

Investigating dynamic functional connectivity during NREM sleep using combined EEG-fMRI

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A thesis submitted to the University of Birmingham for the degree of

DOCTOR OF PHILOSOPHY

School of Psychology
College of Life and Environmental Sciences
University of Birmingham
April 2016

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Abstract

Brain activity recorded with EEG during sleep is shown to be dynamic. However the relationship between this dynamic activity and the brain's functional architecture is not well understood. The aim is to investigate the sensitivity of functional magnetic resonance imaging (fMRI) functional connectivity (FC) in the assessment of dynamic changes in brain function with sleep stage, focussing on the FC of intrinsically connected networks (ICNs) underlying functionality. Firstly the dynamic capability of resting-state fMRI signal fluctuations to calculate FC is investigated, highlighting the benefits of dynamic FC analysis and the validity of measuring FC using thirty-second epochs. Simultaneous EEG-fMRI was then used to investigate the effects of sleep on ICN FC, showing ICN re-organisation with sleep stage, which is modulated by prior wakefulness and sleep history. Furthermore, the network reorganisation was specific to certain ICNs, providing evidence for their association with sleep-wake behaviour. We also show FC variability between epochs of the same sleep stage, suggesting additional dynamic FC changes across the sleep cycle, a promising area of future investigation. Overall, this work demonstrates that dynamic FC is a useful measure of brain activity during sleep and regionally specific functional brain reorganisation can be used as a sensitive marker of the sleeping brain.

Dedication

This thesis is dedicated to the memory of my beloved father, Geoff Wilson, if it had not been for his unwavering belief in me I would not have aspired to achieve and were it not for the constant support of my mother, Janet Nelder, whose love and wisdom I treasure, I could not have succeeded.

I also profoundly thank Richard Shellam for teaching me that the best things in life are worth waiting for.

Acknowledgements

As an undergraduate psychology student several years ago Dr Andrew Bagshaw gave me the chance to write a review of sleep disorders during the summer break. Unbeknownst to me at the time it would mark the beginning of my interest in sleep research and when a PhD opportunity presented itself in Dr Bagshaw's lab the following year it was an easy decision. I am very grateful to Dr Bagshaw for the amazing opportunity. I would also like to thank him for the research inspiration he has provided and for always allowing me the freedom to develop research ideas and skills. His continued encouragement throughout has been fantastic and without it I would not be submitting.

I would also like to thank and acknowledge the contribution of Dr Stephen Mayhew, whose door is always open for help with any issues or discussion, for which I am very grateful. I also wish to thank Dr Mayhew for his support and positivity particularly during the peer-review process.

I also wish to thank Dr Aimée Goldstone who has been a wonderful source of support, encouragement, inspiration, help, advice and most importantly, laughter for which I am very grateful. Finally I would also like to thank everyone on the 'corridor' who has been so supportive over the years.

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List of Abbreviations

ACC	Anterior cingulate cortex
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AUD	Auditory network
BCG	Ballistocardiographic artifact
BOLD	Blood oxygenation level dependent
CBF	Cerebral blood flow
DAN	Dorsal attention network
DMN	Default mode network
EEG	Electroencephalography
EMG	Electromyography
EOG	Electrooculography
EPI	Echo-planner imaging
EPSP	Excitatory post-synaptic potentials
FC	Functional Connectivity
FC-V	Functional connectivity variability
FEAT	FMRIBs expert analysis tool
FLIRT	FMRIBs registration tool
fMRI	Functional magnetic resonance imaging
FSL	FMRIB software library
GABA	<i>Gamma</i> -Aminobutyric acid
GLMM	Generalized linear mixed-model
HIP	Hippocampal network
HRF	Haemodynamic response function
ICA	Independent component analysis
ICN	Intrinsically connected network
IPSP	Inhibitory post-synaptic potentials
LFP	Local field potential
LH	Left hippocampus
LINS	Left insula
LIPL	Left inferior parietal lobe
LIPS	Left interparietal sulcus
LLV	Left lateral visual cortex
LLV	Right lateral visual cortex
LMC	Left motor cortex
LMF	Left middle frontal gyrus
LMTL	Left medial temporal lobe

LPH	Left parahippocampal gyrus
LPV	Left primary visual cortex
LST	Left superior temporal gyrus
LT	Left thalamus
MANOVA	Multivariate analysis of covariance
MELODIC	Multivariate exploratory linear optimised decomposition into independent components
MNI	Montreal neurological institute
MR	Magnetic resonance
NREM	Non-rapid eye movement sleep
PET	Positron emission tomography
PFC	Prefrontal cortex
REM	Rapid eye movement sleep
RETROICOR	Retrospective correction of physiological motion effects in fMRI
RH	Right hippocampus
RINS	Right insula
RIPL	Right inferior parietal lobe
RIPS	Right interparietal sulcus
RMC	Right motor cortex
RMF	Right middle frontal gyrus
RMTL	Right medial temporal lobe
ROI	Region of interest
RPH	Right parahippocampal gyrus
RPV	Right primary visual cortex
RS	Resting-state
RST	Right superior temporal gyrus
RT	Right thalamus
SAL	Saliency network
SENS	Sensorimotor network
SENSE	Sensitivity encoding
SMA	Sensory motor area
STD	Standard deviation
SWA	Slow wave activity
SWS	Slow wave sleep
TE	Echo time
THAL	Thalamus network
TR	Repetition time
VCG	Vectorcardiogram
VIS	Visual network

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Wilson, R. S., Mayhew, S. D., Rollings, D. T., Goldstone, A., Przedzik, I., Arvanitis, T. N., & Bagshaw, A. P. (2015). Influence of epoch length on measurement of dynamic functional connectivity in wakefulness and behavioural validation in sleep. *NeuroImage*, *112*, 169-179.

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Wilson, R. S., Mayhew, S. D., Assecondi, S., Arvanitis, T. N., & Bagshaw, A. P. (2013).

Functional connectivity within the default mode network: the effect of epoch length.

Organisation of Human Brain Mapping, Seattle, USA

Wilson, R. S., Mayhew, S. D., Assecondi, S., Arvanitis, T. N., & Bagshaw, A. P. (2013). The

effect of epoch length on functional connectivity within the default mode network. *Basic and*

Clinical Multimodal Imaging, Geneva, Switzerland

Wilson, R. S., Mayhew, S. D., Rollings, D. T., Goldstone, A., Przedzik, I., Arvanitis, T. N.,

& Bagshaw, A. P. (2014). Differences in functional connectivity between normal and recovery

sleep in major brain networks. *Organisation of Human Brain Mapping*, Hamburg, Germany

Wilson, R. S., Mayhew, S. D., Rollings, D. T., Goldstone, A., Przedzik, I., Arvanitis, T. N.,

& Bagshaw, A. P. (2014). Differences in Functional Connectivity between normal and recovery

sleep in the default mode network. *SLEEP*, Minneapolis, USA

Chapter 1

INTRODUCTION TO SLEEP

1.1 Overview

On average humans spend a third of their lives sleeping. However the function of sleep remains a mystery and the processes within sleep are also not well understood (Mignot, 2008). Much of our knowledge about sleep has been acquired since the beginning of the 20th century with the development of electroencephalography (EEG) and the discovery of changing EEG activity during sleep (Loomis, Harvey, & Hobart, 1937). Sleep research has continued to expand, incorporating researchers from different disciplines.

Our understanding of sleep function has also benefitted from the study of sleep deprivation, where sleep is restricted and wakefulness prolonged. There are different types of sleep deprivation; acute where the event results in sleep being restricted for a long period of time and chronic sleep deprivation which occurs more frequently but for a shorter duration. However both types can have profound physiological and behavioural consequences,

testament to how important sleep is (Banks & Dinges, 2007) and the organ most affected is the brain (Horne, 1985).

Sleep deprivation has been shown to have a harmful impact on a number of physiological systems, including metabolism and endocrine function in humans (Spiegel, Leproult, & Van Cauter, 1999), with links to higher rates of obesity and diabetes due to its impact on appetite regulation (Knutson & Van Cauter, 2008; Leproult & Van Cauter, 2011). In addition, sleep loss has been shown to impair the human immune system, with one study finding a reduction in the amount of antibody produced following an influenza vaccination after sleep loss (Spiegel, Sheridan, & Van Cauter, 2002). The most severely sleep deprived individuals, such as night shift workers, have also been associated with having poorer cardiovascular health (Knutsson, Hallquist, Reuterwall, Theorell, & Akerstedt, 1999).

Furthermore, sleep deprivation has a detrimental impact on waking neurocognitive behavioural performance, particularly in causing a reduction in attention/alertness, which declines cumulatively as sleep restriction increases (Van Dongen, Maislin, Mullington, & Dinges, 2003). As well as increasing the disruption to processes of memory and learning (Goel, Rao, Durmer, & Dinges, 2009; Stickgold & Walker, 2007). Sleep deprivation has also been shown to negatively impact emotion regulation (van der Helm, Gujar, & Walker, 2010; Walker & van der Helm, 2009) and is strongly association with mental health complaints such as depression (Lopresti, Hood, & Drummond, 2013) and other psychiatric or neurogenerative conditions (Wulff, Gatti, Wettstein, & Foster, 2010). Taken together, these findings suggest a profound physiological function of sleep in maintaining the optimum health throughout most systems of the body.

Importantly sleep problems are also the most commonly reported health complaint among men and women according to a UK government survey (Singleton, Bumpstead, O'Brien, Lee, & Meltzer, 2000) with the two most common symptoms reported being excessive daytime sleepiness and insomnia (Ohayon & Sagales, 2010). Insomnia is the inability to fall or remain asleep over a period of several nights. It affects millions of people as either a primary disorder or comorbid condition, making it the most common sleep disorder (Mai & Buysse, 2008). Together with the high levels of excessive sleepiness reported in the general population often caused by work conditions, poor sleep habits, known clinically as sleep hygiene, as well as some medications (Ohayon & Sagales, 2010; Maurice & Ohayon, 2002), it is clear that sleep problems result in large economic costs to both individuals and society (Hillman, Murphy, & Pezzullo, 2006; Skaer & Sclar, 2010). Indeed, given the importance of sleep the justification and need for further sleep research has perhaps never been greater than in our modern '24-hour society'.

1.2 Why do we sleep?

Several theories have been put forward to explain the reason for sleep (Siegel, 2011), although none successfully encompasses the question entirely, and as such it remains frustratingly unanswered. According to evolutionary biology, sleep must fulfil an important adaptive process which aids genetic reproduction and the advantages outweigh the obvious risk of predation during sleep (Siegel, 2009). Researchers have approached this question by assessing sleep in animals, in which sleep is often defined behaviourally due to the absence of features of neuronal activity, as measured by EEG, which provide characteristic measures of

sleep. The few mammals that have been studied demonstrate a form of sleep, many exhibiting similar behavioural signatures and some even showing similar EEG patterns to humans, including rapid eye movement (REM) and non-REM (NREM) sleep cycles (Siegel, 2011). Sleep behaviours have also been seen in several species of bird, fish, reptile and invertebrate, although the exact behavioural definition of sleep remains contentious in the field of animal research which has mainly focused on criteria for mammalian sleep (Siegel, 2011; Tobler, 2011). However studying mammalian sleep quantity has shown some interesting patterns, such as sleep quantity reduces with increased body size, although there are exceptions to this rule (Allada & Siegel, 2008) and carnivorous mammals also generally sleep more than omnivores or herbivores (Siegel, 2005).

1.2.1 Theories of sleep function

There are three main theories of why animals sleep; cellular restoration, adaptive inactivity and consolidation of learning. Each of these theories can explain important aspects of sleep, and it seems likely that their relative importance will vary with species and development. The cellular restoration theory hypothesises that during wake the cerebral glycogen energy and neurotransmitters stores are depleted through demand and sleep is thus required to replenish (Benington & Heller, 1995) or reverse the build-up of neurotoxic waste (Xie et al., 2013). Adaptive inactivity posits that sleep duration and timing have adapted to optimise the timing of environmental factors such as feeding and mating (Siegel, 2009). Supported by NREM requiring lower brain energy expenditure compared to wake when assessing brain metabolism (Buchsbaum et al., 1989; Maquet et al., 1990), this also suggests a purpose of sleep could be to conserve energy for a more advantageous time. However, similar to the cellular restoration

theory, adaptive inactivity is unable to explain the function of REM in conserving energy given it is a high energy state of brain activity comparable to wakefulness (Siegel, 2011).

It has also been suggested that the function of sleep is to consolidate memory and learning with behavioural evidence showing sleep benefits human memory performance, particularly for declarative memory, tasks requiring recall of factors or episodes (Plihal & Born, 1997; Rasch, Büchel, Gais, & Born, 2007) and procedural memory, a practiced or learnt behaviour (Fischer, Hallschmid, Elsner, & Born, 2002; Plihal & Born, 1997; Walker, 2003). It is understood that these improvements are brought about via sleep-dependent memory consolidation and the associated molecular, cellular and network level processes (Stickgold & Walker, 2007; Stickgold, 2005). However none of the theories of sleep function outlined provides a comprehensive explanation for all aspects of sleep, although the synaptic homeostasis model has been suggested as an integrated approach (Tononi & Cirelli, 2006).

Synaptic homeostasis model

A recent theory incorporating ideas from several theories of sleep function is the synaptic homeostasis theory, in which the restorative synaptic process, known as ‘synaptic downscaling’ occur during slow wave sleep (SWS) in order to: conserve energy, save memory space and enable learning to occur (Tononi & Cirelli, 2006). Processes such as long-term potentiation (LTP) are hypothesised to occur predominantly during wakefulness to encode information, resulting in increased overall synaptic strength which is supported by evidence showing increased neuronal plasticity in wakefulness with genetic expression across the sleep-wake cycle (Cirelli & Tononi, 2000). The downscaling acts as a means of thresholding, preserving the synapses strongly potentiated and removing weak potentiation,

arguably improving signal/noise. See Figure 1.2.1 for a schematic depiction of the theory.

Researchers continue to test hypotheses generated by the theory, however while the precise mechanism of ‘synaptic downscaling’ by SWS remains unknown there is considerable support for the memory function of sleep (Diekelmann & Born, 2010).

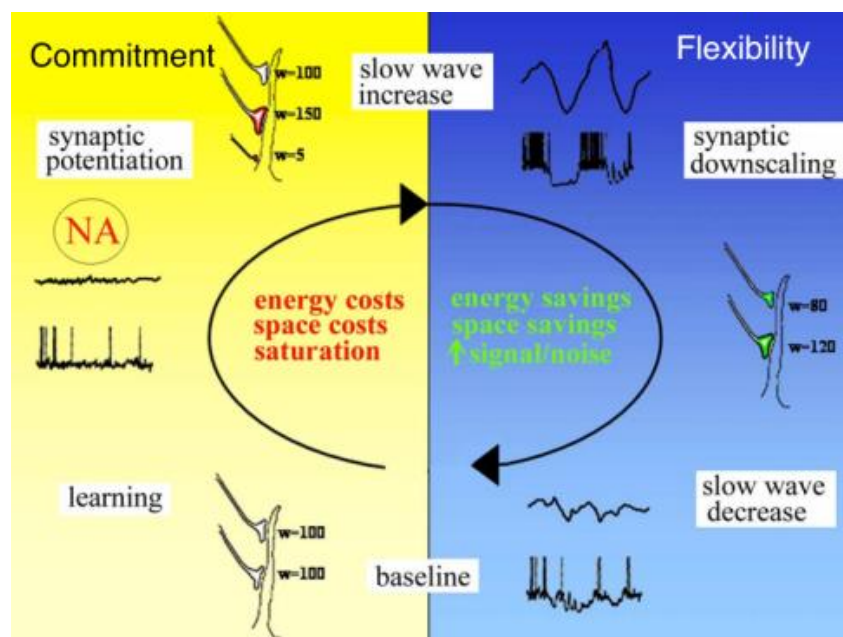


Figure 1.2.1. A schematic depiction of the synaptic homeostasis hypothesis, adapted from Tononi & Cirelli (2006). Wake (yellow) increase in synaptic strength meaning high energy requirement for plasticity. Sleep (blue) triggers slow oscillations ensuring activity is not followed by potentiation, instead synaptic downscaling occurs until it returns to an appropriate baseline.

1.3 Defining human sleep

Sleep is a complex physiological and behavioural phenomenon that is defined behaviourally as a reversible disengagement and unresponsiveness to the environment, including a degree of sleep rebound when sleep is curtailed (Carskadon & Dement, 2011). Classifying the physiological changes that occur during sleep has enabled researchers and clinicians to better define this complex behaviour. Based on discrete physiological parameters provided by EEG, electrooculography (EOG) and electromyography (EMG) two states are known to exist within sleep; REM and non-REM (NREM) (Dement & Kleitman, 1957), see Chapter 2. NREM is itself composed of three distinct stages (N1,N2,N3), while REM is an active state characterised by muscle atonia (AASM, 2007). These two types of sleep are found to alternate, oscillating in an ultradian rhythm every 90-120mins in healthy adults depending on the duration of sleep, resulting in approximately five cycles across the sleep period. This can be visually depicted as a hypnogram (Figure 1.3.1). REM sleep is linked with vivid dreaming, with 80% of individuals woken during REM recalling their dreams (Dement & Kleitman, 1957). NREM contains slow wave activity (SWA) which is associated with sleep homeostasis (Borbély, Baumann, Brandeis, Strauch, & Lehmann, 1981), outlined in more detail below (see section 1.4). Sleep in healthy adults begins initially with NREM after which periods of REM occur. There is also a difference in the quantity of each sleep type throughout the night, with NREM more prevalent at the start with longer periods of REM later in the night (Figure 1.3.1). The sleep criteria are outlined in section 2.1.7.

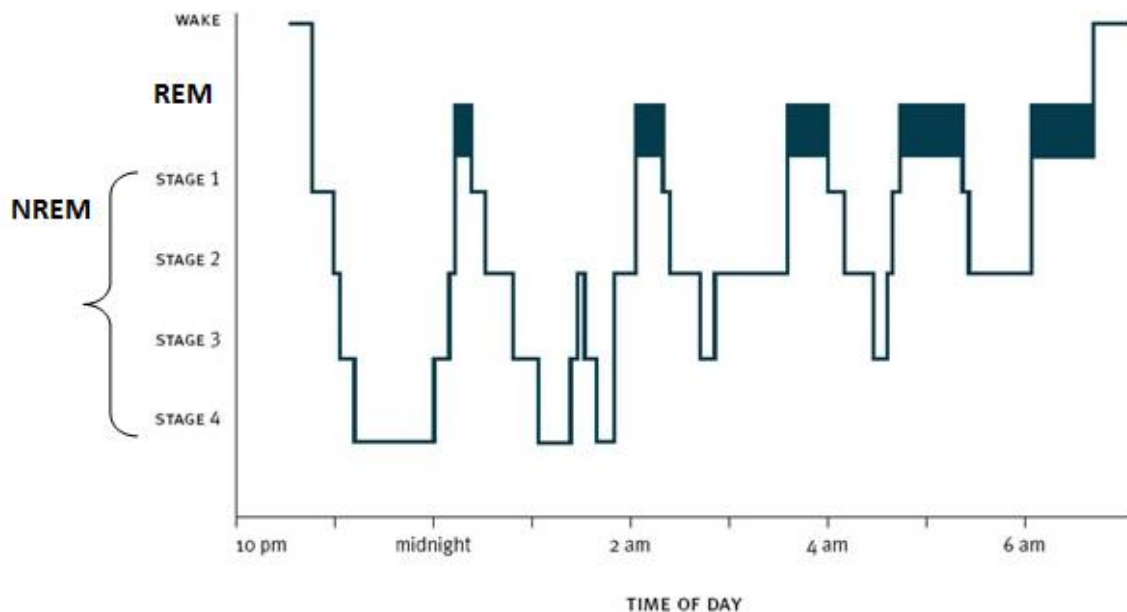


Figure 1.3.1. Schematic hypnogram of sleep cycles across the night in a healthy adult according to the (Rechtschaffen & Kales, 1968)

1.4 Sleep homeostasis: a model of sleep-wake regulation

The most influential theory of human sleep-wake regulation, the two-process model, is outlined below. While there are other models of sleep-wake regulation, these are often highly specialised single-cell, neuronal or cellular network models and are therefore difficult to extrapolate to human sleep-wake behaviour (see Achermann and Borbély (2010) for a review of these). Sleep homeostasis is important for the regulation of the sleep-wake cycle; it is the mediation of sleep pressure or sleep propensity which increases with prolonged wakefulness and reduces with sleep. Borbély and colleagues first modelled sleep homeostasis, known as process-S (Figure 1.4.1), as part of their original two-process model (Borbély, 1982). An important marker of process-S is SWA, defined as low frequency neuronal activity (0.5 to

4.5Hz) recorded during sleep (Borbély et al., 1981; Tobler & Borbély, 1986). The neuronal mechanisms underpinning the homeostatic sleep drive (process-S) are not well understood, although the accumulation of the neuromodulator adenosine with prolonged wake (Halassa et al., 2009), led to speculation that it could be a homeostatic maker of sleep, with the build-up of adenosine increasing the activity of sleep-promoting systems (Saper, Scammell, & Lu, 2005).

The two-process model also incorporates the circadian rhythm, known as process-C (Figure 1.4.1) (Borbély, 1982). Circadian rhythms are controlled by our internal biological clock, maintaining behaviour across the 24-hour period, synchronised by the level of external light and other factors (dietary intake, social cues (Davidson & Menaker, 2003; Mendoza, 2007). Circadian rhythms are discussed in more detail in the following section. According to the two-process model, process-S and process-C interact in order to determine the duration, timing and architectural structure of sleep (Figure 1.4.1). With prolonged wakefulness process-S increases and is then opposed by process-C until the onset of sleep, at which both processes are sleep promoting.

Dissociating the effects of the two processes can be difficult, and requires relatively complex controlled laboratory paradigms, of which the two most common are the forced desynchrony protocol and the constant routine protocol (see section 2.1.5). These can be used to demonstrate that homeostatic and circadian processes can be analysed independently (Dijk & Czeisler, 1995). However, researchers believe both process-S and process-C interact under normal conditions and this interaction is reflected in measures of subjective daytime alertness (Daan, Beersma, & Borbély, 1984) and general neurobehavioural performance (Goel, Van

Dongen, & Dinges, 2011). This has led to other processes being identified and added into the model, including sleepiness-alertness regulation (Åkerstedt & Folkard, 1995; Åkerstedt, Ingre, Kecklund, Folkard, & Axelsson, 2008), inclusive of sleep inertia or process-W which also considers behavioural performance, such as reaction times or subjective sleep measures as important factors in the model. A further process was also identified by Achermann and colleagues, the ultradian rhythm of NREM-REM sleep cycle (Figure 1.4.1). The alteration of these two states is also thought to be linked to the build-up of SWA during NREM counteracted by a REM oscillator triggering a decline in SWA through the night which reduces process-S (Achermann & Borbély, 1990; Achermann, Dijk, Brunner, & Borbély, 1993).

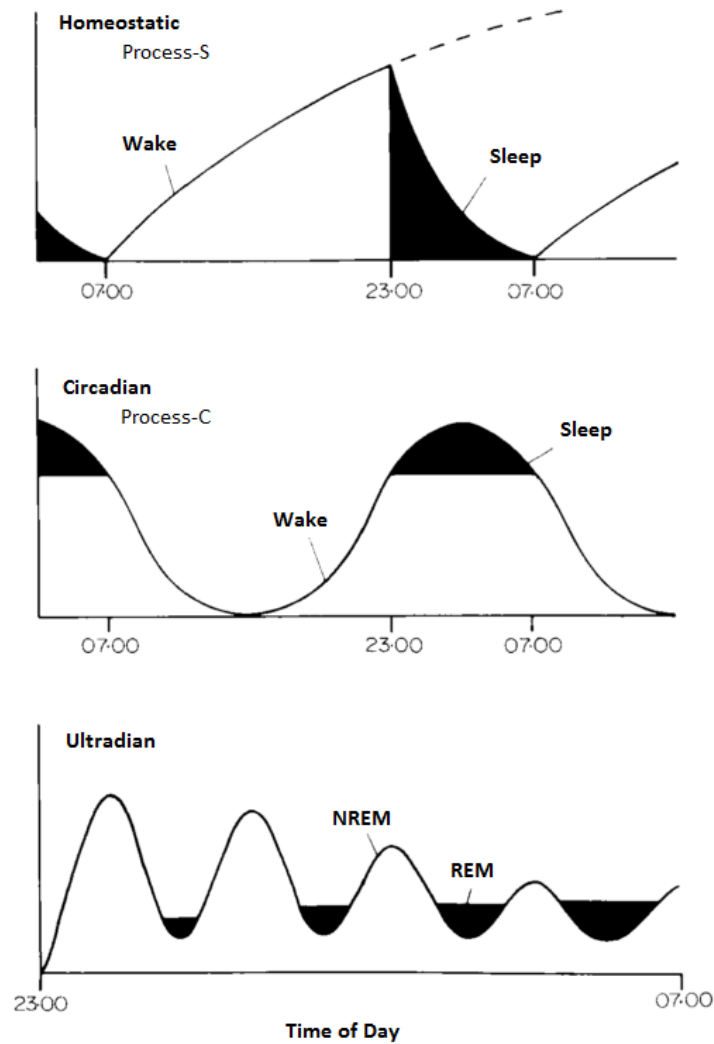


Figure 1.4.1. Schematic representation of the three processes of sleep propensity involved in regulating sleep. Process-S is represented in the top picture, followed by process-C and finally ultradian process. Adapted from Borbély and Achermann, 1992.

1.4.1 Brain structures involved in sleep-wake regulation

This section will briefly review the anatomical structures and neurotransmitter systems supporting both sleep and wakefulness in humans. The wake-promoting system was first identified as a collection of brain regions in the midbrain reticular formation associated with waking EEG (Moruzzi & Magoun, 1949), since then multiple pathways from the pons to the forebrain have been identified, known as the ascending reticular activating system (ARAS) (Saper, Lu, Chou, & Gooley, 2005). According to the ARAS the collective firing of neurons in the brainstem, hypothalamus and basal forebrain that project via the different cholinergic and noncholinergic pathways inputting to the thalamus and cerebral cortex are understood to regulate wake and underpin EEG activity (Jones, 2004). These systems were more active during wake and REM compared to NREM (Steriade, McCormick, & Sejnowski, 1993a), with wake impaired and replaced with a sleep-like state when these areas are lesioned (Saper, Lu, et al., 2005). The ARAS is schematically outlined in Figure 1.4.2A and for a more comprehensive review of the evidence see (Brown, Basheer, McKenna, Strecker, & McCarley, 2012; Saper, Fuller, Pedersen, Lu, & Scammell, 2010). However these systems are thought to be inhibited by a sleep-promoting system, which results in the switching to a sleep state (Saper et al., 2010) and a change in the EEG activity.

The sleep-promoting system is a collection of neurons in the ventrolateral preoptic nucleus (VLPO) within the anterior hypothalamus shown to activate during NREM sleep (Sherin, Shiromani, McCarley, & Saper, 1996) and lesions to the area disrupted sleep regulation (Lu, Greco, Shiromani, & Saper, 2000). The VLPO neurons project to ARAS regions including the TMN, raphe, LC, PPT and LDT, in addition to the hypothalamus and brainstem via inhibitory

neurotransmitters such as gamma-Aminobutyric acid (GABA) (Sherin, Elmquist, Torrealba, & Saper, 1998), thus reducing the activity of the ARAS regions which promote wake. Figure 1.4.2B schematically outlines this system. The ‘flip-flop switch model’ has been put forward to explain the changes between wake and the sleep states (Saper, Chou, & Scammell, 2001), see Figure 1.4.3. According to the flip-flop model the neuro-circuits of the ARAS and sleep-promoting VLPO maintain and reinforce one another resulting in quick transitions, supported by animal VLPO lesions which show a pronounced alteration in switching between states (Lu et al., 2000). REM sleep has also been associated with neurons firing in the pons and forebrain, with the existence of pons lateral geniculate and occipital cortex (PGO waves) (Sakai & Jouvet, 1980). Research continues into these REM sleep pathways, however the lateral and posterior hypothalamus appears to be important in the regulation of both REM and NREM sleep and the flip-flop switch model could also explain the transitions between the types of sleep in the ultradian rhythm (Saper, Scammell, et al., 2005).

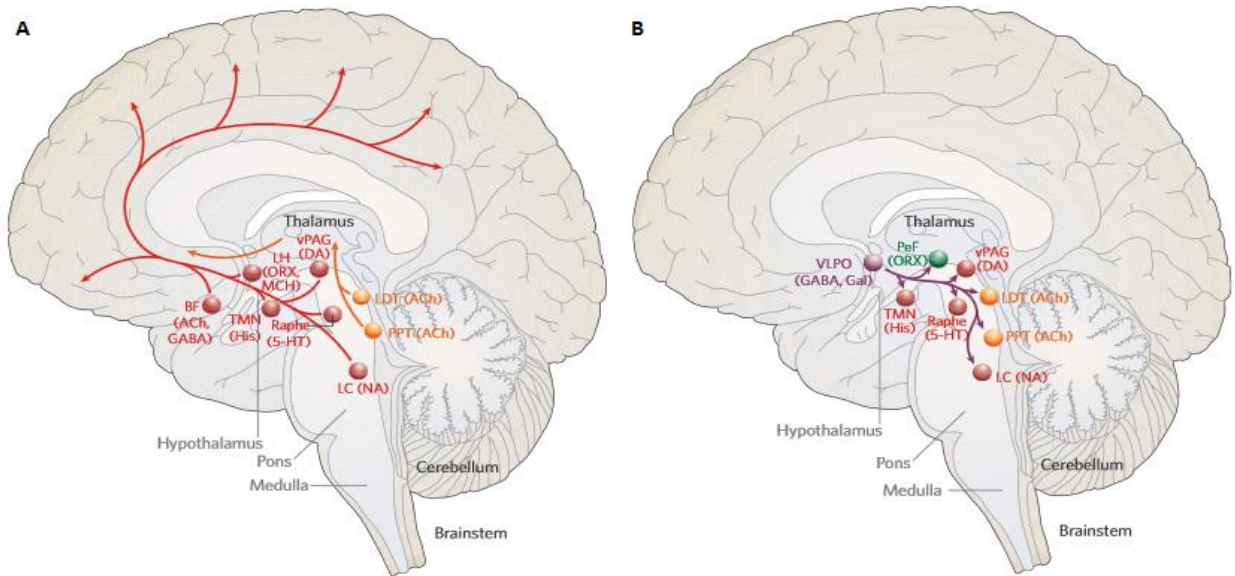


Figure 1.4.2. Schematic of the main anatomy and neuromodulators of the ascending arousal system. **A)** The cholinergic (ACh) pathway (*orange*) input to the reticular nuclei of the thalamus from the pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT). A second pathway (*red*) connects the cerebral cortex via histamine (His) projections from the tuberomammillary nucleus (TMN), dopamine (DA) from raphe, serotonin (5-HT) from the dorsal and median raphe nuclei and noradrenaline (NA) from the locus coeruleus (LC). Lateral hypothalamus (LH) with orexin (ORX) or melanin concentrating hormone (MCH) and the basal forebrain (BF) containing γ -aminobutyric acid (GABA) or ACh also input into the pathway. **B)** Schematic of the GABA ventrolateral preoptic nucleus (VLPO) projections. Monoaminergic cell groups (*red*) including the TMN, raphe and LC. Neurons from the LH and perifornical (PeF) ORX (*green*) and ACh interneurons (*yellow*) from the PPT and LDT. Taken from Saper, Scammell, & Lu (2005).

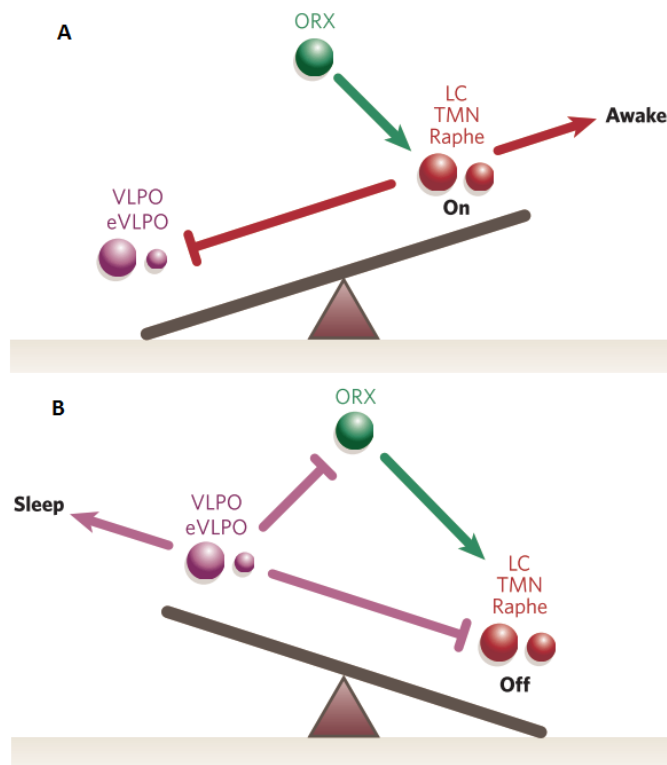


Figure 1.4.3. Schematic of the flip-flop model of wake-sleep state switch. A) In wake monoaminergic nuclei (red) inhibit the VLPO (purple) and stop the inhibition indirectly with the ORX neurons (green). B) In sleep the VLPO neurons inhibits the monoaminergic and orexin neurons. Adapted from Saper, Scammell, & Lu (2005).

Suprachiasmatic nucleus (SCN) and circadian rhythms

Another region within the hypothalamus important for sleep is the SCN. It is the ‘master clock’ in the mammalian brain which regulates all circadian rhythms. Its neurons fire in a 24 hour cycle, set daily by the light exposure received via retinal ganglion cells in the eye and melatonin hormone released from the pineal gland at night which collectively maintain the sleep-wake cycle (Reppert & Weaver, 2002; Saper, Scammell, et al., 2005). The SCN influences cells throughout the body via a molecular transcriptional feedback loop of

translational proteins, including BMAL1 and CLOCK which regulate the transcriptions of genes and control the release of other proteins; Per1, Per2, Per3, Cry1 and Cry2, thus maintaining a molecular oscillation underpinning the circadian rhythm (Reppert & Weaver, 2002). This circadian molecular process is believed to underpin process-C of the two-process model (see section 1.4). The study of circadian oscillators is known as chronobiology and the field focuses on the internal rhythms which regulate the sleep-wake cycle.

Thalamocortical connections

During wake the thalamus relays sensory information to the cortex, however during sleep increased cortical feedback maintains the level of cortical activity via thalamic projections (Destexhe & Sejnowski, 2009). Sleep is characterised by SWA across the cerebral cortex and these oscillations are regulated by important thalamocortical and corticothalamic neuronal loops (Steriade et al., 1993a). In particular sleep spindles, transient neuronal events which form a feature of NREM sleep and are prevalent in stage two sleep, are generated in the thalamic reticular nucleus by GABAergic neurons, which synchronise with inhibitory post-synaptic potentials via glutamatergic projections to the cortex, known as corticothalamic projections (Steriade, 2000). However intracortical networks have also been shown to generate SWA (>1 Hz) in vitro (Sanchez-Vives & McCormick, 2000), suggesting the importance of intrinsic cortico-cortico activity alongside thalamic connectivity during sleep. See Steriade (2014) for a review of the evidence.

Chapter 2

METHODS TO MEASURE AND INVESTIGATE SLEEP

2.1 Measures of sleep

It is clear that sleep is a complex multi-faceted behaviour generated and maintained in the brain (Hobson, 2005). Therefore to investigate and understand sleep in humans non-invasive methods are required to study sleep behaviour and importantly the brain activity that occurs during sleep. There are many different aspects of sleep which can be investigated and quantified using various methods; these include questionnaires, behavioural assessment, actigraphy and neuroimaging, and are often used in combination. However polysomnography (PSG) is the most commonly used and is often considered the ‘gold-standard’, although other measures are often assessed independently or simultaneously with PSG in order to determine additional aspects of sleep. We review many of these measures of human sleep, focusing on the neuroimaging of sleep in section 2.2.

2.1.1 Sleep diary

A daily sleep diary kept over two weeks can be one of the most useful tools in the assessment of sleep behaviour and as an aid in the diagnosis of sleep disorders, due to the low cost and ease of use. Sleep diaries usually ask the person to record the time of their bedtime, the duration of any daytime naps, nightly awakenings and the time of their immediate wake-up. Standardised sleep diaries are used clinically (Carney et al., 2012), although research often requires variations depending on the study hypothesis with some diaries also asking additional questions. For an example of a sleep diary that was used in the studies described in this thesis, see Appendix A. Sleep diaries are often used in combination with other measures such as questionnaires (Smith & Wegener, 2003).

2.1.2 Sleep questionnaires

Self-reported sleep factors such as overall sleep quality and daytime sleepiness are important, subjective, behavioural features of sleep-wake function, with each questionnaire measuring a different aspect and having a threshold of clinical significance based on validated samples, thus allowing their common use as screening tools. However there are many questionnaires used to assess different aspects of sleep behaviour and in different populations (Abrishami, Khajehdehi, & Chung, 2010; Spruyt & Gozal, 2011), although we only review those used in the thesis.

One of the most widely used subjective measures is the Pittsburgh Sleep Quality Index (PSQI), a questionnaire designed to evaluate subjective sleep quality over the previous month by asking questions regarding sleep latency and duration, as well as sleep efficiency. The

higher the PSQI score the worse the sleep quality of the person (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). The Epworth Sleepiness Scale (ESS) which assesses the likelihood of falling asleep in various situations of daily life is also commonly used, and is designed to measure daytime sleepiness (Johns, 1991).

Another subjective questionnaire used both clinically and in research, is the Insomnia Severity Index (ISI) which was originally developed as an outcome measure for insomnia research based on DSM-IV and International Classification of Sleep Disorders criteria for insomnia (Smith & Wegener, 2003). The PSQI, ESS and ISI are each thought to measure subtly different aspects of sleep behaviour (Buysse et al., 2008; Mondal et al., 2013; Smith & Wegener, 2003). In addition, the fatigue Severity Scale (FSS) assesses the impact of fatigue on behaviour (Krupp, 1989). Daytime fatigue and sleepiness are thought to be separate phenomena (Shen, Barbera, & Shapiro, 2006), as well as independent consequences of sleep disorders (Hossain et al., 2005) and other medical conditions (Merkelbach et al., 2006). A copy of each of these questionnaires is available in Appendix B.

2.1.3 Actigraphy

Actigraphy is the 24-hour recording of physical activity, over a period of at least several days and typical two weeks (Van Someren, 2007), which enables us to distinguish between wake and sleep on the basis that movement is generally reduced during sleep compared to wake. The small light-weight device, usually worn on the wrist contains sensors which detect and record movement as well as light (Martin & Hakim, 2011). The activity ‘count’ can then be displayed (Figure 2.1.1) and analysed further. Physical activity or movement is generally

sampled every few seconds and recorded in 1 minute epochs depending on the specifications of the device. In addition to physical activity some devices can also measure light exposure which can be used to further assess the individual's sleep environment and habits. Once the data is recorded algorithms analyse the activity counts over time to give details of circadian rhythmicity, and sleep measures such as total sleep time (TST) for a particular night, on average across the weeks and the variability in TST. These TST sleep measures are used in the thesis. TST as recorded by actigraphy has been shown to correspond with PSG classification of sleep in the healthy adult population (Ancoli-Israel et al., 2003; Sadeh, 2011), with a 90% concordance to EEG classification (Sadeh, Hauri, Kripke, & Lavie, 1995).

Furthermore, measures of sleep efficiency can also be obtained, which is the ratio of overall time spent in bed verses time spent sleeping. Due to actigraphy being unobtrusive and cost effective it has become widely used in the study of sleep in the healthy population and some clinical populations (Sadeh, 2011), often recorded in conjunction with sleep diaries (Morgenthaler et al., 2007). However movement measured with actigraphy is a surrogate marker of sleep, it is unable to distinguish between sleep stages and is not suitable without additional measures of sleep (Martin & Hakim, 2011).

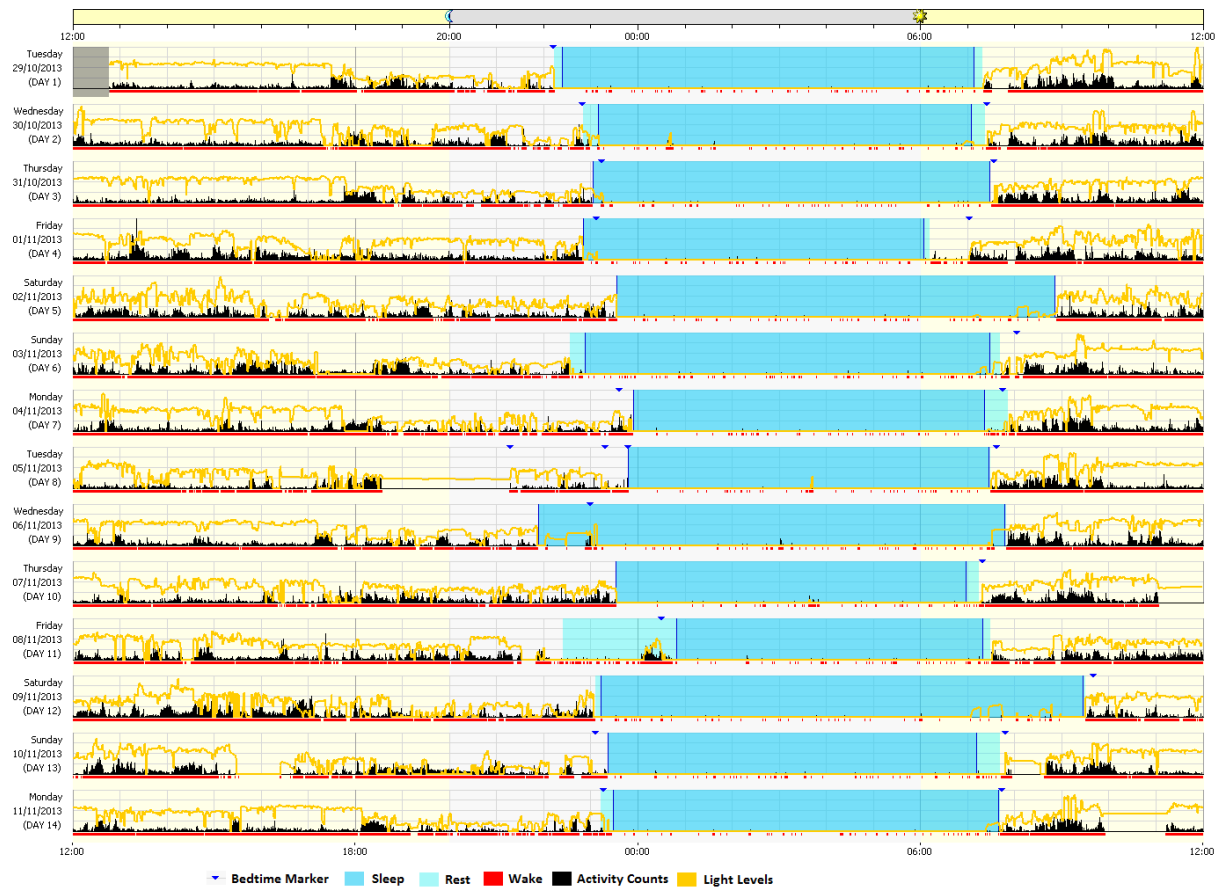


Figure 2.1.1. Example of wrist actigraphy measured for two weeks in a healthy adult with a consistent sleep-wake pattern. Every row represents a separate day and the time of day is indicated on the horizontal axis. The periods in blue represent sleep where there was a low activity count (activity is in black) compared to the high levels recorded during daytime, waking activity enabling the distinction to be made. Single subject taken from data recorded as part of the thesis.

2.1.4 Psychomotor Vigilance Task (PVT)

As previously discussed (see section 1.1) sleep deprivation can be used as an experimental protocol to investigate the function of sleep and the PVT has been a widely used neurobehavioral assessment in these experiments. PVT is a simple task that quantifies reaction times and target hits/misses, which have been shown to closely link with waking

alertness in relation to sleep status, with longer reaction times or lapses associated with sleep deprivation in a ‘dose-response’ fashion when measured repeatedly (Belenky et al., 2003; Van Dongen et al., 2003). It is both reliable and validated in capturing the dynamic changes in performance following sleep loss (Dorrian, Rogers, & Dinges, 2005). As such the PVT can be used as a measure of sleep status via the assessment of waking behavioural performance.

2.1.5 Controlled laboratory experiments

Researchers investigating human sleep have often used controlled laboratory experiments, particularly with sleep deprivation protocols to determine the functionality of sleep (see section 1.1). Controlled experiments are also used by chronobiologists investigating the circadian pacemaker. Furthermore, by controlling many of the factors influencing circadian systems including amount of sleep, social cues and light exposure present in the external environment, researchers are able to directly measure the impact of these factors on the sleep-wake cycle, behaviours, core body temperature and melatonin levels (Czeisler & Buxton, 2011; Herman, 2011). One protocol used is phase-shifting, which is analogous to jet-lag, whereby the subjects sleep-wake phase is moved by the impact of zeitgebers (e.g. light exposure) and the behavioural or biological impact investigated (Rosenthal et al., 1990). Another is constant routine, where the subject is simply monitored across the 24-hour cycle under a fixed routine. The long-night protocol explicitly investigates the impact of long periods of darkness on humans, however more extreme protocols such as forced-desynchrony, where the day length is varied, all attempt to further understand the circadian clock (Herman, 2011).

2.1.6 Multiple Sleep Latency Test (MSLT)

The MSLT was developed to measure the drive of daytime sleepiness (Carskadon et al., 1986). Sleepiness is related to the time taken to fall asleep, a short sleep onset indicating a greater drive to sleep or greater homeostatic sleep pressure ('process-S'). MSLT is used to determine levels of excessive daytime sleepiness in clinical populations, together with other measures such as sleep history (Littner et al., 2005). Subjects are monitored in a controlled laboratory environment, given the opportunity to nap at 2-hour intervals throughout the daytime and the speed with which they fall asleep is measured in each nap opportunity. Subjects are assessed with PSG (see section 2.1.7) during the protocol in order to classify sleep onset. Average sleep onset over the nap opportunities of under five minutes is associated with sleep deprivation in the normal population and a clinically significant threshold for a sleep pathology such as narcolepsy (Carskadon et al., 1986).

2.1.7 Polysomnography (PSG)

The visual scoring of PSG in thirty second epochs remains the 'gold-standard' classification and measurement of sleep (Rechtschaffen & Kales, 1968), although this has since been updated (AASM, 2007). It involves collectively assessing EEG, a scalp measure of the electric field of the brain's neuronal activity (Figure 2.1.2), EMG and EOG which provide a measure of muscle tone and eye movements respectively, both recorded using electrodes placed directly on the skin (Figure 2.1.3). The methodology of these techniques will now be introduced, focusing on EEG before discussing the PSG sleep staging classification.

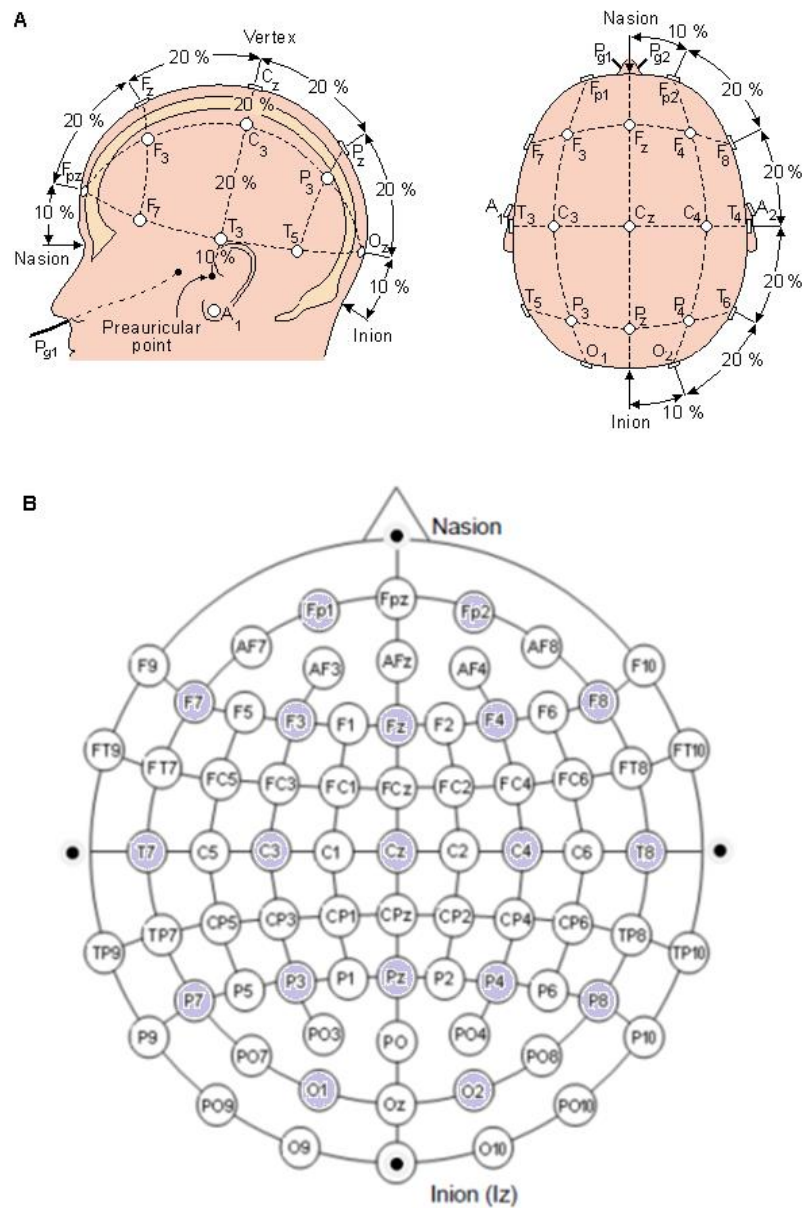


Figure 2.1.2. EEG electrode placement. A) The international standard 10-20 system. B) A 62 channel EEG electrode cap with the electrodes used in the sleep montage (purple). F=frontal, Fp= frontal polar, T=temporal, C=central, P=parietal, O=occipital. Odd numbers denote the left side of the head, even numbers denote right and Z the midline.

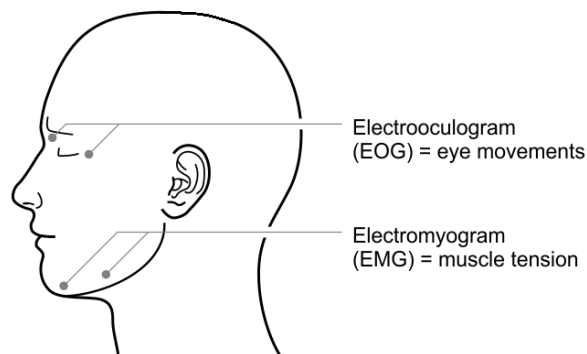


Figure 2.1.3. EOG and EMG electrode placement used for ‘gold-standard’ PSG sleep recording. Adapted from (Stickgold & Walker, 2009).

Electroencephalography (EEG)

EEG non-invasively measures the electric fields generated by the brain’s neuronal activity which conduct to the scalp. The EEG signal is comprised of synchronised post synaptic potentials, both excitatory postsynaptic potentials (EPSP) and inhibitory postsynaptic potentials (IPSP) across billions of neurons. These post-synaptic potentials are created by extracellular current flow, around neuronal cell bodies, for example as a result of action potential transmission. EEG primarily reflects activity of cortical pyramidal neurons which are oriented in parallel alignment close to the scalp, such that they produce electric dipoles of similar spatial orientation, allowing the synchronous electric field to summate across these neurons (Niedermeyer & Lopes da Silva, 2005). Figure 2.1.4 shows a schematic of a pyramidal neuronal dipole whose current is detected by the EEG electrode on the scalp, although in reality the signal will consist of multiple dipoles with the same orientation.

The spontaneous activity, recorded with millisecond temporal resolution by the scalp signal, displays discrete oscillations and temporal and spatial synchrony across neurons which equates to increased signal amplitude (μV). However the EEG signal cannot distinguish whether increased signal amplitude is due to a greater number of activated neurons or simply greater synchrony of activity within a particular neuronal population. The scalp EEG signal cannot be assumed to faithfully represent all of the underlying brain activity because of signal losses affected by the brain's geometry. While the brain itself, the scalp and the skull are electrically conductive, they also scatter the signal, acting as a volume conductor. This means that the signal from deeper brain structures can be weakened due to the distance and interference with other sources of activity. These issues limit the spatial resolution of EEG by creating uncertainty in the ability to localise the underlying neuronal sources of EEG activity.

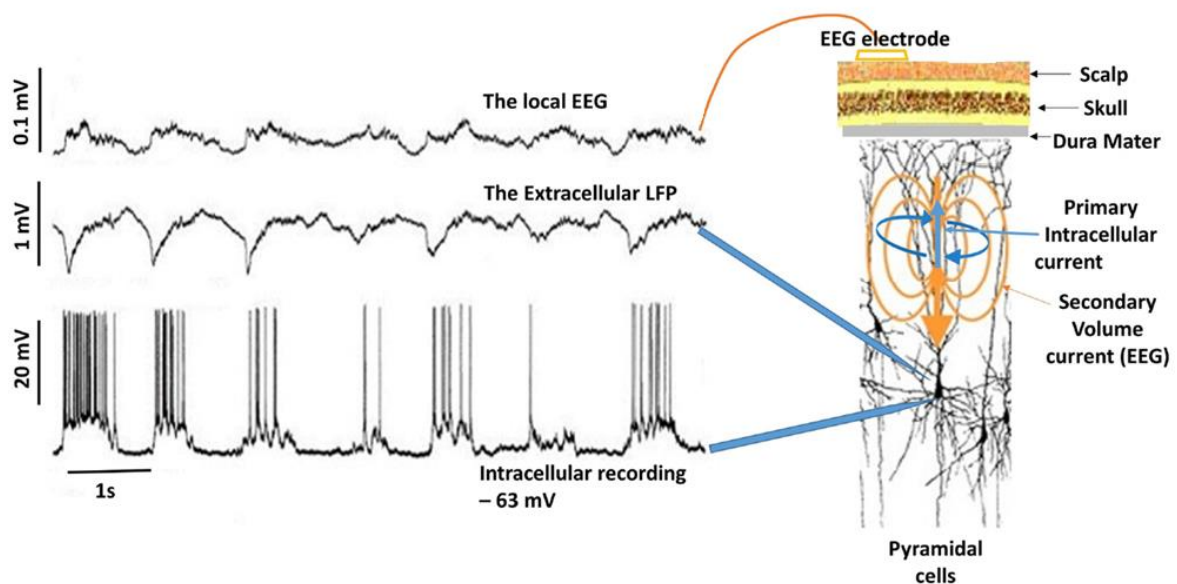


Figure 2.1.4 Pyramidal neuron activity recorded in the somatosensory cortex of a cat during deep sleep. The schematic demonstrates the intracellular and extracellular recordings which underlie but do not map directly onto the local EEG signal due to volume conduction. The EEG records the dipole current on the conductive scalp. Adapted from (Adjamian, 2014; Contreras & Steriade, 1995).

Recording EEG requires placement of individual electrodes, or an EEG cap containing the electrodes, on the head after the area of scalp is cleaned using isopropyl alcohol and conductive jelly applied. The position of the electrodes is standardised by the EEG society (Jasper, 1958) and known as the 10-20 international system, see Figure 2.1.2. The analogue signal from each electrode is amplified and filtered before being digitised and recorded by the computer software. A montage is the grouping of electrodes to allow relative signal measurements to be made and there are two types; bipolar and referential. The referential montage is frequently used, referring the signal of each electrode to that of a common reference electrode AFz. However there are various artefacts which can negatively affect the quality of the EEG signal, these include physiological artefacts such as muscle activity, cardiac-pulse and breathing, in addition to external artefacts such as electrical equipment interference. In order to extract and minimise the ‘noise’ from the EEG signal the signal is amplified and a sampling rate, typically 500Hz for regular EEG is used. The hardware also filters the data as an important signal processing step, usually sampling between 0.016-250 Hz. These processing steps are important before the EEG can be further analysed.

PSG sleep staging

Staging sleep requires the visual scoring of EEG recorded as part of PSG (AASM, 2007; Rechtschaffen & Kales, 1968). It relies on the various parameters used to quantify the EEG signal (Benbadis, 2005). These include; the *amplitude* of the signal's voltage (μV), the *frequency* of which a signal waveform repeats (Hz) and the *duration* of the discharge (ms). The frequency of the EEG is conventionally grouped into bands (Hz), see Figure 2.1.5 for an illustration of the different oscillations. During sleep the EEG signal decreases in frequency

with alpha attenuating with the onset of sleep, an important criterion for stage classification (AASM, 2007; Rechtschaffen & Kales, 1968). The different frequencies correspond to different functional processes or states in the brain and can be generated by different regions (Niedermeyer & Lopes da Silva, 2005), for example the synchrony of the EEG signal has been shown to directly relate to the inhibition of thalamocortical cells (Contreras & Steriade, 1995).

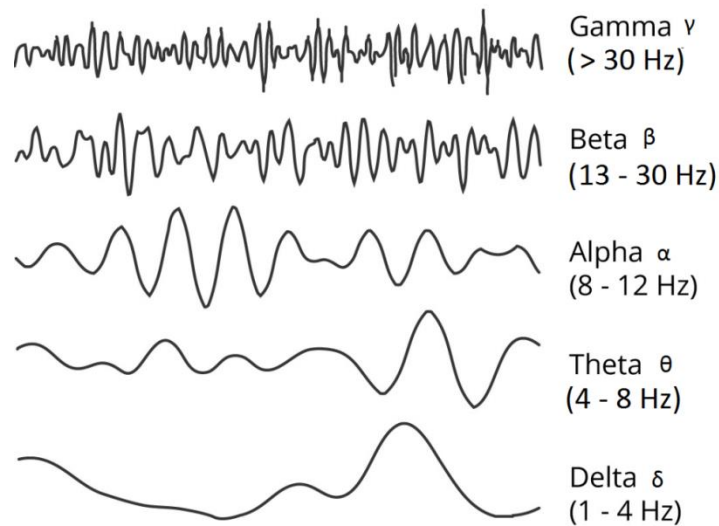


Figure 2.1.5. Illustration of the main EEG rhythms by frequency band

Additionally when dealing with paroxysmal discharges observed during sleep, other more qualitative EEG signal parameters such as; *morphology* the shape and configuration of the discharge, *spatial location* and the delay between events known as the *latency* of the discharge are also important. The presence of EEG discharges (<0.5 seconds) unique to sleep are also used to visually classify stage, these include vertex sharp waves (VSW), K-complexes and

sleep spindles, see Figure 2.1.6. VSW are short contouring waves over the central regions found in N1 (AASM, 2007). K-complexes are negative sharp waves followed by a positive component and periodically occur during slow frequency (<1 Hz) N2 stage of sleep (AASM, 2007; Amzica & Steriade, 2002; Colrain, 2005). Sleep spindles are discrete activity usually between 12-14 Hz and their presence indicates a N2 sleep classification (AASM, 2007).

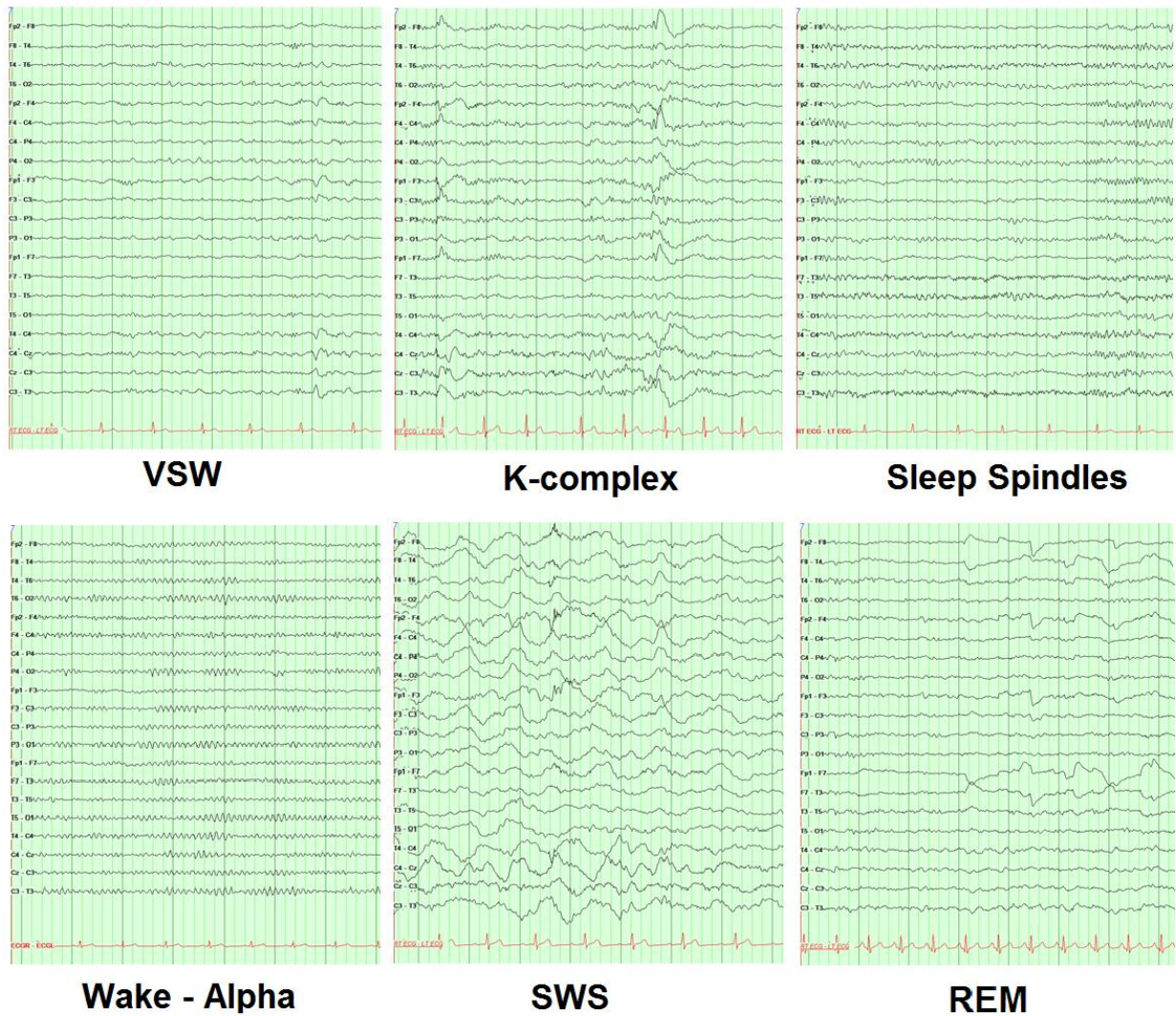


Figure 2.1.6. Examples from a healthy adult of EEG discharges (black signals) associated with sleep and also used to classify sleep stage. VSW= Vertex sharp waves, SWS =slow wave sleep (N3), REM= rapid eye movement sleep.

While staging NREM sleep is largely based on visually inspecting the EEG, staging REM requires the simultaneous recording of EMG and EOG. EOG detects the rapid eye movements and EMG records the irregular transient muscle activity and low baseline activity under the chin which are both unique to REM (AASM, 2007). From the beginning of the recording each thirty second epoch of PSG is then staged as either wake, N1, N2, N3 or REM based on the changing EEG parameters of frequency, amplitude and morphology outlined in Table 2.1.1, as well as EOG and EMG measurements. This can then be plotted visually as a hypnogram providing an indication of sleep architecture (see Figure 2.1.7). However thirty seconds is chosen for historical reasons, previously enabling the sufficient visual inspection on older paper generated EEG systems, although arguably thirty seconds is an arbitrary defined classification, it remains the standard used by researchers and clinicians (AASM, 2007).

Table 2.1.1. Average EEG characteristics for wake and the sleep stages in a healthy adult based on old and new classification criteria

Old Sleep Stage Classification (<i>Rechtschaffen & Kales, 1968</i>)	New Sleep Stage Classification (<i>AASM, 2007</i>)	% Time Asleep	EEG Frequency (Hz)	Amplitude (μ v)	EEG Wave Type
Awake	W	N/A	>12	<30	Beta
Relaxed	W	N/A	8-12	<50	Alpha
Non-REM Stage 1	N1	5%	4-8	50-100	Theta, VSW
Non-REM Stage 2	N2	45%	4-8	50-150	theta, spindles, K-complexes
Non-REM Stage 3	N3 (SWS)	12%	2-4	100-150	delta, theta
Non-REM Stage 4		13%	0.5-2	100-200	delta, theta
REM	REM	25%	>12	<30	Beta

Stages are typically scored in 30-second epochs

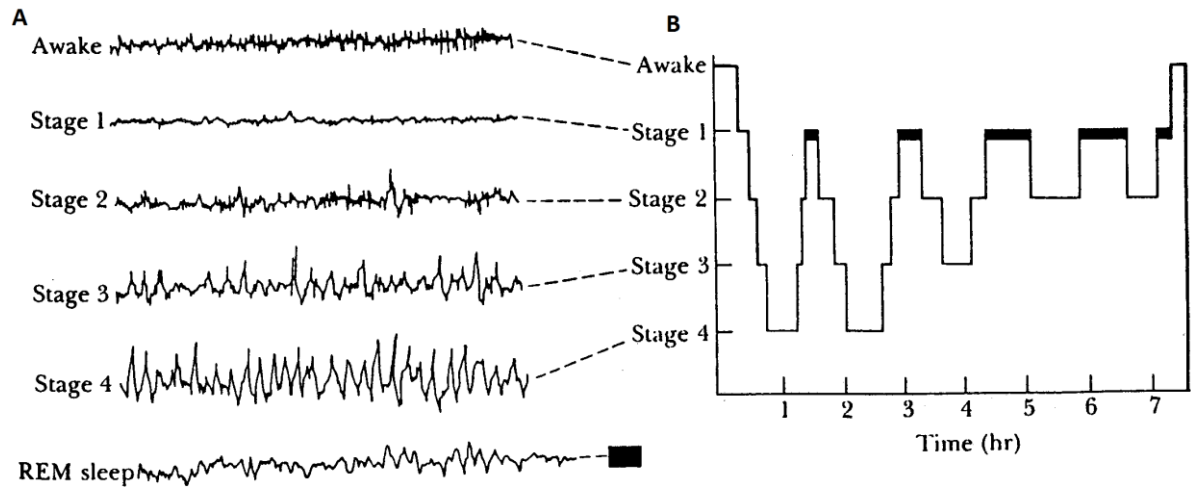


Figure 2.1.7. EEG sleep recording based on Rechtschaffen and Kales classification. A) Example of EEG signal morphology for each type of sleep stage. B) Hypnogram representing the classified sleep architecture over time.

2.2 Measuring sleep using Neuroimaging

The field of neuroimaging is largely concerned with observing and quantifying the highest level of neuronal organisation within the brain. There are multiple techniques which can be applied to investigate a range of human behaviours, including sleep, and the field has greatly expanded in recent years with the development and improvement of methods designed to assess brain function. Neuroimaging of sleep has generally been approached in four ways. Firstly by comparing the brain's activity between sleep states (NREM & REM) or between NREM sleep stages and during wakefulness. Secondly, by focusing on the activity of transient neuronal events occurring during sleep, such as sleep spindles, SWA and K-complexes. Thirdly, comparing sleeping brain activity between a group of either sleep

disordered or other clinical population with that of a healthy population. Finally, the severity and duration of sleep deprivation during wakefulness has also been assessed by comparing the brain's activity during various cognitive tasks between non-deprived and sleep deprived individuals. This thesis primarily focuses on the literature applying the first approach.

The main functional imaging techniques relevant to sleep research are briefly reviewed in this section, beginning with a description of the basic mechanisms underpinning each neuroimaging technique, although it is not designed to be a comprehensive theoretical or methodological overview. Instead, the aim is to provide the reader with a basic understanding of each neuroimaging method and review the important sleep research findings that have applied functional neuroimaging techniques.

2.2.1 Positron emission tomography (PET)

PET is an imaging technique using radiolabeled tracers to generate images of the brain which was pioneered for use clinically in the 1980s. Radioisotopes are injected into the subject and absorbed by the tissue. [^{18}F] fluorodeoxyglucose (^{18}F -FDG) isotope is a commonly used clinical radiotracer to view regional cerebral glucose metabolism, a marker of neuronal activity in the brain through the oxidation of glucose. The spatial distribution of the radioisotope is detected by the PET system by mapping radioactive decay of the isotopes and is often combined with anatomical x-ray computed tomography (CT) known as PET/CT. PET using the ^{18}F -FDG radiotracer has been used to investigate the metabolic changes occurring regionally in the brain during sleep, with studies generally showing that slow-wave sleep metabolism is reduced compared to wake, while periods of REM show higher levels of

metabolic glucose demand than NREM, often similar to wake (Buchsbaum et al., 1989; Maquet et al., 1990).

Another radiotracer used with PET is ^{15}O -water, which is a measure of regional cerebral blood flow (rCBF). rCBF levels in the brainstem, thalamus and basal forebrain have been shown to reduce during SWS compared with wake (Braun et al., 1997) in agreement with the metabolism changes reported above. Additionally rCBF has been shown to increase during REM in areas that were activated during a waking motor reaction task in task trained subjects, suggesting possible activity of memory processes during REM (Maquet et al., 2000). However PET possesses a reduced temporal resolution due to the slow tracer and blood flow kinetics (on the order of minutes). Therefore with the development of alternative functional neuroimaging methods such as MRI in the late 1990s, the application of PET has reduced. The early PET studies greatly contributed to our spatiotemporal understanding of the functional changes during sleep (Maquet & Phillips, 1998), and PET retains the advantages of physiological flexibility and specificity, with radioisotopes existing which can be used for several different physiological processes. For a comprehensive literature review of studies investigating sleep using PET see Maquet (2000).

2.2.2 Functional magnetic resonance imaging (fMRI)

fMRI is based on the phenomenon of nuclear magnetic resonance (NMR) whereby the nuclei of certain atoms 'spin' within an applied magnetic field, which creates a precession around the magnetic field. Hydrogen atoms that are present throughout human body have this property, behaving as dipoles or small magnets. Thus when placed within the static

homogenous magnetic field (B_0 , measured in Tesla (T)), of an MR scanner a proportion of the hydrogen nuclei align with the B_0 field and either precess in parallel (low-energy state) or anti-parallel (high-energy state), reaching an equilibrium of a small, net magnetization. When an additional radiofrequency (B_1) electromagnetic pulse (RF pulse), at a specific frequency that the atoms are resonant with (Larmor frequency), is applied via a radiofrequency coil the hydrogen nuclei absorb this energy, flip their precession state according to the flip angle and spin at the frequency of the RF pulse. Once this RF pulse is removed the atoms emit the acquired energy and relax back to their equilibrium energy state. This process is known as relaxation and the hydrogen atom's emitted energy is measured as MR signal via the receive coil of the MR scanner. Key parameters of the MR sequence are the time between the RF excitation pulses (repetition time (TR)) and; the overall time between the excitation pulse and the data acquisition (the echo time, TE).

There are two types of relaxation with characteristic time constants which are tissue dependent. T1 recovery relates to the relaxation back to alignment with the static field, while T2 decay is dephasing of a population and dependent on local magnetic field properties. Relaxation times are tissue dependent due to their different chemical composition, thus by mapping the relaxation rate spatially it enables us to determine the tissue composition of an MR image (Jezzard, Mathews, & Smith, 2001). However by altering the sequence parameters (TE, TR) for the different relaxation types we can examine different tissue contrasts. A T1-weighted contrast has a short TE and intermediate TR, sensitive to the number of protons within each voxel, and is used for anatomical imaging.

Precise measurement of MR signals from specific spatial locations in the tissue is obtained using 3-dimensional magnetic field gradients which are applied, in addition to the main B_0 field, to tune the resonant MR frequency in a spatially dependent manner. Spatial locations are quantified using voxels, cubic areas that make-up an image, typically millimetres in dimension.

Functional imaging (fMRI) methods examine dynamic changes in the brain's activity and are commonly acquired using the $T2^*$ -weighted contrast. The $T2^*$ -weighted contrast is commonly used to assess brain function due to it being sensitive to the amount of oxygenated haemoglobin in the blood, which changes with metabolic demand. This is known as the blood oxygenation level dependent (BOLD) signal (Ogawa, Lee, & Kay, 1990), whereby increased levels of oxyhaemoglobin in the blood result in increased levels of BOLD signal. The BOLD signal provides an indirect measure of neuronal activity and is understood to primarily reflect local field potentials (Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001) due to neurovascular coupling. Neurovascular coupling is the complex interaction between synaptic signalling and the metabolic action of neurons and glial cells which leads to increased blood flow to a brain region to support the metabolic demand of neuronal signalling (Attwell & Iadecola, 2002).

Initially, task-based fMRI studies mapping brain function defined the canonical haemodynamic response function (HRF), as the typical BOLD response to an external stimulus. A schematic HRF is shown in Figure 2.2.1. There is an initial dip, primary positive response and a post-stimulus undershoot before it returns to baseline. The total temporal length of the signal depends on the duration of stimulation, but to an impulse stimulus can be

between 12-18 seconds. Although the measurement of BOLD signal has considerable dynamic potential (Jezzard et al., 2001) its temporal resolution is limited by the sluggish haemodynamic response. In order to determine regional voxel-wise differences in brain activity further statistical analysis is required depending on the hypothesis and experimental protocol. In task-based fMRI the BOLD response is statistically compared between two conditions; this can be between different experimental conditions, population groups or comparing against a baseline or so-called ‘resting activity’. The regional map of BOLD activity is the statistical difference in BOLD signal between these conditions.

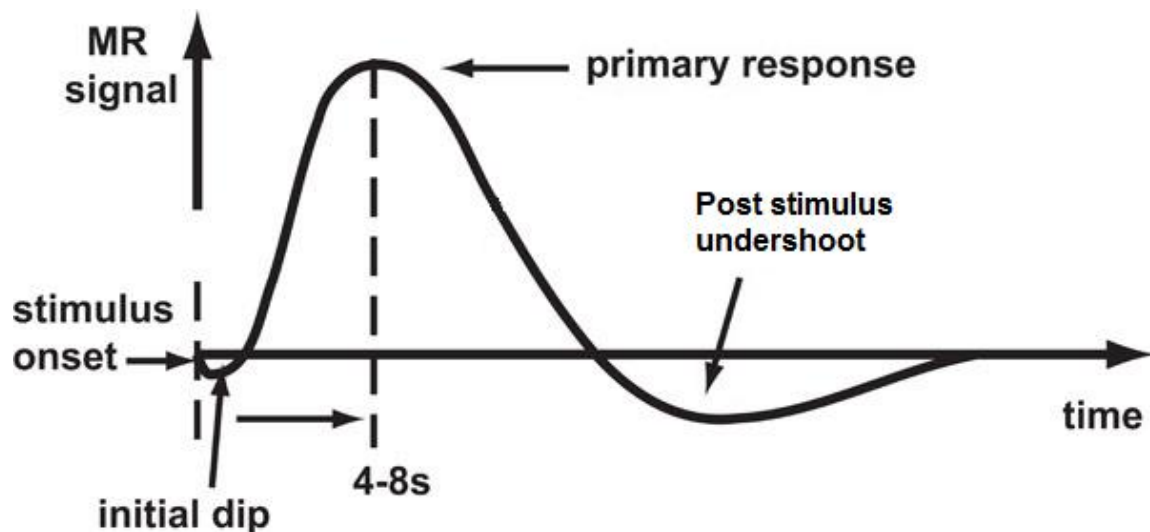


Figure 2.2.1. A schematic of the BOLD haemodynamic response function (HRF) to a visual stimulus during a task-based fMRI protocol.

2.2.3 Multimodal imaging: simultaneous EEG-fMRI

Multimodal imaging combines measures that differ in spatiotemporal resolution or neurophysiological origins; see Figure 2.2.2 for the spatiotemporal resolution of the techniques reviewed. The simultaneous recording of EEG and fMRI has proved to be an informative method, combining the high spatial resolution of MRI (millimetres) with the high temporal resolution of EEG (milliseconds). Both brain imaging tools are thought to relate to local field potential aspects of the underlying neuronal activity, EEG as a direct measure of electrical LFPs and fMRI as an indirect measure via neurovascular coupling (Logothetis et al., 2001). The integration of EEG and fMRI data has been approached in three ways; prediction, constraints and fusion. Integration through prediction uses activity measures derived from the EEG temporal signal to make predictions about the concurrent changes to the fMRI BOLD signal. Integration through prediction is particularly useful in the investigation of sleep which is defined based on EEG signatures, combined EEG-fMRI allows researchers to investigate the regional correlations between the haemodynamic response and EEG activity (Czisch et al., 2004). Integration through prediction is used in this thesis when investigating sleep, using sleep-classification of the EEG signal to arrange the corresponding fMRI data based on sleep stage, an effective method in sleep research (Duyn, 2012). Integration by way of constraints is the opposite of prediction, where the EEG dipole or source location is constrained to an estimate of the fMRI localised activity. Fusion by integration is more complex and poses challenges for the ‘true’ integration of the data, requiring the data to be linked with neuronal activity on the same temporal scale (Kilner & Friston, 2010).

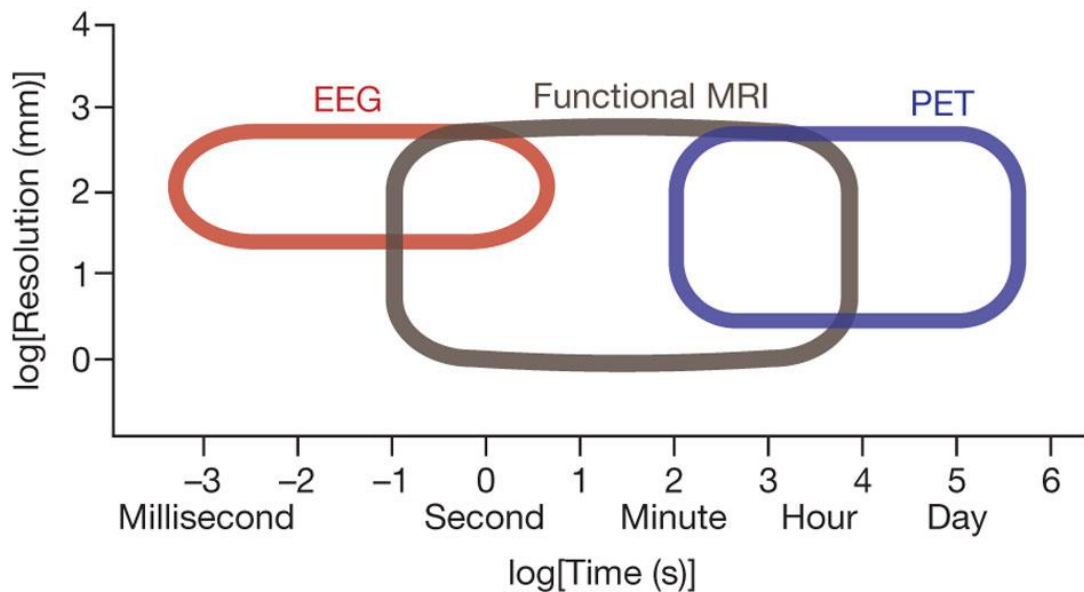


Figure 2.2.2. Diagram of the brain imaging techniques reviewed in respect to their spatial and temporal resolution. Taken from Meyer-Lindenberg (2010).

Simultaneous recording of EEG-fMRI

Specialist instrumentation and set-up are required for the experimental recording of EEG-fMRI due to the magnetic fields within the MR environment and their impact on the EEG equipment and signals (Allen, 2010). Therefore, an MR-compatible EEG cap with nonferrous silver electrodes is used, along with shielded amplifiers, twisted electrode wires and resistors attached to each electrode to minimise the risk of heating during MRI. The two main EEG artefacts that occur during combined EEG-fMRI are the pulse artefact, also known as the ballistocardiographic (BCG), and the MR gradient artefact (Allen, 2010), both arising due to variations in magnetic flux through the EEG electrodes and wires inducing artefactual currents on top of the neuronal signals. The BCG artefact is believed to be primarily induced by the heart-beat causing a small head-nod and movement of blood through the scalp which

moves the EEG electrodes within the MR magnetic field. The MR gradient artefact is induced by high frequency (kHz) switching of magnetic gradients. Both of these EEG artefacts can be satisfactorily corrected after EEG-fMRI recording by template-based, linear subtraction methods (Allen, Josephs, & Turner, 2000).

EEG acquisition during fMRI typically uses amplifiers with a large dynamic range and a sampling rate of $\geq 5\text{kHz}$, which is an order of magnitude higher than normal EEG experiments, to prevent saturation of the EEG amplifiers during scanning and accurately measure the gradient artefacts (Allen et al., 2000). It is also important to synchronise the MR scanner clock to the EEG sampling rate in order to precisely record each artefact repetition and ensure optimal correction (Mandelkow, Halder, Boesiger, & Brandeis, 2006a).

Sleep EEG-fMRI methodological challenges

In order to investigate sleep we must first identify and classify it into stages, which requires EEG (see section 2.1.7), hence combined EEG-fMRI is necessary in fMRI studies investigating spatial patterns of changes in brain activity during sleep and we review the results of these pioneering studies in the next section. However given the necessity for often physically uncomfortable EEG-fMRI, in addition to the reliance on the subject's ability to sleep in the noisy MR scanner, the data attrition rate is high for sleep studies and a significant methodological challenge. As a result of this all previous sleep studies reviewed in this thesis have very small sample sizes included in the final analysis due to the difficulty recruiting successfully sleeping subjects. For example $n=14$ is the largest sample size of sleep EEG-fMRI data used in subsequent analysis in the literature and this was from an original sample of $n=25$ (Dang-Vu et al., 2008). While many other studies have even smaller sample sizes of

sleeping subjects, including n=6 (Larson-Prior et al., 2009; Uehara et al., 2013) or n=10 (Miyauchi, Misaki, Kan, Fukunaga, & Koike, 2009; Spoormaker et al., 2010; Uehara et al., 2013) from significantly larger recruited samples.

EEG-fMRI and pioneering sleep studies

One of the first studies to use EEG-fMRI found reduced BOLD signal or ‘deactivation’ in sensory, as well as non-sensory cortices, when comparing the response to acoustic stimulation during NREM sleep to during wake (Czisch et al., 2004). The authors theorised that the cortical deactivation or suppression of activity could be a fundamental signature of sleep, reflecting a state of reduced sensory stimulation (Czisch et al., 2004). Indeed the response to auditory tones during NREM was also assessed in association with sleep events including sleep spindles, K-complexes (Dang-Vu et al., 2011) and slow waves (Schabus et al., 2012). The activation response to an auditory stimulation was shown to differ depending on whether spontaneous spindle activity was present, with reduced widespread cortical activation when coinciding with spindles (Dang-Vu et al., 2011). Larger activation was also shown in the thalamus, auditory cortex and inferior frontal gyrus when a k-complex was prompted by an auditory stimulation (Dang-Vu et al., 2011). Similar BOLD response differences have also been shown for slow oscillations, where the activity to an auditory response is dependent on the temporal phase of the oscillation (Schabus et al., 2012). These results highlight the complex interaction between induced and spontaneous activity during sleep.

A further fMRI study showed reduced activity during NREM compare to wake in multiple areas including the cerebellum, cingulate cortex, insula and thalamus, although this activity reduced further in SWS compared to wake in the ACC, left insula and hippocampus, showing

less brain activity during sleep (Kaufmann et al., 2006). These findings are in line with PET data, showing reduced global metabolism and rCBF during SWS sleep compared to wake (Braun, 1997). However, fMRI assessment of activity during SWA found regional overlap with regions known to activate during wakefulness, suggesting that SWA could reflect ‘wake-like’ activity and that sleep is not characterised by complete BOLD deactivation (Dang-Vu et al., 2008). This led to the examination of spontaneous sleep events in more temporal detail, often focusing on events within the stage or state (Caporro et al., 2012; Stern et al., 2011).

BOLD activity during individual eye movements in REM has been assessed compared to the tonic state of REM, finding activation in visual areas, thalamus and midbrain structures (Miyauchi et al., 2009; Wehrle et al., 2005). Additionally the patterns of BOLD response to spontaneous spindles and K-complexes were compared to each other in order to determine the regions contributing to the transient activity (Caporro et al., 2012). They found that both sleep spindles and K-complexes resulted in activation of the thalamus, while spindles also showed activation in the posterior cingulate and other cortical areas and K-complexes showed activation of the temporal, occipital and frontal lobes (Caporro et al., 2012). VSW have also shown increased BOLD signal in sensorimotor regions (Stern et al., 2011).

fMRI has also be used during wake to understand sleep by comparing the BOLD signal between sleep deprived and non-deprived individuals, thus enabling the identification of regions affected by sleep debt. Studies have used task-based designs testing performance and regional activity in various cognitive domains including; working memory (Chee et al., 2006), attention (Chee et al., 2008), visual short-term memory (Chuah & Chee, 2008), emotional processing (Yoo, Gujar, Hu, Jolesz, & Walker, 2007) and other higher level cognitive abilities

(see Chee & Chuah (2008) for a review of the fMRI evidence). Results from tasks known to activate the frontal lobes, namely divided attention and verbal short-term memory, largely show increased activation following SD compared to normal sleep suggesting the presence of compensatory mechanisms (Drummond, Gillin, & Brown, 2001; Drummond et al., 2000). However this response increase to SD is not consistent and depends on the cognitive task (Drummond et al., 1999) and more recently has been shown to depend on the individuals' sensitivities to the effects of sleep deprivation (Chuah, Venkatraman, Dinges, & Chee, 2006).

2.2.4 fMRI functional connectivity (FC)

Aside from task-based fMRI other methods of BOLD signal analysis have become increasingly utilised by researchers. FC is defined as the temporal dependency, or correlated neuronal activity, between separate anatomically defined brain regions (Friston, Frith, Liddle, & Frackowiak, 1993). FC analysis has been successfully applied to compare spontaneous BOLD time series between brain regions and it can be performed without a task-based design, a major advantage for many behavioural applications including sleep (see section 2.2.4: *Sleep EEG-fMRI methodological challenges*). Early FC studies show the BOLD signal of right and left motor cortex to be highly correlated, meaning these areas are highly connected, compared to the rest of the brain during spontaneous periods of rest (i.e. no task), known as the 'resting-state' (Biswal, Van Kylen, & Hyde, 1997; Biswal, Zerrin Yetkin, Haughton, & Hyde, 1995). These intrinsic low-frequency (<0.1 Hz) synchronous fluctuations in BOLD signal can be used to map functionally associated regions or networks (Lowe, Dzemidzic, Lurito, Mathews, & Phillips, 2000), including primary sensory networks such as motor, auditory and visual as well as higher cognitive networks (Biswal et al., 1997; Damoiseaux et al., 2006; Fox &

Raichle, 2007; Lowe et al., 2000), often described as intrinsically connected networks (ICNs). One network of regions whose activity is highly correlated in resting-state data is described as the default mode network (DMN), a ‘baseline’ network whose activity is suppressed during task-based or goal-directed behaviours but which is strongly connected during rest (Raichle et al., 2001). Interestingly the spontaneous activity of regions within other ICNs such as the dorsal attention network (DAN), saliency (SAL) and sensorimotor (SENS) have been shown to be anticorrelated with the activity of the DMN (Fox et al., 2005), suggesting that signalling interactions occur between, not just within, ICNs.

FC reflects the spontaneous activity of regions collectively required for a particular function or behaviour (Lowe et al., 2000) and there is a strong relationship between spontaneous activity and task-based activity (Mennes et al., 2010; Tavor et al., 2016), suggesting FC is a meaningful measure of the underlying neuronal activity. While the neuronal basis of this spontaneous activity is still an on-going area of investigation, intracranial electrocorticography (ECoG) recordings of LFPs in the human sensory cortex are shown to correlate with spontaneous fMRI activity in these regions (Nir et al., 2008). Monkeys also show similar patterns of FC in functionally comparable regions suggesting an evolutionary similarity in functional brain organisation (Vincent et al., 2007). Together with evidence from invasive cell recordings in monkeys which also show correlations between fMRI spontaneous activity and both LFPs and action potentials (Shmuel & Leopold, 2008), a neuronal basis of spontaneous activity is recognised (Schölvinck, Maier, Ye, Duyn, & Leopold, 2010).

Furthermore, the observation that functionally similar regions (i.e. left and right primary motor) have greater FC or correlated spontaneous activity compared with other regions is

consistently replicated across both scanning sessions (Damoiseaux et al., 2006) and subjects (Van Dijk et al., 2010), suggests that FC reflects the functional architecture of the brain. This is also supported by studies showing that the structural organisation of the brain overlaps with functional ICNs, which could be an important factor in shaping spontaneous functional activity (Honey, Kotter, Breakspear, & Sporns, 2007; Honey, Thivierge, & Sporns, 2010; van den Heuvel, Mandl, Kahn, & Hulshoff Pol, 2009), although structure is unable to explain all FC believed to be due to indirect connections.

FC acquisition and analysis methods

To assess FC, fMRI data is usually acquired during wakeful rest from subjects instructed to think of nothing in particular, either with their eyes open or closed for durations ranging between 2-30minutes, although shorter scans (~5 minutes) are frequently used (Leonardi & Van De Ville, 2014; Van Dijk et al., 2010). FC can be calculated using spatially independent component analysis (ICA) (Beckmann, DeLuca, Devlin, & Smith, 2005; Beckmann & Smith, 2004) to obtain spatial network components, although this approach assumes spatial stationarity across time (Cole, Smith, & Beckmann, 2010). Alternatively, a seed-based FC analysis is commonly calculated using linear correlation (Pearson or Partial) of the BOLD time series between pre-determined regions of interest (Figure 2.2.3). This has typically been calculated as an average across the whole scan of several minutes, however the benefit of a seed-based approach is it allows dynamic FC analysis using smaller temporal epochs or windows (i.e. 30s) (see Chapter 4 for a review) for each region. No FC methods imply causality between regions activity, simply a temporal relationship between time series.

The BOLD signal also contains non-neuronal factors often considered ‘noise’ arising from fluctuations in heart rate (Chang, Cunningham, & Glover, 2009) and respiratory activity (Birn, Smith, Jones, & Bandettini, 2008), as well as head motion (Van Dijk, Sabuncu, & Buckner, 2012). Therefore prior to seed-based FC analysis it is important to reduce noise or artifacts from the data via pre-processing steps, these include the regression of motion parameters, white matter and ventricular time series (Weissenbacher et al., 2009).

Additionally fluctuations in subject’s breathing and cardiac rhythm are regressed from the BOLD signal prior to FC calculations, which has been shown to reduce FC false positives (Khalili-Mahani et al., 2013). Some studies have also advocated regressing the global mean BOLD signal, known as global signal regression (GSR), which provides greater spatial specificity by removing signal common between regions and has been suggested to improve positive FC correlation (Fox et al., 2005; Weissenbacher et al., 2009), although this pre-processing step remains contentious in the field (Gotts et al., 2013; Keller et al., 2013) with researchers often comparing FC calculated both with and without GSR.

FC is increasingly being investigated across the whole-brain, improving our understanding of the functional organisation of the brain as a large-scale network (van den Heuvel & Hulshoff Pol, 2010), or “connectome” (Finn et al., 2015). In recent years the functional and structural network properties of the brain have been investigated using graph theoretical analysis, with ICNs analysed as a whole network (Bullmore & Sporns, 2009; Sporns, 2011). Graph theory is an area of mathematics focused on defining and analysing graphs (Bullmore & Sporns, 2009), defined for neuroscience as a group of *nodes* (brain regions) connected via *edges* (FC value) which are analysed using various metrics or network measures to quantify the overall system. Measures commonly applied to the FC network include; *node degree* the number of links with

other nodes, *clustering coefficients* the number of links with neighbouring nodes, *path length* is the minimum number of edges connecting each node, centrality is the number of nodes that pass through with the shortest paths and overall *network hierarchy* often using clustering (Bullmore & Sporns, 2009).

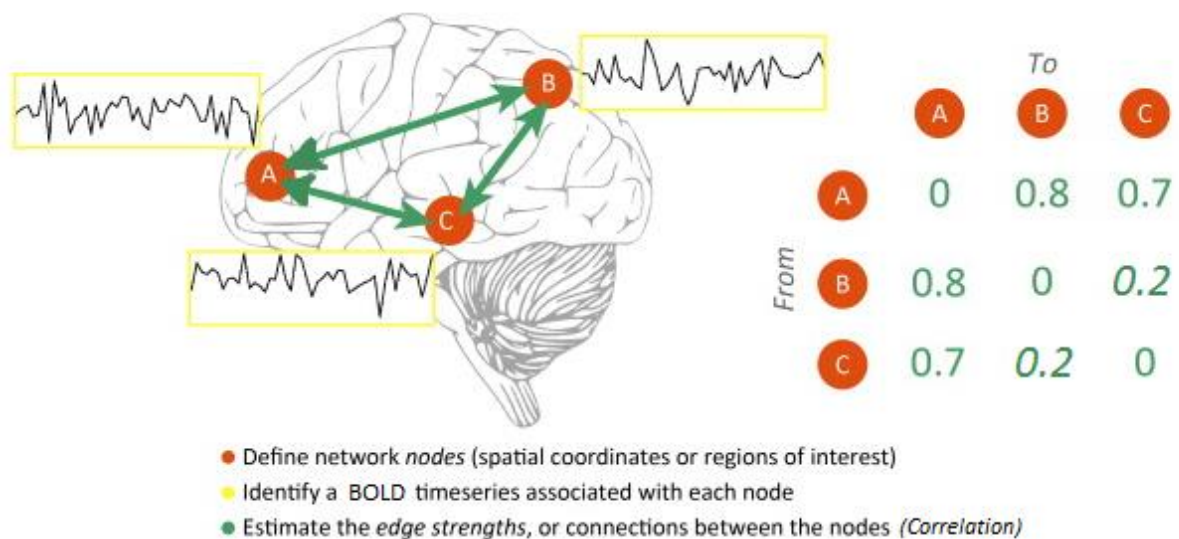


Figure 2.2.3. Schematic of FC analysis using BOLD time series to generate temporal correlation. With the description of graph analysis including; node definition (ROI) and edge estimate (FC). Also outlining the creation of a correlation matrix representing the network's FC.

FC during resting wakefulness and sleep

The resting technique is ideally suited to the study and investigation of spontaneous brain activity during sleep (Picchioni, Duyn, & Horovitz, 2013). In particular the DMN, which is strongly correlated when inwardly 'thinking' and anti-correlated with externally focused networks (Fox et al., 2005), was hypothesised to be a marker of consciousness (Fransson, 2005; Horovitz et al., 2009), as well as being fundamental for overall brain function (Boly et

al., 2008). These findings led researchers to investigate how, or whether, resting-state ICNs changed during sleep using combined EEG-fMRI (see section 2.2.3). Additionally researchers have also focused on the association between waking FC and sleep deprivation, showing complex changes to waking FC strength across the brain following reduced sleep (Bosch et al., 2013; Dai et al., 2015; De Havas, Parimal, Soon, & Chee, 2012; Gujar, Yoo, Hu, & Walker, 2010; Kaufmann et al., 2015; Sämann et al., 2010; Shao et al., 2013; Stoffers et al., 2015; Yeo, Tandi, & Chee, 2015; Zhu et al., 2015) and also in association with other measures of prior sleep such as questionnaires (Killgore, Schwab, & Weiner, 2012; Killgore, 2013; Killgore et al., 2015; Ward et al., 2013) or actigraphy (Khalsa et al., 2016). However the focus of this section is the FC changes during the different sleep stages compared to wakefulness and the studies reviewed in this section are focused on the average FC across EEG classified epochs.

One of the first EEG-fMRI studies to assess FC during NREM sleep showed similar FC strength within the DMN during light sleep (N1) compared to wake and concluded that connectivity was not simply a marker of wakefulness, although they noted higher levels of FC variability in the sensory cortex in light sleep (Horovitz et al., 2008). The same group have also shown a reduction in frontoparietal FC with increased sleep depth in the DMN (Horovitz et al., 2009). Extending the FC analysis to other ICNs showed an increase in FC in the dorsal attention network (DAN) amongst others during sleep compared with wake (Larson-Prior et al., 2009). Sleep was also shown to reduce the FC between DMN regions and the anti-correlated networks, in addition to reductions in the intra-network FC of the DMN between posterior and anterior regions (Larson-Prior et al., 2011; Sämann et al., 2011). FC differences between NREM and REM sleep have also been shown in the DMN, with one study showing

increased FC across the whole network during REM compared with NREM (Ming et al., 2013), while another found FC differences restricted to the medial temporal lobe (MTL) within the DMN (Koike, Kan, Misaki, & Miyauchi, 2011).

There has been a considerable focus on the FC of the DMN and regions within the network are shown to be important during sleep (Uehara et al., 2013). In addition to the DMN whole-brain FC analysis using graph theory also revealed hierarchical network changes in sleep compared with wake (Boly et al., 2012; Spoormaker et al., 2010; Tagliazucchi et al., 2013). Furthermore, thalamocortical FC has been shown to reduce with sleep onset (Hale et al., 2015; Kaufmann et al., 2006), with the reduction in thalamocortical FC shown across the majority of thalamic subdivisions and their associated cortical projections (Hale et al., 2015).

2.3 Thesis motivation and aims

The primary aim of this thesis is to investigate the sleeping brain and the underlying neuronal changes associated with the onset of sleep using neuroimaging. As sleep is a process within the brain neuroimaging is required to non-invasively investigate it, thus moving beyond ‘gold-standard’ PSG methodology to combined EEG-fMRI during sleep, in order gain a better understanding of the regions involved. Firstly the sensitivity of fMRI FC in the assessment of dynamic changes in brain activity with sleep state within cortical and subcortical intrinsically connected networks (ICNs) is assessed. The focus then shifts to whether the FC within these ICNs during sleep is a sensitive measure of other aspects of sleep-wake behaviour such as sleep deprivation and behavioural measures.

Main aims:

- Explore the use of fMRI in the advancement of our understanding of human sleep
- Investigate the sensitivity of FC as a measure of dynamic brain activity during sleep
- Investigate FC changes with sleep stage between normal and recovery sleep
- Investigate the association of FC sleep stage with prior sleep history and behaviour

Chapter 4 investigates the dynamic capability of resting-state BOLD fluctuations in the calculation of FC using short temporal epochs, with and without regression of the mean global BOLD signal. Thus, I attempt to validate FC analysis with NREM sleep stage using thirty-second epochs.

Chapter 5 investigates the FC differences between different types of sleep, namely normal and recovery (~24hours) NREM sleep separately for each of the sleep stages within each of the major ICNs. The purpose of this was to identify the spatial reorganisation of the brain with NREM sleep stage and the impact of increased sleep pressure.

Chapter 6 investigates whether any FC changes that occur with changes in normal NREM sleep stage are associated with prior sleep history, using objective and subjective measures of an individual's sleep history to predict FC changes within the major ICNs. This highlights the networks during NREM sleep which are mediated by measures of prior sleep-wake history.

Chapter 7 investigates the changes in FC for a classified sleep stage based on different temporal groupings for each of the ICNs. Furthermore it explores the dynamic variability in FC strength within sleep stages.

Chapter 3

METHODS AND MATERIALS

3.1 Overview

This chapter introduces the methodology consistently used in the subsequent experimental chapters. Explaining the participant recruitment, experimental procedures, design, data acquisition and analysis used. In addition to highlighting methodological issues and justification for these where appropriate. Any additional methodology unique to each experiment is included in the methods section of that chapter.

3.2 Participant procedure

All subjects gave written informed consent for each experimental study, which were approved by the Research Ethics Board of the University of Birmingham and were paid for their involvement. All subjects were pre-screened via interview for abnormal neurology, sleep or medical conditions and were also asked to restrict their alcohol and caffeine intake 24 hours

prior to all scanning sessions. Subjects' habitual sleep patterns were monitored with a written self-reported sleep diary and wrist actigraphy (Actiwatch2, Philips, Respironics®) for either one or two weeks prior to the scanning session depending on the study. Actiwatches recorded wrist movement based on 1 minute intervals and those intervals with an activity count exceeding 40 were defined as wake.

3.3 EEG-fMRI acquisition

EEG data were recorded from 62 Ag/AgCl MR-compatible EEG electrodes (EasyCap), with electrodes placed below the left clavicle or on their back to record the electrocardiogram (ECG) and an additional electrode placed below the left eye to record the electrooculogram (EOG) during sleep scans. The reference electrode was positioned at FCz, and the ground electrode at AFz. EEG data were acquired at a sampling rate of 5kHz, with hardware filters 0.016-250Hz, using MR-compatible BrainAmp MR-plus EEG amplifiers (Brain Products, Germany). Impedance at all recording electrodes was maintained below 20k Ω . The EEG clock was synchronised to the MR scanner clock to ensure consistent sampling of the gradient artefact (Mandelkow, Halder, Boesiger, & Brandeis, 2006b). A 3T Philips Achieva MRI scanner with an 8-channel head coil was used to acquire T1-weighted anatomical images (1mm isotropic voxels) at the beginning of all scanning sessions before whole brain T2*-weighted fMRI were acquired. Participants wore earplugs and headphones to minimise acoustic noise and their head was supported with foam padding to minimise motion artefacts. Respiratory and cardiac fluctuations were recorded using a pneumatic belt and a vectorcardiogram (VCG) both acquired at sampling rate of 500 Hz by the scanner hardware.

3.4 Regions of interest (ROIs)

3.4.1 Rational for selected ROIs

Sleep is understood to impact the whole brain with important sub-cortical regions of the thalamus understood to propagate widely to areas throughout the cortex according to the ascending reticular activating system (ARAS) controlling or mediating the sleep-wake transition (see Section 1.4.1). Thus investigating a large number of brain ROIs is merited. As mentioned previously in section 2.2.4 a number of functional networks have been identified from resting-state FC analysis of the spontaneous activity, these intrinsically connected networks (ICNs) are shown in cortical and subcortical areas. As such the ROIs forming the ICNs are identified in the current thesis in accordance with previous literature on these ICNs (Damoiseaux et al., 2006), aiding the direct comparison of results to previous FC literature and giving a comprehensive overview of the ROIs across the whole-brain during sleep.

3.4.2 Definition of ROIs

Data from six-minute, waking eyes open, resting-state fMRI scans (3x3x4mm voxels, TR=2000ms, TE=35ms, flip angle 80°, SENSE factor = 2) acquired in a separate cohort of fifty-five subjects (28 male, age 25±4yrs) as part of a previous study (Przezdziak, Bagshaw, & Mayhew, 2013) were used to independently define the spatial location of major brain networks. Using FSL 4.1.8 (www.fmrib.ox.ac.uk/fsl), data were motion corrected, spatially smoothed (5mm), temporally concatenated across subjects and decomposed into 20 spatially-independent components using MELODIC (Beckmann & Smith, 2004). Following previous

methodology (Khalsa, Mayhew, Chechlacz, Bagary, & Bagshaw, 2014), the chosen component was thresholded at a Z-statistic > 4 , to ensure spatial distinction between network regions, and then manually divided into its individual nodes (Figure 3.4.1). The thalamus and hippocampus were anatomically defined from the Harvard-Oxford cortical and subcortical atlases. Each MNI space mask was then registered to the current experimental data of each individual subject using FLIRT (Jenkinson & Smith, 2001). Individual regions of interest (ROIs) were defined as a 5x5x5 voxel cube centred on the maximum Z-statistic voxel for each node (Table 3.4.1). The individual regions were grouped into cortical networks; DMN, DAN, SAL, SENS, AUD and VIS, each region was visually identified from a single component, based on its spatial similarity to previous reports (Damoiseaux et al., 2006), in addition to hippocampus and thalamus networks. The bilateral parahippocampus (PH) as defined using ICA was grouped with the anatomically defined hippocampus from the FSL atlas to create the hippocampus network, although the PH also forms part of the DMN in some analyses. The thalamus network is made up of the bilateral anatomically defined thalamus regions.

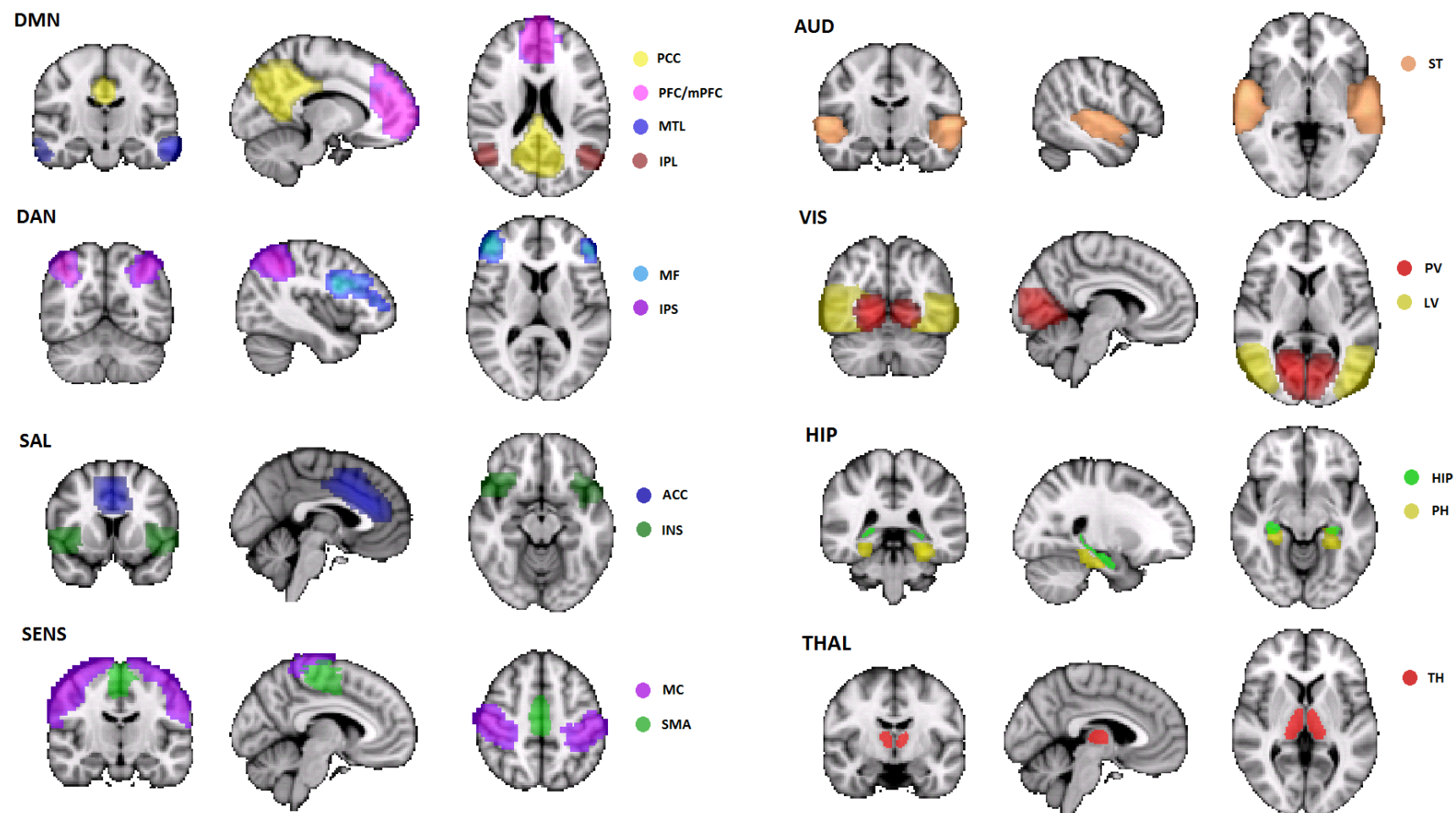


Figure 3.4.1. ICA or FSL anatomical masks for the regions within each network

Table 3.4.1. Peak voxel coordinates for each ROI within each network defined by the group ICA or FSL anatomical masks

Network	Region	MNI Coordinates		
		X	Y	Z
Default mode network (DMN)	Posterior cingulate (PCC)	2	-54	32
	Prefrontal cortex (PFC)	2	50	4
	Left inferior parietal lobe (LIPL)	-50	-70	36
	Right inferior parietal lobe (RIPL)	50	-70	36
	Left medial temporal lobe (LMTL)	-62	-12	-20
	Right medial temporal lobe (RMTL)	54	0	-32
Dorsal attention network (DAN)	Left interparietal sulcus (LIPS)	-42	-50	46
	Right interparietal sulcus (RIPS)	42	-54	48
	Left middle frontal gyrus (LMF)	-50	22	30
	Right middle frontal gyrus (RMF)	50	38	16
Saliency network (SAL)	Anterior cingulate cortex (ACC)	2	24	28
	Left insula (LINS)	-38	14	0
	Right insula (RINS)	38	22	0
Sensorimotor (SENS)	Left motor cortex (LMC)	-42	-18	60
	Right motor cortex (RMC)	46	-18	60
	Sensory motor area (SMA)	2	-22	56
Auditory (AUD)	Left superior temporal gyrus (LST)	-58	-20	4
	Right superior temporal gyrus (RST)	58	-22	4
Visual (VIS)	Left primary visual cortex (LPV)	10	-74	4
	Right primary visual cortex (RPV)	14	-70	4
	Left lateral visual cortex (LLV)	-46	-76	8
	Right lateral visual cortex (RLV)	50	-70	8
Hippocampus (HIP)	Left Hippocampus (LH)	<i>FSL Mask</i>		
	Right Hippocampus (RH)	<i>FSL Mask</i>		
	Left parahippocampal gyrus (LPH) I	-30	-40	-16
	Right parahippocampal gyrus (RPH) I	30	-34	-16
Thalamus (THAL)	Left Thalamus (LT)	<i>FSL Mask</i>		
	Right Thalamus (RT)	<i>FSL Mask</i>		

ROIs in bold are the seed regions used in subsequent functional connectivity analysis of the networks spatial map.

I ROIs also form part of DMN in certain analysis

3.5 fMRI preprocessing

fMRI data were pre-processed according to standard methodology to prepare for FC analysis (Fox et al., 2005), using FSL (FMRIB Software Library, <http://www.fmrib.ox.ac.uk/fsl>, Smith et al. 2004) and Matlab (MathWorks, USA). Data were motion corrected using MCFLIRT, spatially smoothed (using a 6mm Gaussian kernel) and temporally band-pass filtered ($0.009 < \text{Hz} < 0.08$). Some of the data in this thesis also underwent correction for physiological noise using RETROICOR (Glover, Li, & Ress, 2000). Further potential confound signals were removed using multiple linear regression. These included: the six motion parameters of head rotation and translation, white matter and CSF signals. For sleep data head movement was also assessed for each 30-second epoch using the measure of relative movement obtained from motion correction (MCFLIRT), in order to investigate its relationship to sleep stage.

Global signal regression (GSR)

The preprocessing step of mean global signal regression, calculated by averaging the BOLD timeseries across all brain voxels, is thought to remove physiological noise in the data (Fox, Zhang, Snyder, & Raichle, 2009). It was initially applied in Chapter 4, although was omitted from later chapters due to the growing contention in the literature regarding spurious correlations (Fox, Zhang, Snyder, & Raichle, 2009; Gotts et al., 2013; Keller et al., 2013), a potential limitation of the method. Additionally research has shown an effect on global signal as a function of the changes during sleep and the amount of prior sleep deprivation (Yeo et al.,

2015), impacting the interpretation of results. Given that GSR is an on-going area of investigation, researchers often calculate FC with and without the processing step.

3.6 Analysis of EEG-fMRI data

MR gradient and ballistocardiogram artefacts were removed from the EEG using average artefact subtraction in Brain Vision Analyzer 2 (Brain Products, Munich). The VCG R-peak markers were aligned with the EEG data and also used for pulse artifact correction (Mullinger et al. 2008). EEG data were then divided into non-overlapping thirty second epochs and manually sleep staged by an experienced electroencephalographer according to the American Association of Sleep Medicine criteria (AASM, 2007).

Dynamic FC analysis using seconds rather than minutes to calculate FC is used for all fMRI data and is calculated using a non-overlapping 30 second sliding window of the BOLD time-series. The dynamic capabilities are explored further in the Chapter 4. Each ROI was in turn used as the seed region and a Pearson correlation (R-value) was calculated for every 30-second epoch to all other independent target ROIs (Table 3.4.1). Therefore FC was calculated both within and between networks, generating a connectivity matrix for each epoch where each element of the matrix represented the FC strength between the two different ROIs. Each of these 30-second FC matrices were paired to the corresponding EEG defined sleep stage, pooled by stage within subject and then averaged across subjects. ROIs were grouped into eight networks and the composite FC values were calculated from these ROIs for each sleep stage, focusing on the within network FC of each of the eight networks.

Negative FC values

Any negative FC coefficients were removed and set to zero at the subject level in the current thesis, while they were maintained at the epoch level. Negative correlations coefficients were originally shown to be associated with the preprocessing step of GSR, cautioning interpretation of negative FC (Murphy, Birn, Handwerker, Jones, & Bandettini, 2009). However negative FC is maintained without GSR (Chang & Glover, 2009) or other preprocessing steps, which suggests that negative values may not solely be artifacts of the FC analysis (Carbonell, Bellec, & Shmuel, 2011). Although research continues and difficulties interpreting the neurophysiological significance of negative FC is an on-going area of investigation (Carbonell et al., 2011; Chen, Chen, Xie, & Li, 2011; Goelman, Gordon, & Bonne, 2014). As the thesis focuses on the within ICNs positive FC values, negative FC values are set to zero in order aid interpretation.

3.7 Statistical analysis

All statistical analysis of the FC changes were conducted in SPSS (IBM SPSS Statistics, version 21, Chicago, IL) using either a Generalised Linear Mixed Model (GLMM) or an Analysis of Covariance (ANCOVA) depending on the research question and structure of the data.

Chapter 4

INFLUENCE OF EPOCH LENGTH ON MEASUREMENT OF DYNAMIC FUNCTIONAL CONNECTIVITY IN WAKEFULNESS AND BEHAVIOURAL VALIDATION IN SLEEP

Wilson, R. S., Mayhew, S. D., Rollings, D. T., Goldstone, A., Przedzik, I., Arvanitis, T. N., & Bagshaw, A. P. (2015). Influence of epoch length on measurement of dynamic functional connectivity in wakefulness and behavioural validation in sleep. NeuroImage, 112, 169-179.

4.1 Abstract

Conventional FC analysis of fMRI data derives a single measurement from the entire scan, generally several minutes in duration, which neglects the brain's dynamic behaviour and potentially loses important temporal information. Short-interval dynamic FC is an attractive proposition if methodological issues can be resolved and the approach validated. This was addressed in two ways; firstly we assessed FC of the posterior cingulate cortex (PCC) node of

the default mode network (DMN) using differing temporal intervals (8 seconds to 5 minutes) in the waking-resting state. We found that 30-second intervals and longer produce spatially similar correlation topography compared to 15-minute static FC measurements, while providing increased temporal information about changes in FC that were consistent across interval lengths. Secondly, we used NREM sleep as a behavioural validation for the use of 30-second temporal intervals due to the known fMRI FC changes with sleep stage that have been observed in previous studies using intervals of several minutes. We found significant decreases in DMN FC with sleep depth which were most pronounced during stage N2 and N3. Additionally, both the proportion of time with strong PCC-DMN connectivity and the variability in dynamic FC decreased with sleep. We therefore show that dynamic FC with epochs as short as tens of seconds is a viable method for characterising intrinsic brain activity.

4.2 Introduction

FC can be measured using fMRI as the temporal correlation of low-frequency ($<0.1\text{Hz}$) blood-oxygenation-level-dependent (BOLD) signal fluctuations from separate brain regions (Friston, 2011). These brain regions collectively form intrinsically connected networks (ICNs), which are believed to underpin cognitive function (Hellyer et al., 2014; Stevens & Spreng, 2014). As such, FC has increasingly been used to characterise ICNs in the healthy and pathological brain (Fox & Greicius, 2010; Van Dijk et al., 2010). Data is typically acquired during ‘rest’ when an experimental subject is not engaged in a task, and the analysis of resting data generally proceeds by calculating the FC over several minutes. Although this static measure of FC enables a useful insight into the average strength of the functional

relationship between brain regions, the brain is a dynamic system that clearly alters its state on much shorter timescales. In recent years there has been considerable interest in using fMRI to quantify dynamic changes in FC within and between ICNs (Buckner, Krienen, & Yeo, 2013; Hutchison, Womelsdorf, Gati, Everling, & Menon, 2012), which reflect alterations in neuronal and behavioural states with greater temporal precision.

There is considerable evidence that FC fluctuates over an fMRI scan lasting several minutes (Allen et al., 2014; Brodbeck et al., 2012; Chang & Glover, 2010; Gonzalez-Castillo et al., 2014; Hutchison et al., 2012; Tagliazucchi, von Wegner, Morzelewski, Brodbeck, & Laufs, 2012; Zalesky, Fornito, Cocchi, Gollo, & Breakspear, 2014), and that these fluctuations have a neurophysiological underpinning (Brookes et al., 2011; Jerbi et al., 2010; Miller, Weaver, & Ojemann, 2009; Tagliazucchi, von Wegner, Morzelewski, Brodbeck, et al., 2012; Thompson et al., 2013). The quantification of dynamic FC opens up a new dimension for analysis, offering an alternative and complementary methodology to understand the complexities of brain function. For example, interactions between ongoing brain activity and stimulus evoked responses have been reported (Mayhew, Hylands-White, Porcaro, Derbyshire, & Bagshaw, 2013; Sadaghiani, Hesselmann, Friston, & Kleinschmidt, 2010), and a dynamic FC approach would allow for a more complete characterisation of inter-individual and inter-trial variability in the brain's response to individual stimuli. Sleep is another dynamic behaviour which would benefit from improved characterisation using dynamic FC. Sleep is characterised clinically using thirty second EEG epochs, based on robust electrophysiological signatures which represent different sleep stages (AASM, 2007). Understanding what these sleep stages represent in terms of alterations to brain function and specifically the modification of large scale brain networks is challenging with EEG alone, although EEG remains the 'gold

standard' technique to define the sleep stages themselves. As such, investigating sleep using fMRI requires simultaneous EEG-fMRI acquisition (Duyn, 2012). Harnessing the ability of fMRI to characterise brain networks will enable further understanding of the brain processes occurring within a stable sleep stage, but also the transitions between stages, the processes underlying which remain poorly understood.

These questions require the ability to quantify FC at timescales much smaller than those generally used. However, there has been an understandable reluctance to compute fMRI FC using temporal intervals shorter than several minutes due to the risk of introducing spurious correlations (Van Dijk et al., 2010). Electrophysiological recordings clearly demonstrate that averaging several minutes worth of data loses the dynamic interplay between brain regions which is central to the brain's function, thus short-interval dynamic FC is an attractive proposition if methodological issues can be resolved. While some previous work has investigated the appropriate temporal interval for FC calculation for various applications (Leonardi & Van De Ville, 2014; Sakoğlu et al., 2010; Whitlow, Casanova, & Maldjian, 2011; Liégeois et al., 2015), a clear consensus remains to be found. One major difficulty is validating the results in the absence of a gold standard measurement of FC or behavioural correlate of FC.

In this study, we addressed this question by comparing static FC to the mean dynamic FC calculated by averaging the correlation values from each short epoch, using a range of epoch lengths. Our rationale was that if FC values from short epochs are dominated by noise, their average will tend to zero rather than converging on the static value of FC over the same period. To address the question of validation further, we identified a behavioural situation

where FC changes are relatively well characterised and a clear hypothesis of expected results can be formed. We studied changes in FC that have previously been observed in the descent into sleep, with a focus on the default mode network (DMN) as the network that has received the most attention and has the clearest changes (Horovitz et al., 2008, 2009; Larson-Prior et al., 2009; Sämann et al., 2010; Spoormaker et al., 2010).

The DMN is the most widely studied ICN within the brain. It comprises of the posterior cingulate cortex (PCC), medial prefrontal cortex (mPFC), inferior parietal lobe (IPL), medial temporal lobe (MTL) and the parahippocampal gyrus (PH) (Buckner, Andrews-Hanna, & Schacter, 2008). The PCC is generally recognised as the central region of the DMN (Fransson & Marrelec, 2008) and is one of the most globally connected areas of the brain (Cole, Pathak, & Schneider, 2010). A number of different behaviours have been linked with the DMN, including self-referential thought, emotional processing (Gusnard & Raichle, 2001) and disengagement from external stimuli (Fransson, 2005). This has resulted in the DMN becoming a focus of research for many psychiatric disorders (Broyd et al., 2009). In addition, FC of the PCC is believed to play an important role in maintenance of consciousness (Bagshaw & Cavanna, 2013; Cavanna & Trimble, 2006; Heine et al., 2012; Leech, Braga, & Sharp, 2012; Uehara et al., 2013; Vanhaudenhuyse et al., 2010). As such, FC has been investigated in several studies of the most common alteration of consciousness, non-rapid eye movement sleep (NREM) (Horovitz et al., 2008, 2009; Larson-Prior et al., 2009; Sämann et al., 2010; Spoormaker et al., 2010) and rapid eye movement (REM) sleep (Koike et al., 2011).

It has been shown that during light sleep (non-REM stage 1: N1), DMN FC is maintained (Horovitz et al., 2008; Larson-Prior et al., 2009), although as sleep progresses there appears to

be a PCC – mPFC ‘decoupling’ with reduced FC strength between these brain regions (Horovitz et al., 2009; Sämann et al., 2010). This suggests a possible breakdown in long-range FC (Massimini et al., 2005) and a large-scale change in the functional configuration of the brain during sleep (Spoormaker et al., 2010). However, this issue has only been addressed using epoch lengths of several minutes to determine FC strength. The aim of this study is to show that using shorter epoch lengths can replicate these results, which would provide support for using dynamic measures of FC, presenting a much richer and more behaviourally relevant temporal FC profile. We therefore restricted our analysis to the PCC as a seed region and the other DMN nodes as targets in order to focus on providing a detailed investigation of the effect of epoch length on FC within the DMN during wake and sleep.

The current study investigated dynamic FC during wakeful rest and sleep, using sleep not only as a behavioural validation of our methodology but also as a behaviour with temporal variation, as seen on EEG over seconds not minutes, that would benefit from the approach of dynamic rather than static FC. We focussed on the DMN as one of the most well studied ICNs which, in the PCC node, contains one of the brain regions previously linked with the changes in consciousness experienced during sleep (Sämann et al., 2011). We aimed to investigate how FC strength calculated during the awake resting-state varied with decreasing length of data epochs used in a seed-based analysis. Once validated, we applied this short-window approach to EEG-fMRI recordings during sleep to investigate how the temporal dynamics of DMN FC varied between different sleep stages.

4.3 Materials and methods

4.3.1 Experimental design

Eight healthy adult volunteers (5 male, age 32 ± 6 years) participated in the study. Following a normal night of sleep participants attended a single fifteen minute, resting-state fMRI session during the daytime. Participants returned on a later date at their usual bedtime (between 22:00 and 00:00), in order to facilitate natural sleep, and underwent simultaneous EEG-fMRI.

4.3.2 Waking RS fMRI acquisition

During the daytime session participants were instructed to keep their eyes open and not think of anything in particular throughout a 15-minute fMRI scan, T2*-weighted with whole brain coverage (3x3x4mm voxels, TR=2000ms, TE=35ms, 450 volumes, flip angle 80°, SENSE factor = 2).

4.3.3 Sleep EEG-fMRI acquisition

The subjects returned for the second session in which EEG data were recorded (see Chapter 3). The participants were instructed to sleep if they could and to signal to terminate the session once when they were no longer able to sleep. Whole brain T2*-weighted fMRI data (3x3x4mm voxels, TR=2000ms, TE=35ms, 450 volumes, flip angle 80°, SENSE factor = 2) were acquired in consecutive 15-minute scans. Subject's respiratory and cardiac cycles were measured using a pneumatic bellows and pulse oximeter.

4.3.4 fMRI preprocessing

Definition of DMN ROIs and RS fMRI data pre-processing for both the waking and sleep sessions are outlined in Chapter 3. Previous investigation using simulated data identified appropriate FC epoch lengths based on the data properties (Leonardi & Van De Ville, 2014). Given the filtering used in the current study the recommendation of epoch lengths between 12 seconds and 1 minute would be considered appropriate according to their criteria. Therefore all the epoch lengths applied in the current study, excluding the 8-second FC satisfy the simulated quantification by Leonardi and Van De Ville (2014). FC was also calculated without the processing step of global signal regression (GSR), see supplementary section 4.6.1.

4.3.5 Effect of epoch length on DMN FC in waking data

Initially, the resting state data from the waking daytime scan were used to investigate the effect of epoch length on FC strength measured between the PCC and all DMN ROIs using the following seven epoch lengths: the full 15 minute scan duration (conventional, static correlation), 5 minutes, 2 minutes with 1 minute overlap, 1 minute with 30 second overlap, 30 seconds with no overlap, 16 seconds with 8 second overlap and 8 seconds with 4 second overlap. No overlap was used with the thirty second epochs to facilitate direct comparison of fMRI FC to each individual sleep stage epoch in subsequent analyses, since sleep staging of EEG is traditionally performed using non-overlapping 30s epochs (AASM, 2007). We aimed to estimate the minimum time window that could be used to calculate FC without compromising accuracy and introducing spurious correlations. For each epoch, the FC

strength was calculated as the correlation coefficient (Pearson's R-value) between the mean PCC ROI BOLD time series and the mean BOLD time series of each of the DMN target ROIs.

Separately for each epoch length, the correlation coefficients from each epoch were averaged across the entire scan for each subject before calculating a group mean for each ROI. This allowed comparison of the mean strength of dynamic FC between different epoch lengths and with the static value. Maps of the mean voxel-wise R-value across epochs were also computed for each subject and averaged across subjects to enable visualisation of the spatial distribution of dynamic PCC FC for different epoch lengths.

In order to assess the extent to which correlation coefficients could be spuriously produced by unrelated signals, and hence the validity of the dynamic FC values, we used a permutation based testing approach for each of the different epoch lengths. By creating a permuted data set we were able to determine the maximum FC which could occur spuriously by using short epoch calculations and compare this to our real dynamic FC values. The PCC BOLD time series of a single subject was correlated with the DMN ROI time series of all other subjects, using exactly the same epoch lengths as for the real FC calculations. As the time series being correlated were from different subjects, on average there should be zero FC. This test should result in a zero-mean correlation coefficient across all epochs, with any non-zero correlations identified as spurious and an artefact of the process of using short epochs. This procedure therefore places an upper bound on the FC values that can be produced by the epoching approach. This procedure was repeated for each subject and for all epoch lengths. A Generalized Linear Mixed-Model (GLMM) was used to determine whether the average FC

coefficients for each of the epoch durations were significantly different from those generated by the permutation tests, with the fixed-effects of ROI, epoch length and data type (actual v permuted) and the random-effect of subject. In addition a separate GLMM, not including simulated FC data, solely investigated the fixed-effect of epoch length in the real data.

As a second assessment, we investigated the degree of variability in FC strength across the duration of the scan for each of the different epoch lengths, by calculating the percentage of time that each target DMN ROI was positively functionally connected to the PCC (defined as $R\text{-value} > 0.2$). This threshold was chosen based on the permutation tests, which showed over all of the regions and epoch lengths that the maximum correlation that could occur by chance was $R=0.16$. For simplicity, we have rounded this figure up to $R=0.2$. As such, we interpret any correlation above this value to be ‘connected’, or at least unlikely to have occurred as a result of our use of different length epochs. The proportion of time spent with high positive FC was calculated for each epoch length and then averaged across subjects.

Finally, we assessed the cohesion of dynamic FC across all DMN regions which are functionally connected to the PCC and investigated whether this cohesive relationship existed at shorter epochs lengths. We compared the temporal dynamics of PCC FC within the DMN by correlating the time series of FC between the PCC and a given target ROI with the FC between the PCC and all other target ROIs (i.e regions functionally connected to the PCC acting in synchrony). Based on the Fisher-transform for the Pearson’s correlations, the significant correlations were thresholded using a Bonferroni correction accounting for multiple comparisons and then binarized. This was done for all epoch types and summed across subjects in order to determine the consistency of PCC FC.

4.3.6 Analysis of sleep data

The dynamic FC of the DMN between the PCC and each target ROI was then calculated in the sleep fMRI data using the corresponding thirty second sleep staged EEG epochs. For each ROI, FC strength was pooled within sleep-stage.

As wake FC was collected on two separate occasions in this study, we also included the two types of wake (day and night) in subsequent analyses. This was motivated by the belief that a daytime scan may be a more representative sample of true wakefulness compared with a scan performed explicitly to investigate sleep late at night, avoiding the complex variations in drowsiness with frequent transitions between wake and sleep. However due to EEG not being recorded in the day session, we cannot discount the possibility that daytime FC could be contaminated by periods of sleep. Therefore dynamic FC for wake and each sleep stage was averaged over all epochs and subjects and compared to waking FC calculated from 30 second epochs in the daytime resting-state scan from the same subjects.

A GLMM was used to test for differences in DMN FC between ROIs and sleep stages including both types of wake (day and night). The fixed effects of ROI, sleep stage and the ROI by sleep stage interactions were assessed as predictors of the FC. In addition the variability of sleep stage FC for each subject was assessed as the percentage of epochs per sleep stage deemed highly functionally connected ($R > 0.2$), using a GLMM, with the fixed-effects of ROI, sleep stage and the subsequent interaction, with the random-effect of subject.

4.4 Results

4.4.1 Dynamic FC using varying epoch lengths in waking data

DMN FC during waking rest was examined for seven different epoch lengths. Seeding from the PCC to all voxels in the brain produced a typical correlation topology associated with the characteristic regions of the DMN. Although this correlation topology varied considerably between individual epochs, it was highly comparable both in terms of the strength and spatial extent of FC between the group average maps of static and dynamic FC (Figure 4.4.1).

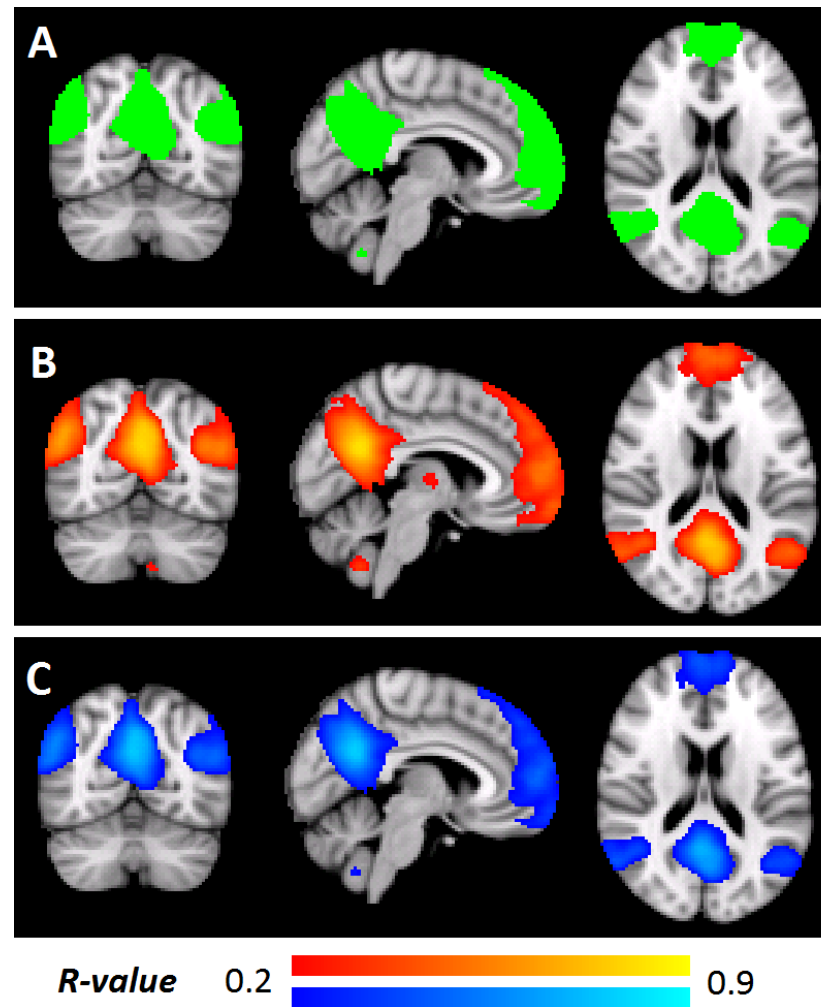


Figure 4.4.1. FC maps comparing between dynamic (30 seconds) and static (15 minutes) methods. The group mean FC topography during waking rest, calculated by seeding from the PCC to all brain voxels. ($x=4$ $y=-60$ $z=30$): A) Common voxels between the static FC and dynamic (Green). Images are displayed according to neurological convention. B) Static FC calculated using the entire 15 minute scan duration. C) Dynamic FC averaged over all 30 second epochs.

The majority of DMN regions exhibited stronger PCC dynamic FC than the permuted data (mean=0.019, STD=0.079) Figure 4.4.2. A GLMM analysis found a significant fixed-effect of ROI ($F(6,472)=59.688$, $p<0.001$), epoch length ($F(6,472)=2.142$, $p<0.047$) and data type ($F(1,441)=1406.257$, $p<0.001$) upon PCC FC and a significant interaction between data type and ROI ($F(6,472)=48.052$, $p<0.001$). In all ROIs except the bilateral PH, PCC FC was significantly stronger in the actual compared with the permuted data. There was also a significant interaction between data type and epoch length ($F(6,472)=5.907$, $p<0.001$) suggesting that FC of some epoch lengths differed more between the actual and permuted data types.

The interaction between epoch length and ROI did not prove significant: ($F(36,472)=0.126$, $p=1.00$), suggesting the effect of epoch length upon PCC FC was not dependent on the regions involved. However, a similar trend in the group average FC was observed across ROIs for all epoch lengths, in that the FC strength decreased as the epoch length became shorter (45.1% decrease from 15 minute to 8 second epochs on average across all ROIs, Figure 4.4.2). This is presumably a result of the increased noise level at shorter epochs. The static FC differed significantly from the mean dynamic FC calculated using the 30 second ($R = -.075$, $t = -2.718$, $p=0.007$), 16 second ($R=-.120$, $t = -4.385$, $p<0.001$) and 8 second ($R = -.167$, $t = -6.129$, $p<0.001$) epoch lengths. No significant difference between the static FC and the mean dynamic FC of 5 minute, 2 minute and 1 minute epoch lengths was observed.

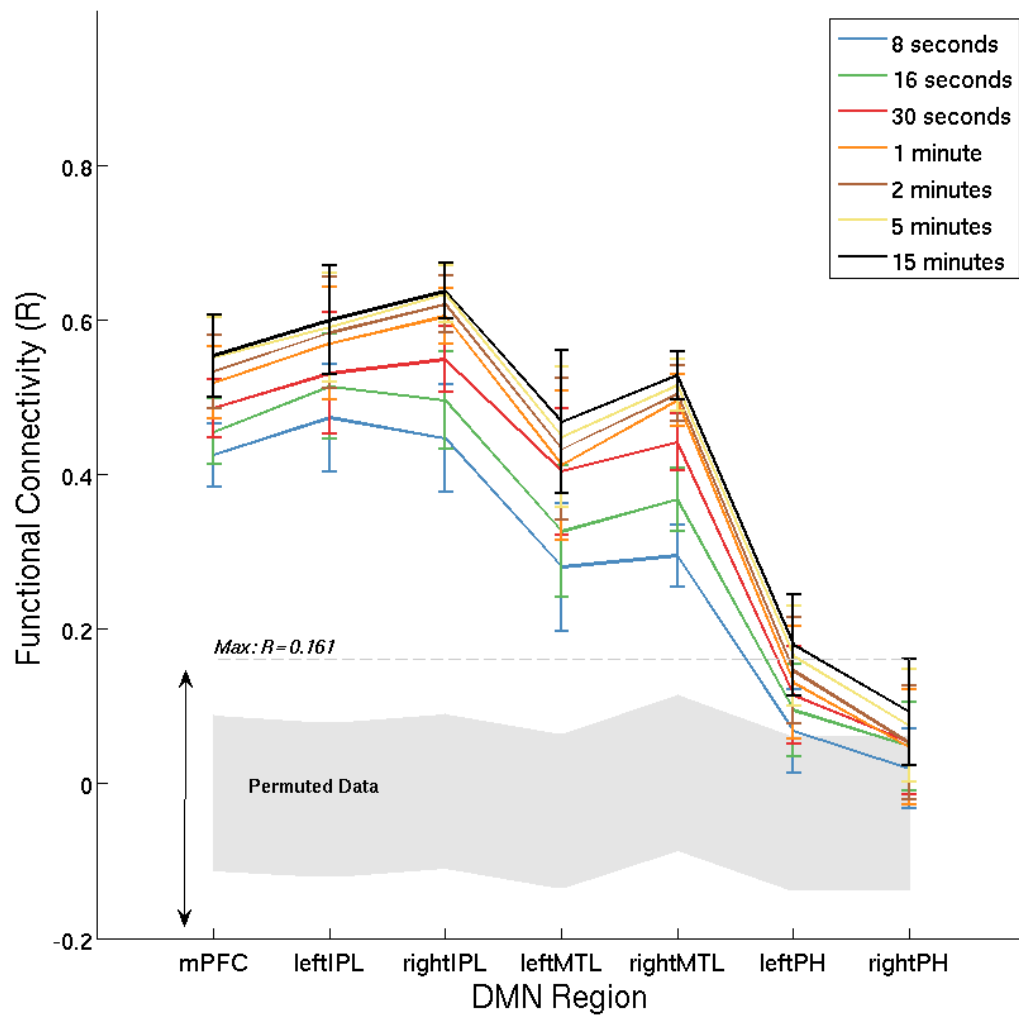


Figure 4.4.2. Group average FC strength between PCC and all other DMN ROIs calculated for each epoch type (solid lines), compared to the permuted data group mean \pm standard deviation (grey shaded box) & the grey dashed line displays the maximum. Error bars reflect the group mean \pm standard error.

The time course of PCC FC for the different epoch lengths are overlaid on the same plot for a representative subject in Figure 4.4.3. Similar results were observed for other DMN regions but only the left and right IPL and mPFC are shown for brevity. Figure 4.4.3. illustrates the dynamic information which is obtained by calculating FC using short temporal windows. We observed that within-subject PCC FC variability increased with decreasing epoch length. This variability was measured using the standard deviation (SD) which on average across the group increased by 85.1% between 2 minute (SD = 0.229) and 8 second (SD = 0.424) epochs.

Figure 4.4.3 also illustrates that regions found to be highly ‘functionally connected’ by static FC, i.e high R-value, do not consistently maintain high strength of connectivity throughout the entire scan duration. Instead, FC fluctuated considerably, often including negative FC values for regions that were positively correlated on average. The overall pattern of the connectivity time series for different epoch lengths was consistent in the sense that, for example, periods of negative FC were replicated at the same time points for different intervals. A similar effect was observed when FC was calculated without global signal regression (see supplementary information). The variability in dynamic FC was further assessed by calculating the percentage of the total scan duration during which each target ROI maintained a strong positive correlation ($R > 0.2$) with the PCC, for each epoch length (Figure 4.4.4). For example, using 30s dynamic FC revealed that during approximately 20% of the epochs the PCC was not strongly connected to the other cortical DMN ROIs. The proportion of the scan time during which positive PCC FC was observed decreased as epoch length became shorter, comparable to how the mean dynamic FC decreased with epoch length. This pattern was consistent across all regions of the DMN.

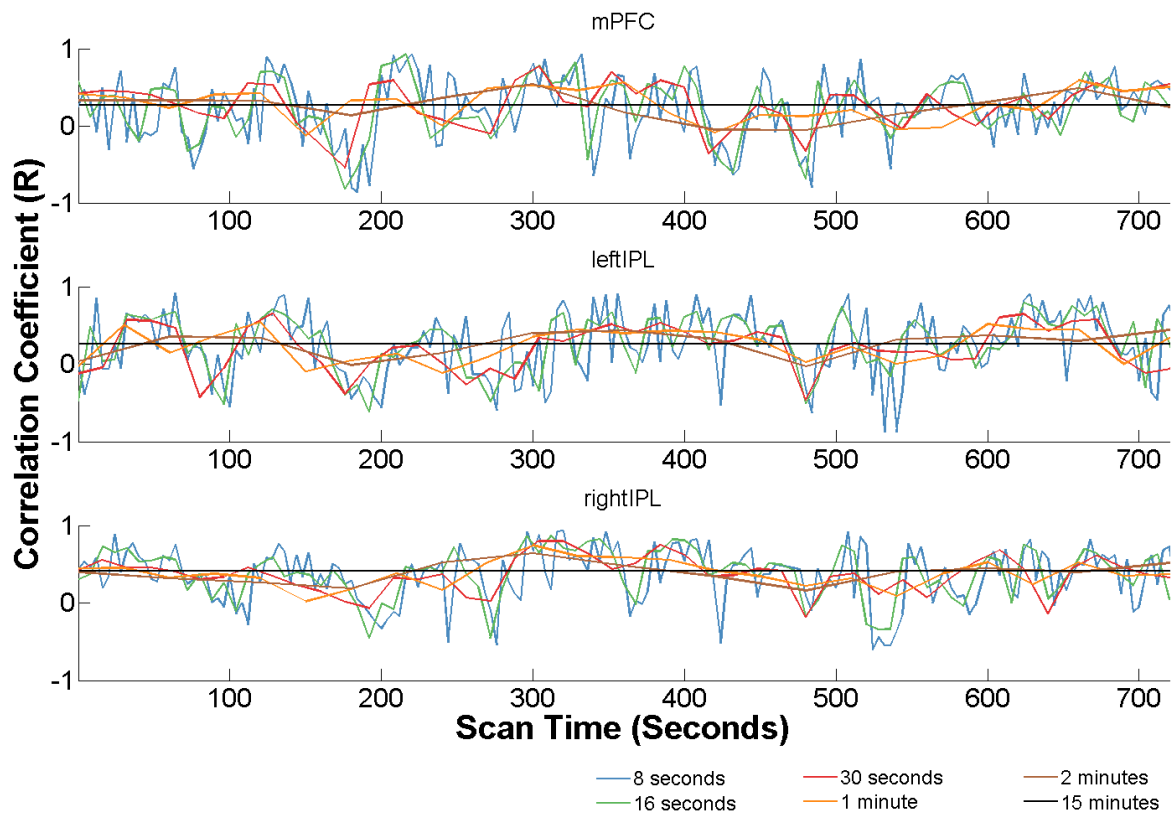


Figure 4.4.3. The time series of dynamic FC between the PCC and three DMN ROIs (mPFC, left & right IPL) in a representative subject. Plotting FC for a selection of epoch lengths, each represented with a different coloured line, allows comparison of the variability in FC between epoch lengths. For clarity the entire 900s of the scan are not shown.

CHAPTER 4: INFLUENCE OF EPOCH LENGTH ON MEASUREMENT OF DYNAMIC FUNCTIONAL CONNECTIVITY IN WAKEFULNESS AND BEHAVIOURAL VALIDATION IN SLEEP

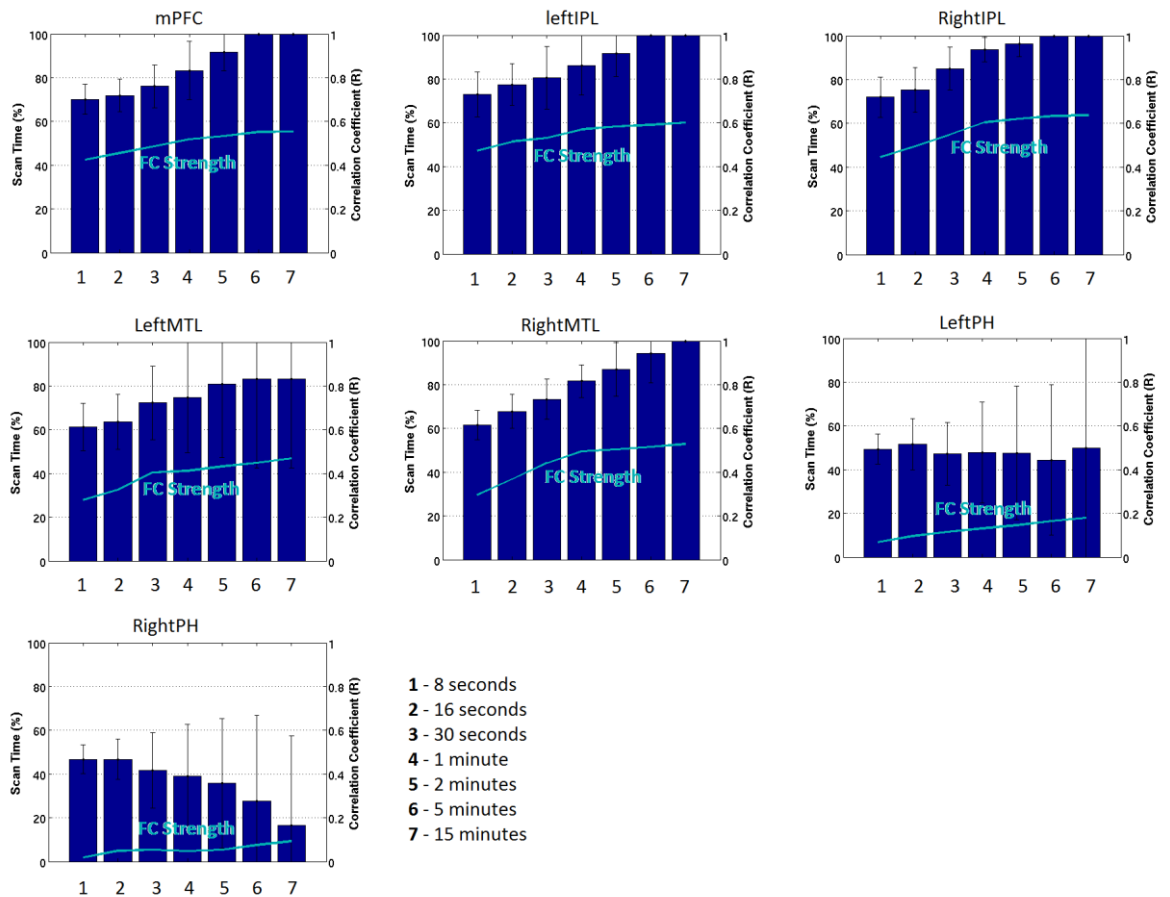


Figure 4.4.4. Each bar chart represents the PCC FC with a DMN region. For each epoch length, the percentage of the entire scan time during which the PCC was strongly positively functionally connected ($R > 0.2$) for each region across subjects is represented as a bar. Error bars represent the standard deviation across subjects. For comparison, the light blue line plots the average correlation coefficient (R-value) of dynamic PCC FC for each epoch length.

We further observed that the temporal dynamics of PCC FC (i.e., the time series shown in Figure 4.4.3) were correlated between target ROIs. For each paired connection, Figure 4.4.5 illustrates the number of subjects who shared a similar cohesive pattern of PCC connectivity (i.e., these are correlations between the time series of dynamic FC, such that each cell represents the number of subjects for whom FC to the PCC from node 1 was significantly correlated with FC to the PCC from node 2). Each matrix shows the calculation for each epoch length. Darker shades of red represent an increasing number of subjects with significant within-DMN FC, and hence a greater consistency of the relationship. Overall, FC to the PCC was significantly correlated between target ROIs, particularly for bilateral regions (i.e. IPL, MTL & PH). Figure 4.4.5 clearly demonstrates that this correlation structure is absent at longer epoch lengths, and indicates the presence of cohesive intra-DMN FC at smaller epochs, with regions functionally connected to the PCC displaying synchronous activity.

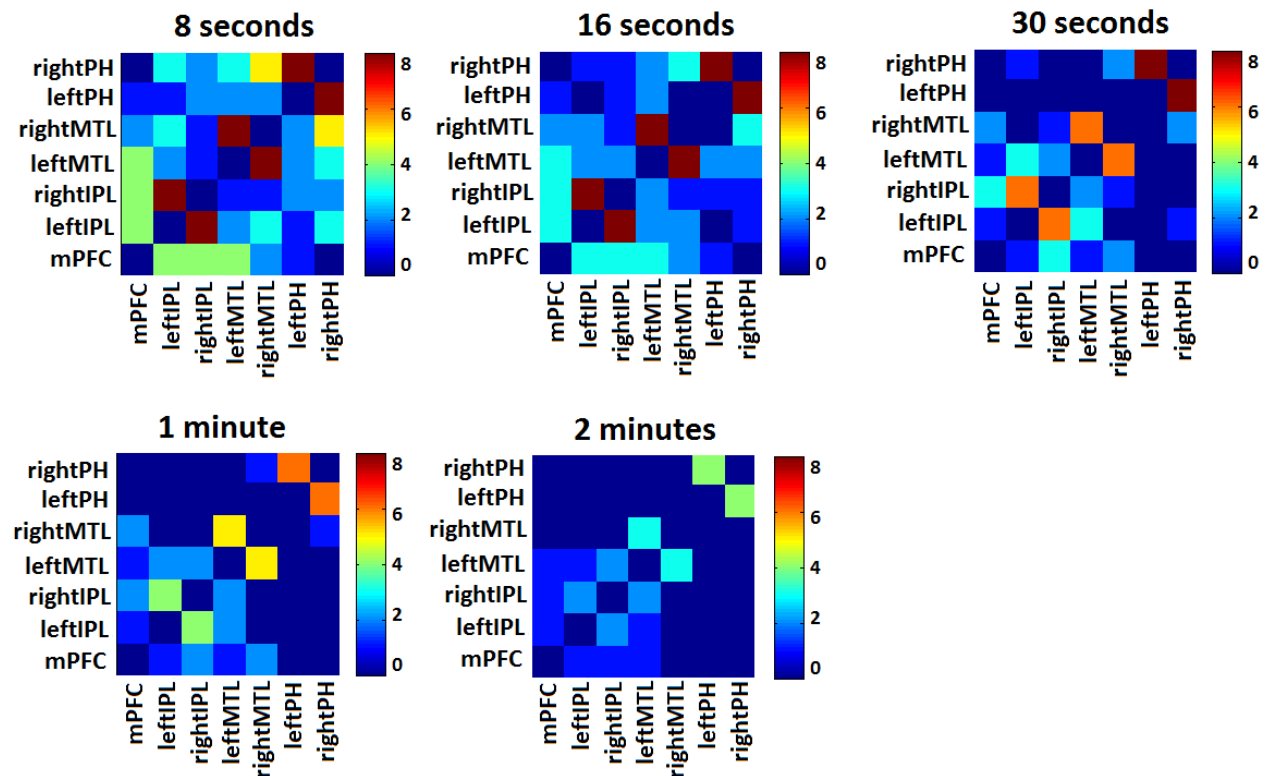


Figure 4.4.5. Group-level summary of the intra-DMN cohesion of the PCC FC during wakefulness. Each matrix entry shows the number of subjects in whom the time series of dynamic PCC FC was significantly correlated between two DMN target ROIs. A separate matrix is plotted for each epoch length. Red indicates consistent significance across subjects.

4.4.2 Changes in PCC FC during sleep

Two subjects were removed from the sleep dataset, one due to a technical problem with the EEG data and the other for failing to sleep. The six remaining subjects (4 male, age 29 ± 4 years) collectively produced a total of 870 thirty-second epochs of data: 178 epochs of wake, 276 epochs of N1, 372 epochs of N2 and 44 epochs of N3. The total number of epochs and the percentage of scan time spent in each sleep stage for each subject are displayed in Table 4.4.1.

Table 4.4.1. The number of consecutive 15 minute scans, sleep staged thirty-second epochs and the percentage of time spent in each sleep stage for each individual subject.

Number of Epochs & Sleep (%)	Subject						Total Epochs
	1	2	3	4	5	6	
<i>Total Scans</i>	6	4	6	4	4	5	
W*	23	47	24	35	1	49	151
N1	36 (20%)	24 (20%)	84 (47%)	28 (23%)	43 (36%)	67 (45%)	282
N2	104 (58%)	35 (29%)	72 (40%)	85 (71%)	63 (53%)	34 (23%)	393
N3	17 (9%)	14 (12%)	0 (0%)	0 (0%)	13 (11%)	0 (0%)	44
Total Epochs	180	120	180	120	120	150	870
Total Sleep	87%	61%	87%	94%	99%	67%	

* Number of epochs in wake recorded during the sleep EEG-fMRI session.

Figure 4.4.6 displays the group average dynamic FC for each sleep stage and both daytime and night time wake for each target ROI. In general, all ROIs apart from PH showed higher PCC FC during the daytime waking scan than during sleep. PH-PCC FC was similar between sleep-stages and showed the lowest FC during daytime wake. PCC FC to the mPFC, IPL and MTL decreased in the night time compared to the daytime wake and continued to reduce with deepening sleep-stage, with the lowest PCC FC observed during N3.

A GLMM with the fixed-effects of ROI, sleep stage and the interaction between these effects was used to test the significance of the FC changes observed during sleep and wake (day and night time). The random effects of subject and subject by sleep stage interaction were also added to the model. The fixed-effect of ROI was found to be significant ($F(6,35)=30.256$, $p<0.001$), suggesting ROI was predictive of the FC (R-value). The fixed-effect of sleep stage alone did not quite reach significance ($F(4,20)=2.863$, $p=0.051$), which can perhaps be attributed to the significant interaction between ROI and sleep stage ($F(24,35)=2.923$, $p=0.002$) suggesting that PCC FC was altered by sleep stage in a regionally dependent fashion.

The FC strength between the mPFC and the PCC was significantly reduced with advancing sleep stage, compared to daytime wake (Figure 4.4.6), with the largest reduction from daytime waking FC observed in N2 ($R = -0.354$, $t = -4.700$, $p<0.001$) and N3 ($R = -0.346$, $t = -3.756$, $p<0.001$). A similar though attenuated effect was also found in comparison to night time wake, with a significant decrease with N2 ($R=-0.119$, $t=-2.626$, $p=0.010$) and N3 ($R=-0.111$, $t=-2.057$, $p=0.042$). The FC between the PCC and the left IPL was significantly reduced compared to the daytime wake in N2 ($R = -0.291$, $t=-2.650$, $p = 0.013$) and N3 ($R = -0.302$, $t = -$

2.245, $p = 0.032$), but not in N1 ($R = -.149$, $t = -1.296$, $p = 0.205$). PCC- left IPL FC was also significantly reduced in comparison with the night time wake for N2 ($R = -0.153$, $t = -2.193$, $p = 0.030$) and N3 ($R = -0.164$, $t = -2.296$, $p = 0.024$). The right IPL-PCC FC was significantly reduced in N1 ($R = -.189$, $t = -2.234$, $p = 0.032$), N2 ($R = -.326$, $t = -4.036$, $p < 0.001$) and N3 ($R = -.338$, $t = -3.417$, $p = 0.002$) between daytime wake, although this did not differ significantly in comparison with night time wake.

The bilateral MTL also exhibited a significant reduction in FC with the PCC with sleep stage compared to daytime wake. The largest reduction occurred in N3 for both the left ($R = -0.299$, $t = -3.027$, $p = 0.005$) and right ($R = -0.387$, $t = -4.871$, $p < 0.001$) MTL. Significantly reduced MTL FC was also observed bilaterally during N1 (left $R = -0.239$, $t = -2.825$, $p = 0.008$; right $R = -0.299$, $t = -4.395$, $p < 0.001$), and during N2 (left $R = -0.282$, $t = -3.500$, $p = 0.001$, right $R = -0.296$, $t = -4.565$, $p < 0.001$). However compared with night time wake the MTL reductions in FC were not as large, only FC of the right MTL in N3 ($R = -0.109$, $t = -2.055$, $p = 0.042$) was significantly reduced (see Figure 4.4.6).

DMN FC strength during the daytime wake epochs was higher on average compared to the night time epochs, although overall there was no significant difference between the two types of wake ($F(1,53) = 1.785$, $p = 0.187$). There were specific regions which did appear to differ significantly in PCC FC strength between daytime and night time wake: these were the right IPL ($R = -0.252$, $t = -3.122$, $p = 0.004$) and bilateral (Left: $R = -0.296$, $t = -3.672$, $p = 0.001$; Right: $R = -0.278$, $t = -4.289$, $p < 0.001$) MTL. There was also no significant difference between N1 and night time wake for any of the regions, demonstrating the increased similarity of night time wake with N1 (see Figure 4.4.6).

In addition, clear decreases in the variability of the PCC FC with increasing sleep depth were observed across all DMN regions (standard deviation: Daytime wake=0.234, Night time wake=0.219, N1=0.170, N2=0.113, N3=0.096). The time spent with a strong positive correlation ($R > 0.2$) was calculated for each sleep stage and wake (day and night), and the subject mean percentages were subsequently computed and plotted in Figure 4.4.7. A GLMM found that ROI ($F(6,43)=17.108$, $p < 0.001$) and sleep stage ($F(4,153)=14.890$, $p < 0.001$) were significant predictors for the percentage of time functionally connected to the PCC. The interaction between ROI and sleep stage was not significant, instead all DMN target ROIs were found to have an overall reduction in the percentage of time spent functionally connected to the PCC as sleep deepened. There was a small reduction in the overall time spent strongly functionally connected in the night time wake compared to wake recorded during the day session, although this was not significantly different ($R = -0.199$, $t = -2.540$, $p = 0.128$).

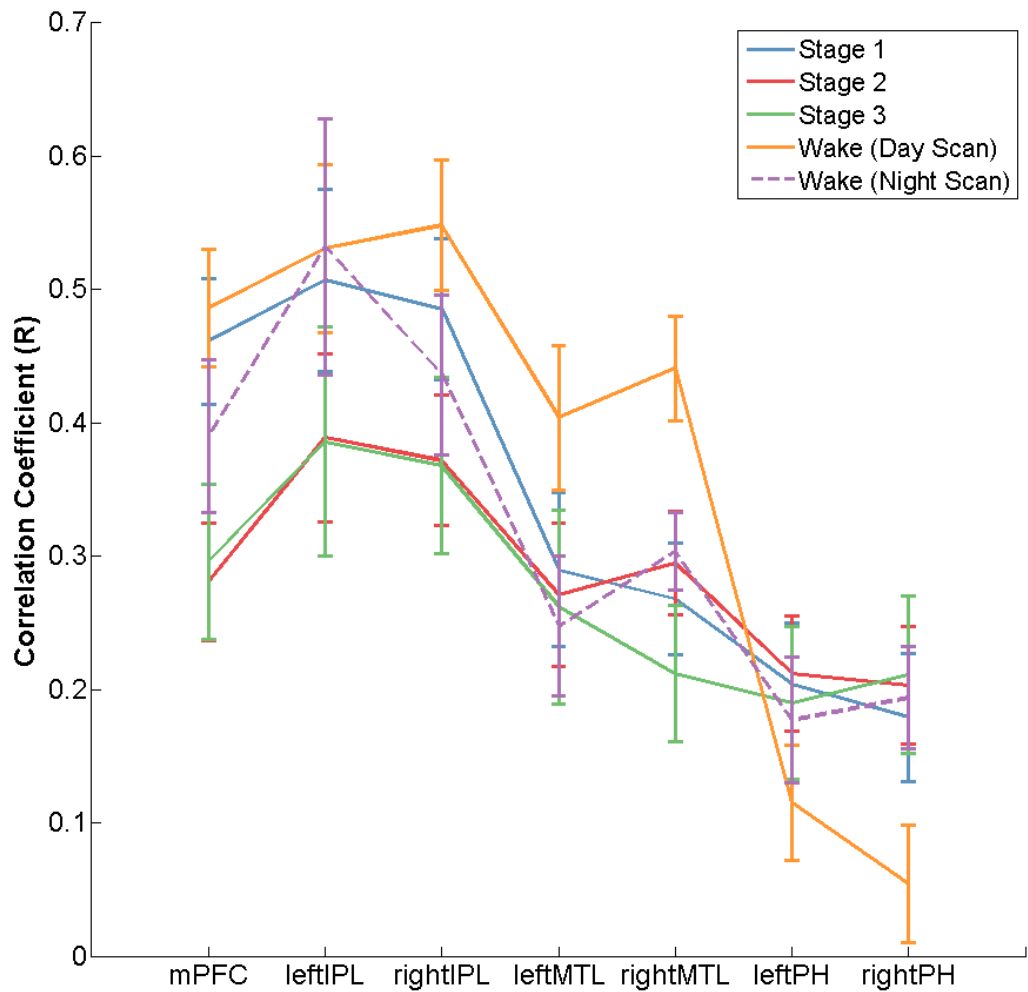


Figure 4.4.6. The effect of sleep stage on the group average dynamic FC between the PCC and all DMN target ROIs. Error bars represent the group mean \pm SE. * Indicates significant change in PCC FC with increasing sleep stage, compared to waking FC calculated from the daytime scan. In all cases FC was calculated using 30 second epochs and seed-based correlation analysis.

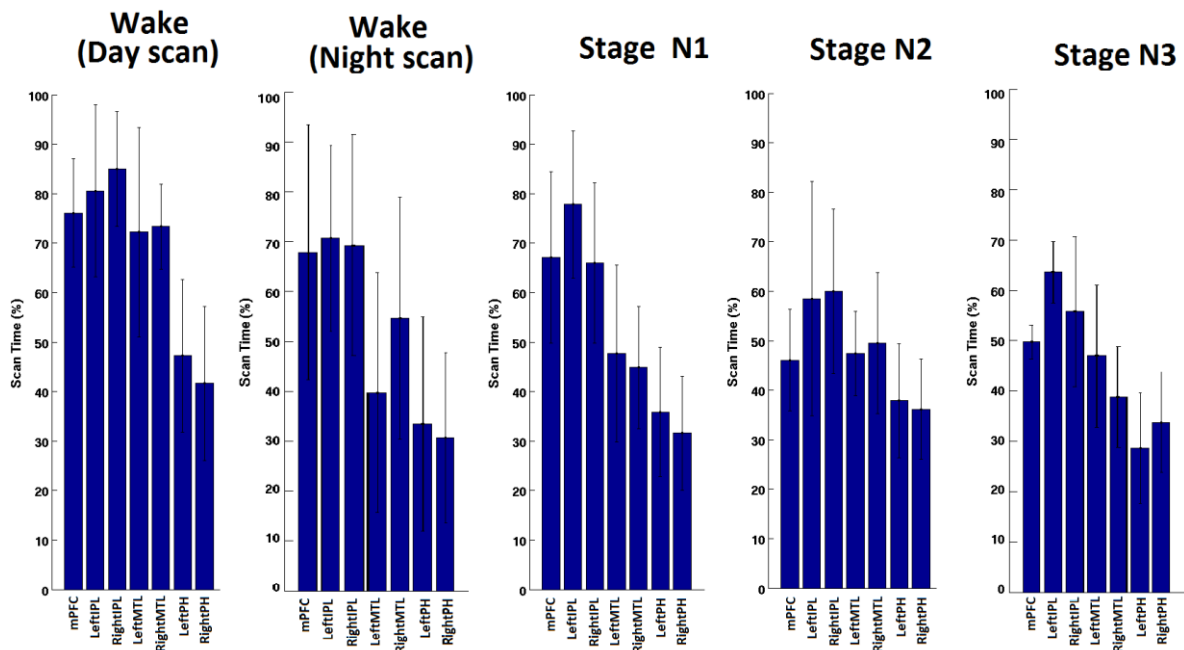


Figure 4.4.7. The proportion of time during which strong functional connectivity ($R > 0.2$) was observed between the PCC and all other DMN ROIs for each sleep stage. Error bars represent \pm standard deviation across subjects.

4.5 Discussion

This study assessed the feasibility of using short duration temporal epochs for the calculation of seed-based FC. Firstly, we assessed how the strength of PCC FC changed as the epoch length was progressively decreased from 15 minutes to 8 seconds, looking at both the spatial topography of FC across the brain and the pattern across regions of the default mode network (DMN). We demonstrated that using 30 second epochs provides a comparable measure to static PCC FC, while providing a substantial increase in the temporal resolution of FC. Secondly, we assessed the variability in dynamic FC for shorter epoch lengths and identified

cohesive relationships in dynamic PCC FC between the other nodes of the DMN, effects which were gradually lost as the epoch length increased.

Finally we took advantage of the increased temporal resolution provided by dynamic FC to study behavioural and physiological correlates of FC at a fine time scale, using the previously reported changes in FC with sleep depth to provide an additional validation of the short epoch approach to characterising FC. We provide the first direct comparison between individual 30s epochs of DMN FC and classical EEG-defined sleep stages by applying 30s dynamic FC analysis to data recorded during natural sleep. This analysis demonstrated the ability to identify alterations in PCC FC that occur with advancing sleep stage. While previous studies have generally examined stable periods of several minutes of a particular sleep stage and often used sleep deprivation to encourage sleep in the MR scanner (Horovitz et al., 2008, 2009; Larson-Prior et al., 2009; Sämann et al., 2010; Spoormaker et al., 2010), we were able to use all of the available sleep data and able to investigate natural sleep. This approach opens up the possibility of investigating FC differences between normal and recovery sleep, which are known to be electrophysiologically and architecturally different (Borbély et al., 1981; Brunner, Dijk, Tobler, & Borbély, 1990; Knoblauch, Martens, Wirz-Justice, & Cajochen, 2003). Overall, this study demonstrates the potential to assess short-term FC changes within the DMN using fMRI, enhancing the potential of FC for the characterisation of dynamic behaviours such as sleep.

4.5.1 The applicability of dynamic FC for studying intrinsic FC during wakefulness

The pattern of PCC FC strength within the DMN was highly comparable when using 15-minute static FC and dynamic FC, although the mean dynamic FC strength progressively reduced with decreasing epoch length, the overall pattern of ROI FC was maintained. After further examination of the FC time series (Figure 4.4.3), it is apparent that this reduction of mean dynamic FC is due to the increase in FC variability with decreasing epoch length. However the reduction in FC strength is not necessarily a confound of this method given that the definitive FC between the neuronal activity of two brain regions (i.e., the ground truth) is not known. Instead we suggest that static FC could in fact be overestimating the true FC, whereas dynamic FC is more representative of the brain's intrinsic properties. Due to the variability of dynamic FC and of the neuronal processes it represents, a single, instantaneous measurement of FC cannot be considered representative of the FC over a period of several minutes. Dynamic FC could provide a very useful tool to investigate the effect of transient connectivity upon a subsequent task response or short term differences between behavioural states, such as we demonstrate in sleep. We suggest that the 30-fold increase in temporal resolution provided by using 30s dynamic epochs, compared to 15-minute static FC, outweighs the proportionally much smaller 15.7% decrease observed in mean PCC FC across all DMN ROIs.

Although there is an increased likelihood of spurious correlations when using shorter epochs (Hutchison et al., 2012), the strength of mean PCC FC was significantly larger than the FC calculated using permuted data in all the highly connected DMN regions (mPFC, bilateral IPL and MTL). Using dynamic measurements of FC has the additional advantage of reducing the

contribution of any transient signal artefact to the FC measurement. Static measurements of FC can be heavily influenced by a small number of infrequent artefacts in the BOLD signal such as head movements which can erroneously increase the apparent correlation between two regions BOLD time series. The effect of such confounds are minimised when using the mean of dynamic FC as the few artefact epochs constitute a relatively small proportion of the total measurements. Furthermore there is the potential to remove artefactual epochs if the timing of the artefact can be accurately identified, possibly saving datasets which would otherwise be discarded.

The average spatial pattern of dynamic FC throughout the brain was also extremely consistent with the static FC measurement, indicating that functionally relevant information can be extracted from the average of individual epochs. Taken together, the findings of this study suggest that dynamic FC provides an equivalent measurement of coherent neurophysiological processes to that obtained from static FC, with the advantage of increased temporal resolution. Indeed previous reports have supported this by showing that the temporal variability of FC is correlated with EEG spectral power, a direct signature of fluctuations in neuronal activity (Chang, Liu, Chen, Liu, & Duyn, 2013; Tagliazucchi, von Wegner, Morzelewski, Brodbeck, et al., 2012). Therefore using dynamic FC enables research to assess the FC of a particular behavioural factor with a finer temporal precision.

The findings of this study suggest that solely relying on the static average FC strength could be restrictive or indeed misrepresentative as temporal fluctuations in FC are indicative of dynamic behavioural changes (Chang & Glover, 2010; Hutchison et al., 2012). Therefore measures of FC variability (Figure 4.4.3) or the percentage of time which a pair of regions

(Figure 4.4.4) or an entire network spend highly connected represents valuable, complementary information alongside FC strength, and may offer further insight into intrinsic brain activity (Garrett et al., 2013).

We were also interested in whether dynamic PCC FC within the DMN is cohesive across regions, i.e. functionally connected regions acting in synchrony. By assessing all epoch lengths we identified certain pairs of regions, mainly bilateral homologous IPL, MTL and PH regions, which consistently shared a similar time series (Figure 4.4.5). The FC stability of these bilateral regions has been shown to be consistent across subjects for 1 minute epochs (Gonzalez-Castillo et al., 2014). Interestingly we find the cohesive stability of bilateral FC within the DMN increases for all subjects at the shortest epoch lengths (30s and below), whereas this consistency across subjects was not apparent with longer epoch lengths (>1 minute). While the data presented here cannot refute the possibility that the differences in cohesiveness are a function of biases introduced by the fundamental properties of the FC time series (e.g., autocorrelation), Figure 4.4.5 does not show evidence of a systematic bias with epoch length, and the permutation testing did not indicate that the amplitude of spurious correlations was determined by epoch length. We would therefore argue based on our analysis that restricting investigations to several minutes worth of data jeopardises the extraction of the intrinsic properties of the FC network.

4.5.2 Dynamic FC during sleep

This study is the first to directly compare classical 30-second sleep stages to fMRI FC calculated dynamically over the equivalent time period during natural sleep. PCC FC assessed

dynamically using 30s epochs was found to be sensitive to the changes in human behaviour observed when descending from wakefulness into sleep and also between subsequent sleep stages. Previous reports using epoch lengths greater than several minutes reported a decrease in DMN FC with advancing sleep stage (Larson-Prior et al., 2009; Sämann et al., 2010), and our dynamic FC analyses support these findings, providing a validation of our approach. In addition this study also demonstrates that a similar decrease in FC exists during natural sleep in the absence of sleep deprivation, an interesting separate finding.

The largest regional reduction in FC with sleep stage occurred between the PCC and the mPFC, corroborating earlier research (Horovitz et al., 2009; Sämann et al., 2011). The strength of PCC FC to the bilateral IPL was also reduced by sleep stage (Figure 4.4.6), although stage N1 was very similar to wake (day and night), N2 and N3 were more comparable in their FC strength. It is also worth noting that PCC FC during wake from the daytime session did not statistically differ from PCC FC during EEG classified wake from the sleep session. However certain regions were significantly different, as shown in (Figure 4.4.6), with FC during daytime wake showing larger FC values in the MTL and smaller FC in the PH compared to wake during the sleep session. Considering only cortical regions, in general lower PCC FC was observed during the night-time wake than during daytime wake, making night-time wake more similar to the FC seen during actual sleep stages. This finding could be due to a greater level of drowsiness in periods of wake during the sleep session reducing the overall FC, or because of the use of (standard) thirty second epochs in the sleep staging which may mix light sleep and wakefulness. Furthermore, FC during periods of wake immediately preceding and following periods of sleep may not accurately reflect waking measures as these differences in brain states are not fully understood. Further research using

dynamic FC methodology is needed to investigate this in more detail. Given the greater PCC FC strength in daytime wake compared to any night time epochs we think it unlikely that this session is significantly influenced by sleep, which is well known to reduce DMN FC from waking levels (Horovitz et al., 2009; Sämann et al., 2011).

Similar to the current study, previous reports have also highlighted the dynamics of resting-state FC during sleep, using classification techniques to stage sleep from fMRI data (Tagliazucchi, von Wegner, Morzelewski, Borisov, et al., 2012). This has enabled the identification of FC changes during the transition from wakefulness into sleep (Tagliazucchi & Laufs, 2014), as well as raising the possibility that what are generally considered waking resting-state scans may contain periods of sleep in around a third of subjects. While this work remains to be replicated, it raises an important point that may influence the interpretation of a large number of studies, including this one. In our case, two independent periods of wake were acquired; the ‘gold standard’ wake data identified during the EEG-fMRI sleep session and the daytime wake scan recorded without EEG. However in our data there was no significant difference in PCC FC between the daytime and night-time wake data sets, suggesting that the daytime session was also predominantly composed of wakefulness. This raises an important issue, and an interesting topic for future work.

It is clear from previous theoretical and empirical research that over the course of prolonged wakefulness sleep-promoting neurotransmitters and neuromodulators build up and are represented by an increase in the EEG slow wave power (Achermann & Borbély, 2010; Borbély, Alexander & Achermann, 1999). Certainly in behavioural terms there is a qualitative difference between wakefulness in the middle of the day and that around midnight (Basner,

Rao, Goel, & Dinges, 2013; Killgore, 2010; McCoy & Strecker, 2011), when the EEG-fMRI session took place. Therefore wakefulness itself could be thought of as a non-stationary state comprising a range of behavioural and functional characteristics where even unambiguous EEG-derived epochs of wake might have different patterns of fMRI FC depending on the time of day that they are recorded. In future studies, the state of wakefulness could be quantified by both sleep status (i.e., time since last sleep) and circadian phase, leading to quantification of a spectrum of waking states. The idea that waking FC is dependent on past sleep history is supported by several studies which have either quantified sleep patterns over several days or used sleep deprivation (De Havas, Parimal, Soon, & Chee, 2012; Killgore, Schwab, & Weiner, 2012). Alterations in the BOLD response to tasks as a function of circadian phase have also been noted (Peres et al., 2011; Schmidt et al., 2012) .

The approach we have taken has several advantages for the investigation of sleep over the more common methodology of using epochs of several minutes to characterise FC. Sleep in the MR scanner is somewhat fragmented, with the noise of the scanner and the inability to move as one would naturally like to during sleep leading to a reduction in sleep quality. The result is a greater number of arousals with an increased number of sleep stage transitions. If the analysis requires stable periods of several minutes of a particular sleep stage a large proportion of the data is excluded, and the ability to investigate explicitly the changes in brain state that accompany sleep stage transitions is lost. Fragmented sleep of this nature is also more common in clinical populations suffering with sleep disorders, as well as in ageing (Carskadon, Brown, & Dement, 1982; Stepanski, 2002). As such this type of fragmented sleep data lends itself to dynamic FC analysis in order to exploit transitional changes in sleep stage which would be removed from the data if epochs of several minutes were used.

In addition to examining FC strength we also compared the variability of FC within the different sleep stages. By calculating the percentage of time during which ROIs were strongly functionally connected to the PCC (Figure 4.4.7) we were able to identify which sleep stages show greater temporal variability in DMN FC. It has been shown that during slow-wave sleep the neuronal activity of the cortex becomes globally more synchronised, with FC variability decreased due to the lack of ‘transient brain states’ which are present in wake (Deco, Hagmann, Hudetz, & Tononi, 2013). Interestingly we find that PCC FC in both periods of wake (day and night) has the greatest temporal variability, with more variability during daytime wake, whereas this is markedly reduced in sleep stage N3 for all regions within the DMN. This reduction in complexity is mirrored by the EEG changes that are used to define the sleep stages, with the mixed frequency EEG of wake giving way to the low frequency slow waves of N3. The relationship between these dynamic FC and EEG signatures of sleep remains to be investigated, as does the relationship between the mean connectivity during wakefulness and its subsequent variance as a function of sleep stage. The current study highlights the advantages of using measures of FC variability defined by dynamic FC as an additional way of investigating the sleeping brain.

The main focus of this study was to look at the methodological aspects of using short epoch dynamic FC, providing a detailed comparison of the effect of epoch length in the waking data and demonstrating some of the issues that can be addressed with a dynamic approach that are not amenable to static analysis (Figure 4.4.3-Figure 4.4.5). While our sample size was relatively small, particularly for the sleep data, the approach concentrates on extracting as much information from individual subjects as possible, which in future work in larger samples can be linked with inter-individual variability in behaviour. The data from the relatively small

number of subjects who had undergone a sleep session was used to provide a validation of this approach. Dynamic FC was able to replicate the observations made with epochs of several minutes reported in previous studies (Heine et al., 2012; Horovitz et al., 2009; Larson-Prior et al., 2009, 2011; Sämann et al., 2011), which is a necessary step prior to applying it for more innovative analyses and those which can only be addressed dynamically.

4.6 Conclusion

Short epochs can be used effectively to calculate dynamic FC as part of a seed-based analysis. This approach is sensitive to FC changes present with advancing sleep stage, offering the possibility of successfully characterising dynamic FC, exploiting the temporal variability that is an intrinsic part of brain function and moving beyond the information that is provided by static FC calculated over several minutes. Dynamic FC with epochs as short as tens of seconds is a viable method for characterising intrinsic brain activity.

4.6.1 Supplementary

The FC analysis was re-run for each of the different epoch lengths using the equivalent methodology but omitting the pre-processing step of global signal regression (GSR). Figure 4.6.1 and Figure 4.6.2 replicate manuscript Figure 4.4.1 and Figure 4.4.2 without GSR. Figure 4.4.1 and Figure 4.6.1 display a very similar pattern of decreasing FC with shorter epoch length across all ROIs. This demonstrates that the effect of epoch length on average PCC FC remains consistent without GSR and has therefore not been artificially induced. As both Figure 4.6.1 and Figure 4.6.2 demonstrate, FC strength appears to be comparatively higher in strength than the manuscript Figure 4.4.1 and Figure 4.4.2, although negative correlation values still remain; the pattern of FC between the epoch lengths is similar regardless of GSR. Therefore the results have not been artificially induced via this pre-processing.

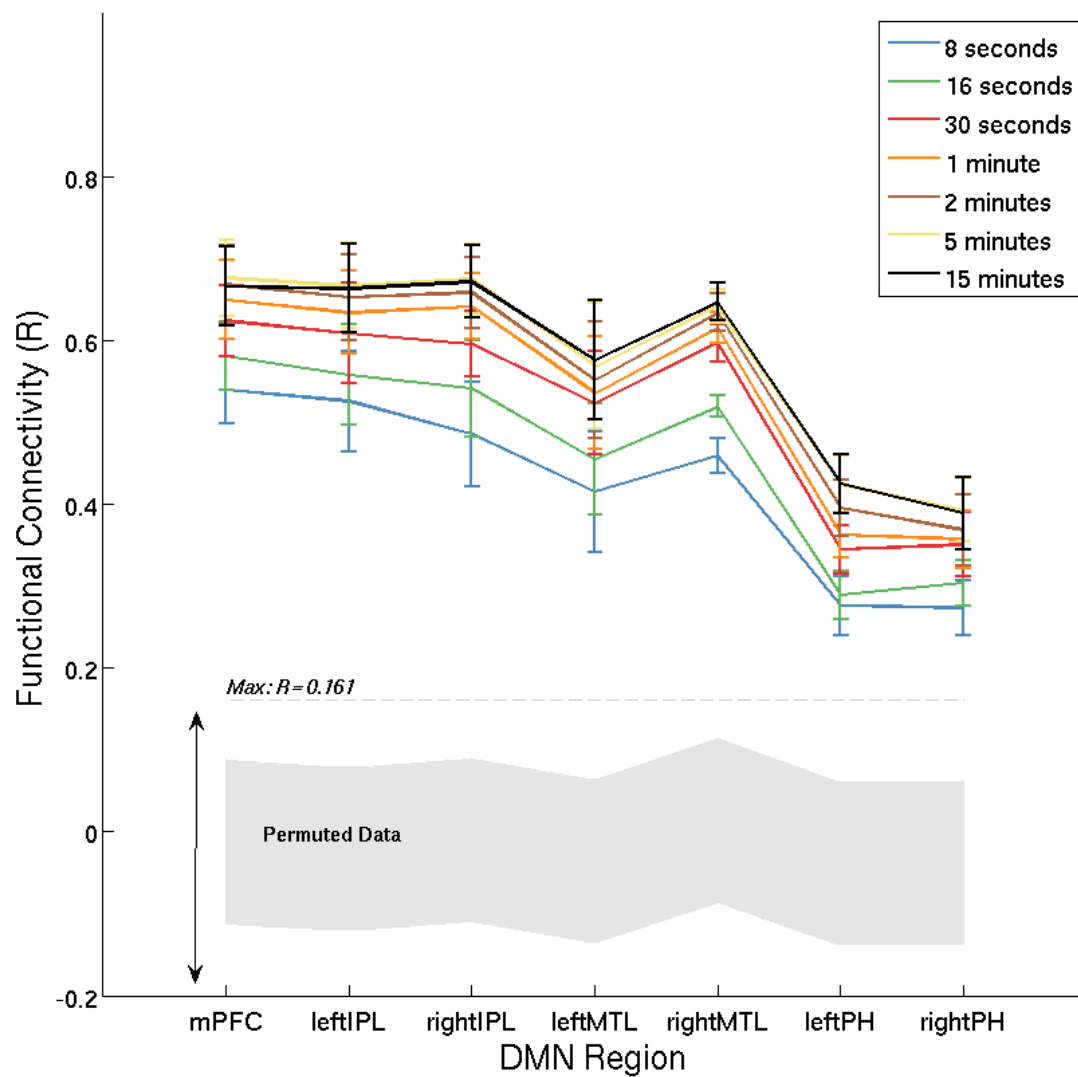


Figure 4.6.1 The group average FC strength, calculated without GSR, between the PCC and all other DMN ROIs calculated for each epoch type (solid line), compared to the permuted data standard deviation (grey shaded box). Error bars reflect the group mean \pm standard error.

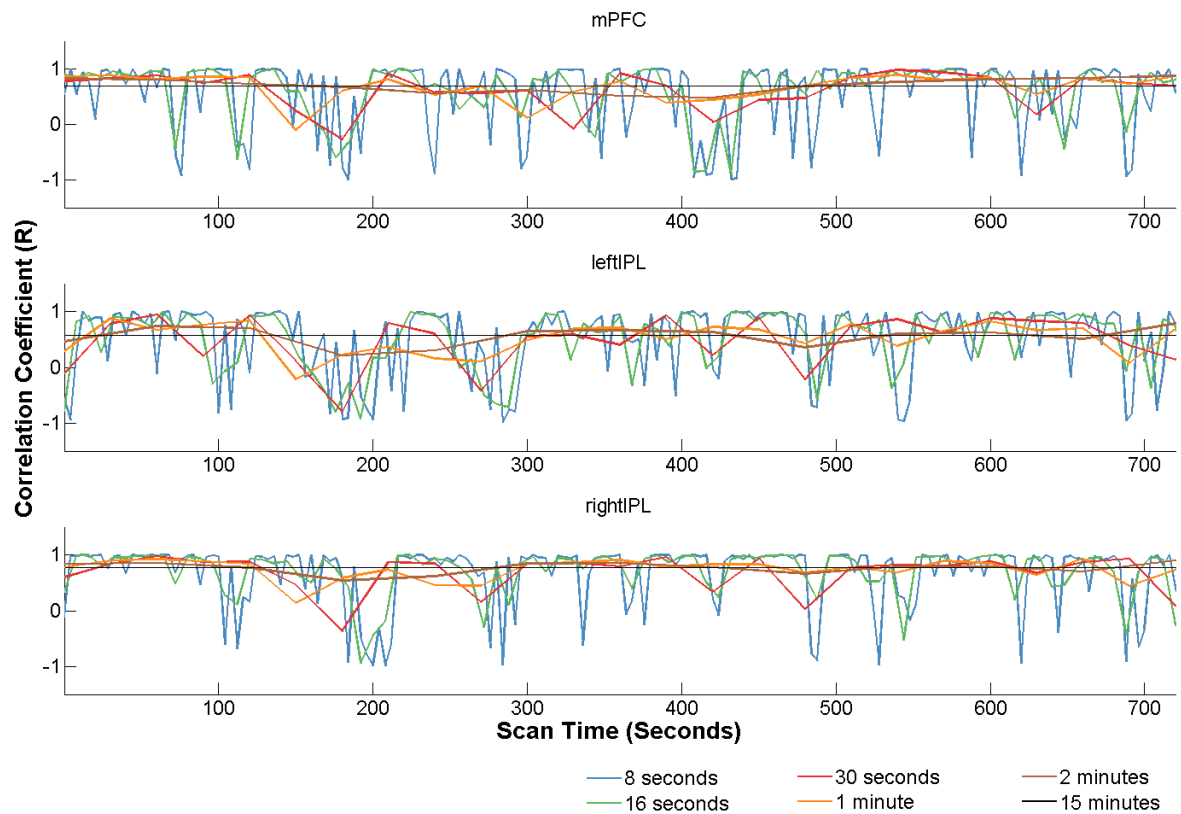


Figure 4.6.2. The timeseries of dynamic FC, calculated without GSR, between the PCC and three DMN ROIs; mPFC, left & right IPL in a representative subject. Plotting FC for a selection of epoch lengths, each represented with a different coloured line, allows comparison of the variability in FC between epoch length.

Chapter 5

PRIOR SLEEP DEPRIVATION AFFECTS FUNCTIONAL CONNECTIVITY DURING NREM SLEEP

5.1 Abstract

Neuroimaging studies are an increasingly important tool for studying the brain during sleep, but the MRI scanner can be a difficult place to initiate sleep. To overcome this, many studies employ sleep deprivation protocols prior to scanning, but the effect of this on the brain's activity during sleep is unknown. Here, we investigated the differences in a small cohort of eight subjects sleeping on two occasions; 1) after 24hrs of sleep deprivation 2) following a normal night of sleep. Dynamic FC was used to assess sleep within eight intrinsically connected networks. FC of large-scale brain networks differed in recovery compared to normal sleep, with effects most pronounced in the default-mode, thalamus and sensorimotor networks, while other sensory and cognitive networks were more resilient to sleep

deprivation. These findings suggest that sleep deprivation should not be used in neuroimaging sleep studies unless recovery sleep is the specific focus.

5.2 Introduction

Human sleep is not well understood, despite its clear importance to waking function and the range of cognitive, behavioural, metabolic and physiological processes that it affects and supports (Banks & Dinges, 2007; Killgore, 2010; McEwen, 2006; Pace-Schott & Hobson, 2002). Neuroimaging studies are becoming an increasingly important tool for studying the brain during sleep (Dang-Vu, 2012; Duyn, 2012) and in particular the combined recording of EEG and fMRI provides the ability to link standard EEG-based sleep staging (AASM, 2007) with changes to brain function. The quantification of FC, the statistical dependencies between brain regions (Friston, 2011), allied with examination of the brain's intrinsic connectivity networks (Fox et al., 2005; Van Dijk et al., 2010), is now a relatively common method for studying brain function, and has demonstrated its utility not only in previous sleep studies (Horovitz et al., 2008; Larson-Prior et al., 2009, 2011; Sämann et al., 2011; Spoormaker, Gleiser, & Czeisler, 2012; Uehara et al., 2013) but in a wide range of brain processes and pathologies (Fox & Greicius, 2010).

One problem with EEG-fMRI for sleep research is that the MRI scanner can be a difficult place to initiate sleep, due to a combination of the noise of the imaging sequences, the presence of scalp electrodes and the requirement to be as still as possible. To overcome this, it is not uncommon for studies to employ partial or total sleep deprivation (SD) prior to scanning (Horovitz et al., 2009; Kaufmann et al., 2006; Sämann et al., 2011; Spoormaker et

al., 2012). However, the effect of this on FC during sleep is unknown. Some indication that preceding SD might affect FC during sleep is provided by the fact that recovery sleep (i.e., sleep following SD) is recognised as neurophysiologically different to normal sleep (Bonnet, 2005), and appears to reverse the cognitive deficits caused by acute sleep deprivation (Banks, Van Dongen, Maislin, & Dinges, 2010; Belenky et al., 2003). Findings from polysomnographic (PSG) studies, the ‘gold-standard’ methodology used to characterise and assess sleep behaviour, indicate consistent changes in the sleep stage architecture (i.e., the sequence and proportion of different sleep stages) of recovery sleep compared to normal sleep (Bonnet, Berry, & Arand, 1991; Bonnet, 2005; Borbély, Alexander & Achermann, 1999; Brunner et al., 1990), as well as increased arousal thresholds in recovery sleep (De Gennaro, Ferrara, & Bertini, 2001) presumably due to the increased homeostatic sleep pressure. For example, a relative increase in slow wave sleep (SWS) is observed during recovery sleep, which may relate to the maintenance aspect of sleep and the build-up of neuromodulators due to prolonged wakefulness (e.g., adenosine) (Benington & Heller, 1995; Porkka-Heiskanen, 1997; Porkka-Heiskanen & Kalinchuk, 2011). In addition to these changes to sleep architecture and the brain’s neurochemistry, it has been suggested that sleep may have a role in maintaining the integrity of large-scale brain networks (Koenis et al., 2013), and that differences in habitual sleep duration affect waking FC (Khalsa et al., 2016) which would suggest that differences may be observed between normal and recovery sleep. However while some studies have explicitly investigated large-scale networks during sleep, to date there has been no comparison between normal and recovery sleep. In this study we seek to investigate this by comparing the FC of the brain’s major ICNs between these two types of sleep.

Considering evidence that sleep deprivation affects FC during wakefulness (Bosch et al., 2013; De Havas, Parimal, Soon, & Chee, 2012; Killgore, Schwab, & Weiner, 2012; Liu, Li, Wang, & Lei, 2014; Shao et al., 2013; Verweij et al., 2014), particularly in the default mode network (DMN, (Gujar et al., 2010; Sämann et al., 2010) alongside the differences in waking FC observed with varying circadian phase (Hodkinson et al., 2014; Shannon et al., 2013) we hypothesise that FC will be different in recovery sleep compared to normal sleep. This will have considerable implications for the use of SD in neuroimaging sleep studies, as well as starting to provide some clues regarding the neurophysiological underpinnings of recovery sleep. Sleep deprivation is increasingly prevalent in society (Bonnet & Arand, 1995; Colten, Altevogt, & Colten., 2006; Goel et al., 2009; Hillman & Lack, 2013), and leads to a range of detrimental waking cognitive and behavioural effects (Durmer & Dinges, 2005; Goel et al., 2009; Killgore, 2010), but whether there are differences in the large scale distributed networks that underlie complex brain function during recovery sleep has not been previously investigated.

Given the degree of variability between definitions of what constitutes recovery sleep (Dijk & Czeisler, 1994), the aim of the current study was not to distinguish between the impacts of homeostatic versus circadian processes upon FC, instead we recognise that recovery sleep features a combination of the two. As such we employed 24 hours of sleep deprivation, occurring through the night at the circadian nadir, with sleep occurring in the morning hours when circadian sleep propensity is low. This design maximises the potential effects as well as allowing comparison with incidences of real world sleep deprivation such as lifestyle choices and night-shift work.

5.3 Materials and methods

5.3.1 Experimental design

Subjects are the same as Chapter 4. Eight healthy adult volunteers (5 male, age 32 ± 6 years) were paid for their involvement in the two experimental sessions of this study. All participants completed the Epworth Sleepiness Scale to ensure a normal level of daytime sleepiness, consistent with the absence of sleep disorders (Johns, 1991). Participants were asked to keep a sleep diary and wear wrist actigraphs (Philips, Respironics® Actiwatch 2) for one week prior to each scan session in order to corroborate a regular sleep-wake pattern and to ensure experimental adherence. For the first session participants were instructed not to sleep the night before scanning (i.e., they were awake for ~24h), after which they underwent EEG-fMRI between 06:00 and 08:00 to facilitate recovery sleep. At least two weeks later the subjects returned for the second EEG-fMRI session after a normal night of sleep, this time at their usual bedtime (between 22:00 and 00:00) to investigate normal sleep. Two subjects had to be excluded: one due to a technical problem with the EEG data and the other for failing to sleep. This resulted in six participants (4 male, age 29 ± 5 years) who completed all parts of the study and were subsequently considered in the analysis.

5.3.2 EEG-fMRI acquisition, preprocessing and analysis

The EEG-fMRI acquisition protocol is outlined in the Chapter 3. T2*-weighted fMRI data (3x3x4mm voxels, TR=2000ms, TE=35ms, 450 volumes, flip angle 80°, SENSE factor = 2) were acquired in consecutive 15-minute scans. During the fMRI scans participants were

instructed to sleep if they could and to signal if they wished to terminate the session by pressing the buzzer once they were no longer able to sleep. fMRI data from both the recovery and normal sleep sessions were pre-processed and analysed as outlined in Chapter 3. Head movements were also assessed; see movement section for further details.

A within-subject ANOVA (factors: sleep type, sleep stage) compared the percentage of scan time across sleep stages between the normal and recovery sleep sessions. FC analysis of the fMRI data was calculated using a non-overlapping 30 second sliding window. This dynamic analysis using seconds rather than minutes to calculate FC has been applied to the investigation of FC changes during sleep in our previous study (Wilson et al., 2015). A FC correlation matrix was created for each sleep stage for each subject, see full methods description (Chapter 3). Exactly the same procedure was carried out for both sleep sessions and FC matrices subsequently used to compare FC between normal and recovery sleep.

As a further analysis designed to investigate spatial variations of the networks with sleep type, FC was examined by seeding from the main region within each of the networks for brevity to all voxels in the brain, generating a separate map of the spatial FC extent for each network. A 30-second non-overlapping sliding window FC analysis was used to calculate a correlation map for each epoch and for each seed (see Table 3.4.1 in Chapter 3). These epoch FC maps were paired to the corresponding EEG defined sleep stage, pooled by stage within subject and then averaged across subjects. This procedure was completed identically for each type of sleep. To reduce the number of seed regions, and hence spatial maps generated, not all network ROIs were used. For the DMN the PCC and PFC were separately used as seed regions to create two DMN FC maps. FC maps of the DAN were calculated by seeding in the

left IPS (LIPS). The anterior cingulate cortex (ACC) was used as the seed region of the SAL and the left motor area (LMC) for the SENS. The left superior temporal gyrus (LST) for the AUD and left primary visual areas (LPV) for the VIS. These regions were chosen for network brevity. The left hippocampus (LH) was the seed region for the HIP, in addition to the left thalamus (LT) for the THAL.

5.3.3 Network FC

Graph theory was applied to the FC correlation matrices in order to summarise FC changes with sleep across the whole-brain. We used an undirected weighted graph to model all regions at the subject level, with all analysis performed in MATLAB® (The MathWorks Inc, Natick, MA, USA) using the brain connectivity toolbox (<http://www.brain-connectivity-toolbox.net/>). The nodes of the graph represented the 28 individual brain ROIs (Table 3.4.1), including the bilateral anatomical hippocampus and thalamus, and positive FC values corresponded to the weights along each edge of the graph. Separate graphs were generated for each participant, sleep stage and sleep type. Analogous to the measure of degree for binary graphs, the nodal strength of a weighted network, s_i , is defined as $s_i = \sum_{j=1}^N a_{ij} w_{ij}$, where a_{ij} and w_{ij} are the adjacency and weight matrices respectively. For each graph the average strength across nodes was calculated as a measure of the extent to which the graph was connected. Differences in the average graph strength between each sleep stage and each sleep type were then assessed at the group-level with a generalized linear mixed-model (GLMM).

We then assessed the within network FC of each of the eight networks as outlined in Chapter 3. The raw correlation values were input into GLMMs for each ICN to determine whether the

FC strength was modulated by stage, sleep type or both. The GLMM included the fixed-effects of ROI, sleep stage, sleep type (normal and recovery) and all subsequent interactions, together with the random-effect of subject. Multiple comparisons were accounted for using Bonferroni correction within the GLMM and only statistically significant effects ($p < 0.05$) are discussed in further detail in the results. Lastly, the mean FC was calculated for each ICN by averaging across all regions for every sleep stage in both normal and recovery sleep.

5.4 Results

The actigraphy report for each subject was visually inspected and all subjects were deemed compliant with the sleep deprived protocol with activity recorded throughout the night time period. The average total sleep time (ATST) and before total sleep time (BTST) for the normal and deprived condition were analysed based on the sleep diaries, together with the Epworth Sleepiness questionnaire scores for each session, see behavioural data in Table 5.4.1. We recorded a total of 870 thirty-second epochs of data during the normal sleep session: 151 epochs of wake, 282 epochs of N1, 393 epochs of N2 and 44 epochs of N3. A total of 840 thirty-second epochs were produced from the same subjects during the recovery sleep session: 57 epochs of wake, 235 epochs of N1, 487 epochs of N2 and 61 epochs of N3. The total number of epochs and the percentage of scan time spent in each sleep stage for each subject are displayed for the normal and recovery sleep sessions in Table 5.4.2. Unsurprisingly the percentage of scan time differed significantly for sleep stage ($F(1,3)=14.998$, $p=0.026$, $\eta^2=0.937$). However there was no significant difference in scan time percentage between the types of sleep ($F(1,5)=0.122$, $p=0.741$, $\eta^2=0.024$) or any interaction between sleep type and

sleep stage ($F(3,3)=1.614$, $p=0.352$, $\eta^2=0.617$). As such, any FC differences observed between sleep stage and type cannot be attributed solely to differences in the quantity of data epochs between the conditions. Although not statistically significant, a higher percentage of scan time was spent in NREM sleep during the recovery session (92%) at the expense of time spent in wake compared to the normal session (83%). This resulted in subtle sleep stage architectural changes, with a decrease in the amount of time spent either awake or during N1 sleep and an increase in the amount of N2 and N3 sleep observed during the recovery condition. This concurs with previous reports of sleep stage transition and duration changes during recovery sleep (Berger & Oswald, 1962; Bonnet, 2005; Borbély, Alexander & Achermann, 1999; Brunner et al., 1990).

Due to the increased possibility of involuntary movements during sleep, absolute head movement was assessed as a function of sleep stage in the current study and epochs confounded by movement were removed (supplementary: Figure 5.6.1). This is an important consideration given head movements within the MR scanner have been shown to affect FC analysis (Satterthwaite et al., 2012; Smith et al., 2014; Van Dijk et al., 2012).

Table 5.4.1 Measures of subjects sleep history included in the analysis (N=6).

Sleep Measures	Mean	Median	SE	SD
<i>Normal sleep session</i>				
Average TST	455.6	461.5	±16.6	±66.2
Before TST	448.8	450	±16.0	±64.1
<i>Recovery sleep session</i>				
Average TST	486.8	496.7	±10.8	±54.1
Before TST	490	510	±12.3	±61.7

TST = Total Sleep Time

Table 5.4.2. The number of sleep-staged, thirty-second epochs and the percentage of time spent in each sleep stage for each individual subject, separately for normal and recovery sleep

Type of Sleep	Number of Epochs & Sleep (%)	Subject						Total Epochs
		1	2	3	4	5	6	
Normal	Scans (<i>15min</i>)	6	4	6	4	4	5	
	Wake	23	47	24	7	1	49	151
	Stage 1 (N1)	36 (20%)	24 (20%)	84 (47%)	28 (23%)	43 (36%)	67 (45%)	282
	Stage 2 (N2)	104 (58%)	35 (29%)	72 (40%)	85 (71%)	63 (53%)	34 (23%)	393
	Stage 3 (N3)	17 (9%)	14 (12%)	0 (0%)	0 (0%)	13 (11%)	0 (0%)	44
	Epochs	180	120	180	120	120	150	870
	Total Sleep	87%	61%	87%	94%	99%	67%	
Recovery	Scans (<i>15min</i>)	4	5	6	2	5	5	
	Wake	15	6	12	21	3	0	57
	Stage 1 (N1)	18 (15%)	78 (52%)	53 (29%)	40 (44%)	11 (7%)	35 (23%)	235
	Stage 2 (N2)	68 (57%)	59 (39%)	115 (64%)	29 (32%)	107 (71%)	109 (73%)	487
	Stage 3 (N3)	19 (16%)	7 (5%)	0 (0%)	0 (0%)	29 (19%)	6 (4%)	61
	Epochs	120	150	180	90	150	150	840
	Total Sleep	88%	96%	93%	77%	98%	100%	

5.4.1 FC across the brain

We found that the strength of FC differed with sleep stage and also between normal and recovery sleep for individual regions of ICNs. The connectivity matrices in Figure 5.4.1A show how the regional pattern of FC was affected by sleep stage and type for all of the assessed ROI comparisons. Qualitatively an overall decrease in FC both within and between ICNs with increasing sleep stage is apparent for both normal and recovery sleep.

The FC differences between the types of sleep were further assessed by subtracting each of the group mean ROI correlation coefficients of normal sleep from the corresponding coefficient during recovery sleep for wake and all sleep stages. The regional FC comparisons between normal and recovery can be seen in Figure 5.4.1B. Much of the region-region FC strength increased in recovery compared to normal sleep stages, although there was a disparity in wake with a reduction in FC for the majority of regions in the recovery session. The increased FC in recovery sleep of the thalamus to cortical regions within the DMN, SAL and SENS was notable through all sleep stages. In N1 during recovery there was a marked increase in the regional FC of the right insula (RINS) within the SAL, to virtually all regions within the DMN, DAN and SENS, as well as greater FC strength with the THAL and HIP. During N2 recovery sleep THAL FC remained higher than in the normal session, particularly to the RINS and SENS regions. Recovery sleep FC strength was considerably increased in N3 between much of the SENS and AUD regions to the DMN and DAN.

In addition to these observations, data from the connectivity matrices were analysed using graph theory to summarise FC across the brain by assessing the average strength across all

regions as a function of sleep type and stage. FC strength appeared higher for recovery than normal sleep for all sleep stages, other than wake which was lower during the sleep deprived session (Figure 5.4.2). A GLMM did not find a significant difference for sleep type ($F(1,16)=0.551$, $p=0.468$), suggesting that graph strength across the brain was similar for normal and recovery sleep. However graph strength across the brain was found to differ significantly as a function of sleep stage ($F(3,15)=15.202$, $p\leq 0.001$), with decreased graph strength observed as sleep deepened (Figure 5.4.2). The interaction between sleep type and stage was not found to be significant ($F(3,14)=0.790$, $p=0.520$), indicating that the decrease in ICN graph strength with advancing sleep stage was a characteristic of both normal and recovery sleep.

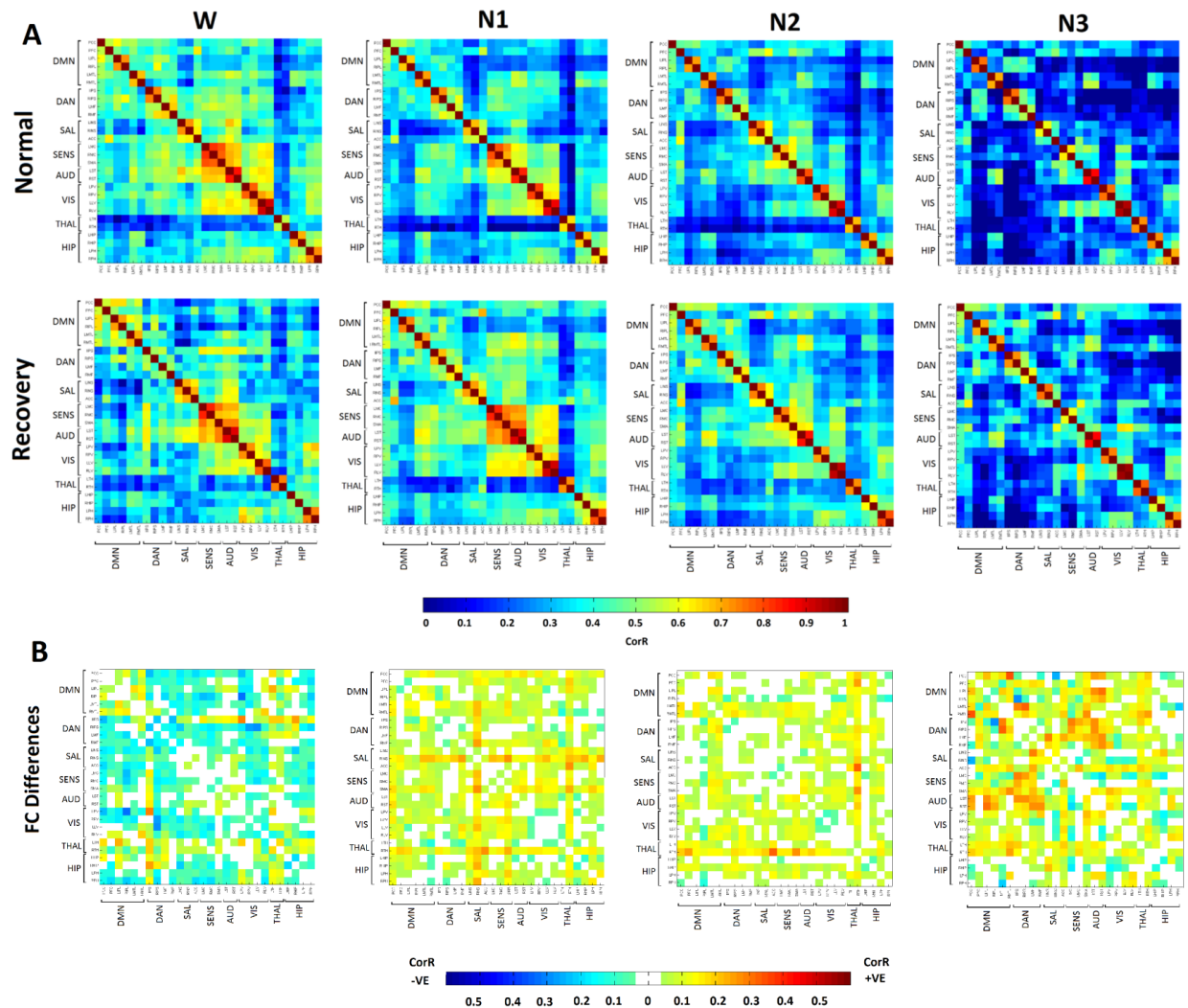


Figure 5.4.1. Group average, uncorrected full correlation matrices showing the Pearson's R value of FC between each pair of ROIs as assessed during wake and each sleep stage. A) The FC correlations for both normal and recovery types of sleep. B) The FC correlation differences between the stages of normal and recovery sleep, the reduced FC during the recovery sleep session compared to the normal session is shown in blue colours and the higher regional FC in recovery sleep is shown in red-yellow colours. ROIs are plotted according to the ICN to which they belong.

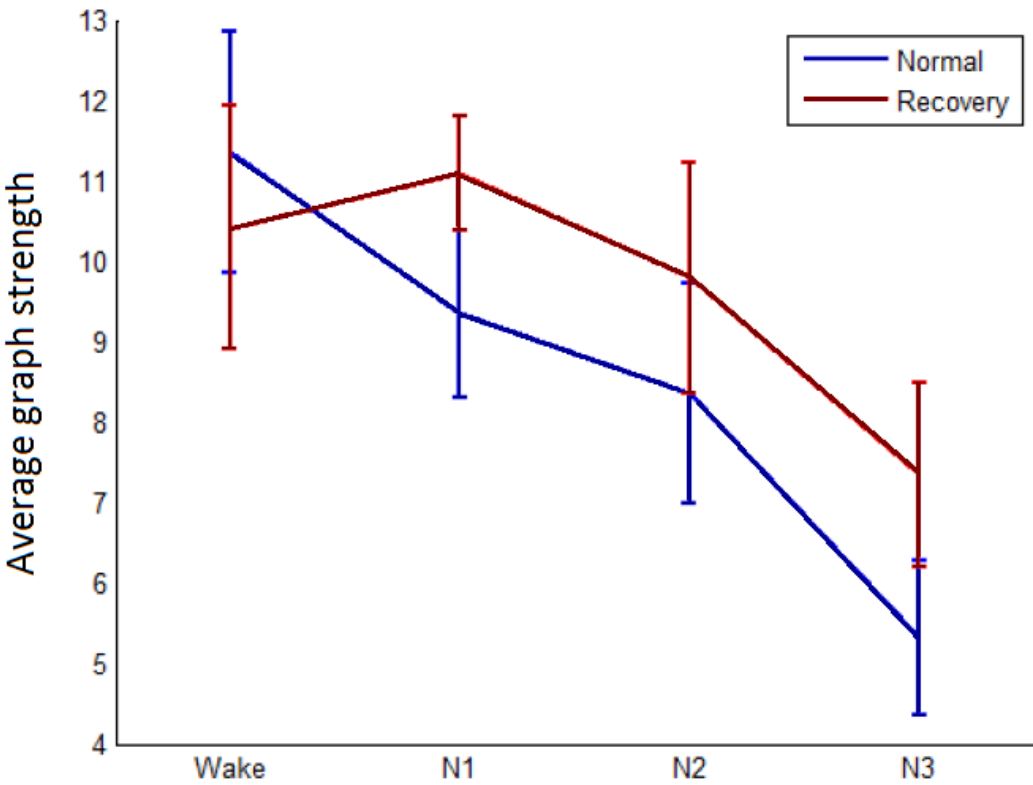


Figure 5.4.2. The average graph strength of nodes for all regions across subjects for sleep stage and for sleep type. Error bars represent \pm SE.

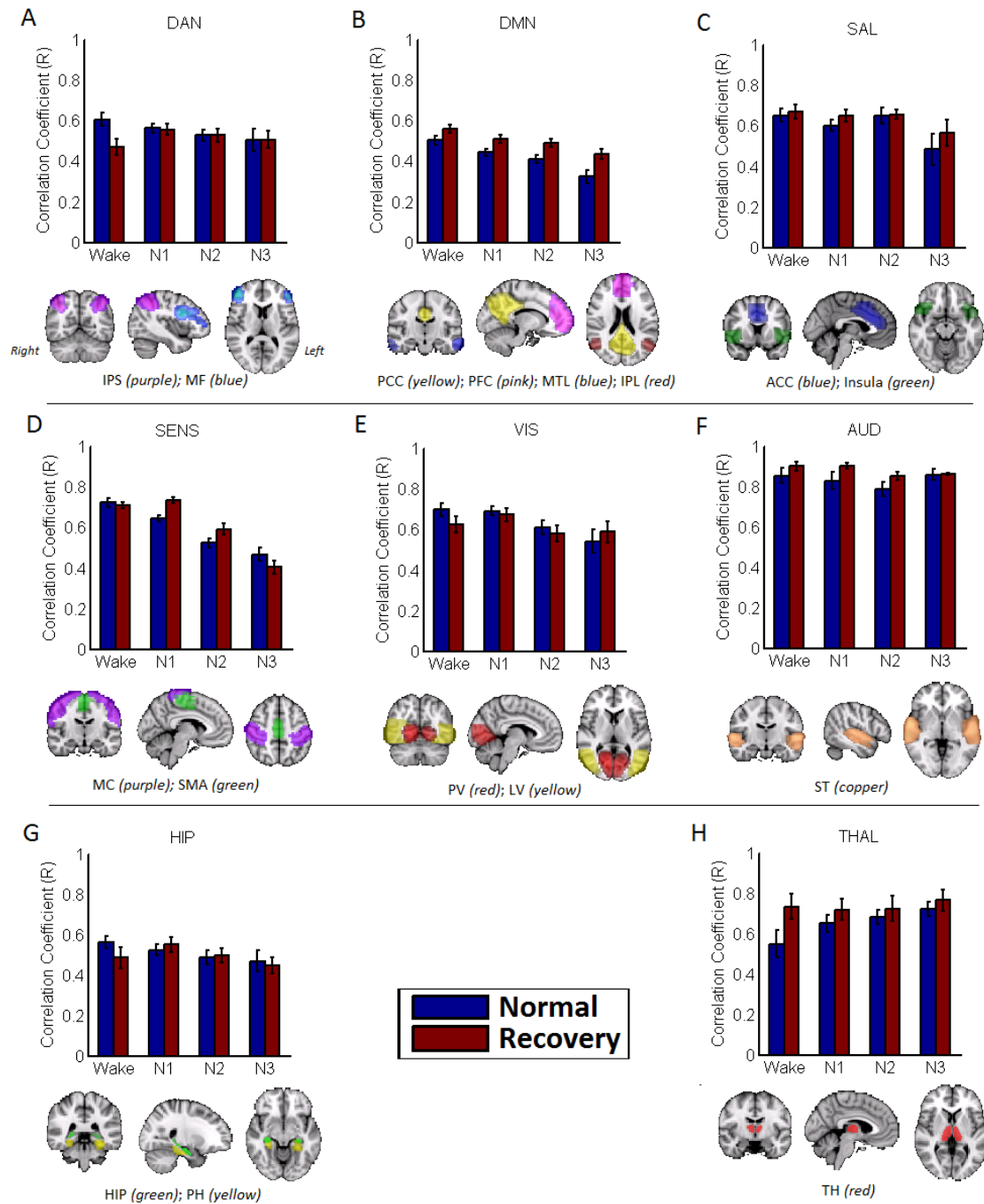


Figure 5.4.3. The group average FC within each of the major ICNs during normal (blue) and recovery (red) sleep for the different sleep stages. Accompanying brain maps illustrate each ICA cluster ROI for each ICN. Error bars represent \pm SE. Abbreviations for the individual ROIs are given in Table 3.4.1

5.4.2 Within ICN FC

Figure 5.4.3 shows that ICNs differed in the effect of sleep stage on their within network FC between normal and recovery sessions. During recovery sleep FC was either not significantly different or significantly higher than that in normal sleep, especially in the DMN and THAL, although there were a few notable exceptions: for example, wake FC was lower in the recovery session for the DAN and in N3 for the SENS. The DAN, DMN, AUD and THAL had the largest differences between the types of sleep, and the DMN was the only network to also be affected by sleep type in a regionally dependent fashion. Sleep stage was found to affect FC most profoundly in the DMN, SENS, VIS and THAL, although the effect of sleep stage was dependent on the type of sleep within the DAN and SENS. These observations are discussed in more detail below.

Dorsal attention network

The main effects of ROI ($F(5,61)=20.498$, $p<0.001$) and sleep type ($F(1,196)=5.786$, $p=0.017$) were significant within the DAN. There was a significant interaction between sleep stage and sleep type ($F(3,196)=3.242$, $p=0.023$) which suggests that overall DAN FC between sleep stage was influenced by type of sleep. This interaction was driven by the reduced DAN FC observed during periods of wake during the recovery session compared to wakefulness in the normal sleep session (Figure 5.4.3A). However the pattern of FC within the DAN was not regionally dependent, given that neither the interaction between ROI and stage ($F(15,61)=1.078$, $p=0.395$) nor ROI and sleep type ($F(5,61)=1.190$, $p=0.325$) were significant.

Default mode network

A GLMM revealed a significant difference in DMN FC strength with ROI ($F(14,64)=50.245$, $p<0.001$), sleep stage ($F(3,469)=17.537$, $p<0.001$) and sleep type ($F(1,464)=43.960$, $p<0.001$). DMN FC strength decreased with advancing sleep stage for both types of sleep, however DMN FC was significantly higher during recovery sleep, see Figure 5.4.3B. There was also a significant interaction between ROI and sleep type ($F(14,64)=2.443$, $p=0.008$), suggesting that sleep type modulated FC within the DMN in a regionally specific manner. In particular the FC of the bilateral MTL with the PCC and LIPL were significantly increased in recovery sleep. With advancing sleep stage there was a decrease in FC for the majority of ROIs in the DMN. As a result, the interaction between ROI and sleep stage failed to reach significance ($F(42,64)=1.253$, $p=0.205$).

Saliency network

The main effects of ROI ($F(2,65)=14.507$, $p<0.001$) and sleep stage ($F(3,98)=4.005$, $p=0.010$) were significant within the SAL network. FC in wake, N1 and N2 did not differ statistically between normal and recovery sleep, but increased FC during recovery sleep was observed within N3, see Figure 5.4.3C. The main effect of sleep type ($F(1,97)=2.509$, $p=0.116$) was not significant within the SAL. The interaction between sleep stage and sleep type was not significant ($F(3,97)=0.428$, $p=0.734$), in addition to no significant ROI interactions with sleep stage ($F(6,65)=0.918$, $p=0.488$) or the type of sleep ($F(2,65)=0.312$, $p=0.733$).

Sensorimotor network

The main effects of ROI ($F(6,57)=21.807$, $p<0.001$) and sleep stage ($F(3,229)=71.742$, $p<0.001$) were significant. Although the main effect of sleep type ($F(1,227)=1.805$, $p=0.180$) and subsequent ROI interactions were not significant there was a sleep stage by sleep type interaction ($F(3,228)=7.425$, $p<0.001$). This network interaction was driven by higher FC in N1 and N2, but lower FC in N3 during recovery sleep compared to normal, as shown in Figure 5.4.3D. Overall, advancing sleep stage caused a large decrease in sensorimotor FC for both types of sleep.

Visual network

The main effects of ROI ($F(5,63)=74.980$, $p<0.001$) and sleep stage ($F(3,166)=7.505$, $p<0.001$) were significant predictors of FC strength within the VIS network. The main effect of sleep type was not significant ($F(1,163)=1.802$, $p=0.181$) nor was the stage by sleep type interaction ($F(3,164)=1.019$, $p=0.386$), see Figure 5.4.3E. There was an ROI and sleep stage interaction ($F(15,63)=2.590$, $p=0.004$), which suggests FC differed regionally with advancing sleep stage. Examining the ROI FC in more detail we found that the FC of the bilateral primary visual area (PV) increased with advancing sleep stage, whilst the FC of the PV to lateral visual areas (LV) decreased, suggesting that the PV is more interconnected bilaterally during sleep but the VIS network as a whole is less functionally connected.

Auditory network

Only the main effect of sleep type on the FC of the bilateral AUD network was significant ($F(1,29)=5.8$, $p=0.023$), with FC increasing in recovery compared to normal sleep (Figure

5.4.3F). The main effect of sleep stage ($F(3,29)=2.209$, $p=0.108$) and the stage by sleep type interaction ($F(3,29)=0.637$, $p=0.597$) were not significant.

Hippocampus network

The main effect of ROI ($F(5,60)=22.414$, $p<0.001$) was significant within the Hippocampus network, however the main effects of stage ($F(3,195)=0.671$, $p=0.571$) and sleep type ($F(1,193)=0.028$, $p=0.866$), in addition to all ROI interactions, were not significant (see Figure 5.4.3G).

Thalamus

The main effects of sleep stage ($F(3,29)=5.861$, $p=0.003$) and sleep type ($F(1,29)=10.582$, $p=0.003$) were significant, indicating that bilateral thalamus FC was modulated by both sleep stage and type of sleep. Figure 5.4.3H shows that an increase in FC was observed with advancing sleep stage in normal sleep, while FC remained consistent in recovery sleep across the sleep stages. There was also an increase in FC strength during recovery sleep compared to normal sleep for all sleep stages (Figure 5.4.3H). In particular waking thalamus FC appeared to be considerably greater during recovery sleep. However no significant interaction between sleep stage and sleep type ($F(3,29)=1.398$, $p=0.263$) was found for the Thalamus.

5.4.3 Spatial extent of FC

The spatial maps of group mean within-ICN FC were compared between each sleep stage and between normal and recovery sleep for descriptive purely purposes. Figure 5.4.4 demonstrates

the robust spatial depiction of the DMN by seeding from either the PCC or the PFC, although spatial differences were observed between sleep conditions. For both PCC and PFC, FC during wake, N1 and N2 was more spatially extensive during the recovery session than during normal sleep. During N3 FC was greatly reduced to the rest of the DMN during recovery sleep compared with normal sleep (see Figure 5.4.4). Figure 5.4.4B illustrates the uncorrected FC maps seeded from the PFC, showing a wider frontal extent during wake in the normal session and conversely greater FC extent around the PCC during recovery sleep. In addition to greater PFC FC extended into areas of the parietal lobe during N1 in recovery sleep (Figure 5.4.4B).

This pattern of increased extent of recovery sleep FC during stage N1, as well as during N2 to a lesser extent, followed by the converse effect of much greater extent of normal sleep FC during N3 was also observed in all other ICNs (Figure 5.4.5-Figure 5.4.7). In N1 and N2 there is a high degree of FC overlap between the types of sleep, although slightly more widespread in recovery sleep, and the FC extended to brain areas outside of the wake defined ICN nodes (Figure 5.4.5-Figure 5.4.7).

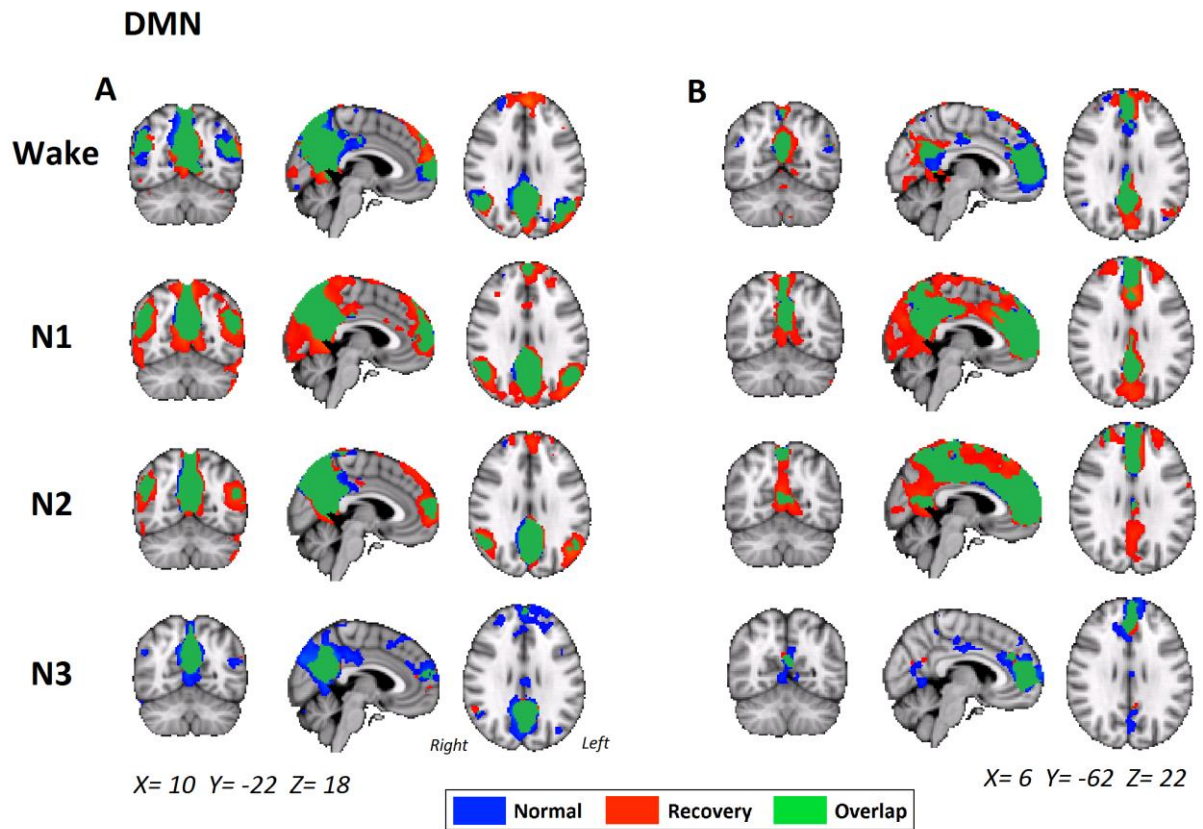


Figure 5.4.4. Maps of group mean DMN FC. A) Seeded in the PCC, B) Seeded in the PFC. Grouped by sleep stage for normal (blue) and recovery (red) sleep (threshold $R>0.4$). Green indicates the common voxels between the two types of sleep

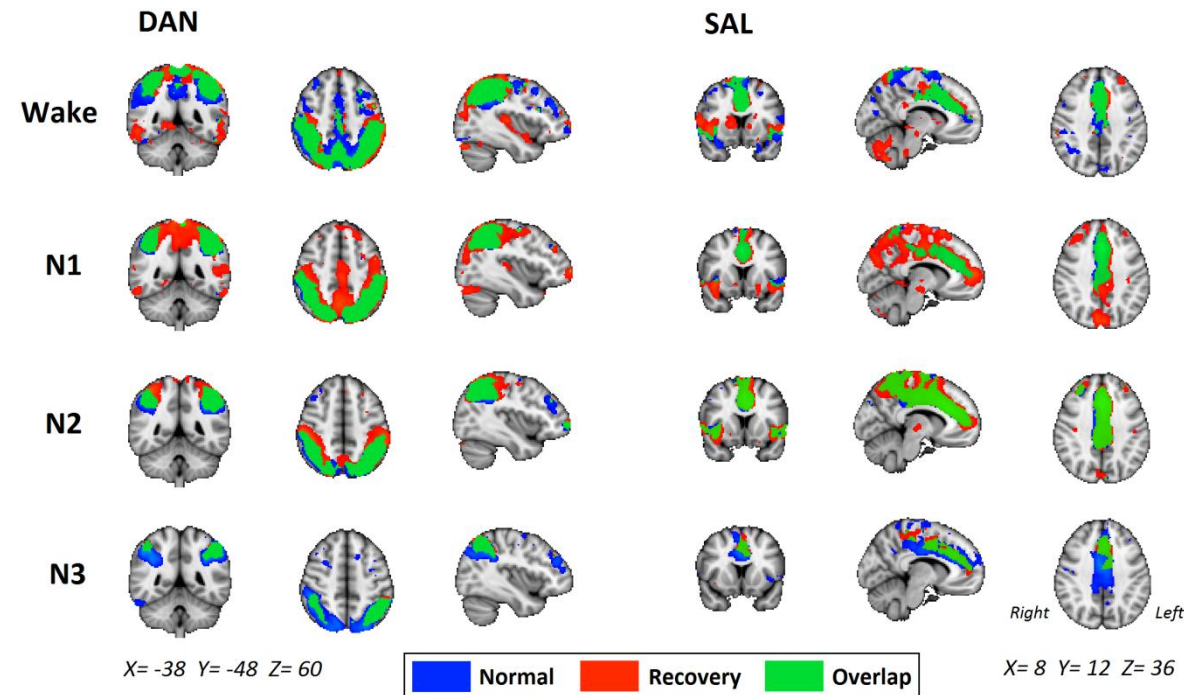


Figure 5.4.5 Maps of the group mean DAN FC seeded from the LIPS and SAL FC seeded from the ACC, grouped by sleep stage for normal (blue) and recovery (red) sleep (threshold $R > 0.4$). Green indicates the common voxels between the two types of sleep

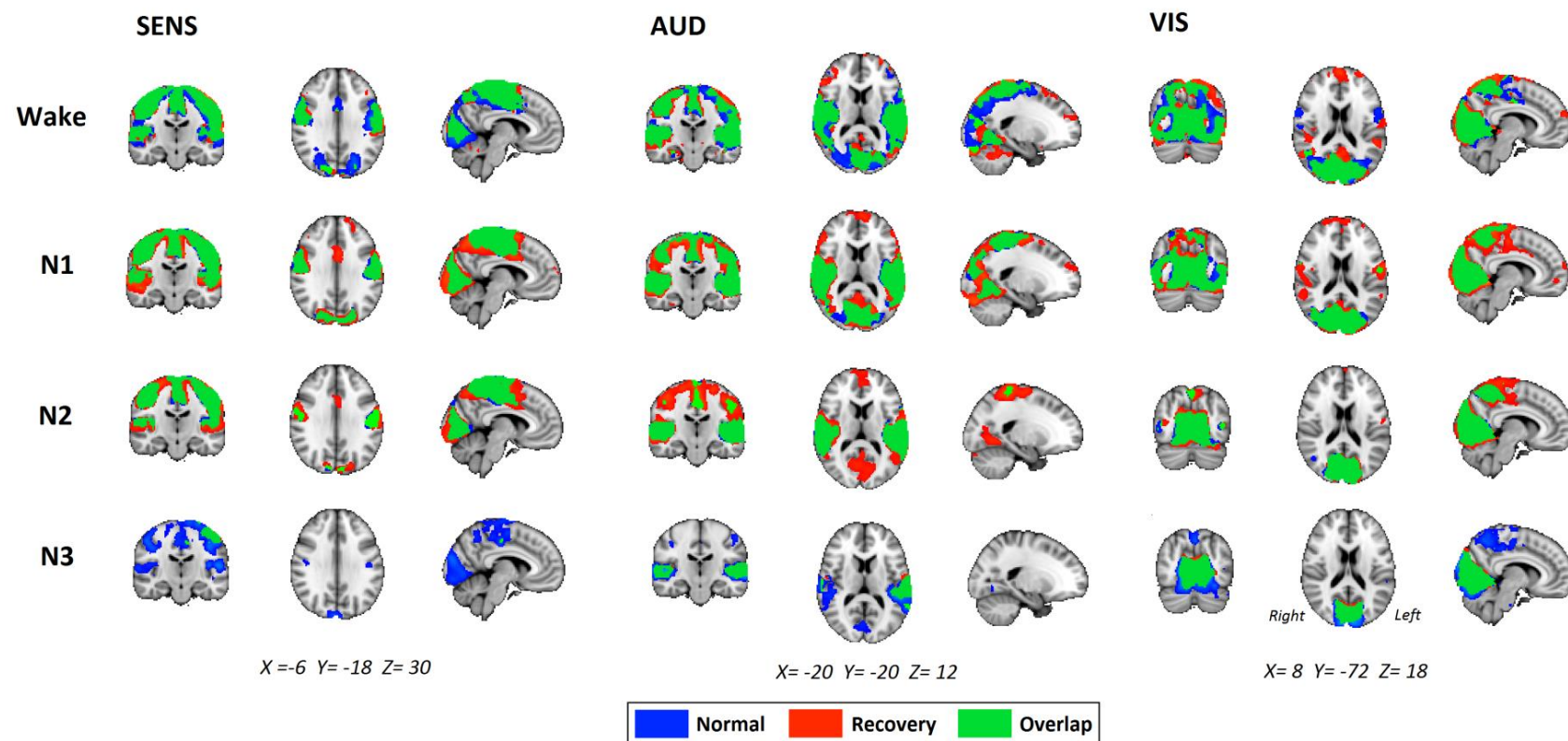


Figure 5.4.6. Maps of the group mean SENS FC seeded from the LMC; AUD FC seeded from LST and VIS-FC seeded from the LPV. Grouped by sleep stage for normal (blue) and recovery (red) sleep (threshold $R>0.4$). Green indicates the common voxels between the two types of sleep

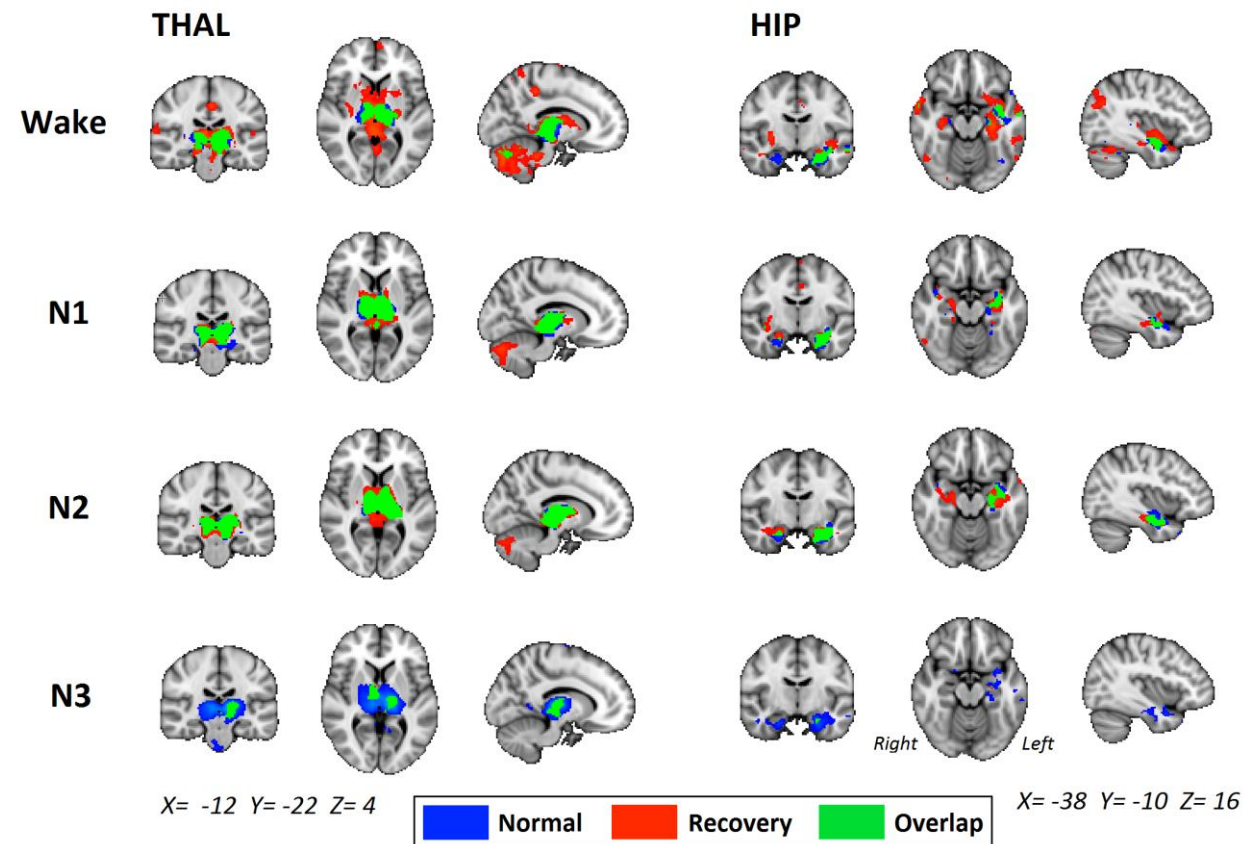


Figure 5.4.7. Maps of the THAL FC seeded from the LTH and HIP FC seeded from the LHIP, grouped by sleep stage for normal (blue) and recovery (red) sleep (threshold $R > 0.4$). Green indicates the common voxels between the two types of sleep.

5.5 Discussion

In the current study, the impact of acute sleep deprivation on the major ICNs was assessed during recovery sleep (following 24 hours of wakefulness) in the morning hours in comparison to normal sleep in the evening hours. We demonstrate that FC during NREM sleep is modulated by prior sleep deprivation in a network-dependent fashion, and to our

knowledge this is the first study to explicitly investigate the quantitative differences between normal and recovery sleep using FC analysis. Although this within subject design is limited by a relatively small sample size ($n=6$), our findings suggest that treating these two types of sleep as equivalent in terms of underlying brain activity is an oversimplification. We show that while large-scale FC, considering connectivity of all individual nodes both within- and between-ICN FC and quantified using graph theory, was similar between normal and recovery sleep for each NREM sleep stage, a profound reorganisation with sleep stage was present independent of prior sleep deprivation. When separately assessing FC within each ICN, differences between normal and recovery sleep sessions specific to individual ICNs and sleep stages were observed which could suggest adjustment of the functionality supported by the stages of sleep. The fact that these differences were observed in our small cohort of subjects indicates the sensitivity of FC as a technique and the extent to which prior sleep history should be taken into account in neuroimaging sleep studies.

5.5.1 Sleep is a multiscale phenomenon

Sleep deprivation increases the propensity to sleep resulting in faster sleep onset (Carskadon & Dement, 1987), and this greater sleep need is accompanied by changes in sleep architecture during subsequent recovery sleep (Tobler & Borbély, 1986). It has been shown that sleep deprivation impacts endogenous homeostatic mechanisms within the brain via interactions with the neurotransmitter systems controlling sleep and wakefulness (Obal & Krueger, 2003). However the exact biochemical mechanisms of recovery sleep are not well understood. It is thought that the build-up of certain molecules during prolonged wakefulness (e.g Adenosine, Nitric oxide, GABAergic neurons) is proportional to the amount of sleep deprivation and as

such is responsible for the high amplitude slow EEG oscillations seen in recovery sleep (Brown et al., 2012; Tobler & Borbély, 1986). Linking these observations with non-invasive brain imaging data is challenging. We find these biological and neurophysiological changes associated with recovery sleep did not translate into alterations in large-scale average graph strength compared with normal sleep. Graph theory has become popular over recent years as a way of understanding and quantifying large-scale FC changes (Bullmore & Sporns, 2009), although in the bid to conceptualise these overall effects, information regarding regional changes can be lost. For this reason, the current study investigated FC changes due to sleep deprivation on a number of different scales, finding an effect of SD only within specific ICNs. Changes during NREM were limited to the default mode network (DMN), thalamus (THAL), auditory (AUD) and sensorimotor networks (SENS), suggesting that factors associated with homeostatic sleep regulation selectivity affect some but not all ICNs. In addition to this, the spatial extent of FC was found to visually alter depending on the sleep stage and between the different types of sleep. Together these findings support the hypothesis that many different interacting spatial and temporal scales within the brain support the various phenomena regulating and occurring during sleep (Vyazovskiy & Delogu, 2014), including the differential effects of sleep homeostasis (Tononi & Cirelli, 2006).

5.5.2 NREM stage FC changes within ICNs during recovery sleep

Falling into NREM sleep and proceeding through the NREM stages was shown to significantly change the large-scale functional network structure of the brain. DMN FC strength has been shown to increase in N1 before reducing with advancing sleep stage compared to wakefulness (Horovitz et al., 2009; Koike et al., 2011; Sämann et al., 2011),

which the current study also finds for both normal and recovery sleep (Figure 5.4.2B). It is thought that these decreases in deeper sleep (i.e N2 & N3) compared to wake represent an underlying change in the network organisation within each stage, or could be seen as further evidence of a ‘breakdown’ in the cortical integration during NREM sleep (Massimini et al., 2005). However, alongside the notable decrease in FC with deepening sleep, we found that for all sleep stages DMN FC during recovery sleep was significantly higher than in the equivalent stage during normal sleep (Figure 5.4.2B). This could reflect the changing functionality of the DMN during recovery sleep, given behavioural evidence from waking studies which suggests increased DMN FC is associated with daydreaming, internal cognition and reduced external focus (Buckner et al., 2008). The higher FC strength in recovery than normal sleep could suggest less external focus and greater internally driven processes, which is consistent with the increased arousal thresholds that have been noted in recovery sleep (De Gennaro et al., 2001; Frederickson & Rechtschaffen, 1978). This suggests that homeostatic processes could moderate DMN disintegration in recovery sleep and as a result may affect the arousal threshold during recovery NREM sleep. This remains to be tested in future studies. In addition the effect of prior SD on DMN FC is shown to be regionally specific, in line with previous research suggesting that certain DMN regions are more susceptible to changes in sleep stage (Sämann et al., 2011) and appear to respond differently during wake to the effects of sleep deprivation (Wang, Liu, Hitchman, & Lei, 2014). As such the findings of the current study suggest the DMN is influenced by the brain's sleep mechanisms.

The thalamus is essential for sleep-wake regulation, controlling sensory input to the cortex via afferent pathways or thalamocortical circuits and affecting arousal levels through these connections (McCormick & Bal, 1997; Steriade & Llinás, 1988; Steriade, McCormick, &

Sejnowski, 1993b; Steriade, 2000). It is a structure of particular interest when assessing the effect of homeostatic sleep pressure. We show FC increased between left and right thalamic hemispheres with advancing sleep stage, the only ICN to show increased FC during NREM sleep (Figure 5.4.2H). In the recovery session THAL FC was larger in wakefulness and NREM compared to the normal session, reflecting even greater thalamic synchrony in recovery sleep. Thalamic FC is arguably associated with sleep regulatory systems and we have previously demonstrated relatively complex regional and sleep stage specific effects of sleep. Further FC investigation particularly in clinical populations suffering with acute or prolonged sleep deprivation is warranted.

FC of the sensorimotor network (SENS) composed of the primary motor and sensory motor areas decreased with NREM stage compared to wake (Figure 5.4.2D). Previous reports have shown FC to be maintained during NREM or increased in light sleep (N1) in these regions (Horovitz et al., 2008; Larson-Prior et al., 2011), although this disparity is likely due to differing analysis methodologies, particularly in regards to cortical parcellation. An early transcranial cortical stimulation study found ‘depressed’ excitability in the motor cortex in slow wave sleep due to the limited movement during sleep (Hess, Mills, Murray, & Schriefer, 1987). Therefore it could be argued that reduced motor excitability may manifest as reduced FC strength, since transcranial direct current stimulation (tDCS) of motor regions have been found to alter topological cortical-cortical FC (Polanía, Nitsche, & Paulus, 2011), as well as cortical-subcortical FC (Polanía, Paulus, & Nitsche, 2012). We find SENS FC changes with sleep stage, in addition to recovery differences, which supports previous research showing increased excitability in sleep and with greater time spent awake (Huber et al., 2012). Meanwhile research has shown that waking activity in the somatosensory cortex impacts EEG

power density in those same areas during subsequent sleep (Kattler, Dijk, & Borbely, 1994), thus demonstrating waking behaviour has a direct impact on sensorimotor activity during sleep. The current study found that waking behaviour in the form of prolonged wakefulness influenced FC in the SENS network during recovery sleep, suggesting sleep regulatory processes could also mediate sensorimotor regions.

In addition the auditory network (AUD), comprising of the bilateral superior temporal gyrus (STG) has been reported to show strong interhemispheric FC during wake (Nir et al., 2008) and maintained FC during light sleep (Larson-Prior et al., 2009), which we also find for all NREM sleep stages. However overall AUD FC was increased in the recovery session compared to normal sleep (Figure 5.4.2F), potentially reflecting changes in auditory arousal thresholds during recovery sleep (Pilon, Desautels, Montplaisir, & Zadra, 2012).

5.5.3 FC within some ICNs is more resilient

The FC of some ICNs does not differ significantly between type of sleep, which could be due to being underpowered or it may suggest a limited impact of prior sleep deprivation or altered circadian phase on FC. So-called task-positive networks such as the DAN and the SAL did not show differences in FC during NREM sleep between the two sessions, suggesting that the processes supported by these networks such as task-engagement and external monitoring when awake (Corbetta & Shulman, 2002; Seeley et al., 2007), may not be modulated by prior SD during NREM sleep. DAN FC remained consistent across all NREM stages, although differences were observed during wake between the normal and recovery sessions. Previous research has shown an increase in FC within the DAN during light sleep in non-deprived

conditions in comparison to wake (Larson-Prior et al., 2009). Whilst we observed this relationship of sleep-related increases in FC in the recovery session it could be due to the wake epochs being differentially distributed across the scan sessions. This is likely to have had an impact on FC, given that waking FC is modulated by prior sleep duration (Killgore et al., 2012, Khalsa et al., 2016). While the focus of this study was NREM sleep, the degree to which sleep pressure modulates waking FC remains a fascinating topic for future investigation. Whereas SAL FC was unchanged for wake, as well as N1 and N2, moderate FC increases were found in N3 during the recovery session which could indicate processing changes within the network associated during slow wave activity. However taken together with the findings for the DAN, FC resilience to homeostatic pressure may indicate the limited impact of external engagement during sleep.

The FC of the visual network (VIS) was not found to alter in recovery sleep suggesting the network organisation is unaffected by prior SD. However we did find FC that decreased with sleep stage in both sessions. Previous reports have shown FC to be maintained in the visual cortex in N1 (Horovitz et al., 2008; Larson-Prior et al., 2009). The change in VIS FC we see with sleep stage could reflect reduced vigilance during sleep which leads to network reorganisation.

One of the functions of sleep is to support neuronal plasticity within the hippocampus-neocortex system through consolidation processes (Abel, Havekes, Saletin, & Walker, 2013), and in particular declarative memory (Diekelmann & Born, 2010). Indeed, FC from the hippocampus to bilateral regions of the DMN has previously been shown to reduce with sleep stage, although higher FC was found in N2 compared to N3 (Andrade et al., 2011). However,

the current study focused on the internal hippocampus network (HIPP) finding unchanged FC during NREM sleep and between sleep sessions, thus the underlying functionality of the HIPP appears resilient.

5.5.4 Differences in the spatial extent of network FC during recovery sleep

We find that the spatial FC extent of ICNs changed with sleep stage. For every ICN we observed greater recruitment from areas both within and outside of the network in N1 and N2 during recovery compared with normal NREM sleep, while the opposite effect is found in N3, where the spatial extent of FC reduced in recovery sleep. The increased spatial extent of FC in N1 and N2 during recovery sleep may reflect changes in excitability as discussed above, although this remains to be tested, or reflect a more general reduction in functional specialisation within increased sleep drive.

5.5.5 Methodological considerations

Circadian phase has an impact on propensity to sleep (Borbély, 1982). Alterations in the BOLD response to tasks as a function of circadian phase have been noted (Peres et al., 2011; Schmidt et al., 2012), as well as the impact of chronotype on FC (Blautzik et al., 2013) and whole system differences in waking FC, between morning or night-time periods (Hodkinson et al., 2014; Shannon et al., 2013). DMN FC strength and spatial extent has been shown to decrease as the day progresses, suggesting the susceptibility in wakefulness of the DMN to homeostatic and circadian influence (Hodkinson et al., 2014). Therefore recovery sleep recorded at a different circadian phase, which influences an individual's sleep propensity

(Gaudreau, 2001), may alter recovery sleep FC. However the current study focused on a 24 hour night-time sleep deprivation period, as such it remains for future work to tease apart the separate impact of the two-processes on FC during recovery sleep.

Combined EEG-fMRI during sleep requires a person to sleep within the MR scanner environment, limiting study to those people who are able to sleep regardless of their environment. Furthermore, possible arousal influences from the scanner noise may have an impact (Muzet, 2007), meaning that even the 'normal' sleep session may not be identical to sleep outside the scanner environment. The current study is also limited to the first few cycles of sleep, which could differ to later cycles in the impact of prior sleep deprivation. In addition the current study did not regress the mean global signal (GSR) due to contention in the field (Gotts et al., 2013; Keller et al., 2013) and uncertainty regarding the change in GSR with sleep stage. While recent work has advocated the use of GSR when assessing sleep deprivation during wake, it was noted that GSR differentially affected the results (Yeo et al., 2015). Coupled with the additional changes to GSR with sleep stage that are not conclusive, the current study omitted this pre-processing step in order to conservatively estimate FC changes between normal and recovery sleep.

Individual differences in terms of sensitivity to sleep deprivation have been shown (Leproult et al., 2003). This has been described as trait-like (Rupp, Wesensten, & Balkin, 2012; Van Dongen, Baynard, Maislin, & Dinges, 2004) and has been shown to differentially affect task-related BOLD responses (Chee & Tan, 2010; Mu et al., 2005). Furthermore, FC patterns in the wakeful resting-state have also been shown to predict an individual's vulnerability to sleep deprivation (Yeo et al., 2015). An interesting area for future work would be to see whether a

person's susceptibility to sleep deprivation could mediate the impact of homeostatic sleep pressure, which has been suggested from predictive models (Van Dongen, Bender, & Dinges, 2012).

5.5.6 Implications and future research

It is thought that the attenuation of sleep could be a consequence of pathological processes resulting in reduced cognition and alertness (Killgore, 2010). However by measuring FC, we show a propagating effect of prolonged wakefulness on the brain's functional processes during sleep. However this effect is not homogenous across all ICNs, instead some networks were more affected, while others appear largely resilient. This suggests waking demands feed directly into and modulate certain large-scale networks in sleep. While previous studies have shown that FC during waking is affected by sleep deprivation (De Havas et al., 2012; Sämann et al., 2010), reduced FC in frontal areas is suggested to be a marker of longer self-reported sleep duration (Killgore et al., 2012) and shorter habitual sleep durations (Khalsa et al., 2016). The current study shows that sleep FC is also affected by prior sleep history. The temporal evolution of FC throughout the sleep-wake cycle could prove an interesting area of future work, revealing how large-scale networks are associated with homeostatic changes.

Thalamocortical FC has been shown to decrease in light sleep compared to wake following minor sleep restriction (Spoormaker et al., 2010), after 36 hours (Kaufmann et al., 2006) and 44 hours of sleep deprivation (Picchioni et al., 2014). These changes are dependent on thalamic region (Hale et al., 2015). While we did not explicitly address thalamocortical FC in the current study, as we instead focus on the FC changes within ICNs, we did notice subtle

FC changes with sleep stage and homeostatic pressure between bilateral THAL and other cortical regions within separate cortical ICNs. This may suggest that thalamocortical FC during sleep could also be mediated by recovery sleep, although this requires further investigation.

5.6 Conclusion

This study has demonstrated that the FC of large-scale brain networks is sensitive to the processes of recovery sleep and that these effects were most pronounced in specific ICNs, namely the DMN, thalamus, auditory and sensorimotor networks, while the FC of other sensory and cognitive networks appeared resilient. FC in recovery sleep was generally more widespread than in normal sleep, as well as demonstrating a reduction in spatial extent with sleep stage. Although our study features a limited number of subjects with sufficient sleep (N=6) we believe our numerous demonstrations of differences in ICN FC between normal and recovery sleep provides evidence that treating these two types of sleep as equivalent in terms of underlying brain activity is an oversimplification and should be used to guide the design of future neuroimaging studies in this field. These findings suggest that future sleep studies should avoid using sleep deprivation to facilitate sleep inside the MRI scanner, unless specifically focussed on recovery sleep. They also have implications for our understanding of recovery sleep and could go some way towards understanding the functional networks underpinning homeostatic sleep pressure and the interacting circadian processes.

Supplementary

Movement

For every scan based on each thirty second epoch the relative movement (RM) from the FSL McFLIRT motion correction (www.fmrib.ox.ac.uk/fsl) was collated across sleep stage for each subject, before being compared between sleep types (normal and recovery). The changes in RM were not found to differ significantly when assessed using a GLMM for the fixed-effects of sleep stage ($F(3,28)=2.488$, $p=0.08$), sleep type ($F(1,1)=0.019$, $p=0.920$) and the subsequent interaction between sleep stage and type ($F(3,29)=0.431$, $p=0.732$). The random effect of subject was included in the model to control for movement variability between subjects. Figure 5.6.1 depicts the average relative movement (mm) and variability for each sleep stage between sleep types. It is also worth noting that the size of these movements are considerably smaller than the smallest voxel dimension used in the current study (3mm), therefore we conclude that the results of the current study are not confounded by movement related physiology.

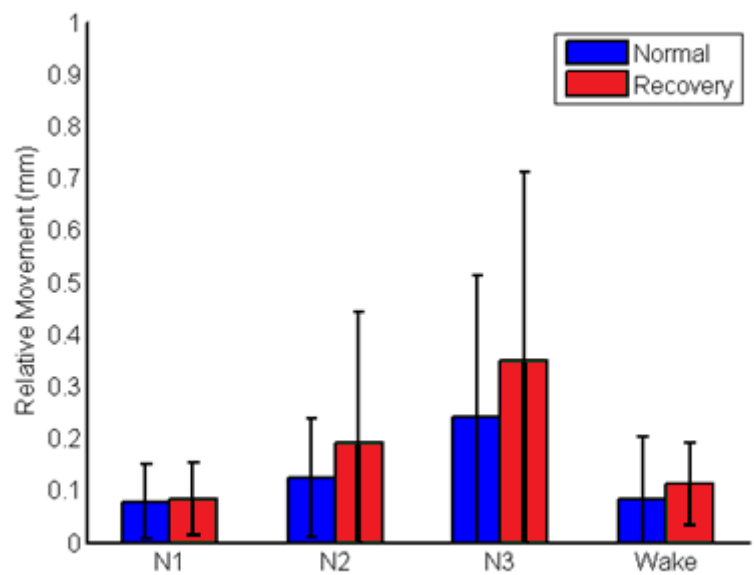


Figure 5.6.1 The group average relative movement of each sleep stage for each type of sleep. Error bars denote the standard deviation.

Chapter 6

THE ASSOCIATION BETWEEN MEASURES OF SLEEP HISTORY AND FUNCTIONAL CONNECTIVITY DURING NREM SLEEP

6.1 Abstract

In this chapter we apply an exploratory analysis to investigate whether changes in ICN FC strength during NREM sleep are affected by the inter-subject variability in prior sleep behaviour using a comprehensive range of subjective and objective sleep measures. We also aimed to replicate our previous FC findings during NREM sleep stages (Chapter 5). We show that both objective and subjective measures of prior sleep-wake behaviour are predictive of FC during subsequent wakefulness and NREM sleep, however the associations are not homogenous across the brain. Therefore this study highlights the need to consider a subject's prior sleep history in all studies utilising FC analysis during wakefulness and sleep.

6.2 Introduction

Across research and clinical settings, both objective and subjective approaches are used to evaluate sleep behaviour. The traditional ‘gold-standard’ objective sleep measure remains laboratory based PSG using EEG electrodes to record signals of neuronal activity, and their changes with sleep, from the scalp. However in recent years neuroimaging has become an increasingly important objective tool for studying the brain during sleep (Dang-Vu, 2012; Duyn, 2012) and in particular the combined recording of EEG and fMRI provides the ability to link standard EEG-based sleep staging (AASM, 2007) with spatially localised changes in brain function. The quantification of FC, the statistical dependency of activity between brain regions (Friston, 2011), together with the examination of the brain's intrinsic connectivity networks (ICNs) (Fox et al., 2005; Van Dijk et al., 2010), is now a common method for studying brain function, and has demonstrated its utility in previous sleep studies which have mainly focused on investigating FC changes with sleep stage (Horovitz et al., 2008; Larson-Prior et al., 2009, 2011; Sämann et al., 2011; Spoormaker et al., 2012; Uehara et al., 2013). However the relationship between other commonly used measures of sleep behaviour, both objective and subjective, and patterns of sleeping FC is not well understood and could help improve our understanding of the underlying brain mechanisms that influence sleep and waking behaviour.

Research has shown past sleep behaviour modulates waking resting-state FC, including habitual cumulative sleep time (Khalsa et al., 2016) and experimentally induced, acute sleep deprivation (De Havas et al., 2012; Gujar et al., 2010; Sämann et al., 2010; Yeo et al., 2015). In addition to those objective measures, the variability of subjective measures has also been

shown to be associated with FC strength during wake, including self-reported total sleep time the previous night (Killgore, Schwab, & Weiner, 2012) and level of daytime sleepiness, which have found increased FC with better reported sleep in regions of the DMN (Ward et al., 2013) and thalamus (THAL) (Killgore et al., 2015). Other ICNs such as the saliency network (SAL) have been a focus of research for the sleep disorder insomnia, with increased FC with bilateral insula (M. C. Chen, Chang, Glover, & Gotlib, 2014). As such we hypothesise that during the different sleep stages the FC within certain ICNs is likely to be differentially influenced by objective and subjective measures. Our findings may help identify correlates of sleep associated with an individual's actual sleep and their own appraisal of their sleep, which could improve clinical understanding of sleep disorders, where subjective self-report is important for diagnosis. In addition, this work investigates whether FC differences may explain why subjective and objective sleep assessments may not necessarily concur.

In this exploratory analysis we investigate whether changes in ICN FC strength during NREM sleep are affected by the inter-subject variability in prior sleep behaviour. Using a comprehensive range of subjective and objective sleep measures enables us to gather a deeper understanding of the relationship between these measures and the change in baseline brain function and FC that occurs with the descent into sleep. In addition to this we also aimed to replicate our previous findings and explore methodological alterations on the overall interpretation.

6.3 Methods and materials

6.3.1 Experimental design

Twenty one healthy subjects (10 male, 25 ± 3 years) with no reported history of neurological, psychiatric or sleep disorders completed the study. Subjects' habitual sleep patterns were monitored with a written self-reported sleep diary and wrist actigraphy for two weeks prior to the scanning session. To assess the subjects' sleep quality, daytime sleepiness, levels of fatigue and insomnia symptoms participants also completed the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989), Epworth Sleepiness Scale (ESS) (Johns, 1991), Fatigue Severity Scale (Krupp, 1989) and the Insomnia Severity Index (Bastien, 2001) questionnaires. Five subjects were excluded for either failing to sleep (3 subjects), excessive movement (1 subject) or technical problems during EEG-fMRI acquisition (1 subject), resulting in a final analysed cohort of sixteen subjects (8 male, 26 ± 3 years).

6.3.2 Sleep measures

Actigraphy data, an objective measure of sleep, was firstly compared with the sleep diary for consistency and several measures of total sleep time (TST in minutes) was calculated for each subject including: 2 week average (ATST), TST the night before scanning (BTST) and 2 week variability (VTST). VTST was calculated as the standard deviation of TST across the 2 weeks, deemed by us to be a measure of consistency in sleep duration. Subjective sleep questionnaires were scored according to each validated procedure (PSQI, ESS, FSS & ISI), with higher scores indicating more sleep difficulties or presence of fatigue and a clinical

significance threshold for each. See Appendix B for the sleep questionnaires. All measures were then mean centred (*z-scored*). Any outliers ($>2\pm SD$) were replaced with the standardised z-score (Grubbs, 1969), however this was only required for one subject for the measure of variability in total sleep time (VTST). See Table 6.3.1 for the sleep measure demographics. All measures were correlated to investigate the association between each of the objective & subjective sleep measures.

Table 6.3.1. Demographic information of objective & subjective sleep measures (N=16).

Sleep Measures	Mean	Median	SE	SD	Range
Objective: Actigraphy (<i>mins</i>)					
Average TST	397.1	402.5	± 10.1	± 40.4	316-457
TST Before	354.1	358	± 23.0	± 92.1	120-481
TST Variability	73.8	63.6	± 7.8	± 31.4	40-160
Subjective: Questionnaires					
PSQI	4.6	4.5	± 0.5	± 1.9	1-7
Epworth	6.4	6.0	± 1.0	± 3.9	0-14
FSS	22.8	21.0	± 2.0	± 8.1	13-38
ISI	4.3	4.0	± 0.7	± 2.7	0-9

6.3.3 EEG-fMRI data

EEG-fMRI data were simultaneously acquired, for methodology details see Chapter 3. Subjects were asked to sleep throughout the scan session and to signal using the internal buzzer if they were unable to do so or when they wished to terminate the session. No sleep deprivation protocol was used and the scanning session took place at the subject's usual

bedtime (between 22:00 and 00:00) to try and promote normal sleep. An initial T1-weighted anatomical was acquired before whole brain T2*-weighted fMRI data (3x3x4mm voxels, TR=2000ms, 32 slices, TE=35ms, 1250 volumes, flip angle 80°, SENSE factor = 2) were acquired in consecutive 40-minute scans. Respiratory and cardiac fluctuations were recorded using a pneumatic belt and a vectorcardiogram (VCG) both acquired at sampling rate of 500 Hz by the scanner hardware. MR gradient and ballistocardiogram artefacts were removed from the EEG using average artefact subtraction in Brain Vision Analyzer 2 (Brain Products, Munich). The VCG R-peak markers were aligned with the EEG data and used for pulse artifact correction (Mullinger et al. 2008). EEG data were then divided into non-overlapping thirty second epochs and manually sleep staged by an experienced electroencephalographer using a standard sleep montage according to the American Association of Sleep Medicine criteria (AASM, 2007).

6.3.4 fMRI pre-processing and FC analysis

For fMRI pre-processing procedure and ROI definition see Chapter 3. Pre-processing was completed with and without regression of the global mean (i.e., global signal regression, GSR) in order to compare the findings in relation to sleep stage. With the likelihood of involuntary movements during sleep, absolute head movement as a function of sleep stage was investigated (see supplementary). Head movements within the MR scanner can affect FC analysis (Satterthwaite et al., 2012; Smith et al., 2014; Van Dijk, Sabuncu, & Buckner, 2012), although none of the included subjects' movement exceeded the minimum voxel size (3mm). This is an important consideration given fMRI data were first co-registered with their anatomical data before registration to MNI standard space using FLIRT in FSL.

FC analysis of the fMRI data was calculated using a non-overlapping 30 second sliding window of the BOLD time-series for each ROI. This dynamic analysis using seconds rather than minutes to calculate FC was applied to the investigation of FC changes during sleep in our previous study (Chapter 4: Wilson et al., 2015) and the further details of the FC analysis is outlined in Chapter 3. Due to the contention regarding the meaningful interpretation of negative FC, we also investigated FC with and without removal of negative correlations. However in line with our previous work (Chapter 5) FC analysis commenced with the removal of any negative FC coefficients which were set to zero and then converted to Z-scores using Fisher's transform. Each of these 30-second FC matrices were paired to the corresponding EEG defined sleep stage, pooled by stage within subject and then averaged across subject epochs.

6.3.5 Statistical analysis

All statistical analyses were conducted on the standardised FC values (*Z-scores*) using SPSS. Data were normally distributed according to tests of normality (Shapiro-Wilk), as such a repeated-measures ANOVA assessed FC strength as a factor of regional paired connections (ROI), sleep stage (wake, N1 & N2) and subsequent ROI-stage interactions within each ICN. Another repeated-measures ANOVA was performed with the added factor of method (with & without GSR) to investigate any differences in methodology by sleep stage. Finally, for each ICN a repeated-measures analysis of covariance with factors ROI, Stage and ROI*Stage was conducted including each sleep measure as a covariate in the model as a predictor of FC strength and a false discovery rate (FDR) calculated to control for multiple comparisons for each ICN (Benjamini & Hochberg, 1995). If the covariate was found to significantly interact

with the overall FC or any of the factors, subjects were median split into two groups for that sleep measure for descriptive purposes. Bonferroni correction was used to control for multiple comparisons for each sleep measure in post-hoc tests. If the Mauchly's test indicated a violation of the assumption of Sphericity within any of the models the Epsilon Greenhouse-Geisser correction was reported.

6.4 Results

All measures of sleep behaviour from Table 6.3.1 were correlated using Pearson correlation. The only significant positive correlation was between the average TST and before TST of subjects ($r=0.515$, $p=0.041$), while all other correlations were non-significant.

We recorded a total of 2433 thirty-second epochs of data during EEG-fMRI; 714 epochs of wake (~5hours), 1209 epochs of N1 (~10hours), 510 epochs of N2 (~4hours) and 69 epochs of N3 (~30minutes) see Table 6.4.1. Due to the limited number of N3 epochs recorded in the current dataset N3 was not analysed. A replication of the results in Chapter 5, regarding regional changes in FC with stage during normal sleep, was initially completed for this data set in order to assess the reliability of the previous findings and determine whether FC changes with sleep stage are present in the latest data set. After which, the relationship between FC and sleep measures is reported within ICNs. Finally, the relationship between FC within ICNs and multiple sleep measures is summarised in Table 6.4.3- Table 6.4.5, although each sleep measure is first individually discussed.

Table 6.4.1. The number of sleep-staged, thirty-second epochs and the percentage of time spent in sleep for each individual subject.

	Subjects																Total Epochs
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Wake	0	19	87	61	57	67	69	77	23	54	53	78	31	0	7	31	714
Stage 1 (N1)	76	44	154	102	21	16	86	43	61	77	36	17	143	117	150	66	1209
Stage 2 (N2)	60	20	3	3	6	16	29	35	24	134	38	0	57	39	46	0	510
Stage 3 (N3)†	30	0	0	0	0	22	0	0	3	14	0	0	0	0	0	0	69
Epochs	166	83	244	166	84	121	184	155	111	279	127	95	231	156	203	97	2502
Total Sleep (%)	<i>100</i>	<i>77</i>	<i>64</i>	<i>63</i>	<i>32</i>	<i>45</i>	<i>63</i>	<i>50</i>	<i>79</i>	<i>81</i>	<i>58</i>	<i>18</i>	<i>87</i>	<i>100</i>	<i>97</i>	<i>68</i>	

† Not included in statistical analysis

6.4.1 Replication of previous findings

A repeated measures ANOVA including all ROIs and sleep stages across all the ICNs indicated a significant effect of ROI upon FC ($F(41,451)=36.759, p<0.001, \eta^2=0.77$). Although there was not a significant overall effect of stage ($F(2,22)=0.582, p=0.567, \eta^2=0.50$) there was a significant interaction between ROI and Stage ($F(82,902)=2.177, p<0.001, \eta^2=0.165$) suggesting the FC changes with sleep stage were not homogenous across the brain. This is a replicated finding from Chapter 5, see Figure 6.4.1. Significant differences in FC with ROI, Stage or ROI*Stage for each of the networks are outlined in Table 6.4.2 and discussed in further detail below for each ICN. We show close correspondence to the findings from the previous dataset, with the larger sample size in the current dataset resulting in additional significant findings with sleep stage and stage and ROI interactions in the DMN, SAL, SENS, VIS, HIP and THAL. All significant effects from Chapter 5 were replicated in the current dataset. However there was some differences noted in the THAL and SENS, particularly in wakefulness.

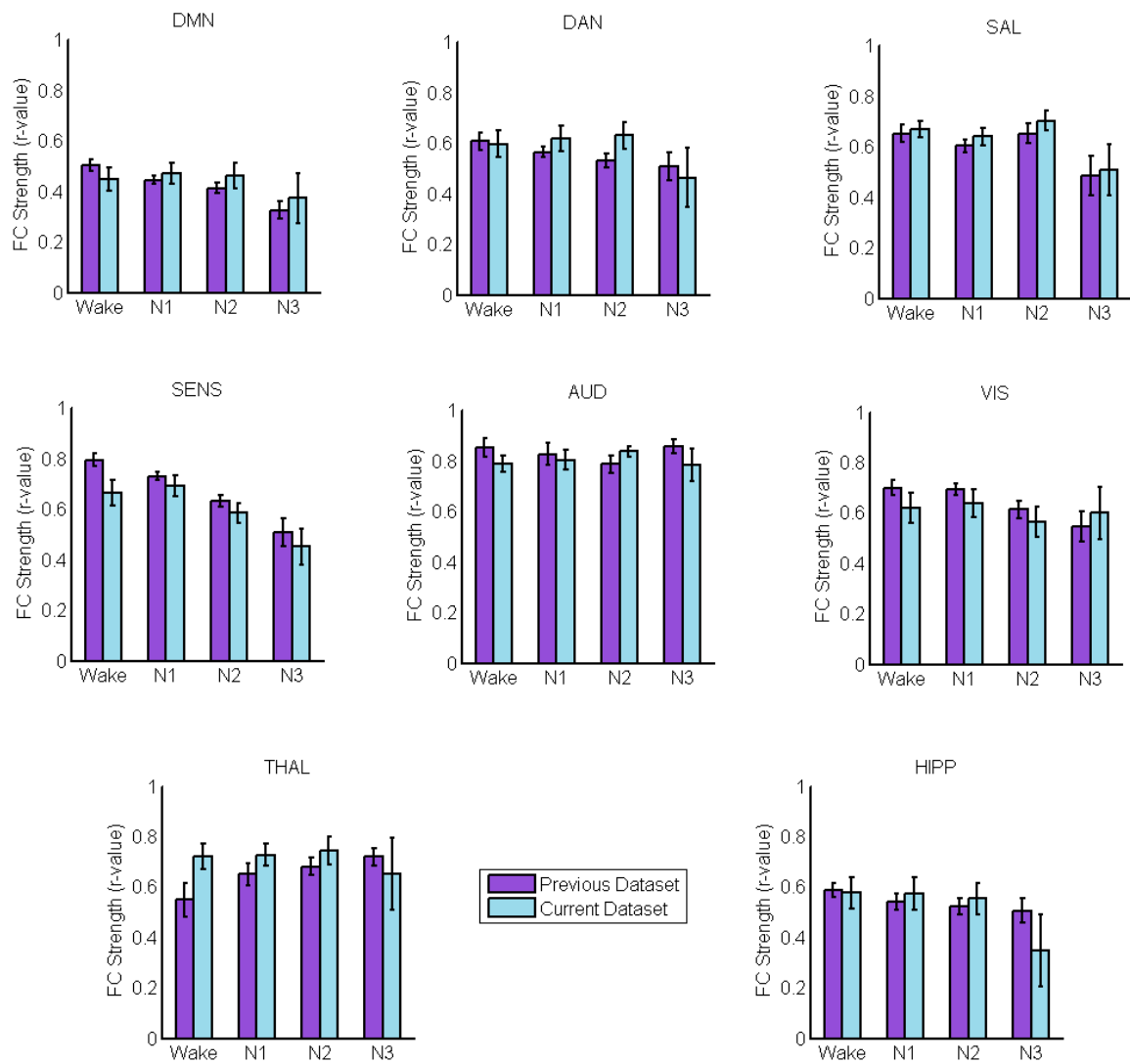


Figure 6.4.1. Replication of the within-network differences in FC between sleep stages as originally investigated in Chapter 5, while N3 is included for visual brevity it does not feature in the statistical analysis given the limited number of N3 epochs in the current dataset. Error bars represent \pm SE.

Table 6.4.2. Statistical comparison within each network for FC changes by ROI and Stage (wake, N1 & N2) for the two NORMAL sleep datasets

Dataset	Network							
	DMN	DAN	SAL	SENS	VIS	AUD	THAL	HIP
Previous ($n=6$)								
ROI	$F(14,70)=$ 9.286, $p<0.001$	$F(5,25)=$ 11.883, $p<0.001$	NS	$F(2,10)=$ 5.415, $p=0.026$	$F(5,25)=$ 23.730, $p<0.001$	-	-	$F(5,25)=$ 5.437, $p=0.002$
Stage	NS	NS	NS	NS	NS	NS	NS	NS
ROI*Stage	NS	NS	NS	NS	NS	-	-	NS
Current ($n=16$)								
ROI	$F(14,154)=$ 22.151, $p<0.001$	$F(2.17,23.8$ 8)=36.477, $p<0.001$	$F(2,22)=$ 22.479, $p<0.001$	$F(2,22)=$ 24.762, $p<0.001$	$F(12,132)=$ 2.540, $p=0.005$	-	-	$F(5,55)=$ 37.279, $p<0.001$
Stage	NS	NS	NS	$F(1.34,14.5$ 9)=6.498, $p=0.017$	NS	NS	$F(1.56,17.1$ 5)=5.311, $p=0.022$	NS
ROI*Stage	$F(28,308)=$ 1.921, $p=0.004$	NS	$F(4,44)=$ 3.88, $p=0.009$	NS	$F(6,66)=$ 164.912, $P<0.001$ -	-	-	NS

Significant ($p<0.05$)

† Duplicate subject data was removed

Note: N3 was excluded from statistical tests

6.4.2 FC changes with sleep stage within ICNs

DMN

An ANOVA found a significant effect of ROI on the FC of the DMN ($F(14,154)=22.151$, $p<0.001$, $\eta^2=0.67$). Although Stage was not found to be significant ($F(2,22)=2.521$, $p=0.103$, $\eta^2=0.19$) there was a significant ROI*Stage interaction ($F(28,308)=1.921$, $p=0.004$, $\eta^2=0.15$) suggesting that DMN FC differences with sleep stage are regionally specific. Pairwise comparisons revealed a significant FC increase for the PCC-MTL between N1 and N2 ($p=0.024$), although FC decreased for the mPFC-lIPL between wake and N1 ($p=0.028$) and N2 ($p=0.043$) and the mPFC-rIPL between wake and N1 ($p=0.018$). However the bilateral MTL significantly increased FC between wake and N2 ($p=0.03$).

DAN

The main effect of ROI upon FC was significant for the DAN ($F(2.17,23.88)=36.477$, $p<0.001$, $\eta^2=0.768$), however neither the main stage ($F(1.28,14.10)=0.447$, $\eta^2=0.039$) or the ROI and stage interaction ($F(3.16,34.81)=1.509$, $p=0.228$, $\eta^2=0.121$) were found to be significant.

SAL

There was a significant main effect of ROI ($F(2,22)=22.479$, $p<0.001$, $\eta^2=0.671$) on SAL FC, although the main effect of Stage failed to reach significance ($F(1.26,13.87)=2.668$, $p=0.120$, $\eta^2=0.195$). There was a significant interaction between ROI and Stage ($F(4,44)=3.88$, $p=0.009$,

$\eta^2=0.261$) suggesting that sleep stage had a different effect on the FC across ROIs within the SAL. However pairwise comparisons failed to reach significance for any particular paired connection by Stage, although the bilateral Insula (rINS-IINS) trended towards significance with increased FC between N1 and N2 ($p=0.093$).

SENS

There was a significant main effect of ROI ($F(2,22)=24.762, p<0.001, \eta^2=0.692$) and Stage ($F(1.34,14.59)=6.498, p=0.017, \eta^2=0.371$) indicating overall FC within the SENS differed depending on Stage. Pairwise comparisons revealed FC reduced significantly between N1 and N2 ($p=0.001$). However this reduction was not specific to a particular ROI paired connection indicated by the none significant ROI*Stage interaction ($F(4,44)=1.191, p=0.328, \eta^2=0.098$).

AUD

There was no significant effect of Stage on FC within the AUD ($F(2,22)=1.219, p=0.315, \eta^2=0.100$).

VIS

There was a main effect of ROI on the FC within the VIS ($F(6,66)=164.912, P<0.001, \eta^2=0.937$). Although Stage was not significant as a main effect ($F(2,22)=2.337, p=0.120, \eta^2=0.175$) there was a significant interaction with ROI ($F(12,132)=2.540, p=0.005, \eta^2=0.188$) suggesting that changes with Stage were regionally dependent within the VIS. In particular the FC between the rLV and the bilateral PV areas significantly decreased their FC strength during N2 compared with N1.

HIP

There was a significant main effect of ROI ($F(5,55)=37.279, p<0.001, \eta^2=0.772$) upon FC of the HIP, however the main effect of Stage ($F(1.52,16.71)=1.204, p=0.312, \eta^2=0.099$) and the Stage and ROI interaction ($F(10,110)=0.254, p=0.989, \eta^2=0.023$) were not significant within the HIP suggesting that sleep stage was not affecting the FC of the network.

THAL

There was a significant main effect of stage on FC within the THAL ($F(1.56,17.15)=5.311, p=0.022, \eta^2=0.326$), with an overall increase in FC strength with advancing sleep stage.

6.4.3 FC methodological differences

FC with and without GSR

Differences in FC strength due to the regression of global signal were investigated across both ROIs and sleep stages, see Figure 6.4.2. A repeated-measures ANOVA assessed the effect of methodology (with and without GSR) on FC strength for all ROIs, sleep stages and all subsequent interactions. There was a significant main effect of method on the FC strength ($F(1,11)=108.546, p<0.001, \eta^2=0.908$), with GSR causing a decrease in FC strength. There was a significant interaction between method and ROI ($F(48,528)=9.471, p<0.001, \eta^2=0.463$) suggesting that GSR differentially effects the FC of paired regional connections and is therefore not homogenous across the brain, which is to be expected based on previous research (Fox et al., 2009). However, importantly there was no significant interaction between

method and sleep stage ($F(2,22)=1.531, p=0.239, \eta^2=0.122$) suggesting that the changes in FC strength with sleep stage may not be dependent on regression method. Given the significant interaction between ROI and Stage ($F(96,1056)=2.048, p<0.001, \eta^2=0.157$) mentioned above, the three-way interaction of ROI, Stage and method was also not found to be significant ($F(6.72,73.90)=1.489, p=0.187, \eta^2=0.119$), therefore any significant differences in regional specificity by Stage may not be affected by GSR. Taken together these results suggest similar overall findings are obtained from both methods of analysis (with or without GSR) in terms of the FC differences between Stage and ROIs. As such for this chapter we will omit the processing step of GSR in order to directly replicate, compare and build on the findings from Chapter 5.

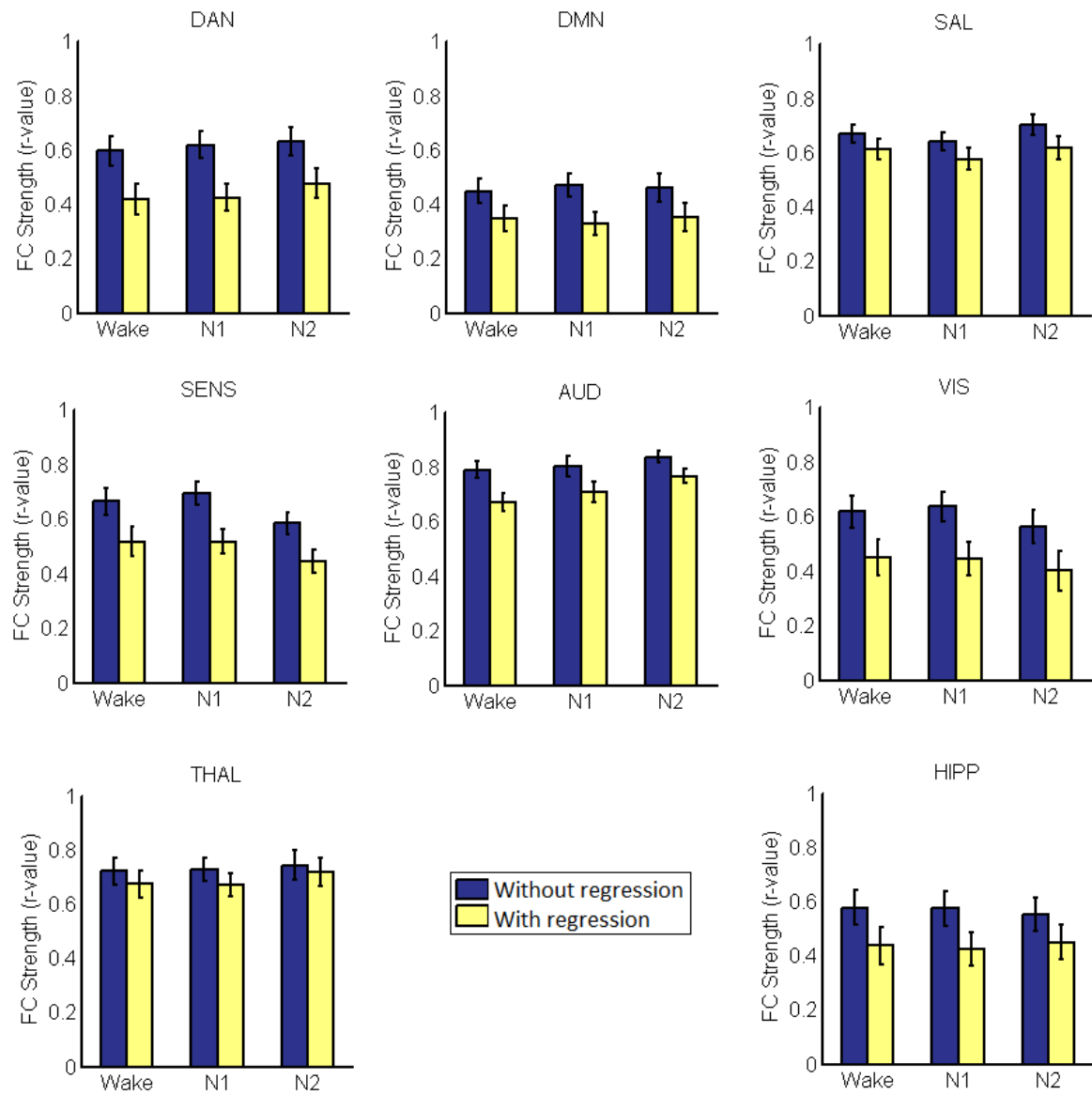


Figure 6.4.2 Within network FC for each of the ICNs and every sleep stage both with (white) & without (blue) GSR. Error bars represent \pm SE.

FC with and without the removal of negative coefficients

Due to the contention regarding the meaningful interpretation of negative FC (Van Dijk et al., 2010), particularly that positive correlations may be more reliable than negative (Shehzad et al., 2009). We also investigated the ICN FC with and without removal of negative correlations (see Figure 6.4.3). The overall pattern of FC changes within the ICNs by sleep stage is largely consistent whether negative correlations were included or removed, while the FC strength increased with the removal of the negative values. As such in order to allow direct comparison to our previous work (Chapter 5) any negative FC coefficients were set to zero in subsequent analyses.

CHAPTER 6: THE ASSOCIATION BETWEEN MEASURES OF SLEEP HISTORY AND FUNCTIONAL CONNECTIVITY DURING NREM SLEEP

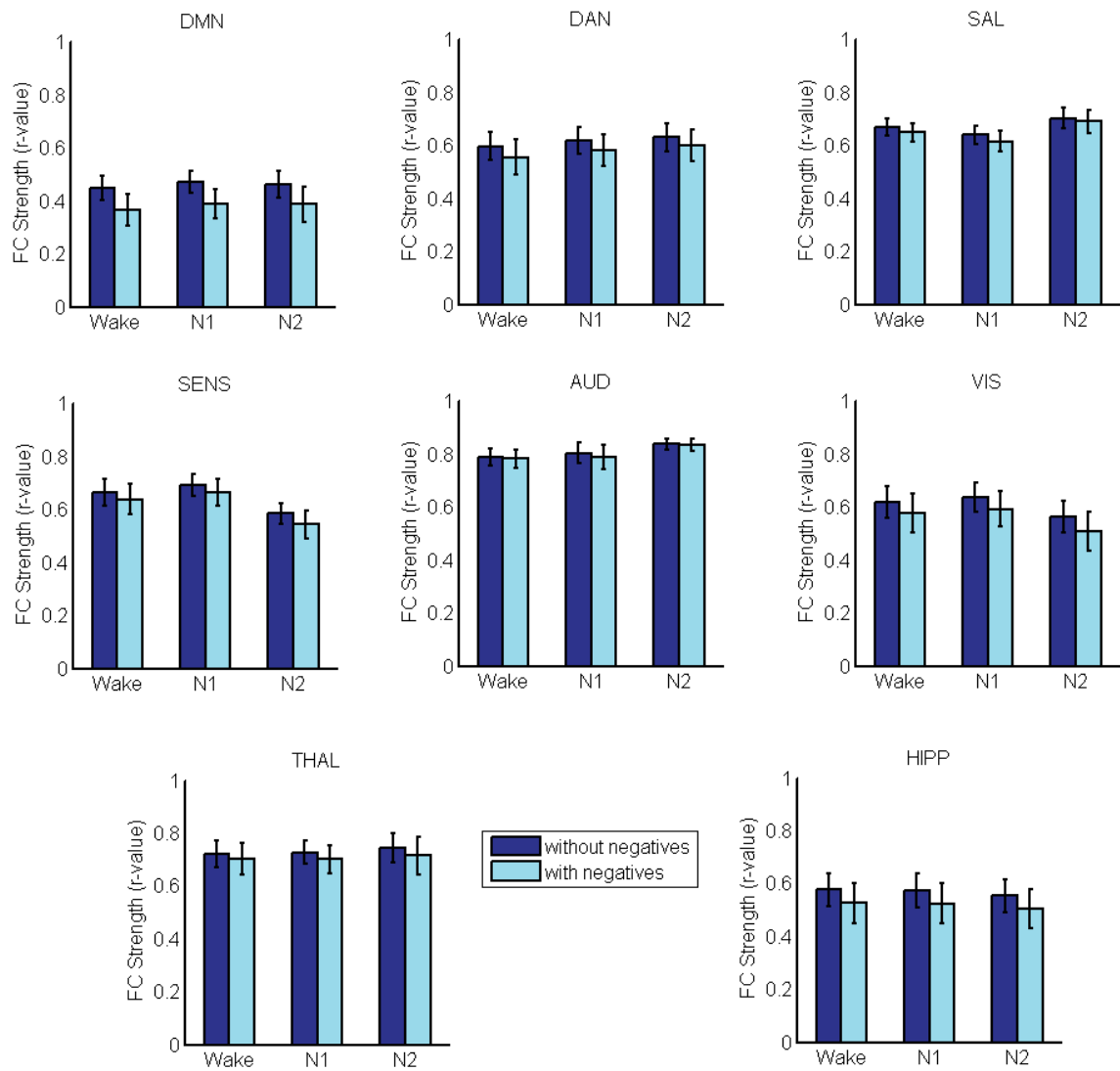


Figure 6.4.3 FC within ICNs with and without removal of negative coefficients. Error bars represent \pm SE.

6.4.4 FC association with objective measures of sleep history

Average total sleep time (ATST)

The ATST across the 2 weeks prior to the sleep scan was not significantly associated with the FC within any ICNs, suggesting that the average nightly sleep duration may not be an influencing factor in FC changes during subsequent sleep.

Night before total sleep time (BTST)

A measure of sleep duration during the night before the sleep scan (BTST) was significantly related to the inter-subject FC within the DMN and HIP. However the FC of other ICNs was not related to BTST, suggesting that the influence of BTST is network dependent. Within the DMN we found BTST significantly interacted only with the main effect of ROI ($p=0.002$), with pairwise comparisons finding significantly altered FC restricted to the PCC, and specifically PCC connections to mPFC, bilateral IPL regions and rMTL (see Figure 6.4.4). For these regions reduced BTST resulted in greater FC, regardless of sleep stage.

FC within the HIP was also significantly associated with BTST ($p=0.045$), however this overall effect was present regardless of ROI or Stage, see Figure 6.4.5, suggesting BTST has a larger influence on HIP FC than sleep itself.

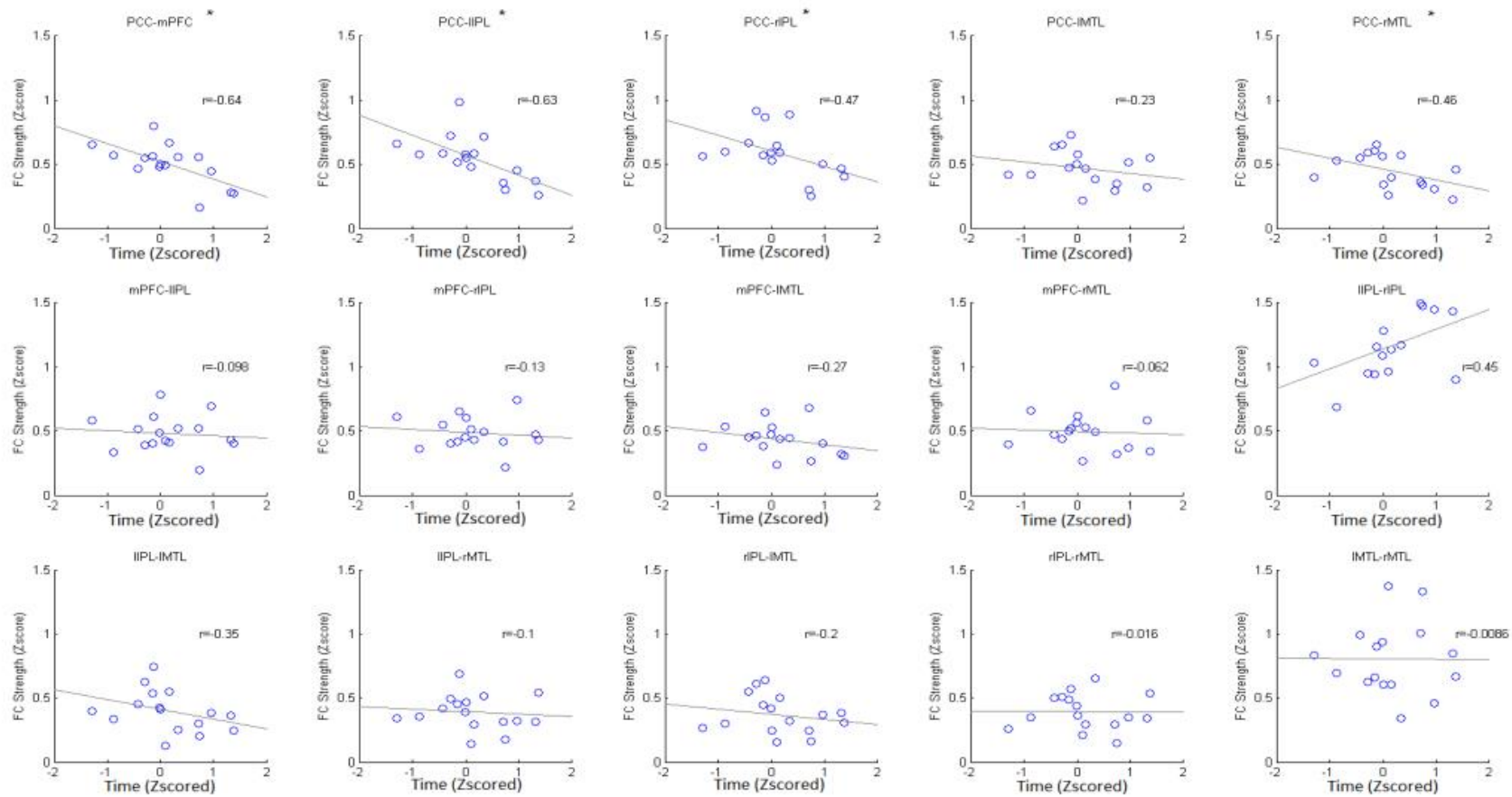


Figure 6.4.4 BTST and ROI interaction within the DMN, *Significant pairwise connection $P < 0.05$ after Bonferroni correction

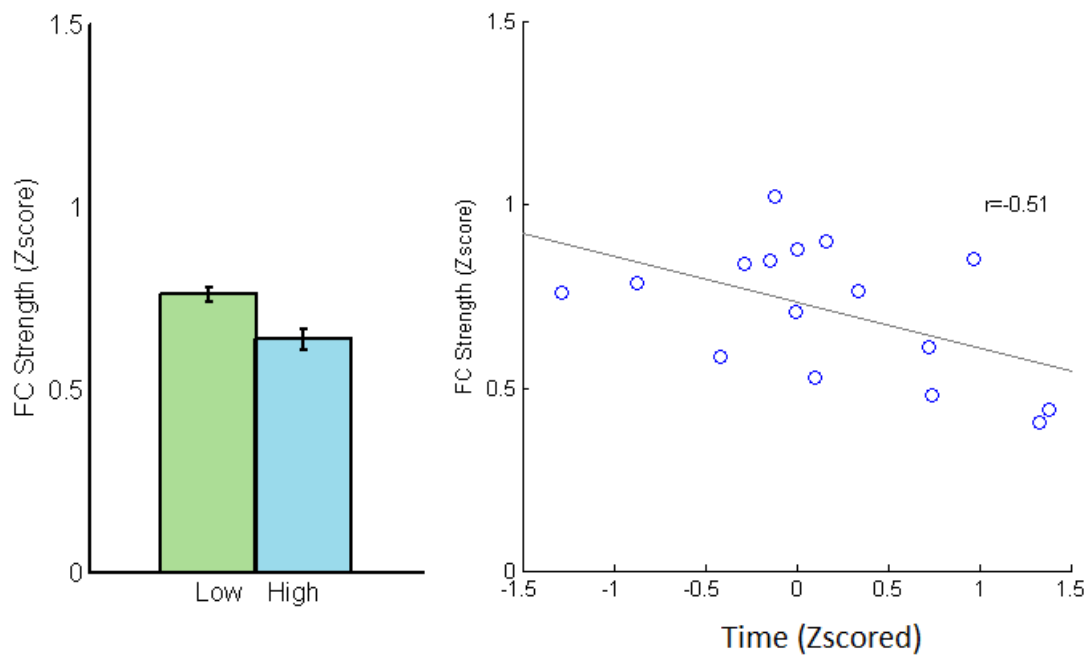


Figure 6.4.5 Overall FC within the HIP and the relationship with BTST. Median split for display. Error bars represent \pm SE.

Variability in total sleep time (VTST)

The VTST significantly related to FC changes with Stage exclusively within the SAL, with higher FC during N2 associated with individuals who were more consistent in their TST (i.e., lower VTST) ($p=0.040$), see Figure 6.4.6.

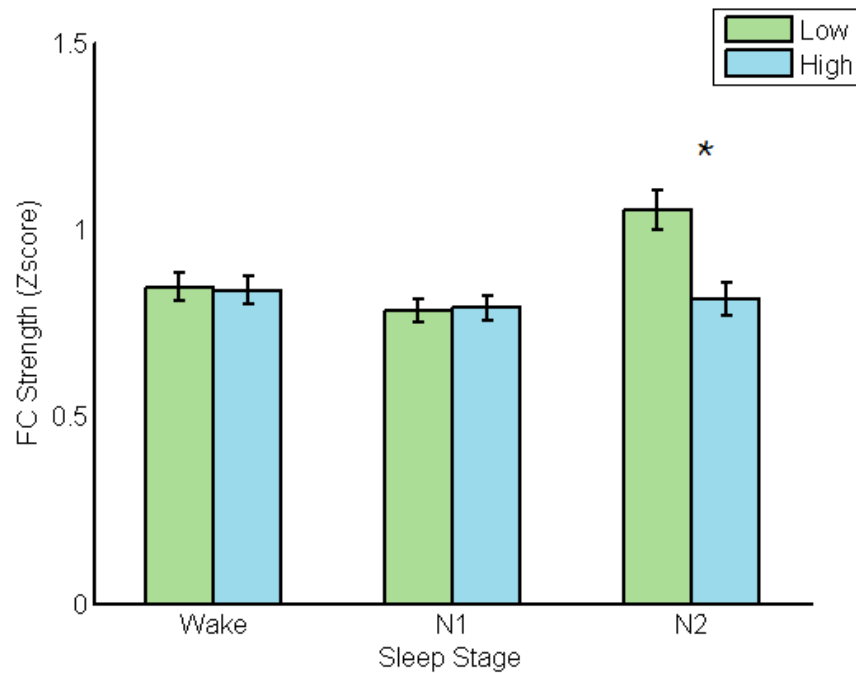


Figure 6.4.6. Effect of VTST within the SAL, indicating the significant interaction with Stage. *Significant pairwise difference $p < 0.05$ after Bonferroni correction. Error bars represent \pm SE.

6.4.5 ICN FC association with subjective sleep measures

Pittsburgh Sleep Quality Index (PSQI)

FC related to PSQI significantly within the SAL where we identified a three-way interaction between ROI, Stage and PSQI. Pairwise comparisons revealed this effect to be significantly different for the ACC-IINS FC in N1 ($p = 0.037$) showing that FC was greater in those subjects reporting better sleep quality (i.e. lower PSQI score), see Figure 6.4.7.

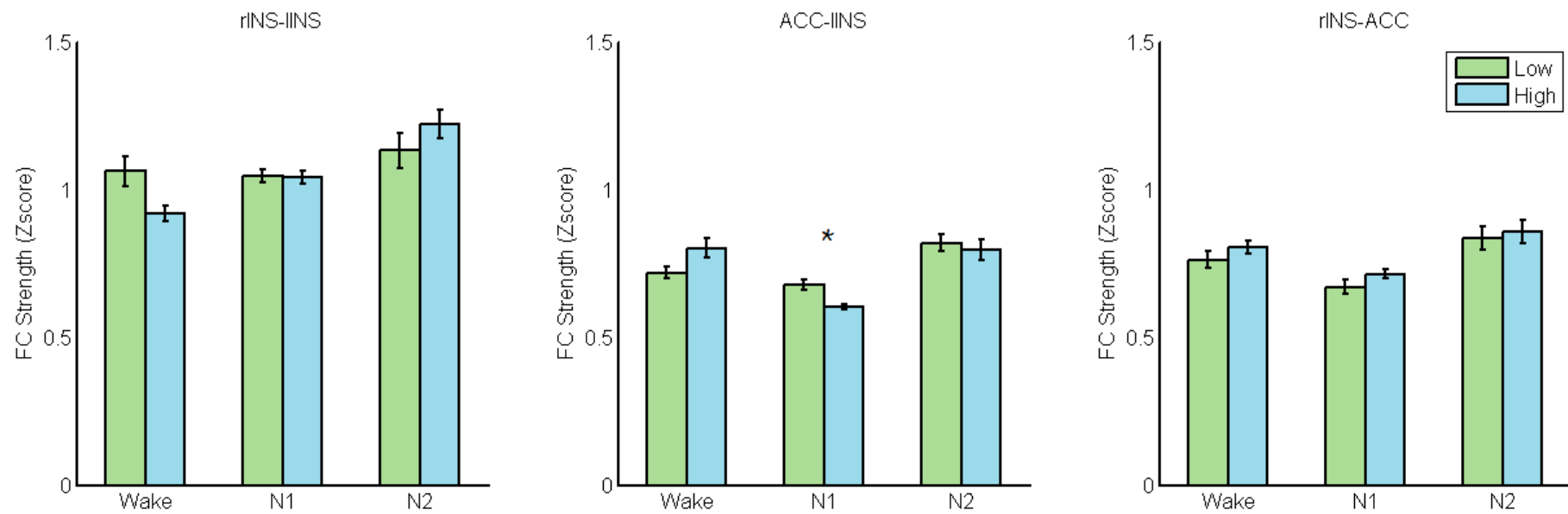


Figure 6.4.7. SAL FC significant three-way interaction with ROI, Stage and PSQI. *Significant pairwise difference $p < 0.05$ after Bonferroni correction. Error bars represent \pm SE.

Epworth Sleepiness Scale (ESS)

ESS scores did not significantly associate with the FC within any of the ICNs, suggesting that self-reported sleepiness may not influence FC changes during sleep.

Fatigue severity scale (FSS)

We observed a significant three-way interaction within the DMN of ROI, Stage and FSS suggesting that a subject's FSS score is linked to the changing FC of certain regions, between the different sleep stages (see Figure 6.4.8). Pairwise comparisons revealed significant differences ($p < 0.05$) for the PCC-lMTL, mPFC to the bilateral IPL, the lIPL-rMTL, rIPL-lMTL, rIPL-rMTL and the bilateral MTL, between those scoring low or high on the FSS. These effects were limited to either wake or N2. Overall FC was significantly higher in the aforementioned paired connections in those who reported less fatigue on the FSS, with the notable exception of the bilateral FC of the MTL which increased for those reporting higher fatigue on the FSS.

Insomnia Severity Scale (ISI)

The ISI score was significantly related to the FC within the DMN alone, in particular interacting with ROI ($p = 0.007$), suggesting that the ISI score associated differentially with the FC of DMN regions. Pairwise comparisons revealed that the rMTL FC (to all other DMN regions) significantly decreased with increasing ISI score, see Figure 6.4.9.

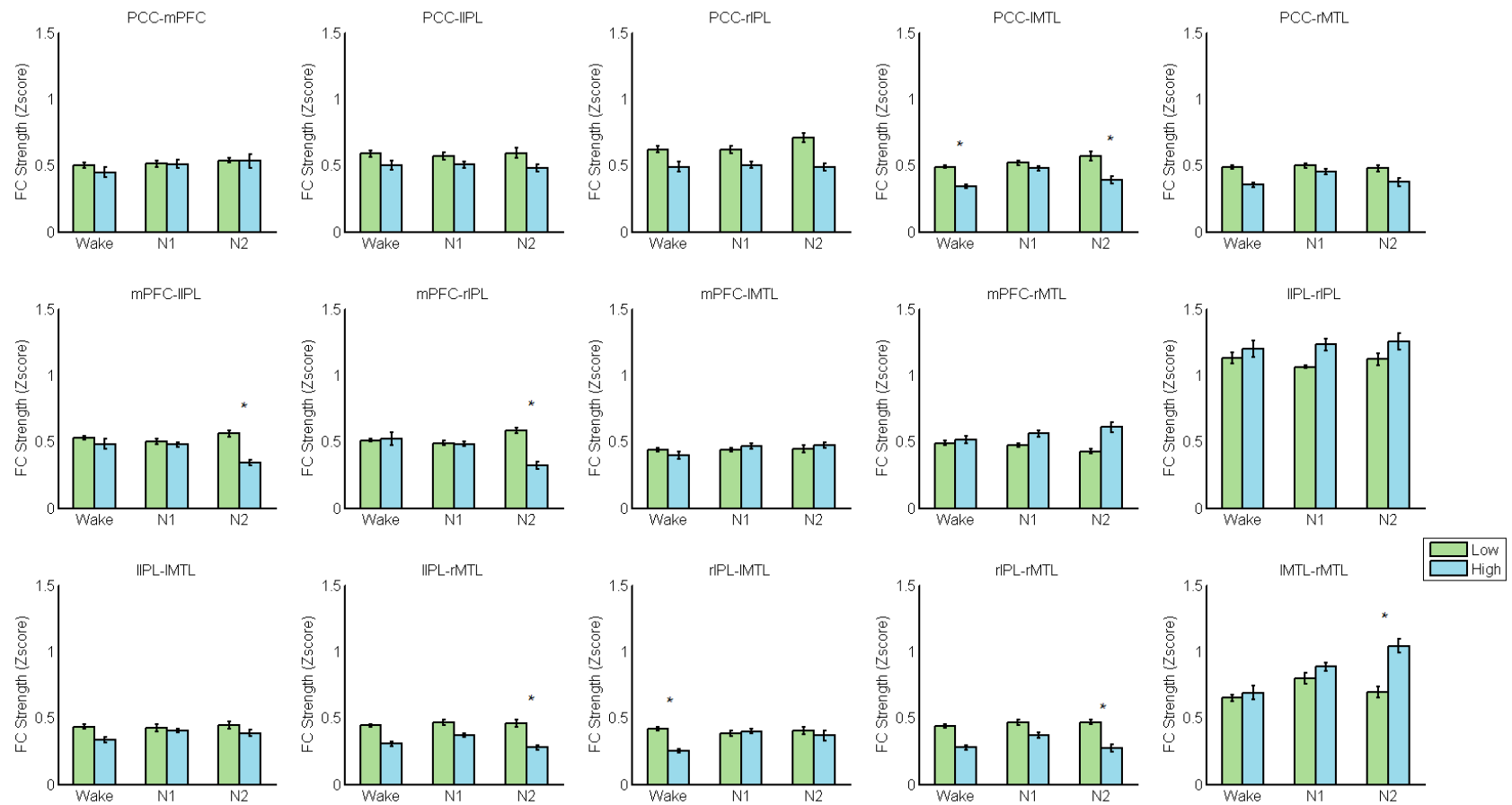


Figure 6.4.8. FSS*ROI*STAGE interaction within the DMN, *Significant pairwise difference $p < 0.05$ after Bonferroni correction. Error bars represent $\pm SE$.

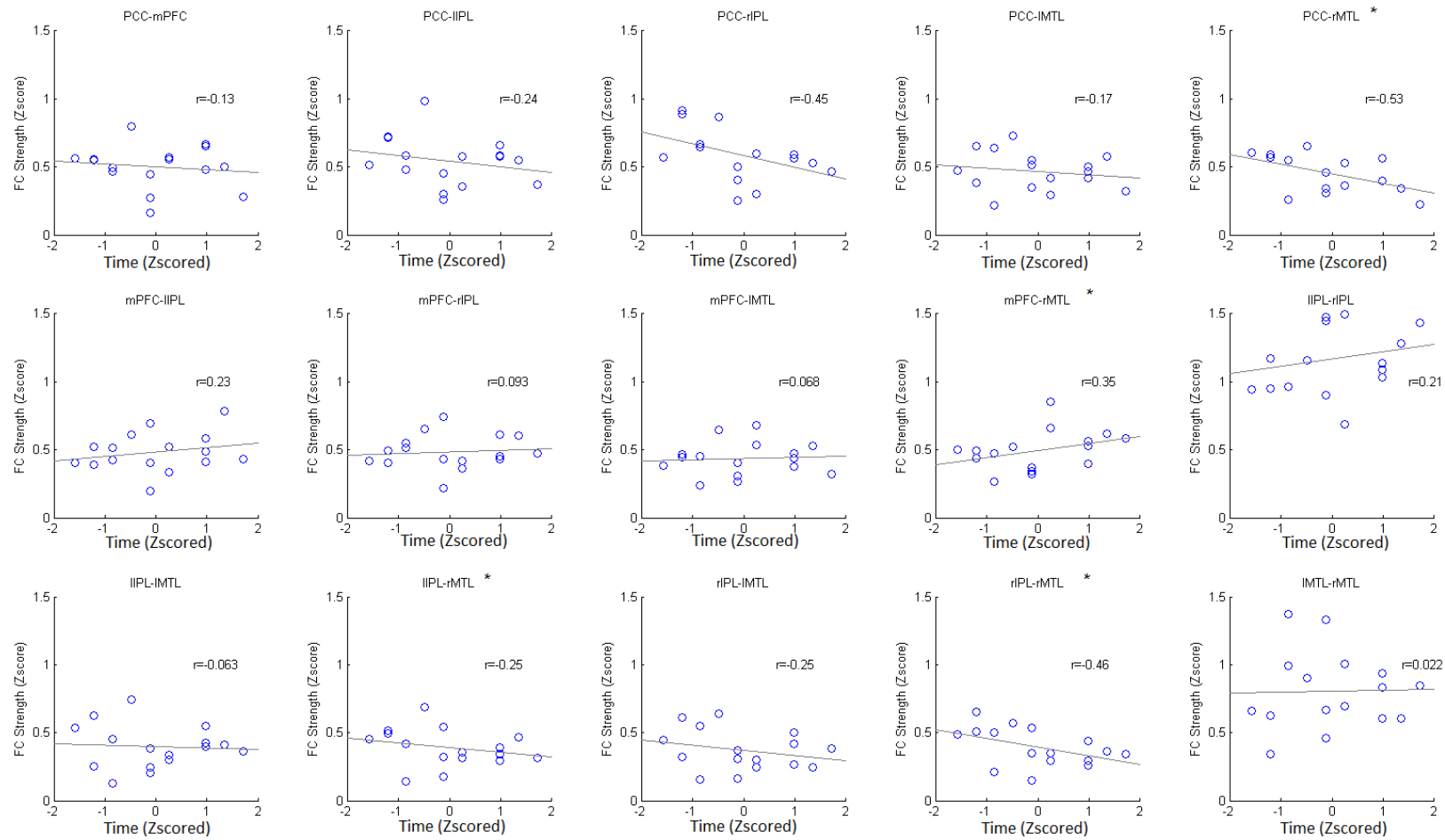


Figure 6.4.9. ISI*ROI interaction within the DMN, *Significant pairwise connection $P < 0.05$ after Bonferroni correction.

Table 6.4.3. Results of covariance analysis with DMN FC as the dependent variable and ROI and stage as factors for each sleep measure predictor. No significant results survived FDR calculations.

Predictor	Overall FC		ROI [‡]		Stage		ROI*Stage [‡]	
	F-value <i>F(1,10)</i>	p-value	F-value <i>F(14,140)</i>	p-value	F-value <i>F(2,20)</i>	p-value	F-value <i>F(28,280)</i>	p-value
ATST	0.001	p=0.971	1.256	p=0.242	2.383	p=0.118	0.776	p=0.787
BTST	1.122	p=0.314	2.668	<u>p=0.002</u>	0.421	p=0.663	0.523	p=0.979
VTST	3.640	p=0.085	1.567	p=0.098	0.961	p=0.400	0.944	p=0.551
PSQI	0.272	p=0.614	0.274	p=0.996	0.206	p=0.815	0.444	p=0.994
ESS	0.150	p=0.707	0.303	p=0.993	1.716	p=0.205	0.597	p=0.949
FSS	2.600	p=0.138	1.692	p=0.064	0.219	p=0.805	1.563	<u>p=0.039</u>
ISI	0.177	p=0.683	1.996	<u>p=0.022</u>	0.090	p=0.915	0.462	p=0.901

[‡]Significant factor for ICN ANOVA

Significant ($p<0.05$)

Table 6.4.4. Results of ANCOVA with SAL FC as the dependent variable and ROI and stage as factors for each sleep measure predictor. No significant results survived FDR calculations.

	Overall FC		ROI [‡]		Stage		ROI*Stage [‡]	
Predictor	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
ATST	F(1,10)= 0.089	p=0.771	F(2,20)= 0.001	p=0.999	F(1.26,12.64)=0.048	p=0.881	F(4,40)= 0.1099	p=0.370
BTST	F(1,10)= 0.532	p=0.483	F(2,20)= 0.969	p=0.396	F(1.24,12.44)=0.305	p=0.639	F(4,40)= 0.384	p=0.819
VTST	F(1,10)= 1.898	p=0.198	F(1.31,13.08)= 0.501	p=0.540	F(2,20)= 5.3445	<u>p=0.014</u>	F(4,40)= 0.080	p=0.988
PSQI	F(1,10)= 0.234	p=0.639	F(2,20)= 0.755	p=0.483	F(1.23,12.31)=0.148	p=0.759	F(4,40)= 3.673	<u>p=0.028</u>
ESS	F(1,10)= 1.250	p=0.290	F(2,20)= 0.046	p=0.955	F(1.26,12.63)=0.071	p=0.849	F(4,40)= 1.244	p=0.308
FSS	F(1,10)= 0.877	p=0.371	F(2,20)= 0.075	p=0.928	F(1.22,12.18)=0.310	p=0.632	F(4,40)= 0.839	p=0.509
ISI	F(1,10)= 1.506	p=0.248	F(2,20)= 0.978	p=0.393	F(1.18,11.85)=0.364	p=0.594	F(4,40)= 0.1326	p=0.287

[‡] Significant factor for ICN without covariate
Significant ($p<0.05$)

Table 6.4.5. Results of covariance analysis with HIP FC as the dependent variable and ROI and stage as factors for each sleep measure predictor. No significant results survived FDR calculations.

Predictor	Overall FC		ROI [‡]		Stage		ROI*Stage	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
ATST	F(1,10)= 1.976	P=0.190	F(5,50)= 0.563	P=0.728	F(1.6,15.5)= 0.586	P=0.527	F(10,100)= 1.071	P=0.391
BTST	F(1,10)= 5.233	<u>P=0.045*</u>	F(5,50)= 1.051	P=0.399	F(1.5,15.2)= 0.065	P=0.894	F(10,100)= 0.842	P=0.488
VTST	F(1,10)= 0.913	P=0.362	F(5,50)= 0.166	P=0.974	F(1.5,14.9)= 0.467	P=0.580	F(10,100)= 1.886	P=0.056
PSQI	F(1,10)= 0.794	P=0.394	F(5,50)= 1.452	P=0.222	F(1.6,15.6)= 1.465	P=0.257	F(10,100)= 0.651	P=0.767
ESS	F(1,10)= 0.001	P=0.977	F(5,50)= 0.189	P=0.966	F(1.5,12.4)= 1.170	P=0.323	F(10,100)= 0.599	P=0.636
FSS	F(1,10)= 1.543	P=0.242	F(5,50)= 0.152	P=0.979	F(1.3,13.4)= 0.929	P=0.381	F(10,100)= 1.377	P=0.202
ISI	F(1,10)= 1.322	P=0.277	F(5,50)= 1.125	P=0.359	F(1.5,15.2)= 0.003	P=0.989	F(10,100)= 0.611	P=0.801

[‡] Significant factor for ICN without covariate.

Significant ($p < 0.05$)

6.5 Discussion

We have shown that both objective and subjective measures of prior sleep-wake behaviour are associated with FC during subsequent wakefulness and NREM sleep, suggesting functional changes in the waking and sleeping brain are associated with between-individual's variability in preceding sleep behaviour. In addition, just as changes in FC with sleep stage were not homogenous across the brain, neither were the associations between sleeping FC and prior sleep-wake behaviour. This link was network or regionally specific, which could have implications for our understanding of the mechanisms underpinning sleep, as well as its functional role. The FC within the DMN, SAL and HIP were related to prior sleep-wake behaviours, while the FC in other sensory networks (SENS, AUD & VIS) and the THAL were not. Interestingly, in several cases FC related to sleep measures independently of stage suggesting FC as an independent measure may be a correlate of historical sleep behaviour, regardless of the current changes in FC with sleep stage. It is in many ways surprising that the changes to FC as a function of prior sleep history are as pronounced as the changes associated with increasing sleep depth. Therefore this study highlights the need to consider a subject's prior sleep history in all studies utilising FC analysis during wakefulness and sleep.

FC changes with sleep stage have been well documented (Horovitz et al., 2009; Larson-Prior et al., 2009, 2011; Spoormaker et al., 2010; Spoormaker, Czisch, Maquet, & Jancke, 2011), and in accordance with these previous reports and those from Chapter 5 we found FC changes with sleep stage within the DMN, SAL, SENS, VIS and THAL, providing a replication of these effects on independent data. We also demonstrated that common methodological

choices (i.e., GSR, negative correlations) do not have a profound impact on the overall pattern of FC with sleep stage. However the primary aim of the current study was to investigate whether these changes in FC during normal NREM sleep (i.e not following experimental sleep deprivation) are related to prior sleep in order to highlight FC markers of sleep history and to better understand the potential causes of dynamic changes in the FC during sleep.

6.5.1 Objective sleep measures and the relationship to ICN FC during sleep

Wrist actigraphy was the main objective measurement used in the current study to assess sleep-wake behaviour. From actigraphy we investigated the interaction between changes in within-ICN FC with sleep stage and three measures of total sleep time: the average two-week sleep duration (ATST), sleep duration on the night prior to scanning (BTST) and variability in average sleep duration (VTST). A previous study showed cumulative sleep duration measured with actigraphy across two weeks prior to scanning predicted waking resting-state FC, in particular the FC of the mPFC and insula with regions within the DMN (Khalsa et al., 2016). The current study also found actigraphy measures to be associated with the FC between regions within the DMN during sleep, supporting the general principle that habitual sleep patterns are related to the FC of RSNs. However the association between FC during sleep and previous sleep history has not to our knowledge been investigated, as such this is the first study to demonstrate a link with what happens in the brain during sleep and previous sleep-wake history. As waking behaviour has been shown to impact changes in the brain during sleep, with slow wave activity across cortical areas being linked to the level of prior activity in wake (Huber et al., 2006), similarly we suggest sleep could be modulated by prior sleep behaviours measured objectively with actigraphy.

BTST was related to the FC within the DMN, with increased FC associated with reduced sleep duration in line with the findings from Chapter 5. This effect was regionally predictive, with the PCC FC to other DMN regions related to BTST regardless of sleep stage. The importance of prior sleep for PCC FC is supported by previous research showing alterations in PCC FC with increased sleep pressure from partial sleep deprivation (Sämann et al., 2010), as well as alterations of DMN FC more generally with sleep deprivation (Gujar et al., 2010; Yeo et al., 2015). We show that this associated decrease in DMN FC with increased sleep pressure is different during sleep, with less sleep the night before (i.e. more wake) resulting in higher FC. This also supports our finding of higher FC during recovery sleep, a more extreme curtail of sleep duration (see Chapter 5). Recent research has shown that both the time of day and sleep deprivation have an impact on the FC of wake for the DMN with increased FC strength in wake following sleep deprivation (Kaufmann et al., 2015). However the current study is the first to suggest that FC during sleep is also influenced by sleep pressure, with the DMN FC again being shown to correspond to changing sleep pressure or sleep-wake history. Therefore previous research taken together with these findings indicates an important role for DMN regions in sleep-wake regulation.

In addition, BTST was predictive of FC within the HIP, incorporating the bilateral hippocampi and parahippocampal gyri regardless of sleep stage and therefore including wake. The increase in HIP FC strength associated with a reduction in sleep duration suggests HIP processes are altered by prior sleep behaviour. Interestingly increased HIP FC following sleep deprivation has previously been shown in wake following sleep deprivation (Kaufmann et al., 2015), suggesting HIP FC changes could be a reflection of sleep pressure. Indeed HIP FC was also shown to change as a function of sleep stage, increasing in N2 compared to other

stages, as well as decreases in HIP-DMN FC with advancing sleep stage (Andrade et al., 2011). Additionally the role of HIP activity in memory consolidation during sleep is well established (Abel, Havekes, Saletin, & Walker, 2013), as is the involvement of the MTL which is also important for episodic memory (Andrews-Hanna, Reidler, Sepulcre, Poulin, & Buckner, 2010). Thus, the relationship with BTST and FC in both the DMN and HIP is in line with previous reports indicating a role for these networks, both working in isolation and together, in regulating memory during sleep.

ATST was not predictive of FC during sleep for any of the ICNs, suggesting that it may be more recent sleep which impacts FC (i.e., BTST), which is supported by the cognitive performance literature which shows that the amount of prior sleep has an immediate effect on performance of waking cognition more so than historical sleep (Banks et al., 2010), although other means of quantifying habitual sleep not assessed in the current study have been shown to associate with FC during wake (Khalsa et al., 2016). Indeed the variability in the duration of total sleep time (VTST) (a measure determining the ‘consistency’ of sleep duration in the two weeks prior to recorded sleep), was found to relate to the FC changes with sleep stage, although this effect was only observed in the SAL. Our findings show an associated reduction in FC across the SAL in N2 in those individuals who had the least consistent nightly sleep duration. Increased variability in sleep duration and fragmentation, as measured using actigraphy, has been shown to relate to increased levels of stress (Mezick et al., 2009). Furthermore, the SAL has been implicated in previous neuroimaging studies investigating the hypothesis of hyperarousal in people suffering from insomnia (M. C. Chen et al., 2014), a condition known to interact with stress (Basta, Chrousos, Vela-Bueno, & Vgontzas, 2007; Linton & Bryngelsson, 2000; Ohayon, 2002). As such the current study is an interesting

additional finding linking SAL activity with the mechanisms of habitual sleep behaviour, which could be mediated or influenced by stress in line with the insomnia literature.

6.5.2 Subjective measures and the relationship to ICN FC during sleep

There are several ways of quantifying subjective sleep and the current study uses four of the most common ones used clinically, the Pittsburgh sleep quality index (PSQI) (Buysse et al., 1989), Epworth sleepiness scale (ESS) (Johns, 1991), fatigue severity scale (Krupp, 1989) and the insomnia severity index (Bastien, 2001). We found that the scores on the PSQI, FSS and ISI were related to DMN and SAL FC during sleep. PSQI was predictive of the FC within regions of the SAL where we observed an association between reduced ACC – left INS FC during N1 for individuals who reported worse overall sleep quality. Functionally the SAL network, and particularly the insula, are thought to regulate the dynamic ‘switching’ between internal and external stimuli required for normal brain functioning (Menon & Uddin, 2010). Thus our observed differences in FC could reflect alterations in SAL ‘switching’ during sleep which are influenced by previous sleep quality. Indeed behavioural evidence suggests that sleep quality measured using the PSQI is predictive of poor behavioural performance in some cognitive domains for older adults (Nebes, Buysse, Halligan, Houck, & Monk, 2009; Schmutte et al., 2007). It remains for future research to investigate whether the relationship between FC during N1 in the SAL and prior sleep quality is also a marker for alterations in waking cognitive performance, although it is clear that the FC of the SAL may be important for the sleeping brain just as it is for waking resting-state.

Self-reported insomnia symptoms were measured with the Insomnia severity index (ISI) questionnaire (Bastien, 2001) and the individual score assessed as a predictor of FC changes with sleep in each ICN. We have shown DMN FC to be associated with the inter-subject ISI variability, namely the right medial temporal lobe (rMTL) FC to all regions within the DMN is reduced in subjects reporting greater level of insomnia symptoms. Interestingly it has previously been proposed that DMN FC could be linked with insomnia (Marques, Gomes, Clemente, Moutinho dos Santos, & Castelo-Branco, 2015), which is supported in the current study. The association with ISI scores and the MTL FC, a region involved in memory function (Alvarez & Squire, 1994), specifically declarative memory (Kahn, Andrews-Hanna, Vincent, Snyder, & Buckner, 2008) and the requirement of sleep in order to consolidate these memories (Diekelmann & Born, 2010) is interesting given that studies have found impairment in declarative memory performance in insomniacs (Backhaus et al., 2006; Nissen et al., 2006, 2011). With this in mind our finding of reduced FC of the MTL (within the DMN) with greater reported symptoms of insomnia seems fitting and requires further investigation. However it is important to note that none of the participants exceed the criterion for insomnia, the individual variability in scores alone predicts FC across all sleep stages suggesting intrinsic FC is related to the subjective measure of Insomnia symptoms in both wake and sleep. However the current study did not assess slow wave sleep which is understood to be important for memory consolidation (Diekelmann & Born, 2010), therefore the assessment of MTL FC in SWS and the relationship with ISI is an interesting area for future investigation.

Fatigue was also considered in the current study as a predictor of FC given its recognised distinction from sleepiness (Hossain et al., 2005; Shen et al., 2006). As with ISI, levels of fatigue did not exceed the threshold for clinical diagnosis, but individual variability was found

to be predictive of within DMN FC changes with sleep stage. We have shown that during N2 FC was reduced between many of the DMN regions with increased reported levels of fatigue. This association between FSS and DMN FC was similar to that seen with the ISI. This may not be surprising given that these measures are related and considering that excessive daytime fatigue has also been reported by people suffering from insomnia (Lichstein, Means, Noe, & Aguillard, 1997; Morin, LeBlanc, Daley, Gregoire, & Merette, 2006). Taken together our findings suggest a possible FC underpinning of fatigue in the brain during wake and sleep which could have implications for our understanding of insomnia.

6.5.3 Methodological considerations and future research

The current study is limited to the first few cycles of sleep and FC could differ in the relationship to prior sleep measures in later cycles, indeed fMRI differences have been noted within N1 (Picchioni et al., 2008). Therefore future work to assess the variability in FC within an EEG classified sleep stage is an important question and can be assessed using dynamic analysis. In addition, extending scanning to later in the night or continually across the whole night would enable an even greater idea of the FC changes occurring during sleep and their relationship with prior sleep behaviour, although this is difficult in the scanner environment as good or usual sleep is disrupted.

We also assessed the differences in FC with and without mean global signal regression (GSR) for all regions and sleep stages due to the contention in the field (Gotts et al., 2013; Keller et al., 2013) and uncertainty regarding GSR changes with sleep stage. However we found that GSR did not change as a function of sleep stage, while we do show GSR effects are regional.

Recent work advocates the use of GSR in sleep deprivation during wake (Yeo et al., 2015), although in the current study we choose not to complete GSR in order to directly compare with our previous findings.

6.6 Conclusion

The FC of the DMN and SAL during sleep are shown to be associated with multiple objective actigraphy measures and subjective self-report questionnaires including the PSQI, ISI and FSS. As well as FC within the HIP being found to associate with an objective measure of TST. We also show that sleep alters the FC of these ICNs and that some of these FC changes are predicted by the characteristics of that individual's prior sleep. This finding has implications for our understanding of sleep, its underlying mechanisms and the brain function associated with it. In addition this work improves our understanding of dynamic FC and the factors which may modulate it both in sleep and wake. We show FC to be a useful measure in sleep research, albeit requiring further dynamic investigation.

Chapter 7

THE VARIABILITY IN FUNCTIONAL CONNECTIVITY WITHIN AN EEG CLASSIFIED SLEEP STAGE DURING NREM SLEEP

7.1 Abstract

This chapter explores whether the different strengths of ICN FC that are observed between sleep stages, are consistently maintained across those stages. By assessing the temporal variability of FC strength we investigate whether within-ICN FC fluctuates or is stable within a standard thirty-second duration, EEG-classified stage, independent of where the stage is in the sleep cycle. We show that differences in both FC strength and FC variability exist between multiple epochs of the same stage, further suggesting that dynamic FC could be a sensitive marker in sleep research beyond ‘gold-standard’ EEG stage classification, expanding our knowledge of sleep and the underlying intrinsic processes. Additionally, these results provide support for FC as a useful, complementary measure in sleep research.

7.2 Introduction

Standardised visual classification of human sleep using discrete thirty-second epochs of EEG data has undoubtedly improved research and clinical practice within the field of sleep medicine (AASM, 2007). However, thirty seconds is a long period of time when assessing dynamically changing brain activity. Averaging across an epoch is arguably simplistic, mixing together temporal fluctuations and thus over or under estimating important functional processes occurring within the length of the defined sleep stage. These temporal changes in functional processes within a stage have been shown to be particularly important during the process of falling asleep, an especially transitory time (Ogilvie, 2001). Research assessing changes in brain activity within a sleep stage has mainly focused on this hypnogenic state of falling asleep, with EEG spectra changing spatially before, during and after a manually defined stage of N1 when using a five second classification (Hori, 1985; Ogilvie, 2001), which suggests that a classified thirty second sleep stage could encapsulate a multitude of activity and functionality. Furthermore, standardised scoring of a defined stage regardless of where in the sleep cycle it falls, either early on or later in the course of the night, could further obscure valuable information and differences in the brain activity between sleep cycles (Borbély et al., 1981).

To date few researchers have focused on within stage differences in brain activity across the sleep cycle, as wake, NREM and REM are classified in the same way throughout sleep, treated no differently whether they occur early or later on in the night. Currently the best technique we have to investigate the spatiotemporal activity within a thirty second classified stage of sleep is combined EEG-fMRI, enabling the assessment of large-scale networks

during sleep (Duyn, 2012). Using fMRI to compare FC across all the sleep staged epochs we are able to show changes in the overall FC within many of the large-scale ICNs (Chapters 5 & 6). A previous EEG-fMRI study focused on the spatial differences in activated regions within EEG classified N1 sleep stages between epochs recorded early and those later in the night (Picchioni et al., 2008). The study showed activity differences for regions overlapping with the DMN and hippocampus (Picchioni et al., 2008). Therefore this previous work cast doubt on the robustness of standard EEG staging (AASM, 2007) which classifies a sleep stage regardless of where it lies in the sleep cycle. The current study aims to determine the FC consistency for each stage based on where they occur in the sleep cycle, whether that be at the start of sleep or in the middle of other stages, comparing the FC between these different staged groupings.

EEG microstates are brief transient states (~second in duration) and have previously been explored within a classified sleep stage, suggesting temporal variations in the frequency and spatial pattern of EEG activity within a stage (Borbély et al., 1981; Hori, 1985; Morikawa, Hayashi, & Hori, 1997; Ogilvie, 2001). These microstate differences within stage are grouped together and undetected when assessing across all epochs of an EEG classified stage.

Therefore, microstates are likely to differ within a classified stage, although the degree to which this is reflected in the intrinsic activity measured using FC is not well understood.

fMRI studies have investigated transitory events within a sleep stage, such as sleep spindles, which have shown spatial differences in the haemodynamic response depending on the frequency of the spindles (Schabus et al., 2007) and K-complexes, which also show specific regional involvement (Caporro et al., 2012). Thus FC differences are entirely possible within a thirty second classified EEG sleep stage.

This chapter will explore the differences in within-ICN FC between sleep stages grouped depending on whether they occur; either early on or later in the sleep cycle. The changes in average FC strength between EEG classified sleep stages previously noted (Horovitz et al., 2008; Larson-Prior et al., 2011, Chapters 5 & 6) and the increased use of dynamic measures of FC (Allen et al., 2014; Tagliazucchi & Laufs, 2015; Tagliazucchi, von Wegner, Morzelewski, Brodbeck, et al., 2012; Chapter 4) will allow us to investigate whether these FC changes with sleep stage are uniform across classified stage. By assessing the variability of FC strength we will investigate whether within-ICN FC fluctuates or is stable within a standard EEG thirty-second classified stage, independent of where the stage is in the sleep cycle or prior stage pattern. The aim of our study was to determine whether FC is homogenous within an EEG classified stage regardless of the timing of that stage in the sleep cycle or whether FC differences emerge despite the same EEG ‘classification’. This has the potential to improve our understanding of the intrinsic spatiotemporal nature of the brain’s activity during sleep and provide support for FC as a useful complementary measure in sleep research.

7.3 Method

The experimental design and subjects are the same as Chapter 6; see Table 6.3.1 for demographic information. Chapter 3 also outlines the procedure for EEG-fMRI acquisition, pre-processing and definition of ROIs. Only additional methodology which has not been previously outlined will be reported below.

7.3.1 FC analysis

FC analysis of the fMRI data was calculated using a non-overlapping 30 second sliding window of the BOLD time-series for each ROI. This dynamic analysis using seconds rather than minutes to calculate FC has been applied to the investigation of FC changes during sleep in our previous study (Wilson et al., 2015). Each ROI was in turn used as the seed region and a Pearson correlation (R-value) was calculated for every 30-second epoch to all other independent target ROIs. Any negative FC coefficients were removed and set to zero and the coefficients were then converted to Z-scores using Fisher's transform, as used in Chapter 6.

We seek to investigate within-stage ICN FC variability (FC-V). For example, whether the FC during a stage of wake early in the scan is similar or different to the FC of a stage of wake that occurs later in the scan after a period of sleep. The FC of N1 early on will also be compared to FC of N1 after a longer period of sleep (i.e. after N2), in addition to comparing the FC of N2 before and after a period of wake.

Each of the 30-second FC matrices were paired to the corresponding EEG defined sleep stage. The epochs for each stage were then grouped according to where in the sleep cycle, either before (B) or after (A) a stage change within the sleep cycle. Due to the limited number of N3 epochs (69) across the whole group, data from stage N3 was removed from all subsequent analysis. An epoch of wake, N1 or N2 occurring at the beginning of the scan transitioning sequentially (i.e. wake-N1-N2) was grouped as *before*, while epochs of a stage after 2.5 minutes (5 epochs) of a different stage were grouped as *after*, see Figure 7.3.1 which shows the groupings (A & B). All epochs are included and grouped accordingly. The 5 epoch cut-off

was chosen to maximise data included and 2.5 minutes was deemed a sufficient length of time to show FC changes.

There were a total of six groups, before and after for each stage (B0, B1, B2, A0, A1, A2). FC values for these groups were then pooled and averaged across epochs and subjects. In order to separately investigate FC-V the standard deviation was calculated across epochs for each of the six groups in turn. For each group these were then averaged across subjects to calculate a group mean variability matrix.

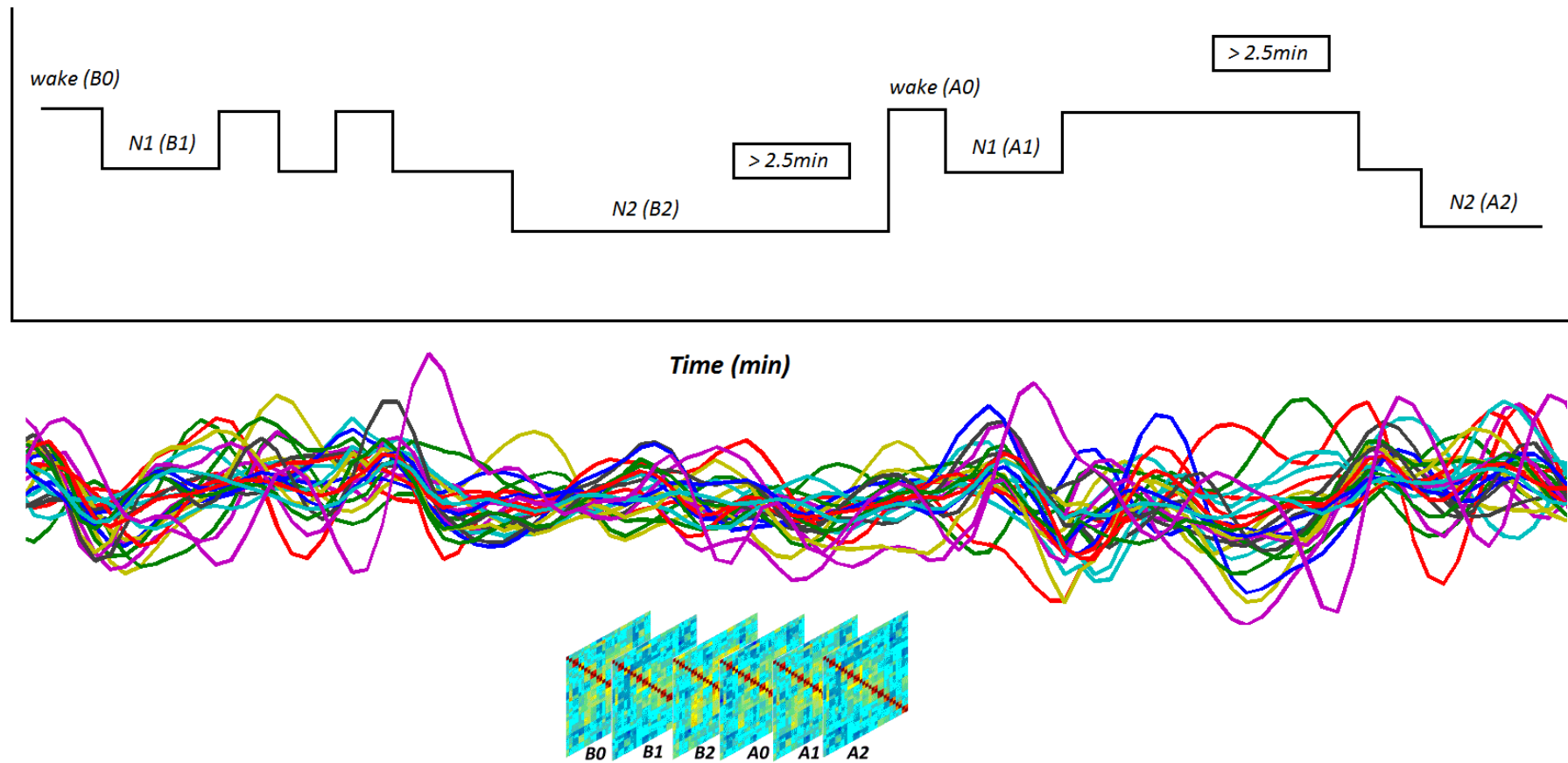


Figure 7.3.1. Methodology schematic. Sleep hypnogram with before and after groupings for each stage, indicating when the 2.5minute distinction would be applied. The FC is then calculated for each thirty second epoch and averaged into a single FC matrix per group, (six groups in total). All epochs are grouped as either *before* or *after*.

7.3.2 Statistical analysis

All statistical analysis was conducted on the standardised FC values (*Z-scores*) using SPSS (IBM SPSS Statistics, version 21, Chicago, IL) with an alpha criterion level of $p < 0.05$. We assessed the within network FC of each of the eight networks. The standardised FC values were input into GLMMs for each ICN to determine whether the FC strength was different for each stage before and after a stage change. A similar GLMM analysis was also completed to investigate the FC-V (standard deviation) instead of FC strength. The GLMM included three fixed-effects of: ROI, WITHIN STAGE grouping (before & after) and sleep stage as well as all subsequent interactions, together with the random-effect of subject. Multiple comparisons were adjusted for by Bonferroni correction within the GLMM and only statistically significant effects ($p < 0.05$) are discussed in further detail in the results. Lastly, the mean FC was calculated for each ICN by averaging across all regions for each WITHIN STAGE grouping for every sleep stage, before and after a stage change.

7.4 Results

We recorded a total of 2502 thirty-second epochs of EEG-fMRI data including: 676 epochs of wake (~5hours) of which 497 preceded stage change B0; 1209 epochs of N1 (~10hours) of which 791 preceded stage change B1; 510 epochs of N2 (~4hours) of which 359 preceded stage change B2see Table 7.4.1. Measures of FC strength and FC-V within each ICN was compared between *before* and *after* a stage change as a function of each sleep stage, paired ROI and all subsequent interactions.

7.4.1 FC strength across the brain within an EEG classified sleep stage

We found that the strength of FC differed within sleep stage when comparing epochs before and after a stage change. Furthermore we found that this effect occurred differentially for ICNs and regionally within networks. The connectivity matrices in Figure 7.4.1 show how the regional pattern of FC was affected by the different stage groupings for all of the assessed ROI comparisons. Qualitatively, for each stage there were regionally specific increases and decreases in intra- and inter- network FC noted between before and after stage change measurements. These were further assessed by subtracting (After – Before) the group mean ROI connectivity matrices for each WITHIN STAGE group individually for wake and all sleep stages (Figure 7.4.1). Some of the largest differences in FC strength are seen for regions in the DMN, SAL and HIP. In particular, the PCC FC increased considerably in wake after sleep (A0) compared to wake before (B0), as did the HIP FC which increased in N1 and N2 after a change in stage (A1 & A2) compared with before (B1 & B2 respectively), while the SAL regions reduced FC strength after a change in stage compared with before, most prominently in N2.

Table 7.4.1. The number of sleep-staged, thirty-second epochs divided into before & after sleep stage based on <2.5mins of stable stage classification and the percentage of time spent in sleep for each individual subject.

	Subject																
Number of Epochs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total Epochs
Total Wake	0	19	87	61	19	67	69	77	23	54	53	78	31	0	7	31	676
Before (B0)	0	9	87	61	19	18	32	59	5	26	53	78	15	0	4	31	497
After (A0)	0	10	0	0	0	49	37	18	18	28	0	0	16	0	3	0	179
Total N1	76	44	154	102	21	16	86	43	61	77	36	17	143	117	150	66	1209
Before (B1)	16	29	154	102	21	10	54	34	30	28	33	17	42	117	38	66	791
After (A1)	60	15	0	0	0	6	32	9	31	49	3	0	101	0	112	0	418
Total N2	60	20	3	3	6	16	29	35	24	134	38	0	57	39	46	0	510
Before (B2)	32	20	3	3	6	61	29	28	24	25	38	0	21	39	30	0	359
After (A2)	28	0	0	0	38	0	0	7	0	109	0	0	36	0	16	0	234
Stage 3 (N3) [†]	30	0	0	0	0	22	0	0	3	14	0	0	0	0	0	0	69
Epochs	166	83	244	166	84	121	184	155	111	279	127	95	231	156	203	97	2502
Total Sleep (%)	100	77	64	63	32	45	63	50	79	81	58	18	87	100	97	68	

[†] Not included in statistical analysis

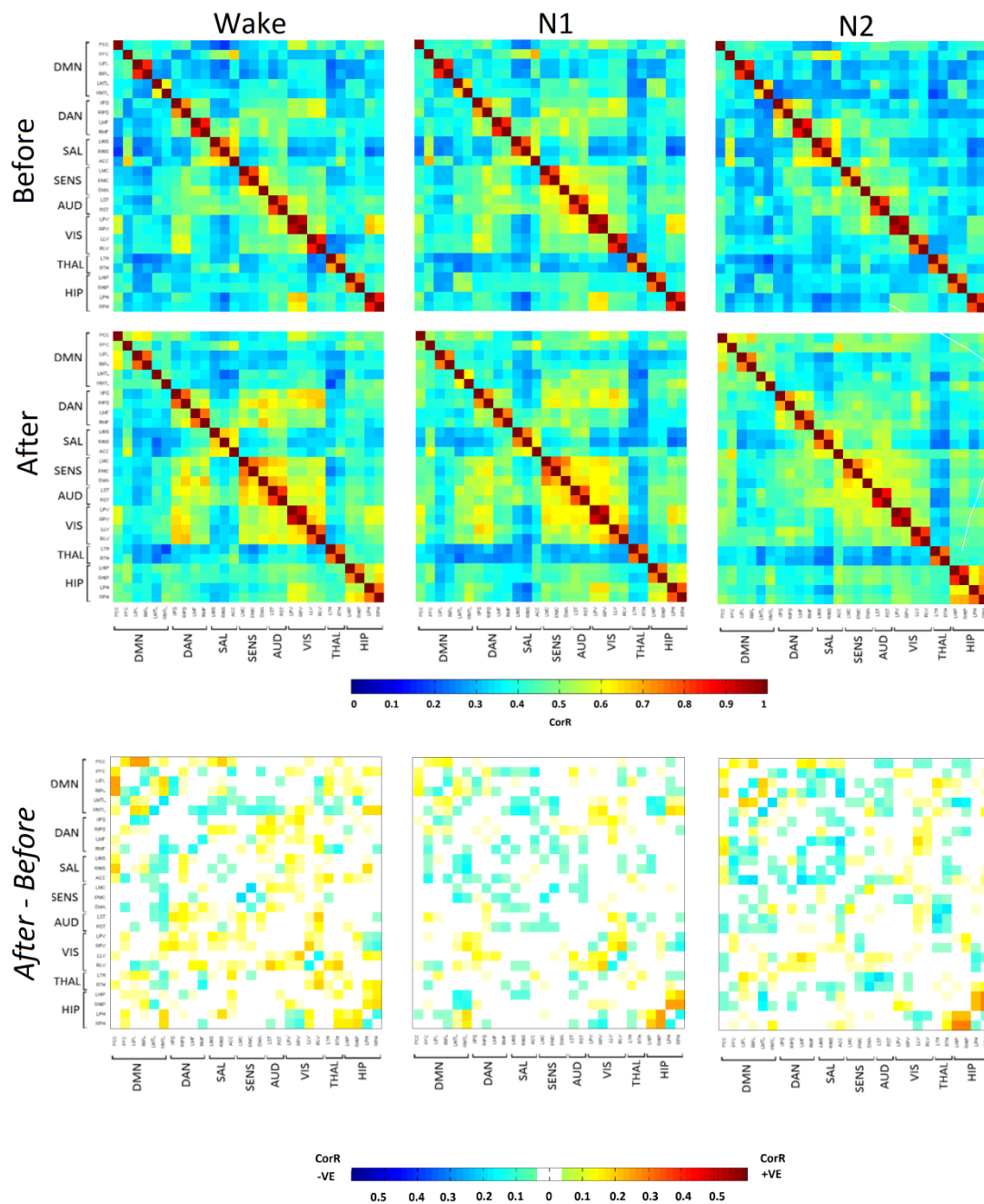


Figure 7.4.1. Average connectivity matrices for each sleep stage for WITHIN STAGE grouping across all subjects. Each square represents a paired ROI connection. ROIs are plotted according to the ICN to which they belong. The FC differences between stage groupings (After – Before) are shown with reduced FC *after* compared to FC *before* shown in blue colours and the higher regional FC *after* a stage change shown in red-yellow colours, *white* indicates a <0.05 FC change or minimal FC change. *CorR*= *Correlation coefficient*

7.4.2 Variability of ICN FC strength within an EEG classified stage

Figure 7.4.2 shows that ICNs differed in the extent to which their FC strength changed between epochs occurring before and after a stage change. For all investigated stages (wake, N1, N2), greater FC strength was noted in epochs after a stage change compared to before a stage change for many of the networks, including the DMN, SENS, VIS, THAL and HIP. For example, DMN FC is greater in epochs of wake, N1 and N2 that occurs after a 2.5 mins ‘steady state’, than it is during an epoch of N2 which occurs at the beginning or before a stage change. The notable exception was the SAL, which largely showed reduction in FC strength after a stage change compared to before. While the DAN and AUD were stable in terms of whole ICN FC strength with no overall differences seen within sleep stage, some individual regions within these ICNs were significantly different within stage. However, for all sleep stages a difference in FC strength was found between epochs before and epochs after a stage-change for the majority of ICNs, suggesting changes in FC are not confined to a particular stage. The statistics for each of the ICNs are outlined in Table 7.4.2 and discussed in more detail below. However the DAN, AUD and THAL GLMM main effects or subsequent interactions were not significant and as such are not discussed further.

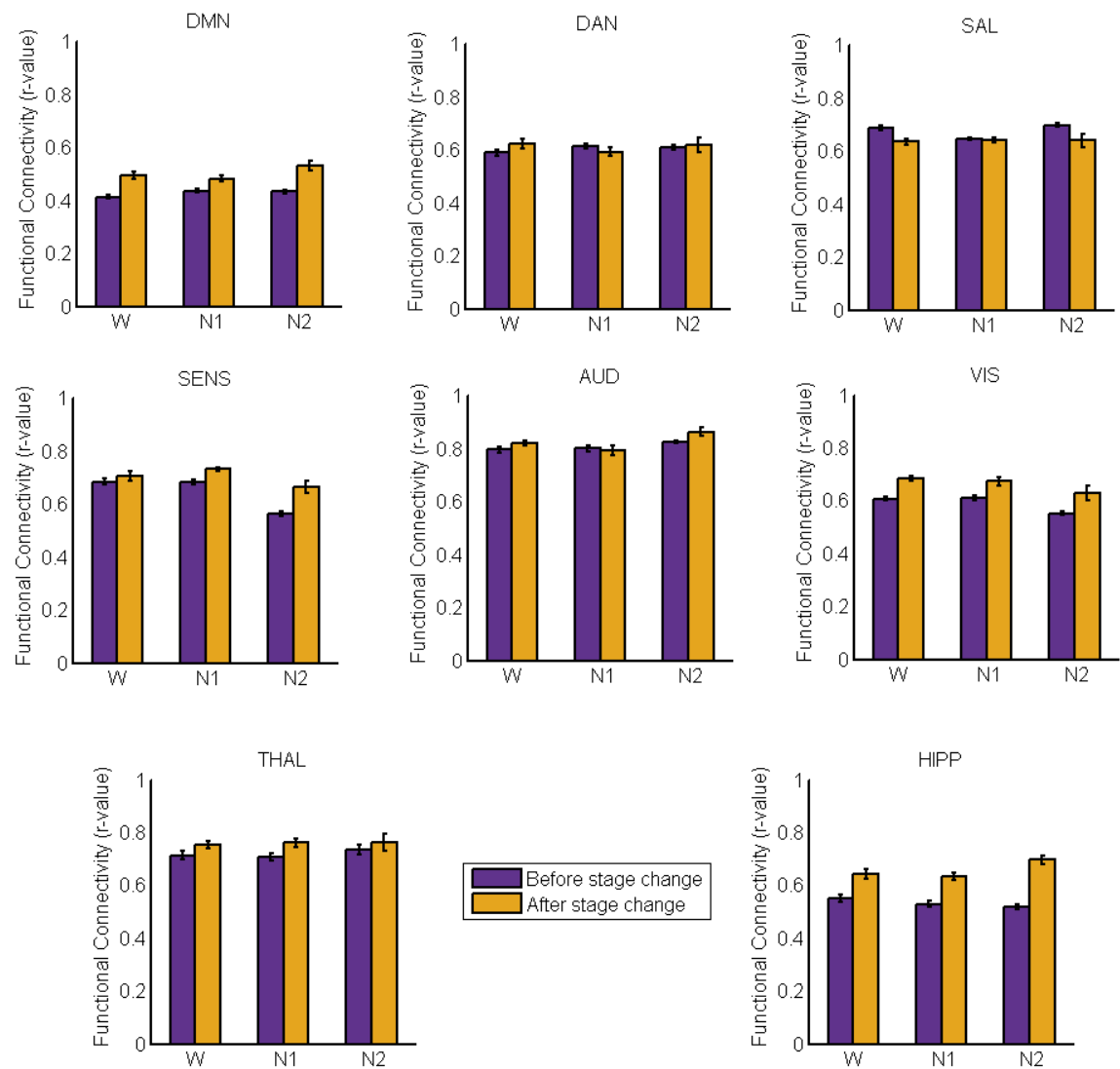


Figure 7.4.2. Group mean FC strength for each ICN within each sleep stage, before and after a change in sleep stage. Error bars represent \pm SE.

Table 7.4.2. Statistical comparison within each network for FC strength (Z-SCORED) changes by ROI, Stage (wake, N1 & N2) and WITHIN STAGE (Before & After), including all subsequent interactions for each ICN.

Factor	Network							
	DMN**	DAN	SAL*	SENS*	VIS*	AUD	THAL	HIP**
WITHIN STAGE	F(1,889) =42.977, p<0.001	F(1,344) =0.443, p=0.506	F(1,180) =5.093, p=0.025	F(1,175) =2.199, p=0.140	F(1,328) =0.708, p=0.401	F(1,52) =0.036, p=0.851	F(1,13) =0.048, p=0.830	F(1,313) =26.388, p<0.001
ROI*WITHIN STAGE	F(14,109) =4.084, P<0.001	F(5,96) =2.006, p=0.085	F(2,103) =1.146, p=0.322	F(2,118) =4.258, p=0.016	F(5,97) =3.697, p=0.004	-	-	F(5,94) =7.708, p<0.001
Stage*WITHIN STAGE	F(2,869) =1.424, p=0.241	F(2,331) =2.643, p=0.073	F(2,173) =1.062, p=0.348	F(2,170) =0.147, p=0.864	F(2,312) =0.320, p=0.797	F(2,47) =0.147, p=0.864	F(2,41) =0.372, p=0.692	F(2,295) =0.562, p=0.571
ROI*Stage* WITHIN STAGE	F(28,109) =0.260, p=1.000	F(10,96) =0.224, p=0.993	F(4,103) =0.212, p=0.931	F(4,118) 0.375, p=0.826	F(10,97) =0.698, p=0.817	-	-	F(10,94) =0.234, p=0.992

* Networks showing significant effects ($p<0.05$) ** $p<0.001$

Note: N3 was excluded from statistical tests

DMN

We found a significant main effect of the factor WITHIN STAGE upon FC before and after a sleep stage change. Specifically we found an overall increase in the strength of FC across all stages and regions within the DMN in epochs after a change in stage compared to epochs before ($p < 0.001$). In addition to the main effect there was a significant interaction with ROI suggesting FC differences within a sleep stage were regionally specific. Pairwise contrasts (Bonferroni adjusted, see Table 7.4.3) demonstrated that PCC and IPL increased strength to other regions within the DMN after a sleep stage change, except for bilateral FC of the IPL (rIPL-lIPL) which reduced in strength.

Table 7.4.3. DMN pairwise contrasts WITHIN STAGE FC strength (before stage change – after stage change) differences by ROI

ROIs	<i>Contrast Mean</i>	<i>Std. Error</i>	<i>t</i>	<i>p-value</i>
PCC-mPFC	-0.162	0.052	-3.134	$p=0.045$
PCC-IIPL	-0.123	0.043	-2.883	$p=0.075$
PCC-rIPL	-0.176	0.046	-3.844	$p<0.001$
PCC-lMTL	-0.070	0.035	-1.986	$p=0.052$
PCC-rMTL	-0.094	0.044	-2.136	$p=0.555$
mPFC-IIPL	-0.023	0.038	-0.607	$p=0.546$
mPFC-rIPL	-0.018	0.045	-0.402	$p=0.689$
mPFC-lMTL	-0.035	0.038	-0.911	$p=0.366$
mPFC-rMTL	-0.033	0.033	-0.985	$p=0.329$
IIPL-rIPL	0.078	0.031	2.473	$p=0.255$
IIPL-lMTL	-0.136	0.043	-3.203	$p=0.03$
IIPL-rMTL	-0.130	0.039	-3.322	$p=0.03$
rIPL-lMTL	-0.142	0.044	-3.195	$p=0.03$
rIPL-rMTL	-0.135	0.040	-3.399	$p=0.015$
lMTL-rMTL	0.079	0.049	1.618	$p=0.111$
Significant($p<0.05$)				

SAL

There was a significant effect of WITHIN STAGE FC, with an overall decrease in the strength of FC across all stages within the SAL in epochs recorded after a change in stage compared to epochs before a stage change ($p=0.025$).

SENS

There was a significant main effect of Sleep Stage, indicating a reduction in strength of FC with advancing sleep stage across all ROIs ($p<0.001$). There was also a significant interaction between ROI and WITHIN STAGE FC, suggesting the within stage FC differences were regionally specific. Pairwise contrasts revealed a significant difference in FC for the SMA-IMC ROI paired connection, which increased in strength after a change in sleep stage independently of stage ($t= -3.085$, $p=0.003$).

VIS

There was a significant effect of Sleep Stage, indicating a reduction in the strength of FC with advancing sleep stage, particularly in N2 compared to the other sleep stages, across all ROIs ($p=0.001$). There was also a significant interaction between ROI and WITHIN STAGE FC, suggesting that the within stage FC differences were regionally specific. Pairwise contrasts revealed a significant FC difference for the rPV-ILV ($t=2.555$, $p=0.013$) with increased FC strength after a sleep stage change, while the bilateral PV (IPV-rPV) decreased in FC strength after a stage change ($t=-2.287$, $p=0.026$).

HIP

We found a significant effect of WITHIN STAGE FC, with an overall increase in the strength of FC within the HIP, across all stages, in epochs recorded after a change in stage compared to before ($p < 0.001$). There was also a significant interaction between ROI and WITHIN STAGE, with paired connections (lPH-lHIP, rPH-lHIP, lPH-Rhip & rPH-rHIP) significantly increasing in FC strength after a sleep stage (all ROIs: $p < 0.001$). The bilateral parahippocampal gyrus (rPH-lPH) reduced FC after a change in sleep stage ($p = 0.007$).

7.4.3 Variability of ICN FC within an EEG classified stage

Figure 7.4.3 shows that ICNs differed in FC variability (FC-V) within an EEG classified sleep stage. Firstly, we note FC-V differences within stage for the DMN and SAL across all stages of sleep, with increased FC in both networks. While the FC of the majority of ICNs did not significantly differ in the FC-V within a stage, the VIS was the only ICN in which WITHIN STAGE FC-V was dependent on the stage of sleep. However differences in FC-V within stage were largely homogenous across regions within ICNs, the only exception being the DAN in which differences were regionally dependent. Finally, FC-V across ROIs was regionally dependent, in particular bilateral FC-V was significantly reduced compared with other connections within most ICNs. The statistical differences for each of the ICNs with regard to the FC-V are outlined in Table 7.4.4 and these observations are discussed in more detail below for each ICN. The GLMM main effects or subsequent interactions were not significant for the THAL or AUD, as such these networks will not be discussed further in this section.

CHAPTER 7: THE VARIABILITY IN THE FUNCTIONAL CONNECTIVITY WITHIN AN EEG CLASSIFIED SLEEP STAGE DURING NREM SLEEP

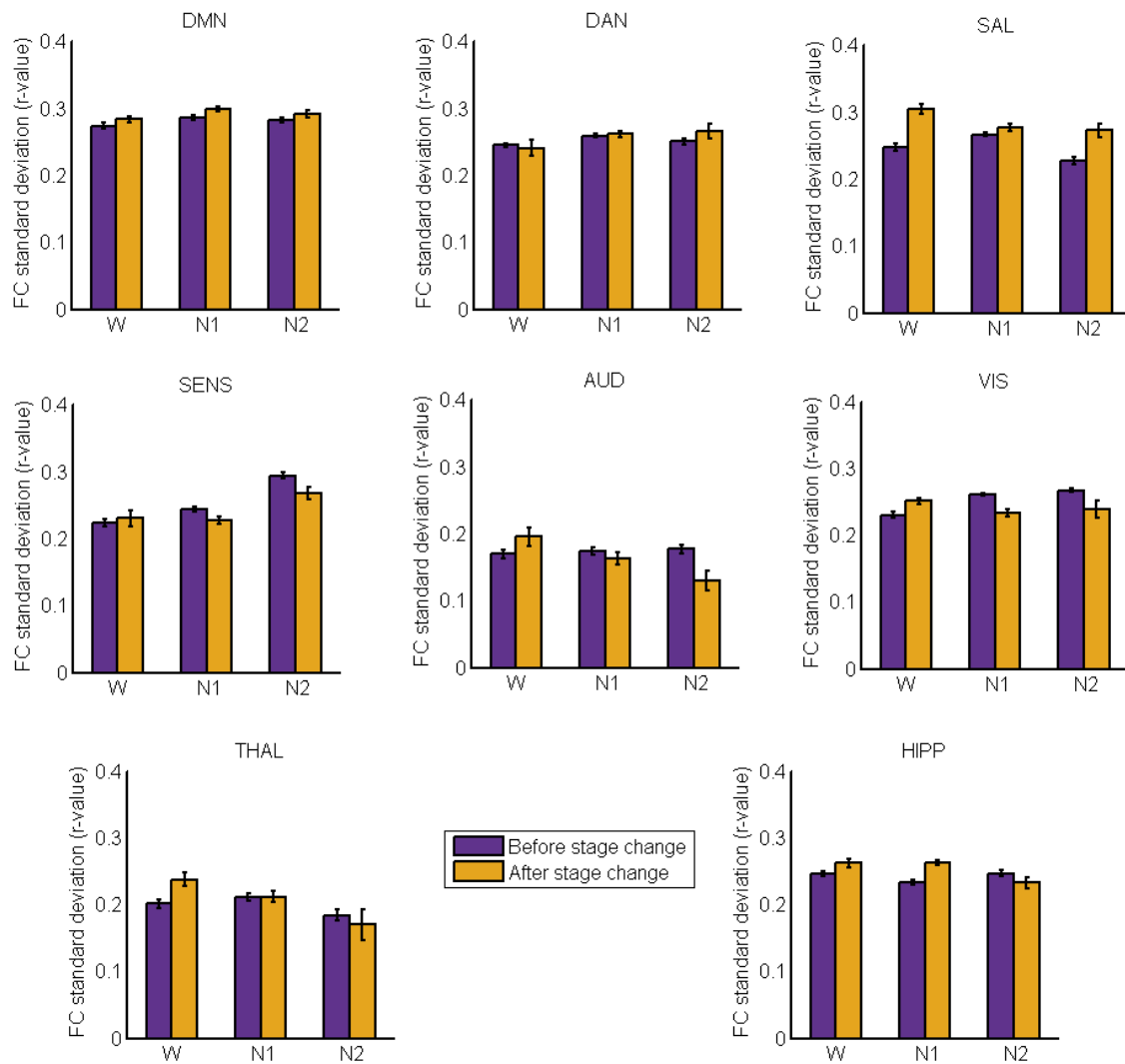


Figure 7.4.3. Group mean variability (standard deviation) in the strength FC for each ICN within each sleep stage, before and after a change in sleep stage. Error bars represent \pm SE.

Table 7.4.4. Statistical comparison within each network for FC-V changes by ROI, Stage (wake, N1 & N2) and WITHIN STAGE (pre- & post-), including all subsequent interactions for each ICN.

Factor	Network							
	DMN*	DAN*	SAL*	SENS	VIS*	AUD	THAL	HIP
WITHIN STAGE	F(1,866) =8.460, p=0.004	F(1,364) =0.595, p=0.441	F(1,178) =9.394, p=0.003	F(1,177) =0.189, p=0.664	F(1,373) =0.484, p=0.487	F(1,53) =0.036, p=0.851	F(1, 51) =0.478, p=0.492	F(1,366) =1.805, p=0.180
ROI*WITHIN STAGE	F(14,110)= 1.134, p=0.337	F(5, 115) =2.927, p=0.016	F(2,126) =1.498, p=0.228	F(2,122) =0.375, p=0.688	F(6,120) =1.633, p=0.144	-	-	F(5, 105) =1.858, p=0.108
STAGE*WITHIN STAGE	F(2,831) =0.043, p=0.957	F(2,355) =1.258, p=0.285	F(2,168) =1.540, p=0.217	F(2,171) =0.510, p=0.601	F(2,352) =6.047, p=0.003	F(2,47) =0.693, p=0.505	F(2,48) =0.820, p=0.447	F(2,354) =1.724, p=0.180
ROI*STAGE*	F(28,110)= 0.603, p=0.938	F(10,115) =0.449, p=0.919	F(4,126) =0.261, p=0.903	F(4,122) =0.550, p=0.699	F(12,120)= 1.118, p=0.352	-	-	F(10,105)= 0.218, p=0.994

* Significant Networks ($p < 0.05$)

Note: N3 was excluded from statistical tests

DMN

There was a significant main effect of ROI upon the FC-V within the DMN ($F(14,107)=5.023$, $p<0.001$), with pairwise comparisons showing that FC-V of the rIPL-lIPL paired connection to be significantly reduced compared to all other connections within the DMN, suggesting more consistent FC between rIPL-lIPL. There was also a significant main effect of STAGE on FC-V ($F(2,930)=3.928$, $p=0.020$), with increased overall FC-V in N1 compared with wake ($t=-2.761$, $p=0.018$). We also found a significant main effect of WITHIN STAGE ($F(1,866),p=0.004$), with an increase in FC-V after a change in stage.

DAN

There was a significant effect of ROI on the FC-V within the DAN ($F(5,116)=19.073$, $p<0.001$), with pairwise comparisons showing FC-V to differ regionally within the network. The bilateral paired connections (rIPS-lIPS & rMF-lMF) were significantly less variable in their FC compared with the other DAN ROIs. There was also a significant main effect of STAGE with an increase in variability with advancing sleep stage ($F(2,357)=3.249$, $p=0.040$). While the main effect of WITHIN STAGE failed to reach significance there was a significant interaction between ROI and WITHIN STAGE FC-V ($F(5,115)=2.927$, $p=0.016$). In particular pairwise comparisons revealed a significant increase in the variability of the bilateral MF (rMF-lMF) after a change in sleep stage, with other DAN ROIs remaining more stable in FC-V within a stage.

SAL

There was a significant effect of ROI on the FC-V within the SAL ($F(2,102)=8.560$, $p<0.001$), with the bilateral insula significantly less variable in FC compared to other SAL ROIs (ACC-Lins: $t=-3.956$, $p<0.001$ & ACC-rINS: $t=-3.373$, $p=0.002$). The main effect of WITHIN STAGE was also found to be significant ($F(1,178)=9.394$, $p=0.003$) with an overall increase in variability after a stage change compared to before.

SENS

We found a significant effect of ROI on the FC-V within the SENS ($F(2,107)=9.133$, $p<0.001$). The variability of FC between bilateral motor cortex (rMC-lMC) was significantly reduced compared with the other ROIs of the SENS (SMA-rMC: $t=-2.752$, $p=0.014$ & SMA-lMC: $t=-4.245$, $p<0.001$). There was also a significant main effect of STAGE ($F(2,172)=10.374$, $p<0.001$) with an increase in FC-V with advancing sleep stage with the greatest increase in N2 compared to wake ($t=-4.133$, $p<0.001$) and N1($t=-3.980$, $p<0.001$).

VIS

There was a significant effect of ROI on the FC-V within the VIS ($F(6,120)=150.080$, $p<0.001$), with bilateral ROIs (rPV-lPV & lLV-rLV) significantly reduced in their FC-V compared with other paired connections. This finding suggests that VIS bilateral FC was more consistent than other paired visual connections. In addition there was a significant STAGE by WITHIN STAGE interaction ($F(2,352)=6.047$, $p=0.003$), suggesting FC-V between the before and after within stage measures were dependent on the stage of sleep. Pairwise comparisons revealed a significant increase in FC-V with the WITHIN STAGE FC

variable in wake after a stage change ($t=2.341$, $p=0.020$) and a decrease in FC-V N1 after a change in stage ($t=-2.175$, $p=0.030$), however there was no significant difference in N2.

HIP

We found a significant effect of ROI on the FC-V within the HIP ($F(5,105)=13.353$, $p<0.001$), with the variability of the bilateral ($r_{HIP-lHIP}$ & r_{PH-lPH}) FC significantly reduced compared to other connections within the HIP.

7.5 Discussion

We have shown that functional connectivity differs in both strength and variability within the NREM sleep onset, suggesting that the spatiotemporal characteristics of large-scale intrinsic networks are not homogenous across an EEG classified sleep stage. The intrinsic processes of sleep measured with FC within intrinsically connected networks, appear to transcend the traditional EEG based classification of stages, revealing network and regionally specific FC changes between epochs of the same NREM stage that were recorded at different time points. This could have implications for our understanding of the mechanisms underpinning sleep. Interestingly, we found that FC differences within stages were not isolated to a particular stage, instead they were uniform across sleep, suggesting FC changes may reflect advancing sleep or time asleep. Therefore this study highlights that classical sleep staging is only partly reflected in changes to intrinsic processes as measured using fMRI FC which should be further explored across the sleep cycle in order to extract additional information.

The concept that there is spatiotemporal variability of brain activity within a classified sleep stage has previously been explored in the EEG literature using epochs of <30seconds (Hori, 1985; Ogilvie, 2001) and differences are also noted within a stage when comparing across sleep cycles (Borbély et al., 1981). The differences in FC strength and variability between epochs within the same EEG classified stage shown by the current study fit with EEG reports outlining greater variability within a thirty second epoch as suggested by Hori and colleagues (1985) and suggests intrinsic processes may also be changing during sleep. In particular, the DMN, SAL and HIP networks all showed within stage changes in FC strength and variability for all stages when comparing between epochs before and after a stage change. We have previously shown that the FC strength within these ICNs during wake and NREM sleep is predictive of a subjects' sleep history, measured both subjectively and objectively (Chapter 6). In addition to this, the only fMRI study to explicitly explore consistency of brain activity within a sleep stage investigated N1 and found differences in the BOLD activity patterns for the DMN and HIP between early and late sleep cycle epochs (Picchioni et al., 2008). Although it is not clear how their measures translate to connectivity, the change in FC of these same regions found in the current study is interesting. Therefore, when taken together, these findings suggest a possible mechanistic function of these ICNs in sleep-wake behaviour requiring further investigation.

We also show regionally specific differences in FC strength and variability within a defined stage for the DMN and HIP, in addition to sensory networks including the SENS and VIS, ICNs which have all previously shown FC changes with sleep stage (Chapter 5 & 6).

However we found the differences in FC before and after a change in stage within these ICNs to be predominantly in bilateral paired connections, which reduced their FC strength and

variability after a stage change while other, non-bilateral homologous ROIs tended to increase FC. For example within the DMN, FC increased across the majority of paired connection with the only reductions shown in bilateral connectivity (lMTL-rMTL & lIPL-rIPL), suggesting inter-hemispheric connectivity differences as sleep progresses. While changes to the laterality of activity during N1 sleep have previously been shown in the hippocampus (Picchioni et al., 2008), the reduced FC in most bilateral regions later in the sleep phase (after a change in stage), coupled with increased FC between other regions suggests a re-organisation of the brain during sleep, arguably leading to changes in the global integration or hierarchy of the brain (Spoormaker et al., 2012). Even though we did not explicitly address changes in the whole brain network or inter-network FC, as we instead focus on the FC changes within ICNs, we did notice alterations in the FC between regions belonging to different ICNs, again suggesting intrinsic re-organisation during sleep.

The reason for FC differences within an EEG classified sleep stage is not known. We suggest it could be due to intrinsic changes occurring across different temporal scales; either differences in transitory activity (vertex sharp waves, K-complexes, sleep spindles) driving intrinsic activity changes within a stage or longer rhythms of intrinsic change across the whole night. While these hypotheses are speculative and require further investigation, there is some evidence that waking FC changes across time following circadian rhythms (Blautzik et al., 2013; Hodkinson et al., 2014) and is also altered diurnally before and after sleep deprivation (Kaufmann et al., 2015). Indeed it is possible that sleep FC also varies dynamically in a similar fashion to wake. Behavioural changes over time in wakefulness and sleep have been shown with the two-process model (Borbély, 1982), thus the timing of sleep stages or cycle could also impact on intrinsic rhythms across the night, which is suggested by

the current finding that sleep stage FC within the temporality of the sleep cycle is not uniform.

Additionally sleep onset is understood to be a gradual transition (Ogilvie, 2001) and is not directly mirrored by the sharp change in EEG defined stages (Hori, 1985). Therefore the heterogeneity in FC that we found across the hypnogram supports the notion of a gradual change in the brain's intrinsic activity, particularly in the period of sleep onset represented in our data which is characterised by fragmented early cycle sleep with multiple sleep onsets. Additionally sleep onset is understood to be a gradual transition (Ogilvie, 2001) and is not directly mirrored by the sharp change in EEG defined stages (Hori, 1985). Therefore the heterogeneity in FC that we found across the hypnogram supports the notion of a gradual change in the brain's intrinsic activity, particularly in the period of sleep onset represented in our data which is characterised by fragmented early cycle sleep with multiple sleep onsets.

In recent years machine learning has been used to classify sleep stage from fMRI FC data alone (Tagliazucchi & Laufs, 2014). Our findings could have implications for sleep classifiers using connectivity parameters and might explain their inability to predict sleep stage with absolute accuracy, given the variability in intrinsic activity across epochs of the same stage as we demonstrate. It is also important to note that individual differences could be driving changes in intrinsic behaviour or 'trait-like' affects. FC studies support trait differences in the effect of sleep deprivation (Rupp et al., 2012) and recently these differences have been shown to predict FC during wake (Yeo et al., 2015). The large differences in FC shown between the groupings of a stage (before & after) could be an indication of a 'good sleeper' as all the

participants included in this study would arguably be defined as such for their ability to initiate sleep in the MR scanner.

To our knowledge this is the first study to investigate FC consistency during sleep and within sleep stage, however there are important methodological considerations to be addressed in future work. Indeed the method of grouping stage and averaging used in the current study could further ‘mix’ intrinsic activity, over or under estimating FC resulting in the loss of temporal information. Future studies could use an alternative approach by grouping stage differently, regressing the FC against time or using a data driven approach such as k-means clustering in order to investigate the within stage FC and the consistency of this across a whole night of sleep.

7.6 Conclusion

The FC strength between conventional EEG defined sleep stages has been previously shown to change by stage within ICNs when multiple epochs are averaged across a particular EEG defined sleep stage (Horovitz et al., 2009; Larson-Prior et al., 2009, 2011; Spoormaker et al., 2010, 2011; Chapters 4, 5 & 6). The current study expands on these findings suggesting that they may be a simplification of intrinsic processes occurring in sleep. We find that FC differences in strength and variability exist between multiple epochs of the same stage, suggesting dynamic FC could be a sensitive marker in sleep research beyond ‘gold-standard’ EEG stage classification, expanding our knowledge of sleep and the underlying intrinsic processes underlying it.

Chapter 8

THESIS SUMMARY AND CONCLUDING REMARKS

8.1 Overview

FC during the waking resting-state has been increasingly used in recent years to investigate the intrinsic activity of separate brain regions which form ICNs (Friston, 2011). This research has focused on the properties of FC, namely the dynamic temporal variability that is an intrinsic part of brain function (Hutchison et al., 2013) and the association between FC and behavioural variables such as the amount of prior sleep (Kaufmann et al., 2015). Additionally the FC changes during sleep compared with wake have also been investigated (Horovitz et al., 2009; Larson-Prior et al., 2011; Spoormaker et al., 2010). However, this requires combined EEG-fMRI to appropriately determine sleep using ‘gold-standard’ thirty second epochs of EEG, as well as dynamic FC analysis. In Chapter 4 we use combined EEG-fMRI with a dynamic, seed-based FC approach, validating its use in sleep. This method was then applied in Chapters 5, 6 & 7 to further explore FC as a sensitive measure of NREM sleep.

8.2 Significant findings and implications of the thesis

We show that FC is not homogenous across time or a thirty-second stage. Therefore classification of sleep based on thirty-second stages may be a simplification of intrinsic processes occurring in sleep and when the thesis findings are considered together we conclude that dynamic FC could be a sensitive measure in sleep research beyond EEG stage classification, expanding our knowledge of sleep and the underlying intrinsic processes.

First we show there are clear benefits to dynamic FC analysis, enabling researchers to gain greater temporal understanding of changing brain activity and the intrinsic network organisation of behavioural function, particularly during sleep (Chapter 4). Following this, we investigated the dynamic FC differences with sleep stage between normal and recovery sleep (Chapter 5). The FC was shown to differ between the types of sleep, however these FC differences were most pronounced in specific ICNs, namely the DMN, thalamus, auditory and sensorimotor networks, while the FC of other sensory and cognitive networks appeared resilient. Therefore treating these two types of sleep as equivalent in terms of underlying brain activity is an oversimplification and has implications for sleep researchers using sleep deprivation protocols. These findings also improve our understanding of the functional brain networks underpinning homeostatic sleep pressure and the interacting circadian processes.

In addition we show that FC during NREM sleep is influenced by the preceding sleep-wake behaviour of the individual, which to our knowledge has not been previously assessed in sleep, although prior sleep behaviour such as sleep deprivation has recently been shown to be associated with FC in wakefulness (Bosch et al., 2013; Dai et al., 2015; De Havas, Parimal, Soon, & Chee, 2012; Gujar, Yoo, Hu, & Walker, 2010; Kaufmann et al., 2015; Sämann et al.,

2010; Shao et al., 2013; Stoffers et al., 2015; Yeo, Tandi, & Chee, 2015; Zhu et al., 2015).

When taken together with the findings of the thesis there is support for an association between dynamic changes of regional FC and sleep behaviour, whether that is directly recorded in the FC changes between sleep stages or as a function of prior sleep. In particular the FC of the DMN and SAL during sleep are shown to be predicted by multiple objective actigraphy measures and subjective self-report questionnaires including the PSQI, ISI and FSS. The regional differences in the association of these sleep measures and FC could improve our understanding of the effects of prior sleep behaviour on our current sleep.

Furthermore, the changes we show in FC with sleep and as a result of sleep behaviour arguably have wider implications beyond the field of sleep research. Indeed, researchers using FC as a measure should also appropriately control for sleep during resting waking scans where the subject could potentially fall asleep (Tagliazucchi & Laufs, 2014), which we have shown alters the FC of certain regions compared to wake. Additionally researchers investigating the FC differences between behavioural conditions and clinical populations should also appropriately control for the modulating factor of sleep behaviour which we have shown to be closely associated with the intrinsic FC patterns. This is particularly important when assessing the FC of clinical populations, many of which have comorbid disrupted sleep patterns or sleep behaviour (Lopresti et al., 2013; Stein, Belik, Jacobi, & Sareen, 2008; Wulff et al., 2010).

8.3 Theories of sleep, mental health and FC

The link between sleep problems and psychiatric disorders is well established (Benca, 1992). In particular there is a strong relationship between disrupted sleep patterns and major depression (Lopresti et al., 2013; Tsuno, Besset, & Ritchie, 2005), leading to sleep difficulties being included as part of the diagnosis for depression (ICD-10 & DSM-IV). Additionally sleep deprivation also shows a paradoxical improvement in the reported symptoms of depression (Borbély & Wirz-Justice, 1982), suggesting an association or similarity in the systems governing sleep and mood, with process-S in the two-process model of sleep hypothesised to regulate both systems (Borbély & Wirz-Justice, 1982; Borbély, 1987). Research continues to investigate mood and sleep concurrently. As such the findings from the sleep research community may go some way towards improving our understanding of depression. Therefore the FC findings within the depression literature are briefly reviewed in this section and assessed alongside the results of the thesis.

For example, studies comparing the FC between clinically depressed subjects and healthy controls in wakefulness revealed increased FC in the anterior DMN, particularly within the subgenual anterior cingulate cortex of depressed individuals (Mulders, van Eijndhoven, Schene, Beckmann, & Tendolkar, 2015; Rodríguez-Cano et al., 2014). As well as increased FC in depressed subjects between regions of the DMN and amygdala within the Salience network (SAL) (Davey et al., 2015), there is also altered FC within the DMN itself with reduced FC or so-called ‘disassociation’ between the anterior and posterior DMN (Mulders et al., 2015), particularly with the medial prefrontal cortex itself (MPFC) (Murrough et al., 2016). These findings are similar to ours, namely the reduced FC as a function of sleep stage

between the anterior and posterior regions of the DMN suggests these regions may be implicated in both sleep and mood. However the novel finding of increased DMN FC with recovery sleep, sleep following sleep deprivation, in addition to increased FC of the PCC as a function of the amount of prior sleep (before total sleep time) is significant. Suggesting functional overlap in the areas showing increased FC during sleep and increased waking FC in depression. This could have implications for our understanding of the complex relationship between sleep and mood, requiring further investigation.

8.4 Future directions

The significance and implications of the main findings have been outlined. However there are a number of methodological considerations and additional research questions that arise out of the work that has been presented in this thesis.

We discuss some of the aims for future work, which are important for our understanding of the sleeping brain as measured using FC. The intra-network FC of the major functional ICNs has been the focus of this thesis, in order to enable the interpretation and comparison with waking FC studies which mainly concentrate on the within-network FC changes between conditions or variables. While we did analyse the FC between all ROIs by calculating a connectivity matrix, this was only explored qualitatively. The consistent finding that sleep induces a re-organisation of the within-network FC of certain ICNs depending on stage is likely to also extend to inter-network re-organisation. This is also supported by the changing FC spatial extent between sleep stages and sleep type, which is arguably direct evidence of changing regional organisation beyond a network (Chapter 5). As such future research could

further investigate the inter-network FC during sleep, perhaps initially focusing on those regions which show significant intra-network FC changes with sleep stages, such as ROIs of the DMN, SENS or THAL. Previous studies investigating the inter-network FC changes with sleep stage have focused on thalamocortical FC, showing pronounced differences between stages in the strength of FC to regions outside the thalamus across multiple cortical ICNs (Hale et al., 2015; Picchioni et al., 2014). This is an interesting future direction in the understanding of FC during sleep and the relationship with the underlying homeostatic mechanisms.

The methodology used throughout the thesis is a seed-based FC sliding window analysis, as discussed in more detail in section 2.2.4. However, this is not the only method used to calculate FC across the brain during rest (Cole et al., 2010). Indeed, several of the analysis steps in the pre-processing and FC calculation are methodological choices and the literature presents several alternatives. For example, brain parcellation or ROI definition in which *a priori* anatomical or functionally defined regional segmentation can be employed at varying degrees of detail (number of voxels included or shape) have all been shown to differentially affect FC calculations (Fornito, Zalesky, & Bullmore, 2010). However there is no gold-standard for regional parcellation of the brain and while future investigation using a more detailed parcellation may improve regional sensitivity (Hayasaka & Laurienti, 2010) it presents an ever greater challenge for functional interpretation.

Other techniques such as clustering algorithms could also be applied in future studies investigating the intrinsic changes in dynamic brain activity during sleep, particularly within epochs of sleep stage or across stage transitions where there are likely to be different FC

‘states’, as previously shown in resting wakefulness (Allen et al., 2014). Similarly machine learning techniques have also been applied to dynamic FC in wake to detect transitioning into sleep, by identifying the temporal trends associated with sleep (Tagliazucchi & Laufs, 2014). These approaches could be applied to investigate FC more dynamically, moving beyond a thirty second EEG staged classification of sleep and further exploring the sensitivity of FC as a measure of sleep.

However the limited temporal resolution of fMRI restricts the analysis of dynamic FC, although accelerated multiband/multislice EPI sequences could increase the temporal capacity of fMRI (Boyacıoğlu et al., 2014; Moeller et al., 2010) and has been shown to reliably detect FC of the ICNs (Liao et al., 2013). Thus future studies could benefit from faster fMRI acquisition during sleep in order to focus on the dynamic FC changes seen in sliding-window analysis (Chapter 4) which would presumably be more robust given the increases in data points.

The study of circadian oscillators is known as chronobiology and the field focuses on the internal rhythms which regulate the sleep-wake cycle. Resting-state FC patterns have been shown to correspond to daily rhythmicity particularly in the regions of the DMN (Hodkinson et al., 2014) and sensorimotor regions (Blautzik et al., 2013), which incidentally are the same regions we have shown significantly change their FC with sleep compared to wake. In addition, significant individual differences in circadian rhythm are referred to as chronotype and individual variability results in grouping into two chronotype extremes: early types or ‘larks’ who wake early; and late types or ‘owls’ who wake later (Roenneberg, Wirz-Justice, & Mellow, 2003). Chronotype is thought to be genetically determined and furthermore has been

shown to be dependent on both age and gender, changing across the life-cycle (Roenneberg et al., 2007). Chronotype is also associated with different activity patterns during a motor task measured using BOLD (Peres et al., 2011). However we do not consider the circadian rhythms of individuals throughout the thesis which future studies should consider in order to disentangle the FC changes associated with the onset of sleep and the overall circadian modulation of FC.

Besides assessing the individual differences in chronotype, the genetic vulnerability to sleep deprivation was also shown to affect the FC of regions of the reticular formation to cortical thalamic regions during a waking reaction time task at night, with those more resilient to sleep loss increasing the strength of FC and those more genetically vulnerable reducing FC (Maire et al., 2015). Future studies investigating sleep should also control for the vulnerability to sleep deprivation which has also been shown to predict the FC of ICNs, in particular increasing the anti-correlation of networks (Yeo et al., 2015). Although individual measures of prior sleep history were assessed in the thesis, combining FC with genetic or behavioural performance could provide an additional understanding of the individual differences and their link to sleep.

8.5 Limitations

The main limitation of the research presented in the thesis is the reliance on small sample sizes in the statistical analysis; this is due to the difficulty in recruitment for EEG-fMRI sleep studies. The methodological challenges are discussed more explicitly in section 2.2.3; *Sleep EEG-fMRI methodological challenges*. However the replication of similar findings across

samples in Chapter 6 is reassuring, suggesting that the results may not be entirely spurious, although caution is still warranted when interpreting the results. As such a larger sample is recommended in future sleep EEG-fMRI studies.

As previously discussed (see section 2.1.7) visual sleep staging using thirty seconds is limited by the subjectivity of the EEG classification. Thus the disagreement or uncertainty of certain epochs, particularly in the transition between stages could introduce a degree of ‘noise’ into the data. Again, a larger dataset may offset this by increasing the statistical power.

Controlling for multiple comparisons is necessary in the current thesis given the large number of statistical tests and comparisons. Although Bonferroni correction and False Discovery Rate (FDR) (Benjamini & Hochberg, 1995) are two techniques employed to reduce the likelihood of Type I errors (*false positive results*) they have their own limitations and can be overly conservative masking true ‘significance’. Therefore reducing the number of comparison at the design stage of the experiment and assessing the data with and without corrections for multiple comparisons may be useful for interpretation.

Finally it is clear that sleep inside the noisy and cramped environment of an MR scanner is more fragmented than PSG recorded outside the scanner, raising the question of how representative of normal, everyday sleep this experimental protocol truly is. As such it is likely that the FC recorded during sleep in the scanner could differ to that of ‘normal’ sleep, although it is unknown how this manifests as intrinsic changes to activity. It may be informative to determine the impact of sleep fragmentation verses ‘steady state’ sleep on the brain’s FC by investigating the stage transitions. Alternatively a habituation night could be introduced into the protocol to reduce disruption, in which the subject can learn to adjust to

the MR environment, therefore potentially improving their sleep in the subsequent night of scanning.

8.6 Summary Conclusion

The primary aim of this thesis was to investigate the sleeping brain and the underlying neuronal changes associated with the onset of sleep using neuroimaging. The sensitivity of FC as a measure of dynamic brain activity during sleep was investigated, in addition to the association of FC changes with sleep stage, types of sleep and sleep history. When the thesis findings are considered together we conclude that dynamic FC could be a sensitive measure in sleep research, expanding our knowledge of sleep and the underlying intrinsic processes.

Appendix A

Sleep Diary

Sleep Diary Sheet

Name

Date of Birth

Start Date

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Alcohol consumed (in units)							
What time did you go to bed?							
What time did you turn off the lights?							
What time did you wake up?							
What time did you get up?							
How many times did you wake during the night, if any?							
How would you rate your nights sleep? <i>Poor; Satisfactory; Good; Excellent</i>							
Did you have any naps during the day? If so, for how long?							

Appendix B

Sleep Questionnaires

Epworth Sleepiness Scale

Name: _____ Today's date: _____

Your age (Yrs): _____ Your sex (Male = M, Female = F): _____

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired?

This refers to your usual way of life in recent times.

Even if you haven't done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the **most appropriate number** for each situation:

- 0 = would **never** doze
- 1 = **slight chance** of dozing
- 2 = **moderate chance** of dozing
- 3 = **high chance** of dozing

It is important that you answer each question as best you can.

Situation	Chance of Dozing (0-3)
Sitting and reading _____	_____
Watching TV _____	_____
Sitting, inactive in a public place (e.g. a theatre or a meeting) _____	_____
As a passenger in a car for an hour without a break _____	_____
Lying down to rest in the afternoon when circumstances permit _____	_____
Sitting and talking to someone _____	_____
Sitting quietly after a lunch without alcohol _____	_____
In a car, while stopped for a few minutes in the traffic _____	_____

Sleep Quality Assessment (PSQI)

What is PSQI, and what is it measuring?

The Pittsburgh Sleep Quality Index (PSQI) is an effective instrument used to measure the quality and patterns of sleep in adults. It differentiates "poor" from "good" sleep quality by measuring seven areas (components): subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over the last month.

INSTRUCTIONS:

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

During the past month,

1. When have you usually gone to bed?
2. How long (in minutes) has it taken you to fall asleep each night?
3. What time have you usually gotten up in the morning?
4. A. How many hours of actual sleep did you get at night?
B. How many hours were you in bed?

5. During the past month, how often have you had trouble sleeping because you	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
A. Cannot get to sleep within 30 minutes				
B. Wake up in the middle of the night or early morning				
C. Have to get up to use the bathroom				
D. Cannot breathe comfortably				
E. Cough or snore loudly				
F. Feel too cold				
G. Feel too hot				
H. Have bad dreams				
I. Have pain				
J. Other reason (s), please describe, including how often you have had trouble sleeping because of this reason (s):				
6. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
9. During the past month, how would you rate your sleep quality overall?	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)

Scoring

Component 1	#9 Score	C1
Component 2	#2 Score (<15min (0), 16-30min (1), 31-60 min (2), >60min (3)) + #5a Score (if sum is equal 0=0; 1-2=1; 3-4=2; 5-6=3)	C2
Component 3	#4 Score (>7(0), 6-7 (1), 5-6 (2), <5 (3))	C3
Component 4	(total # of hours asleep) / (total # of hours in bed) x 100 >85%=0, 75%-84%=1, 65%-74%=2, <65%=3	C4
Component 5	# sum of scores 5b to 5j (0=0; 1-9=1; 10-18=2; 19-27=3)	C5
Component 6	#6 Score	C6
Component 7	#7 Score + #8 score (0=0; 1-2=1; 3-4=2; 5-6=3)	C7

Add the seven component scores together _____ Global PSQI _____

A total score of "5" or greater is indicative of poor sleep quality.

If you scored "5" or more it is suggested that you discuss your sleep habits with a healthcare provider

Fatigue Severity Scale (FSS) of Sleep Disorders

The Fatigue Severity Scale (FSS) is a method of evaluating the impact of fatigue on you. The FSS is a short questionnaire that requires you to rate your level of fatigue.

The FSS questionnaire contains nine statements that rate the severity of your fatigue symptoms. Read each statement and circle a number from 1 to 7, based on how accurately it reflects your condition during the past week and the extent to which you agree or disagree that the statement applies to you.

- A low value (e.g., 1) indicates strong disagreement with the statement, whereas a high value (e.g., 7) indicates strong agreement.
- It is important that you circle a number (1 to 7) for every question.

FSS Questionnaire							
During the past week, I have found that:	Disagree <-----> Agree						
My motivation is lower when I am fatigued.	1	2	3	4	5	6	7
Exercise brings on my fatigue.	1	2	3	4	5	6	7
I am easily fatigued.	1	2	3	4	5	6	7
Fatigue interferes with my physical functioning.	1	2	3	4	5	6	7
Fatigue causes frequent problems for me.	1	2	3	4	5	6	7
My fatigue prevents sustained physical functioning.	1	2	3	4	5	6	7
Fatigue interferes with carrying out certain duties and responsibilities.	1	2	3	4	5	6	7
Fatigue is among my three most disabling symptoms.	1	2	3	4	5	6	7
Fatigue interferes with my work, family, or social life.	1	2	3	4	5	6	7
	Total Score:						

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Scoring your results

Now that you have completed the questionnaire, it is time to score your results and evaluate your level of fatigue. It's simple: Add all the numbers you circled to get your total score.

The Fatigue Severity Scale Key

A total score of less than 36 suggests that you may not be suffering from fatigue.

A total score of 36 or more suggests that you may need further evaluation by a physician.

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