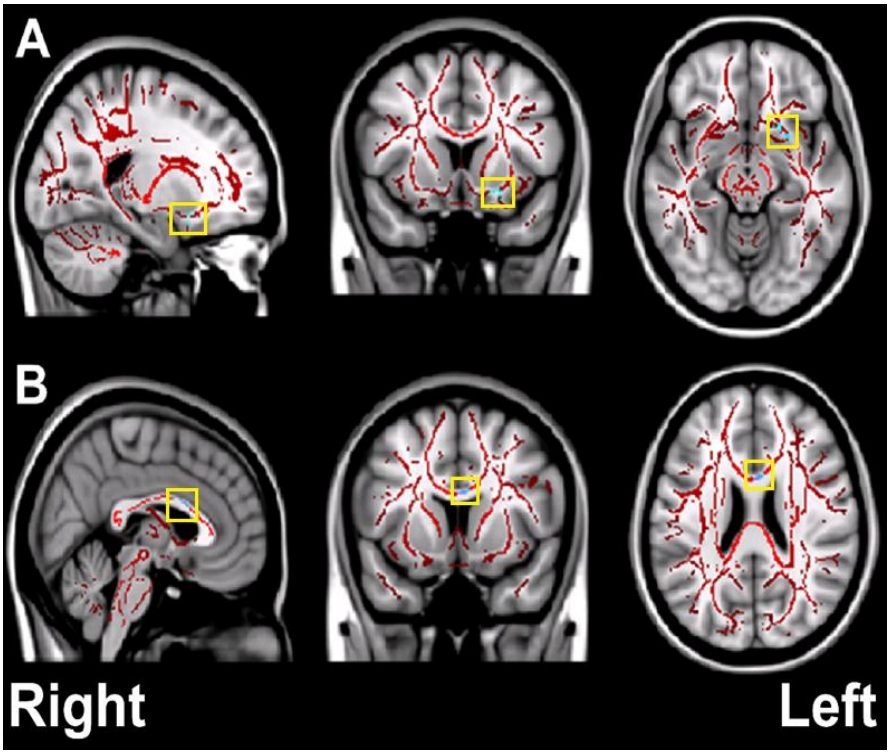

Figure 5.7. Differences in MD between poor and good sleepers. The mean all sleepers MD skeleton (red) is projected onto the standardised T1 MNI 1mm brain image. The light blue regions (highlighted in yellow boxes) show statistically significant ($p < 0.05$) increases in the mean MD of poor sleepers compared to good sleepers. The significant increases correspond to (A) the left orbito-frontal white matter tracts and (B) the left anterior cingulum bundle (tracts identified using Mori et al 2011).

Regions of white matter with significant increases in mean MD	MNI Co-ordinates X	Y	Z
left orbito-frontal WM	-20	29	-6
left anterior cingulum	-6	18	18

Table 5.5 shows the MNI co-ordinates for the significant group differences at p=<0.05 in mean MD between poor and good sleepers shown in figure 5.7 above, from the TBSS analysis. (Supplementary material shows all the DTI metrics for the regions demonstrating significant differences).

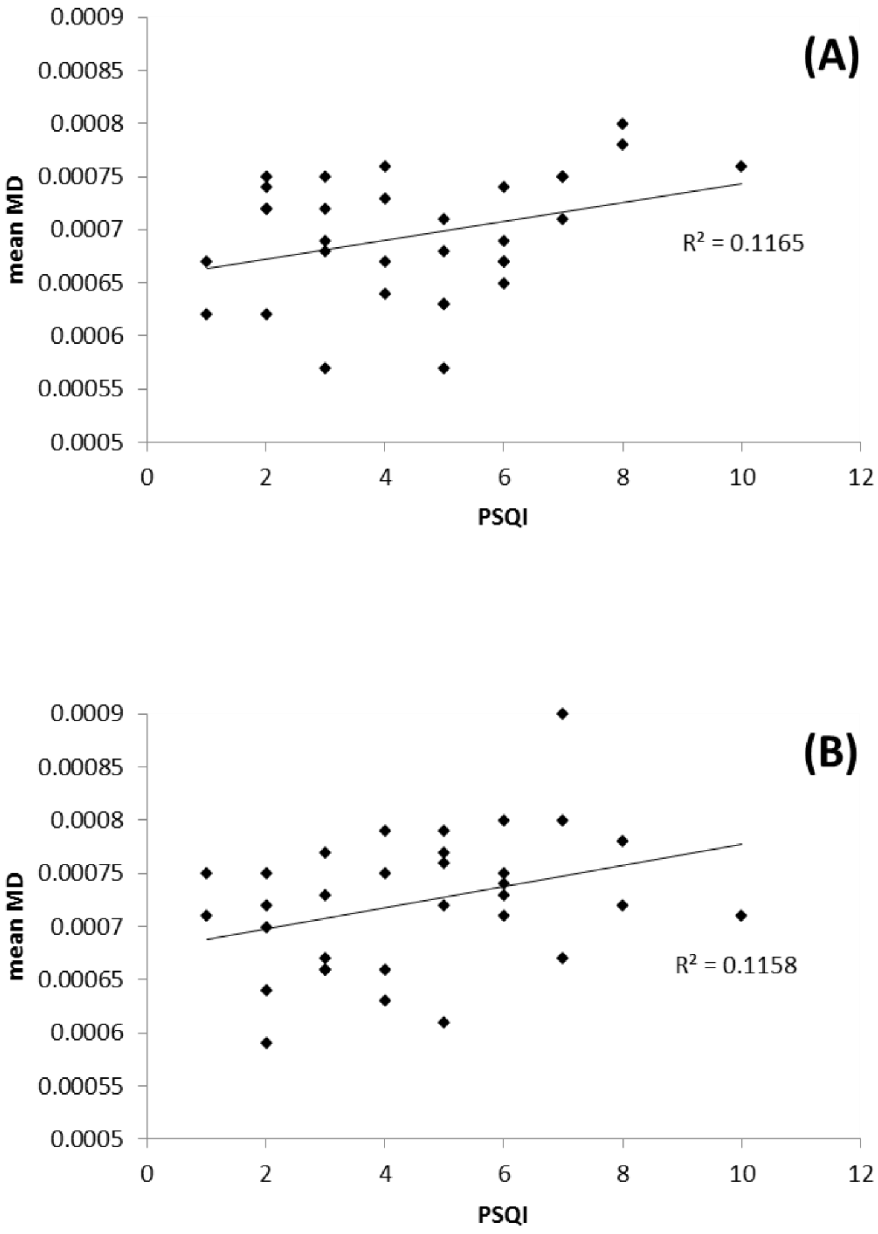

Figure 5.8. Correlational plots of the significant differences in mean FA between good (squares) and poor (dots) sleepers. For A) left orbito-frontal region (p= <0.01), B) the left anterior cingulum (Cingulate, p= < 0.05).

DISCUSSION

We used TBSS to investigate whole brain changes in white matter architecture in relation to habitual sleep patterns, as quantified by cTST, and sleep quality, as quantified by the PSQI. In both cases, we were able to identify specific white matter tracts which differed in FA or MD, demonstrating for the first time that objective and subjective measures of habitual sleep are associated with changes to brain structure. The differences when comparing the 'poorer' sleep group (i.e., shorter cTST or higher PSQI) with the 'better' sleep group (i.e., longer cTST or lower PSQI) indicated lower FA and/or higher MD. While many physical and physiological factors contribute to the quantification of these DTI measures (Jones et al 2013), both reductions in FA and increases in MD have generally been linked with reductions in behavioural and cognitive performance (see below). Our observations of these changes to brain structure in relation to sleep would therefore be consistent with the considerable literature on the behavioural and cognitive effects of acute or chronic sleep deprivation, which does not point towards improved performance with poor sleep. The differences in brain structure that we see could therefore form the underlying substrate of the behavioural and cognitive effects of poor sleep patterns.

We found a significant reduction in mean FA values for the shorter sleepers compared to the longer sleepers in the left orbito-frontal region, right superior corona radiata and right inferior longitudinal fasciculus. The changes observed in the orbito-frontal regions of white matter are consistent with regions known to be affected by sleep deprivation and habitual sleep durations in functional MRI studies (Bosch et al 2013, De Havas et al 2012, Gujar

et al 2010, Khalsa et al 2016,Tomasi et al 2009, Verweij et al 2014). With respect to the corona radiata, there is a considerable body of literature which has identified alterations to their structural properties and linked them with behavioural performance deficits, particularly in relation to attention and cognitive control (Durston et al 2006, Leite et al 2013, Liston et al 2011, Nogi et al 2010, Thillainadesan et al 2012). FA decreases in the anterior corona radiata also suggest possible disruption of thalamocortical connective relays of the frontal cortex (Nogi et al 2010). We also found a reduction in FA in the right inferior longitudinal fasciculus in short sleepers, which is known to have numerous projections to the superior temporal regions and also to the long fibres of the posterior cingulum bundle (Catani et al 2003). Such reductions may suggest subtle disruption to relays from posterior cingulate/parietal and other cortical areas to the temporal lobe which may in turn affect functional interactions between these cortical regions resulting in subtle changes such as memory impairment (Hayes et al 2012). Haller et al (2013) have reported group level decreases in FA using TBSS analysis for the right inferior longitudinal fasciculus in patients with mild cognitive impairment. A study by Orbitus et al (2012) suggests an association between reduced FA in the inferior longitudinal fasciculus and object recognition deficits in children with visual impairment compared to normal controls. They found the severity of clinical impairment was reflected in the degree of FA reductions within the inferior longitudinal fasciculus. This suggests the inferior longitudinal fasciculus plays a role in object recognition. Studies investigating the effect of sleep deprivation on object recognition and memory consolidation in rodents (Palchykova et al 2006, 2006) and in humans (Chee et al 2010) have shown impairment in object recognition and memory for the sleep deprived subjects compared to normal controls. It is possible that

the reductions in FA of the longitudinal inferior fasciculus that we report here in association with short sleep durations may underlie the subtle reductions in the cognitive processes involved with object recognition and memory consolidation. Overall, it is plausible that changes in the structure of tracts such as the orbito-frontal regions, superior corona radiata and the inferior longitudinal fasciculus may contribute to attentional and other cognitive impairments which are commonly seen as a function of sleep deprivation and poor sleep quality (Alhola et al 2007, Babkoff et al 2005, Banks and Dinges 2007, Belenky et al 2003, Dinges et al 1997, Van Dongen et al 2003).

Significant increases in MD for short sleepers compared to long sleepers were found in the right orbito-frontal white matter and the right inferior longitudinal fasciculus, the latter in agreement with the findings discussed above in relation to FA. The increases in mean MD to the right orbito-frontal white matter are also consistent with regions know to be affected by sleep deprivation and cumulative sleep time in functional MRI studies (Bosch et al 2013, De Havas et al 2012, Gujar et al 2010, Tomasi et al 2009, Verweij et al 2014).

We also investigated whole brain changes in white matter architecture in relation to subjective sleep quality, and demonstrated significant, regionally specific changes in subjects with subjectively poorer sleep compared to good sleepers. For subjectively poor sleepers we found statistically significant lower FA values for the white matter regions associated with the left caudate, left orbito-frontal region, left anterior cingulum bundle and the right insula compared to subjectively good sleepers. It has been suggested from functional MRI studies that the caudate nucleus is linked to neural networks involved in the regulation of executive function, sleep and arousal (Stoffers et al 2014). Reduced functional connectivity recruitment of the left caudate has been reported in patients with

insomnia during executive function tasks (Stoffers et al 2014) and it has been suggested that reduced input from the left orbito-frontal cortex may contribute to altered caudate recruitment (Stoffers et al 2014). Lesions to the caudate in animal studies (Villablanca et al 1976b) induced restlessness and hyper-arousal which indicate failing inhibitory modulation of sensory inputs. Therefore from our findings we can postulate that poor subjective sleepers may demonstrate subtle reductions in inhibitory modulation compared to good subjective sleepers due to the comparatively reduced white matter integrity in the orbito-frontal regions and caudate white matter. This may suggest a subtle state of hyper-arousal in poor subjective sleepers, and may partly explain the impairment of left caudate recruitment during executive function (Stoffers et al 2014), and the state of hyper-arousal which has been reported (Bonnet and Arand 2010) in subjects with sleep pathology such as insomnia.

We also found significant reductions in FA in the left anterior cingulum bundle and white matter associated with the right insula, as well as increases in MD in left orbito-frontal white matter and the left anterior cingulum. These white matter regions would suggest a connection with the corresponding cortical areas that form part of the salience network (Seeley et al 2010) which consists of the anterior cingulate and right and left insula regions. The right anterior insula is also thought to act as a control switch between the central executive network and the default mode network (Sridharan et al 2008) and is involved in the brain's attention system (Eckert et al 2009). A recent study has shown the functional connectivity between the right insula and the mesial prefrontal cortex to co-vary with cTST (Khalsa et al 2016), therefore suggesting a link between saliency and quantitative measures of habitual sleep status. The current study extends these findings,

and although we did not directly measure them it is possible that subjects with poor subjective sleep who demonstrated lower FA values may have a comparatively reduced level of salience and attention, again consistent with previous behavioural studies of short sleep (Belenky et al 2003, Dinges et al 1997, Van Dongen et al 2003).

TBSS is a useful and effective tool for group wise comparisons of DTI metrics but some limitations need to be considered. Firstly TBSS conceptually derives anisotropic values from the centre of any given white matter tract assuming maximum anisotropy, and a gradual reduction in anisotropic diffusion is assumed the further from the tract centre the measurement is taken. This assumption is not true for all regions. For example where two or more tracts cross, converge or diverge a more complex methodology is required (Smith et al 2006). Also it is possible for the morphological properties of the mean FA skeleton to alter in tracts adjacent to the ventricles, for example the posterior cingulum bundle, due to cerebral spinal fluid causing partial volume effects. Therefore although we report significant FA and MD changes in various regions of white matter there may be other regions with significant changes which have not been identified with TBSS. Secondly due to convergence of numerous tracts in certain brain regions and the low inherent spatial resolution of DTI, it is not possible to absolutely discern fibres of one tract from another. The inferior longitudinal fasciculus for example is known to connect the occipital pole to the anterior part of the temporal lobe, however a second fibre bundle connects the occipital cortex to the frontal lobe (the fronto-occipital fasciculus) and spatially overlaps with the inferior longitudinal fasciculus along parts of its pathway (Ashtari 2012). Diffusion spectrum imaging (DSI) tractography and related methods may be more appropriate to image complex distributions of intravoxel fibre orientations (Wedeen et al 2008), and

would provide an additional level of detail compared to the analysis we were able to perform.

In conclusion, our findings report for the first time that reduced habitual cTST and poor subjective sleep quality may result in subtle white matter micro-architectural changes. It is possible that these changes may in turn result in grey matter network functional disruptions which result in cognitive deficits such as those we find as a function of sleep deprivation. The regions we identified as being related to habitual sleep patterns were restricted to the frontal and temporal lobes, and the functions they support are consistent with those which have previously been demonstrated as being affected by short sleep durations. Examining how brain structure and function are linked with inter-individual differences in habitual sleep patterns could help to shed light on individual susceptibility to short sleep durations, as well as potentially the networks and systems responsible for variations in sleep patterns themselves.

CHAPTER 6

The relationship between brain functional connectivity and objective and subjective assessments of sleep quantity and quality

ABSTRACT

The primary purpose of this study was to investigate whether the total mean FC of the pairwise connections between all nodes within a network (whole or overall ICN FC) from three higher cognitive brain networks (DMN, SN, CEN) was differentially related to subjective and quantitative chronic habitual sleep measures in the form of PSQI, sleep diary data and actigraphy metrics. From previous seed based FC analysis in chapter 4 it was found that specific bivariate FC correlations between node pairs within and between networks were more susceptible to reduced habitual sleep durations (mPFC node couplings for example). Here we wanted to build on these findings and investigate whether other sleep metrics associated with chronic habitual sleep status, as well as sleep time could effect whole network connectivity and whether overall ICN FC could be related to quantitative or subjective sleep measures. Investigating whole network FC in relation to chronic habitual sleep behaviour is of interest as it has not previously been investigated and theories of wake state instability suggest totally sleep deprived individuals try to resist the onset of sleep by using increasingly greater compensatory effort to maintain consciousness. Application of biological energy resource allocation theories to SD points to wake state instability being a consequence of changes to energy resource allocation. We propose, it is possible that such wake state instability and changes in energy resource allocation may be characterised within whole networks as alterations to ICN FC in association with chronic habitual sleep status as defined by subjective and quantitative sleep metrics. We used factorial ANOVA statistical analysis to determine the possibility of any significant main effects between chronic habitual sleep metrics and overall network FC.

The results of this study demonstrated a significant interaction effect of subjective sleep efficiency on overall network FC for the CEN. There were no significance of main effects of the overall network functional connectivity for any of the sleep measures investigated in relation to the DMN or SN. This suggests, in general, that overall DMN, SN whole network FC is not characterised differentially by quantitative or subjective chronic habitual sleep measures. Therefore changes within ICNs with respect to chronic habitual sleep status may in general, only be region specific as reported in chapter 4.

INTRODUCTION

Sleep is a complex, multiscale phenomenon that can be assessed and quantified with a wide range of techniques. It has subjective and objective components which are often not highly correlated (Landry et al 2015, Van Den Berg et al 2008). For example, when receiving inadequate sleep, an individual's assessment of their cognitive or behavioural impairment is generally poor compared to more objective assessments (Blackwell et al 2011), and even subjective assessments of sleep duration tend to be inaccurate (Åkerstedt et al. 2002, Backhaus et al 2002, Buysse et al. 1991, van den Bergh 2008). The reason for this is unclear, but given the importance of distributed brain networks for cognition generally (Bressler, 1995, Bressler and Menon 2010, Luna et al 2001) and the evidence that sleep deprivation (De Havas et al 2012, Tomasi et al 2009, Verweij et al 2014) and variability in habitual sleep patterns (chapter 4, Khalsa et al 2016) can affect FC, it would be expected that networks would be differentially related to subjective and objective markers of sleep.

As well as region specific studies (Khalsa et al 2016), investigating the effect of habitual sleep measures across whole network FC is also important.

The need to sleep is resisted by sleep deprived individuals who use greater compensatory effort to remain awake (Doran et al 2001). Inevitably the homeostatic need for sleep prevails and leads to slower responses and rapid uncontrolled sleep initiation (i.e. lapses, Priest et al 2001). These lapses become longer and longer with increasing SD, until the subject can no longer resist the need to sleep. Lie et al 2015 looked at how 36 hours of SD effected cognitive capacity in relation to increased sleep pressure using a sleep pressure index (as a measure of homeostatic sleep drive). Lie's group used fMRI and ICA with correlational analysis to define ICNs and measure whole network FC and found that 36 hours of SD leads to alterations in whole network FC correlations between the DMN, SN and CEN. Their findings suggest that wake state instability is a consequence of changes to energy resource allocation between networks due to sustained SD and progressive increases in sleep pressure. Therefore whole network modulation may be taking place when individuals are sleep deprived. This being the case it is possible whole network FC modulation may be apparent within networks in relation to chronic habitual sleep measures. This may be the result of progressive increases in sleep pressure in subjects with short chronic habitual sleep status.

There are a range of standard tools to investigate and quantify habitual sleep patterns (Åkerstedt et al 1990, Buysse et al 1989, Johns 1991, Kushida et al 2001, van de Water et al 2011). Actigraphy allows the assessment of habitual sleep over extended periods of time and therefore the characterisation of habitual sleep patterns (see chapter 1). There are several quantitative sleep variables which can be extracted from actigraphy analysis.

Examples include total sleep time (TST) which has been shown to have strong correlations with TST measured from PSG studies (Ancoli-Israel et al 2003), Wake after sleep onset (WASO) is a measure of the degree of sleep fragmentation and can therefore be an indicator of sleep quality with PSG and correlates well in normal sleepers (Marino et al 2013). Sleep efficiency can also be extracted, but has been shown not be particularly accurate when compared against PSG sleep efficiency data (Ancoli-Israel et al 2003, Marino et al 2013). In this study we used TST and WASO as quantitative variables.

The Pittsburgh sleep quality index (PSQI, Buysse et al 1989) is a self-reported retrospective subjective sleep measure with relatively good psychometric properties that is useful for clinicians and researchers to assess a variety of sleep disturbances that might affect sleep quality, and differentiate good sleepers from poor sleepers (Buysse et al 1989, see chapter 1 for further details). It has been reported that retrospective self-reported sleep measures are not as accurate as prospective subjective sleep measures such as sleep diaries in terms of recording sleep time (e.g. the subjective sleep time component of the PSQI). Comparison has been made between PSQI and sleep diaries (Backhaus et al 2002) which suggest that retrospective subjective estimates of sleep duration are subject to bias due to the subjects being assessed, focusing more on nights when they had particularly poor sleep and subsequently reporting long sleep latencies and underestimated sleep durations (Backhaus et al 2002). This might be even more important in cases of habitual poor sleep (e.g., insomnia). However, the use of measures such as the PSQI to characterise habitual sleep status is common in clinical settings, as the PSQI is designed to give an overall subjective estimate of a subject's sleep quality and not specifically for the characterisation of individual sleep variables (Buysse et al

1989). The PSQI has also been used in conjunction with other sleep measures and fMRI for sleep research studies characterising the relationship between FC and cognitive changes associated with sleep status and pathological conditions such as insomnia, for example (Dai et al 2014, Li et al 2014, Minkel, et al 2012).

There has only been one study which has investigated the relationship between habitual cumulative sleep duration and brain network FC (chapter 4, Khalsa et al 2016), and while that examined the bivariate pairwise FC correlations within and between network nodes in three ICNs it looked at a very limited range of sleep variables. Whole network correlational analysis of FC of the DMN, SN and CEN has been used to investigate SD in the context of increased sleep pressure (Lie et al 2015), but there are no studies which have investigated whole intra-network FC correlations in relation chronic to habitual sleep status.

The primary purpose of this study was to investigate whether overall ICN FC from three higher cognitive brain networks (DMN, SN, CEN) was differentially related to subjective and objective sleep measures in the form of PSQI and sleep diary data and actigraphy metrics. We investigated the relationship between overall mean network FC and sleep quality and sleep duration for each network using six sleep measures. We hypothesized that differences in overall network functional connectivity for short/long, poor/good sleepers would be seen between subjective and objective measures of sleep quality, and that these differences would affect different networks.

METHODS AND MATERIALS:

Subjects:

Data was from the same subjects used for the study in chapter 4 . (For further details refer to chapter 2 and chapter 4).

SUBJECTIVE SLEEP MEASURES

Sleep diaries: subjective habitual prospective sleep time assessment:

Subjects were asked to complete a 14 day sleep diary in which they logged their sleep patterns, time they settled for bed, how long they thought it took them to fall asleep and time they awoke. The daily sleep time was calculated from the time settled for bed to the awake time the next morning .

Definition of short and long sleepers (dTST):

The short and long sleeper groups for diary sleep time (dTST) were defined by a median split of the 32 subjects based on dTST . The split was done independently and therefore the two groups of 16 subjects were not necessarily the same individuals. The 16 subjects with the shortest dTST comprised the short sleepers group, and the 16 subjects with the longest dTST made up the long sleepers group respectively

Sleep Patterns and Questionnaires:

Participants also completed the following questionnaires: Epworth Sleepiness Scale (ESS, Johns 1991), Depression, Anxiety and Stress Scale-21 (DASS, Lovibond and

Lovibond 1995), Karolinska Sleepiness Scale (KSS, Åkerstedt and Gillberg 1990). (See chapter 2 for further details).

Definition of poor and good sleepers (PSQI):

PSQI global scores for the assessment of sleep quality were used to define subjectively poor or good sleepers; 32 subjects were split into two groups. The 16 subjects with the lowest PSQI global scores comprised the good sleepers group, and the 16 subjects with the highest PSQI global scores represented the poor sleepers group. By definition the lower the global PSQI score the better the subjective sleep quality for each subject.

Definition of poor and good sleepers (SSE):

Sleep efficiency is the time in bed spent asleep expressed as a percentage of the total time in bed. It can be used as a measure of sleep quality, with greater sleep efficiency indicating better quality of sleep. The subjects were split into good and poor sleepers based on their subjective sleep efficiency score from the SSE component of the PSQI in the same way as described above.

QUANTITATIVE SLEEP MEASURES (Actigraphy data)

Definition of short and long sleepers (cTST):

The short and long sleeper groups (cTST) were defined by a median split of the 32 subjects based on the cTST . The split was done independently and therefore the two groups of 16 subjects were not necessarily the same individuals. The 16 subjects with the shortest cTST comprised the short sleepers group, and the 16 subjects with the longest cTST made up the long sleepers group .

Definition of poor and good sleepers (WASO):

WASO scores from the actigraphy metrics were used to define poor and good sleepers. Sleepers with higher WASO indicating greater sleep fragmentation and therefore poorer sleep quality compared to those subjects with lower WASO scores. Subjects were split into two groups based on WASO scores in the same way as described above.

IMAGE ACQUISITION AND PRE-PROCESSING

Subjects underwent a single resting-state fMRI session in the early afternoon during which they were instructed to lie still in the scanner and relax with eyes open. All participants confirmed that they remained awake and alert through the scanning session. (See chapter 2 for details).

Defining regions of interest:

Regions of interest (ROI) representing the nodes of the DMN, CEN and the SN were created from data from a separate cohort of 55 subjects from a previous study (Przezdzik et al 2013, 28 male, age 25±4yrs). (See chapter 2 for further details).

Measuring DMN, CEN and SN FC:

Following previous methodology (Khalsa et al 2014, 2016) we used seed-based FC analysis performed according to standard methods (Fox MD et al 2005) using in-house MATLAB code (Mathworks, USA). (See chapter 2 for more details).

The ROIs for each network as described above were used in turn as the seed to measure the strength of FC to all other DMN, SN and CEN ROIs. The average FC across all paired

connections in each ICN was calculated to define overall mean FC for each ICN for the overall network FC analysis.

Statistical Analysis:

IBM SPSS Statistics for windows (version 21.0) was used to conduct, two-way factoral ANOVAs .The first factor for the analysis was sleep status measured as the objective or subjective sleep metric (cTST, dTST, SSE, WASO, SE, PSQI) with two levels (low/high, short/long or poor/good). The second factor was network which had 3 levels: DMN, CEN and SN. The dependent variable was network FC. Further testing was performed in the form of pairwise comparisons to determine the exact significance of the main effect.

RESULTS:

There was a significant within subject main effect of SSE on overall network FC from the ANOVA analysis ($F(2,30) = 3.682$, $P = 0.033$, $\eta^2=0.109$), therefore suggesting overall brain network connectivity was not homogenously related to all sleep measures (see table 6.1 below for full results).

Independent Variables	Overall FC F-value	Partial eta squared (η^2)	Level of significance (p)
cTST	0.883	0.029	0.409
SE	0.527	0.019	0.549
WASO	0.371	0.012	0.671
dTST	0.731	0.024	0.475
SSE*	**3.682***	**0.109***	**0.033***
PSQI	0.695	0.023	0.490

Table 6.1 results of ANOVA analyses of within subject effects *significant at p=<0.05.

A significant main effect was found from the ANOVA analysis for SSE in relation to overall network FC. Therefore further split plot analysis was performed in order to interpret the main effects. The marginal means from the split plot ANOVA analysis were plotted to observe the main effects in a graph to highlight which network in particular demonstred the significant main effect (figure 6.1).

From the graph in figure 6.1 we can see that for the DMN there is higher network FC in relation to low SSE compared to high SSE, but the difference is small. For the CEN, low SSE is associated with substantially lower overall network connectivity compared to

high SSE. For the SN again we can see that low SSE corresponds to higher FC compared to high SSE, but again the difference is smaller compared to FC of the CEN.

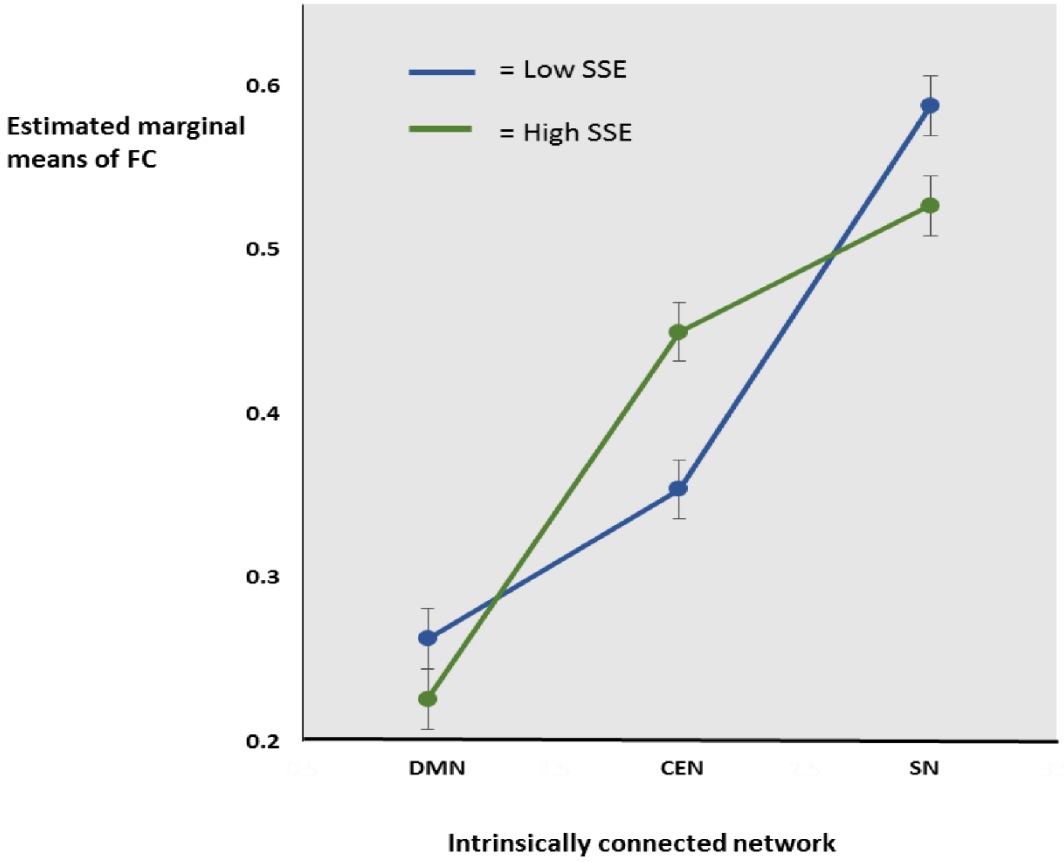

Figure 6.1. split plot from the ANOVA analysis demonstrating a significant main effect of SSE and overall network FC. Low SSE and High SSE plotted against estimated marginal means of the overall network FC on the y-axis for the three networks on the x-axis (error bars represent standard error, the data points are connected in order to visualise the interaction effects).

All other sleep metrics (subjective and objective) did not demonstrate any significant main effects (see table 6.1).

DISCUSSION:

The aim of this study was to investigate whether overall mean ICN FC from three higher cognitive brain networks (DMN, SN, CEN) was differentially related to subjective and objective sleep measures in the form of PSQI, sleep diary data and actigraphy metrics. We investigated the relationship between mean network FC and sleep quality/duration metrics, including three subjective measures (PSQI, SSE, dTST) and three objective measures (WASO, SE, cTST). SSE demonstrated a significant relationship with overall FC within the CEN, but overall, the majority of these metrics did not explain variance in FC in the ICNs, indicating either that mean whole network FC is not a sensitive metric, or that the sleep measures do not have strong relationships with overall network FC in the contex of habitual sleep status.

Significant differences in whole network mean FC between low SSE and high SSE were seen in the CEN (figure 6.1 and table 6.1). High SSE demonstrated significantly higher FC than low SSE. We know the DMN and CEN demonstrate anticorrelation in well rested subjects (Fox et al 2005). The CEN is activated (demonstrates increased FC) while the DMN demonstrates a reduction in FC in response to cognitive tasks or saliency and this modulation of networks is regulated by the SN (Sridharan et al 2008, Seely et al 2007). In sleep deprived subjects the CEN demonstrates reduced overall FC and DMN demonstrates greater FC in response to varying degrees of increased homeostatic sleep pressure (Lei et al 2015). Our findings demonstrate increased CEN FC in relation to high SSE. A recent study by Lei et al (2015), demonstrated faster reaction time responses in relation to CEN activation and slower responses and greater errors with increases in mPFC FC (a key node of the DMN) and found this correlated with subjective sleepiness

scores in sleep deprived subjects. The associations we report may suggest that greater activation of the CEN is associated with greater levels of alertness and lower levels of sleepiness. Our significant findings of SSE in relation to CEN FC demonstrate increased FC of the CEN in relation to higher SSE suggesting lower levels of sleepiness in comparison to lower CEN FC in subjects with low SSE (figure 6.1).

There were no significant effects associated with the mean overall network functional connectivity for any of the sleep measures investigated with respect to the DMN This suggests in general, that overall DMN whole network FC is not characterised differentially by poor, good, short or long sleepers whether that be objectively or subjectively. From previous region of interest studies the effects of short habitual sleep are known to affect the mPFC cortex of the DMN resulting in reduced mPFC FC when ROI FC analysis is performed (Khalsa et al 2016). However, whole network FC analysis (which was performed in this study) may result in offsetting this reduced frontal FC to some degree. For example the hippocampal regions are known to be involved in moderating connectivity patterns within and between networks and have complex and dynamic connectivity patterns with other cortical networks and demonstrate fluctuations in FC under various conditions (Hartzell et la 2015). For example, Hartzell and colleagues found the DMN whose connectivity was determined by features of the current resting-state of the subject demonstrated lateralized FC of the hippocampus to the right frontal gyrus. Following a passive task the network FC of the left hippocampus was weaker than the right. While following an attentive task the FC of left hippocampus was stronger compared to the right. Hartzell's findings suggest that ongoing hippocampal FC networks mediate information integration across networks at multiple temporal scales, with hippocampal

laterality moderating these connectivity patterns and this may be another factor influencing overall DMN network connectivity.

The findings for the SN did not give any significant results from the ANOVA analysis either. Again from ROI studies it is thought the right anterior insula (rAI) demonstrates increased inter-network connectivity in order to increase the level of saliency within sleep deprived individuals (Menon and Uddin 2010, Sridharan et la 2008,Uddin et al 2009). It is possible the complex interactions of the rAI and other ICNs may offset any clear significant relationships between the whole SN network FC and sleep measures.

We performed whole network FC analysis in this study to investigate the effect of quantitative and subjective sleep measures on overall network FC. This approach was preferred to the ROI FC analysis used in chapter 4 as using such methods to assess the FC for each paired connection across all networks would subsequently lead to a huge multiple comparisons issue. Also whole network correlational analysis has been previously used in SD studies (Lei et al 2015). An alternative to the approach that we used in this chapter for the assessment and summarising of whole network connectivity may be graph theory (Bullmore and Sporns 2009), which characterises brain networks as a collection of nodes and edges. Nodes indicating basic elements within a system such as ROIs and edges indicating associations among those elements (e.g. FC, SC). Various characteristics of network behaviour and interaction can be determined using graph methodology (Bullmore and Sporns 2009). Graph theory application to whole brain network connectivity is still a developing feild and further reseach is needed to provide clear interpretation of graph generated data. Due to the intricacy of graph generated data, interpretation and analysis can be complex.

In conclusion, this study demonstrates some significant differences in FC between subjective measures; subjective sleep efficiency (SSE) which may possibly be associated with the modulatory effects of networks as discussed above.

Investigating overall whole network FC changes in relation to habitual sleep status is important as it is has been shown previously in whole network FC SD studies (Lei et la 2015) that total sleep deprivation effects overall network connectivity. This in turn has general effects on alertness and attention as highlighted by the wake state instability hypothesis (Doran et al 2001). Our overall findings here in general, suggests that FC changes within ICN in relation to chronic habitual sleep status do not involve whole network FC and therefore we believe them to be region specific (chapter 4, Khalsa et al 2016). It is possible that gradual increases in sleep pressure, such as those observed in prolonged sleep deprivation may result in these initial regions specific changes to become more widespread within networks involved in internal cognitive processing, saliency, attention and higher external cognitive functioning. The need to understand to what degree these changes are applicable to habitual sleep status measures will allow a greater understanding of the possible whole brain effects of chronic cumulative sleep restriction.

CHAPTER 7

GENERAL DISCUSSION

The purpose of this final chapter is to provide an integrated discussion of the research contained in this thesis. Specific discussion for each experiment can be found in the relevant chapters. This chapter starts by presenting a summary of the overall findings. It then goes on to discuss the potential impact of the findings in the field of neuroimaging sleep research and potential clinical applications. It subsequently goes on to look at the limitations and future directions of this work and ends with a thesis conclusion.

Summary of thesis:

The aim of the work presented in this thesis was to investigate the bi-variate correlational relationship between chronic habitual sleep status and functional and structural changes in higher order ICNs using fMRI and DTI imaging modalities in normal awake adult control subjects. The hypothesis for this work is based on the premise that the integrity of ICNs, in terms of both their functional and structural connectivity, may be a sensitive marker of prior chronic habitual sleep history.

The results presented provide evidence of ICN functional and structural connectivity changes, which are associated with chronic habitual sleep durations and in some cases sleep quality. The results demonstrate modulation of higher ICNs, with shorter habitual sleepers demonstrating altered intra-network FC within the DMN compared to longer chronic habitual sleepers. These FC changes were not global but region specific to the

frontal regions (mPFC) of the DMN. Furthermore inter-network FC changes were examined and the rAI of the SN was seen to demonstrate co-variance with DMN mPFC FC activity with a negative correlational relationship to habitual sleep time. Reduced sleep time significantly correlated with greater inter-network connectivity between the mPFC and the rAI. This indicates direct associations of the SN and mPFC FC to reduced chronic habitual sleep durations. Although it is not possible to confirm whether such changes are compensatory without addition behavioural assessments. Subsequent investigations of SC revealed that shorter habitual sleepers also demonstrated SC correlations in relation to habitual sleep status in regions associated with FC changes, in particular the frontal regions, compared to longer habitual sleepers. These findings further reinforce earlier observations from chapter 3, that there is covariance between SC and FC changes within ICNs and that this co-variance is specific and graded. Further global assessment of FC across networks as a whole in comparison to subjective and quantitative sleep metrics demonstrated no significant main effects between quantitative sleep metrics and whole network FC, but the subjective sleep efficiency measure did demonstrate a significant main effect on the FC for the CEN. No such significance was found between subjective sleep measures and the DMN or SN. Such findings demonstrate that ICN FC changes in relation to quantitative sleep measures are region specific, but subjective measures may to some degree be associated whole network FC.

The results presented in this thesis introduce important and novel findings to the field of neuroimaging sleep research when investigating functional or structural changes within neural networks in the context of sleep status. The findings highlight the importance of

chronic habitual sleep status, an area within the field which has been completely overlooked in neuroimaging investigations of sleep status in waking adult control groups.

Significance and implications:

The importance of investigating chronic habitual sleep status within the general population cannot be stressed enough, with modern day to day living leaving less time for sleep due to work commitments and extended leisure times as well as individuals having to work various hours and shifts depending on their occupation (Härmä et al 1998). These increasing demands on our time may lead to reduced habitual sleep durations. Although not all literature points this conclusion (Yetish et al 2015) and temperature changes may play a major role in sleep behaviour. This may result in the disruption of higher order ICN FC and SC which increases the possibility of potential neurobehavioural cognitive deficits similar to those found in sleep deprived subjects (Belenky et al 2003, Dinges et al 1997,Van Dongen et al 2003), as a direct result of the chronic habitual seep status of these individuals. Although several neuroimaging investigations examining ICN FC have been performed looking at totally sleep deprived individuals or partially sleep deprived subjects (De Havas et al 2012, Gujar et al 2009, , Samaan et al 2010, Yeo et la 2015, Lei et al 2015), there are no neuroimaging studies (except for the studies published resulting from the work in this thesis, Khalsa et al 2016) that have investigated the effect of chronic habitual sleep status on ICN FC or performed investigations to identify FC markers that allow prediction of an individual subject's behavioural vulnerability to SD. In terms of SC there are very few neuroimaging studies published investigating changes in SC in relation to sleep deprivation (Elvsashagen et al 2014, Piantoni et al 2013, Rocklage

et al 2009) and none at all to our knowledge investigating SC in relation to habitual sleep status.

The aim of the first study (chapter 3) was methodological. It showed clear evidence that a direct correlation between the strength of SC and the strength of FC within a single ICN exists, despite considerable differences in terms of the methodology used to quantify SC. This is also evident between the two measures of SC strength defined from deterministic and probabilistic tractography. Previous studies have reported regions that are functionally connected tend to demonstrate structural connectivity (Hagmann et al 2008, Honey et al 2009, Margulies et al 2009). The findings from chapter 3 further extend neuroimaging research of FC and SC of ICNs by showing that there is a specific and graded relationship whereby regions which have stronger structural connections also have stronger FC. This issue has received much less attention in the literature than the more general question of whether regions which are functionally connected are structurally connected (Skudlarski et al 2008). The findings from the work in chapter 3 give a better understanding of how structural connections relate to functional connectivity. This is important not only to enhance our understanding of changes in SC and FC that occur as a result of chronic habitual sleep status in control subjects, but also for FC and SC changes associated with neurological or sleep disorders.

Chapter 4 investigated the functional connectivity of ICNs and habitual sleep status, which has not previously been investigated, despite habitual sleep patterns being relatively stable within individuals but different between individuals (Roenneberg et al 2007). The results provided evidence that changes in habitual sleep durations demonstrate a significant intra-network FC correlational relationship with the prefrontal regions. A

significant reduction in mPFC FC strength with reduced habitual sleep duration was found. Importantly the more extreme case of SD and partial sleep deprivation has been found to cause reduced FC strength of the mPFC by others (De Havas et al 2012,Gar et al 2009, Horovitz et al 2009, Samaan et al 2010, Samaan et al 2011). This is consistent with and lends weight to our findings, which indicate individuals with short chronic sleep habitual durations demonstrate co-variance with network FC changes and demonstrate similar FC network changes to those seen in sleep deprived subjects. Behavioural studies investigating chronic partial sleep deprivation have reported deficits in behavioural and cognitive performance (Belenky et al 2003, Dinges et al 1997, Van Dongen et al 2003) primarily involving the prefrontal cortex (Harrison et al 2000, Horne 1993, Naghavi and Nyberg 2005, Thomas et al 2000). We suggest the network changes reported in this thesis with respect to chronic habitual sleep status may result in these cognitive performance deficits and future research will need to investigate such relationships to build on the preliminary findings presented in this thesis. Other important findings from chapter 4 indicate that short habitual sleep durations disrupt rAI connectivity to the DMN and therefore, the ability to switch between internal and external modes, which may also have an effect on widespread cognitive and behavioural domains. The important findings of chapter 4 show evidence that despite habitual sleep behaviour being relatively stable within individuals, variance of sleep behavior between individuals demonstrates marked differences in intra and inter network ICN FC connectivity. We propose from these findings that this in turn may result in cognitive and behavioural performance deficits similar to those observed in sleep deprived subjects.

The work in chapter 5 addresses a gap in the field of sleep neuroimaging. No studies investigating chronic habitual sleep status in relation to SC have been preformed previously. The work in chapter 5 supports the idea that ICNs are affected by habitual sleep status and that this has consequences for both SC as well as FC. Structural connectivity is important to consider in the context of habitual sleep status as ICN FC changes may possibly be modulated by SC therefore the long term effects of reduced chronic habitual sleep may be associated with structural changes to white matter pathways associated with ICN FC regulation and consequently lead to ICN network disruption. This could lead to neurobehavioral cognitive deficits like those mentioned above. Structural studies on control subjects have primarily focused on characterising the relationship between SC-FC. In chapter 5 we have shown that the structural connectivity correlated to chronic habitual sleep status metrics is comparable to FC regions within ICNs. This is in agreement with existing SC-FC studies to the extent that such studies performed on well rested control subjects have shown that there is a significant relationship between SC and FC (Greicius et al 2009, Hagmann et al 2008, Skudlarski et al 2008, Van den Huevel et al 2010).

Structural connectivity of the prefrontal cortex has been shown to play a part in N3 (slow wave sleep expression). A TBSS study (Rosenberg et al 2014) investigating the relationship between SC and EEG sleep demonstrated a significant correlational relationship between N3 slow wave sleep oscillations and prefrontal white matter connectivity. These findings suggest structural white matter tracts which represent the structural backbone of neural network connectivity (Hagman et al 2007) are important in determining the expression of sleep oscillations in individuals and therefore individual

sleep characteristics, which in turn determine sleep status. We have shown that short chronic habitual sleep status demonstrates reduced frontal structural white matter integrity as characterised by FA and MD metrics. We propose, that the changes we report in frontal white matter in relation to sleep status may therefore influence N3 slow wave sleep architecture, subsequently resulting in changes in relation to chronic habitual sleep status and thus influence sleep pressure in the awake state and possible neurobehavioural changes as a consequence of regional changes (such as those reported in chapter 4) to ICN FC which may be due to the effects of the white matter changes as discussed above. Further integrated multimodal work is needed to build on our initial findings and to elucidate the relationship between structural changes, chronic habitual sleep status and neurobehavioural measures in order to give credence to the above hypothesis.

Chapter 6 provides evidence that whole network connectivity does not demonstrate a significant association with quantitative sleep metrics in relation to habitual sleep status. There is some evidence which indicates behavioural measures such as SSE demonstrate a significant main effect with overall ICN FC, for example a significant effect CEN FC and SSE.

The majority of sleep measures subjective or objective did not a demonstrate significant main effect with overall network connectivity in chapter 6. This points towards the idea that the effects of reduced chronic habitual sleep durations are region specific (chapter 4, Khalsa et al 2016). We also deduce from our findings that rather than overall network changes (chapter 6), in general, shorter chronic habitual sleep durations cause region specific changes in higher ICNs both in terms of FC and SC (chapters 4 and 5). This idea

lends weight to and expands on Horne's 'prefrontal hypothesis' which is based on behavioural cognitive studies and suggests that impairment to cognitive functioning in the prefrontal cortex, including higher order functions, executive functions, saliency and attention are most affected as a result of sleep deprivation. The preliminary findings of chapter 6 are important as they inform us that reduced quantitative measures of habitual sleep status are not associated with overall whole network modulation to ICN network connectivity and this complements the work in chapters 4 and 5

We propose, based on the findings in this thesis (chapters 3-6), that the impairments to the regions suggested by Horne (as mentioned above) in response to sleep deprivation, (which have been mirrored in ICN changes, by network FC SD neuroimaging studies, De Havas et al 2012, Gujar et al 2009) are also evident in relation to short chronic habitual sleep status and are reflected in SC as well as FC. Our overall findings suggest that subjects with shorter chronic habitual sleep times may in fact be subject to increased cumulative sleep pressure. This would explain ICN FC and SC changes seen in shorter chronic habitual sleepers, which we report for the first time in this thesis, corresponding to similar FC changes seen in sleep deprived individuals (Bosch et al 2013, De Havas et al 2012, Gujar et al 2010, Sämann et al 2011, Tomasi et al 2009, Verweij et al 2014). Therefore, neurobehavioral cognitive deficits reported in sleep restricted individuals and sleep deprived subjects may also be evident in habitually chronic short sleepers. This area will require further investigation.

Potential clinical applications:

This work has the potential for clinical applications, and to be further extended to investigate network modulation and behavioural cognitive performance changes within

patient groups suffering from sleep pathology such as insomnia, periodic limb movement disorder or restless legs syndrome (Ohayon and Roth 2002). These latter two disorders are both characterised by abnormal leg movements which effect the quality of an individuals sleep and result in varying degrees of sleep deprivation, Hening et al 1999) . This would allow investigation of whether the degree of sleep disruption and subsequent reduced habitual sleep duration may be reflected in ICN network disruption and possible neurobehavioural cognitive performance impairment (Pearson et al 2006, Neikrug et al 2009). Such investigations performed on a large scale involving hundreds (or more) subjects over a prolonged period (e.g. several years) may allow the acquisition of enough quality data to produce a clinical referential database which may aid the clinician when assessing patients with reference to the degree of pathology and subsequent network disruption and neurocognitive behavioural deficits. In the short term there would be scope to introduce fMRI scanning as a diagnostic tool once an understanding of the nature of the relationship between ICN FC and sleep behaviour was established. Over time, the need for sleep clinicians to send all such patients for scanning would not be necessary as the clinician could use wrist agtrigraphy (King et al 2005) and sleep questionnaires to characterize an individual patient's sleep patterns. This data could be used to compare with the neuroimaging network database corresponding to quantitative sleep metrics such as actigaphy to determine to which degree the patient may be susceptible to ICN disruption and possible associated behavioural cognitive deficits. This would allow appropriate tailoring of clinical treatment for each specific patient. Application and development of the research findings from this thesis could be applied to determine medication efficacy or even cognitive behavioral therapy efficacy in patients with sleep

pathology such as PLMs and insomnia. Patients could be compared before and after treatments using actigraphy, sleep questionnaires and neuroimaging FC and SC data to determine the degree of efficacy of treatment with respect to chronic habitual sleep duration, ICN recovery and neurobehavioral cognitive performance. The use of actigraphy in the assessment of various sleep disorders such as PLMs and insomnia has already been established (Sadeh, 2011). At present most assessment is done using actigraphy before and after treatment (Sadeh, 2011), we propose to extend such assessment to include neuroimaging data assessment of ICN recovery and subsequent neurobehavioral improvements could be assessed in conjunction with improvements of chronic habitual sleep status with respect to clinical sleep pathology such as PLMs.

Limitations and future directions:

In chapter 3, we investigated structural and functional connectivity of an ICN at the macroscale with a few reasonably large ROIs and therefore this probably represents the lower bounds of the true FC-SC connectivity. Other methodologies need to be considered which may allow a more intricate consideration of SC-FC relationships. Graph theory is one such methodology (Bullmore and Sporns 2009, Sporns et al 2004, Van Wijk, Stam and Daffertshofer 2010). Human brain networks are complex and analysis of complex networks therefore forms an important methodological tool for intricate network systems analysis. According to graph theory, structural and functional brain networks can be considered as graphs consisting of nodes (vertices) which represent network brain regions or neuronal elements such as grey matter voxels, connected by edges which represent white matter architectural connections derived from DTI data in structural studies or FC in functional investigations. Connected networks or connectomes (Smith et

al, 2013, Sotiropoulos et al, 2013,) can be generated using graph theory and analyzed at various levels of resolution. For example, dense connectomes define connectivity between small volumes or surface elements while parcellated connectomes provide a more compact description of structural and functional regions and their interconnections (Bullmore and Sporns, 2009). Studies using graph methodology have identified key hub nodes among parietal and prefrontal regions (Hagmann et al, 2008). Using such methods to characterise functional and structural connections in relation to habitual sleep status are future areas of research worth considering and may provide additional detailed information on the network modulation associated with habitual sleep status. However, it must be stated that there is no commonly agreed approach or ideal method for characterising brain FC and SC. Although future studies using more detailed compartmentalisation methods may enhance region specific sensitivity it poses a greater challenge to SC and FC interpretation.

It has been shown that individual sleep status is determined by a number of factors. As well as habitual sleep duration, circadian phenotype needs to be considered. In this thesis, we scanned our subjects at a single time point, and did not characterise their circadian phenotype, hence we did not take in to consideration circadian oscillation effects. Resting state FC within the DMN has been shown to correspond to circadian rhythmicity (Hodkinson et al 2014). For future work scanning in the morning, mid-day and evening may be more appropriate in order to account for circadian phenotypical differences which cause certain subjects to be more alert in the mornings and others to be more alert in the evenings (i.e. larks and owls, Roenneberg et al 2007). Also subjects could be categorised as larks or owls by measuring melatonin levels. Melatonin is a

hormone, which is produced by the pineal gland, is controlled by the master circadian clock, the suprachiasmatic nucleus of the hypothalamus (SCN, Dawson and Encel, 1993). It is known to be secreted during the late evening about 2 hours before habitual bedtime with the peak occurring during the middle of the night (Dijk and Cajochen, 1997). Saliva samples could be taken to measure the onset of melatonin prior to sleep through the use of a dim light melatonin onset (DLMO) testing procedure, which is a marker of the patient's individual circadian timing (Pandi-Perumal et al 2007, Zisapel, 2007). This could potentially be combined with other circadian considerations such as genetic vulnerability to sleep restriction (Maire et al 2015). Clear differentiation of habitual sleep status from circadian and chronotypical effects is a complex issue.

For example when considering how to quantify the level of sleep debt. Sleep debt can be described as the increased sleep pressure on an individual acquiring an inadequate amount of normal physiological sleep (Geol et al 2009). Therefore, sleep debt implies some fundamental duration of sleep below which waking deficits begin to accumulate. The basal sleep need (which is defined as habitual sleep duration in the absence of pre-existing sleep debt) has been reported as 8 hours a day based one study in which prior sleep debt was completely eliminated through repeated nights of long duration sleep. Despite this most researchers agree that there are considerable interindivdual differences in sleep durations. Epidemiological studies have shown large numbers of adults with self reported sleep durations of less than 8 hours a night (Kripke et al 2002) report form a survey of more than 1.1 million Americans that approximately 20% had sleep durations of 6.5 hours or less per-night. By considering the above limitations and future directions

a better understanding of habitual sleep status in the context of ICN modulation may be possible.

The work in this thesis shows clear changes in FC and SC in prefrontal areas associated with habitual sleep status and we infer from these findings that region specific FC and SC changes in turn may be responsible for neurobehavioral cognitive changes such as those reported in previous behavioral studies investigating sleep restriction (Belenky et al 2003, Dinges et al 1997, Van Dongen et al 2003). fMRI has also been used to investigate FC changes in sleep disorders, for example insomnia, during wakefulness (Spiegelhalder et al 2013). Other studies have found reduced FC activation in the brains of patients with insomnia in comparison to control subjects during cognitive testing while awake in the scanner (Altena et al, 2008, Drummond et al, 2013, Stoffers et al, 2014), thus complementing and giving support to the daytime findings of impairment reported by insomniac patients. Future work will need to incorporate neurobehavioral cognitive measures in order to consolidate the link between habitual sleep status, ICN functional and structural modulation and neurobehavioral cognition. Data collection of FC and SC in relation to chronic sleep status and behavioral measures will also allow us to better understand how variation in network connectivity in relation to chronic sleep status relates to variations in behaviour in general.

Thesis conclusion:

The primary aim of this thesis was to investigate the relationship between higher ICN FC and SC and sleep status in control groups using fMRI and DTI. The main findings suggest that short cumulative habitual sleep durations in particular are related to non-homogenous

changes in higher ICNs in terms of intra-network and inter-network FC, and such changes are reflected in SC. These changes in general are region specific and correspond to cortical regions involved with cognition. Our approach of investigating higher order multiple ICNs and the functional and structural interactions between them in relation to chronic habitual sleep status has the potential to provide a more detailed mechanistic explanation for why some cognitive functions are affected by sleep status, while others are not, and also for the individual differences that are seen in the effects of sleep deprivation for habitually shorter sleepers compared to habitually longer sleepers. Chronic habitual sleep status and its relationship with FC and SC of ICNs has not previously been examined. This thesis has made an important and original contribution to knowledge in the field of neuroimaging sleep research.

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APPENDIX 1

Supplementary material for chapter 4:

All non-significant intra-network results with habitual cTST as the dependent variable:

Table A4.1(a)
ACC (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	113.696	10.897		10.433	.000	
Age	.097	.240	.075	.405	.689	-.010
LIN	-66.975	49.930	-.262	-1.341	.191	-.324
RIN	-87.137	60.512	-.270	-1.440	.162	-.349

R^2=0.178 F=1.883 P=0.157

Table A4.1(b)
lAI (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	96.565	11.611		8.316	.000	
Age	.017	.230	.013	.075	.941	-.015
RIN	84.862	47.944	.339	1.770	.088	.182
ACC	-125.339	56.354	-.423	-2.224	.035	-.299

R^2=0.188 F=2.003 P=0.138
Non-significant results of the regression analysis between habitual cTST (dependent variable) and SN(seeded) intra-network connectivity (table S4.1 (a) and (b) above).

Table A4.2(a)
PCC (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	86.370	9.916		8.710	.000	
Age	-.133	.345	-.117	-.387	.703	-.003
Lipc	17.922	34.014	.215	.527	.603	.223
Lmtl	23.767	34.851	.253	.682	.502	.082
LHC	-10.152	53.729	-.067	-.189	.852	-.045
mPFC	62.765	31.497	.510	1.993	.058	.451
rIPC	-22.033	33.380	-.226	-.660	.516	.102
rMTL	21.371	37.708	.181	.567	.576	.218
rHC	-77.804	54.713	-.470	-1.422	.168	-.198

R^2=0.411 F= 2.010 P=0.91

Table A4.2(b)
lIPC (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	82.668	14.367		5.754	.000	
Age	-.021	.316	-.016	-.065	.949	.026
rIPC	38.251	64.987	.139	.589	.562	.044
mPFC	58.918	38.995	.323	1.511	.145	.266
PCC	27.217	39.738	.154	.685	.501	.079
rMTL	7.203	101.157	.023	.071	.944	.204
lMTL	74.377	77.123	.294	.964	.345	.181
rHC	59.180	68.429	.179	.865	.396	.203
LHC	-10.152	53.729	-.067	-.189	.852	-.045

R^2=0.198 F=0.777 P=0.613

Table A4.2(c)
lMTL (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	102.259	16.017		6.384	.000	
Age	-.126	.299	-.098	-.421	.678	-.015
rIPC	37.465	56.433	.155	.664	.514	.320
lIPC	-8.738	44.766	-.047	-.195	.847	.255
LHC	141.411	79.174	.535	1.786	.089	.296
mPFC	-.017	30.849	.000	-.001	1.000	.213
PCC	59.971	33.379	.417	1.797	.087	.541
rMTL	-1.806	52.057	-.008	-.035	.973	-.178
rHC	-138.338	96.984	-.442	-1.426	.168	.018

R^2=0.416l F=1.871 P=0.119

Table A4.2(d)
LHC (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	71.379	17.604		4.055	.001	
Age	.056	.283	.043	.197	.846	-.015
Lipc	157.730	72.970	.589	2.162	.042	.165
Lmtl	24.873	103.214	.077	.241	.812	.215
mPFC	23.597	38.249	.126	.617	.544	.014
PCC	75.540	50.246	.336	1.503	.148	.106
Ripc	-192.782	87.206	-.596	-2.211	.038	-.173
Rmtl	21.180	100.810	.062	.210	.836	.174
rHC	141.979	91.508	.325	1.552	.136	.185

R^2=0.324 F=1.260 P=0.315

Table A4.2(e)
rIPC (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	86.959	21.301		4.082	.001	
Age	.222	.391	.168	.568	.576	.260
Lipc	.752	31.797	.005	.024	.981	.047
Lmtl	29.545	44.411	.230	.665	.513	.371
LHC	-24.677	62.015	-.149	-.398	.695	.165
mPFC	25.025	27.497	.205	.910	.373	.273
PCC	-10.176	25.066	-.144	-.406	.689	.265
rMTL	17.111	45.189	.129	.379	.709	.354
rHC	25.420	79.103	.123	.321	.751	.220

R^2=0.195 F=0.637 P=0.739

Table A4.2(f)
rMTL (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	87.072	20.880		4.170	.000	
Age	.215	.383	.162	.560	.581	.260
Lipc	.040	31.117	.000	.001	.999	.047
Lmtl	39.622	34.855	.308	1.137	.268	.371
LHC	-21.727	60.314	-.131	-.360	.722	.165
mPFC	27.286	26.313	.224	1.037	.311	.273
PCC	-7.092	23.240	-.101	-.305	.763	.265
Rhc	19.162	75.836	.093	.253	.803	.220
Ripc	17.111	45.189	.129	.379	.709	.354

R^2=0.190 F=0.736 P=0.644

Table A4.2(g)
rHC (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	66.978	15.375		4.356	.000	
Age	.172	.297	.134	.578	.570	-.041
Ripc	-7.508	90.474	-.021	-.083	.935	-.027
Lipc	35.664	34.620	.208	1.030	.315	.272
LHC	162.193	76.155	.427	2.130	.045	.395
Lmtl	36.701	149.226	.102	.246	.808	-.135
Mpfc	20.671	37.801	.121	.547	.590	.007
PCC	39.954	70.025	.190	.571	.574	.181
rMTL	-81.145	94.149	-.203	-.862	.398	-.187

R^2=0.280 F=1.019 P=0.452
Non-significant results of the regression analysis between habitual cTST (dependent variable) and DMN(seeded) intra-network connectivity (table A4.2(a) - (g) above).

Table A4.3(a)
ldlPFC (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	104.012	13.036		7.979	.000	
Age	.017	.274	.013	.060	.952	-.007
RdlPFC	-33.459	64.508	-.120	-.519	.609	-.078
Lipl	-6.937	81.800	-.026	-.085	.933	.107
Ripl	52.738	99.228	.165	.531	.600	.125

R^2=0.027 F=0.173 P=0.950

Table A4.3(b)
lIPL (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	99.523	13.424		7.414	.000	
Age	-.074	.291	-.058	-.255	.801	.026
LdlPFC	-32.926	74.047	-.130	-.445	.660	-.160
RdlPFC	-27.480	90.856	-.085	-.302	.765	-.129
Ripl	5.212	72.781	.019	.072	.943	.044

R^2=0.031 F=0.200 P=0.936

Table A4.3(c)
rdlPFC(seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	101.954	10.092		10.102	.000	
Age	-.487	.393	-.376	-1.239	.227	-.023
LdlPFC	42.001	26.225	.410	1.602	.122	.213
Lipl	-9.454	23.854	-.086	-.396	.695	-.060
Ripl	-9.567	39.929	-.065	-.240	.813	-.058

R^2=0.105 F=0.734 P=0.578

Table A4.3(d)
rIPL (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	75.280	15.395		4.890	.000	
Age	.489	.274	.370	1.787	.086	.260
LdlPFC	-6.880	27.998	-.052	-.246	.808	-.075
Lipl	11.242	24.886	.082	.452	.655	.047
rdlPFC	40.078	21.046	.378	1.904	.068	.232

R^2=0.191 F=1.480 P=0.238
Non-significant results of the regression analysis between habitual cTST (dependent variable) and CEN(seeded) intra-network connectivity (table A4.3 (a) - (d) above).

All non-significant inter-network results with cTST as the dependent variable:

Table A4.4(a)
ACC (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	92.385	16.209		5.700	.000	
Age	-.048	.370	-.038	-.131	.897	-.010
Lipc	35.090	66.122	.147	.531	.601	.181
rIPC	-45.863	94.977	-.129	-.483	.634	.092
lMTL	-97.148	134.810	-.305	-.721	.479	-.111
LHC	-28.593	84.586	-.106	-.338	.739	-.196
mPFC	58.431	55.810	.296	1.047	.308	.237
PCC	-26.471	47.067	-.151	-.562	.580	.052
rMTL	-14.346	119.899	-.048	-.120	.906	-.130
rHC	-71.106	92.847	-.237	-.766	.453	-.143

R^2=0.178 F= 1.883 P=0.157

Table A4.4(b)
lAI (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	93.788	13.091		7.165	.000	
lIPC	-45.551	79.802	-.225	-.571	.574	-.070
lMTL	-24.651	85.666	-.090	-.288	.776	-.102
LHC	-53.480	120.582	-.179	-.444	.662	-.236
mPFC	2.082	39.332	.012	.053	.958	.075
PCC	12.165	49.222	.062	.247	.807	.009
rIPC	-19.356	78.564	-.076	-.246	.808	-.169
rMTL	.368	91.175	.001	.004	.997	-.060
rHC	-74.645	177.770	-.208	-.420	.679	-.201
Age	-.058	.379	-.045	-.154	.879	-.015

R^2=0.124 F= 0.314 P=0.961

Non-significant results of the regression analysis between habitual cTST (dependent variable) and SN(seeded) inter-network connectivity with the DMN (table AS4 (a) and (b) above).

Table A4.5(a)
PCC (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	98.721	8.157		12.102	.000	
Age	.027	.260	.024	.103	.918	-.003
Lai	55.988	58.169	.390	.963	.344	.092
ACC	24.777	32.174	.248	.770	.448	.106
rAI	-67.824	48.291	-.556	-1.404	.172	-.035

R^2=0.082 F= 0.601 P=0.665

Table A4.5(b)
lIPC (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	90.091	11.371		7.923	.000	
Age	.024	.247	.019	.096	.924	.026
ACC	78.311	63.553	.237	1.232	.229	.203
Lai	28.080	48.396	.119	.580	.567	-.056
rAI	-103.801	58.731	-.363	-1.767	.089	-.296

R^2=0.149 F= 1.098 P=0.397

Table A4.5(c)
lMTL (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	96.565	9.214		10.480	.000	
Age	-.021	.251	-.016	-.083	.934	-.015
ACC	50.189	55.002	.192	.912	.370	.043
Lai	-9.148	41.220	-.047	-.222	.826	-.153
Rai	-67.416	45.766	-.335	-1.473	.153	-.272

R^2=0.107 F=0.749 P=0.568

Table A4.5(d)
LPH (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	97.772	9.103		10.740	.000	
Age	-.113	.290	-.087	-.388	.701	-.015
ACC	-18.954	82.387	-.055	-.230	.820	-.108
Lai	37.874	59.385	.163	.638	.529	-.055
rAI	-97.572	79.903	-.327	-1.221	.233	-.234

R^2=0.075 F=0.505 P=0.732

Table A4.5(e)
rIPC (seed)

Model	B	Std. Error	β	T	P	Zero-order
(Constant)	77.663	12.116		6.410	.000	
Age	.608	.336	.460	1.812	.082	.260
ACC	11.793	34.006	.112	.347	.732	-.006
Lai	-12.337	44.440	-.074	-.278	.784	-.029
rAI	-18.499	21.700	-.250	-.852	.402	.013

R^2=0.117 F=0.832 P=0.518

Table A4.5(f)
rMTL (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	77.663	12.116		6.410	.000	
Age	.608	.336	.460	1.812	.082	.260
ACC	11.793	34.006	.112	.347	.732	-.006
Lai	-12.337	44.440	-.074	-.278	.784	-.029
rAI	-18.499	21.700	-.250	-.852	.402	.013

R^2=0.118 F=0.830 P=0.517

Table A4.5(g)
RPH (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	101.545	9.337		10.876	.000	
Age	-.153	.254	-.119	-.603	.552	-.041
ACC	-7.289	56.978	-.025	-.128	.899	-.090
Lai	17.018	54.544	.069	.312	.758	-.131
rAI	-102.296	60.819	-.378	-1.682	.105	-.330

R^2=0.123 F=0.877 P=0.492
Non-significant results of the regression analysis between habitual cTST (dependent variable) and DMN(seeded) inter-network connectivity with the SN (table A4.5(a) - (g) above).

Table A4.6(a)
ACC (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	111.877	13.021		8.592	.000	
Age	-.118	.255	-.091	-.463	.648	-.010
Lipl	3.047	68.542	.013	.044	.965	.181
Ripl	67.509	93.875	.190	.719	.479	.092
ldlPFC	69.041	108.353	.202	.637	.530	-.067
rdlPFC	-143.702	94.607	-.474	-1.519	.142	-.275

R^2=0.137 F=0.765 P=0.584

Table A4.6(b)
rAI (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	115.389	14.223		8.113	.000	
Age	-.522	.355	-.404	-1.472	.154	-.023
Lipl	7.523	36.249	.042	.208	.837	-.093
Ripl	47.219	29.177	.611	1.618	.119	.300
LdlPFC	-25.226	34.186	-.298	-.738	.468	.151
RdlPFC	23.241	22.552	.301	1.031	.313	.228

R^2=0.213 F=1.298 P=0.298

Table A4.6(c)
lAI (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	96.827	12.302		7.871	.000	
LdlPFC	-15.800	81.325	-.057	-.194	.848	-.073
Lipl	16.289	68.374	.080	.238	.814	-.070
rdlPFC	-18.685	87.138	-.058	-.214	.832	-.146
rIPL	-46.684	79.591	-.184	-.587	.563	-.169
Age	-.029	.271	-.023	-.108	.915	-.015

R^2=0.038 F=0.192 P=0.963
Non-significant results of the regression analysis between habitual cTST (dependent variable) and SN(seeded) inter-network connectivity with the CEN (table A4. 6(a),(b),(c) above).

Table A4.7(a)
ldlPFC (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	113.926	9.646		11.810	.000	
Age	-.098	.230	-.076	-.428	.672	-.023
ACC	-70.465	33.889	-.371	-2.079	.048	-.403
Lai	18.770	31.681	.119	.592	.559	-.064
Rai	-51.767	33.988	-.307	-1.523	.140	-.312

R^2=0.239 F=1.968 P=0.130

Table A4.7(b)
lIPL (seed)

Model	B	Std. Error	β	T	P	Zero-order R
(Constant)	77.663	12.116		6.410	.000	
Age	.608	.336	.460	1.812	.082	.260
ACC	11.793	34.006	.112	.347	.732	-.006
Lai	-12.337	44.440	-.074	-.278	.784	-.029
rAI	-18.499	21.700	-.250	-.852	.402	.013

R^2=0.117 F=0.832 P=0.518

Table A4.7(c)
rdlPFC (seed)

Model	B	Std. Error	β	t	P	Zero-order R
(Constant)	113.926	9.646		11.810	.000	
Age	-.098	.230	-.076	-.428	.672	-.023
ACC	-70.465	33.889	-.371	-2.079	.048	-.403
lAI	18.770	31.681	.119	.592	.559	-.064
Rai	-51.767	33.988	-.307	-1.523	.140	-.312

R^2=0.239 F=1.968 P=0.130

Table A4.7(d)
rIPL (seed)

Model	B	Std. Error	β	t	P	Zero-order R
(Constant)	77.663	12.116		6.410	.000	
Age	.608	.336	.460	1.812	.082	.260
ACC	11.793	34.006	.112	.347	.732	-.006
Lai	-12.337	44.440	-.074	-.278	.784	-.029
Rai	-18.499	21.700	-.250	-.852	.402	.013

R^2=0.117 F=0.832 P=0.518
Non-significant results of the regression analysis between habitual cTST (dependent variable) and CEN(seeded) inter-network connectivity with the SN (table A4.7(a) - (d) above).

Table A4.8(a)
mPFC (seed)

Model	B	Std. Error	β	t	P	Zero-order R
(Constant)	105.398	6.566		16.051	.000	
Age	-.351	.190	-.313	-1.845	.076	-.048
rdlPFC	-43.627	25.700	-.319	-1.698	.101	-.297
ldlPFC	48.276	30.745	.334	1.570	.128	-.131
rIPL	43.848	31.135	.441	1.408	.170	.479
lIPL	22.468	26.099	.267	.861	.397	.469

R^2=0.402 F=3.765 P=0.01

Table S4.8(b)
PCC (seed)

Model	B	Std. Error	β	t	P	Zero-order R
(Constant)	98.869	8.742		11.309	.000	
Age	-.349	.372	-.304	-.939	.357	-.003
rIPL	4.002	34.823	.041	.115	.909	.102
lIPL	53.689	33.892	.661	1.584	.125	.131
rdlPFC	34.233	32.382	.350	1.057	.300	.096
ldlPFC	15.097	30.659	.128	.492	.627	.128

R^2=0.142 F=0.858 P=0.522

Table A4.8(c)
rIPC (seed)

Model	B	Std. Error	β	t	P	Zero-order R
(Constant)	75.280	15.395		4.890	.000	
Age	.489	.274	.370	1.787	.086	.260
LdlPFC	-6.880	27.998	-.052	-.246	.808	-.075
Lipl	11.242	24.886	.082	.452	.655	.047
rdlPFC	40.078	21.046	.378	1.904	.068	.232
rIPL	4.002	34.823	.041	.115	.909	.102

R^2=0.191 F=1.480 P=0.238

Table A4.8(d)
rMTL (seed)

Model	B	Std. Error	β	t	P	Zero-order R
(Constant)	75.280	15.395		4.890	.000	
Age	.489	.274	.370	1.787	.086	.260
ldlPFC	-6.880	27.998	-.052	-.246	.808	-.075
lIPL	11.242	24.886	.082	.452	.655	.047
rdlPFC	40.078	21.046	.378	1.904	.068	.232
rIPL	5.212	72.781	.019	.072	.943	.044

R^2=0.191 F=1.480 P=0.283

Table A4.8(e)
rHC (seed)

Model	B	Std. Error	β	t	P	Zero-order R
(Constant)	97.577	9.776		9.982	.000	
Age	-.114	.271	-.089	-.420	.678	-.041
ldlPFC	37.411	99.463	.097	.376	.710	-.070
lIPL	56.094	33.828	.326	1.658	.110	.272
rdlPFC	-94.260	74.640	-.348	-1.263	.219	-.160
rIPL	-15.795	69.257	-.044	-.228	.822	-.027

R^2=0.142 F=0.797 P= 0.563

Table A4.8(f)
lIPC (seed)

Model	B	Std. Error	β	t	P	Zero-order R
(Constant)	99.523	13.424		7.414	.000	
Age	-.074	.291	-.058	-.255	.801	.026
ldlPFC	-32.926	74.047	-.130	-.445	.660	-.160
rdlPFC	-27.480	90.856	-.085	-.302	.765	-.129
rIPL	5.212	72.781	.019	.072	.943	.044
lIPL	54.094	32.828	.316	1.458	.107	.252

R^2=0.031 F=0.200 P= 0.936

Table A4.8(g)
lMTL (seed)

Model	B	Std. Error	β	t	P	Zero-order R
(Constant)	87.742	9.558		9.180	.000	
Age	.002	.236	.001	.007	.994	-.015
ldlPFC	-33.699	81.186	-.109	-.415	.682	-.246
rdlPFC	-106.066	98.011	-.253	-1.082	.290	-.296
rIPL	59.378	67.790	.245	.876	.390	.320
lIPL	21.935	52.778	.118	.416	.681	.255

R^2=0.210 F=1.277 P= 0.306

Table A4.8(h)
LHC (seed)

Model	B	Std. Error	β	t	P	Zero-order R
(Constant)	103.344	9.035		11.438	.000	
Age	-.309	.275	-.240	-1.122	.273	-.015
ldlPFC	-114.504	96.864	-.351	-1.182	.249	-.240
lIPL	115.998	74.371	.433	1.560	.132	.165
rdlPFC	2.803	88.958	.009	.032	.975	-.343
rIPL	-146.602	85.773	-.453	-1.709	.100	-.173

R^2=0.235 F=1.472 P= 0.236

Non-significant results of the regression analysis between habitual cTST (dependent variable) and DMN(seeded) inter-network connectivity with the CEN(table A4.8(a)-(h) above).

Table A4.9(a)
ldlPFC (seed)

Model	B	Std. Error	β	t	P	Zero-order R
(Constant)	90.748	15.210		5.966	.000	
Lipc	2.169	116.568	.008	.019	.985	.107
rIPC	-6.037	111.736	-.019	-.054	.957	.125
Age	.022	.409	.017	.053	.958	-.007
lMTL	-75.936	128.804	-.245	-.590	.562	-.370
LHC	-19.108	135.394	-.064	-.141	.889	-.191
mPFC	-20.084	49.861	-.110	-.403	.691	-.248
PCC	-1.047	41.594	-.007	-.025	.980	.118
rMTL	-21.468	112.030	-.072	-.192	.850	-.278
rHC	-34.628	195.200	-.077	-.177	.861	-.228

R^2=0.166 F=0.441 P= 0.987

Table A4.9(b)
lIPL (seed)

Model	B	Std. Error	β	t	P	Zero-order R
(Constant)	82.668	14.367		5.754	.000	
Age	-.021	.316	-.016	-.065	.949	.026
rIPC	38.251	64.987	.139	.589	.562	.044
mPFC	58.918	38.995	.323	1.511	.145	.266
PCC	27.217	39.738	.154	.685	.501	.079
rMTL	7.203	101.157	.023	.071	.944	.204
lMTL	74.377	77.123	.294	.964	.345	.181
rHC	59.180	68.429	.179	.865	.396	.203
rIPC	5.212	72.781	.019	.072	.943	.044

R^2=0.198 F=0.777 P= 0.613

Table A4.9(c)
rIPL (seed)

Model	B	Std. Error	β	t	P	Zero-order R
(Constant)	90.748	15.210		5.966	.000	
lIPC	2.169	116.568	.008	.019	.985	.107
rIPC	-6.037	111.736	-.019	-.054	.957	.125
Age	.022	.409	.017	.053	.958	-.007
lMTL	-75.936	128.804	-.245	-.590	.562	-.370
LHC	-19.108	135.394	-.064	-.141	.889	-.191
mPFC	-20.084	49.861	-.110	-.403	.691	-.248
PCC	-1.047	41.594	-.007	-.025	.980	.118
rMTL	-21.468	112.030	-.072	-.192	.850	-.278
rHC	-34.628	195.200	-.077	-.177	.861	-.228

R^2=0.166 F=0.441 P= 0.897

Table A4.9(d)
rdlPFC (seed)

Model	B	Std. Error	β	t	P	Zero-order R
(Constant)	105.718	14.892		7.099	.000	
Age	-.187	.543	-.144	-.344	.734	-.023
lIPC	.460	24.784	.004	.019	.985	-.060
lMTL	91.470	46.464	.687	1.969	.063	.437
LHC	19.366	70.223	.118	.276	.786	.188
mPFC	-51.709	26.484	-.417	-1.952	.065	-.315
PCC	-14.400	40.582	-.161	-.355	.726	-.187
rIPC	33.498	50.558	.226	.663	.515	-.058
rMTL	-29.235	48.624	-.210	-.601	.554	.217
rHC	-23.650	73.362	-.137	-.322	.751	.300

R^2=0.387 F=1.405 P= 0.251
Non-significant results of the regression analysis between habitual cTST (dependent variable) and CEN(seeded) inter-network connectivity with the DMN (table A4.9(a)-(d) above).

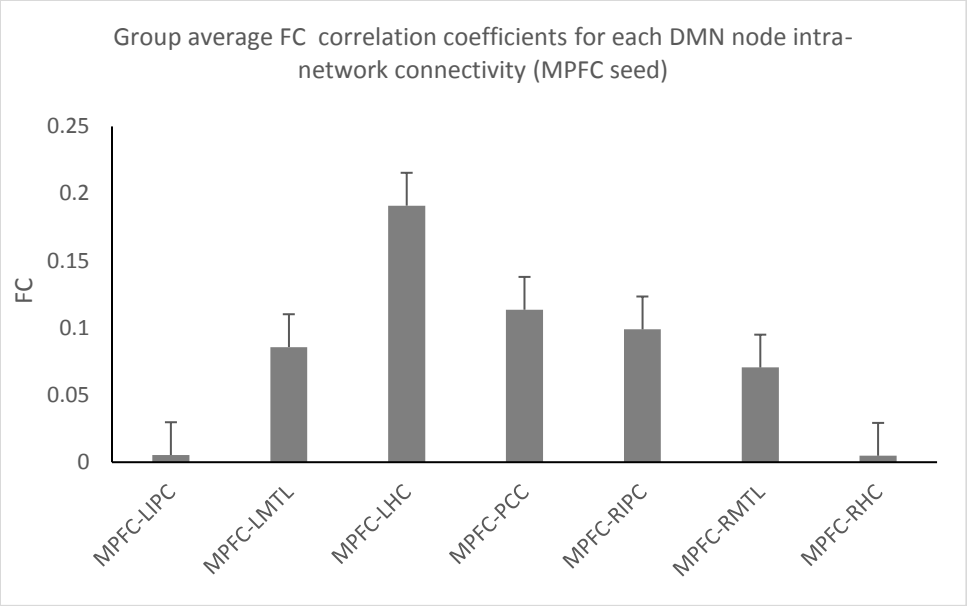


Figure A1.1

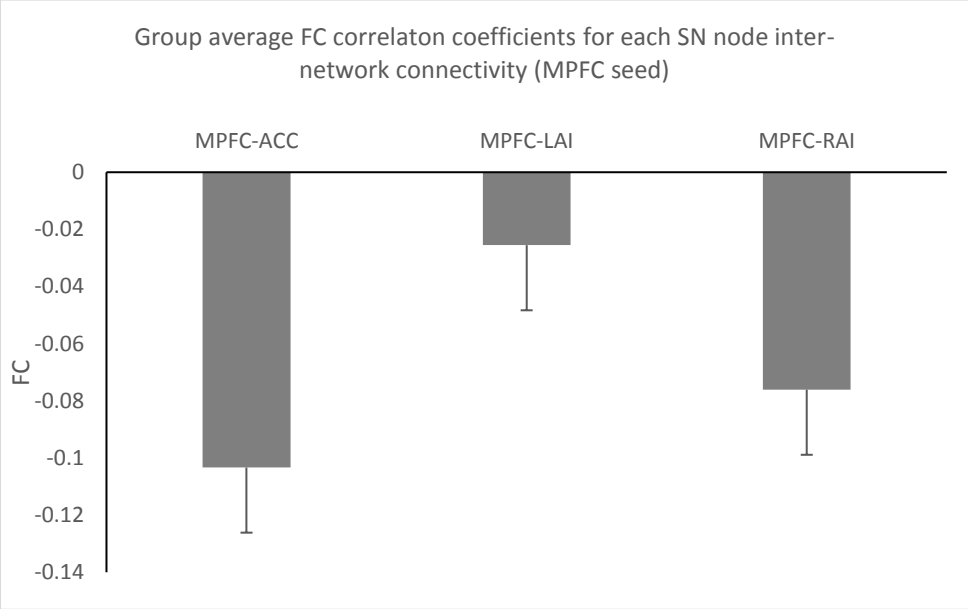


Figure A1.2

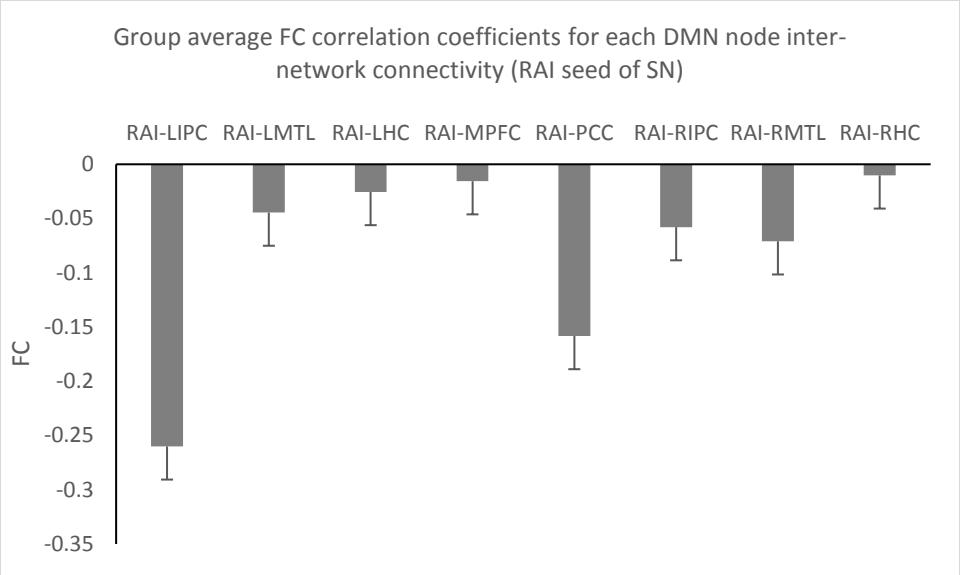


Figure A1.3

Figure A1.1.

Group correlation coefficient intra-network FC for each of the nodes of the DMN to the mPFC seed. Error bars represent standard deviation.

Figure A1.2.

Group correlation coefficient inter-network FC of the SN to the mPFC seed of the DMN. Error bars represent standard deviation.

Figure A1.3.

Group correlation coefficient inter-network FC of the DMN to the rAI seed of the SN. Error bars represent standard deviation.

APPENDIX 2

Basic neurobiology of wakefulness, sleep, and sleep-wake regulation

In this appendix section, I give a brief overview and historical perspective of the neurobiological processes involved in promoting wakefulness, sleep, and wake-sleep transitions in order to inform the reader of the basic neurobiology regulating sleep, awake and sleep-wake transitions, which can complement our knowledge and understanding of sleep status.

The Romania neurologist Constantin von Economo was the first to identify brain regions controlling sleep and wakefulness (Von Economo 1930). He investigated (post-mortum) patients suffering a form of encephalitis that was prevalent in Europe and the United states in the early twentieth century. He discovered in these encephlopathic patients, there were two types of presentation. In the first type patients were excessively sleepy, sleeping for extended periods of time and wakening only to eat and perform bodily functions. In the second type patients had the inverse problem, they were unable to sleep or did not manage to maintain sleep for a significant period (severe insomnia). None of these patients demonstrated any obvious cognitive deficits (Saper et al 2005). On closer examination, Von Economo found the excessive sleepiness was related to lesions in brain stem and posterior hypothalamus. While the insomnia was caused by lesions in the anterior hypothalamus and basal forebrain. Proceeding studies by Moruzzi and Magoun found that the ascending reticular activating system (ARAS) which originates in the brain stem plays a major and essential role in maintaining wakefulness and arousals (Moruzzi

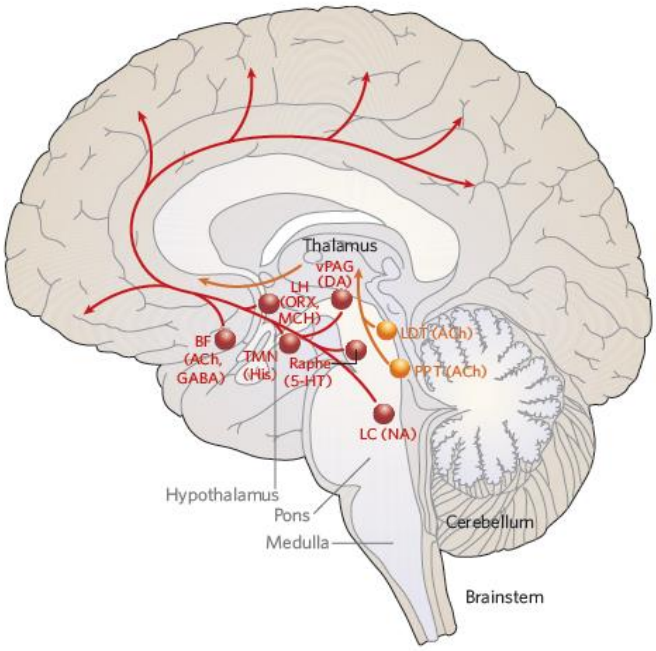
and Magoun, 1949). More recent research (Saper et al 2005) has identified neumerous cell groups and nuclei which contribute to sleep wake regulation in the brainstem, hypothalamus, basal forebrain and thalamus.

Pathways involved in wakefulness:

The ARAS consists of interconnected regions within the brainstem, hypothalamus, basal forebrain and reticular nuclei of the thalamus. ARAS is responsible for promoting wakefulness in humans and animals (Saper et al 2005). Upper brain stem neurons send signals to the ARAS and these are relayed to the thalamus and subsequently from the thalamus to all other cortical regions. The ARAS consists of two major pathways consisting of well defined cell groups with specific neurotransmitters (cholinergic and non-cholinergic). The ascending pathway to the thalamus activates thalmocortical neurons. Two cell groups in the cholinergic pathway which are primary inputs to the thalamic reticular nucleus and to the thalamic relay nuclei are the pendunculo pontine (PTT) and laterodorsal tagmental nuclei (LDT). These nuclei project to the thalamus, hypothalamus and basal forebrain (Jones and Cuello 1989). The firing rate of these two cell groups is fast during the waking state and these cholinergic neurons are also active during REM sleep (Maloney et al 1999).

The second pathway within the ARAS originates from monaminergic neurones in the upper brain stem and posterior hypothalamus including essential neuromodulators such as noradrenaline from the locus coeruleus (LC), serotonin (5-HT) from the dorsal raphe nucleus (DR), dopaminergic periaqueductal grey matter (DA) and histamine from the tuberomamillary neurons (TM). Peptidergic neurons in the lateral hypothalamus (LHA)

which contain melanin-concentrating hormone (MCH) or orexin (also known as hypocretin), and basal forebrain neurons which contain acetylcholine or gamma buteric acid (GABA) extend input throughout the cerebral cortex. Studies have shown lesions in the LHA can produce coma and prolonged forms of sleepiness. The Orexin neurons in the LHA fire the fast in the awake state as do the neurons of the monoaminergic nuclei in this pathway.



Fiugure A2.1: Main neurotransmitters involved in the ascending reticular activating system.: One ascending arousal pathway (Red) includes noradrenergic (NA) neurons in the locus coeruleus (LC), serotonergic (5-HT) neurons in the raphe nuclei, histaminergic (His) neurons in the tuberomammillary nucleus (TMN) and dopaminergic (DA) neurons in the ventral periaqueductal grey matter (vPAG). This pathway receives contributions from neurons in the lateral hypothalamus (LH), which contains orexin (ORX) and melanin-concentrating hormone (MCH), as well as from basal forebrain (BF) neurons that contain acetylcholine (ACh) and gamma-aminobutyric acid (GABA). A second ascending arousal pathway (orange) comprises cholinergic neurons in the pedunculopontine nucleus (PPT) and laterodorsal tegmental nuclei (LDT) that activate thalamic relay neurons resulting in cortical activation. (from Seral et al 2005).

Pathways involved in promoting sleep:

The neurons of the GABAergic ventrolateral preoptic area (VLPO) and median preoptic neucleus (MnPO) and more active during sleep than in wakefulness and contain sleep promoting cells. They contain the inhibitory neurotransmitters galanin and GABA (Saper et al., 2005; Sherin et al., 1996; Szymusiak and McGinty, 2008). Lesions of the VLPO causes insomnia.

Wake promoting activity from neurons in the ARAS and lateral hypothalamic regions can be inhibited via projections from the VLPO and MnPO to the those wake promoting cell groups (Sherin et al., 1998; Suntsova et al., 2007). Conversly the neuromodulators of the ARAS can inhibit the sleep promoting neurons of the VLPO and MnPO (Gallopin et al., 2000; Manns et al., 2003). Saper et al 2010 proposed the flip-flop circuit which describes a sleep-wake switch based on the mutal inhibitory influence between the ARAS and the VLPO.

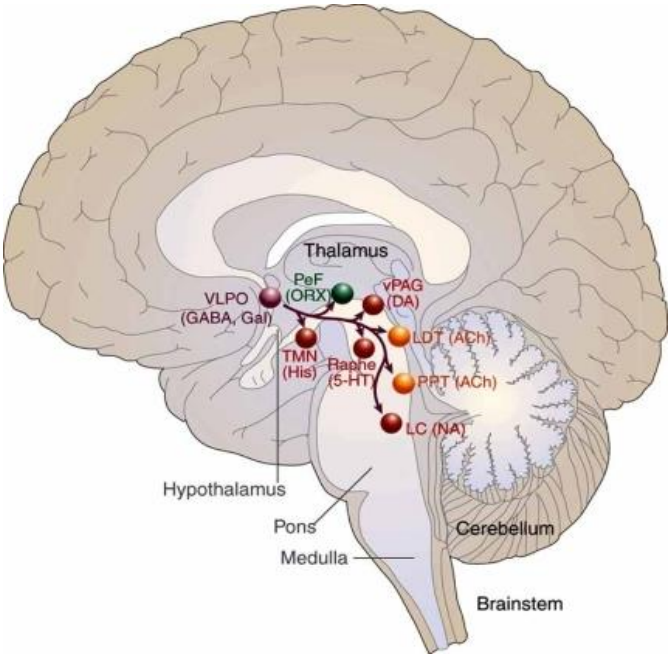


Figure A2.2 The neuronal projections involved in sleep promoting pathways. The ventrolateral preoptic nucleus (VLPO) inhibits the monoaminergic cell bodies (red) such as the tuberomammillary nucleus (TMN), the ventral periaquaductal gray matter (vPAG), the raphe and the locus coeruleus (LC). It also innervates neurons in the lateral hypothalamus (LHA; green), including the perifornical (PeF) orexin (ORX) neurons, and cholinergic (ACh) interneurons (yellow), the pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT). (from Saper et al(2005)).

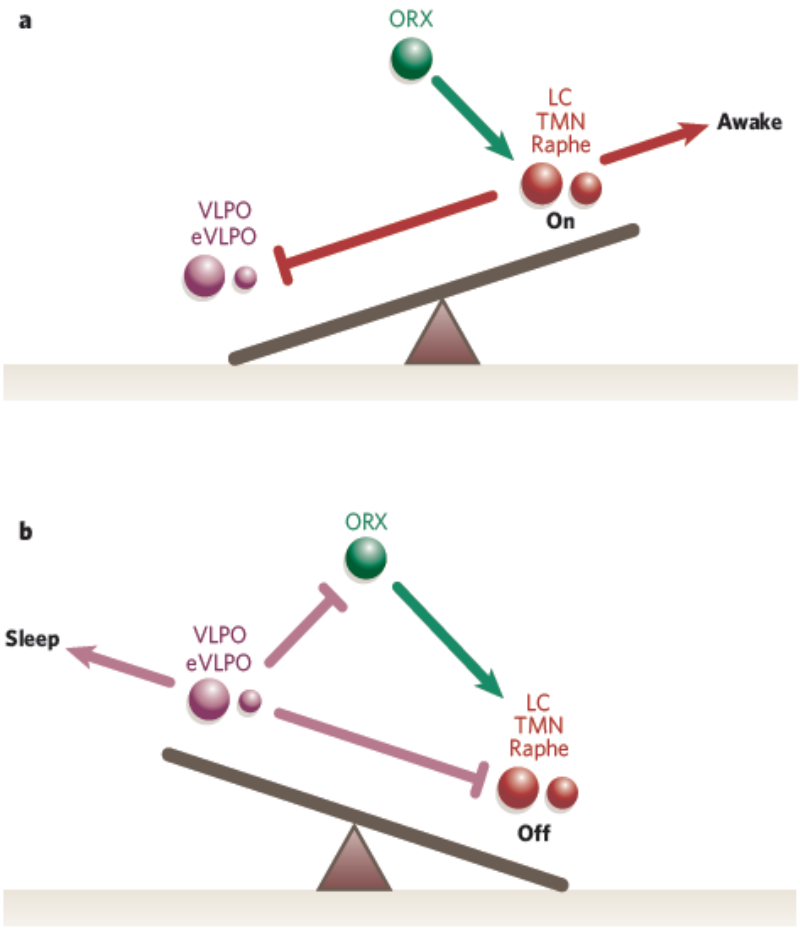


Figure A 2.3 The diagram of the the flip-flop switch model. The flip-flop switch prevents the existence of intermediate states between sleep and arousal, but instead produces abrupt transitions between awake and sleep states. a) when awake monoaminergic nuclei (red) inhibit VLPO neurons (purple) and indirectly prevent inhibition with the ORX neurons (green). b). During sleep state the VLPO inhibits the monoaminergic and orexin neuronal cell groups. from Saper et al (2005).