"*Citius, Altius, Fortius*"

The Impact of Circadian Phenotype and Sleep on The Brain's Intrinsic Functional Architecture, Well-Being & Performance

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Abstract

A major challenge facing our rapidly developing 'round the clock' society is in understanding how disruptions to sleep and endogenously driven biological clocks influence many aspects of our lives. This thesis combines the fields of chronobiology, sleep and neuroimaging to investigate the impact of Circadian Phenotype and time of day on the brain's intrinsic architecture (using functional MRI), well-being and performance. Brain function changes during wakefulness and sleep, as well as in a range of neurological and psychiatric disorders.

The results show, for the first time, clear differences in intrinsic functional architecture between Early and Late Circadian Phenotypes (ECP/LCP), as well as variations depending on the time of day. In general, ECPs have higher functional connectivity than LCPs to the majority of regions identified, and these differences can predict better outcomes in cognitive and physical performance measures. Performance also shows significant diurnal variations within Circadian Phenotypes. A wider investigation in the LCP group showed that a phase advance in a real world setting using non-pharmacological interventions has a positive impact on mental well-being and performance.

In summary, this thesis supports the need to consider both Circadian Phenotype and time of day in neuroimaging and performance research. It also highlights that chronic disruptions often associated with LCPs could have intrinsic neural origins. Furthermore, it provides a novel intervention strategy for LCPs to enhance well-being and performance during a societal constrained day. These findings could have significant implications in clinical, research and real world settings.

Dedications:

I would like to dedicate this thesis to all my family members who are no longer with us. Grandma, Grandad, George, Nic Nocs and ma meilleure amie Mémee. Without each of you I would not be who I am today.

As we come to the end of a long chapter in my journey, I can look back and smile, because I know that this is just the beginning.

"Citius, Altius, Fortius"
Faster, Higher, Stronger

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List of Abbreviations

ACC	Anterior cingulate cortex
ANOVA	Analysis of variance
AWL	Actiwatch light
BOLD	Blood oxygenation level dependent
CAR	Cortisol awakening response
CBF	Cerebral blood flow
CBT	Core body temperature
CMAT	Chrono Memory and Attention Test
CR	Constant routine
CRSDs	Circadian Rhythm Sleep Disorders
CSF	Cerebral spinal fluid
DAN	Dorsal attention network
DASS	Depression Anxiety and Stress Scale
DLMO	Dim light melatonin onset
DMN	Default mode network
DQ	Diet Questionnaire
DTI	Diffusion tensor imaging
DVARS	Temporal deviation of time courses and movement variance over voxels
ECG	Electrocardiography
ECP	Early Circadian Phenotype
EEG	Electroencephalography
EMG	Electromyography
EOG	Electrooculography
EPI	Echo-planner imaging
ESS	Epworth Sleepiness Scale
F	ANOVA F test statistic
FC	Functional connectivity
FD	Forced desynchrony
FrD	Framewise displacement
fMRI	Functional magnetic resonance imaging
FQ	Freidman's statistic
GEE	Generalised estimating equation
GM	Grey matter
GSR	Global signal regression
HRT	Heart rate variability
ICN	Intrinsically connected network
IPGRCs	Intrinsically photosensitive retinal ganglion cells
LCP	Late Circadian Phenotype

LM1	Left primary motor cortex
MCTQ	Munich Chronotyping questionnaire
MEQ	Morningness-Eveningness questionnaire
MN	Motor Network
MNI	Montreal neurological institute
MPFC	Medial pre-frontal cortex
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MVC	Maximum voluntary contraction
NREM	Non-rapid eye movement sleep
PCC	Posterior cingulate cortex
PER	Period
PET	Positron emission tomography
PFC	Prefrontal cortex
POMS	Profile of Mood States
PSG	Polysomography
PSQI	Pittsburgh Sleep Quality Index
PVT	Psychomotor Vigilance Task
RAI	Right anterior insula
REM	Rapid eye movement sleep
RETROICOR	Retrospective correction of physiological motion effects in fMRI
RIA	Radioimmunoassay
RM1	Right primary motor cortex
ROI	Region of interest
RS-FC	Resting-state functional connectivity
RVT	Respiratory volume per time
SCN	Suprachiasmatic nucleus
SD	Standard deviation
SEM	Standard error of the mean
SMA	Supplementary motor area
SN	Saliency network
SPM	Statistical parametric mapping
T	T test statistic
TE	Echo time
TOD	Time of day
TR	Repetition time
TTFL	Transcriptional translational feedback loop
U	Mann Whitney U Test Statistic
UF2C	User friendly functional connectivity toolbox
VNTR	Variable number tandem repeat
W	Wald test statistic
WM	White matter

Publications

Work Not Presented in this Thesis

Facer-Childs E. et al., (2015) The impact of circadian phenotype and time since awakening on diurnal performance in athletes. Curr Biol 25(4):518–22. doi:10.1016/j.cub.2014.12.036

Facer-Childs E. et al., (2015) Circadian phenotype composition is a major predictor of diurnal physical performance in teams. Front. Neurol. 6:208. doi: 10.3389/fneur.2015.00208

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CHAPTER 1

Introduction

Timing is everything. From being able to anticipate the best time to conserve energy to identifying time of peak performance in order to win an Olympic gold medal; from recognising when attention may be sub-optimal to finding the most effective time to take crucial drugs; the temporal organisation of human nature impacts on all aspects of our lives. However, our internal timing systems are rapidly being disrupted due to increasing economic and social demands of modern day society (Rajaratnam and Arendt, 2001). The majority of physiological and behavioural processes are heavily influenced by endogenous 'biological clocks' but one manifestation of rhythmicity, the sleep/wake cycle, has become the focus of much scientific research. This is not surprising since one third of our lives is spent in a state of 'unconsciousness' (Foster and Wulff, 2005), and sleep is essential for the waking brain to function effectively (Hobson and Pace-Schott, 2002).

Disruptions to both biological rhythms and sleep impair daily functioning by impacting on our biology, such as the immune function (Lange et al., 2010), our psychology including mental health (Wulff et al., 2010), and our capability such as cognitive performance (Dijk et al., 1992). Ignoring the importance of our rhythms puts a strain on us as individuals and on society by causing prominent health issues such as shift work disorder (Rajaratnam and Arendt, 2001), as well as accumulating substantial economic costs due to decreased productivity in the workplace (Van Dongen and Belenky, 2009), and increased risk of accidents (Horne and Reyner, 1999). On top of this, individual differences in physiology and behaviour create yet another angle of complexity when investigating the adverse outcomes of these disruptions.

The purpose of this thesis is primarily to explore how individual differences in sleep and biological clocks can impact behaviour and performance. There is also a need to integrate functional imaging techniques into these fields to increase our understanding of the neuronal basis behind disruptions and how they relate to performance. Therefore, the secondary purpose of this thesis is to use functional magnetic resonance imaging (fMRI) to investigate central mechanisms of cognitive and physical performance measures. Furthermore, our rapidly evolving 'round the clock' society is both increasing disruptions to our rhythms but at the same time, providing opportunities for flexibility in the management of these rhythms. The final purpose of this thesis is to consider how individuals could benefit from changes in their lifestyles.

1.1 Chronobiology & Sleep

All organisms exhibit endogenously driven biological rhythms which allow the anticipation of environmental changes to modify behaviour accordingly. Although biological rhythms are synchronised to environmental factors, the endogenous nature of these oscillations mean they will continue in the absence of exogenous cues (Vitaterna et al., 2001). Temporal variations in biological rhythms result in categorisations depending on the length of oscillations (Table 1.1). For example, birds migrate on an annual basis (circannual), the human menstrual cycle occurs on a monthly basis (circa-lunar) and the human sleep/wake pattern repeats on a daily basis (circadian). This thesis will focus on near 24 hour (h) circadian rhythms in mammals, and more specifically, humans.

Table 1.1 Categorisation of temporal variations in biological rhythms.

Type of Rhythm	Period	Example
Ultradian	Shorter than 24 hours	Sleep
Infradian	Longer than 24 hours	Seasonal Affective Disorder
Circadian	Approx. 24 hours	Core body temperature
Circannual	Approx. 1 year	Seasonal migration
Circatidal	12.4 hours	Vertical migration
Circalunar	29.5 days	Menstrual cycle

1.1.1 Circadian Rhythms

The word circadian originates from the Latin '*circa*' which means around and '*diem*' which means day. Therefore, circadian cycles are defined as any biological rhythms that oscillate to a near 24 h period. In addition to the readily observed sleep/wake cycle, numerous physiological and behavioural processes are circadian regulated including: core body

temperature (CBT) (Krauchi, 2002, Refinetti and Menaker, 1992), gene expression (Roenneberg and Merrow, 2003), hormone levels (Lewy et al., 1999), and cognitive performance (see Blatter and Cajochen (2007) for a review). In mammals, circadian rhythms are orchestrated by a specific area of the brain called the suprachiasmatic nuclei (SCN), which is a subset of around 20,000 cells in the anterior hypothalamus sitting on top of the optic chiasm (Gritton et al., 2013). There is individual variability in circadian period, with the average sleep/wake pattern showing a 24.2 h cycle (Klerman et al., 1998). Therefore, these rhythms are synchronised to an exact 24 h period by zeitgebers (time-givers) through a process called entrainment. The SCN, which is entrained by the light/dark cycle, is referred to as the 'master clock' and is crucial in maintaining daily rest and activity rhythms (LeGates et al., 2014). Photoreceptors in the eye known as intrinsically photosensitive retinal ganglion cells (ipRGCs), register cyclic variations in the light/dark cycle and in turn transmit signals down the retinohypothalamic tract (RHT) to the SCN (Takahashi et al., 1984). These photoreceptors are not part of the visual system, supported by the fact that visually blind individuals are still able to entrain to the light/dark cycle if the ipRGCs are intact, whereas those with no photoreceptors become arrhythmic (Vandewalle et al., 2013). Entrainment to the light/dark cycle creates a diurnal pattern of rest and activity, with activity usually occurring during light hours and rest periods occurring during dark hours in humans. Diurnal rhythms refer to oscillations during the period of wakefulness and, although many are endogenously driven, they are not to be confused with circadian rhythms that are measured over the full 24 h period (LeGates et al., 2014).

Although the master circadian pacemaker is located in the brain, individual organs and cells are able to maintain their own circadian oscillations independently of the SCN

(Yamazaki et al., 2000). For example, the clock in the liver has been shown to be entrained by food intake (LeSauter et al., 2009, Stokkan et al., 2001, Stephan, 2002). Although these rhythms can persist in isolated tissues, the SCN has been shown to play the major role in synchronising and coordinating the 24 h temporal organisation of mammals (Vitaterna et al., 2001). Therefore, the SCN works collectively with peripheral clocks throughout the body to regulate and synchronise physiology and behaviour. At the molecular level these oscillations are driven by a number of clock genes that make up a system called the transcription translational feedback loop (TTFL) (Weber, 2009). The details of the TTFL are beyond the scope of this thesis but many complex cellular interactions allow organisms to regulate their activity (summarised in Figure 1.1).

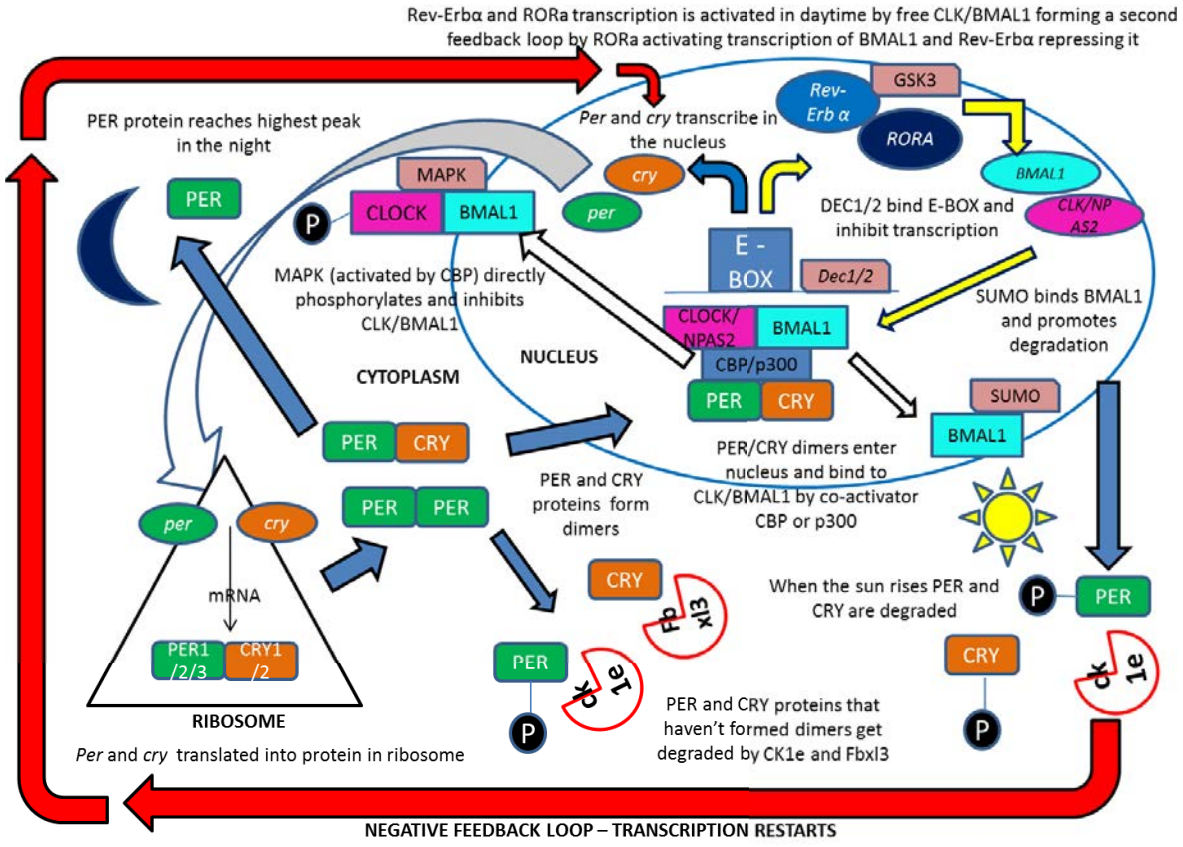

Figure 1.1. Schematic diagram to summarise cellular rhythm generation in mammals highlighting the key processes involved in the TTFL.

1.1.2 Sleep

During the rest period of the circadian cycle, mammals generally sleep. The complex process of sleep was once regarded as a simple "passive state of unconsciousness" in which the body shuts down. However, with the observation of highly controlled fluctuations in neuronal activity during sleep using electroencephalography (EEG), this view has long been replaced (Niedermeyer and da Silva, 2005). EEG measures electrical activity of the brain through surface electrodes, and shows distinct differences in wakefulness and sleep states depending on the frequency of waveforms produced (measured in Hertz, Hz). These signal changes have allowed the classification of the structural organisation of sleep or 'sleep architecture' (Rechtschaffen, 1968). Sleep architecture involves different sleep stages which can be categorised into rapid eye movement (REM), and non-rapid eye movement (NREM) sleep (Dement and Kleitman, 1957). NREM sleep is further classified into stages one, two and three (previously known as stages three and four separately). The details of how to measure sleep will be covered in the 'methods used in sleep and circadian research' section of this Chapter.

These landmark discoveries, originally explored by Nathaniel Kleitman and William Dement in the 1950s, revolutionised sleep research. Since then, sleep has been shown to be integral to consolidation of memory and learning (Diekelmann and Born, 2010), and disruptions of sleep have drastic physiological and behavioural consequences (Banks and Dinges, 2007). Although we now acknowledge the importance of sleep for everyday functioning, health and well-being, the reasons behind why we sleep still remain elusive (Foster and Wulff, 2005). Due to the circadian systems regulating the temporal organisation of the sleep/wake cycle the study of sleep could fall under the field of chronobiology. Yet, the field of sleep research was initiated independently of

chronobiology (Dement, 2000). Some of what is known about this natural, cyclic state that causes temporary lack of consciousness can be explained by two mechanisms (Vitaterna et al., 2001). Timings of rest/activity are largely regulated by the circadian alerting system, a mechanism that enables the anticipation of environmental cues, as discussed previously. However, sleep and wakefulness are also under the control of homeostatic mechanisms that drive the need to sleep through a build-up of sleep promoting neurotransmitters such as adenosine. The complex interaction of wakefulness and sleep are controlled by opposing neural systems. In brief, sleep promoting neurons within the brain are found in the ventrolateral preoptic area (VLPO) of the hypothalamus whilst wake promoting neurons originate in the brainstem. These wake promoting regions activate cerebral cortex through either the reticular nuclei of the thalamus via thalamic relay neurons or the upper brain stem via monoaminergic neurons. Collectively, this pathway has been called the reticular activating system (Moruzzi and Magoun, 1949). The opposing sleep-promoting neurons in the VLPO have inhibitory effects on this circuitry resulting in a switch like transition between wake and sleep states (Saper et al., 2005). This phenomenon is referred to as the flip-flop circuit model, for which orexin neurons in the lateral hypothalamus have been shown to play a key stabilising role (Lin et al., 1999). The importance of these systems has been shown by lesions causing profound impairments to sleep and wake states (Saper et al., 2005). This complex process of sleep has gained much interest in research as well as the general public since the negative consequences of disrupted sleep have been revealed (Banks and Dinges, 2007).

1.1.3 Sleep and Circadian Rhythms

As both physiology and behaviour are influenced by changes in circadian regulation as well as sleep-dependent factors, there is a need to study the interaction of these two fields to ultimately understand the impact on human health and performance. A number of models exist that attempt to link homeostatic and circadian control of sleep to understand behaviour, although only one will be discussed in detail as it covers the most relevant issues in this thesis.

A pivotal model that has dominated sleep and circadian research is the two process model of sleep regulation, originally proposed by Borbély (1982) (Figure 1.2). This model suggests that the human cycle of activity and sleep is regulated by a homeostatic drive for sleep, which builds up during time spent awake (Process S), and a circadian alerting process which endogenously follows a near 24 h oscillation (Process C). As sleep pressure (Process S) gradually increases to reach a peak at the start of the biological night, Process C reaches a minimum and sleep is initiated. In turn, this dissipates Process S and allows the circadian drive to rise during sleep and reach acrophase at the end of the biological night, thus aiding waking. The original model has since been re-appraised whilst highlighting new evidence that is not incorporated such as brain differentiation, the influence of sleep pressure on molecular circadian clockwork and the continuous interaction of Process S and C (Borbely et al., 2016). Although modifications of this model are constantly emerging, the interaction of these two systems is undoubtedly vital to maintenance of good quality sleep and waking performance. Therefore, this model provides a clear framework within which to consider the interaction between circadian rhythmicity and sleep.

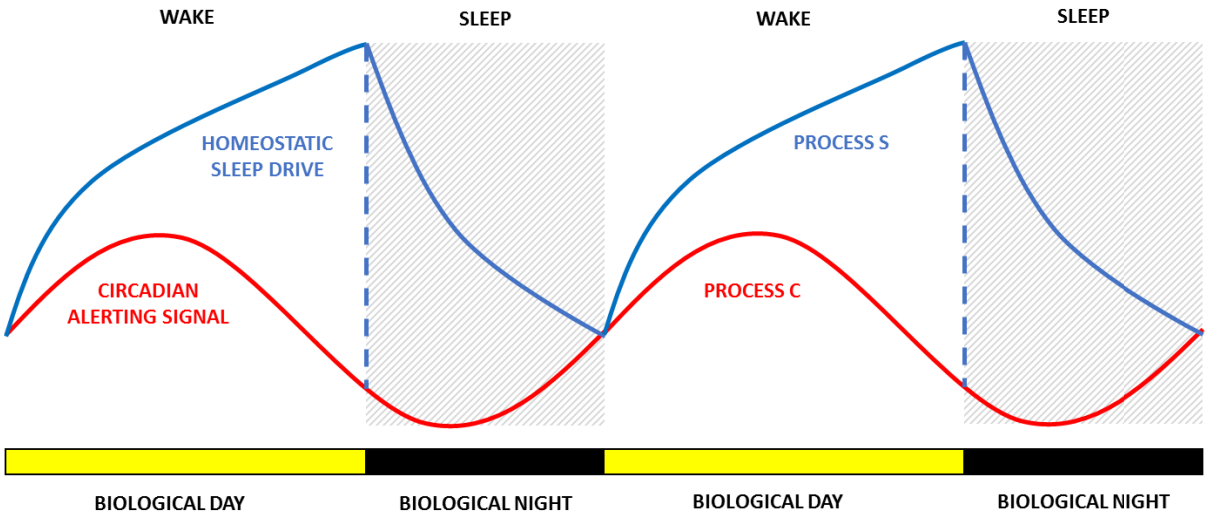

Figure 1.2. Two process model of sleep regulation.

1.2 Individual Variability in Sleep and Circadian Rhythms

'Optimum' versus 'non-optimum' times of day are hugely impacted by lifestyles and environmental influences e.g. work, school, family and social commitments, as well as individual differences in physiology and preference. There is a wide spectrum between those individuals who prefer the morning and report better alertness and performance during the early hours and those who are shifted to more of an evening optimum (Roenneberg et al., 2003). The groups at either end of this spectrum can be referred to as Early Circadian Phenotypes (ECPs), Late Circadian Phenotypes (LCPs) and those in between: Intermediate Circadian Phenotypes (ICPs). Circadian Phenotypes are determined by a combination of environmental (Roenneberg and Merrow, 2007), genetic (Allebrandt and Roenneberg, 2008, Lane et al., 2016), and physiological factors (Brown et al., 2008b). At the behavioural level i.e. assessing sleep patterns, subjective self-report measures can define one's 'Chronotype' using the Munich Chronotyping Questionnaire (MCTQ) (Roenneberg et al., 2003), or diurnal preference using the Morningness-Eveningness Questionnaire (MEQ) (Horne and Ostberg, 1976). However, not only do ECPs and LCPs

have very different sleep patterns but it has been shown that they are also starkly different in their physiology and behaviour. Circadian phase markers melatonin (Burgess and Fogg, 2008, Voultsios et al., 1997) and cortisol (Bailey and Heitkemper, 2001, Kudielka et al., 2006) peak earlier in ECPs which is mirrored by the circadian rhythm of CBT (Baehr et al., 2000) and measures of subjective sleepiness (Lack et al., 2009). There have also been genetic links suggesting variation in molecular clockwork and phase of clock gene expression between ECPs and LCPs (Archer et al., 2008, Nováková et al., 2013). On top of this, diurnal variations in physical and cognitive performance have been uncovered with ECPs performing better earlier in the day and LCPs in the evening (Facer-Childs and Brandstaetter, 2015a, Schmidt et al., 2012).

Given that the majority of physiological processes are circadian regulated it is unsurprising that adverse effects arise when endogenous biological rhythms become disrupted or out of synchrony with environmental cues (Zhu and Zee, 2012). These negative outcomes span from sleep disorders to health problems such as cardiovascular disease, cancer and impairments to cognitive functioning (Klerman, 2005). For example, irregular shift work contributes to desynchronisation of biological clocks and is linked to higher sensitivity to health issues such as cancer (Costa et al., 2010, Erren et al., 2010), fatigue (Åkerstedt and Wright, 2009, Yumang-Ross and Burns, 2014), and cognitive impairments (Devore et al., 2013, Marquie et al., 2015). Additionally, individual differences in sleep and circadian regulation contribute to considerable differences in human behaviour, with major influences shown in performance variations. Therefore, the following sections will discuss cognitive and physical performance respectively and the link to sleep and circadian research.

1.3 Cognitive Performance

Cognitive processes are essential for basic functioning, with disruptions to sleep and the circadian system resulting in impairments to cognitive functioning and performance (Rajaratnam and Arendt, 2001). When investigating cognitive performance, it is imperative to recognise that performance can be influenced by the individual, the environmental conditions e.g. light and temperature, as well as the simplicity, complexity and duration of the task in hand (Blatter and Cajochen, 2007). Research into sleep and circadian effects on cognitive performance has focussed on three main areas; attention/reaction times, memory and executive function (Figure 1.3). Simple tasks on attention and reaction time include the sign cancellation test to measure vigilance (Bougard et al., 2009), cognitive inhibition (Garcia et al., 2012), attention network test (Clarisse et al., 2010) and the sustained attention task (Escribano and Francisco Diaz-Morales, 2014). More complex tasks have been developed to examine executive function e.g. the continuous performance test, the digital span test, controlled word association test and the Wisconsin card sorting test (Bennett et al., 2008). Measurements of memory have steered towards tasks such as the artificial grammar learning task and the N-back working memory task (Delpouve et al., 2014). The psychomotor vigilance task (PVT) is the most widely used cognitive test within circadian and sleep research, most likely due to the simplicity of the task and sensitivity to sleep loss and circadian misalignment (Dinges and Powell, 1985). The individual has to look at a screen for a certain period of time and press a button when a light appears, thereby allowing the measurement of speed, reaction time and sustained attention by noting lapses of concentration. The PVT is a standard clinical tool to measure daytime sleepiness, and has been shown to exhibit circadian rhythmicity (Kline et al., 2010). Since the PVT is a simple measure, more complex tasks have also been explored e.g. the Stroop

colour word test. This is a task in which participants are presented with names of colours and must identify the colour displayed whilst ignoring the meaning. Both congruent i.e. the word 'green' presented in green, and incongruent i.e. the word 'green' presented in red terms can be used (Jensen and Rohwer, 1966). This task measures attention and cognitive flexibility through interference due to conflict processing and has been shown to fluctuate in a circadian manner (Ramirez et al., 2012).

There is a large base of research linking sleep and cognition, with unanimous findings that disruptions to sleep yield both physiological and behavioural deficits (Banks and Dinges, 2007). Synaptic consolidation of memory and learning has been shown to be largely dependent on sleep (Diekelmann and Born, 2010), whilst sleep pressure and partial sleep deprivation of as little as 2 h can also negatively impact cognitive performance (Jarraya et al., 2014b, Shochat et al., 2014). Therefore, it is clearly established that disruptions to sleep quality, duration or timings can cause adverse effects on cognitive functioning and, at their most severe, are often associated with clinical sleep disorders (Altevogt and Colten, 2006). Variations in sleep timings are closely linked with circadian influences due to the temporal regulation of the sleep/wake cycle. However, unlike the robust findings into the impact of sleep on cognitive performance, there have been contradictory results into diurnal variations depending on which cognitive abilities are being studied as well as individual differences in circadian rhythmicity (Schmidt et al., 2007).

Circadian fluctuations in cognitive performance show general trends of peak performance during daytime and cognitive impairments during the biological night, which are accentuated with sleep deprivation (Valdez et al., 2014, Valdez et al., 2008). However, many investigations into time of day differences in cognitive performance have produced

differing results. Behavioural studies looking at diurnal variations in PVT performance suggest attention is worst in the morning and increases throughout the day (Mollicone et al., 2008). Vigilance has been reported to peak in the afternoon (Atkinson and Davenne, 2007, Kline et al., 2010, Kraemer et al., 2001), and reaction time in the morning (Jarraya et al., 2014a). Stroop tasks, on the other hand, often show no diurnal fluctuations (Bratzke et al., 2012). Sustained attention has been shown to be best when performed at 'optimal times of day' (Lara et al., 2014), whilst others suggest learning and memory are better at non optimal times of day (Delpouve et al., 2014, May et al., 2005). Key issues with these differing conclusions seem to relate to the lack of circadian phenotyping and discrepancies in the task being considered i.e. measuring different cognitive domains. For example, differences have been suggested to be more pronounced in complex tasks compared to simple tasks (Bennett et al., 2008), as shown in Figure 1.3. When Circadian Phenotypes are considered there seems to be more of a common thread with ECPs performing better during morning hours and LCPs during afternoon/evening hours in attentional tasks (Clarisse et al., 2010, Matchock and Mordkoff, 2009). Executive functions have shown the same pattern as the previous studies, suggesting outcomes are dependent on both time of day and Chronotype (Hahn et al., 2012). This highlights the need to categorise individuals into Circadian Phenotypes to prevent the misinterpretation of group results.

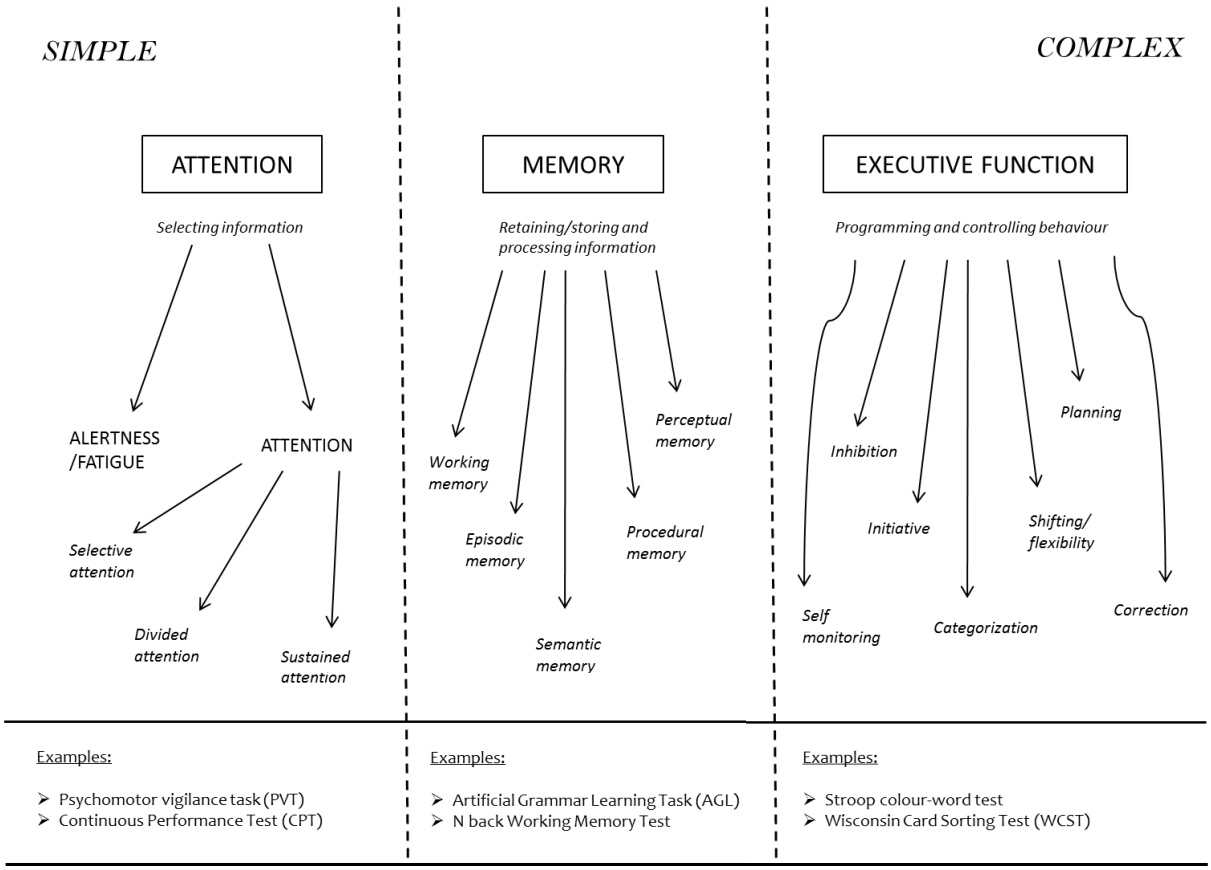

Figure 1.3. The main cognitive performance measures used within sleep and circadian research. Adapted from (Schmidt et al., 2007).

1.4 Physical Performance

All athletes and coaches are looking for slight competitive edges to improve performance (Thun et al., 2015). In the sports world, times of training and competitions largely determine an athlete's daily schedule, and yet time of day and sleep effects on physical performance are rarely taken into account. A large variety of factors have been documented to influence physical performance, including training (Gabbett, 2010, Smith et al., 2003), fitness (Arnason et al., 2004), motivation/competition (Hull et al., 2003, Marcora et al., 2009), nutrition (Smith, 1984), tactics (da Costa et al., 2011), management (Espitia-

Escuer and García-Cebrián, 2006), and psychology e.g. perceptions of training (Barreiros et al., 2011). Research into the effect of physiological functions on performance also highlights the impact of cardiac function, resting blood pressure (Cornelissen and Fagard, 2005), hormone levels e.g. cortisol (Paccotti et al., 2005), oxygen uptake and heart rate (Vianna et al., 2014).

All professional sports teams have specific daily training schedules as well as regular fitness testing sessions to monitor progress and performance at certain times of day (Ingebrigtsen et al., 2012). Although physical performance is complex and can be measured in multiple ways, it is generally reported that athletic performance fluctuates daily, increasing throughout the day and peaking in the late afternoon/early evening, following the circadian rhythm of CBT (Taylor et al., 2011, Souissi et al., 2010b). Different sports have been investigated such as tennis (Atkinson and Speirs, 1998), badminton (Edwards et al., 2005), swimming (Deschodt and Arsac, 2004, Pallares et al., 2014, Rae et al., 2015) and football (Reilly et al., 2007, Rahnama et al., 2009). Other studies have focused on specific measures e.g. muscle strength (Blonc et al., 2010, Coldwells et al., 1994, Giacomoni et al., 2005, Pereira et al., 2011, Squarcini et al., 2013), anaerobic capacity (Hachana et al., 2012, Gholamhasan et al., 2013, Racinais, 2010, Racinais et al., 2010) and aerobic capacity (Bessot et al., 2006, Brown et al., 2008a, Facer-Childs and Brandstaetter, 2015a, Hobson et al., 2009). The majority of the findings of these studies report peak performance to occur in the evening, apart from when Circadian Phenotype is considered (Facer-Childs and Brandstaetter, 2015a, Rae et al., 2015). The consideration of Circadian Phenotypes has also uncovered differences in the range of diurnal performance between ECPs and LCPs. Performance from morning to evening is accentuated in the LCPs suggesting a wider range

of performance difference for LCPs in both muscle strength (Tamm et al., 2009), and aerobic capacity (Facer-Childs and Brandstaetter, 2015a).

The importance of the relationship between sleep and exercise has also been uncovered as disruptions to sleep can impact on physical performance (Atkinson and Davenne, 2007). One night of sleep deprivation significantly impairs anaerobic performance, as well as reducing a significant improvement from morning to evening observed after a normal night's sleep (Souissi et al., 2003). However, it has been emphasised that individual variability in response to sleep loss can create complications when evaluating sleep effects (Meney et al., 1998). Since many of the studies investigating elements of physical performance, time of day and sleep failed to consider Circadian Phenotypes in the study design, these complications could be due to differences in 'optimal' performance times masking true results. The close interaction of circadian and sleep mechanisms that regulate physiology brings to light the fact that research into the influence of Circadian Phenotypes on physical performance is lacking and additional investigations are needed.

So far, this introduction has discussed how chronobiology and sleep research relates to physiological and behavioural processes, with particular emphasis on cognitive and physical performance. However, the extensive variations deliberated in this introduction raises the question of what can be done to manipulate these factors and if there are any benefits of this, which will be explored in the next section.

1.5 Phase-Shifting

The need to 'fit' into our working society has raised concerns because of the constant mismatch of internal and external time, termed social jetlag (Wittmann et al., 2006). There is a constant fight between endogenous biological clocks and the social time system. This 'desynchronisation' occurs when there are changes in exposure to the light/dark cycle (shift work, jet lag), or changes in the environment and behaviour. This is a predominant issue for individuals who naturally tend towards more of a 'late' preference i.e. LCPs due to being forced to follow earlier schedules for work/school timings and reverting back to late schedules on unconstrained days. This mismatch results in adverse effects on health and well-being due to chronic sleep restriction (Banks and Dinges, 2007), and disruptions to circadian rhythms (Zelinski et al., 2014). Circadian misalignment and sleep disturbances have been shown to increase the risk of cancer (Reiter et al., 2007, Savvidis and Koutsilieris, 2012), diabetes (Reutrakul and Van Cauter, 2014), and have a negative impact on immune function (Bryant et al., 2004, Lange et al., 2010). Therefore, there is a motivation to increase our understanding of how these disruptions can be treated to minimise the negative impact on health and well-being.

As well as basic sleep hygiene i.e. control of sleep environment, much attention surrounding treatments of these disruptions has focused on shifting the circadian phase of individuals. Photic entrainment (light), is often used to shift phase since light is the major zeitgeber of the circadian system (Boivin and James, 2002a, Boivin and James, 2002b, Czeisler et al., 1990, Santhi et al., 2008). Bright light during the morning phase advances the circadian system causing dim light melatonin onset (DLMO) to rise earlier and sleep onset to become advanced (Crowley and Eastman, 2015). Conversely, light exposure

during the biological night creates a phase delay shown by a later DLMO (Kelly et al., 1997, Zeitzer et al., 2014). Non-photic forms of entrainment have also been researched to try and shift circadian phase (Mistlberger and Skene, 2005). For example, food has been proposed as a zeitgeber of the circadian system (Stephan, 2002, Stokkan et al., 2001). This was supported by a study showing that stomach cells secreting the appetite hormone ghrelin are entrained by timed food intake (LeSauter et al., 2009). Furthermore, physical exercise has also been suggested to aid phase shifts (Miyazaki et al., 2001), by altering CBT (Eastman et al., 1995), and melatonin rhythms (Buxton et al., 2003). Others have established that exogenous melatonin can also be used in treatments to shift phase (Lewy et al., 1992).

Although this has been explored within the realm of night shift work (Boivin and James, 2002a), and circadian rhythm sleep disorders (CRSDs, (Zhu and Zee, 2012)), little has been done to translate this into general society. Since general distributions of Circadian Phenotypes suggest between 60-80% of our society falls into the ICP and LCP groupings (Roenneberg et al., 2007), it would be of interest to explore if healthy individuals could benefit from a phase advance in a real world setting. The main conclusions from this research suggest that using a combination of morning timed light exposure and afternoon melatonin administration is the most effective way to advance circadian phase and is often used in the treatment of Delayed Sleep Phase Syndrome (DSPS) and shift work disorder (Burke et al., 2013, Crowley and Eastman, 2015, Paul et al., 2011, Revell et al., 2006). However, timing of drug administration or ingestion of artificial substances can have a negative impact on individuals. For instance giving stimulants like caffeine at the wrong time of day or during the night could affect performance and cognition the following day (Sherman et al., 2011). Long term effects of exogenous melatonin administration are still

unknown, so stimulate the need to carry out longitudinal studies (Lewy et al., 1992). On top of this, the side effects of drugs used in clinical settings can be vast and diverse e.g. ramelteon can exacerbate insomnia and sedative hypnotics can have soporific effects at the wrong times. Consequently, this could result in more problems (Roth, 2012). Therefore, being able to achieve a phase advance using purely non-pharmacological interventions is more realistic and feasible in non-clinical populations e.g. LCPs (Sharkey et al., 2011). There is a need to further understand the benefits of a phase advance in the real world on clinically healthy individuals. As of yet, there are no reports that have specifically investigated the effects of a phase advance on the mental well-being combined with cognitive and physical performance in LCPs. This topic will be discussed further in Chapter 5.

1.6 Methods used in Sleep and Circadian Research

1.6.1 Protocols

Due to the complex interaction of circadian and sleep dependant influences on behaviour, exploring the relative contributions of each is challenging. The development of strict protocols has allowed an insight into how each factor affects physiology and performance by disentangling the two. Constant routine (CR) or forced desynchrony (FD) protocols are often used in sleep and circadian research (Hofstra and de Weerd, 2008), and have uncovered the endogenous nature in many behavioural and physiological processes. For example, CR was used to determine circadian phase differences between ECPs and LCPs (Lack et al., 2009), and FD has been used to show the intrinsic period of melatonin for adolescents is greater than 24 h (Carskadon et al., 1999).

CR protocols, originally proposed by Mills et al. (1978), are performed in a controlled laboratory setting and aim to remove exogenous influences that can 'mask' a circadian rhythm. Masking factors can be internal, such as motivation and mood, or external such as temperature, light, food intake and noise (Czeisler et al., 1986). The CR protocol requires participants to remain in a constant condition i.e. no food, sleep or movement, intravenous glucose and constant light, for more than 24 h. Although during the CR period sleep homeostasis is still increasing, it removes the effect of rhythmic variations in activity and physiology induced by the sleep-wake cycle and therefore reflects the interaction of the two. An extension to this protocol, called the ultra-short sleep/wake cycle, introduces multiple sleep opportunities during the same CR conditions (Lavie, 1986). This results in a low homeostatic sleep pressure which allows 'less noisy' circadian profiles to be displayed (Blatter et al., 2006). FD protocols completely separate the sleep and circadian mechanisms by desynchronising endogenous rhythms from external influences. As mentioned previously, individual endogenous periods tend to diverge from an exact 24 h rhythm and are therefore synchronised to the 24 h light dark cycle following the earth's rotation. Enforcing a sleep/wake schedule that deviates significantly from this 24 h rhythm (e.g. 20 h or 28 h) causes the endogenous circadian clock to disengage and follow its own period, a process called 'free running' (Folkard and Akerstedt, 1989). The desynchronisation of internal and external time allows the differentiation between circadian and sleep homeostatic processes, and thereby results in the observation of endogenous circadian rhythms (Blatter and Cajochen, 2007, Schmidt et al., 2007). FD protocols were initially developed by Kleitman in 1987 and have since been used to study circadian variations in a variety of measures including cognitive performance (Wright et al., 2002), sleepiness (Silva et al., 2010), and motivation (Hull et al., 2003). However, there is increasing need to study

individuals in their home environment because behaviour and performance are ultimately impacted by both sleep and circadian mechanisms. In addition, lab based protocols are largely unrealistic and thus hold poorer external validity when relating results to everyday functioning (Zee et al., 2014). Although real world conditions do not allow for the control of exogenous influences, the integrated approach may help to shed light on how both biological and environmental factors affect behaviour, physiology and performance. There are advantages and disadvantages to each of these protocols which are summarised in Table 1.2.

Table 1.2. Advantages and disadvantages of the protocols used in circadian and sleep research.

	Forced De-synchrony	**Constant Routine**	**Real World**
Truly circadian effects?	Yes	No	No
Controlled environment?	Yes	Yes	No
Setting	Lab Based	Lab Based	Home Environment
Cost	High	High	Low
Time consuming?	Yes	Yes	No
Masking factors?	No	Yes	Yes
Hard to implement?	Yes	Yes	No

1.6.2 Characterising Sleep

There are several ways to measure sleep and circadian processes, including techniques that require individuals to visit the lab/clinical facilities and those designed to monitor factors in the home environment (Iber et al., 2004). To explicitly investigate the structural organisation of sleep (sleep architecture), brain activity, muscle activity and eye movements need to be measured. The combined overnight assessment of these

physiological activities is called polysomography (PSG) which includes multiple techniques. EEG is used to measure electrical activity and generally consists of eight electrodes, two of which are used as references. These are placed on the surface of the scalp and cover the frontal, central and occipital regions of the brain according to the International 10-20 guidelines (Klem et al., 1999). The frequencies of the signal measured in EEG vary depending on sleep stage (Niedermeyer and da Silva, 2005). For example, the beta wave type (15-30Hz) is a typical characteristic of wakefulness and also seen during REM sleep. The theta band is often observed in stages 1 and 2 NREM sleep (4-8Hz), and the delta band (2-4Hz) is seen in stage 3 or slow wave sleep. Electrocardiography (ECG) measures heart rate using two or three electrodes which are typically placed under the collar bone. Electromyography (EMG), and electrooculography (EOG), measure muscle and eye activity respectively. EMG uses four electrodes, one on each leg and the remaining two under the chin. A loss of muscle tension recorded by these electrodes indicates periods of REM sleep. The two electrodes used in EOG are placed just above the right eye and below the left. This allows characteristics of different sleep stages to be observed, such as rolling eye movement in stage 3 or slow wave sleep (SWS), and rapid eye movements in REM sleep. Respiratory function is also monitored with pressure transducers and pulse oximetry. PSG, originally developed by Rechtschaffen (1968) and updated by Berry et al. (2012b), is well documented as being the 'gold standard' in the analysis of sleep. Staging of sleep using PSG is done by visually scoring PSG recordings in 30 second epochs based on the frequency, duration and amplitude of the signal produced (Berry et al., 2012a, Rechtschaffen, 1968). Despite PSG being costly and time consuming as it is recorded in a laboratory environment and participants generally require an acclimatisation night, it is

currently the best way to study sleep and diagnose sleep disorders, and is therefore used commonly across sleep research (Bloch, 1997, Vaughn and Giallanza, 2008).

1.6.3 Actigraphy

As PSG is costly, time consuming and difficult to implement in field studies, alternative methods have been developed to study sleep/wake patterns in the home environment. Wrist actigraphy can be used to provide a reliable and accurate overview of sleep/wake patterns and behaviour over long periods of time. Actiwatches or actigraphs, which have been validated against some PSG parameters such as total sleep time, sleep efficiency and wake after sleep onset (de Souza et al., 2003), were developed to monitor 24 h activity of individuals in their home environment and provide a cheaper and easier alternative to methods like PSG (Kushida et al., 2001). These small wrist worn devices are triaxial accelerometers and contain 4D motion sensors which can detect and record both light and activity/movement in given time frames e.g. epochs of 2 seconds up to 1 minute, to distinguish between activity and lack of (which is assumed to be sleep). Actigraphs are used extensively in sleep and circadian research as well as having more clinical uses in respiratory medicine, mental health and other fields (Ancoli-Israel et al., 2003). However, despite the many advantages using movement as a surrogate marker can cause inaccurate classifications of sleep versus rest.

1.6.4 Sleep Diaries

Subjective recording of sleep and wake times in the form of a 'sleep diary' are often used in studies to keep a record of activity patterns and are frequently used alongside other measures such as actigraphy. Despite some sleep diaries being available clinically for

diagnosis of sleep disorders e.g. insomnia, many research studies use adapted versions to take into account other factors such as alcohol intake, daytime naps and physical activity (Carney et al., 2012).

1.6.5 Questionnaires

Questionnaires are a useful tool in within these research fields as they allow the investigator to gather a wide range of information (Lomeli et al., 2008). In addition to the questionnaires to categorise individual differences in circadian phase and preference, a huge number of questionnaires have been developed in sleep research to study sleep timings, quality, and subjective sleepiness. The details of those which are relevant to the current work will be given in the Materials and Methods Chapter. Furthermore, the need to assess diet and timing of food intake has become a key topic in sleep and circadian research due to studies suggesting that both type of food as well as timings can affect sleep and circadian rhythmicity (Arble et al., 2009, Potter et al., 2016).

1.6.6 Physiological Methods

Physiological markers are often used to determine the circadian phase of an individual, for example, through hormonal rhythms. Two of the main hormones involved in the regulation of the sleep wake cycle are melatonin, which rises in the evening to reach acrophase in the middle of the night, and cortisol which peaks in the morning (Nakagawa et al., 1992). Levels of these hormones change depending on the time of day, which gives an objective indication of the phase of the biological rhythms (Czeisler et al., 1999). Other hormones such as the appetite hormones ghrelin and leptin also show diurnal fluctuations (LeSauter et al., 2009). Saliva allows easy, safe and noninvasive collection from which these

hormones can be analysed by radioimmunoassay (RIA) without the need to extract any cellular material. RIA is a sensitive and specific assay that detects concentrations of antigens in biological samples e.g. blood, plasma or saliva, through antigen-antibody reactions involving radiolabelled isotopes. One of the most commonly used isotopes is iodine (^{125}I) (Carter and Shieh, 2015). There are many protocols that have been developed with the main two techniques being coated tube or double antibody RIA. Coated tube RIA involves monoclonal antibodies being coated to the preparation tube or microtiter plate, thereby allowing any unbound antigens to be removed by disposing of the supernatant (the liquid on top of a solid residue following centrifugation). Double antibody RIA uses a second antibody to bind with the primary antibody which allows the unbound antigens to be removed by washing. Radioactivity is measured in a gamma counter that measures radioactivity counts per minute. From this a calculation of antigens present in the sample can be made due to this value being inversely proportional to the radioactivity. A summary of the RIA technique is given in Figure 1.4.

1.6.7 Genetic Methods

As mentioned earlier, there has been an increase in research into the cellular mechanisms involved with individual differences in sleep patterns and circadian phase. Mutations within clock genes involved in the TTFL have been identified and linked to individual differences in circadian rhythmicity (Katzenberg et al., 1998), although only one will explicitly be discussed within this thesis. The most widely researched gene associated with differing Circadian Phenotypes is the period 3 (per3) gene and can be easily identified using polymerase chain reaction (PCR) (Archer et al., 2003). Variable number tandem repeat polymorphisms within the coding region of this gene result in either a 4 repeat or 5

repeat segment that has been associated with circadian preferences and vulnerability in homeostatic sleep pressure (Lazar et al., 2012). Homozygous $per3_{(5/5)}$ individuals show a preference for morning and have a higher vulnerability to sleep loss, whereas homozygous $per3_{(4/4)}$ individuals show a preference for the evening (Archer et al., 2003).

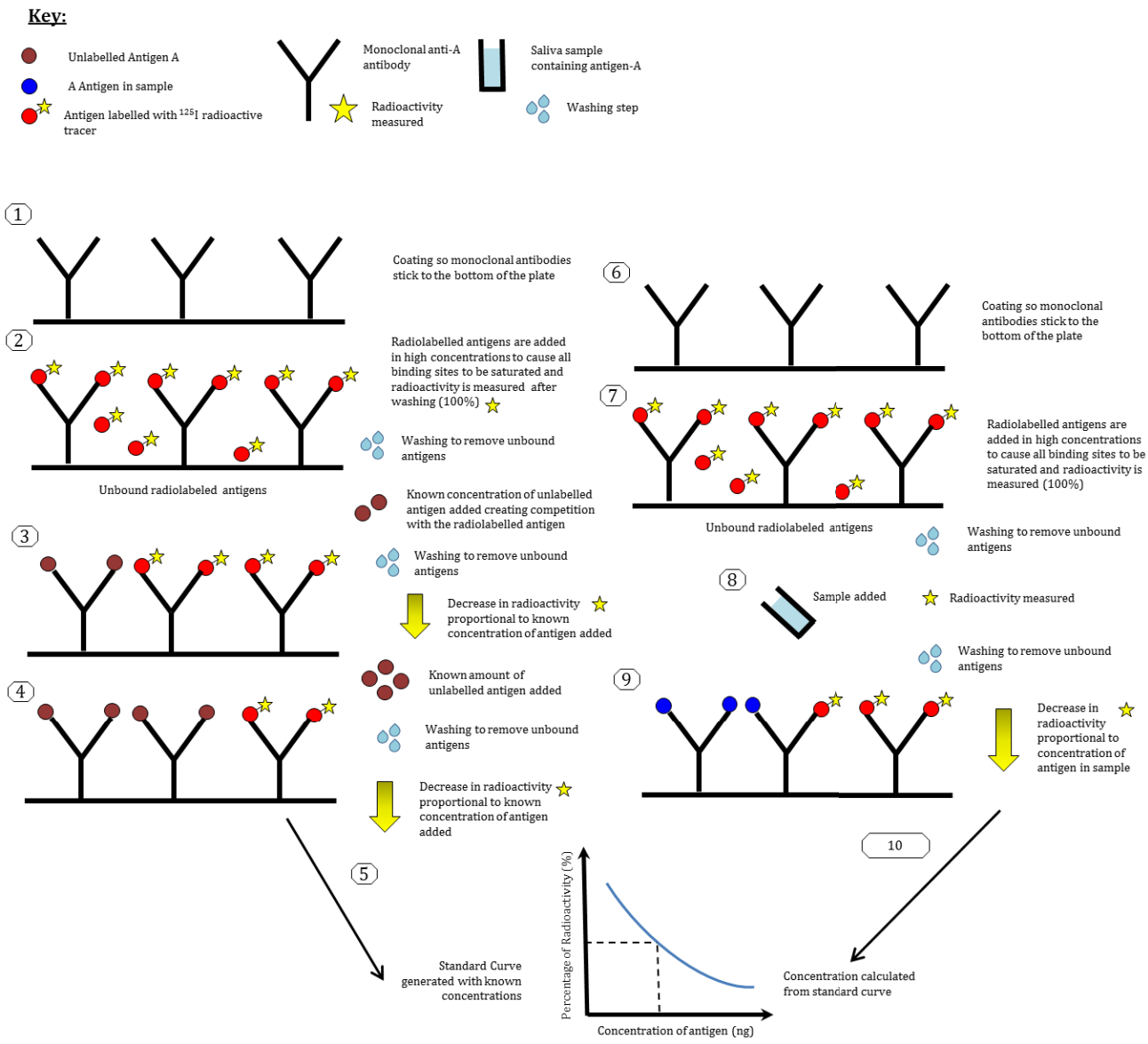

Figure 1.4. Schematic diagram of radioimmunoassay technique showing how RIA is performed to detect antigen concentrations in human biological samples.

1.7 Neuroimaging

Given that the brain is integral to basic functioning, it is remarkable that the use of brain imaging is still lacking in sleep and circadian research. There is a need to increase our understanding of the functional contributions behind Circadian Phenotypes and how they link to behaviour and performance. Therefore, this section will give an overview of human brain mapping, with particular focus on resting state functional magnetic resonance imaging (rs-fMRI).

Imaging the human brain was initially performed in the 1970s using X-ray computed tomography (Hounsfield, 1973). Since then, imaging of the brain through techniques such as positron emission tomography (PET) and magnetic resonance imaging (MRI), has allowed the crucial link between brain function and behaviour to be explored in the field of cognitive neuroscience (see Raichle (2009) for a review). Furthermore, the past decade has seen a rapid expansion in the use of functional brain imaging techniques like electroencephalography (EEG), magnetoencephalography (MEG) and functional MRI (fMRI), on top of the development of newer methods in optical imaging e.g. near-infrared spectroscopy (NIRS). While each of these procedures share the aim of visualising the human brain non-invasively, this thesis will focus explicitly on the most common imaging technique, MRI, which was also used in this thesis.

1.7.1 MRI Physics

MRI is derived from nuclear magnetic resonance (NMR), in which non-ionising radiation is used to create images in multiple planes (Figure 1.5). The physical principles of MRI involve the behaviour of atoms in water, whereby nuclei of atoms precess within an

applied magnetic field at a frequency proportional to the field strength (Larmor frequency). There is an abundance of water and fat molecules throughout the human body which contain hydrogen atoms behaving as dipoles. An MR scanner uses a strong homogeneous magnetic field measured in Tesla (T, B_0), which causes the atoms to align and precess in a low energy (parallel) or high energy state (anti-parallel) to the magnetic field creating a net magnetisation. Additionally, gradient fields in the x, y and z planes are used to vary the strength of the magnetic field within the scanner which changes the precession frequency of the atoms in a spatially-dependent manner. Appling a radiofrequency electromagnetic (B_1, RF) pulse via a radiofrequency coil, distorts this equilibrium and excites the atoms causing them to absorb the energy and flip their low/high energy state accordingly. Removal of this RF pulse results in the atoms returning to their original state of equilibrium through a process called relaxation. Tissue dependent differences in energy measured are defined by the rate of relaxation and depend on the re-alignment of nuclei and the phase coherence. By applying field gradients and RF pulses, excitation of atoms can be confined to specific locations or slices measured in 3D cubic areas called voxels. As a result, time dependent changes in relaxation can be used to determine different tissue compositions as a function of the local environment. T1 relaxation measures the time taken for atoms to return to equilibrium within a longitudinal static field whilst T2 relaxation relates to the time constant for the loss of phase coherence (decay). Since the chemical compositions of tissues vary, different signal intensities can be identified depending on the sequence parameters used, thereby creating contrasts. The time between RF pulses is referred to as the repetition time (TR), while the time from RF pulse to data acquisition is called the echo time (TE). Other sequence parameters include the flip angle which depends on the duration of RF pulse, resolution, field of view,

bandwidth and signal to noise ratio (SNR). T1-weighted contrasts have short TR and TE, whereas T2-weighted contrasts have a long TR and TE. Both contrasts are used to gather anatomical details with tissues showing opposite signal intensities in each e.g. water appears dark in T1 but bright in T2 (Figure 1.5).

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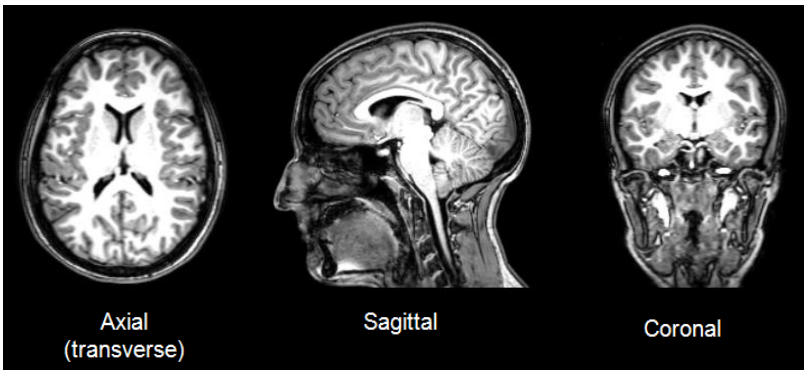


Figure 1.5. Cross section views of a T1 weighted image to show the different planes in MRI.

T2*-weighted contrasts involve contributions from local magnetic field inhomogeneities causing the T2 dephasing to occur more rapidly. Functional MRI (fMRI) is a technique using T2* weighted imaging to map dynamic variations in regional cerebral blood flow (rCBF), blood oxygenation and blood volume. Since the discovery of magnetic properties in haemoglobin was applied to neuroimaging, it has been possible to measure rCBF in the brain without contrast agents (Thulborn et al., 1982). The blood oxygenation level dependent (BOLD) signal is sensitive to field distortions due to changes in the relative concentrations of oxyhaemoglobin and deoxyhaemoglobin in the blood. Increases in the BOLD signal have been linked to increases in metabolic demand due to higher concentrations of oxyhaemoglobin resulting from an influx of oxygenated blood (Ogawa et al., 1990). FMRI is used to study how parts of the brain are activated (activation), or connected (functional connectivity). Typically, an experiment using BOLD-fMRI would

involve performing a task within the scanner and statistically comparing the results to period of rest i.e. when the task is not being performed. Response to the task causes cortical and subcortical activation resulting in an increase in rCBF to compensate for the brain's metabolic requirements (Bandettini et al., 1992). This in turn decreases the concentrations of deoxyhaemoglobin in activated areas leading to the signal change detected from local field potentials. The time between neuronal activation and indirect changes in rCBF is known as the haemodynamic response function which shows an initial dip, a high peak and is followed by a post-stimulus undershoot (Buckner, 1998). The BOLD signal does not directly measure neuronal activity but infers it through the process of neurovascular coupling (Attwell and Iadecola, 2002). Therefore, interpretation of this BOLD signal relies on an understanding of metabolic and physiological processes regulating CBF (Hillman, 2014). Research over the past decade has proposed a number of mechanisms and cell groups which are thought to contribute to neurovascular coupling including astrocytes, synaptic signalling and vasculature (Hillman, 2014). Although there is dispute into the relative importance of each mechanism, the complex interactions support the idea of increased CBF relating to metabolic demand of neuronal activity (Figure 1.6).

The majority of research in neuroimaging has been through studying activation of brain in response to tasks (Van Den Heuvel et al., 2009). However, connectivity within the brain is also fundamental to elucidating the neuronal basis of brain function. Connectivity can be referred to in relation to anatomical links, functional connections (temporal correlations), or effective connections (casual interactions). Understanding the anatomical and functional organisation of the brain is crucial to the study of neural activity, since activation is constrained by connectivity (Horwitz, 2003). Although much could be discussed on the

variations in connectivity, this thesis will focus on functional connectivity (FC), and in particular resting state functional connectivity (rs-FC).

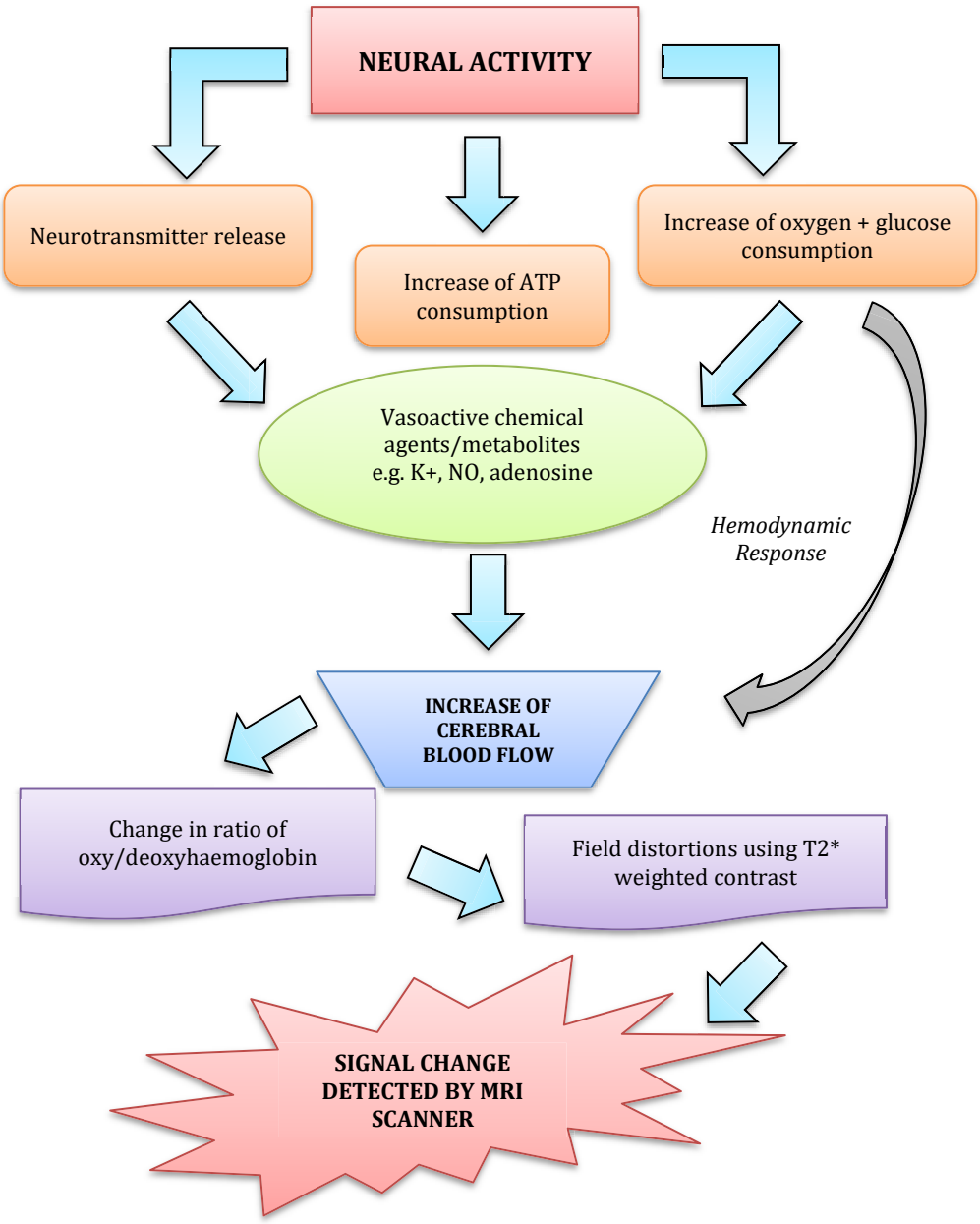


Figure 1.6. Schematic diagram summarising the process of neurovascular coupling.

1.7.2 Resting State Functional Connectivity (rs-FC)

The common misconception that the brain is not 'active' during periods of rest has become the focus of much scientific research since spontaneous fluctuations in low frequency oscillations were found at rest. These low frequency (<0.1Hz) spontaneous fluctuations in BOLD signal observed at rest have allowed the classification of widely distributed intrinsically connected networks (ICNs). ICNs are identified as being functionally related through temporal correlations and reflect the neuronal 'baseline' of the brain (Damoiseaux et al., 2006). Rs-FC was originally identified by Biswal et al. (1995), who published a study showing FC of the motor cortex at rest. A large number of ICNs have been identified, with differing anatomical locations and each being linked to particular functions. However, this thesis will focus on two ICNs, the default mode network (DMN) and the motor network (MN).

1.7.2.1 The Default Mode Network (DMN)

Regions of the DMN have consistently been shown to exhibit increased activation at rest, which decreases upon task demands (Buckner et al., 2008). The original identification of the DMN stemmed from neuroimaging studies identifying specific brain regions which were 'task negative' (Shulman et al., 1997) (Figure 1.7). These regions consist of the posterior cingulate cortex (PCC) and precuneus, medial pre-frontal cortex (mPFC), bilateral angular gyri at the temporal parietal junction and often include bilateral medial temporal lobes (MTL) (Greicius et al., 2003).

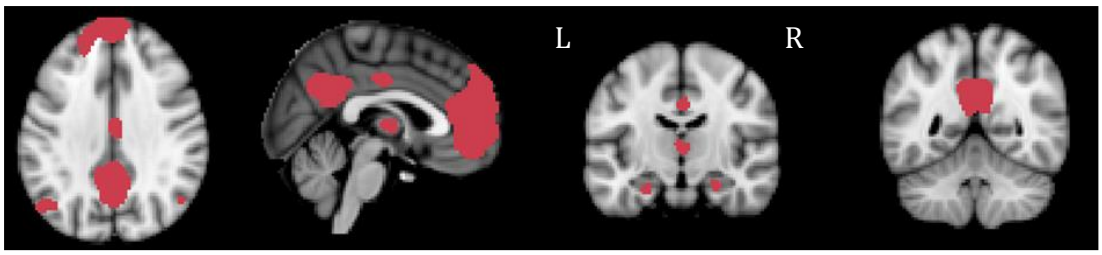


Figure 1.7. Regions of the Default Mode Network (DMN) identified by Shirer et al. (2012).

Whilst a significant function of the DMN is to maintain and regulate consciousness and cognitive function (Picchioni et al., 2013), extensive work has now been carried out to define the role of the DMN in sleep. Studies have shown that FC of the DMN varies depending on sleep stage, persisting during light sleep (Larson-Prior et al., 2009), and disconnecting during deeper slow wave sleep (Horovitz et al., 2009). This highlights the fact DMN activity is not unique to resting wakefulness and supports the idea that the DMN is closely involved in regulating consciousness, which does not stop immediately with light sleep but progressively reduces until awareness and consciousness are at a minimum in deep sleep (Buckner and Vincent, 2007). Rs-FC of the DMN has also been shown to be affected by sleep disturbances and may exhibit diurnal rhythmicity (Blautzik et al., 2013). As well as decreased rs-FC observed following sleep deprivation (Chee and Thomas, 2013, De Havas et al., 2012), lower rs-FC has also been linked to cumulative total sleep time (Khalsa et al., 2016), and reduces with a build-up of sleep pressure (Sämann et al., 2010). Although the regions of the DMN are spatially distant from one another some studies have examined anatomical links between them. Greicius et al. (2009) hypothesised that there would be anatomical links between the anterior and posterior midline structures of the DMN (the PCC and mPFC). The results revealed clear structural connections between PCC and mPFC as well as between PCC and bilateral MTL (hippocampus and parahippocampus)

but no clear links from mPFC to MTL suggesting that these regions may be connected via an intermediate region like the PCC (Greicius et al., 2009).

Rs-FC has become a useful and novel tool for clinical applications with many studies suggesting the role of DMN in the pathophysiology of mental disorders (Broyd et al., 2009). In addition, studies have shown the potential to use rs-FC of the DMN as a biomarker of neurodegenerative diseases such as Alzheimer's disease (Greicius, 2008). A recent paper by Cole et al. (2016) also highlighted the relevance of ICNs, including the DMN, on predicting cognitive tasks activations. A more detailed discussion of how the DMN is affected by sleep and circadian mechanisms, and how it links to cognitive performance, will be given in Chapter 3.

1.7.2.2 The Motor Network (MN)

The rs-MN is comprised of the same regions activated in motor tasks (Figure 1.8). These areas include the bilateral precentral gyri, which make up the left and right primary motor cortices (LM1 and RM1), the supplementary motor area (SMA), bilateral thalami and cerebellum (Shirer et al., 2012).

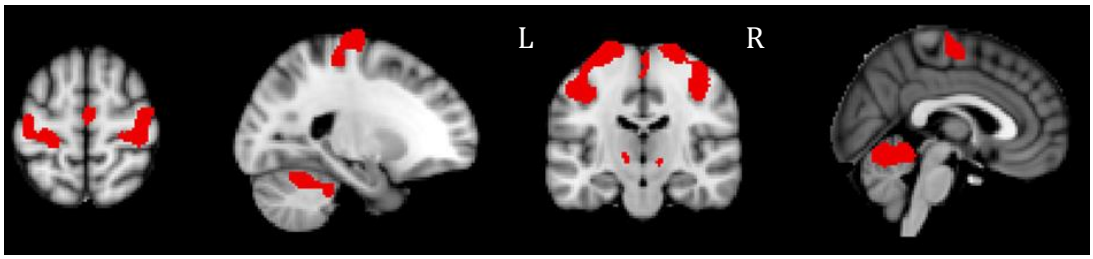


Figure 1.8. Regions of the resting state Motor Network (MN) identified by Shirer et al. (2012).

Rs-FC of the MN has a number of applications in clinical work. Much attention has been given to using the MN to study recovery to stroke (Andrew James et al., 2009, Carter et al., 2012), thereby allowing a greater understanding of functional organisation and developing novel therapies. The motor system is also the focus of neurodegenerative disorders that impact on motor function such as Parkinson's disease (Luo et al., 2014, Wu et al., 2009, Wu et al., 2011).

The relationship between the MN and motor function has also been widely studied. The MN is highly dynamic as switching between resting and task states is required for effective functioning (Jiang et al., 2004). This is supported by decreases in rs-FC of the bilateral primary motor cortex being detected with muscle fatigue (Peltier et al., 2005). In addition, increased rs-FC of the MN has been shown to promote more efficient switching when performing a task is required (Jiang et al., 2004). The MN has also been the focus of research into extensive motor training, which suggests rs-FC increases after a period of training (Ma et al., 2011, Taubert et al., 2011), coupled with a decrease in activation (Xiong et al., 2009). This finding highlights the need to interpret activation and connectivity with caution as increased FC of the MN could relate to more fundamental changes in the brain i.e. synaptogenesis or metabolic capacity, whereas decreases in activation could relate to lower metabolic demand for the same output following a period of training (Xiong et al., 2009). The MN is reported to show daily rhythmicity (Blautzik et al., 2013), and follows circadian modulation during tasks (Peres et al., 2011). Peres et al. (2011) also showed that peak neural activity can be predicted by Circadian Phenotype. However, little research has specifically investigated the MN in relation to sleep and circadian mechanisms linked to physical performance variables. Therefore, Chapter 4 of this thesis will explore this further.

1.8 FMRI in Circadian & Sleep Research and Performance

Since FC is required for daily functioning, and rs-FC has been shown to be affected by sleep, time of day and differs in various pathological states, it brings to light the need to understand more about the how the functional significance of ICNs relates to both cognitive and physical performance measures in healthy populations.

The studies that have explicitly used fMRI to investigate sleep and circadian impacts on performance have presented some interesting results, with the majority using task based fMRI. Sleep dependent mechanisms are more widely studied than circadian mechanisms and generally show deficits following sleep disruptions (Basner et al., 2013). For example, lack of sleep has an impact on task based FC, with many studies showing decreases in FC and cognitive performance following sleep deprivation (Basner et al., 2013, De Havas et al., 2012, Goel et al., 2013). When it comes to rs-FC, sleep deprivation has been shown to diminish morning rs-FC of several ICNs including the DAN and DMN, an observation not present if sleep was permitted (Kaufmann et al., 2016). Chronotype specific activation patterns have been shown in attentional networks with ECPs showing increased activation of the left anterior insula compared to LCPs (Reske et al., 2015). Differences in activations between ECPs and LCPs have also been found in both PVT (Schmidt et al., 2009) and Stroop tasks (Schmidt et al., 2012), showing that ECPs have lower activation than LCPs during the evening which is associated with vulnerability to sleep pressure. These studies highlight the interaction of homeostatic and circadian processes on neuronal activity in ECPs and LCPs. Some research is also beginning to reveal the effect of time of day on rs-FC. Increased rs-FC has been reported in regions associated with memory retrieval during the evening compared to morning (Shannon et al., 2013), and diurnal rhythms of rs-FC have been

observed in a number of ICNs including the DMN and the MN (Blautzik et al., 2013, Hodkinson et al., 2014).

Therefore, variations in rs-FC have been found to be influenced by time of day, in addition to tasked based fMRI showing differences between Circadian Phenotypes. However, no one has, as far as we know, combined these findings to investigate rs-FC between Circadian Phenotypes at different times of day and linked it to cognitive and physical performance measures.

1.9 Aims

The information presented in this Introduction highlights the importance of considering individual differences in both circadian and sleep factors when researching human behaviour and performance. The use of neuroimaging in these fields is lacking and more work is needed to be able to investigate central mechanisms affecting performance in sleep and circadian research. While the investigation into the impact of sleep and time of day on rs-FC is growing, there is a gap in the literature that has yet to be investigated – the effect of Circadian Phenotype on rs-FC and its link to performance.

These ideas are reflected in the research questions presented in this thesis:

1. Are there differences in rs-FC between ECPs and LCPs?
2. Does rs-FC vary depending on time of day?
3. Can differences in rs-FC predict cognitive and physical performance variables?
4. Can LCPs be phase advanced in a real world setting, and what effect does this have on mental well-being and performance?

The next section shows a schematic of the thesis structure to provide an overview of what is included in each Chapter (Figure 1.9). These details will be expanded in bridging paragraphs between each Chapter shown in italics. Many of the methods used within this thesis have been defined and discussed throughout the Introduction. However, Chapter 2 details the specific methodology used throughout this thesis.

1.10 Outline of Thesis Structure

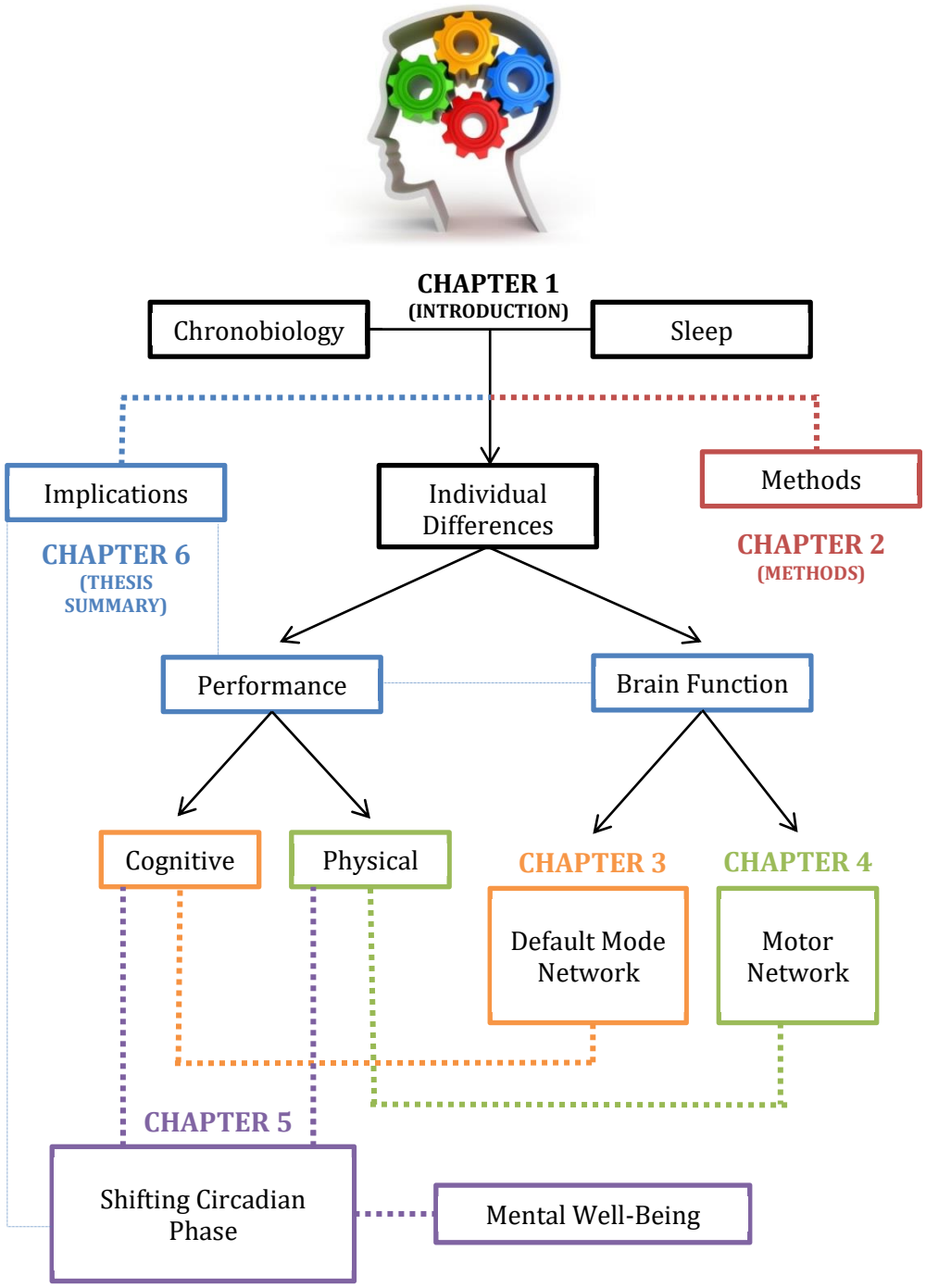


Figure 1.9. Schematic diagram of thesis structure. Chapters 1 and 6 are the Introduction and Thesis Summary respectively, Chapter 2 is the Methods and Chapters 3, 4 and 5 are Experimental Chapters. Boxes and arrows indicate key topics of the thesis. Thick dotted lines show what elements are included for each Chapter and thin dotted lines show what was discussed in Chapter 6.

CHAPTER 2

Materials and Methods

This Chapter will describe the methods used across all experimental Chapters, giving details of the ethics, participants, study protocol, and all techniques used within the study. Specific information about methods used for the variety of differential analysis will be expanded on in individual Chapters.

2.1 Ethics

The study was approved by the University of Birmingham Research Ethics Committee. Participants gave written consent before involvement and all details provided were given on a voluntary basis. Participants were compensated for taking part and were free to withdraw at any time throughout the study. Information sheets and consent forms were sent via email or provided in person and participants were given at least 48 h before written consent was provided at the first meeting.

2.2 Participants

2.2.1 Recruitment

Participants were initially recruited through emails and research posters which were displayed the in communal areas of the Schools of Biosciences and Psychology. Interested individuals (N=204) were invited to meet with the postgraduate researcher where they were asked to complete a medical history form, an MR safety screening form and the MTCQ (paper version). This allowed each participant to be screened for any contraindications to inclusion in the study based on medical history and MR safety (inclusion and exclusion criteria can be found in section 2.2.2). MCTQ scores were collected by calculating corrected mid sleep on free days (MSF_{sc}). This calculation is done by adding half of the sleep duration to the sleep onset time. Individuals who fell into the 'Early' (MSF_{sc} below or equal to 4.00) or 'Late' (MSF_{sc} above or equal to 5.50) Chronotype categories and also passed all inclusion criteria were invited to take part in the main study (Figure 2.1).

2.2.2 Inclusion and Exclusion Criteria

Participants were selected based on no prior diagnoses of sleep, neurological or psychiatric disorders, were not taking any medications that affect sleep and did not have any physical impairment that would prevent them completing a simple hand grip task. Details were also gathered about handedness, exercise and contraceptive information for females. All left-handed individuals (n=5) were excluded from the analysis of Chapter 4 due to the fact this Chapter explores the relationship between rs-FC of the MN and isometric grip strength. None of the participants had any physical impairment. Six female participants reported themselves to be on contraception but this data was only used for informative purposes.

2.2.3 Participant Details

A total of 38 participants took part in Phase One of the study (N = 38, 24 female). Average age was 22.7 ± 4.2 years (mean ± standard deviation). Of the 38 participants, 16 comprised the ECPs group (age 24.7 ± 4.6 years, 9 female, MSF_{sc} 02:24 ± 00:10) and the remaining 22 comprised the LCPs group (age 21.3 ± 3.3 years, 15 female, MSF_{sc} 06:52 ± 00:17) (Figure 2.1). Phase Two solely involved the LCPs who were randomly spilt into an experimental (n=12, age 21.7 ± 2.8 years, 9 female, MSF_{sc} 07:15 ± 00:27) and control (n=10, age 20.9 ± 3.9 years, 6 female, MSF_{sc} 06:24 ± 00:14) group. Details of age, gender and MSF_{sc} are given in Table 2.1. Groups did not differ in gender distribution, height or weight. There was a small but significant difference in age between ECPs and LCPs (p=0.028).

Table 2.1. Summary table of participant variables. Table shows sample sizes for each group, count of males/females, percentage of males/females, age and corrected mid sleep on free days (MSF_{sc}).

	Sample Size	Count of Males/Females		Percentage of Males/Females (%)		Age (mean ± SD)	MSF_{sc} Score (mean ± SEM)
ECPs	N=16	M=7	F=9	M=43.75	F=56.25	24.7 ± 4.6	02:24 ± 00:10
LCPs	N=22	M=7	F=15	M=31.82	F=68.18	21.3 ± 3.3	06:52 ± 00:17
LCPs Experimental	N=12	M=3	F=9	M=25.00	F=75.00	21.7 ± 2.8	07:15 ± 00:27
LCPs Control	N=10	M=4	F=6	M=40.00	F=60.00	20.9 ± 3.9	06:24 ± 00:14
Phase One	N=38	M=14	F=24	M=36.84	F=63.16	22.7 ± 4.2	04:58 ± 00:24
Phase Two	N=22	M=7	F=15	M=31.82	F=68.18	21.3 ± 3.3	05:49 ± 00:19

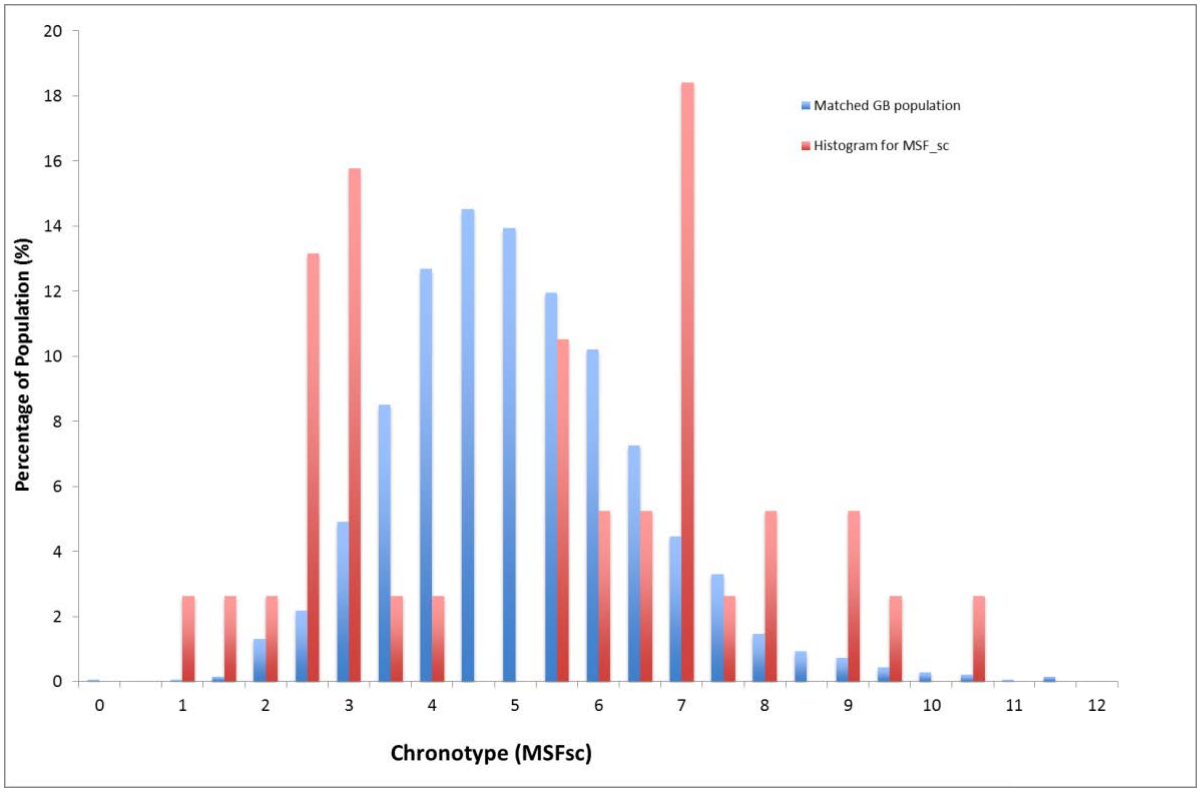


Figure 2.1. Histogram of Chronotype distribution. Participants involved in this study are shown in red and data of a matched (age and sex) population is shown in blue. Early Chronotypes have a MSFsc < 4 and Late Chronotypes > 5.5. X axis shows Chronotype (MSF_{sc}) and y axis shows percentage of the population (%).

2.3 Study Protocol

As mentioned in the Introduction, often the best way to monitor individual's behaviour including their sleep/wake cycles, psychology and performance is to combine multiple techniques (Hofstra and de Weerd, 2008). These can span from control periods in the lab including overnight sleep analysis, to remote monitoring and subjective assessment depending on the type of study being carried out e.g. lab versus real world.

This study was based on a real world circadian typology protocol, which involved multiple phases detailed below and summarised in Figure 2.2 and Table 2.2. This protocol was selected specifically to be able to monitor individuals in their home environment whilst following their normal preferred routines. As discussed previously, despite the difficulties in controlling the influencing factors, this protocol allows a real world assessment of the impact of Circadian Phenotype on rs-FC and performance. It also allows the effects acute sleep deprivation to be removed from all but the morning test session (08:00 h), as the LCPs were not constrained to any schedules. Firstly, an overview of the study protocol will be discussed proceeded by more descriptive details under relevant section headings.

2.3.1 Acclimatisation

The selected participants attended a primary meeting to fill out several sleep related questionnaires (section 2.4.1), receive training for taking saliva samples (section 2.6) and to be set up with an actiwatch (section 2.4.2), sleep diary (section 2.4.3), smart phone app (section 2.4.4) and participant pack (section 2.5). During the two weeks of acclimatisation participants were asked to complete the Chrono-Memory and Attention Test (CMAT, section 2.7.1) at least three times before their first testing session to familiarise themselves with the test. Compliance was monitored remotely.

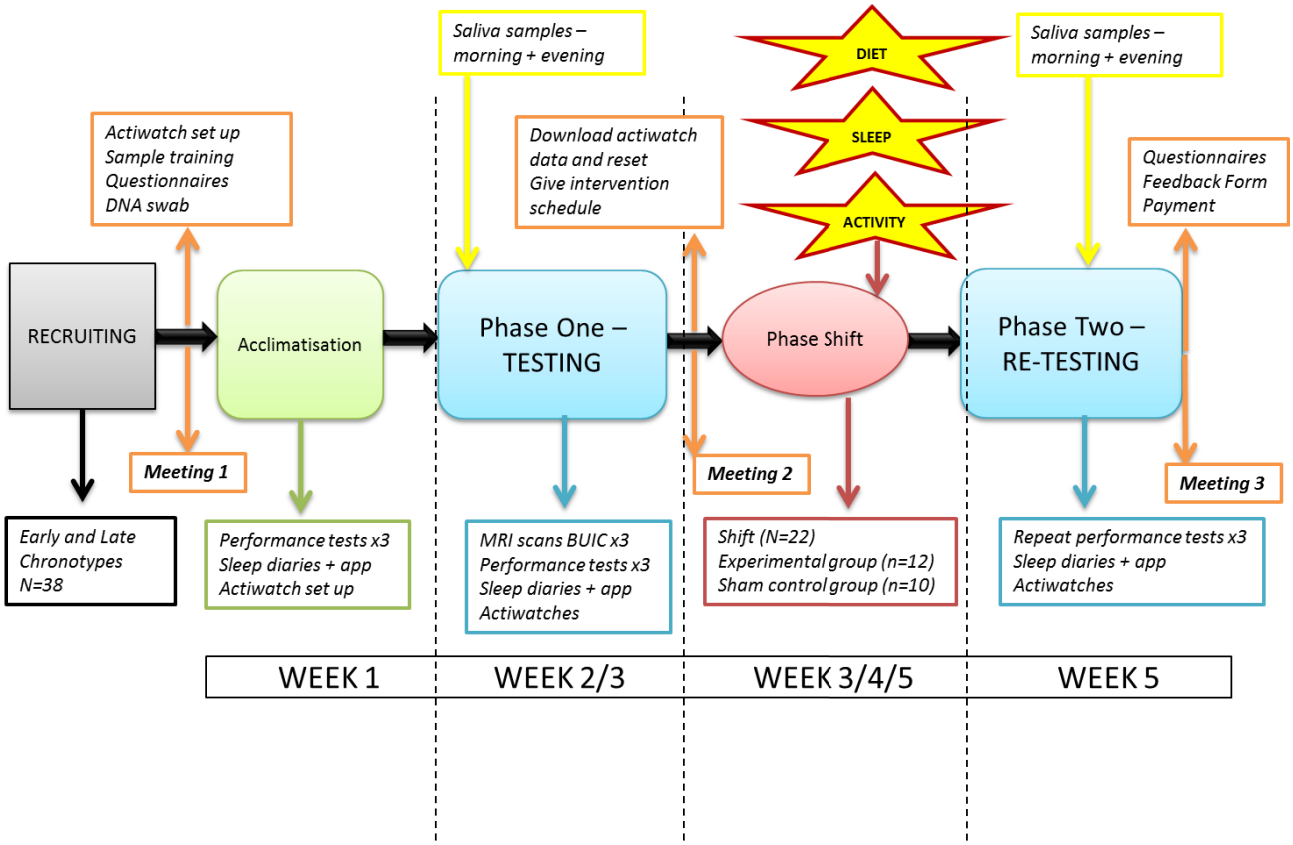


Figure 2.2. The full study protocol including recruitment, acclimatisation, Phase One and Phase Two. Details of meetings and testing sessions are also given along with timings for physiological sampling and behavioural monitoring throughout.

2.3.2 Phase One

Following at least two weeks' acclimatisation and monitoring of sleep patterns participants visited the Birmingham University Imaging Centre (BUIC) to undergo testing at 14:00 h, 20:00 h and 08:00 h (GMT) the following morning (details of testing sessions: sections 2.7 & 2.8).

2.3.3 Phase Two

The group of LCPs (N = 22) entered Phase Two of the study. Participants were randomly assigned to the experimental (n = 12) or control (n = 10) group prior to involvement in the

study. At the third testing session at the end of Phase One, both groups were given a schedule to follow for three weeks before repeating all procedures described in Phase One, except for the neuroimaging. Schedules were based on non-pharmacological interventions e.g. changes in sleep timings, exercise and diet and aimed to phase advance individuals (experimental) or not affect phase (control). The experimental group was asked to modify behaviour by moving bed/sleep and wake/get up times earlier, not consuming caffeine in the evenings and keeping a regular schedule. To give the control group something to do that would not have affected their circadian phase participants were asked to eat lunch at the same time each day. Further details are given in Chapter 5.

Table 2.2. Required involvement throughout the study for ECPs (red) and LCPs (blue). Through weeks 1 to 5 boxes are coloured in where participants were required to carry out the tasks detailed in the headings.

ECTs	Actiwatch	Sleep Diary	Sleep App	Testing Session	Performance testing	Physiological Sampling	Genetic Swab	Schedule	Meetings	Questionnaires
Week 1										
Week 2										

LCTs	Actiwatch	Sleep Diary	Sleep App	Testing Session	Performance testing	Physiological Sampling	Genetic Swab	Schedule	Meetings	Questionnaires
Week 1										
Week 2										
Week 3										
Week 4										
Week 5										

2.4 Behavioural Measures

2.4.1 Questionnaires

A battery of questionnaires was completed by all participants at the first meeting. The individuals who also completed Phase Two of the study were asked to repeat the battery of questionnaires at the last testing session to allow a comparison before and after non-pharmacological interventions.

2.4.1.1 Munich Chronotype Questionnaire (MCTQ)

The MCTQ, originally developed by Till Roenneberg in 2003, assesses individual differences in sleep times, light exposure and other behaviour on work and free days (Roenneberg et al., 2003). This allows the calculation of MSF_{sc} and further classification into 'Chronotypes' from extreme Early to extreme Late (see Figure 2.1 and Figure 2.3).

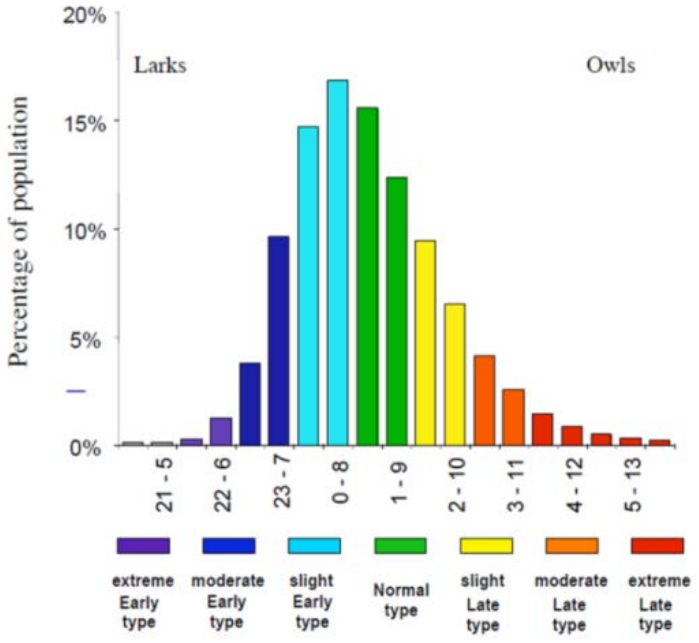


Figure 2.3. Distribution of chronotypes shown by percentage of population from extreme Early type to extreme Late type (taken from https://www.euclock.org/).

2.4.1.2 Epworth Sleepiness Scale (ESS)

The ESS is a self-reported questionnaire that assesses daytime sleepiness (Johns, 1991). Participants are asked to rate the likelihood of falling asleep in eight different situations from 0 'no chance of dosing' to 3 'high chance of dosing'. The scores are collated to give a value from 0-24 which relates to general levels of daytime sleepiness, with a higher score indicating more daytime sleepiness.

2.4.1.3 Karolinska Sleepiness Scale (KSS)

The KSS gives an indication of current sleepiness using a nine point Likert scale from 1: 'extremely alert' to 9: 'fighting sleep' (Åkerstedt and Gillberg, 1990). Subjective sleepiness measured by the KSS has been shown to correlate with objective sleepiness using EEG through PVT, the Karolinska Drowsiness tests and alpha attenuation tests (Kaida et al., 2006). KSS score will be referred to as daytime sleepiness throughout this thesis and was recorded before and after each testing session.

2.4.1.4 Pittsburgh Sleep Quality Index (PSQI)

Subjective assessment of sleep quality can be measured with the PSQI (Buysse et al., 1989). The PSQI asks a set of 19 self-rated questions about sleep/wake behaviour over the past month to allow a calculation of sleep onset, duration and latency as well as taking into account sleep efficiency and daytime dysfunction. A standard calculation is made using these variables, which results in a global score between 0-21. Higher scores are associated with poorer sleep quality.

2.4.1.5 Depression Anxiety and Stress Scale (DASS)

The DASS is a self-administered questionnaire that aims to measure the emotional states Depression, Anxiety and Stress (Lovibond and Lovibond, 1995). There is a long version consisting of 42 questions as well as a shortened 21 question version that was used in this study. Subjects are asked to rate how much statements apply to them over the past week from 0 'did not apply' to 3 'applied to me most of the time'. Both versions give separate scores for depression i.e. motivation, self-esteem and mood, anxiety i.e. fear, panic and alertness, and stress i.e. how unstable and tense the individual feels. As well as scores for the three individual measures, these scores can be combined to give an overall DASS score from 0-126. In addition, the DASS21 was used to uncover anyone reporting an unusually high score for depression, anxiety and stress. According to Lovibond and Lovibond (1995), a score above 21, 15 and 26 infers severe/extremely severe depression, anxiety and stress respectively. Therefore, any participant scoring higher than these on any scale was excluded. This resulted in one ECP and one LCP being excluded from the DASS analysis.

2.4.1.6 Profile of Mood States (POMS)

The POMS is a scale made up of 65 words to assess mood states. The participants, using a five point Likert scale, must rate each word from 'not at all' to 'extremely' based on their mood. The results are split into six different mood classifications including depression, tension, anger, vigour, fatigue and confusion (McNair D M, 1971). An overall 'mood disturbance' value was calculated by adding scores for depression, tension, anger, fatigue and confusion and subtracting the vigour score. One ECP and two LCPs were excluded from POMS analysis due to insufficient completion of the questionnaire in Phase One and one LCP in Phase Two.

2.4.1.7 Diet Questionnaire

Diet questionnaires are relatively easy to administer and can be used to assess food types consumed and timings through self-report. A food frequency questionnaire developed at the University of Surrey was used to collect information about the types of food consumed by each of the participants throughout the study as well as average meal times. Two ECPs and two LCPs did not answer the questionnaire correctly so were not included in the analysis.

2.4.1.8 Feedback Questionnaire

A feedback questionnaire was developed specifically for this study due to its longitudinal nature and close regular participation from subjects. This allowed an insight into participants' experience of the study including subjective ratings of sleep and wake related factors, subjective productivity and performance changes before and after the study. Two ECPs did not complete the feedback questionnaire.

2.4.2 Actiwatch Light (AWL)

For the duration of the study, participants were asked to wear wrist activity monitors to gather activity, sleep and light (1-32,000 lux) data throughout the time period. Each participant was allocated with an actigraph (Actiwatch® Light, AWLs, 2006, Cambridge Neurotechnology Ltd) during the initial meeting and given details of how to use them, including removing for bathing/showering and preventing sleeves covering them to allow light data to be gathered. Actiwatches were worn on the non-dominant wrist. Due to a maximum recording length of 22 days, data was collected at the third scanning session and AWLs were reset for participants entering Phase Two of the study. Actigraphy data was acquired from AWLs in 1-minute epochs (medium sensitivity setting) and analysed using

Sleep Analysis 7 Software (version 7.23, Cambridge Neurotechnology Ltd). Details of daily bed time and get up times were extracted from sleep diaries/smart phone app and inputted to the manufacturer's software which allowed in built algorithms to calculate sleep onset and wake up times per night, as well as other sleep and activity parameters (Table 2.3). Non Parametric Circadian Rhythms Analysis (NPCRA) was calculated from a spreadsheet designed by Eus Van Someren (Van Someren et al., 1999) and adapted by Benita Middleton, University of Surrey (Table 2.3). Example actograms for an ECP and a LCP can be found in Figure 2.4.

As a result of staggered testing sessions over the course of the study the number of days of actigraphy data collected varied between individuals. For this reason, between 13-16 days before the first testing session (Phase One) and between 13-16 days before the last testing session (Phase Two) were used in the analysis. Due to inaccurate recording or loss of actiwatch, one subject had the first phase of data excluded and two had the second phase of data excluded.

Table 2.3. Parameters calculated from Actiwatches using Sleep Analysis in manufacturer's software and Non-Parametric Circadian Rhythms Analysis (NPCRA).

Sleep Analysis			**Non-Parametric Circadian Rhythm Analysis (NPCRA)**
Actograms	Actual wake (%)	Moving time (mins)	Inter-daily Stability Index
Bed time	Sleep efficiency	Moving time (%)	Intra-daily Variability
Get up time	Sleep latency	No. of immobile phases	L5
Time in bed	Sleep bouts	Mean length immobility	L5-onset-phase
Sleep start	Wake bouts	One Minute immobility	M10
Sleep end	Avg wake movement	One Min immobility (%)	M10-onset-phase
Assumed sleep	Mean sleep bout time	Total activity score	Amplitude
Actual sleep time	Mean wake bout time	Mean activity score	Relative Amplitude
Actual sleep (%)	Immobile time (mins)	Mean score in active periods	
Actual wake time	Immobile time (%)	Fragmentation index	

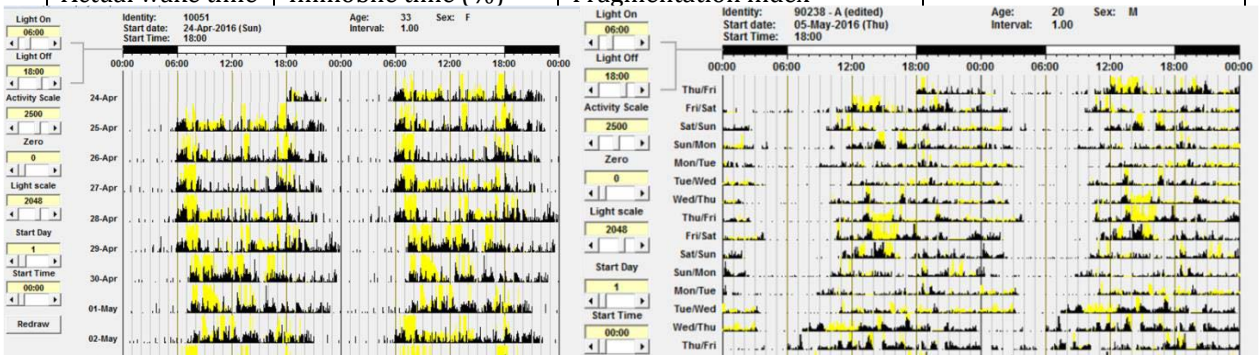


Figure 2.4. Example actograms for an ECP and a LCP using Actiwatch Light (CamTech) and Sleep Analysis 7 software. Left panel shows recordings from an ECP and right panel from a LCP. Black lines indicate activity and yellow indicates light exposure.

2.4.3 Sleep Diaries

In combination with actigraphy and to facilitate actigraphy analysis, each participant was given a sleep diary which they were asked to fill out on a daily basis. This allowed information to be gathered on bed times, sleep times, wake up times, alcohol intake, sleep quality, naps and times when actiwatches were removed. This data was used in conjunction with the actigraphy (Appendix A).

2.4.4 Research Sleep Diary App

A smart phone app, developed by researchers at the University of Michigan, was used in this study to gather data allowing an analysis and validation of smart technology against actigraphy. Participants were instructed to download the app on the TestFlight platform (iOS) or download an attachment (android). Data gathered from the app included daily information about bed times, get up times, light exposure, meal times, caffeine intake and exercise (Figure 2.5). All data was anonymised by using ID numbers when setting up the

app. Bed time and get up times were extracted and used in addition to sleep diaries to calculate actigraphy parameters.

2.4.5 Automatic SMS Service

To try and maximise compliance and minimise drop out, an online SMS software programme (https://www.esendex.co.uk/) was used to send automatic text messages to participants at various points throughout their involvement in the study. The details of these text messages included:

- Reminders of initial set up meetings and locations
- Reminders to complete acclimatisation tests
- Details of saliva sample collections (dates/times)
- Reminders to export smart phone app data
- Reminders to fill out daily sleep/wake diaries and smart phone app
- Reminders of when to take saliva samples and to follow the protocol
- Reminders that testing sessions were during the following week
- Reminders for repeat testing sessions
- A reminder 2 h before each testing session
- Reminders not to drink caffeine or alcohol during the specified period before testing

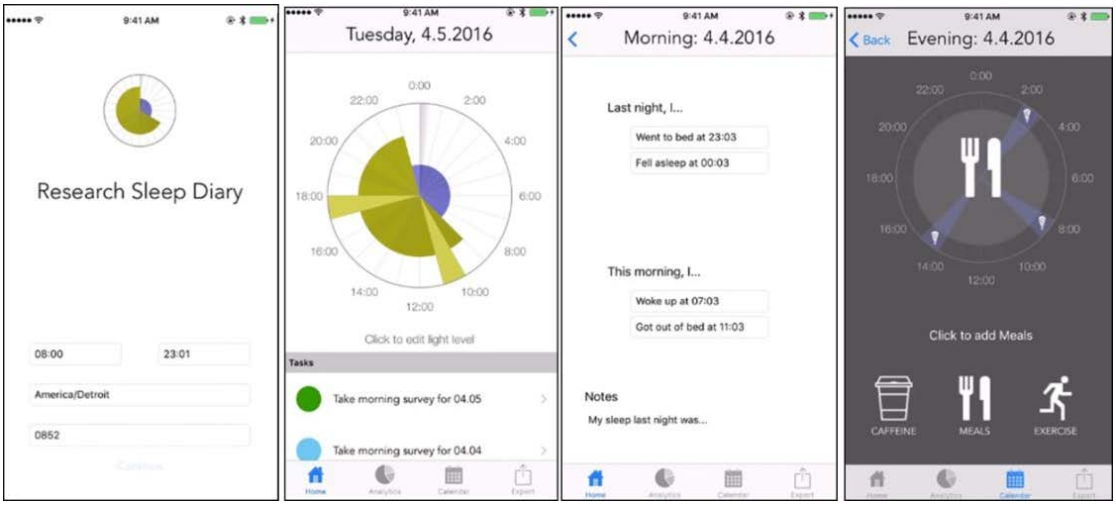


Figure 2.5. The smart phone interface of the sleep research app used during the study.

2.5 Participant Pack

All participants were provided with an individual pre-prepared folder at the start of the study that included all relevant documentation and equipment for the duration of the experiment. Details of the 'participant pack' are given below (Figure 2.6 & Appendix F).

2.5.1 Important Information

Documents were provided detailing contact details of the researchers, start and end date of involvement in the study, directions to BUIC, dates booked for testing sessions and serial number of actiwatch.

2.5.2 Weekly Tick Lists

Participants had weekly tick lists placed chronologically through the folder that gave information on what tasks needed to be completed each week e.g. sleep diary, smart phone app, physiological sampling, testing sessions. There were also options to give information on any reason data could have been affected during the week in question.

2.5.3 Sampling Protocols and Packs

The participant pack also contained the relevant protocols for physiological sampling (Appendix B&C). Pre-prepared labelled sampling packs were placed in wallets at the back of the participant pack folder. To try to control the effects of light exposure and noise on sleep, all participants were given an eye mask and ear plugs (Figure 2.6).

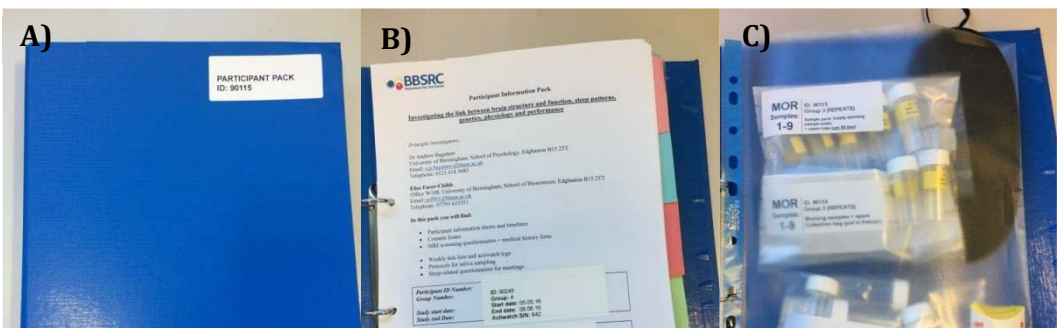


Figure 2.6. An example participant pack showing A) Front cover of folder labelled with ID number B) Front page with details on emergency contacts, study details and important information and C) Physiological sampling packs in a plastic wallet at the back of the participant pack with eye mask and ear plugs.

2.6 Physiological and Genetic Measures

For physiological and genetic data collection, COSHH risk assessments and biological assessment forms were completed and approved by the University of Birmingham's Advisory Group on the Control of Biological Hazards. All samples were anonymised.

2.6.1 Physiological Samples Collection

Participants were asked to provide saliva samples to measure hormonal (cortisol and melatonin) rhythms. These were done in their home environment by spitting into pre-labelled polypropylene collection tubes (7ml plastic bijou, Figure 2.7). Prior to sampling participants were trained to take the samples in a meeting with the postgraduate researcher and given specific protocols to follow (Appendix B&C). Participants were asked to choose two days within the week of testing where they could commit to giving saliva samples during one evening (to measure DLMO) and during one morning (to measure the cortisol awakening response, CAR). Collections were carried out on the week of testing but not on testing days as evening and mornings were disrupted by attendance at BUIC. For

analysis of the DLMO participants were instructed to collect saliva samples during one evening every 30 minutes from 3-4 h before habitual bedtime until one hour after. For analysis of cortisol rhythms participants were instructed to collect saliva samples over a 3 h period during one morning (upon wake up, every 15 minutes for the first hour and every 30 minutes for the following 2 h). Samples were collected during Phase One and repeated during Phase Two. Once collected, participants were asked to seal the tubes and place the samples in pre-labelled grip seal bags at -20˚C for storage so no contact with samples would be made until processing. The samples were collected on ice within 48 h. They were kept at -20˚C and shipped on dry ice to the University of Surrey for processing (where they were rendered acellular) within 7 days. Shipping followed all transport regulations and samples were sent in double layered styrofoam boxes with dry ice class 9 labels, together with a material transfer document which recorded the details in line with the Human Tissue Act 2004.

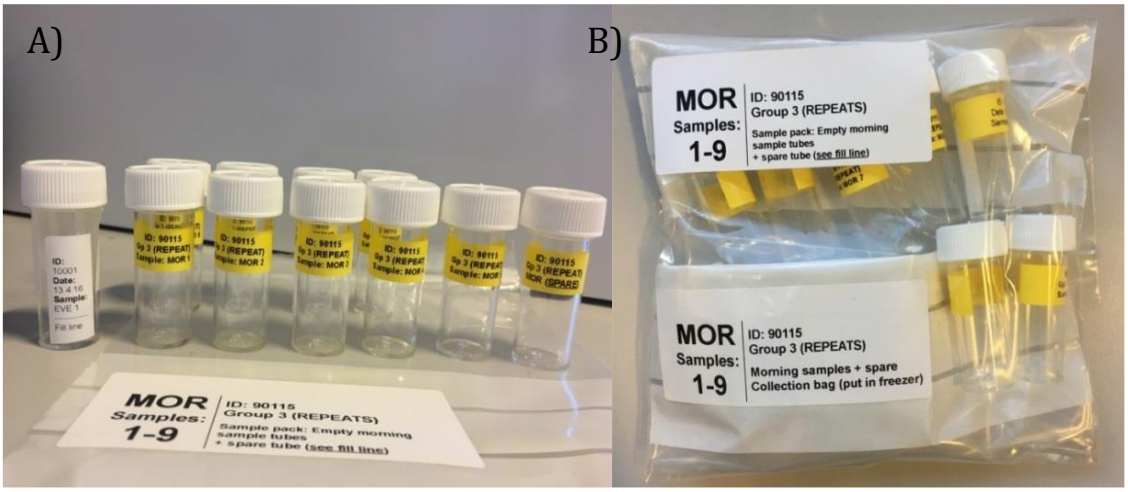


Figure 2.7. Images of an example physiological sampling pack showing A) Pre-labelled tubes for morning samples 1 to 9, and B) Pre-packaged morning sample kit complete with labelled tubes in grip seal bag and collection bag for collected samples.

2.6.2 Physiological Samples Analysis

Radioimmunoassays (RIA) of melatonin and cortisol in human saliva were performed (Stockgrand Ltd, University of Surrey) using an Iodine^{125} radioactive labelled tracer and solid phase separation. The procedure followed that published in Moreno et al. (2015). Quality controls (QCs) and intra-assay coefficients of variation (CVs) can be found in Table 2.4. In RIA QCs are used to confirm accurate and reliable results, as well as uncover any random error (Drew, 1985). In this study in house QCs (salivary melatonin QCs are not available commercially) were used at the beginning and end of each assay to check for drift (time lag from adding reagents). A standard curve was created with known concentration of antiserum for each assay. CVs are often used in immunoassay analysis to describe the reliability and reproducibility of the tests. CV is calculated by dividing the standard deviation by the mean (Schultheiss and Stanton, 2009). This can be used to show consistency between multiple measures e.g. different assay plates (inter-assay CV, values <15% is acceptable), or within sample measures e.g. duplicates of the same sample (intra-assay CV, <10% is acceptable).

Table 2.4. Summary table of quality controls and intra assay coefficients of variance for salivary cortisol and melatonin assays.

	Low QC mean	**Medium QC mean**	**High QC mean**	**Average**
Salivary Melatonin Inter-assay CV	9.0 pg/ml SD = 1.1 CV = 12.2%;	20.1 pg/ml SD = 2.0 CV = 9.9%;	44.4 pg/ml SD = 4.2 CV = 9.4%.	10.5%
Salivary Cortisol Inter-assay CV	3.0 nmol/l SD = 0.3 CV = 9.8%;	15.9 nmol/l SD = 1.0 CV = 6.1%	48.0 nmol/l SD = 3.9 CV = 8.3%.	8.1%
	Low	**Medium**	**High**	
Salivary Melatonin Intra-assay CV	5.8 pg/ml CV = 9.1%	25.9 pg/ml CV = 10.1%	42.8 pg/ml CV = 9.1%	9.4%
Salivary Cortisol Intra-assay CV	3.3 nmol/l CV = 10.8%	6.4 nmol/l CV = 8.8%	24.7 nmol/l CV = 5.3%.	8.3%
Cortisol Limit of Detection	Mean = 0.45nmol/L			

2.6.2.1 Melatonin Analysis

DLMO was calculated by using the mean of the baseline concentration values plus two standard deviations of the mean. This gave a concentration for DLMO which could then be used to calculate the timing of melatonin onset. Peak time and concentration of melatonin were calculated by identifying the time at which the highest concentration of melatonin was measured per participant. Phase angle (minutes) was calculated by subtracting DLMO from average sleep onset time gained from actigraphy analysis. Due to insufficient or contaminated samples, DLMO values were unable to be calculated for two ECPs and four LCPs in Phase One and five LCPs in Phase Two.

2.6.2.2 Cortisol Analysis

Cortisol analysis included the calculation of:

1) The CAR (percentage change between first and third samples)
2) Peak cortisol concentration (highest concentration recorded)
3) Time of peak cortisol concentration (time point of highest concentration recorded)
4) Total area under the curve (AUC_t) with respect to baseline (0.5nmol/l) (Fekedulegn et al., 2007)
5) Area under the curve for the first hour (AUC_1) with respect to baseline (0.5nmol/l) (Fekedulegn et al., 2007)

2.6.3 Genetic Analysis

Participants were asked to give a non-invasive buccal mucosa (cheek) swab to assess genetic traits linked to sleep patterns, specifically the identification of the variable number tandem repeat (VNTR) polymorphism in per3 clock gene. The buccal DNA swabs were taken in a meeting with the postgraduate researcher and followed a specific protocol

(Appendix D). Participants were asked to wash their mouth out with water before taking the swab. Each individual then took a pre-labelled buccal mucosa swab and rubbed each side of their mouth 10 times before inserting the swab into a sterile tube (Sarstedt). They were then placed into a -20˚C freezer until being shipped on dry ice to the University of Surrey for analysis. Genotyping assays were carried out by Professor Simon Archer at the University of Surrey using PCR and followed a previously published procedure (Archer et al., 2003). One ECPs genotype could not be determined in the analysis (Table 2.5).

Table 2.5. Distribution (count and percentage) of per3 genotypes in ECPs and LCPs.

Group	**Per3 (4/4)**		**Per3 (4/5)**		**Per3 (5/5)**	
ECPs	n=10	66.67%	n=4	26.67%	n=1	6.67%
LCPs	n=14	63.64%	n=8	36.36%	n=0	0.00%

2.7 Performance Measures

2.7.1 Cognitive Performance

2.7.1.1 Chrono-Memory and Attention Test (CMAT)

At each testing session, participants were taken into a separate testing room and asked to complete a short cognitive test on a desktop computer (DQ67OW, Intel® Core™ i7-2600 processor, 4GB RAM, 32-bit Windows 7). The CMAT is a tool designed by occupational psychology firm Team Focus Limited (based on https://teamfocus.co.uk/memory-attention-test-mat/), and is available on an online platform (https://www.profilingforsuccess.com). The CMAT includes measurements of reaction time through the PVT (Dinges, 1995), reaction time with interference through the Stroop colour-word task (Stroop, 1935), short term memory through a pairs task and executive

function through a more complex task (Figure 2.8). The test also includes a short questionnaire before and after the test to gather details of sleep/wake timings the day of the test as well as hours since eating, consuming caffeine and exposure to natural light. The variables used in this thesis (PVT and Stroop) will be referred to as measures of cognitive performance throughout experimental Chapters.

2.7.1.2 Psychomotor Vigilance Task (PVT)

As discussed, the PVT has been widely used in many fields of research as well as clinically (Dinges, 1995, Dinges and Powell, 1985). It assesses reaction time (s) under sustained attention and is the most widely used cognitive performance test in sleep and circadian research (Blatter and Cajochen, 2007). It requires the subject to look at a blank screen and respond whenever a stimulus is presented (Figure 2.8A). A recorded response in the absence of a stimulus is measured as a 'false start' (Dinges and Powell, 1985). Numerous studies have linked both long and short versions of the PVT to sleep deficits e.g. sleep deprivation (Basner et al., 2011, Dinges et al., 1997) as well as circadian disruption (Goel et al., 2013, Kline et al., 2010). These studies show negative impacts on PVT performance with sleep loss and circadian misalignment. PVT is therefore a useful tool to capture an element of waking performance in studies involving time of day and sleep patterns.

2.7.1.3 The Stroop Task

The Stroop task is a reaction time test that introduces interference causing the need to process irrelevant information as well as the information that requires a response. The test is based on a phenomenon called the Stroop effect that was presented by J. R. Stroop (1935), although its origins date back to the 19th Century (Cattell, 1886). Many versions of the Stroop

test have now been developed but the basic principles remain the same. This requires a subject to decide on a response when presented with two stimuli simultaneously. A word stimulus is given in the name of a colour and a colour stimulus is given by the colour of the said word e.g. the word RED presented in blue ink (Figure 2.8B). Individuals can be asked to report either the name of the colour or the colour itself. It is thought that the brain automatically registers the word and its semantic meaning but must process the information further to determine the colour creating a delay in response time (Jensen and Rohwer, 1966, Stroop, 1935). Stroop tasks are often used as a measure of cognitive performance in sleep and circadian research (Ramirez et al., 2012, Schmidt et al., 2012).

2.7.1.4 Short Term Memory Task

To gain a measure of short term memory participants were asked to complete a matching pairs task (Figure 2.8C). A 6 x 6 block design was presented containing 36 squares. Participants were asked to uncover squares by clicking on them which would show an image. If a pair was not uncovered both images would disappear. Once a pair was found these images would remain uncovered. Time until completion of task was taken as a measure of short term memory.

2.7.1.5 Executive Function Task

The final task in the CMAT required a higher level of cognitive processing due to the need to retain information required for the task. Subjects were presented with a screen containing a number of different coloured shapes and asked to complete a task based on a rule e.g. click on all the blue diamonds. Following this a subsequent, more complex rule was given e.g. click on all the red circles unless there is a black square. There were a total of five

different rules with five trials for each that needed to be completed (total of 25 trials). The basic principles remained with the complexity of the rule increasing from first to last. Time until completion of task was taken as a measure of executive function (Figure 2.8D).

Due to the CMAT being repeated multiple times the trials within each task were randomised so that the tests could not be learnt. For example, PVT stimuli were presented with randomised times between trials, Stroop words were presented in a randomised order, placement of images in the pairs task were randomised and rules in the executive function test were changed each time.

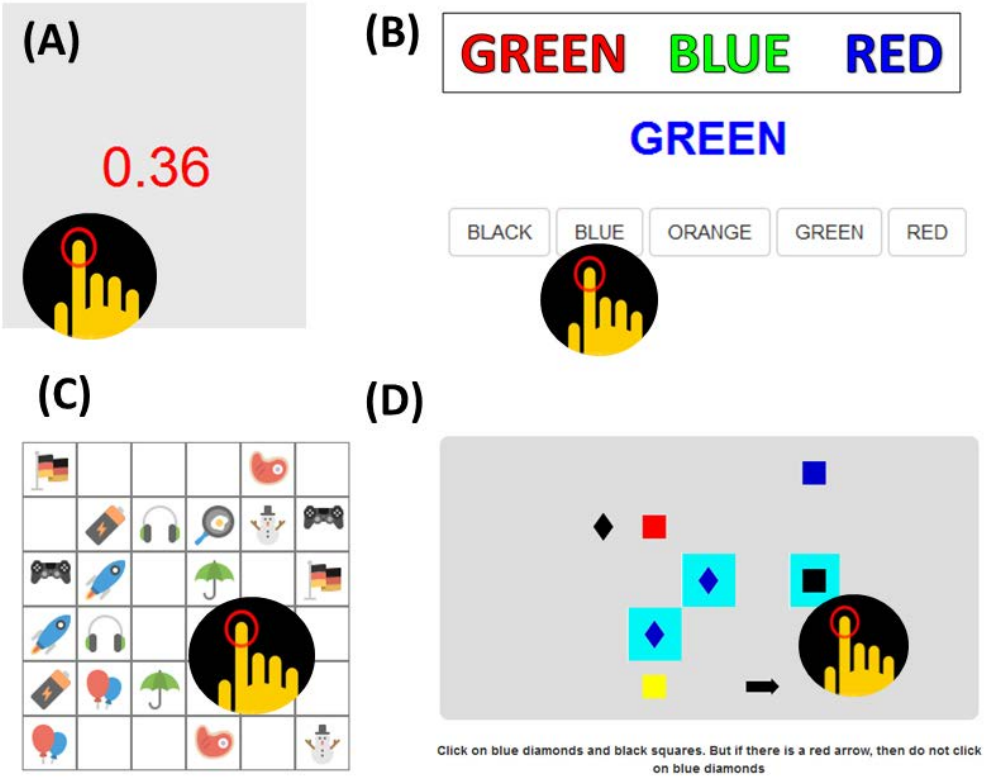


Figure 2.8. Example of stimuli presented the Chrono-Memory and Attention Test (CMAT). A) Psychomotor vigilance task (PVT). B) Stroop colour-word test. C) Pairs memory task. D) Executive function task.

2.7.2 Physical Performance

2.7.2.1 Isometric Grip Strength

After completion of the CMAT participants were asked to do a muscle strength test to gather a simple index of physical performance (Bohannon, 2001). Maximum Voluntary Contraction (MVC) was measured using the six second isometric grip strength test using an electronic hand dynamometer (EH101, CAMRY). Participants were standing with the elbow fully extended at 180˚ and used their dominant hand in a pronated position to apply as much grip pressure as possible on a dynamometer for six seconds. The maximum value was recorded in kilograms (Kg). This process was repeated three times with two minute intervals between. The maximum score achieved was used in the analysis. Due to motivation affecting performance (Marcora, 2008) a strict protocol was adhered to with a script being read out each time to encourage the participant to try their best (protocol can be found in Appendix E). To keep the protocol standardised, the same researcher assessed the task for all participants. MVCs of isometric grip strength will be referred to as a measure of physical performance throughout experimental Chapters.

2.8 Neuroimaging

Rs-fMRI data is relatively easy to acquire due to there being no task demands in the scanner and therefore increased compliance from subjects. However, analysing the data remains far from simple. Therefore, the first part of this section will discuss confounding factors affecting fMRI data and what can be done to minimise these effects. The second part will provide details of fMRI data acquisition and analysis carried out in this study.

2.8.1 Confounding Factors in fMRI

Being able to detect small changes in BOLD signal that are coming from fluctuations in neuronal activity is challenging due to a number of confounding i.e. non neuronal factors that have been shown to contribute to variations in the BOLD signal (Birn, 2012). There are a several steps required to prepare the data for analysis and remove any signal changes from 'non-neuronal' sources, discussed below. These factors, which include movement, scanner variability (7T, 3T and 1.5T), global signals and physiological noise, need to be measured and removed during preprocessing (Figure 2.9).

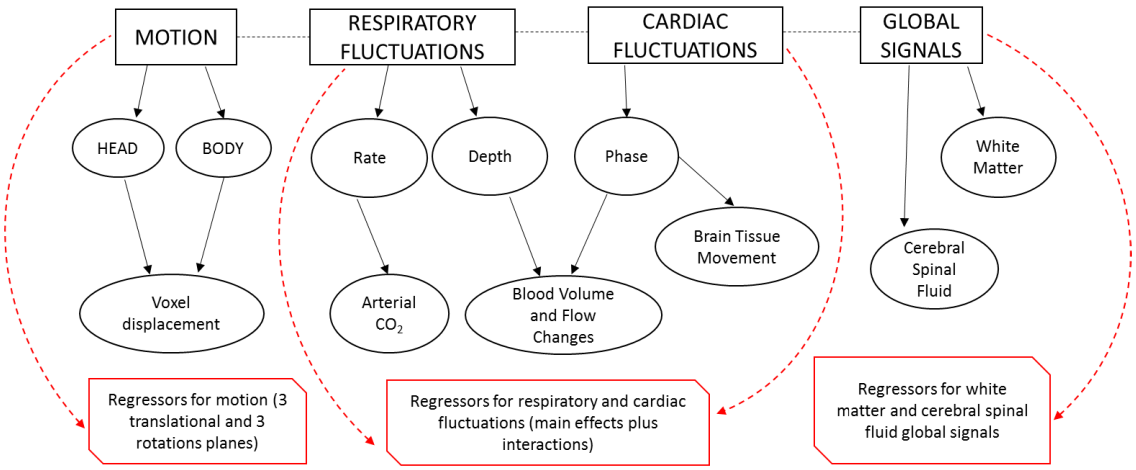


Figure 2.9. Confounding factors affecting BOLD signal in fMRI data . Details are given to show how these factors can be accounted for during preprocessing of data and added as nuisance regressors to minimise contributions of non-neuronal sources.

2.8.1.1 Physiological Noise

Fluctuations in cardiac and respiratory rates have been shown to influence BOLD signals in fMRI and therefore need to be taken into account in resulting analysis (Birn et al., 2006). Changes in respiratory and cardiac output e.g. breathing and heart rate can cause up to 60% of variation in fMRI data and affect many different parts of the brain and blood vessels (Hutton et al., 2011). Low frequency oscillations of less than 0.1Hz caused from physiological noise can induce global BOLD changes which introduce correlations in signals from widespread regions. This signal can be mistaken for FC and has been shown in a

number of resting-state networks including the DMN (Birn, 2012). Retrospective Correction of Physiological Motion Effects in fMRI, also known as RETROICOR, is a method that uses Fourier expansion to correct for physiological noise in the image domain. This technique, which was originally explored by Josephs et al. (1997), was further developed by Glover et al. (2000) and is widely used to reduce the unwanted effects of cardiac and respiratory fluctuations on fMRI data. PhysIO is a Matlab based toolbox (http://www.translationalneuromodeling.org/tnu-checkphysretroicor-toolbox/) that utilities cardiac and respiratory data recorded from ECG, pulse oximeters and pneumatic belts to model physiological noise. The outputs can be added to general linear model (GLM) analysis as regressors of no interest, thereby reducing the effects of physiological noise on the BOLD signal (Figure 2.10). Within PhysIO, modelling includes RETROICOR (Glover et al., 2000), heart rate variability (HRV) i.e. the time difference interval between heart beats, and respiratory volume per time (RVT) i.e. variation between breaths, through cardiac and respiratory response functions (Birn et al., 2006, Chang et al., 2009).

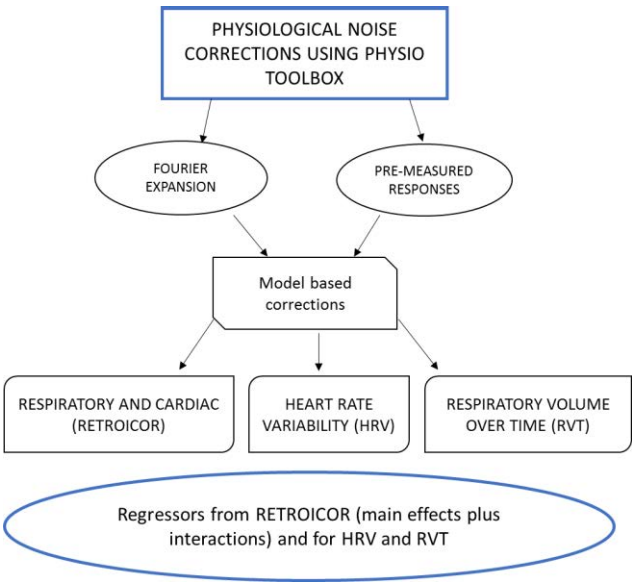


Figure 2.10. Flow chart describing the data acquired from the PhysIO toolbox to model physiological noise in fMRI data.

2.8.1.2 Head Motion

Another factor that can create spurious correlations in fMRI data is head motion. Along with movement causing potential voxel misalignment between images it can also cause signal fluctuations leading to misinterpretation of results (Van Dijk et al., 2012). Conventionally, data is motion correction during preprocessing routines and nuisance regressors from three translational (x, y and z) and three rotational (pitch, roll and yaw) axes are created to add to the GLM (Jenkinson et al., 2002). To correct for movement in space individual subject movement is estimated and volumes realigned using rigid body transformations at each time point e.g. a 15 minute scan with a TR of 2 seconds creates 450 volumes. These values can be summarised into a single measurement which can be used to compare motion between subjects or groups as well as exclude those over specified thresholds. However, recent work by Power et al. (2012), has suggested that these methods do not account for total signal changes due to head movement and have proposed an additional approach to motion corrections that uses two more detailed measurements of motion. The first, named framewise displacement (FrD), calculates image by image changes in movement for both translational and rotational planes. FrD converts degrees to millimetres for rotational displacements by calculating the average distance from cortex to centre as if the brain were a sphere (radius of 50mm). The second measure determines the "temporal derivation of time courses and movement variance over voxels" (DVARS). This allows a measure of whole brain signal intensity change, image by image, related to the previous image. Taking these extra methods into account provides a more comprehensive picture of the effects of head motion on BOLD signal and should therefore be considered when analysing rs-fMRI data (Power et al., 2012, Power et al., 2014).

2.8.1.3 Global Signals

There is an assumption that the BOLD signal measured comes from neuronal activity through the theory of neurovascular coupling, as discussed previously (Attwell and Iadecola, 2002). Along with physiological changes in respiratory and cardiac function and movement, oscillations in white matter (WM) and cerebral spinal fluid (CSF) are also attributable to the numerous non-neuronal sources discussed previously and thus need to be removed when preprocessing fMRI data (Windischberger et al., 2002). This is done by creating an average signal (all voxels) from both WM and CSF time series. These can then be used as nuisance regressors in the GLM. Furthermore, creating a time course of the spontaneous fluctuations which are shared across regions and averaging it across the brain is known as the global resting state signal (Saad et al., 2012). Global signal changes have been associated with differential BOLD responses (Murphy et al, 2009), and have been shown to introduce stronger anti-correlations between resting state networks e.g. the DMN and the DAN (Buckner et al., 2013, Fox et al., 2009).

Removing these through global signal regression (GSR), even though they may be of neuronal origin (Schölvinck et al., 2010), remains a hot topic of controversy within rs-fMRI literature. Up until 2009 research had shown that using GSR results in very clear networks that are anti-correlated at rest e.g. DMN and DAN (Fox et al., 2005, Fransson, 2005). However, in recent years many studies have highlighted the pitfalls of using GSR suggesting that it can alter FC results from individual to group level and increase the anti-correlation between ICNs or even introduce artificial correlations (Gotts et al., 2013, Murphy et al., 2009, Saad et al., 2013). Therefore, GSR was not used within this study, which will be discussed further in the final Chapter under limitations (section 6.6).

2.8.2 Scanning Sessions

Imaging data were acquired using a Philips Achieva 3 T MRI scanner with a 32-channel head coil at the BUIC. 15-minute resting state scans were obtained at each scanning session preceded by a five minute T1 scan. Whole brain coverage gradient echo-planar imaging (EPI) data were acquired parallel to the AC-PC line with the following parameters: 450 volumes, TR=2000ms, TE = 35ms, flip angle = 80˚, 3x3x4mm voxels. Standard high resolution anatomical T1-weighted scans (1mm isotropic) were also collected to facilitate co-registration. Respiratory and cardiac fluctuations were recorded with the pulse oximeter and pneumatic belt provided by the scanner manufacturer. Standard BUIC operating procedures were followed for the MRI safety screening and during the scanning sessions, and participants were not asked to perform any task. Subjects were talked to before and after each scan to confirm that they were comfortable and willing to continue and were free to terminate the sessions at any time. Subjects were instructed to lie still, not cross arms or legs and keep eyes open for the duration of the scan. Foam pads were placed around the head to maximise comfort and minimise movement. All participants were given ear plugs and ear defenders to minimise noise within the scanner. They were also provided with a button with which they could alert the researchers if they wanted to stop the session at any time. It has been proposed that differences in FC can arise when individuals close their eyes during rs-fMRI (Patriat et al., 2013), and it has also been suggested that there is a danger of subjects falling asleep during a supposedly waking scan (Tagliazucchi and Laufs, 2014). For that reason, a camera was placed in the scanner during each session to monitor participants' eyes and confirm that sleep had not been initiated. If eye closure exceeded fifteen seconds, which is half a 30s epoch according to the standard sleep staging approach by Berry et al. (2012a), the scan was re-started. This occurred in one scan from one participant.

2.8.3 Neuroimaging Preprocessing & Analysis

FMRI preprocessing and analysis was performed using UF²C (de Campos et al., 2016, http://www.lni.hc.unicamp.br/app/uf2c/), PhysIO (http://www.translationalneuromodeling.org/tnu-checkphysretroicor-toolbox/, Kasper et al., 2017), and SPM12 (http://www.fil.ion.ucl.ac.uk/spm/, Penny et al., 2011), toolboxes implemented in MATLAB (MathWorks, USA).

2.8.3.1 FMRI Preprocessing

Preprocessing was carried out in UF²C using standardised methodologies and general linear modelling implemented in SPM12. Data were re-orientated to the anterior commissure as origin, motion corrected using rigid body transformations (three translational and three rotational planes) and spatially smoothed with a 6mm Gaussian kernel. Scans were co-registered to Montreal Neurological Institute (MNI) space following high pass temporal filtering (>0.01 Hz). Physiological noise corrections (RETROICOR, HRV, RVT) were modelled using the PhysIO toolbox (Kasper et al., 2017), and added to preprocessing routines as nuisance regressors in UF²C, along with whole brain signals for WM and CSF. Framewise displacement (FrD) and deviation of variance (DVARS) were calculated (Power et al., 2012, Power et al., 2014), and any scan with an average FrD value above 0.5mm was excluded. This resulted in one scan (ECP, 14:00 h) being removed from further analysis. Head movement (translational, rotational, FrD and DVARS) did not differ significantly between the groups or between times of day (Figure 2.11 & Figure 2.12).

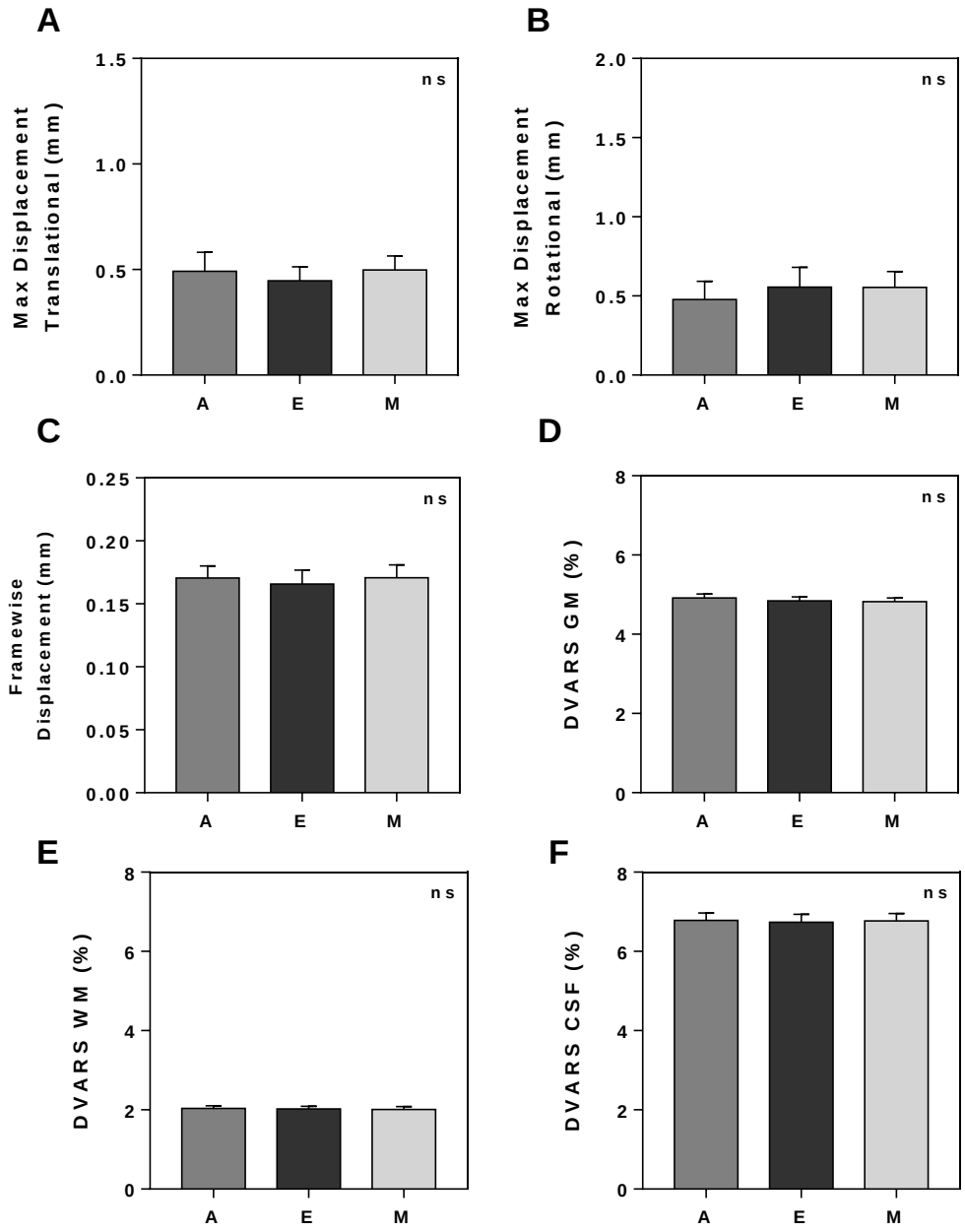


Figure 2.11. Head movement analysis as a function of time of day (A – 14:00 h, E – 20:00 h, M – 08:00 h). A) Maximum displacement value in the translational plane. B) Maximum displacement value in the rotational plane. C) Framewise displacement. D) Deviation of variance for grey matter. E) Deviation of variance for white matter. F) Deviation of variance for cerebral spinal fluid.

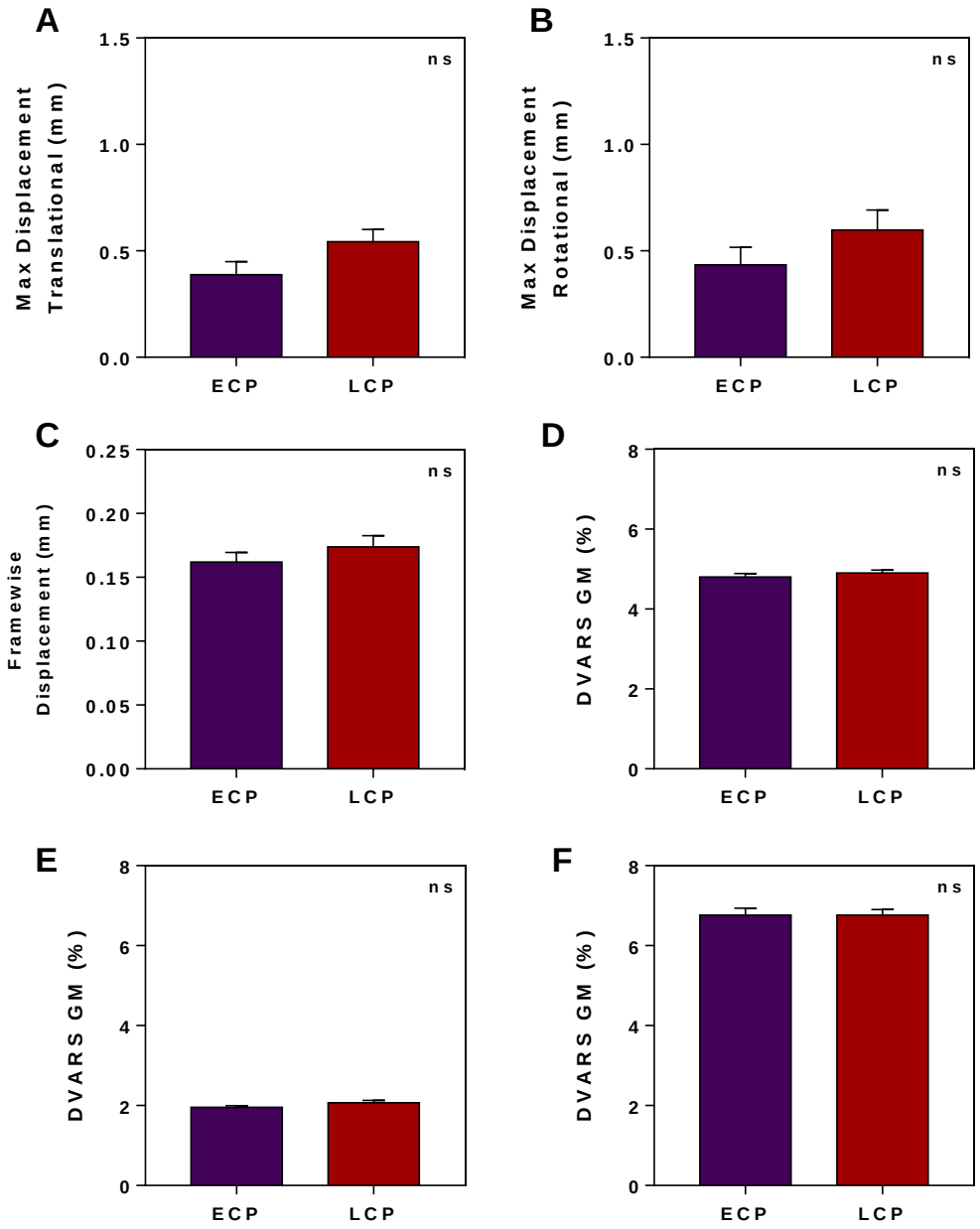


Figure 2.12. Head movement analysis as a function of Circadian Phenotype (ECP – Early, LCP - Late). A) Maximum displacement value in the translational plane. B) Maximum displacement value in the rotational plane. C) Framewise displacement. D) Deviation of variance for grey matter. E) Deviation of variance for white matter. F) Deviation of variance for cerebral spinal fluid.

2.8.3.2 Functional Connectivity Analysis

A seed based FC approach was used to analyse the data. Using a number of predefined seeds (https://findlab.stanford.edu/functional_ROIs.html, Shirer et al., 2012), whole brain correlation maps were created using UF^{2}C, which were then smoothed and normalised (Fishers z transformation). Seeds used in Chapter 3 were the mPFC and PCC regions of the DMN and the seed used in Chapter 4 was the LM1 (left primary motor cortex) of the MN. The mPFC and PCC seed were chosen as key hubs of the DMN which has been linked to maintenance of consciousness and regulating cognitive processing (Greicius et al., 2003) and is of interest when looking at the relationship between rs-FC and cognitive performance. The LM1 seed was chosen to investigate the link between rs-FC of the MN and isometric grip strength in right handed individuals. Further details for why each of these seeds was chosen are expanded upon in individual Chapters. These maps represented the output from correlating the average BOLD time series from the seed with BOLD time series of all other voxels, resulting in individual correlation maps for each scan and each seed. Pearson correlation maps were then converted to z-maps. Only positive correlations were used in the analysis. Details of ROIs used can be found in Figure 2.13 and Table 2.6. Using the GLM implemented in SPM12, second level group analysis were performed using a flexible factorial design in SPM12 (Figure 2.14). Subject, group and time of day were added as factors and the model was set up for the main effect of group (ECPs and LCPs), the main effect of time of day (morning; 08:00, afternoon; 14:00 and evening: 20:00 h) as well as the interaction of group and time of day. All subject variability including age and gender were accounted for by adding subject as a factor. Significant clusters that were different between groups were identified at a significance level of $p<0.05$ corrected for family wise error (FWE). Extent thresholds were chosen based on a fifth of the largest identified cluster for reporting purposes. This was defined at 100 voxels for mPFC seed,

150 for PCC and 30 for LM1 seed. Coordinates of significant clusters were taken and average correlation values between these ROIs and the seed were extracted using in house Matlab code. This resulted in a single correlation value representing rs-FC per participant for each scan between specific regions or average for all clusters identified. These values were used to explore the relationships between rs-FC and other variables collected in the study. Throughout this thesis peak t scores, cluster size (voxels) and MNI coordinates are given (reported as [x y z] which represents the location of the cluster centroid).

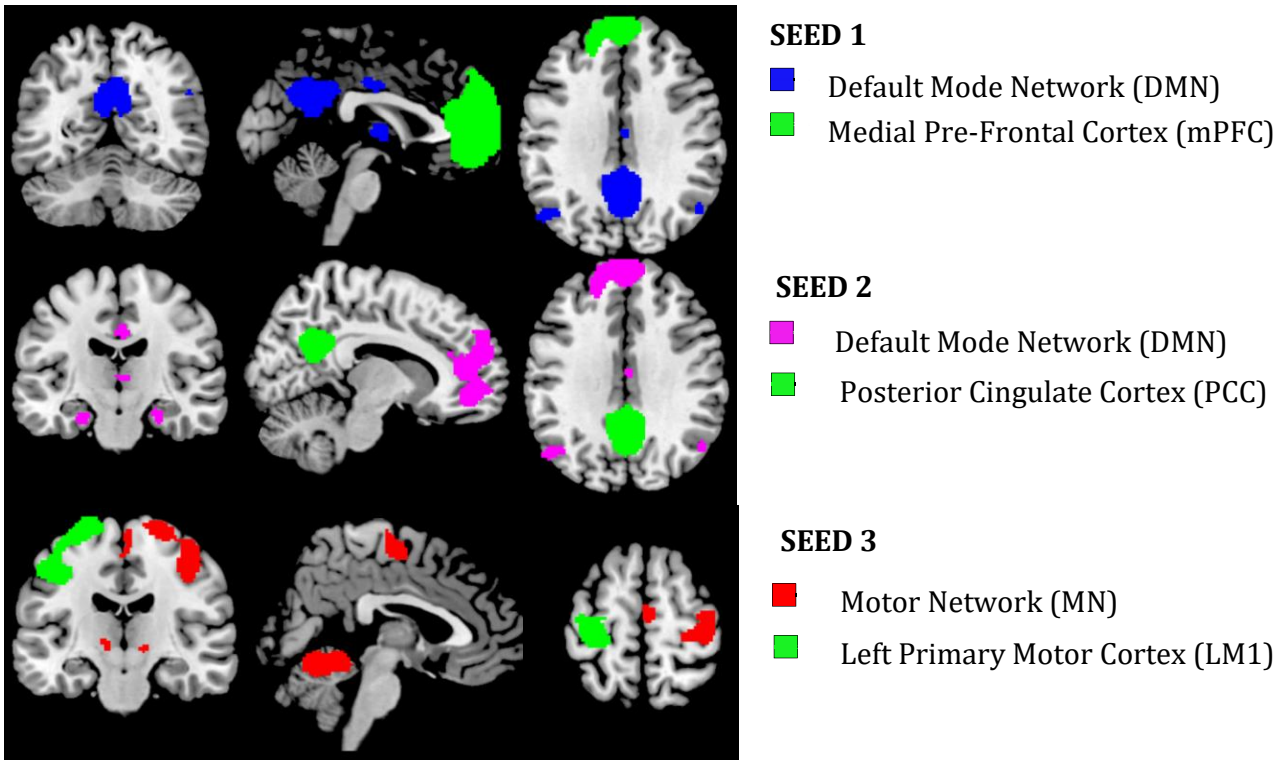


Figure 2.13. Details of ROIs used in seed based analysis. ICA networks (findlabs) are shown in blue and pink (default mode), red (sensorimotor) and the seed is shown in green. ROIs are overlaid on a brain extracted template image (MRIcron).

Table 2.6. Origins of ROIs used in seed based analysis.

Network	Region	Origin of ROI
Default mode network (DMN)	Medial prefrontal (mPFC)	FindLabs Dorsal Default Mode Network, (https://findlab.stanford.edu/functional_ROIs.html)
	Posterior cingulate (PCC)	
Sensorimotor (MN)	Left primary motor cortex (LM1)	FindLabs Sensorimotor Network, (https://findlab.stanford.edu/functional_ROIs.html)

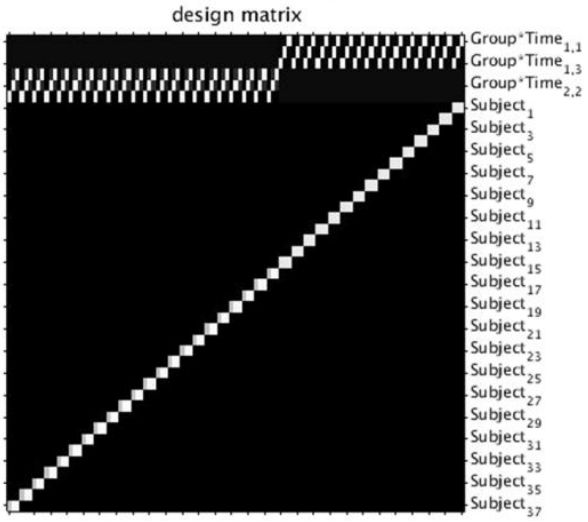


Figure 2.14. Design matrix of flexible factorial design created in SPM12. Factors added were subject, group and time of scan (three scans per subject).

2.9 Statistical Analysis

Statistical analysis specific to each Chapter is detailed in individual Chapter methods sections. In brief, statistical comparisons of behavioural data between the groups (Phase One; ECPs and LCPs, Phase Two; Experimental and Control) were performed in GraphPad Prism (version 7, https://www.graphpad.com/scientific-software/prism/) and SPSS (IBM SPSS Statistics, version 24, Chicago) using two sample unpaired t-tests, Mann-Whitney U tests, Fisher's exact test and linear regression after testing for equality of means with Levene's test. Non-parametric tests were implemented where data did not follow a normal distribution. All p-values were FDR corrected to control for multiple comparisons using the Benjamini Hochberg methods (Benjamini and Hochberg, 1995). Diurnal variations were analysed using either One-Way or Two-Way Analysis of Variance (ANOVA) for repeated measures with Tukey's or Sidak's multiple comparison tests. Friedman's tests were used where data was not normally distributed with Dunn's post hoc tests. Test statistics are shown as 't' for t-tests, 'U' for Mann-Whitney tests, 'F' for ordinary ANOVA and 'FQ' when Friedman's tests were used.

After exploring different nonlinear curve fits, diurnal variations in performance and sleepiness variables were plotted using second order regression curves.

To explore the predictive effects of rs-FC on performance variables and daytime sleepiness an extension of the generalised linear model – generalised estimating equations (GEEs) were used in SPSS (IBM SPSS Statistics, version 24, Chicago, USA). GEEs account for repeated measures and within subject variability and do not assume normal distribution or linear relationships. GEEs are often used in studies with time of day data to model the average effect. GEEs have been used in sleep and circadian research to model the relationship between insomnia, depression and Chronotype (Alvaro et al., 2014) as well as in studies on sleep durations (Hasler et al., 2004, Lauderdale et al., 2008) and circadian patterns in epilepsy (Ramgopal et al., 2012). Wald Chi-Squared test statistics are referred to as 'W'. When interaction terms were not significant they were removed from the model and analysis re-run. Corrected quasi likelihood under independence model criterion (QICC) values were used to choose the best fit for models.

Throughout the thesis, significance levels are displayed as ns = not significant, $p<0.05$*, $p<0.01$=**, $p<0.001$=***, $p<0.0001$****. Values are represented as the mean ± standard error of the mean (SEM) unless specified otherwise e.g. age is given as mean ± standard deviation (SD). Exact p values are given to two significant figures, apart from when significance is identified as less than 0.0001, in which case $p<0.0001$ is reported. Times of day are presented as hh:mm. Testing sessions are referred to as 08:00 h or 'morning', 14:00 h or 'afternoon' and 20:00 h or 'evening'. This phrasing will be used interchangeable throughout the thesis. Functional anatomy terminology used throughout the thesis is summarised in Figure 2.15.

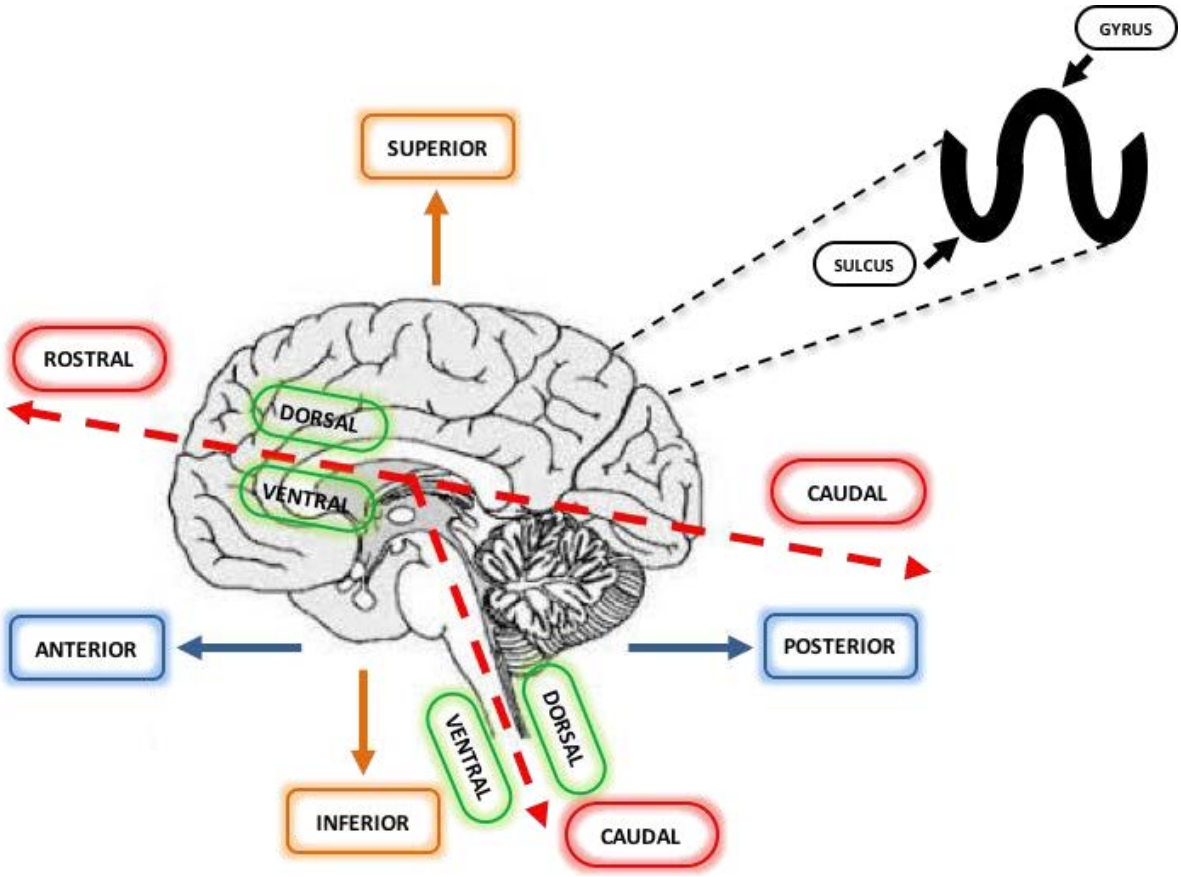


Figure 2.15. Schematic diagram to show functional and structural terminology used throughout this thesis.

The first experimental Chapter explored rs-FC of the DMN, using seeds in the posterior and frontal regions of the DMN. This investigation builds on previous research suggesting that longer cumulative total sleep time results in higher connectivity with the mPFC (Khalsa et al., 2016). Differences between ECPs and LCPs were investigated and the analysis was extended to explore the predictive effects rs-FC of the DMN can have on measures of cognitive performance (PVT and Stroop) and daytime sleepiness (KSS).

As many of the methods and study design were similar in Chapter 3 and 4, the limitations of these studies are discussed in Chapter 6 section 6.6.

CHAPTER THREE

Intrinsic Functional Architecture of the Default Mode Network in Circadian Phenotypes, Cognitive Performance and Daytime Sleepiness

Abstract

The aim of this study was to investigate waking FC associated with the DMN in ECPs and LCPs. 38 participants took part (14 male, age 22.7± 4.2 years), categorised into two groups by the MCTQ (ECP n=16, LCP n=22). After completing sleep related questionnaires, physiological sampling (melatonin and cortisol) and actigraphy, participants were tested at 14:00 h, 20:00 h and 08:00 h (GMT).

The results show that, at the group level, ECPs had higher rs-FC compared to LCPs in seven regions from the mPFC seed, and eight regions from the PCC seed (FWE corrected at $p<0.05$), while LCPs had higher FC than ECPs in two and one region respectively. For both seeds, the regions identified as different between the Circadian Phenotypes were primarily within the DMN. Time of day did not significantly modulate FC from either seed, and there was no interaction between Circadian Phenotype and time of day. For both mPFC and PCC seeds, regions with higher rs-FC in ECPs were predictive of better cognitive performance and lower daytime sleepiness. FC of regions higher in LCPs did not predict cognitive performance or daytime sleepiness.

In summary, Circadian Phenotype is a significant predictor of rs-FC within the DMN in the waking human brain. These differences in the brain's functional architecture at rest are predictive of differences in cognitive performance and daytime sleepiness, and may represent the underlying mechanism by which Circadian Phenotype affects performance.

3.1 Introduction

In our technology driven society the need to be at our best is crucial. However, this 'always switched on' approach, permitted by using artificial light at night, is becoming increasingly detrimental to our health and well-being by disrupting basic physiological processes such as sleep (Foster and Wulff, 2005) and circadian rhythmicity (Potter et al., 2016).

As discussed in the Introduction, endogenously driven 24 h oscillations in human physiology and behaviour, which are synchronised to the external light/dark cycle, allow the anticipation of exogenous cues and are essential to maintenance of health (Rajaratnam and Arendt, 2001). However, since the discovery of artificial light, utilising light at night has become a typical characteristic of society. Despite the benefits of being able to extend our days into the dark hours, this can disrupt both circadian and homeostatic sleep processes (Shochat, 2012). Although some have shown no differences in sleep parameters in pre-industrial societies (Yetish et al., 2015), the negative consequences of 'chronodisruption' are gradually being revealed (Reiter et al., 2007).

Diurnal variations have been identified in both sleepiness and cognitive performance, providing evidence for cyclic patterns in the risk of traffic accidents (George, 2004). There are also a number of catastrophes e.g. Chernobyl and motor incidents linked to disruptions in sleep, in particular sleep deprivation (Mitler et al., 1988). This may be predicted given that increased sleepiness leads to increased number of errors (Lyznicki et al., 1998).

Since brain function is integral to maintenance of consciousness which is regulated by circadian processes (Baars and Gage, 2010), as well as distinct electrical brain activity being observed during sleep (Dement and Kleitman, 1957), research into the impact of these disruptions on intrinsic brain function and time of day has not yet been fully exploited.

One way of investigating how sleep and circadian mechanisms affect brain function is using FC. FC is used to describe relationships between brain regions that are temporally correlated, therefore 'functionally connected' (Aertsen et al., 1989). Intrinsic properties of brain function have been identified in a number of functionally connected regions called ICNs that appear in distinct networks at rest, but can also be observed during tasks (Biswal et al., 1995, Smith et al., 2009). A widely studied ICN, the default mode network (DMN), reflects activity whilst in the absence of external cognitive demand (Gusnard and Raichle, 2001). The DMN has been associated with a multitude of functions, mainly surrounding self-referential processing (Raichle et al., 2001) and maintaining consciousness (Danielson et al., 2011). Considerable research has also indicated that this network is heavily involved in regulating cognition (Leech and Sharp, 2013), attention (Gui et al., 2015), and working memory (Esposito et al., 2009). Furthermore, the DMN has been shown to be modified in disorders such as Alzheimer's disease (Greicius et al., 2004) and depression (Sheline et al., 2009) as well as being widely affected by sleep deprivation (De Havas et al., 2012, Gujar et al., 2010) and sleep stages (Horovitz et al., 2009, Sämann et al., 2010). It is therefore unsurprising that this network, representing the 'default state' of the brain, has become the motivation of much neuroimaging research in cognitive neuroscience.

3.1.1 Individual Differences

Even though neuroimaging is becoming increasingly used as a technique in sleep and circadian research, inter-subject variability brings another level of complexity that needs to be accounted for. It is well established that there are individual differences in circadian rhythmicity (Horne and Östberg, 1977). At the extreme end of the continuum, these different groups of individuals can be identified into ECPs and LCPs.

It has been suggested that ECPs have less disrupted sleep (Giannotti et al., 2002, Taillard et al., 2003), make healthier food choices (Maukonen et al., 2017, Kanerva et al., 2012), thereby minimising risks of obesity and diabetes (Ross et al., 2016), and even reach higher standards in the sports world (Lastella et al., 2016, Roden, 2017). Conversely, LCPs have been linked to greater daytime sleepiness (Owens et al., 2016), increased alcohol consumption and substance abuse (Hasler et al., 2012b, Wittmann et al., 2010), decreased psychological well-being through higher rates of depression (Merikanto et al., 2013a), sleep disorders (Alvaro et al., 2014, Hasler et al., 2012a) and negative health outcomes such as obesity (Roenneberg et al., 2012), diabetes (Merikanto et al., 2013b, Reutrakul et al., 2013, Reutrakul and Van Cauter, 2014) and cardiovascular disease (Wong et al., 2015). The fact that the LCPs seem to be associated with these adverse effects has been linked to the constant desynchronisation of their biological clocks through trying to 'fit' to external societal time i.e. work/school schedules. This persistent fight against endogenous rhythms is known as 'social jetlag' (Wittmann et al., 2006).

The effect of Circadian Phenotype and time of day on rs-FC has rarely been explored. Park et al. (2012) looked into the stability of ICNs over a 24 h time period and found that some networks exhibit rhythmic patterns and others retain a state of stability. Blautzik et al. (2013) explored daily rhythmicity of rs-FC and found that the central executive network was stable across the day but the DMN and MN had a rhythmic nature. However, despite controlling for internal time using the MCTQ, they only used Intermediate-Late Chronotypes, eliminating the potential of investigating differences between Early and Late groups. Hodkinson et al. (2014), showed a decrease of global rCBF from morning to evening within the DMN in healthy participants. However, no studies have, as far as we

know, explored differences in rs-FC of the DMN in Circadian Phenotypes and linked it to aspects of alertness and cognitive performance.

Despite the building knowledge of these consequences, we are still searching for ways to help understand the effect of these disruptions with the hope that we can implement change in society to minimise health risks and maximise productivity and performance (Rajaratnam and Arendt, 2001). This raises the question: 'is the impaired behaviour and the adverse associations seen in LCPs, who are in a constant state of chronodisruption or 'social jetlag' associated with changes to rs-FC?' Given that the DMN is evidently vital to basic maintenance of consciousness, implicated in sleep alterations, and plays a role in cognitive functioning, it was used as the network of interest in this study to examine whether Circadian Phenotype was able to explain differences in rs-FC of the DMN. The anterior (mPFC) and posterior (PCC) regions of the DMN were used as seed regions to gather information about the intra and inter-network functional integrity of the DMN at rest and how it is linked to cognitive performance and sleepiness outside of the MRI scanner.

This study explored rs-FC in two groups of Circadian Phenotypes, Early and Late, over three separate testing sessions during the course of a typical societally constrained day (08:00 h to 20:00 h). The main aims were to uncover any differences in rs-FC between these two groups, and if so whether the differences can predict variability in daytime sleepiness or performance in cognitive tasks.

3.2 Materials and Methods

Methods unique to this Chapter are given below and the study design is summarised in Figure 3.1. A more detailed description of methods used across all experimental Chapters is given in Chapter 2.

3.2.1 Participants

All 38 healthy individuals (14 male, 22.7 ± 4.2 years) were included in this study. Information on ethics, consent and inclusion/exclusion criteria was given in Chapter 2.

3.2.2 Questionnaires and Sleep Analysis

Subjects completed MCTQ to initially identify ECPs and LCPs. Corrected mid-sleep on free days was used to categorise the groups, of which 16 were ECPs (age 24.7 ± 4.6 years, nine female, MSF_{sc} 02:24 ± 00:10 h) and 22 were LCPs (age 21.3 ± 3.3 years, 15 female, MSF_{sc} 06:52 ± 00:17 h). Groups were of comparable height and weight (Table 3.1).

Actigraphy data coupled with sleep diaries and daily interaction with the smart phone app was collected for between 13-16 days prior to testing sessions to monitor sleep and activity patterns. Participants also provided saliva samples during one morning and one evening the week of testing to determine circadian phase through melatonin and cortisol rhythms. Procedure and analysis is described in Chapter 2. After completing the questionnaires, physiological sampling and between 13-16 days of actigraphy, participants attended BUIC for testing sessions at 14:00 h, 20:00 h and 08:00 h (GMT) the following morning. Summary details of participants' questionnaire, actigraphy and physiological data can be found in Table 3.1.

3.2.3 Neuroimaging Acquisition & Analysis

Acquisition and analysis were carried out as described in Chapter 2 using predefined seeds for the frontal (mPFC) and posterior (PCC) regions of the DMN (https://findlab.stanford.edu/functional_ROIs.html, Shirer et al., 2012). Descriptions of significant findings (FWE, $p<0.05$), are presented as total voxels, peak t score and peak MNI centroid cluster coordinates [x y z]. Average correlation values from mPFC and PCC to all clusters identified as different between ECPs and LCPs will be referred to as 'total' or 'average' rs-FC.

3.2.4 Cognitive Performance & Sleepiness

A two-minute PVT and a Stroop Colour-Word Task were completed as part of the CMAT. Reaction time values were used from the PVT (Dinges and Powell, 1985), and the Stroop colour word task (Jensen and Rohwer, 1966, Stroop, 1935) as indices of cognitive performance. The KSS was completed before and after the cognitive tests were performed.

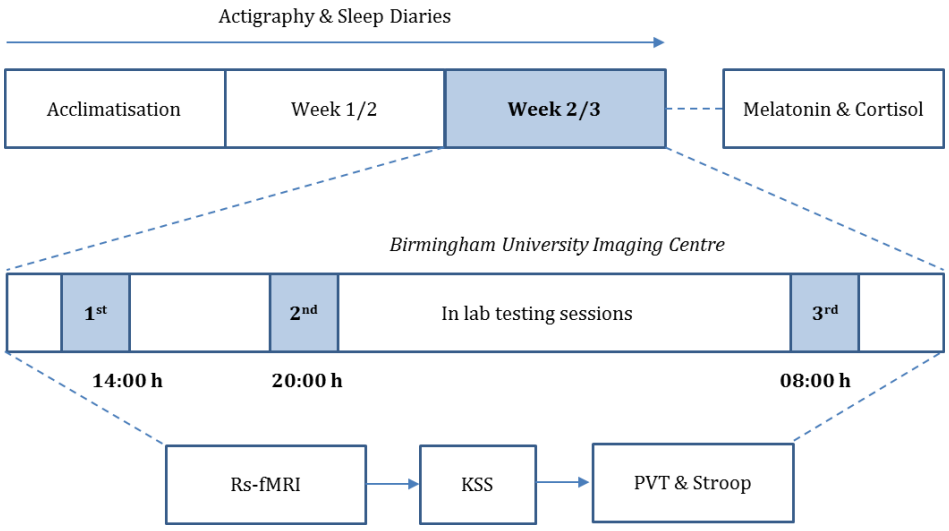


Figure 3.1. Schematic illustration of experimental protocol. Actigraphy combined with sleep diaries/app were completed for 13-16 days prior to testing as well as physiological sampling for melatonin and cortisol rhythms. Participants attended in lab testing sessions at Birmingham University Imaging Centre at 14:00 h (1st), 20:00 h (2nd) and 08:00 h the following morning (3rd). At each testing session participants underwent a resting-state fMRI scan followed by subjective sleepiness ratings with the KSS before and after cognitive tests.

3.2.5 Statistical Analysis

Data used in GEE analyses were; rs-FC correlation values between clusters identified to be different between ECPs and LCPs, cognitive test results, KSS values and times of testing (08:00 h, 14:00 h and 20:00 h). A scale linear response GEE with identity link function was used to model the independent effects of rs-FC on cognitive performance. A negative binomial GEE with log link function was used to model the effects of rs-FC on sleepiness. Both models were designed adding Subject ID as a subject variable, Circadian Phenotype (two groups) and time of day (three time points), as a within-subject variables. Time of day was also added as a fixed factor. If an interaction term was not significant it was removed from the model and the analysis was re-run.

3.3 Results

3.3.1 Circadian Phenotyping

The individuals who were initially categorised into Early (n=16) and Late (n=22) Chronotypes using the MCTQ were confirmed as ECPs and LCPs by analysis of DLMO, time of peak morning cortisol concentration in addition to sleep start and wake up times automatically calculated from actigraphy analysis (Figure 3.2 & Table 3.1). MSF_{sc} differed significantly between the two groups ($t(36)=12.2$, $p<0.0001$). ECPs had an average MSF_{sc} of 02:24 ± 00:10 h whilst LCPs averaged 06:52 ± 00:17 h. DLMO and peak time of morning cortisol also differed significantly ($t(30)=6.8$, $p<0.0001$ and $t(36)=8.0$, $p<0.0001$ respectively) with DLMO being at 20:27 ± 00:16 h for ECPs and 23:55 ± 00:26 h for LCPs and peak cortisol at 07:04 ± 00:16 h for ECPs and 11:13 ± 00:23 for LCPs (Figure 3.2A&B). These results were consistent with sleep onset and wake up times calculated from

actigraphy data, both significant ($t(34)=8.9$, $p<0.0001$ and $t(34)=9.9$, $p<0.0001$). Sleep onset for ECPs was 22:57 ± 00:10 h and 02:27 ± 00:19 h for LCPs. Wake up time was 06:33 ± 0.10 h and 10:13 ± 00:18 h for ECPs and LCPs respectively (Figure 3.2C&D).

As expected, each of these parameters were significantly correlated with MSFsc. Figure 3.4 shows significant relationships resulting from linear regression analysis between MSFsc and DLMO ($R^2 = 0.65$, $p<0.0001$), peak time of morning cortisol ($R^2 = 0.75$, $p<0.0001$), sleep onset ($R^2 = 0.80$, $p<0.0001$) and wake up time ($R^2 = 0.86$, $p<0.0001$). Other sleep parameters gathered from actigraphy were not significantly different between ECPs and LCPs (Table 3.1). Sleep duration was calculated as 7.59 ± 0.18 h for ECPs and 7.70 ± 0.14 h for LCPs ($t(34)=0.5$, $p=0.72$). ECPs had a sleep efficiency of 79.29 ± 1.96 % whilst LCPs sleep efficiency was 77.23 ± 1.14 % ($t(34)=0.9$, $p=0.47$). The time it took individuals to get to sleep (sleep onset latency) was 00:25 ± 00:06 h for ECPs and 00:25 ± 00:03 h for LCPs ($U(34)=118.5$, $p=0.31$). Group average curves for melatonin and cortisol rhythms are shown in Figure 3.3. Phase angle was not significantly different between the groups being 02:28 ± 00:16 h for ECPs and 02:34 ± 00:18 h for LCPs ($t(30)=0.3$, $p=0.84$). All participants in this study were following their preferred schedules for the duration of the experiment so were not sleep deprived and LCPs did not differ from ECP in any objective sleep parameters apart from timings (Table 3.1). These results support the classification into Circadian Phenotypes and demonstrate that these two groups are behaviourally and physiologically different in sleep timings and circadian phase but not in other sleep parameters.

To be able to confirm that basic cognitive abilities between the groups were not different, the results from tests completed during the acclimatisation phase were analysed. There were no significant differences in cognitive performance variables or sleepiness between

groups, confirming comparable cognitive abilities. For PVT performance, ECPs average reaction time was 0.39 ± 0.01s and 0.42 ± 0.01s for LCPs ($t(58)=2.0$, $p=0.09$). Average Stroop performance i.e. reaction times were 1.12s ± 0.04 for ECPs and 1.14s ± 0.03 for LCPs ($U(62)=442$, $p=0.63$). KSS score for ECPs was 4.27 ± 0.31 and 4.65 ± 0.30 for LCPs ($t(61)=0.91$, $p=0.49$). This will be discussed further in the overall discussion found in Chapter 6.

Table 3.1. Summary of demographic, actigraphy and physiological details for ECPs and LCPs. Values are shown as mean ± SEM unless specified. Significance is shown with [a]unpaired two sample t-tests, [b]non-parametric Mann-Whitney or [c]Fisher's exact test. All p values are FDR corrected.

Variable Measured (mean ± SEM)	**ECPs**	**LCPs**	**Significance**
Sample Size	N=16	N=22	n/a
Number of Scans/Testing Sessions	N=48	N=66	n/a
Percentage of Males/Females (%)	M: 43.75	M: 31.82	ns[c]
	F: 56.25	F: 68.18	ns[c]
Age (years) (mean ± SD)	24.69 ± 4.60	21.32 ± 3.27	p=0.028[a]
Height (cm)	171.30 ± 1.97	171.10 ± 2.38	ns [a]
Weight (kg)	66.44 ± 2.78	67.05 ± 2.10	ns [a]
MCTQ Score (hh:mm)	02:24 ± 00:10	06:52 ± 00:17	p<0.0001[a]
Sleep Onset (hh:mm)	22:57 ± 00:10	02:27 ± 00:19	p<0.0001[a]
Wake Up Time (hh:mm)	06:33 ± 0.10	10:13 ± 00:18	p<0.0001[a]
Sleep Duration (h)	7.59 ± 0.18	7.70 ± 0.14	ns[a]
Sleep Efficiency (%)	79.29 ± 1.96	77.23 ± 1.14	ns[a]
Sleep Onset Latency (hh:mm)	00:25 ± 00:06	00:25 ± 00:03	ns[b]
Phase Angle (hh:mm)	02:28 ± 00:16	02:34 ± 00:18	ns[a]
Dim Light Melatonin Onset (hh:mm)	20:27 ± 00:16	23:55 ± 00:26	p<0.0001[a]
Cortisol Peak Time (hh:mm)	07:04 ± 00:16	11:13 ± 00:23	p<0.0001[a]

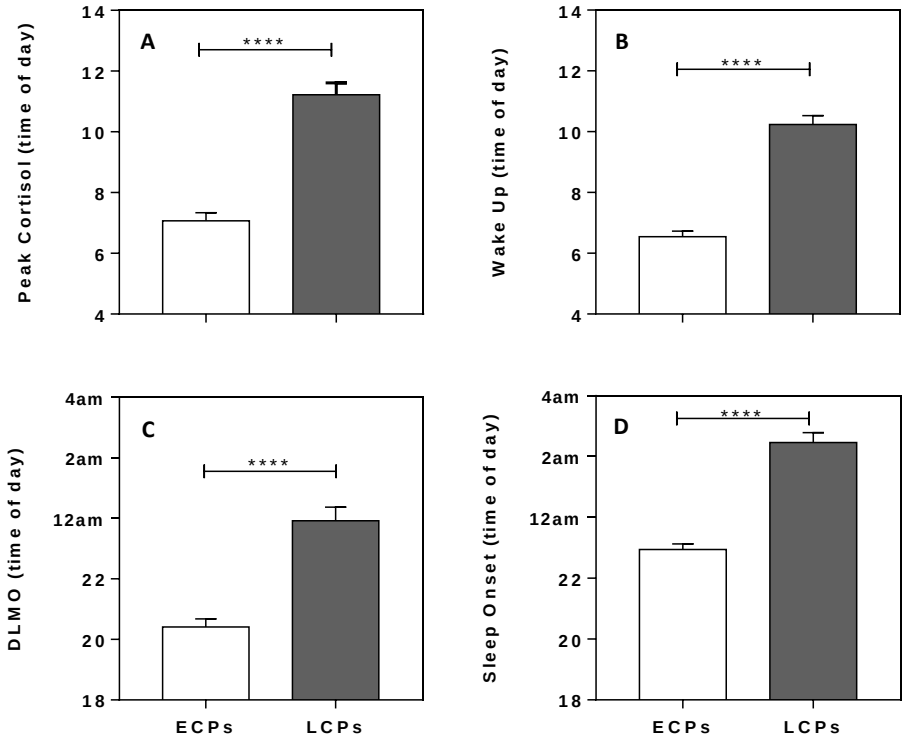


Figure 3.2. Significant differences in behavioural and physiological parameters between ECPs and LCPs. A) Peak time of cortisol, B) Wake up time, C) Dim light melatonin onset, D) Sleep onset. Mean for ECPs (white) and LCPs (grey) is shown plus SEM.

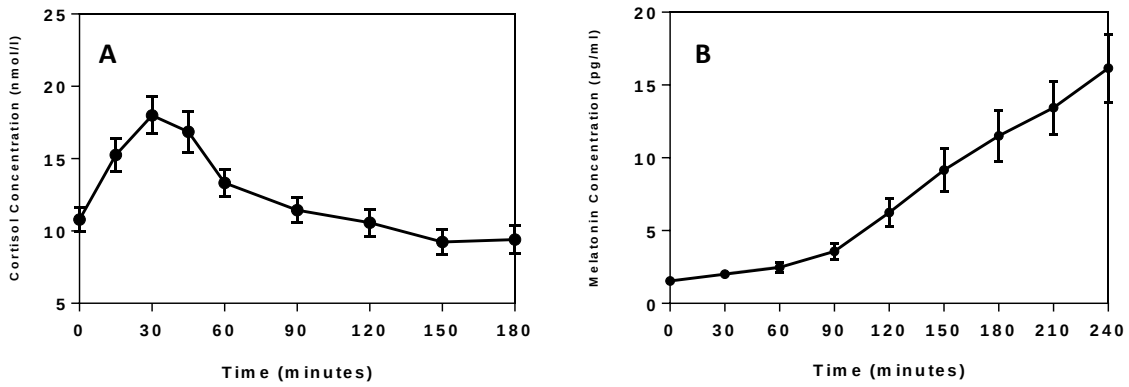


Figure 3.3. Group average curves for Cortisol Awakening Response and Dim light melatonin Onset. A) Cortisol concentration (nmol/l) was measured upon waking followed by 15 minute intervals for 1hr then 30minutes for 2 h. B) Melatonin concentration (pg/ml) was measured every 30 minutes from 4 h before individual habitual bed time until 1 hour after. Time (minutes) is displayed on the x axis and concentration on y axis.

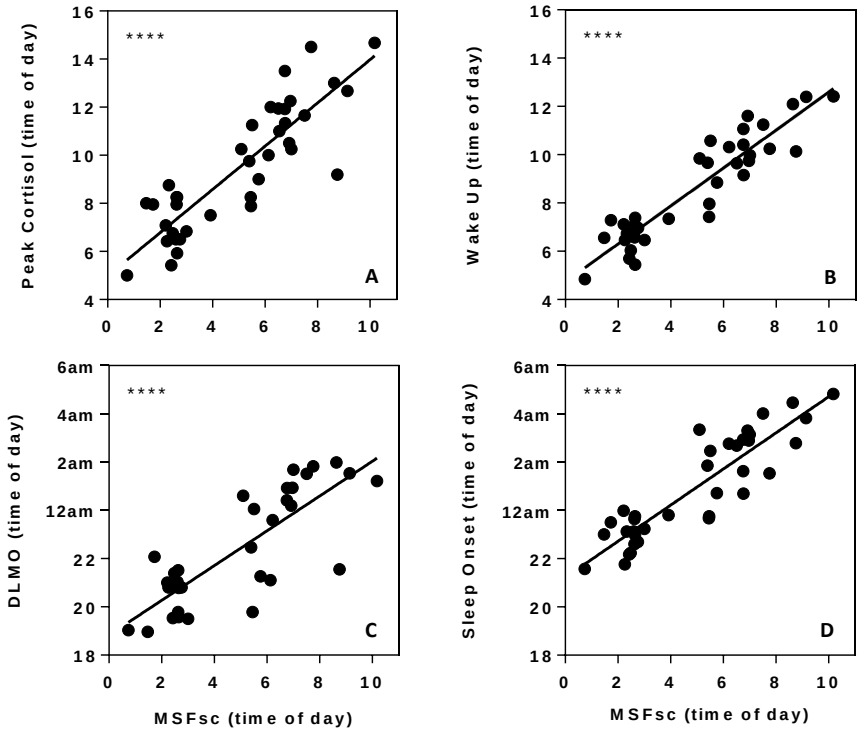


Figure 3.4. Linear relationships between corrected MSF_{sc} to validate circadian phenotyping. A) Dim light melatonin onset, B) Sleep onset, C) Time of peak cortisol concentration, D) Wake up time. MSF_{sc} is displayed on the x axis.

3.3.2 Resting State Functional Connectivity in Circadian Phenotypes

When all subjects were grouped together, seeding in the medial-pre frontal (mPFC) and posterior cingulate (PCC) cortices led to a clear DMN being observed, as expected (Figure 3.5 & Figure 3.6). Significant FC (corrected FWE $p<0.05$) was observed between all major components of the DMN including the PCC/precuneus, mPFC, bilateral angular, temporal

gyri and cerebellum. Details of clusters larger than 350 voxels which survived FWE correction at $p<0.05$ for each seed are given in Table 3.2 & Table 3.3.

Table 3.2. Summary of significant brain regions for the whole sample when seeding in the mPFC.

Region	Cluster size (voxels)	MNI Centroid Coordinates [x y z]	Maximum t-score
Frontal Lobe	35300	[0 48 12]	94.36
PCC/Precuneus	4375	[6 -50 24]	55.07
Left Angular Gyrus	1921	[-50 -60 30]	47.81
Right Angular Gyrus	1182	[58 -60 32]	44.11
Left Medial Temporal Lobe	398	[-66 -28 -4]	27.88

Table 3.3. Summary of significant brain regions for the whole sample when seeding in the PCC.

Region	Cluster size (voxels)	MNI Centroid Coordinates [x y z]	Maximum t-score
Precuneus	17811	[4 -54 26]	145.01
Frontal Lobe	12944	[0 62 -4]	63.05
Temporal Lobe	3040	[60 -8 -18]	56.13
Left Angular Gyrus	2333	[-40 -68 38]	61.79
Right Angular Gyrus	1952	[48 -62 26]	65.24
Cerebellum	860	[-6 -54 -44]	40.09
Left Cerebellum	543	[-28 -80 -34]	29.36

When comparing differences between ECPs and LCPs, contrasts were defined using the SPM12 contrast manager to either look at ECPs > LCPs or LCPs > ECPs. Results showed that ECPs had increased FC compared to LCPs at all times of day in all but three clusters identified (FWE corrected at $p<0.05$). When seeding in the mPFC there was significantly higher FC from the seed to seven individual clusters including: the frontal lobe (384 voxels, t=10.99, [2 70 6]), bilateral insula (left; 378 voxels, t=8.87, [-26 14 -24] and right; 241 voxels, t=9.36, [26 18 -20]), left medial frontal lobe (160 voxels, t=9.62, [-44 16 56]), left angular gyrus (134 voxels, t=10.19, [-56 -58 18]), left superior frontal gyrus (111 voxels,

t=8.96, [-4 68 28]), and right medial temporal lobe (108 voxels, t=6.15, [68 -12 -8]) (Table 3.4 & second and third rows of Figure 3.5).

Table 3.4. Summary of significant brain regions between ECPs and LCPs when seeding in the mPFC.

Region	**Contrast**	**Cluster size (voxels)**	**MNI Centroid Coordinates [x y z]**	**Maximum t-score**
Medial Pre-Frontal Cortex	ECPs > LCPs	384	[2 70 6]	10.99
Left Anterior Insula	ECPs > LCPs	378	[-26 14 -24]	8.87
Right Anterior Insula	ECPs > LCPs	241	[26 18 -20]	9.36
Left Medial Frontal Lobe	ECPs > LCPs	160	[-44 16 56]	9.62
Left Angular Gyrus	ECPs > LCPs	134	[-56 -58 18]	10.19
Left Superior Frontal Gyrus	ECPs > LCPs	111	[-4 68 28]	8.96
Right Medial Temporal Lobe	ECPs > LCPs	108	[68 -12 -8]	6.15
Anterior Cingulate	LCPs > ECPs	233	[22 44 10]	7.20
Right Superior Frontal Gyrus	LCPs > ECPs	161	[22 42 52]	6.55

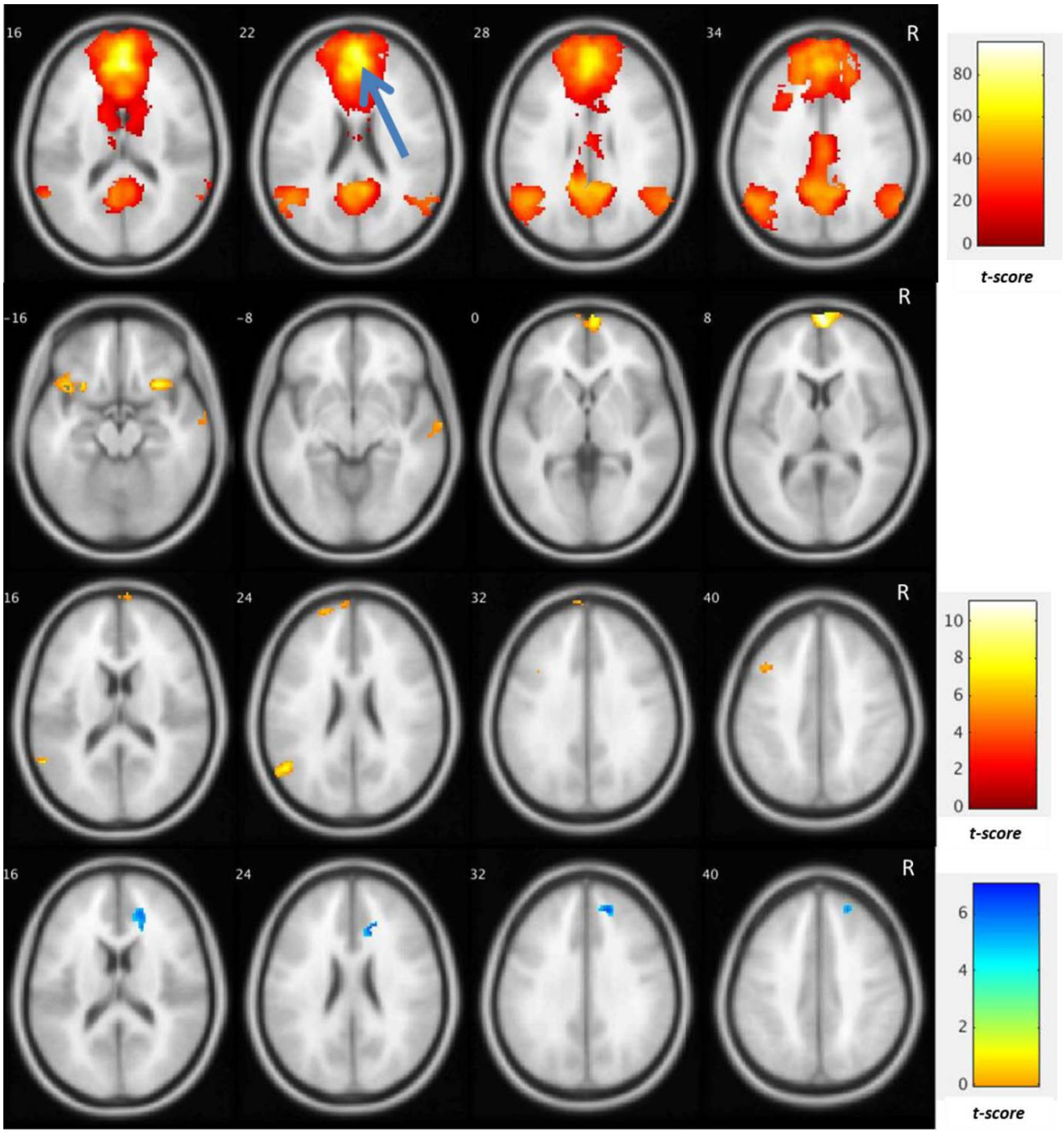
When seeding in the PCC there was, again, significantly higher FC for ECPs from PCC to the precuneus (431 voxels, t=9.73, [0 -64 18]), bilateral angular gyri (right; 481 voxels, t=8.14, [46 -68 26] and left; 257 voxels, t=14.75, [-54 -62 18]), left medial temporal lobe (237 voxels, t=7.94, [-58 -6 -24]), and cingulate gyrus (150 voxels, t=18.90, [-16 -42 26]). The largest cluster was found in the mPFC (739 voxels, t=13.71, [-2 72 12]) along with two other clusters in the left medial frontal (173 voxels, t=8.71, [-46 16 56]) and superior frontal lobe (212 voxels, t=7.91, [-18 60 26]) (Table 3.5 & second and third rows of Figure 3.6).

When comparing LCPs > ECPs two clusters survived FWE correction at $p<0.05$ when seeding in the mPFC and only one significant cluster when seeding in the PCC. From mPFC seed LCPs shows higher FC to the anterior cingulate cortex (233 voxels, t=7.20, [22 44 10])

and right superior frontal gyrus (161 voxels, t=6.55, [22 42 52]) (Table 3.4 & & bottom row of Figure 3.5). From PCC seed, there was higher FC to the left angular gyrus (428 voxels, t=16.29, [-32 -54 26]) (Table 3.5 & bottom row of Figure 3.6).

Table 3.5. Summary of significant brain regions between ECPs and LCPs when seeding in the PCC.

Region	**Contrast**	**Cluster size (voxels)**	**MNI Centroid Coordinates [x y z]**	**Maximum t-score**
Medial Pre-Frontal Cortex	ECPs > LCPs	789	[-2 72 12]	13.71
Right Angular Gyrus	ECPs > LCPs	481	[46 -68 26]	8.14
Precuneus	ECPs > LCPs	431	[0 -64 18]	9.73
Left Angular Gyrus	ECPs > LCPs	257	[-54 -62 18]	14.75
Left Medial Temporal Lobe	ECPs > LCPs	237	[-58 -6 -24]	7.94
Left Superior Frontal Gyrus	ECPs > LCPs	212	[-18 60 26]	7.91
Left Medial Frontal Lobe	ECPs > LCPs	173	[-46 16 56]	8.71
Cingulate Gyrus	ECPs > LCPs	150	[-16 -42 26]	18.90
Left Angular Gyrus	LCPs > ECPs	428	[-32 -54 26]	16.29


16
22
28
34
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t-score
-16
-8
0
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10
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t-score
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Figure 3.5. Resting state functional connectivity maps using the mPFC seed. Seed region is indicated by the blue arrow. Top row shows whole sample group maps. Second and third rows show regions higher in ECPs (ECPs > LCPs) and fourth row shows regions higher in LCPs (LCPs > ECPs). Significant clusters (FWE $p<0.05$, extent threshold = 100 voxels) are indicated in yellow for ECPs and blue for LCPs. T score scales for each contrast is shown on the right.

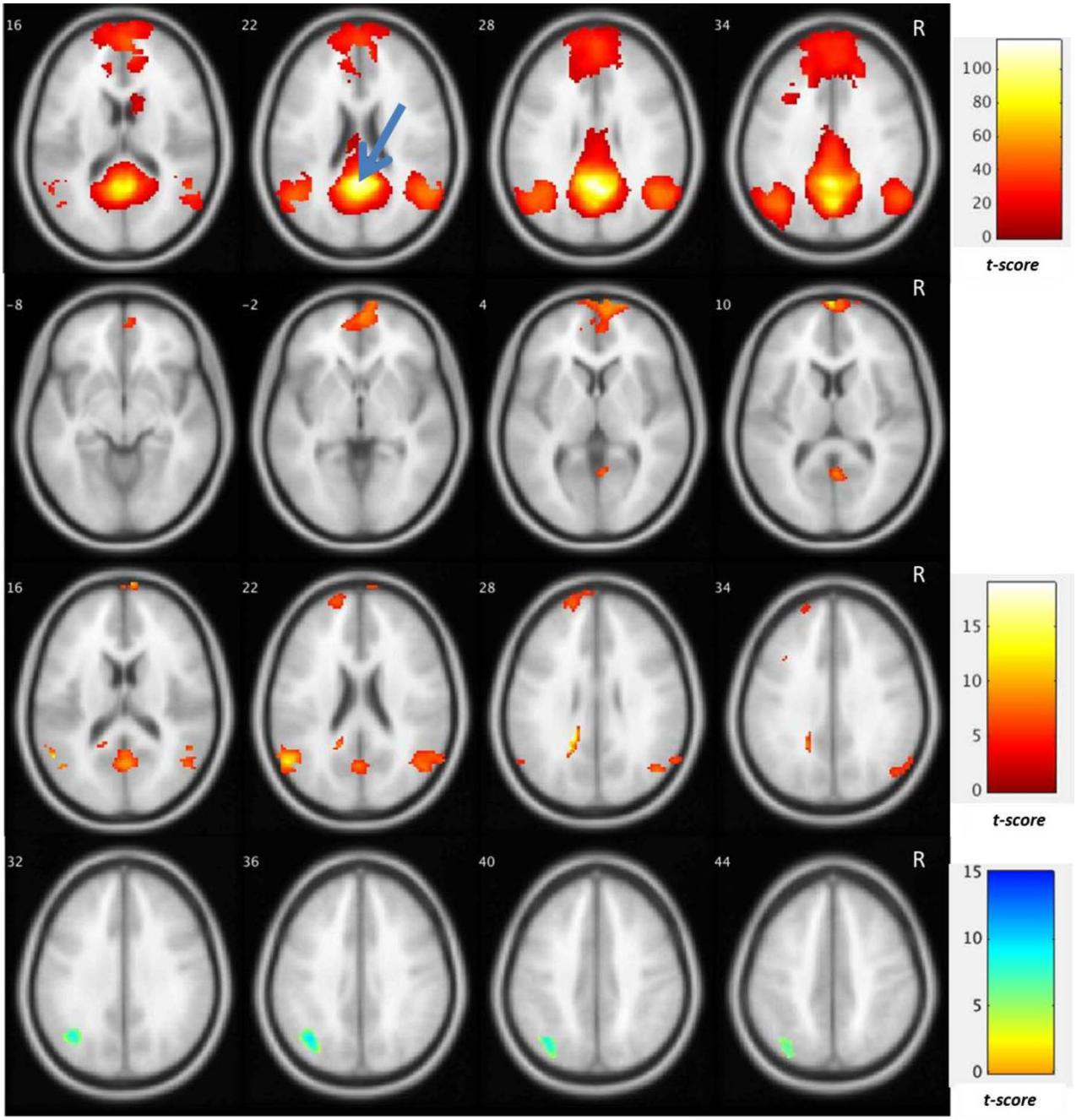


Figure 3.6. Resting state functional connectivity maps using the PCC seed. Seed region is indicated by the blue arrow. Top row shows whole sample group maps. Second and third rows show regions higher in ECPs (ECPs > LCPs) and fourth row shows regions higher in LCPs (LCPs > ECPs). Significant clusters (FWE $p<0.05$, extent threshold = 150 voxels) are indicated in yellow for ECPs and blue for LCPs. T score scales for each contrast is shown on the right.

3.3.3 Cognitive Performance

When solely looking at time of day effects as a whole group, PVT and Stroop performance showed significant diurnal variations (FQ=9.5, p=0.0088 and FQ=7.4, p=0.025 respectively). Morning (08:00 h) to afternoon (14:00 h) was significantly different for both measures (PVT: p=0.02 and Stroop: p=0.0067), as well as 08:00 h to 20:00 h for PVT performance (p=0.0037). When investigating diurnal variations in separate groups, PVT performance was not significant in ECPs but was in LCPs (FQ=12.1, p=0.0024) with morning to evening differing significantly (p=0.0005). Stroop performance was significant in ECPs (FQ=6.9, p=0.032) from morning to afternoon and evening (both p=0.023), but not in LCPs (Figure 3.7).

Using results from regions higher in ECPs, GEE analysis showed that rs-FC of the mPFC (ECPs > LCPs) could independently predict PVT performance (W=14.5, $p<0.0001$) and Stroop performance (W=9.0, p=0.003). Time of day within the model could also predict PVT but not Stroop performance (W=9.2, p=0.01). Rs-FC of the PCC (ECPs > LCPs) could predict PVT performance (W=6.4, p=0.012) but not Stroop performance. Independently of rs-FC, time of day was a significant predictor of PVT and Stroop Performance (W=6.3, p=0.042 and W=7.1, p=0.028 respectively).

Rs-FC from mPFC regions identified as being higher in the LCPs (LCPs > ECPs), could not predict any performance variables. Time of day however, could predict PVT (W=7.2, p=0.027) and Stroop (W=6.9, p=0.033) performance, as seen in previous models. The same

was seen for rs-FC of the PCC that was higher in LCPs (LCPs > ECPs), with only time of day being able to predict PVT and Stroop performance (W=9.0, p=0.011 and W=7.3, p=0.026 respectively) (Figure 3.8).

3.3.4 Sleepiness

As a whole group, there was no significant effect of time of day on sleepiness assessed by KSS score (F(3,38)=2.1, p=0.34). However, a significant time of day effect was found when looking at ECPs and LCPs independently (ECPs F(3,16)=8.0, p=0.018 and LCPs F(3,22)=15.6, p=0.0004 respectively). This diurnal variation showed ECPs to be least sleepy in the morning (3.13 ± 0.35), and most sleepy in the evening (4.88 ± 0.42). LCPs showed the inverse relationship being most sleepy in the morning and least in the evening (6.41 ± 0.30 to 4.00 ± 0.33). Post hoc tests revealed significant differences in LCPs between 08:00 h and 14:00 h (p=0.0026) as well as 08:00 h to 20:00 h (p=0.0009) (Figure 3.7).
GEEs analysis using results from regions higher in ECPs (ECPs > LCPs) showed that the interaction of rs-FC and time of day could predict daytime sleepiness for both models using mPFC (W=14.5, p=0.001) and PCC (W=8.7, p=0.013) seeds. The main effect of time of day was also significant for both mPFC (W=17.1, p<0.0001) and PCC models (W=11.1, p=0.004). In the PCC model rs-FC could also independently predict daytime sleepiness (W=6.0, p=0.015).
No significant effects were found for rs-FC higher in LCPs (LCPs > ECPs) for either mPFC or PCC seeds (Figure 3.8).

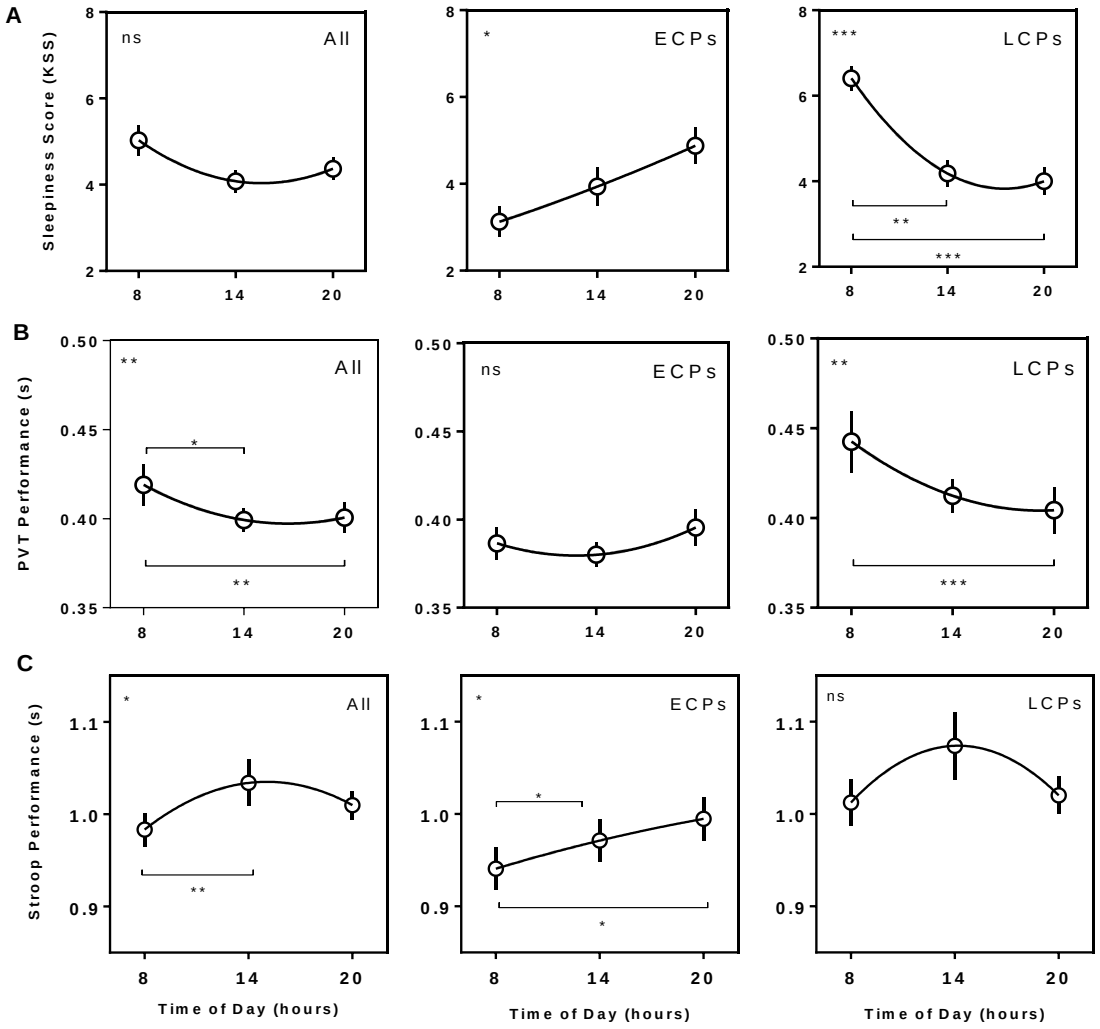


Figure 3.7. Nonlinear regression curves to show diurnal variations in PVT, Stroop and Sleepiness for whole group (All), ECPs and LCPs. A: KSS, B: PVT performance, C: Stroop performance. Time of test is shown on the x axis for each parameter.

3.3.5 Predicting Cognitive Performance and Daytime Sleepiness

A summary of results from the GEEs for both ECPs > LCPs and LCPs > ECPs is shown in Figure 3.8. In brief, average rs-FC of the mPFC, identified as being higher in ECPs, predicts better cognitive performance i.e. faster reaction times in both PVT and Stroop performance. Similarly, higher rs-FC of the PCC could predict better PVT performance and lower daytime sleepiness but not Stroop performance. The interaction of time of day and rs-FC can predict daytime sleepiness for mPFC and PCC seeds. Time of day can independently predict cognitive performance and sleepiness variables in both models.

For the regions that showed higher rs-FC in LCPs, only time of day could predict PVT and Stroop performance. No predictive effects of rs-FC for either seed on cognitive performance or sleepiness were found.

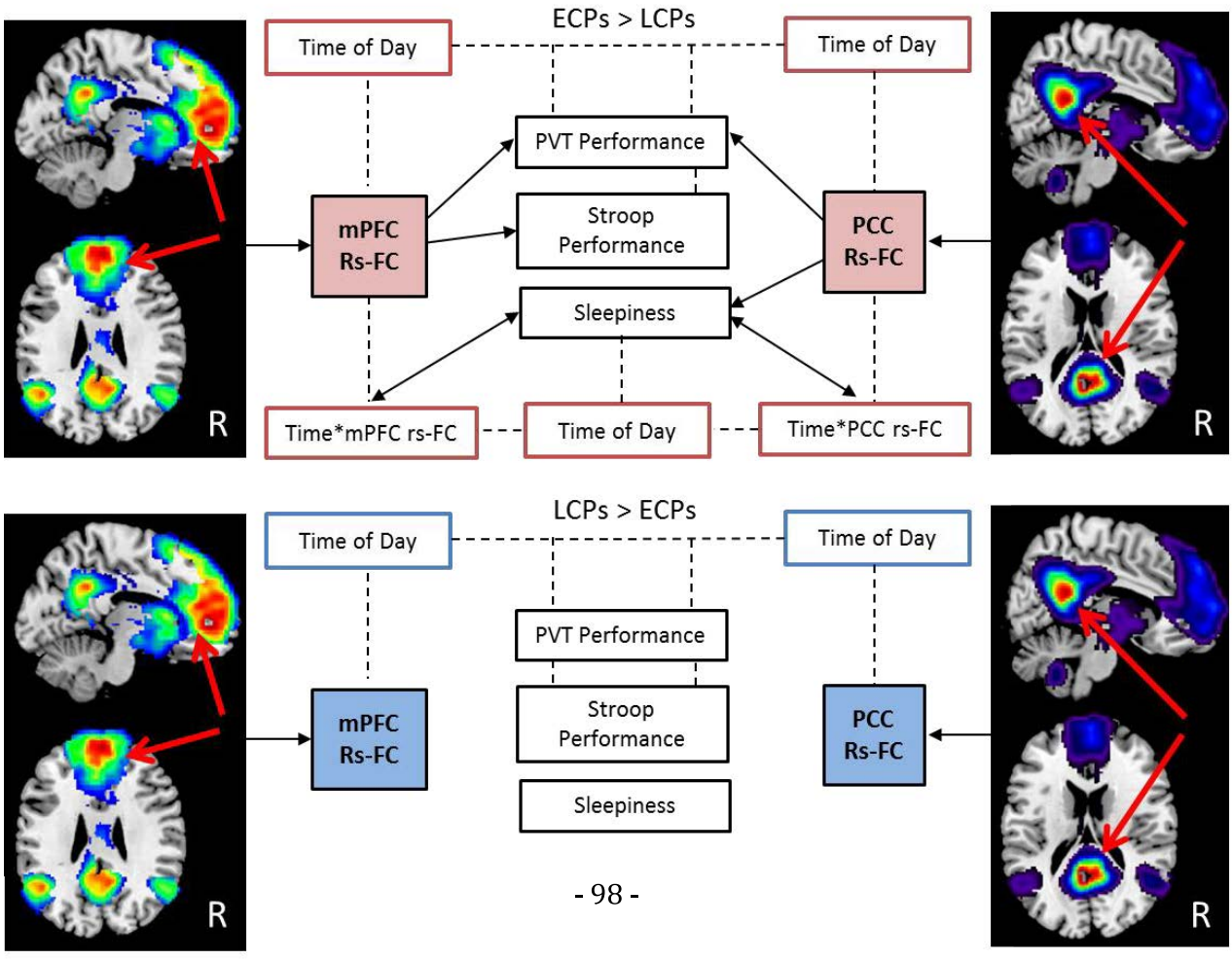


Figure 3.8. Summary of Default Mode Network GEE analysis using rs-FC values higher in ECPs (red) and LCPs (blue) from mPFC and PCC to predict cognitive performance and daytime sleepiness (black boxes). Arrows indicate the predictive effects of rs-FC on performance and sleepiness variables. Dotted lines and red/blue boxes indicate where time of day or the interaction of time of day and rs-FC was also found to be a significant factor.

3.4 Discussion

According to Roenneberg et al. (2007), only around 15% of the population falls into extreme or moderate Early Chronotypes (going to sleep from between 20:30 – 23:00 h and waking between 04:30 – 07:00 h), meaning the majority would usually not fit into a working schedule, preferring to sleep and wake up later. Consequently, many individuals, in particular LCPs, are constantly fighting their innate sleep patterns to fit into societal routines.

Here we show, for the first time, fundamental differences in intrinsic functional architecture between ECPs and LCPs during a societally constrained working day (08:00 h – 20:00 h). Regardless of time of day, ECPs have higher resting state-FC in the majority of regions identified within the DMN but also between mPFC, PCC and other brain regions. These differences are predictive of cognitive performance and subjective sleepiness suggesting higher rs-FC between certain brain regions results in better performance in tasks. Of the 18 regions identified, only three of them were higher in LCPs suggesting ECPs have higher overall rs-FC from mPFC and PCC seeds. Our results suggest the differences observed could be due to Circadian Phenotype and not acute sleep differences, since both groups had similar sleep durations. As mentioned, LCPs tend to be heavily disrupted throughout their lifetimes but were able to follow their own preferred routines throughout this study and had comparable sleep parameters to ECPs (duration, efficiency) with only

sleep timings differing significantly. Therefore, it is likely that the chronic effect of this long term circadian misalignment may continue to impact intrinsic brain properties even when individuals are able to follow their own schedules for a period of two weeks. This would support the notion of LCPs showing adverse effects when persistently following an earlier schedule during the work week and trying to compensate on 'free days' (Wittmann et al., 2006).

These data shed light on our understanding of how Circadian Phenotypes differ in terms of intrinsic FC and how rs-FC can predict cognitive performance as well as sleepiness. We show that 1) There are differences in rs-FC of the DMN between ECPs and LCPs. 2) The differences identified as having higher FC in ECPs are associated with intra and inter-DMN connectivity and can predict better cognitive performance, and lower sleepiness. 3) There are significant diurnal variations in cognitive performance and sleepiness.

3.4.1 Circadian Phenotyping

We investigated two groupings of Circadian Phenotypes, those that are Early and Late, and show clear behavioural and physiological differences in their sleep homeostasis and circadian phase. It has been independently recognised that these groups have vastly different sleep patterns (Roenneberg et al., 2003) which was confirmed in our study through actigraphy. There was a near 4 h difference between both sleep start and wake up times with ECPs going to sleep at 22:57 h and waking up at 06:33 h and LCPs sleeping from 02:27 to 10:13 h. Interestingly, despite the literature showing that LCPs tend to have differing sleep durations (Roepke and Duffy, 2010), sleep efficiency (Selvi et al., 2010) and longer sleep latency (Tzischinsky and Shochat, 2011) we found all these parameters not to be significantly different between the groups (Table 3.1). Phase angle (time between DLMO

and sleep start) was also similar between both groups as shown in Mongrain et al. (2008). As mentioned earlier, this finding is probably due to the fact that our participants were following their preferred sleep/wake cycles and not constrained to a schedule. Although this may minimise the impact of the acute sleep deprivation that LCPs experience, it allows the potential to tease apart the sleep homeostatic and circadian effects. All participants were choosing their own sleep/wake times, resulting in their overall sleep being very similar. It was, however, the timing of their sleep that differed significantly, paralleled by the differences in circadian phase measured by DLMO (Figure 3.2 & Table 3.1). A near 4 h shift in endocrine rhythms (melatonin and cortisol) is similar to the 4 h difference in clock gene expression shown by Nováková et al. (2013), endorsing the molecular differences between ECPs and LCPs. ECPs DLMO was at 20:27 h compared to 23:55 h in LCPs and peak timing of cortisol 07:04 h for ECPs and 11:13 h for LCPs.

3.4.2 Resting State Functional Connectivity in Circadian Phenotypes

Differences in rs-FC between ECPs and LCPs have not been shown before. The differences in sleep timings observed between the groups were emulated by circadian timings in physiological processes (melatonin and cortisol) but no other sleep parameters. This certainly raises the question of the functional significance of these differences. Given that both groups were following their own schedules and there were no time of day effects in rs-FC identified, these differences could relate to the outcome of long term disruptions. The group of LCPs, who may be chronically affected by long term misalignment of sleep and circadian phase, have lower rs-FC in the majority of regions identified from mPFC and PCC over the 'working day' (Figure 3.5 & Figure 3.6).

This study used seed based fMRI analysis to look at rs-FC changes between ECPs and LCPs. For both seeds the DMN was clearly visible when all individuals were grouped together. However, ECPs showed higher rs-FC in 15 of the 18 identified regions across the brain as being different between the groups. These regions were not exclusive to the DMN but from the mPFC, included the right and left anterior insula (rAI and lAI), two main regions of the salience network (SN). Due to the fact that the clusters identified are linked to cognitive function and salient control, further analysis was done to explore the relationship between collective rs-FC of these regions and cognitive performance measures.

3.4.3 Cognitive Performance

The consequences of sleep and circadian disruption on health and cognitive performance are well established (Foster and Wulff, 2005, Wulff et al., 2010), along with this area gaining much interest in the general population and media. However, the use of fMRI in this area is still lacking and much of the literature surrounding the relationship between FC and cognitive performance has been mainly focused on task based fMRI leaving only a few focusing on rs-FC. Song et al. (2008) linked rs-FC to intellectual performance, whilst others have shown that the efficiency of networks and quality of structural links between spatially diverse regions are associated with greater intelligence (Chiang et al., 2009, Van Den Heuvel et al., 2009). This research marks the importance that neuroimaging could play in predicting cognitive performance. To take it one step further, a handful of studies have been carried out to try and link FC, sleep, Circadian Phenotype and cognitive performance. Most of these investigations used task based fMRI and controlled for the effect of Circadian Phenotype by scheduling testing based on internal time e.g. every 4 h starting 1.5 h after waking, preventing the exploration these effects throughout a societally constrained day

(Anderson et al., 2014, Barclay and Myachykov, 2017, Marek et al., 2010, Matchock and Mordkoff, 2009).

Using GEEs, we were able to inspect the predictive effects that rs-FC had on post-scan completed cognitive performance and daytime sleepiness. Our analysis revealed rs-FC can independently predict task performance in both PVT and Stroop. This suggests that the higher strength of rs-FC between these regions the better an individual does in a cognitive task. Since our analysis used seeds within the DMN, one could infer that the functional integrity of connections within and but also between the DMN are important for performance on a task. As previously mentioned the DMN is important in maintenance of consciousness and is also linked with many cognitive functions (Greicius et al., 2003). These cognitive domains are generally sub-served by the frontal cortex and include memory, attention and executive function. Hampson et al. (2006) showed that strength of connections between the posterior and anterior regions of the DMN at rest are positively correlated with working memory performance. Therefore, strength of rs-FC could facilitate task performance. This would be supported by the fact that mPFC seed showed a significant connection with bilateral anterior insula, known as the functional hubs of the salience network and linked to higher level cognitive control (Menon and Uddin, 2010). The fact that we show predictive effects of rs-FC on task performance lends promise to the utilisation of rs-FC for examining neurobiology non-invasively in humans. This provides important information about how intrinsic lifestyle factors are reflected in the brain's functional architecture.

3.4.4 Sleepiness

Alertness and vigilance are crucial for achieving best cognitive performance, with both decreases in objective alertness (Danker-Hopfe et al., 2001) and increases in subjective sleepiness (Babkoff et al., 1991, Dinges et al., 1997) being linked to inferior cognitive performance and increased risk of errors (Dinges, 1995). There were significant diurnal variations in the KSS observed within the groups, with ECPs reporting sleepiness to increase from morning to evening and LCPs the inverse. These results show that ECPs are least sleepy in the morning and most sleepy in the evening, whereas LCPs show the opposite relationship with highest sleepiness in the morning which decreases throughout the day, as shown in earlier work (Taillard et al., 2011) (Figure 3.7). In the GEEs analysis, time of day could predict sleepiness as well as an interaction of rs-FC and time. It is interesting to find significant differences in subjective sleepiness ratings despite the fact that ECPs and LCPs did not differ in objective measures of sleep provided by actigraphy. Subjective measures have however, been shown not to always correlate with objective sleep recordings (Baker et al., 1999). Increased sleepiness has been associated with decreases in brain metabolism and function. Thomas et al. (2000) showed global decreases in the brain's metabolic rate through PET scans, with increased sleepiness leading to cognitive decline. It is also widely accepted that increased sleep pressures due to sleep deprivation results in reduced rs-FC, particularly in areas of the DMN (Sämann et al., 2010, Yeo et al., 2015). GEE analysis showed that rs-FC can predict daytime sleepiness. These

results suggest that changes in rs-FC from mPFC and PCC may be associated with differences in subjective sleepiness, despite similarity in objective sleepiness. This may be expected since the growing research using rs-FC to investigate sleep and alertness (Thomas et al., 2000).

3.4.5 Conclusions

Previous research has shown diurnal variations in rs-FC (Blautzik et al., 2013, Hodkinson et al., 2014). Although clear diurnal variations were found in cognitive performance and sleepiness measures, surprisingly this study found that the effect of group was much clearer than an effect of time of day, suggesting a potential underlying intrinsic difference between these two groups. It is important to note that these data were gathered during common working hours (08:00 h – 20:00 h) which could have resulted in failure to record time points in which LCPs could have shown higher FC and better cognitive performance. However, as discussed throughout this Chapter, LCPs are under constant pressure to fight again their endogenously driven circadian rhythms to fit into societies imposed schedules. This could cause them to be in a state of 'perpetual chronodisruption' despite being able to follow their preferred schedules for the duration of this study. This requires further longitudinal investigations. A remedy to this issue could lie with phase advancing LCPs to enable this group to fit into a 'normal working day'. Chapter 5 will explore this idea in more details.

In summary, we have taken two groups of individuals, one of which (LCPs) have to fight against their internal clocks during regular societal working days and the other that fits into this routine. We find that:

1. There are fundamental differences in intrinsic functional architecture between ECPs and LCPs during a societally constrained working day (08:00 h to 20:00 h).
2. Rs-FC from mPFC and PCC (higher in ECPs) predicts cognitive performance measures and subjective sleepiness, which is also modulated by time of day.
3. Effects on cognition may be mediated by disruption to ICNs.
4. This could provide potential evidence of a neural basis to performance differences between ECPs and LCPs in the real world.

The implications of these findings are far reaching. Firstly, Circadian Phenotype should be a factor that is taken into account when using fMRI for research and clinical applications (discussed in Chapter 6), as should habitual sleep status (Khalsa et al., 2016). Secondly, we provide potential understanding to the neuronal basis of individual differences that may be resulting in negative outcomes linked to LCPs. Finally, LCPs are impaired during normal socially constrained days which lead to diminished cognitive performance. This suggests a need to be more conscious about how to manage time to maximise productivity and minimise health risks.

Since Chapter 3 considered how rs-FC is associated with cognitive performance measures, the next Chapter focused on the physical performance aspect. To do this, the resting state MN was

explored, seeding in the contralateral primary motor cortex of right handed individuals. The impact of Circadian Phenotype and time of day on muscle strength and rs-FC of the MN was investigated, as well as the predictive effects of rs-FC on this index of physical performance (MVC of isometric grip strength).

CHAPTER 4

Intrinsic Functional Architecture of the Motor Network in Circadian Phenotypes and the link with Physical Performance

Abstract

FC of the motor network (MN) is often used to investigate how intrinsic properties of the brain are associated with elements of physical performance. In addition, the MN is a key feature in clinical work to map the recovery to stroke and aid the understanding of neurodegenerative disorders. Diurnal variations in muscle strength have been widely reported, with the majority of studies showing peaks in the evening, but others reporting highest muscle strength in the morning. These contradictory findings could be a result of not classifying individuals into Circadian Phenotype groups. Furthermore, functional imaging techniques are rarely included in sleep and circadian research on physical performance.

This Chapter investigated rs-FC between ECPs/LCPs, and time of day in 32 healthy, right handed individuals (13 male, 23.1 ± 4.2 years). The predictive effects of rs-FC of the MN on an index of physical performance (MVC using isometric grip strength), were explored using GEEs. Significant diurnal variations in physical performance measures were identified as a whole group and in each Circadian Phenotype group. Clear significant differences were found in rs-FC of the MN between ECPs and LCPs, as well as between different times of day. These differences were able to predict MVC.

These results support the need to include clear assessments of Circadian Phenotype and time of day in neuroimaging, specifically when using the MN as a network of interest. They also show that lack of consideration for Circadian Phenotype and time of day could result in misinterpretation of data on physical performance variables.

4.1 Introduction

4.1.1 Diurnal Variations in Physical Performance

Circadian rhythmicity influences many physiological processes involved in physical performance, including plasma levels of hormones, glucose tolerance, CBT, blood pressure, and performance variables such as reaction times, alertness and memory speed (Atkinson and Reilly, 1996, Atkinson et al., 2010, Chtourou and Souissi, 2012, Reilly et al., 1996, Schwartz et al., 1997, Wever, 1979, Facer-Childs and Brandstaetter, 2015a). Muscle strength, for example, was reported to be highest in the early evening in several independent studies (Callard et al., 2000, Gauthier et al., 1996, Nicolas et al., 2005, Souissi et al., 2002) and personal best performance in the evening was confirmed across various different sports including cycling (Atkinson et al., 2007b), tennis (Atkinson and Speirs, 1998) and swimming (Kline et al., 2007, Arnett, 2002, Rodahl et al., 1976) with peak performance times sometimes as late as 23:00 h. This is surprising given the well documented variability in sleep cycles, genetics, lifestyle, age, gender and activity patterns between individual ECPs and LCPs, with ECPs generally reporting higher activity in the morning and LCPs being more active in the evening (Horne and Ostberg, 1976, Roenneberg et al., 2003). A close inspection of previous physical performance studies reveals that many of these studies failed to distinguish between these different Circadian Phenotypes, as discussed in the Introduction Chapter (Atkinson and Speirs, 1998, Arnett, 2002, Conroy and O'Brien, 1974, Gauthier et al., 1996, Kline et al., 2007, Rodahl et al., 1976, Souissi et al., 2002).

Grip strength is a simple measure of muscle strength, which is used as an evaluation of muscle function in sports and exercise settings as well as clinical practice (Roberts et al., 2011). Due to its ease of implementation, grip strength has been a major technique in the

study of stroke rehabilitation (Sunderland et al., 1989), sarcopenia (Roberts et al., 2011) and muscle fatigue (Taylor and Gandevia, 2008). MVCs are a standardised method to study grip strength. MVC of isometric grip strength offers a robust approach to investigating contributions from central and peripheral mechanisms because the ability to produce maximal force relies on the capability of the muscle as well as the activation from the central nervous system (Tamm et al., 2009).

4.1.2 The Motor Network and Physical Performance

The motor system, which is part of the CNS, consists of pyramidal i.e. voluntary movement, and extrapyramidal i.e. involuntary movement tracts (Rizzolatti and Luppino, 2001). The MN, on the other hand, refers to specific brain regions associated with motor function that are activated during tasks and connected at rest. Amongst the key ICNs is the resting state sensorimotor network (rs-MN), originally detected by Biswal et al. (1995). Within the sports world, exploring the motor system has been a fundamental part of understanding muscle fatigue, which is influenced by peripheral and central mechanisms (Taylor and Gandevia, 2008). Central fatigue refers to suboptimal force produced despite maximal effort, and is mainly associated with dysfunction of upper motor neurons and cortical output (Taylor and Gandevia, 2008). This suggests that if cortical and subcortical output from the motor system is not at an optimal level, maximum muscle force cannot be achieved. Peripheral fatigue, on the other hand, is defined as the loss of lower motor neuronal or muscle responsiveness over time creating a reduction in force production and therefore suboptimal performance. As opposed to central fatigue, peripheral fatigue is more localised to lower motor neurons and muscle fibres (Taylor and Gandevia, 2008). Incorporating rs-FC into these investigations has allowed cortical and subcortical signals of the MN to be studied non-invasively in the absence

of task demand, and has led to rs-FC of the MN being proposed as an index of recovery in muscle fatigue (Peltier et al., 2005).

Understanding the neuronal origins of physical performance is a key motivation in the exploration of central mechanisms relating to motor function. While rs-FC has been comprehensively used to study muscle fatigue, research into specific effects of rs-FC on motor function in physical performance is lacking. Studies investigating diurnal variations in rs-FC are limited, although there is evidence to suggest ICNs are highly rhythmic (Blautzik et al., 2013, Jiang et al., 2016). The difficulty in examining time of day effects is the number of confounding factors that could impact on the results. For example, the consideration of individual differences in Circadian Phenotype could result in altered diurnal patterns, as mentioned previously. Clinical work has often used rs-FC to track recovery from stroke or concussion (Johansen-Berg et al., 2002, Zhu et al., 2015). If individuals were scanned at a 'non-optimal' time of day this could result in a variation in interpretation of the results. Despite growing evidence for time of day and Circadian Phenotype effects on resting state neuronal activity, there is a shortage of studies investigating how these differences link to measures of physical performance.

The objectives of this study were two-fold. Firstly, we wanted to investigate diurnal variations in MVC between ECPs and LCPs. Secondly, we wanted to explore the potential contribution of intrinsic central mechanisms underlying diurnal variation in performance using rs-FC from a seed in the LM1, whether these vary between Circadian Phenotype groups and if they can predict physical performance variables.

Our key questions were:

1) Are diurnal variations in MVCs different between ECPs and LCPs?

2) Does rs-FC of the MN differ between ECPs and LCPs?
3) Does rs-FC of the MN vary as a function of time of day?
4) If there are differences, are they able to predict MVC?
5) Are diurnal variations in MVC influenced by intrinsic central mechanisms?

4.2 Materials and Methods

Methods for this Chapter follow those given in Chapter 3, other than the information given below. A more detailed description of methods used across all experimental Chapters is given in Chapter 2.

4.2.1 Questionnaires and Sleep Analysis

32 right handed healthy individuals (13 male, 23.1 ± 4.2 years) took part, who were the right-handed participants from the overall cohort described in Chapter 2. Groups (ECPs and LCPs) were categorised based on corrected mid-sleep on free days from the MCTQ. ECPs (n=12, 6 male) had an average age of 25.0 ± 5.2 years and a MSF_{sc} of 02:31 ± 00:10 h. LCPs' (n=20, 7 male) average age was 21.3 ± 3.2 years and MSF_{sc} was 06:59 ± 00:17 h. Age, height and weight were not significantly different between the groups (Table 4.1). Actigraphy data and saliva samples were collected and analysis as described in Chapter 3 and summarised in Table 4.1.

4.2.2 Neuroimaging Acquisition and Analysis

Imaging data was collected as described in Chapter 3. Preprocessing and analysis of fMRI data was performed as described in Chapter 2, using the left primary motor cortex (LM1) as a seed. To explore the difference between ECPs and LCPs a flexible factorial model was used in SPM to include the effect of group, time and subject. A further analysis was done independently of Circadian Phenotype group, using predefined regions of the sensorimotor network (FindLab, https://findlab.stanford.edu/functional_ROIs.html (Figure 4.1). In house matlab code was used to extract correlation values from LM1 to clusters identified as different between Circadian Phenotypes, and to the pre-defined regions.

Therefore, analysis of diurnal variations in rs-FC and predictive effects on physical performance variables were explored by two means:

1) **Using identified regions between Circadian Phenotypes**

To investigate time of day differences in rs-FC identified as different between Circadian Phenotypes, the correlation values from the regions of the primary motor network (LM1, RM1) as well as the average value from LM1 to all significant clusters identified as different between ECPs and LCPs, were used in the analysis.

2) **Using pre-defined regions of the Sensorimotor Network (Findlabs)**

To allow further investigation into time of day differences in rs-FC of the MN, pre-defined regions of interest for the MN were used independently of Circadian Phenotype (Figure 4.1). Specifically, correlation values were calculated between seed region (LM1) and RM1, SMA (Figure 4.2) as well as an average value from LM1 to whole MN, which included the thalamus and cerebellum.

Descriptions of significant findings (FWE, $p<0.05$), are presented as total voxels, peak t score and peak MNI centroid cluster coordinates [x y z]. Average values from LM1 in both sets of analyses will be referred to as 'total' or 'average' rs-FC.

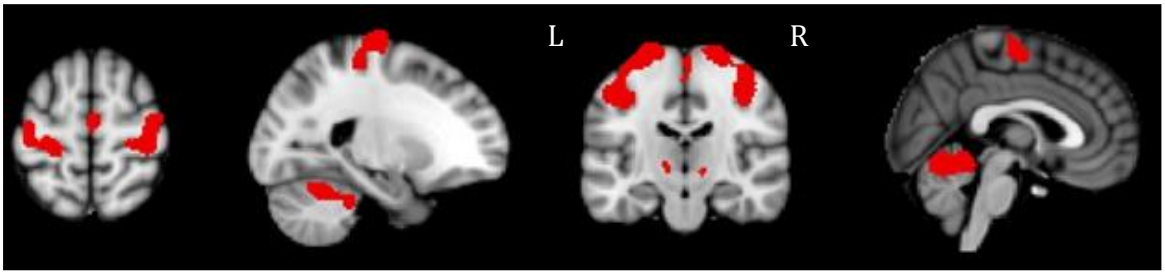


Figure 4.1. Findlab's Sensorimotor Network shown in axial, sagittal and coronal views (Shirer et al., 2012).

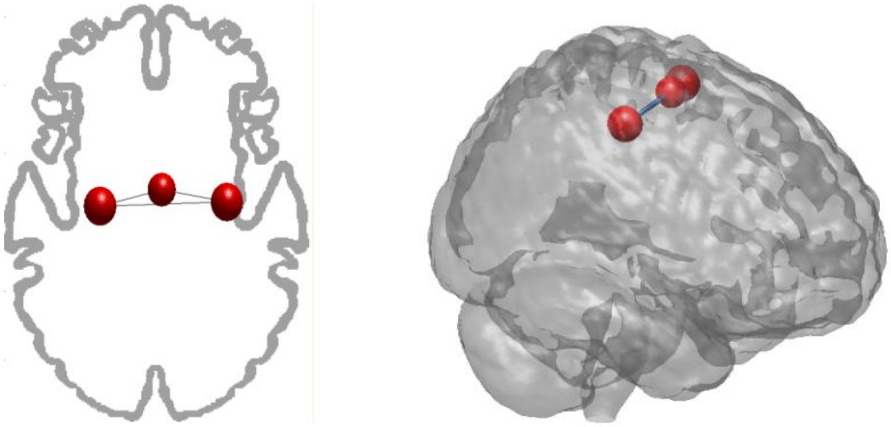


Figure 4.2. 2D and 3D images to show the positive correlations between areas of the primary motor network used in the analysis which include LM1, RM1 and SMA.

4.2.3 Physical Performance

MVC was measured using the six second isometric grip strength test using an electronic hand dynamometer (EH101, CAMRY). Protocol followed the description given in Chapter 2. MVC and percentage of best MVC will collectively be referred to as physical performance variables in this Chapter.

4.2.4 Statistical Analysis

Raw grip strength (MVC) data includes subject variability in fitness, general muscle strength etc. and is a measure that combines both peripheral and central contributions to performance outcome. To normalise these effects, percentages of performance maximum were used in addition to the raw MVC scores. The raw data were transformed into percentages using the time that best performance was achieved as 100% and other values were calculated as a percentage decrease relative to this score. Normalising the data reduces between subject variability, as well as making the groups comparable for analysis of time of day differences. Therefore, both raw and percentage scores were used in the analysis. These results were combined with rs-FC correlation values using GEEs to explore the predictive effects of rs-FC and time of day on physical performance variables. A gamma response with a log link function GEE was used to model the effects of rs-FC on physical performance. Any interaction terms that were not significant in the model were removed and analysis re-run.

4.3 Results

The results are presented as:

1) Circadian phenotyping (as described in Chapter 3).
2) Diurnal variations in physical performance.
3) Rs-FC differences between ECPs and LCPs and between time of day.
4) Rs-FC of pre-defined regions of the sensorimotor network and time of day.
5) Using rs-FC to predict motor performance using GEEs.

4.3.1 Circadian Phenotyping

Right handed individuals were categorised into ECPs (n=12) and LCPs (n=20), as described in Chapter 3. Age, height and weight were comparable in both groups with no significant differences found. MSF_{sc}, sleep onset, wake up, DLMO and time of peak cortisol were all significantly different between the groups, whilst sleep duration, efficiency, latency and phase angle were not (Table 4.1).

Table 4.1. Summary of demographic, actigraphy and physiological details for ECPs and LCPs. Values are shown as mean ± SEM unless specified. Significance is shown with [a]unpaired two sample t-tests, [b]non-parametric Mann-Whitney or [c]Fisher's exact test. All p values are FDR corrected.

Variable Measured (mean ± SEM)	**ECPs**	**LCPs**	**Significance**
Sample Size	N=12	N=20	n/a
Number of Scans/Testing Sessions	N=36	N=60	n/a
Percentage of Males/Females (%)	M: 50	M: 35	ns[c]
	F: 50	F: 65	ns[c]
Age (years, mean ± SD)	25.00 ± 5.21	21.25 ± 3.18	ns[a]
Height (cm)	173 ± 2.56	172 ± 2.54	ns [a]
Weight (kg)	66.50 ± 2.86	67.50 ± 2.32	ns [a]
MCTQ Score (hh:mm)	02:31 ± 00:10	06:59 ± 00:17	p<0.0001[a]
Sleep Onset (hh:mm)	22:57 ± 00:11	02:35 ± 00:20	p<0.0001[b]
Wake Up Time (hh:mm)	06:33 ± 0.10	10:20 ± 00:19	p<0.0001[b]
Sleep Duration (h)	7.58 ± 0.23	7.66 ± 0.16	ns[a]
Sleep Efficiency (%)	78.50 ± 2.29	76.77 ± 1.12	ns[a]
Sleep Onset Latency (hh:mm)	00:23 ± 00:07	00:25 ± 00:02	ns[b]
Phase Angle (hh:mm)	02:27 ± 00:23	02:28 ± 00:20	ns[a]
Dim Light Melatonin Onset (hh:mm)	20:23 ± 00:20	00:10 ± 00:27	p<0.0001[b]
Cortisol Peak Time (hh:mm)	07:11 ± 00:17	11:24 ± 00:24	p<0.0001[a]

4.3.2 Diurnal Variations in Physical Performance

Significant time of day differences in MVC were found for the whole sample ($F(2,57)=10.16$, $p=0.0002$), as well as for ECPs ($F(2,19)=5.68$, $p=0.015$) and LCPs ($FQ=21.70$, $p<0.0001$) (Figure 4.3A). In the ECPs group, MVC was 41.68 ± 2.76 kg at 08:00 h, 43.18 ± 2.91 kg at 14:00 h and 40.52 ± 2.45 kg at 20:00 h. A significant difference was found between afternoon and evening values ($p=0.0036$). LCPs' lowest score was at 08:00 h (33.43 ± 2.40 kg) and increased in the afternoon (36.45 ± 2.39 kg) and evening (38.12 ± 2.48 kg). The difference between morning and afternoon as well as morning to evening was found to be significant ($p=0.008$ and $p<0.0001$ respectively). These significant findings remained when values were converted to percentage of maximum MVC per individual (Figure 4.3B).

For the whole group, significant diurnal variations were found ($FQ=13.18$, $p=0.0014$), with 08:00 h to 14:00 h and 20:00 h remaining significant ($p=0.0022$ and $p=0.015$ respectively). There was a 5.61% difference between best and worst percentage of maximum performance in ECPs ($FQ=8.98$, $p=0.011$). Average performance in the morning was 96.00 ± 1.48 %, 99.43 ± 0.30 % in the afternoon and 93.82 ± 0.95 % in the evening. Afternoon to evening performance was significantly different ($p=0.0092$). LCPs showed a 12.24% difference over the course of the day ($FQ=21.70$, $p<0.0001$). Worst performance was seen in the morning (86.32 ± 1.92 %) and best performance in the evening (98.56 ± 0.62 %) ($p<0.0001$). Afternoon performance for LCPs was 94.32 ± 1.37 % and also differed significantly compared to morning ($p=0.008$).

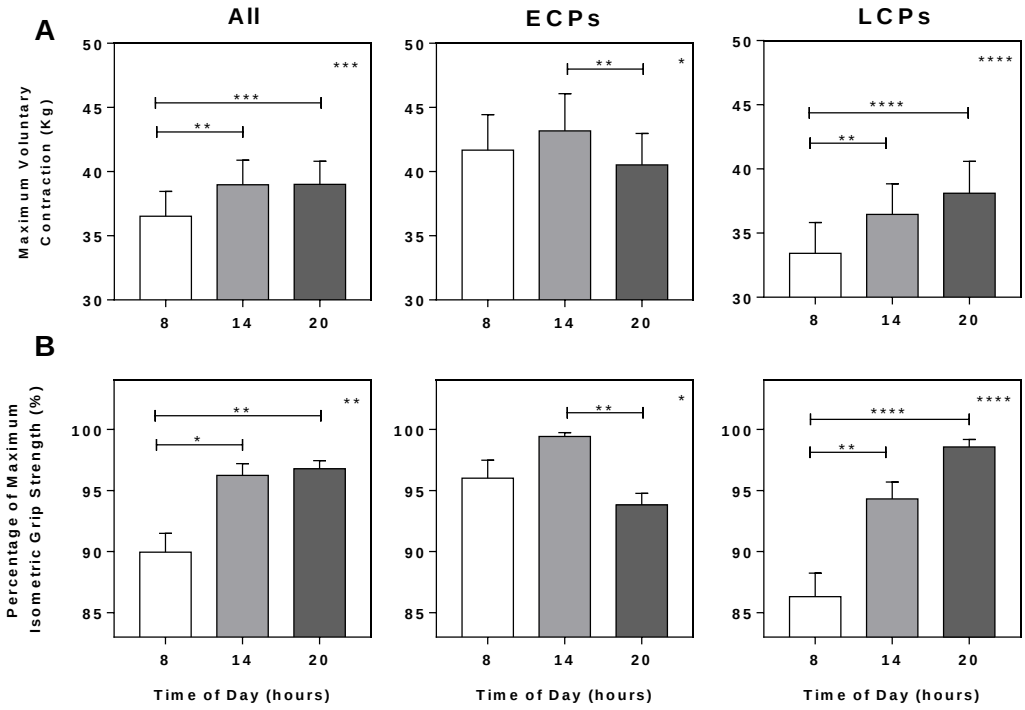


Figure 4.3. Time of day differences in maximum voluntary contraction (MVC) and percentage of MVC for all participants, ECPs and LCPs. A) MVC (kg) for All, ECPs and LCPs B) Percentage of maximum MVC (%) for All, ECPs and LCPs.

4.3.3 Impact of Circadian Phenotype on rs-FC of the Motor Network

The main regions of the rs-MN were identified with whole group analyses seeded from LM1 (ECPs + LCPs contrast, corrected FWE $p<0.05$, top row of Figure 4.4 & Table 4.2). The largest cluster encompassed the majority of MN regions and included bilateral primary and supplementary motor areas (41111 voxels, t=58.04, [28 -24 70]). The left and right thalamus were also clearly shown (322 voxels, t=23.74, [-12 -22 0] and 252 voxels, t=22.83, [14 -20 0] respectively). The left and right cerebellum were visible (262 voxels, t=20.84, [-12 -60 -24] and 113 voxels, t=19.45, [12 -62 -26]), as well as a cluster in the right parahippocampal gyrus (123 voxels, t=15.51, [24 -40 0]) and the right temporal lobe (34 voxels, t=13.52, [52 -32 -12]).

Table 4.2. Summary of significant brain regions for whole sample when seeding in the LM1.

Region	Cluster size (voxels)	MNI Centroid Coordinates [x y z]	Maximum t-score
Motor Network	41111	[28 -24 70]	58.04
Left Thalamus	322	[-12 -22 0]	23.74
Right Thalamus	252	[14 -20 0]	22.83
Left Cerebellum	262	[-12 -60 -24]	20.84
Right Cerebellum	113	[12 -62 -26]	19.45
Right Parahippocampal Gyrus	123	[24 -40 0]	15.51
Right Temporal Lobe	34	[52 -32 -12]	13.52

When comparing the effect of group, eight significant clusters were identified as having significantly higher rs-FC in ECPs (second and third rows of Figure 4.4 and Table 4.3). A cluster was found within the seed region of LM1 and in the contralateral RM1 (LM1, 68 voxels, t=8.28, [-24 26 82] and RM1, 31 voxels, t=11.60, [44 -20 28]), as well as the right paracentral lobule (35 voxels, t=6.36, [4 -36 52]). The largest cluster was located in RM1 and included the SMA (147 voxels, t=11.95, [30 -24 80]). The remaining clusters were located in the left superior temporal gyrus (72 voxels, t=8.58, [-24 14 -26]), right insula (51 voxels, t=8.15, [30 -34 16]), left temporal lobe (46 voxels, t=8.32, [-38 -40 6]) and right inferior frontal gyrus (41 voxels, t=6.81, [14 10 -22]). No significant clusters were identified as higher in LCPs (LCPs > ECPs contrast).

Table 4.3. Summary of significant brain regions higher in ECPs when seeding in the LM1.

Region	Cluster size (voxels)	MNI Centroid Coordinates [x y z]	Maximum t-score
Right Primary Motor Cortex & SMA	147	[30 -24 80]	11.95
Left Superior Temporal Gyrus	72	[-24 14 -26]	8.58
Left Primary Motor Cortex	68	[-24 -26 82]	8.28
Right Insula	51	[30 -34 16]	8.15
Left Temporal Lobe	46	[-38 -40 6]	8.32
Right Inferior Frontal Gyrus	41	[14 10 -22]	6.81
Right Paracentral Lobule	35	[4 -36 52]	6.36
Right Primary Motor Cortex	31	[44 -20 28]	11.60

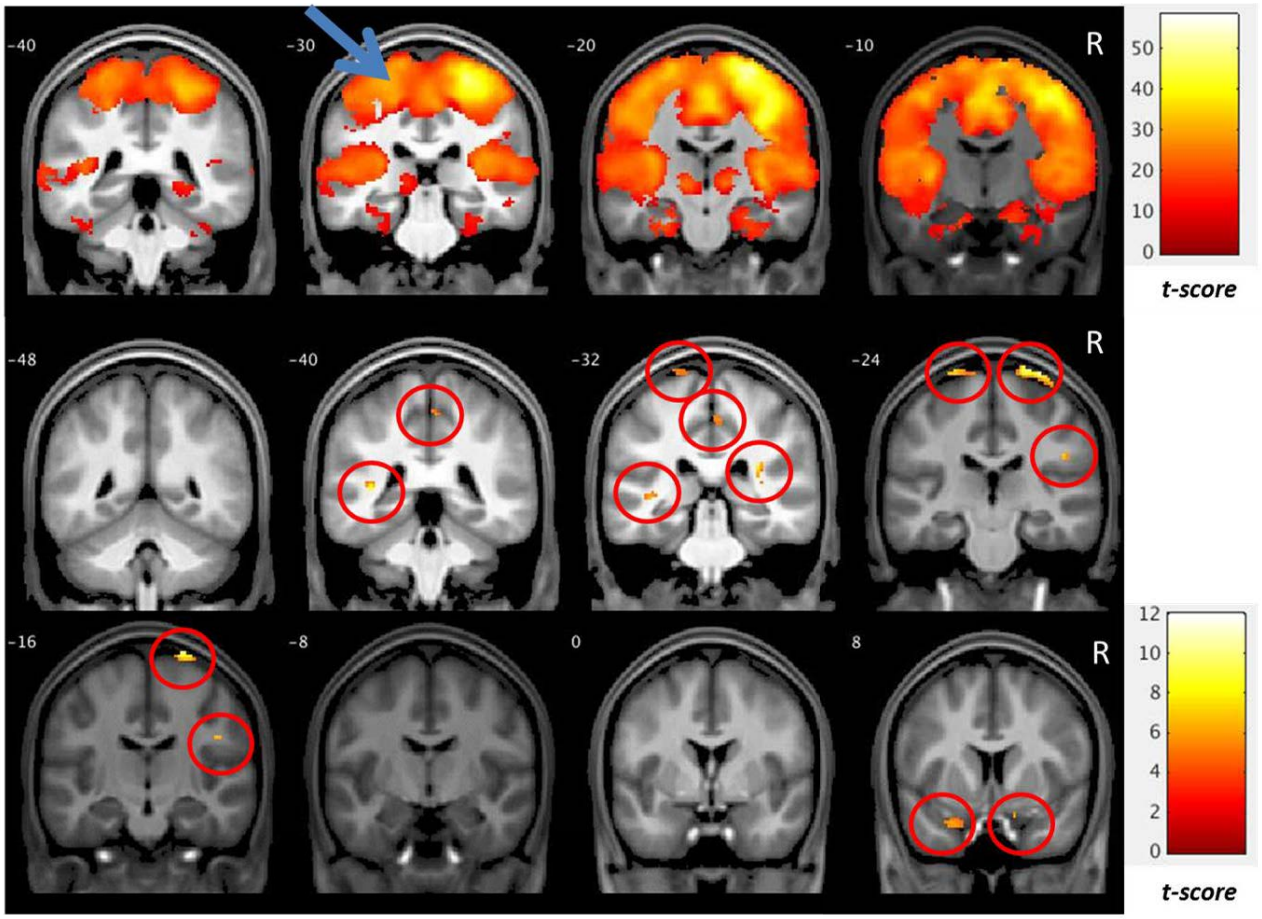


Figure 4.4. Resting state functional connectivity maps using the LM1 seed. Seed region is indicated by the blue arrow. Top row shows whole sample group maps. Second and third rows show regions higher in ECPs (ECPs > LCPs). Significant clusters (FWE $p<0.05$, extent threshold = 30 voxels) are shown in yellow and circled in red. T score scales for each contrast are shown on the right.

4.3.4 Identified Regions between Circadian Phenotypes

There were no significant diurnal variations found for average rs-FC (from the LM1 to all significant regions) in the whole group, ECPs or LCPs (Figure 4.5A). From LM1 to the

largest cluster identified (RM1), a significant time of day difference was found for all subjects (F=3.21, p=0.049), with post hoc tests showing a significant increase from morning to afternoon (p=0.029). This difference was also seen for LCPs with rs-FC increasing from morning and afternoon (p=0.021). There were no significant time of day differences in ECPs (Figure 4.5B). Rs-FC from seed to LM1 (within seed region), demonstrated significant diurnal variations for all subjects (F=5.01, p=0.010), and LCPs (F=8.89, p=0.0009) but not ECPs (Figure 4.5C). Rs-FC was lowest at 08:00 h with significant increases between morning and afternoon (p=0.023 for all subjects and p=0.006 for LCPs). Evening rs-FC was also significantly higher than morning (p=0.041 for all subjects and p=0.007 for LCPs). Nonlinear regression curves show that rs-FC remained stable for ECPs with all measures showing no significant time of day differences (Figure 4.6). The only significant diurnal variations were found in LCPs with rs-FC between LM1 and RM1 and within LM1. Rs-FC from LM1 to RM1 was lowest in the morning (0.23 ± 0.01), highest in the afternoon (0.25 ± 0.01) and decreased again in the evening (0.24 ± 0.01). Rs-FC for LCPs within LM1 was, again, lowest at 08:00 h (0.17 ± 0.01), increasing throughout the day from afternoon (0.22 ± 0.02) to evening (0.23 ± 0.02).

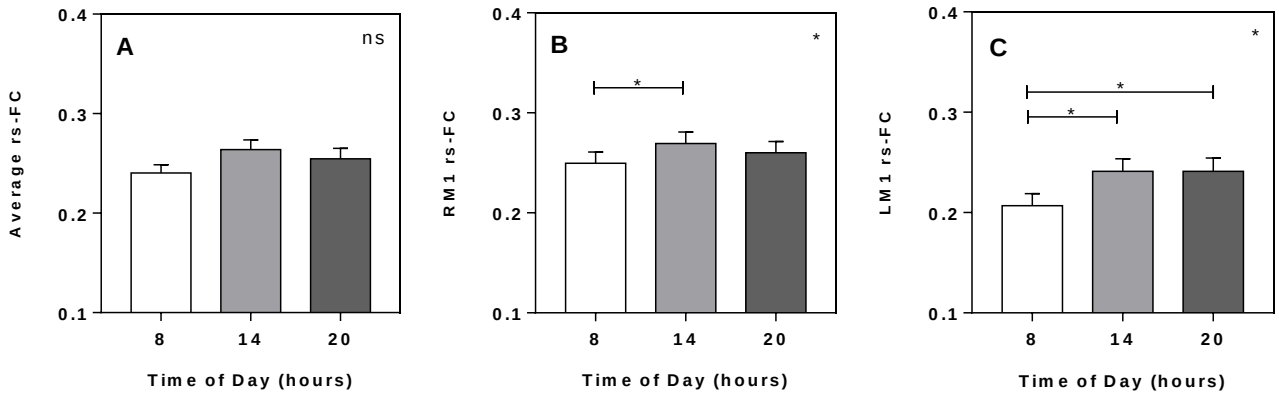


Figure 4.5. Diurnal variations in rs-FC to regions higher in ECPs (ECPs > LCPs) for whole population from seed to A) Average of all identified clusters, B) RM1 and C) LM1.

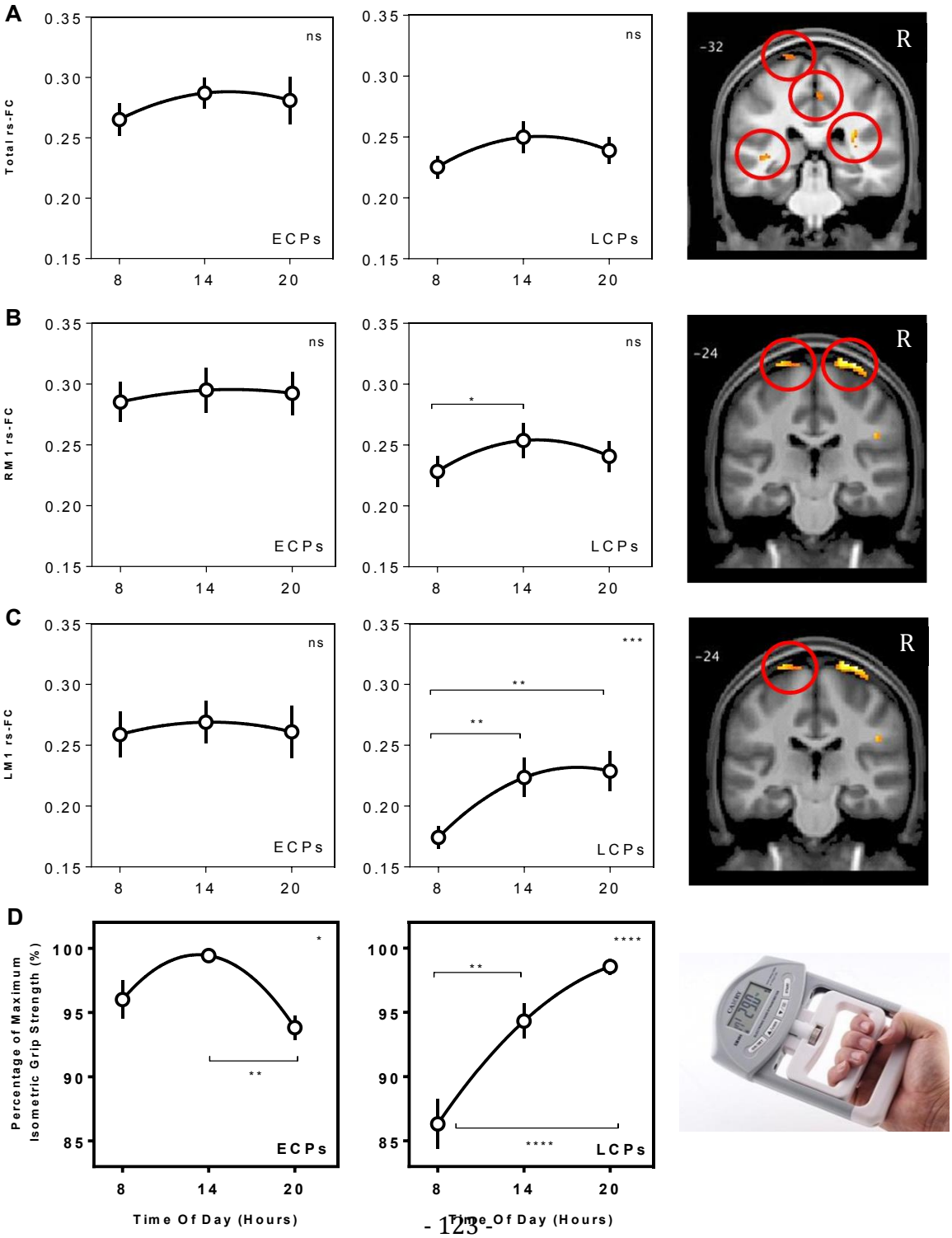


Figure 4.6. Nonlinear regression curves of rs-FC from seed (LM1) to regions identified as different between Circadian Phenotypes (ECPs > LCPs) and percentage of MVC.
A) Average rs-FC between LM1 and all identified regions, B) Rs-FC between LM1 and RM1, C) Inter-seed rs-FC in LM1, D) Percentage of best MVC performance. Red circles identify regions used.

4.3.5 Pre-Defined Regions of the Sensorimotor Network

Measures used in the analysis were:

1) Average rs-FC from LM1 to whole predefined sensorimotor network (total).
2) Rs-FC from seed to RM1.
3) Rs-FC from seed to SMA.

Significant diurnal variations were found for average rs-FC from LM1 to the whole sensorimotor network for all subjects ($FQ=6.44$, $p=0.040$) and LCPs ($F(2, 38)=3.94$, $p=0.028$) (Figure 4.7A). For both groups, average FC was significantly lower in the morning compared to the afternoon ($p=0.037$ for all subjects and $p=0.038$ for LCPs). Rs-FC from LM1 to RM1 was significantly affected by time of day at the whole group level ($FQ=6.81$, $p=0.033$) and for LCPs ($F(2, 38)=5.48$, $p=0.008$) but again, not for ECPs (Figure 4.7B). Post hoc tests revealed significant increases from morning to afternoon ($p=0.023$) and morning to evening ($p=0.026$) in LCPs. A similar pattern was seen for rs-FC from LM1 to SMA (Figure 4.7C). There were significant time of day differences for all subjects ($FQ=6.44$, $p=0.040$) and LCPs ($F(2,38)=6.95$, $p=0.003$). Both groups showed a significantly higher rs-FC in the afternoon compared to morning ($p=0.037$ for all subjects and $p=0.006$ for LCPs), and LCPs also had higher rs-FC in the evening compared to morning LCPs ($p=0.012$). Nonlinear regression analysis using correlation values from predefined regions showed significant diurnal variations in LCPs but not ECPs for rs-FC of RM1 (Figure 4.8B), SMA

(Figure 4.8C) as well as average rs-FC (Figure 4.8A). Average rs-FC from LM1 to all regions of the MN was highest at 14:00 h for ECPs and LCPs. Lowest values, however, were at 20:00 h for ECPs and 08:00 h for LCPs. Rs-FC to RM1 followed a very similar pattern with highest values identified at 14:00 h. Rs-FC for ECPs was similar at 08:00 h and 20:00 h, whereas in LCPs there was a significant difference between rs-FC at 08:00 h and 20:00 h. This significant difference in LCPs was also seen between 08:00 h and 14:00 h. For rs-FC to SMA no significant diurnal variations were found in ECPs, whose highest values were at 14:00 h and 08:00 h and lowest at 20:00 h. There were significant differences in LCPs between 08:00 h and 14:00 h as well as between 08:00 h and 20:00 h.

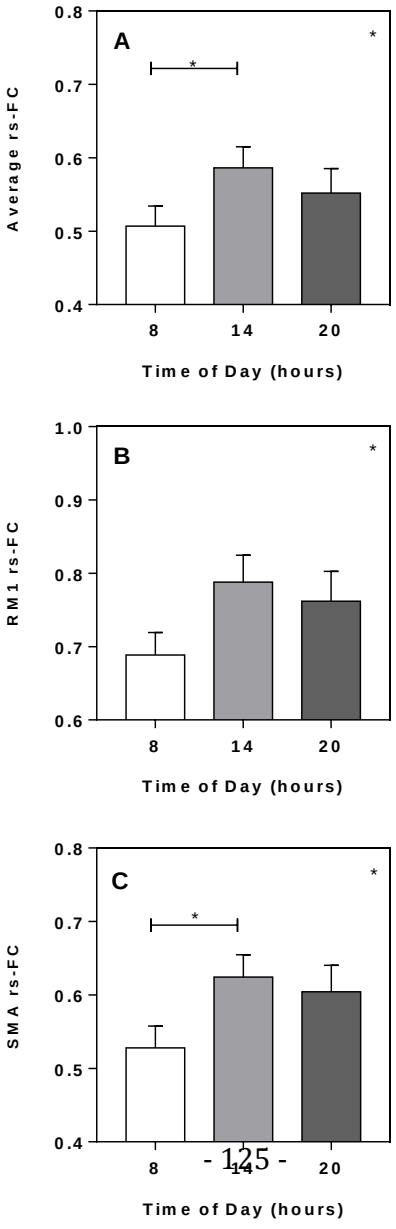


Figure 4.7. Diurnal variations in rs-FC (pre-defined regions) of the sensorimotor network for the whole population from seed to A) Average from LM1 to whole network, B) RM1 and C) SMA.

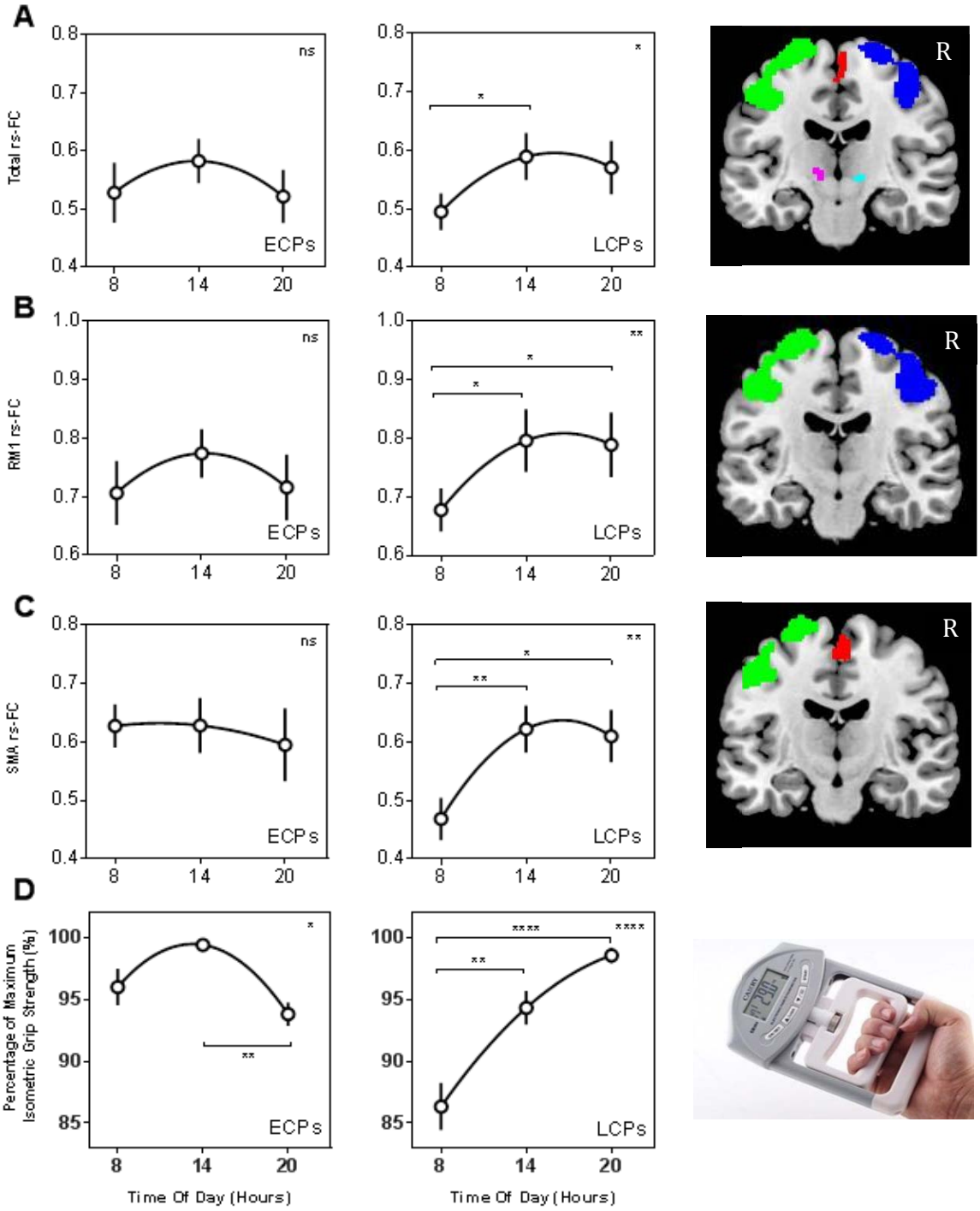


Figure 4.8. Nonlinear regression curves of rs-FC from seed (LM1) to pre-defined regions and percentage of MVC. A) Average rs-FC between LM1 and motor network, B) Rs-FC between LM1 and RM1, C) Rs-FC between LM1 and SMA, D) Percentage of best MVC performance. LM1 region is shown in green, RM1 in blue, SMA in red and bilateral thalamus in pink/aqua. Cerebellum is not shown but included in average rs-FC values.

4.3.6 Predicting Performance

The previous analyses have demonstrated diurnal variations in motor performance and rs-FC as a whole group, as well as differences in both of these factors between ECPs and LCPs. GEEs were further used to investigate if rs-FC could predict raw MVC and percentage MVC. The two levels of analysis were used in separate GEE analysis:

1. Rs-FC values extracted from regions identified as different between Circadian Phenotypes (ECPs > LCPs) (Figure 4.9A).
2. Rs-FC extracted from predefined regions of the sensorimotor network (Figure 4.9B)

4.3.6.1 Identified Regions between Circadian Phenotypes

Average rs-FC from LM1 to all identified clusters (ECPs > LCPs) as well as rs-FC from LM1 to largest cluster found in the RM1 (ECPs > LCPs) were used. Both models showed that the interaction of time of day and rs-FC could predict percentage of best MVC meaning that the ability of rs-FC to predict percentage of MVC depends on the time of day (average rs-FC; W=9.0, p=0.011 and RM1; W=8.9, p=0.012). Rs-FC to RM1 could also predict MVC (W=4.6, p=0.033). Independently, time of day was able to predict MVC, the model using average rs-FC (W=7.4, p=0.025) and rs-FC to RM1(W=7.8, p=0.020). The same was seen for percentage of MVC (average rs-FC model; W=10.3, p=0.006, and RM1; W=10.3, p=0.006).

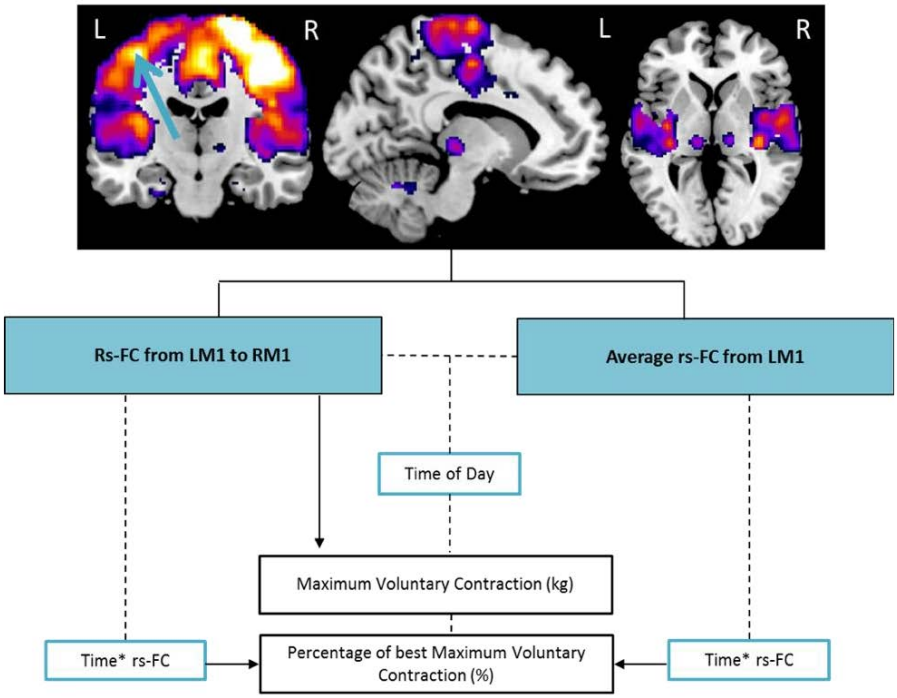
4.3.6.2 Pre-defined Regions (Sensorimotor Network)

To allow an analysis independently of Circadian Phenotype, defined regions of the MN (whole network, RM1 and SMA) were used to explore these relationships further. In all the models time of day could independently predict percentage of MVC (LM1 to: whole network; W=18.3, p<0.0001, RM1; W=18.8, p<0.0001 and SMA; W=18.5, p<0.0001). Time of day could also independently predict raw MVC score in the model using SMA rs-FC (W=7.5, p=0.024). In the RM1 and SMA models rs-FC could independently predict raw MVC (W=5.2, p=0.022 for RM1 and W=12.2, p<0.0001 for SMA) but not percentage of MVC. The interaction of rs-FC and time of day was significant in the SMA model (W=7.0, p=0.030).

4.3.6.3 Summary of GEEs

GEE analysis shows that rs-FC can predict physical performance variables independently of time of day from regions identified as higher in ECPs (RM1, Figure 4.9A), and pre-defined regions (RM1 and SMA, Figure 4.9B). These clusters encompass the same regions of the primary motor network. Therefore, despite regions being identified in different ways, they show a very similar result. These results point towards the conclusion that higher rs-FC values can predict better performance achievement in MVCs.

(A)


L
R
L
R
Rs-FC from LM1 to RM1
Average rs-FC from LM1
Time of Day
Maximum Voluntary Contraction (kg)
Time* rs-FC
Percentage of best Maximum Voluntary Contraction (%)
Time* rs-FC

(B)

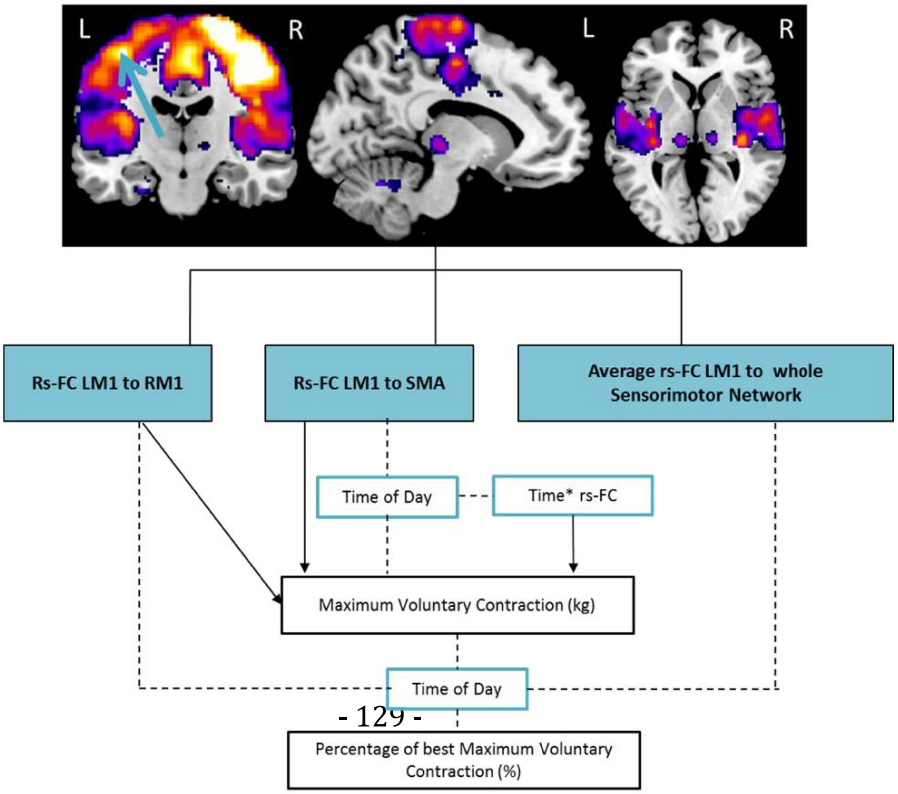

L
R
L
R
Rs-FC LM1 to RM1
Rs-FC LM1 to SMA
Average rs-FC LM1 to whole Sensorimotor Network
Time of Day
Time* rs-FC
Maximum Voluntary Contraction (kg)
Time of Day
Percentage of best Maximum Voluntary Contraction (%)

Figure 4.9. Summary of Motor Network GEEs analysis using A) Identified regions between Circadian Phenotypes (ECPs > LCPs) and B) Predefined regions of the sensorimotor network. Blue boxes show what correlation values were used in the models. Dotted lines indicate when time of day or the interaction of time of day and rs-FC were significant. Arrows show when a variable of rs-FC was able to significantly predict performance.

4.4 Discussion

Factors affecting optimal performance span from cognitive and physical abilities to expert skills, training, and experience. One factor that is frequently overlooked in the study of diurnal variations in muscle strength is individual differences in circadian and sleep patterns. In addition, there is a need for a greater understanding of central contributions on physical performance measures in this field. As previously discussed, there are diurnal differences in physiology and behaviour between ECPs and LCPs (Baehr et al., 2000, Burgess and Fogg, 2008). The underlying differences in intrinsic functional architecture of the MN, shown in this Chapter, reinforces that these factors should be taken into account when investigating elements of physical performance. The results presented also highlight the importance that Circadian Phenotype, time of day and rs-FC could have on predicting MVC measured with isometric grip strength. Here we show:

1) Significant diurnal variations in MVC between ECPs and LCPs.
2) Significant differences in rs-FC from LM1 between ECPs and LCPs.
3) These differences, identified as higher in ECPs, vary depending on time of day.
4) Significant time of day differences in the rs-FC of the pre-defined MN.
5) Rs-FC can predict performance variables.

4.4.1 Diurnal Variations in Physical Performance

Clear differences in sleep patterns and physiology confirm these groups as ECPs and LCPs as described in Chapter 3. Previous discoveries of time of day effects on muscle strength were not surprising given the multitude of physiological functions that are circadian regulated. Circadian and diurnal rhythms in MVCs have been reported in a number of studies, with results predominantly showing acrophase in the early evening in line with the CBT rhythm (Atkinson and Reilly, 1996). The increase in CBT during the evening has been suggested to enhance the capacity of muscle force (Bernard et al., 1998). However, more recent research has indicated that CBT cannot be the main reason for these differences since larger fluctuations in temperature are required to affect muscle function. Consequently, many other factors may be attributed to these times of day variations (Tamm et al., 2009). The ability to generate MVC relies on both peripheral and central mechanisms to activate the muscle. Martin et al. (1999) proposed that the diurnal variation in muscle force does indeed peak in the evening, but that this cannot be solely explained by a rise in CBT. The authors suggest peripheral factors may play a more dominant role in time of day fluctuations. We show clear diurnal variations in MVC for both ECPs and LCPs (Figure 4.3). Both raw and percentage MVC score was highest at 14:00 h for ECPs and 20:00 h for LCPs. These distinctly different patterns support the studies showing variation between Circadian Phenotypes, which contradicts the long-established view of muscle strength peaking in the evening. In fact, if the results are grouped together peak performance would occur on average at 17:30 h, in line with previous literature (Callard et al., 2000, Martin et al., 1999). Moreover, there was a much larger range in performance observed in LCPs, who consistently show more significant diurnal variations. Best and worst performance varied by 5.61% in ECPs and 12.24% in LCPs (Figure 4.6D). These

results alone emphasise the importance of controlling for Circadian Phenotype differences in future research looking at physical aspects of performance.

4.4.2 Impact of Circadian Phenotype on rs-FC of the Motor Network

The extent to which central mechanisms impact on physical performance remains largely unknown. However, being able to study the motor system *in vivo* has allowed the potential to study the central contributions to motor function. Despite the rise in neuroimaging techniques being used for clinical and research purposes, time of day and Circadian Phenotype are factors that are rarely considered. A handful of studies have explicitly looked at the effects of time of day or Circadian Phenotype on FC. The main outcomes show that ICNs fluctuate over the course of the day with varying degree. The DMN and MN seemed to be highly rhythmic whereas the executive network remained reasonably stable over an 18 h period (Blautzik et al., 2013). Conversely, Schmidt et al. (2012), did show time of day influences in the central executive network during tasks. Others have proposed Chronotype as a predictor of MN modulation during tasks (Peres et al., 2011).

Our results show distinctive differences in rs-FC from LM1, with ECPs having higher connectivity to all identified regions (Figure 4.4). The majority of these regions are components of the MN and include both RM1 and LM1. What is more, these regions identified as having higher FC to LM1 in ECPs compared to LCPs also show time of day differences (Figure 4.5). Interestingly, the diurnal variations are much clearer in LCPs who have noticeably lower rs-FC during the morning, discussed below. LCPs in this study were

not acutely sleep deprived as they had similar sleep durations to ECPs. However, as discussed in Chapter 3, the differences observed with ECPs having higher rs-FC may be down to a more chronic build-up of circadian and sleep disruption prior to taking part in the study (Wittmann et al., 2006).

It has been suggested that higher rs-FC of the MN could help modulate the change to task dependent behaviour (Jiang et al., 2004). Given that the MN is highly dynamic, higher connectivity at rest could result in the brain being 'more prepared' to switch between resting and task states (Jiang et al., 2004). One could speculate that as ECPs have higher rs-FC from LM1 than LCPs, their ability to perform in motor tasks is enhanced. If the rs-MN is 'primed', the central mechanisms of motor function could have more influence on behavioural output. This would be supported by the reduced variation in performance for ECPs compared to LCPs.

4.4.3 Impact of Time of Day on rs-FC of the Motor Network

The rs-MN has been shown to be highly rhythmic over the course of the day during various motor tasks as well as at rest (Blautzik et al., 2013). The results of this Chapter have highlighted that diurnal variations in rs-FC of the MN between ECPs and LCPs show distinct differences.

Over a societally constrained day (08:00 h to 20:00 h), LCPs show much clearer diurnal variations in rs-FC of the MN. ECPs remain relatively stable with no significant differences from morning to evening for both group-defined (Figure 4.6) and pre-defined regions (Figure 4.8). LCPs, however, show significant rhythmicity. In both analyses (group and pre-defined regions) time of day differences were identified between LM1, RM1 and SMA. The largest effect was observed for the morning session, with afternoon and evening FC

showing comparable results. There were remarkable similarities in the diurnal variation of rs-FC for the MN and isometric grip strength. MVC and percentage of best MVC showed clear time of day differences with ECPs performing their best at 14:00 h and worst at 20:00 h. LCPs on the other hand performed their worst at 08:00 h which increased throughout that day to best performance at 20:00 h.

As this study was designed to measure individuals at specific clock times as opposed to internal time, the 08:00 h testing session occurs during a 'negative biological time' for LCPs. Average wake up and MSF_{sc} times for LCPs were 10:20 h and 06:59 h respectively, meaning the morning testing was carried out at -2.33 h internal time or an hour after MSF_{sc}. If LCPs were measured during their 'biological night', the impact of the sleep homeostat may be a large factor accounting for the decreased rs-FC at 08:00 h. However, the fact that there were no time of day differences found in the initial flexible factorial fMRI analysis suggests that the impact of Circadian Phenotype is much larger than that of time of day. As no significant diurnal variations were seen for average connectivity of all group defined regions it makes sense that time of day was not identified in the second level analysis (Figure 4.5). Nonetheless, it seems that rs-FC from LM1 to specific regions does show fluctuations over the course of the day. These regions include LM1, RM1 and SMA, which are central components of the primary motor network.

These results were confirmed by pre-defining MN regions independently of Circadian Phenotype and observing similar patterns in diurnal rhythmicity between LM1 and RM1 (Figure 4.8). In addition, pre-defining regions uncovered further time of day differences between LM1 and SMA, as well as for the MN as a whole (average rs-FC from LM1 to all regions). Although these analyses show similar results, there are slight differences. This is

presumably because the regions used are themselves marginally different, which would need to be explored further to uncover why these regions may be behaving differently.

The fact that physical performance at 08:00 h is also the most 'impaired' suggests that the disruption to central mechanisms could be impacting on motor function. Sleep deprivation is known to affect rs-FC, with the general view that lack of sleep and disruptions of sleep reduce rs-FC, although this relationship may differ between different ICNs. MVC, on the other hand, has been shown not to be particularly affected by sleep deprivation indicating an endogenous circadian component to time of day effects (Jasper et al., 2009). These results could reinforce the idea that the circadian system plays a more dominant role than the sleep homeostat. As these two processes were not measured separately e.g. in a CR protocol, this remains speculative and cannot be confirmed without further studies.

4.4.4 Predicting Physical Performance

Both levels of GEE analysis (using group-identified and predefined regions), confirm that both rs-FC and time of day contribute to the variability in performance measured by raw and percentage MVC scores (Figure 4.9). The interactions shown for both sets of analyses suggest that the relationship between rs-FC and physical performance variables differs depending on the time of day. One could propose that these findings could be driven by the significantly lower rs-FC and performance values recorded in the morning. Both analyses clearly show that rs-FC between seed (LM1) and RM1 plus SMA in the morning is significantly lower than other times of day, which is paralleled by the performance variables (Figure 4.6 & Figure 4.8).

Independently, time of day was a more significant predictor when using percentage of performance maximum, which is supported by the significant diurnal variations discussed

previously, whereas rs-FC played a more dominant role in predicting raw MVC score. This means that time accounted for more of the variability in performance when converted to percentages, but rs-FC could account for a different part of the variance in raw scores. Although the hypothesis of transforming the data to percentages could account for more central mechanisms contributing to performance, these results suggest that it is the raw MVC score that can be better predicted by rs-FC. It is interesting that these predictive effects seem to be from rs-FC between specific regions, and not the MN as a whole, shown by average rs-FC in both analyses being unable to independently predict raw MVC. This implies that sub-regions of the MN are behaving differently, which minimises the effects of averaging rs-FC across regions.

These results are supported by research on task based fMRI showing increased activation of the primary and supplementary motor cortices on contralateral and ipsilateral sides with stronger grip strength (Ismail et al., 2014). Others have concluded that higher rs-FC between left and right LM1 as well as SMA can be detected after four weeks of motor training (Xiong et al., 2009). Grip strength depends on many factors, but if individuals are able to generate more force it may be due to increased training/exercise, which would be reflected by higher rs-FC. Although peripheral mechanisms were not measured specifically, with EMG for example, the variation in time of day of rs-FC of the MN suggests that central mechanisms may be contributing to the variation in MVC performance. However, it is important to consider the feedback effects of peripheral mechanisms on neuronal networks. There is a constant flow of information between the central nervous system and distal lower motor neurons that make up the peripheral nervous system. Therefore, peripheral mechanisms feedback to the central nervous system to facilitate the ability to perform and could in turn impact on FC of the MN. The relative contributions of the peripheral and central mechanisms remain to be uncovered and require future work into

this area. For example, use of cortical muscular coherence with EEG and MEG would provide more concrete evidence on the relationship between central and peripheral mechanisms (Kilner et al., 2000). Nevertheless, outcomes of this Chapter provide preliminary evidence that strength of connectivity in the MN at rest could help facilitate motor function.

4.4.5 Conclusions

Firstly, we show significant diurnal variations in MVC between ECPs and LCPs. This stresses the importance of considering the Circadian Phenotype of athletes within the sports world, as well as anyone involved in a motor study. Secondly, we have highlighted that LCPs are more affected by time of day in both rs-FC and performance. Higher rs-FC between regions used within this study results in higher scores when grip strength is performed outside of the scanner. The differences shown between ECPs and LCPs as well as the general time of day differences in rs-FC of the sensorimotor network could have many implications.

1) Circadian Phenotype and time of day needs to be taken into account when studying the MN.
2) Time of day is a major predictor of rs-FC of the MN.
3) Rs-FC of the MN and time of day can be used to predict MVC.

These results do not only hold significant repercussions for the study of motor function and performance in sports, but there are also links to clinical research. Applications of rs-fMRI are becoming more frequent in clinical practice and research because brain connectivity exhibits distinct differences in healthy and pathological states (Lee et al., 2013). For example, rs-fMRI has now been used to study Alzheimer's disease (Wang et al., 2007), schizophrenia (Hoptman et al., 2010) and rs-FC of the MN has gained considerable interest

in the study of Parkinson's (Luo et al., 2014, Wu et al., 2009, Wu et al., 2011) and Huntington's disease (Muller et al., 2016). In addition, FC is widely used to measure recovery to stroke (Andrew James et al., 2009, Carter et al., 2012). The interpretation of such data could be considerably affected by Circadian Phenotype or time of day and should therefore be taken into account.

Despite the need for future work, this study certainly supports the idea that intrinsic central mechanisms contribute to diurnal variations in performance and the functional integrity of the rs-MN influences the ability to perform in a motor task.

The final experimental Chapter in this thesis diverged from the previous Chapters as it did not include any functional imaging. Instead this Chapter focused on the question of phase advancing LCPs in a real world setting and the impact on mental well-being and performance.

CHAPTER FIVE

Phase Shifting 'Night Owls': Effects on Sleep and Physiology & Impact on Mental Well-Being and Performance

Abstract

In addition to the 24 h light/dark cycle, research suggests that human circadian rhythms can be phase shifted by other non-photic stimuli e.g. food, exercise and social cues. In this study, we investigated whether LCPs can be phase advanced using non-pharmacological interventions (sleep timings, exercise and diet) in a real world setting. The LCP group described previously continued in the study (N = 22, 7 male, age 21.3 ± 3.3 years). Participants were randomly assigned to an experimental (n = 12) or control (n = 10) group prior to involvement in the study. All individuals completed sleep-related questionnaires, provided saliva samples to measure melatonin and cortisol at the start and end of the study and wore actigraphs throughout. After 2-weeks acclimatisation participants visited the BUIC to undergo testing at 14:00 h, 20:00 h and 08:00 h. Testing consisted of subjective sleepiness measures as well as cognitive (PVT) and physical (MVC) performance testing. Both groups were then given a schedule to follow for three weeks before returning for repeat testing at the same time points.

Actigraphy data showed a significant phase advance in both sleep and wake up times of the experimental group, coupled with a phase advance in both DLMO and cortisol rhythms. DASS and POMS scores significantly decreased in the experimental group after the intervention. PVT performance was significantly better at 08:00 h following intervention, along with a significant decrease in sleepiness being observed. MVC performance was significantly better at 08:00 h and 14:00 h after interventions. There were no significant changes in the control group for any of the parameters investigated.

This study has shown that we are able to successfully phase advance the sleep/wake cycle and physiology of LCPs and that this phase advance has a positive effect on mental

well-being and performance. This could provide exciting new behavioural techniques to aid in optimising performance and well-being in the real world.

5.1 Introduction

Everyone is constantly seeking ways to better understand the constraints of society with the hope of making better choices to improve health, quality of life and performance (Organization, 2000). However, the complex interaction of environmental and biological constraints means a perfect solution is near impossible to find. Better understanding of how these factors affect behaviour and physiology in the real world could provide invaluable evidence in the search for improvements to well-being and performance. Despite there being numerous biological and environmental constraints worth discussing, this thesis has focused on circadian and sleep impacts on performance, which will therefore be continued throughout this Chapter.

5.2 Biological and Environmental Constraints

Environmental influences on circadian rhythms arise from both social and natural factors. Cyclic variations in the light/dark cycle, which is the strongest zeitgeber of the human circadian clock, allow the resetting of biological clocks through entrainment (Czeisler et al., 1981). Conversely, this can have adverse effects on health and well-being when there is exposure to light at the wrong times, as so often documented in shift work and jet lag (Kolla and Auger, 2011). Social influences such as work/school schedules, meal times and diet have also been shown to affect biological clocks and in turn behaviour (Duffy et al., 1996).

The majority of our society has stringent work and schooling hours requiring attendance between the hours of 09:00 h and 17:00 h. Despite these traditional environmental requirements, there has been a shift towards understanding biological constraints by allowing flexibility of working hours (Joyce et al., 2009), as well as attempts to move school start times to fit to adolescents' notoriously 'late' running biological clocks (Kelley et al., 2015). However, perhaps there is a need to compromise by using both environmental and biological factors to maintain better health and optimise performance.

Figure 5.1 summarises a projected model of environmental and biological constraints in chronobiology and sleep research. The bottom right hand panel indicates situations which have a high environmental and low biological constraint e.g. traditional working hours. The top left hand box shows a high biological and low environmental constraint situation e.g. choosing own working hours or flexi time. When both environmental and biological constraints are high this results in greater risk of disruption. For example, flying across time zones causes desynchronisation of biological clocks and changing environmental conditions resulting in jet lag (Reilly et al., 2005). The best fit compromise, which will be experimentally explored in this Chapter, is shown in the bottom left panel and indicates the potential to utilise the environment to adapt/shift biology and behaviour.

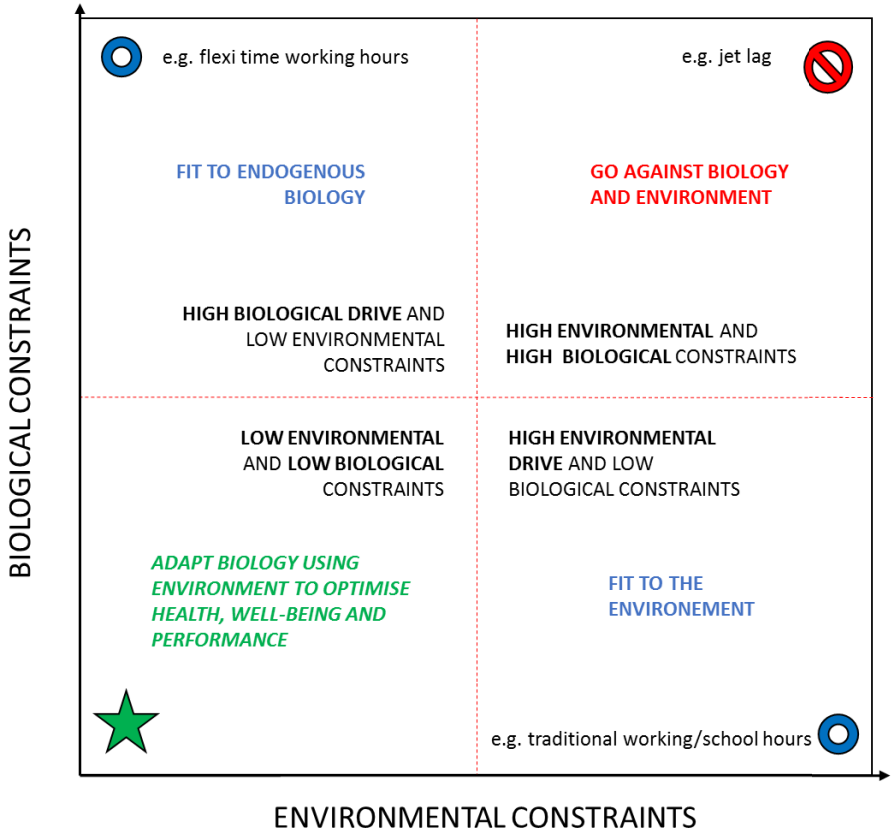


Figure 5.1. Model of biological and environmental constraints in the context of chronobiology and sleep research.

5.2.1 Individual differences

Individual differences (ECPs/LCPs) in biological rhythms which are influenced by physiological (Burgess and Fogg, 2008, Kudielka et al., 2006), genetic (Archer et al., 2008) and behavioural (Roenneberg et al., 2007) factors, as well as a difference in diurnal preference (Horne and Östberg, 1977), create another level of complexity when trying to balance and manage constraints. Using the constraints model discussed in Figure 5.1, LCPs would feature in the 'high environmental and high biological' constraints box due to being in constant conflict between biology and environment (Wittmann et al., 2006). ECPs would be placed near the centre as the environment and biology follow a similar pattern. At their most extreme these differences result in clinical diagnoses of CRSDs. Advanced Sleep Phase

Syndrome (ASPS) is a condition more common in older people, in which sleep phase is severely advanced coupled with an advance in melatonin and CBT rhythm (Jones et al., 1999). The opposing disorder, DSPS is more common in younger individuals and displays symptoms of extremely late sleep initiation and difficulty in morning awakening (Zhu and Zee, 2012). The genetic vulnerability in both ASPS and DSPS has been identified by variations in clock genes involved in the cellular rhythm generation, as discussed in the Introduction (Archer et al., 2003, Toh et al., 2001). Research into the shared neurobiology between circadian rhythms and mood have resulted in DSPS being associated with mood disorders such as depression (Shirayama et al., 2003). This group of individuals also tend to be restricted by social factors such as work/school routines which shorten sleep resulting in an accumulation of 'sleep debt'. This causes excessive sleepiness during the day and impairment of cognitive functioning (Zhu and Zee, 2012). Whether these links are a direct result of underlying Circadian Phenotype or disruption to sleep remains unknown. While clinical assessment is needed to diagnose DSPS, many of the symptoms associated with it are shared with LCPs. LCPs are categorized based on late sleep/wake timings and a delay in DLMO, and have been associated with higher scores for depression (Merikanto et al., 2013a), decreased morning cognitive performance and excessive daytime sleepiness (Giannotti et al., 2002). Since around 25% of a given population would fall into a 'Late Chronotype' category (sleeping after 1.30am and waking after 9.30am), according to Roenneberg and Merrow (2007), one could propose that these individuals are compromised by having a delayed circadian rhythm. This group has a high biological drive and a high environmental constraint and is therefore forced to constantly go against their biology to fit to the environment. The consequence of this is a state of chronic sleep restriction and persistent circadian misalignment called social jetlag (Wittmann et al., 2006).

5.2.2 Diurnal Performance

Since there are large differences in physiology and behaviour between ECPs and LCPs, investigating the impact of time of day on performance could influence the results if Circadian Phenotypes are not taken in account. This could cause performance to be skewed depending on the composition within a given population (Facer-Childs and Brandstaetter, 2015b). As such, diurnal variations in cognitive performance have reported differing results with some suggesting attention and vigilance peak in the afternoon (Kline et al., 2010), and others showing best reaction time and attention in the morning (Jarraya et al., 2014a). A key issue when studying this area is the type of task being performed as well as individual differences (Schmidt et al., 2007). Simple tasks such as reaction time may have different diurnal profiles to more complex tasks requiring higher cognitive functioning (Blatter and Cajochen, 2007). It has also been widely reported that physical performance fluctuates over the course of the day with performance increasing throughout the day and peaking in the late afternoon/early evening, following the circadian rhythm of CBT (Taylor et al., 2011, Souissi et al., 2010b). However a few studies are now showing that diurnal variations in physical performance may be more complex than this generalised view due to individual differences in sleep and circadian rhythmicity (Facer-Childs and Brandstaetter, 2015a).

Diurnal variations in both cognitive and physical performance have been shown to vary between Circadian Phenotypes, with LCPs often have difficulties fitting into traditional working hours. It would therefore be interesting to explore if these performance differences can be manipulated to maximise performance at certain times of day.

5.2.3 Phase Shifting

When investigating how to alter the circadian or diurnal phase of individuals there are two ways in which biological clocks can be shifted. One way is to phase advance the clock, thus pushing it earlier, and the other is to phase delay the clock by pushing it later (Minors et al., 1991). A phase shift can be targeted using behavioural methods, pharmacological methods or a combination of the two. Behavioural targets i.e. non-pharmacological interventions, include: timed bright light therapy (Mishima et al., 1994), altering sleep/wake cycles (Petit et al., 2014) and timed physical exercise (Miyazaki et al., 2001). A range of pharmacological agents have also been developed to try and treat CRSDs. For example, the melatonin receptor agonist ramelteon is used to treat insomnia (Simpson and Curran, 2008), caffeine is used to promote night-time alertness (Ker et al., 2010) and exogenous melatonin is used to promote sleep (Folkard et al., 1993). As discussed in the Introduction, the human circadian system is most responsive to light, which allows sleep/wake activity and physiology to adapt to the 24 h light dark cycle (Zeitzer et al., 2014). As a result light, or lack of light, is a major target to try and phase shift biological clocks through a process called photic entrainment (Minors et al., 1991). Morning bright light therapy has been used to improve sleep and behaviour in clinical situations (Mishima et al., 1994, Rosenthal et al., 1990, Van Someren et al., 1999) as well as phase advances being used to try and treat mood disorders such as depression (Wehr et al., 1979). Other forms of entrainment that do not involve light e.g. food and exercise, are called non-photic entrainment (Mistlberger and Skene, 2005). Phase shifts are routinely used in the treatment of CRSDs such as DSPS (Rosenthal et al., 1990) and shift work disorder (Folkard et al., 1993).

Alternatively, a phase shift could be used to try and alter diurnal performance profiles thereby enhancing performance at non-optimal times of day. For example, Petit et al. (2014) used a 5 h phase advance in a laboratory setting over two days to show that

physical performance is not impaired by the earlier adjustment. However, there are no reports specifically on how phase advancing LCPs in their home environment could benefit measures of physical performance.

5.2.4 Aims

In summary, there is conflict between fitting to our biology or fitting to our environment, with misalignment of these resulting in negative consequences to health and performance (Klerman, 2005). Being able to shift biology to adapt to the environment could provide ways to optimise performance in the real world. This brings into question whether a true phase advance can be achieved by healthy individuals in their home environment, in the absence of any pharmacological agents, and if any positive outcomes can be observed on mental well-being and performance. Therefore, this study took a group of LCPs, and attempted to phase advance them in a real world setting using non-pharmacological interventions. The influence of this shift on mental well-being and performance variables (PVT and MVC) was monitored.

5.3 Materials and Methods

For detailed methods please refer to Chapter 2.

5.3.1 Participants

The LCPs group discussed in previous Chapters continued into this phase of the study (n=22, 7 male, 21.3 ± 3.3 years). Individuals were randomly assigned to the experimental (n=12, 3 male) or control (n=10, 4 male) groups at the start of the study.

5.3.2 Study Design

The study was conducted over six weeks for each participant and took place between April and June 2016. All participants wore actigraphs and completed sleep diaries as well as a smart phone app for the duration (details given in Chapter 2). Acclimatisation was used to assess habitual sleep patterns and gather questionnaire data. During the acclimatisation participants were asked to complete the cognitive test three times. The testing results discussed in previous Chapters, which included the neuroimaging, were used as baseline measurements. Participants were then given a schedule to follow for the next two-three weeks before returning to repeat all testing sessions and physiological sampling. Details of the study design can be found in Figure 5.2.

5.3.3 Behavioural Data

As discussed in Chapter 2, a set of questionnaires was completed by each participant at the start and repeated at the end of the study. Questionnaires included and used in the analyses were the ESS (Johns, 1991), PSQI (Buysse et al., 1989), POMS (McNair D M, 1971), DASS (Lovibond and Lovibond, 1995), and a Diet Questionnaire. Participants were also asked to complete a feedback questionnaire at the end of the study to assess subjective

ratings of sleep quality, alertness, productivity and moods. This was to control for any "Hawthorne effects" (Adair, 1984). The Hawthorne effect is a common problem in field studies and refers to the modification of behaviour due to simply being observed as opposed to any treatments/interventions themselves (Adair, 1984). Due to insufficient completion of questionnaires, three individuals' results were not recorded for POMS, two for DASS and two for the food frequency questionnaire in this Chapter.

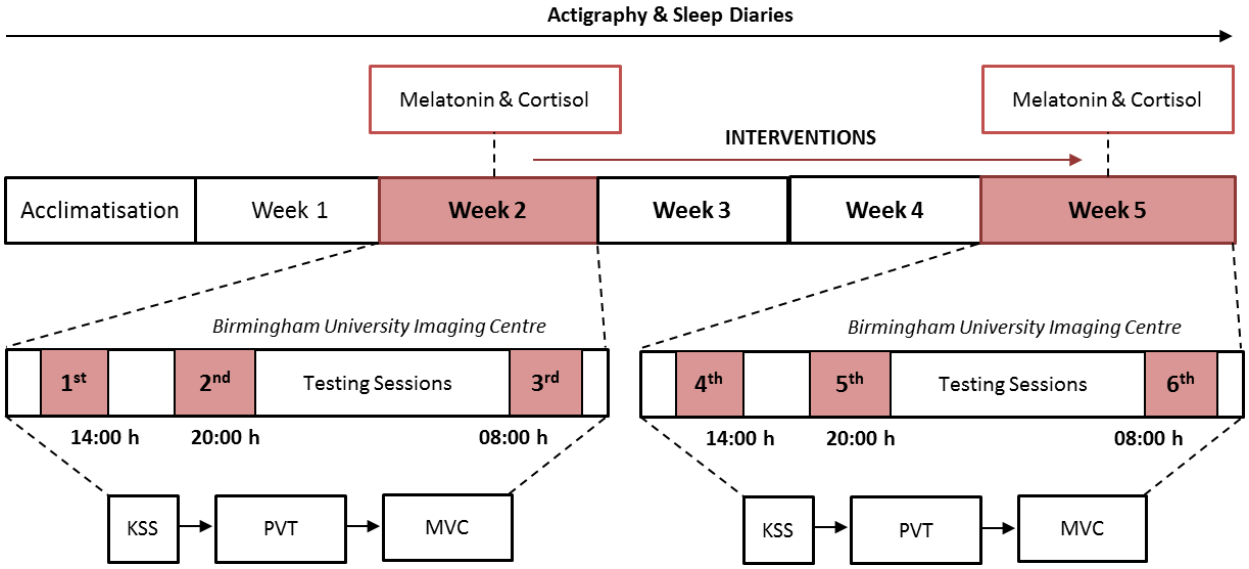


Figure 5.2. Schematic illustration of experimental protocol. Actigraphy combined with sleep diaries/app were completed for the duration of the study as well as physiological sampling for melatonin and cortisol rhythms in weeks 2 and 5. Participants attended testing sessions at 14:00 h (1st), 20:00 h (2nd) and 08:00 h the following morning (3rd), and repeated them in week 5 (4th, 5th and 6th). At each session participants completed a PVT and MVC test, as well as subjective sleepiness ratings with the KSS.

5.3.4 Actigraphy Data

Actigraphs (AWLs) were worn on the non-dominant wrist for the duration of the study and participants completed daily sleep diaries as described in Chapter 2. Parameters used from the NPCRA and specifically discussed in this Chapter were inter-daily stability (IS), intra-daily variability (IV) and relative amplitude (RA). IS is a measurement that reflects the strength of the activity rhythm by calculating the variability between days. A score of 0-1 is produced, with higher values representing a more stable i.e. consistent, rhythm. IV provides an indication of rhythm fragmentation i.e. changes between rest and active periods. Scores span from 0 (least fragmented) to 2 (most fragmented). RA gives a value for the rhythm amplitude (0: low amplitude and 1: high amplitude). NPCRA was also used to calculate the start time of the lowest five hours of activity (L5 onset) and the start time of the highest 10 hours of activity (M10 onset) (Van Someren et al., 1999).

5.3.5 Physiological Data

Participants provided saliva samples during one morning and one evening during the first week of testing and repeated them during the final week to determine circadian phase through melatonin and cortisol rhythms, as discussed in Chapter 2.

5.3.6 Performance Data

At each testing session, a two minute PVT was completed followed by a six second MVC test for isometric grip strength as defined in Chapter 2. For this Chapter, PVT and MVC scores were normalised by converting into percentage of maximum performance achieved relative to each individual at baseline and after interventions. This was done to quantify increases/decreases in performance relative to each individual due to the interventions. Percentage change from the best score of the three time points (100%) was calculated. For

the purpose of consistency and simplicity PVT performance, assessed by reaction time (s), will be referred to as cognitive performance and MVC performance, assessed by isometric grip strength, will be referred to as physical performance throughout this Chapter. KSS score will be referred to as sleepiness.

5.3.7 Non-Pharmacological Interventions

Non-pharmacological interventions were used to attempt an advance in circadian phase in order to investigate the effects on behaviour and performance. These interventions followed fairly standard sleep hygiene suggestions and targeted light exposure and sleep (through a change in wake up times), diet and physical exercise. The experimental group was given a schedule asking individuals to go to bed and wake up 2-3 h earlier than their habitual sleep/wake times. If individuals drank caffeine they were asked not to drink any caffeine during the afternoon. If individuals engaged in physical exercise they were asked to schedule this in the mornings where possible. The control group was asked to eat lunch at the same time each day with the intention that there would be no differences in sleep timings and hence no alterations in light exposure. This schedule was therefore, specifically aimed to act as a placebo with a hypothesis that it would not affect circadian phase.

5.3.8 Statistical Analysis

Statistical comparisons were performed using GraphPad Prism (version 7, https://www.graphpad.com/scientific-software/prism/) and SPSS (IBM SPSS Statistics, version 24, Chicago). Non-parametric tests were implemented where the distribution was non-normal. Initial comparisons between the groups (experimental and control) were performed using unpaired two sample t-tests, Mann-Whitney tests, Fisher's exact test and linear regression after testing for equality of means with Levene's test. Time of day effects (between 14:00 h, 20:00 h and 08:00 h) were analysed using Two Way (Time of day and Group i.e. experimental and control) Mixed ANOVA with Tukey's post hoc tests. Results of the interventions within each group were performed using paired t-tests and Wilcoxon signed-rank tests. To correct for multiple comparisons, all p-values were corrected for false discovery rate (FDR) using the Benjamini Hochberg method (Benjamini and Hochberg, 1995). Diurnal variation was analysed using Two Way (Time of day and Condition i.e. baseline and after interventions) repeated measures ANOVA with Tukey's or Sidak's post hoc tests corrected for multiple comparisons. After exploring different nonlinear curve fits, second order polynomial regression curves were chosen to plot the data as a function of time of day. The 08:00 h test will be described as morning, 14:00 h as afternoon and 20:00 h as evening.

5.4 Results

5.4.1 Baseline Results

To confirm that the groups were comparable, all data were initially compared at baseline and no significant differences were found in any of the parameters measured (Table 5.1). Experimental and control groups were of similar age (21.7 ± 2.8 and 20.9 ± 3.9 years), height (168 ± 4.02 and 173 ± 2.27 cm) and weight (65.7 ± 3.41 and 68.4 ± 2.58 kg). MCTQ scores were comparable with the experimental group reporting a mid-sleep on free days of 07:15 h ± 00:27 and control group 06:02 h ± 00:14. Mental well-being, physiological and actigraphy data were all not significantly different between the groups (Table 5.1).

Table 5.1. Summary of demographic, mental well-being, actigraphy and physiological details for experimental and control groups.Values are shown as mean ± SEM. Significance is shown with [a]unpaired two sample t-tests, [b]non-parametric Mann-Whitney or [c]Fisher's exact test.

Variable Measured (mean ± SEM)	Experimental Group	Control Group (Con)	Significance
Sample Size	N = 12	N = 10	ns[a]
Demographic Variables			
Age (years) (mean ± SD)	21.7 ± 2.8	20.9 ± 3.9	ns[b]
Percentage of Males/Females (%)	M: 25	M: 40	ns[c]
	F: 75	F: 60	
Height (cm)	168.73 ± 4.02	173.80 ± 2.27	ns[a]
Weight (kg)	65.70 ± 3.41	68.40 ± 2.58	ns[a]
MCTQ Score (hh:mm)	07:15 ± 00:27	06:24 ± 00:14	ns[a]
Mental Well-Being Variables			
Pittsburgh Sleep Quality Index (PSQI)	4.83 ± 0.71	5.30 ± 0.80	ns[a]
Profile of Mood States (POMS)	10.33 ± 6.15	8.50 ± 5.74	ns[a]
Epworth Sleepiness Scale (ESS)	7.08 ± 1.16	9.00 ± 0.99	ns[a]
Depression Anxiety and Stress Scale (DASS)	19.83 ± 3.36	13.78 ± 3.66	ns[a]
Average days per week eating breakfast (days)	4.09 ± 0.62	4.70 ± 0.84	ns[a]
Average breakfast time (hh:mm)	10:33 ± 00:25	10:01 ± 00:34	ns[a]
Actigraphy Variables and Non-Parametric Circadian Rhythm Analysis (NPCRA)			
Bed Time (hh:mm)	02:19 ± 00:25	01:16 ± 00:30	ns[a]

Get Up Time (hh:mm)	10:46 ± 00:23	09:54 ± 00:31	ns[a]
Sleep Onset (hh:mm)	02:46 ± 00:26	01:37 ± 00:30	ns[a]
Wake Up Time (hh:mm)	10:31 ± 00:23	09:37 ± 00:29	ns[a]
Sleep Duration (h)	7.75 ± 0.20	7.81 ± 0.20	ns[a]
Sleep Efficiency (%)	76.80 ± 1.48	78.26 ± 1.91	ns[a]
Sleep Latency (hh:mm)	00:27 ± 00:04	00:21 ± 00:03	ns[a]
Fragmentation Index	34.86 ± 3.63	30.47 ± 2.27	ns[b]
Inter-daily Stability	0.38 ± 0.03	0.38 ± 0.05	ns[b]
Intra-daily Variability	0.85 ± 0.05	0.79 ± 0.06	ns[a]
L5 Onset (hh:mm)	03:57 ± 00:27	03:03 ± 00:34	ns[a]
M10 Onset (hh:mm)	12:43 ± 00:37	12:14 ± 00:36	ns[a]
Relative Amplitude	0.83 ± 0.03	0.82 ± 0.03	ns[b]
Physiological Variables			
Dim Light Melatonin Onset (DLMO) (hh:mm)	23:56 ± 00:34	23:18 ± 00:54	ns[a]
Phase Angle (h)	2.94 ± 0.29	2.47 ± 0.72	ns[b]
Peak Melatonin Concentration (pg/nl)	26.89 ± 3.98	21.02 ± 5.85	ns[a]
Peak Time of Melatonin (hh:mm)	02:06 ± 00:28	02:01 ± 00:33	ns[a]
Cortisol Peak Time (hh:mm)	11:19 ± 00:31	11:05 ± 00:36	ns[a]
Peak Cortisol Concentration (nmol/l)	23.31 ± 2.39	22.64 ± 3.57	ns[b]
Cortisol Awakening Response (%)	113.16 ± 33.71	112.37 ± 45.28	ns[b]
Area Under the Curve (total time)	98.83 ± 10.47	104.41 ± 14.01	ns[a]
Area Under the Curve (1st hour)	62.89 ± 8.51	64.55 ± 9.18	ns[b]

5.4.2 Circadian Phenotyping

Significant linear regressions were observed for MSF_{sc} plotted against DLMO ($R^2 = 0.22$, F=4.61, p=0.04), peak time of morning cortisol ($R^2 = 0.39$, F=12.65, p=0.002), sleep onset ($R^2 = 0.40$, F=12.69, p=0.0021) and wake up time ($R^2 = 0.53$, F=21.21, p=0.0002). These results support the classification into LCPs originally identified as Late Chronotypes from the MCTQ (Figure 5.3 & Table 5.1).

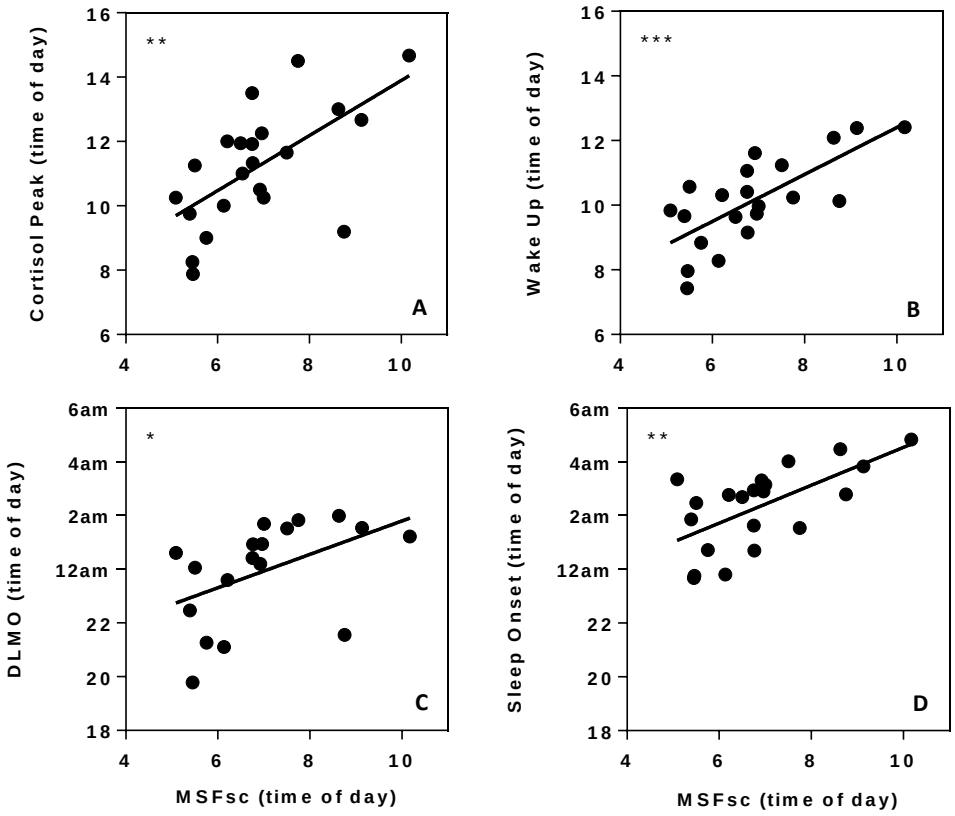


Figure 5.3. Linear relationships to validate circadian phenotyping between MSF_{sc} and physiological/actigraphy parameters from MCTQ. A) Time of peak cortisol concentration, B) Wake up time, C) Dim light melatonin onset, D) Sleep onset. Time of day is displayed on the y axis and MSF_{sc} on x axis.

5.4.3 Diurnal Variations in Performance and Sleepiness at Baseline

There was no significant main effect of group or the interaction of group*time of day for any parameters showing that groups were comparable at baseline (Figure 5.4). Post hoc tests also revealed no significant differences at each time point between the experimental and control groups for any variables measured.

There was, however, a significant main effect of time showing diurnal variations for KSS ($F(2,40)=18.43$, $p<0.0001$), MVC($F(2,40)=27.06$, $p<0.0001$) and PVT($F(2,40)=7.58$, $p=0.0016$) (Figure 5.4A). Average KSS score was highest at 08:00 h (6.41 ± 0.30), lowest at 20:00 h (4.00 ± 0.33) and 4.18 ± 0.31 at 14:00 h (Figure 5.4D). There were significant differences from 08:00 h to 14:00 h and 20:00 h (both $p<0.0001$) (Figure 5.4A). Physical performance was lowest in the morning (86.32 ± 1.75%) increasing throughout the day with 94.43 ± 1.25% in the afternoon and 98.69 ± 0.57% in the evening (Figure 5.4E). Significant time of day differences were observed between all times; morning to afternoon and evening (both $p<0.0001$) and afternoon to evening ($p=0.04$) (Figure 5.4B). Cognitive performance followed the same pattern with worst performance at 08:00 h (87.79 ± 2.52%) and best at 20:00 h (97.55 ± 1.22%). Average performance at 14:00 h was 95.03 ± 1.09%. Again, significant diurnal variations were found from 08:00 h performance to afternoon ($p=0.021$) and evening performance ($p=0.0012$) (Figure 5.4C&F).

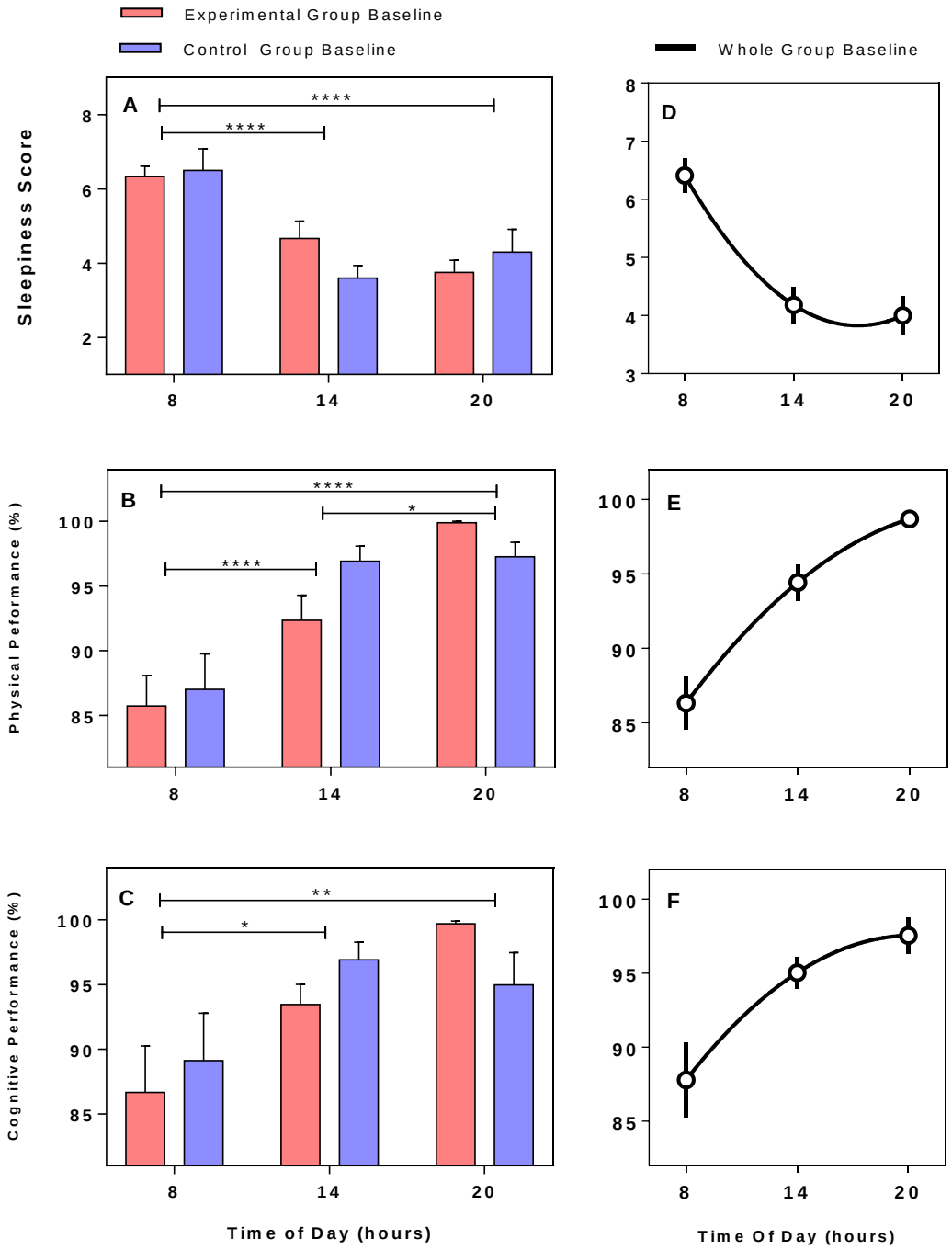


Figure 5.4. Diurnal variations in sleepiness, cognitive and physical performance. For A-C; Red bars represent the experimental group and blue bars the control group. A&D: sleepiness, B&E: cognitive performance and C&F: physical performance**.** Time of test is shown on the x axis. D-F: nonlinear regression curves for each parameter. Values are shown as the mean ± SEM.

5.4.4 Impact of Interventions on Phase Advance

Results from the experimental group will be presented in relevant sections below (Table 5.2). Due to the fact that none of the observed parameters for questionnaire, physiological or actigraphy data were significantly different from baseline to after interventions in the control group they will not be discussed explicitly in the Results section (Table 5.3), although the data are included in figures.

5.4.4.1 Sleep/Wake Cycles

Actigraphy analysis showed a significant advance in both sleep and wake times, but no significant changes in sleep duration, efficiency or latency (Figure 5.5). There was a near 2 h advance in sleep timings with bed time shifting by 1.76 h ± 0.18 from 02:19 h to 00:34 h ($p=0.00075$) and get up time by 1.81 h ± 0.18 from 10:46 h to 08:58 ($p=0.0006$). This difference was replicated in both sleep onset and wake up times (Figure 5.5A&B). Sleep onset became earlier by 1.73 h ± 0.22 from 02:46 h to 01:03 h ($p=0.0005$), and wake up time by 1.92 h ± 0.20 from 10:31 h to 08:36 h ($p=0.00043$). Sleep duration remained similar being 7.75 h ± 0.20 before and 7.55 h ± 0.20 after shift, as well as sleep efficiency (76.80 ± 1.48 % before, 75.40 ± 1.25 % after), sleep latency (00:27 ± 00:04 h before, 00:28 ± 00:02 h after) and fragmentation index (34.86 ± 3.63 before, 35.62 ± 4.08 after) (Figure 5.5C-F). NPCRA revealed no significant changes in inter-daily stability (0.38 ± 0.03 before, 0.39 ± 0.03 after), intra-daily variability (0.85 ± 0.05 before, 0.91 ± 0.07 after) or relative amplitude (0.83 ±0.03 before, 0.87 ± 0.04 after) (Figure 5.5G&H). There was a significant advance in L5 onset from 03:57 ± 00:27 h before, to 02:21 ± 00:22 after ($p=0.038$), but not M10 onset (12:43 ± 00:37 before, 12:07 ± 00:49 after).

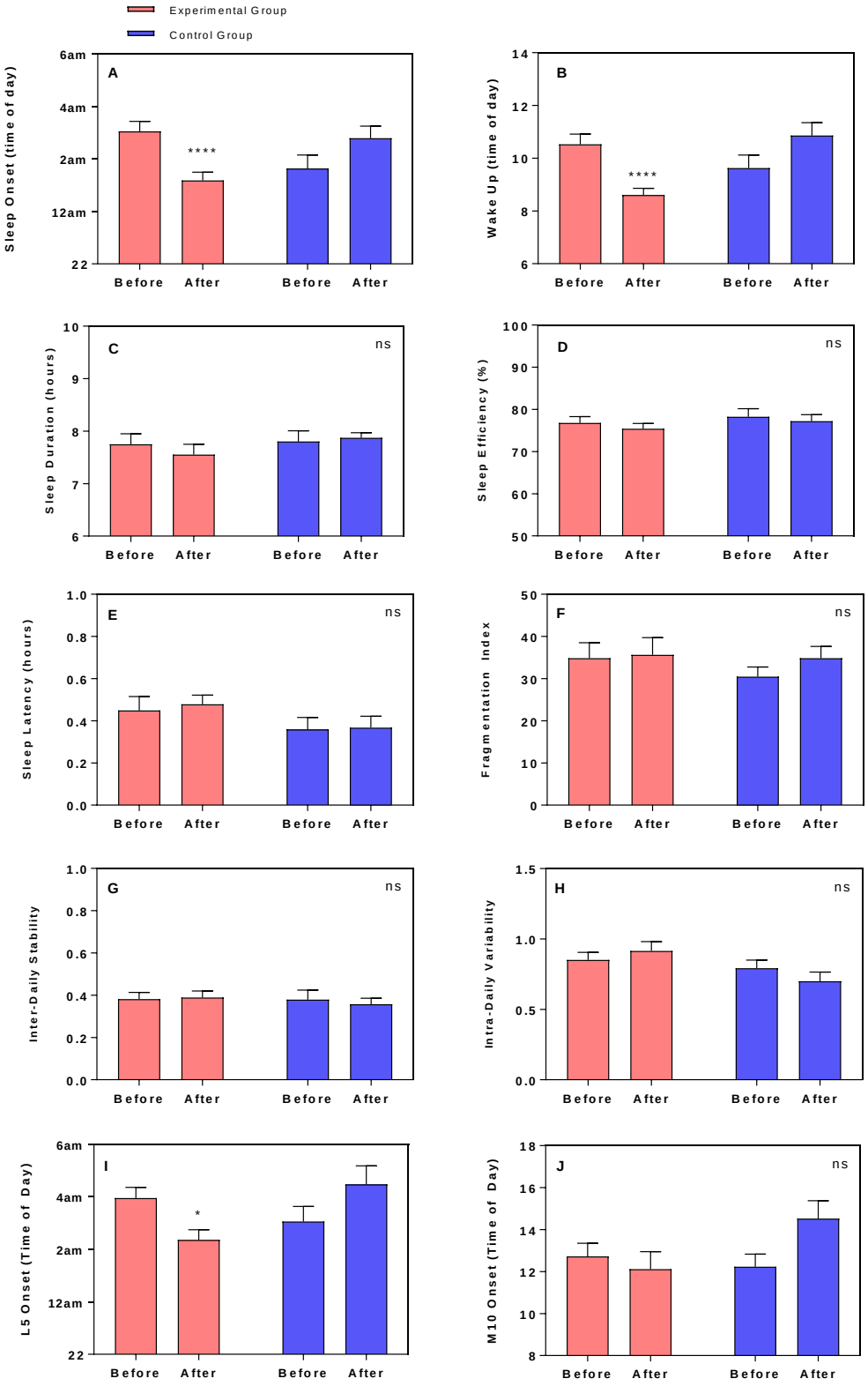


Figure 5.5. Actigraphy data at baseline and after interventions for experimental (red) and control (blue) groups. A) Sleep onset, B) Wake up time, C) Sleep duration, D) Sleep efficiency, E) Sleep latency, F) Fragmentation index, G) Inter-daily variability, H) Intra-daily variability, I) L5 onset, J) M10 onset.

5.4.4.2 Physiology

A phase advance was observed for both DLMO (p=0.024) and peak time of cortisol (p=0.001) (Figure 5.7A&B). DLMO was advanced by 1.60 h ± 0.47, shifting from 23:56 h to 22:07 h (Figure 5.6E). Time of peak cortisol advanced by 2.22 h ± 0.45, from 11:19 h to 9:06 h (Figure 5.6F). There was no significant change in phase angle (2.99 ± 0.29 h before, 2.80 ± 0.18 h after), peak concentration of melatonin and cortisol (28.02 ± 4.22 pg/ml before, 22.11 ± 4.01 pg/ml after and 23.31 ± 2.39 nmol/l before, 24.55 ± 2.86 nmol/l after, respectively), cortisol awakening response (113.16 ± 33.71 % before, 118.69 ± 37.66 % after) or area under the curve for total time (98.83 ± 10.47 % before, 107.70 ± 12.15 % after) or first hour (62.89 ± 8.51 % before, 60.74 ± 4.77 % after) (Figure 5.7C-H).

5.4.5 Impact of Interventions on Mental Well-Being

Analysis of questionnaire data revealed significant differences following the interventions in the experimental group for MSF_{sc}, DASS, POMS and average breakfast time (Figure 5.8A,B,C,F). No significant differences were observed for PSQI or ESS (Figure 5.8D&E). MSF_{sc} was shifted earlier by 2.57 ± 0.35 h from 07:15 to 04:40 h (p=0.003). POMS score decreased by 13.22 ± 4.48 points from 10.33 to -2.89 (p=0.045), coupled with an 8.67 ± 2.38 point decrease in DASS score from 19.83 to 11.17 (p=0.013). Splitting DASS into depression, anxiety and stress scores separately revealed a significant effect of condition ($F(1,33)=17.52$, p=0.0002), and significant decreases in the depression and stress elements but not anxiety. Depression was reduced from 5.50 ± 1.08 to 1.27 ± 0.49 (p=0.036), and stress from 9.5 ± 2.18 to 5.67 ± 1.86 (p=0.0089) (Figure 5.9A). Average breakfast time shifted by 1.11 h from 10:33 to 09:25 (p=0.0015). Average number of days' breakfast was

eaten per week increased from 4.09 days before to 5.36 days after but did not quite reach significance after FDR corrections (p=0.07).

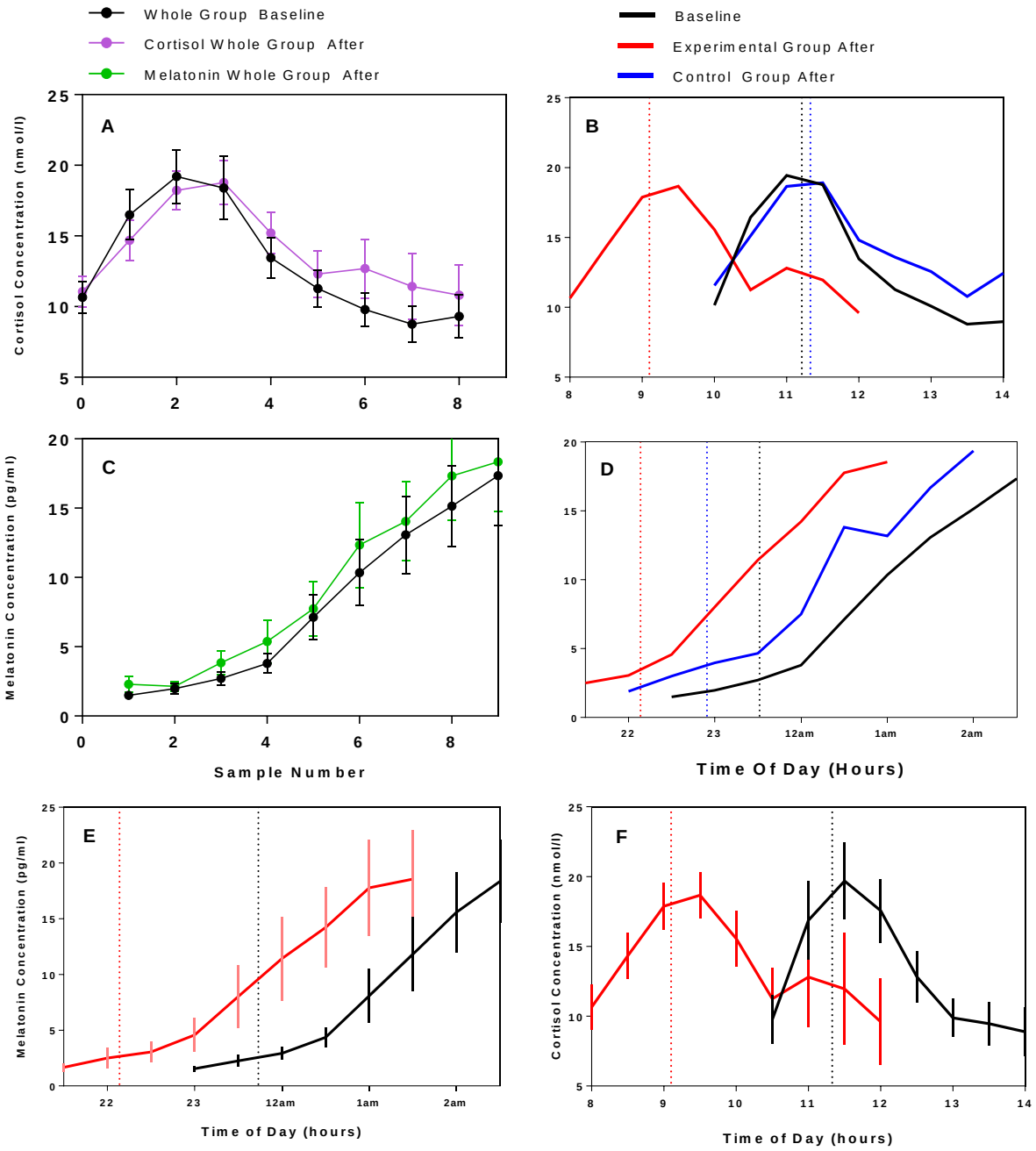


Figure 5.6. Physiological sampling curves at baseline and after interventions. Whole group sampling curves for cortisol (A) and melatonin (C). B) Cortisol curves relative to time of day, D) DLMO curves relative to time of day, E) Experimental group DLMO at baseline (black) and after (red) interventions, F) Experimental group cortisol at baseline (black) and after (red) interventions. Dotted lines indicate average peak time of cortisol and DLMO for baseline (black line), control group (blue line) and experimental group (red line).

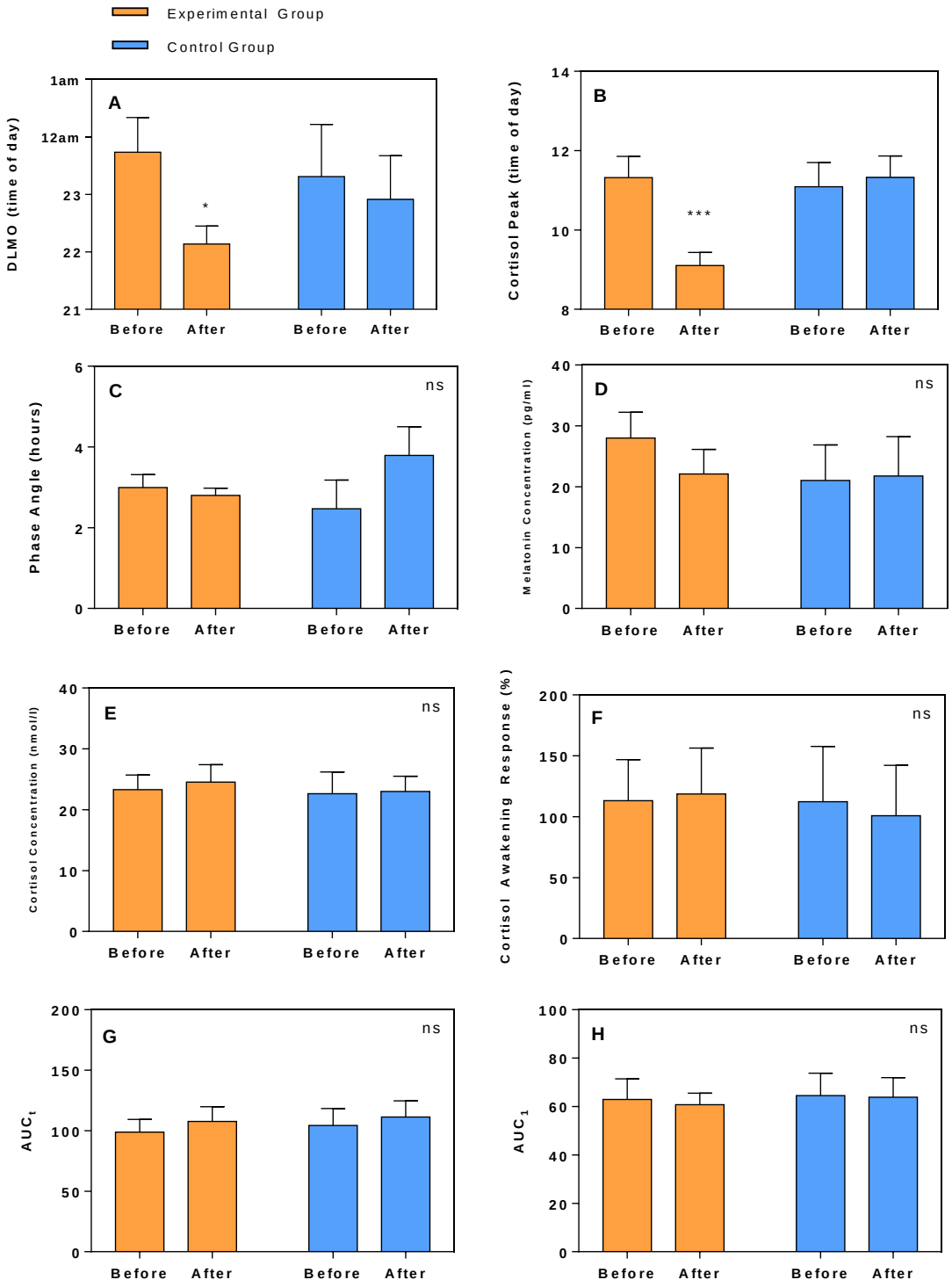


Figure 5.7. Physiological data at baseline and after interventions for experimental (orange) and control (blue) groups. A) DLMO, B) Time of peak cortisol, C) Phase angle, D) Peak concentration of melatonin (pg/ml), E) Peak concentration of cortisol (nmol/l), F) Cortisol awakening response (%), G) Area under the curve measured for the total time of sampling (AUC_t), H) Area under the curves measured for the 1st hour (AUC_1).

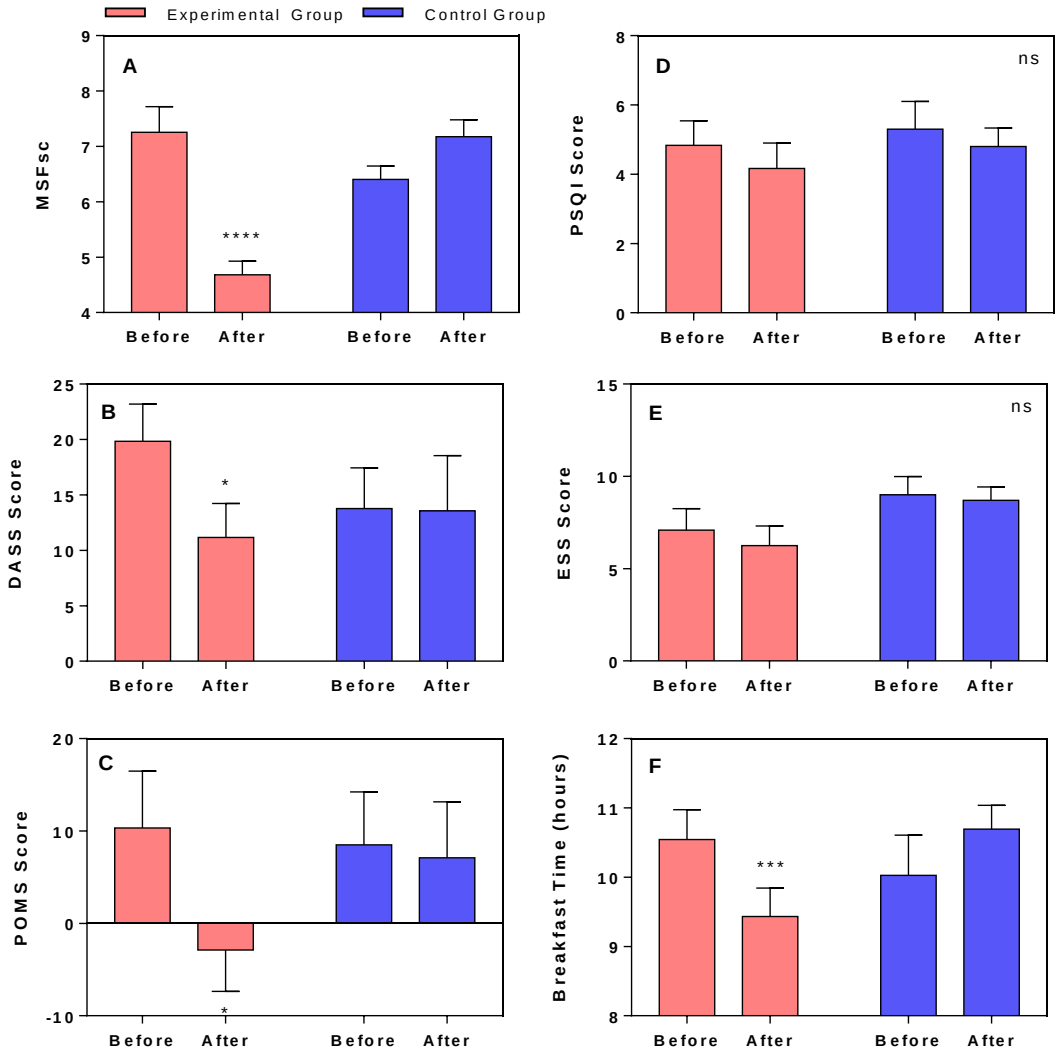


Figure 5.8. Mental well-being data at baseline and after interventions for experimental (red) and control (blue) groups. A) MSF_{sc}, B) DASS, C) POMS, D) PSQI, E) ESS, F) Average time of breakfast.

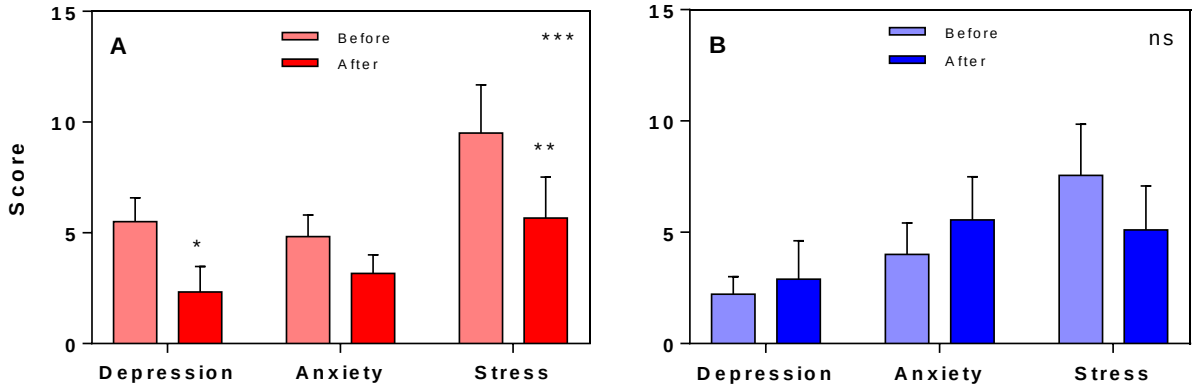


Figure 5.9. DASS analysis at baseline and after interventions for A) experimental (red) and B) control (blue) groups before and after interventions.

Table 5.2. Summary of all variables for the experimental group at baseline and after interventions**.** Significance is shown with [a]paired two sample t-tests or [b]non-parametric Wilcoxon signed rank test. All p-values are FDR corrected ($p<0.05$) using the Benjamini Hochberg method.

Variable Measured (mean ± SEM)	Baseline	After Interventions	Significance
MCTQ Score (hh:mm)	07:15 ± 00:27	04:40 ± 00:15	p=0.003[a]
Mental Well-Being Variables			
Pittsburgh Sleep Quality Index (PSQI)	4.83 ± 0.71	4.17 ± 0.74	ns[a]
Profile of Mood States (POMS)	10.33 ± 6.15	-2.89 ± 4.46	p=0.045[a]
Epworth Sleepiness Scale (ESS)	7.08 ± 1.16	6.25 ± 1.07	ns[a]
Depression Anxiety and Stress Scale (DASS)	19.83 ± 3.36	11.17 ± 3.07	p=0.013[a]
Average days per week eating breakfast (days)	4.09 ± 0.62	5.36 ± 0.47	ns[b]
Average breakfast time (hh:mm)	10:33 ± 00:25	09:24 ± 00:24	p=0.0015[a]
Actigraphy Variables and Non Parametric Circadian Rhythm Analysis (NPCRA)			
Bed Time (hh:mm)	02:19 ± 00:25	00:34 ± 00:18	p=0.00075[a]
Get Up Time (hh:mm)	10:46 ± 00:23	08:58 ± 00:17	p=0.0006[a]
Sleep Onset (hh:mm)	02:46 ± 00:26	01:03 ± 00:18	p=0.0005[a]
Wake Up Time (hh:mm)	10:31 ± 00:23	08:36 ± 00:15	p=0.00043[a]
Sleep Duration (h)	7.75 ± 0.20	7.55 ± 0.20	ns[a]
Sleep Efficiency (%)	76.80 ± 1.48	75.40 ± 1.25	ns[a]
Sleep Latency (hh:mm)	00:27 ± 00:04	00:28 ± 00:02	ns[a]
Fragmentation Index	34.86 ± 3.63	35.62 ± 4.08	ns[b]
Inter-daily Stability	0.38 ± 0.03	0.39 ± 0.03	ns[a]
Intra-daily Variability	0.85 ± 0.05	0.91 ± 0.07	ns[a]
L5 Onset (hh:mm)	03:57 ± 00:27	02:21 ± 00:22	p=0.038[a]
M10 Onset (hh:mm)	12:43 ± 00:37	12:07 ± 00:49	ns[a]
Relative Amplitude	0.83 ± 0.03	0.87 ± 0.04	ns[b]
Physiological Variables			
Dim Light Melatonin Onset (DLMO) (hh:mm)	23:56 ± 00:34	22:07 ± 00:18	p=0.024[a]
Phase Angle (h)	2.94 ± 0.29	2.80 ± 0.18	ns[b]
Peak Melatonin Concentration (pg/nl)	28.02 ± 4.22	22.11 ± 4.01	ns[a]
Peak Time of Melatonin (hh:mm)	02:06 ± 00:28	01:19 ± 00:12	p=0.0038[a]
Cortisol Peak Time (hh:mm)	11:19 ± 00:31	09:06 ± 00:19	p=0.001[a]
Peak Cortisol Concentration (nmol/l)	23.31 ± 2.39	24.55 ± 2.86	ns[b]
Cortisol Awakening Response (%)	113.16 ± 33.71	118.69 ± 37.66	ns[b]
Area Under the Curve (total time)	98.83 ± 10.47	107.70 ± 12.15	ns[b]
Area Under the Curve (1st hour)	62.89 ± 8.51	60.74 ± 4.77	ns[a]

Table 5.3. Summary of all variables for the control group at baseline and after interventions**.** Significance is shown with [a]paired two sample t-tests or [b]non-parametric Wilcoxon signed rank test. All p-values are FDR corrected ($p<0.05$) using the Benjamini Hochberg method.

Variable Measured (mean ± SEM)	Baseline	After Interventions	Significance
MCTQ Score (hh:mm)	06:02 ± 00:14	07:10 ± 00:18	ns[b]
Mental Well-Being Variables			
Pittsburgh Sleep Quality Index (PSQI)	5.30 ± 0.80	4.80 ± 0.53	ns[a]
Profile of Mood States (POMS)	8.50 ± 5.74	7.10 ± 6.05	ns[a]
Epworth Sleepiness Scale (ESS)	9.00 ± 0.99	8.70 ± 0.73	ns[a]
Depression Anxiety and Stress Scale (DASS)	13.78 ± 3.66	13.56 ± 4.99	ns[a]
Average days per week eating breakfast (days)	4.70 ± 0.84	4.40 ± 0.75	ns[b]
Average breakfast time (hh:mm)	10:01 ± 00:34	10:31 ± 00:21	ns[a]
Actigraphy Variables and Non Parametric Circadian Rhythm Analysis (NPCRA)			
Bed Time (hh:mm)	01:16 ± 00:30	02:25 ± 00:27	ns[a]
Get Up Time (hh:mm)	09:54 ± 00:31	11:05 ± 00:30	ns[a]
Sleep Onset (hh:mm)	01:37 ± 00:30	02:47 ± 00:27	ns[a]
Wake Up Time (hh:mm)	09:37 ± 00:29	10:51 ± 00:29	ns[a]
Sleep Duration (h)	7.81 ± 0.20	7.87 ± 0.10	ns[a]
Sleep Efficiency (%)	78.26 ± 1.91	77.20 ± 1.54	ns[a]
Sleep Latency (hh:mm)	00:21 ± 00:03	00:22 ± 00:03	ns[a]
Fragmentation Index	30.47 ± 2.27	34.83 ± 2.80	ns[a]
Inter-daily Stability	0.38 ± 0.05	0.36 ± 0.03	ns[b]
Intra-daily Variability	0.79 ± 0.06	0.70 ± 0.06	ns[a]
L5 Onset (hh:mm)	03:03 ± 00:34	04:28 ± 00:42	ns[a]
M10 Onset (hh:mm)	12:14 ± 00:36	14:31 ± 00:51	ns[a]
Relative Amplitude	0.82 ± 0.03	0.82 ± 0.02	ns[a]
Physiological Variables			
Dim Light Melatonin Onset (DLMO) (hh:mm)	23:18 ± 00:54	22:54 ± 00:45	ns[b]
Phase Angle (h)	2.47 ± 0.72	3.79 ± 0.71	ns[a]
Peak Melatonin Concentration (pg/nl)	21.02 ± 5.85	21.78 ± 6.45	ns[a]
Peak Time of Melatonin (hh:mm)	02:01 ± 00:33	02:07 ± 00:37	ns[a]
Cortisol Peak Time (hh:mm)	11:05 ± 00:36	11:19 ± 00:32	ns[a]
Peak Cortisol Concentration (nmol/l)	22.64 ± 3.57	22.99 ± 2.51	ns[b]
Cortisol Awakening Response (%)	112.37 ± 45.28	100.87 ± 41.37	ns[b]
Area Under the Curve (total time)	104.41 ± 14.01	111.40 ± 13.28	ns[a]
Area Under the Curve (1st hour)	64.55 ± 9.18	63.86 ± 8.11	ns[b]

5.4.6 Impact of Interventions on Performance

Changes in diurnal rhythms for sleepiness, cognitive and physical performance were analysed with Two Way Repeated Measures ANOVA using time of day and condition (baseline and after interventions) as factors as well as the interaction of time of day*condition (Figure 5.10 & Figure 5.11). As reported previously, baseline sleepiness score was highest in the morning and decreased throughout the day to its lowest in the evening. Diurnal variation for both cognitive and physical performance followed a similar trend with worst performance achieved in the morning and best in the evening (Figure 5.10A,C&E).

5.4.6.1 Sleepiness (KSS)

A significant interaction of time of day and condition was found for sleepiness (KSS score) in the experimental group ($F(2,22)=3.44$, $p=0.049$) as well as main effects of time of day ($F(2,22)=11.41$, $p=0.0004$) and condition ($F(2,11)=5.36$, $p=0.041$). Following interventions, sleepiness was lower at 08:00 h (4.58 ± 0.56 vs 6.33 ± 0.28) and 14:00 h (3.58 ± 0.50 vs 4.67 ± 0.47) but these differences were only significant at 08:00 h ($p=0.0061$, Figure 5.10A). A significant main effect of time of day was found for the control group ($F(2,18)=8.86$, $p=0.0021$) but not for condition or the interaction. Post hoc tests did not show any significant changes from baseline after interventions in the control group (Figure 5.10B).

5.4.6.2 Physical Performance (MVC)

The experimental group showed a significant main effect of time of day on physical performance ($F(2,22)=21.73$, $p<0.0001$), as well as a significant main effect of condition ($F(1,11)=4.94$, $p=0.048$) and an interaction of time of day and condition ($F(2,22)=9.19$, $p=0.0013$). Post hoc tests revealed that performance at both 08:00 h and 14:00 h had significantly improved following interventions ($p=0.015$ and $p=0.0075$ respectively, Figure 5.10C). Performance did not significantly improve for the control group at any time point and only the main effect of time of day was found as significant ($F(2,18)=14.73$, $p=0.0002$) (Figure 5.10D).

5.4.6.3 Cognitive Performance (PVT)

There was a main effect of time of day ($F(2,22)=3.85$, $p=0.037$) but not condition on cognitive performance. The interaction of time and condition was found to be significant ($F(2,22)=7.93$, $p=0.0026$). Post hoc tests revealed that performance at 08:00 h significantly increased after interventions ($p=0.017$) but not at 14:00 h or 20:00 h (Figure 5.10E). The main effect of time of day was significant for the control group ($F(2,18)=3.63$), $p=0.048$) but no main effects of condition or interactions were found (Figure 5.10F).

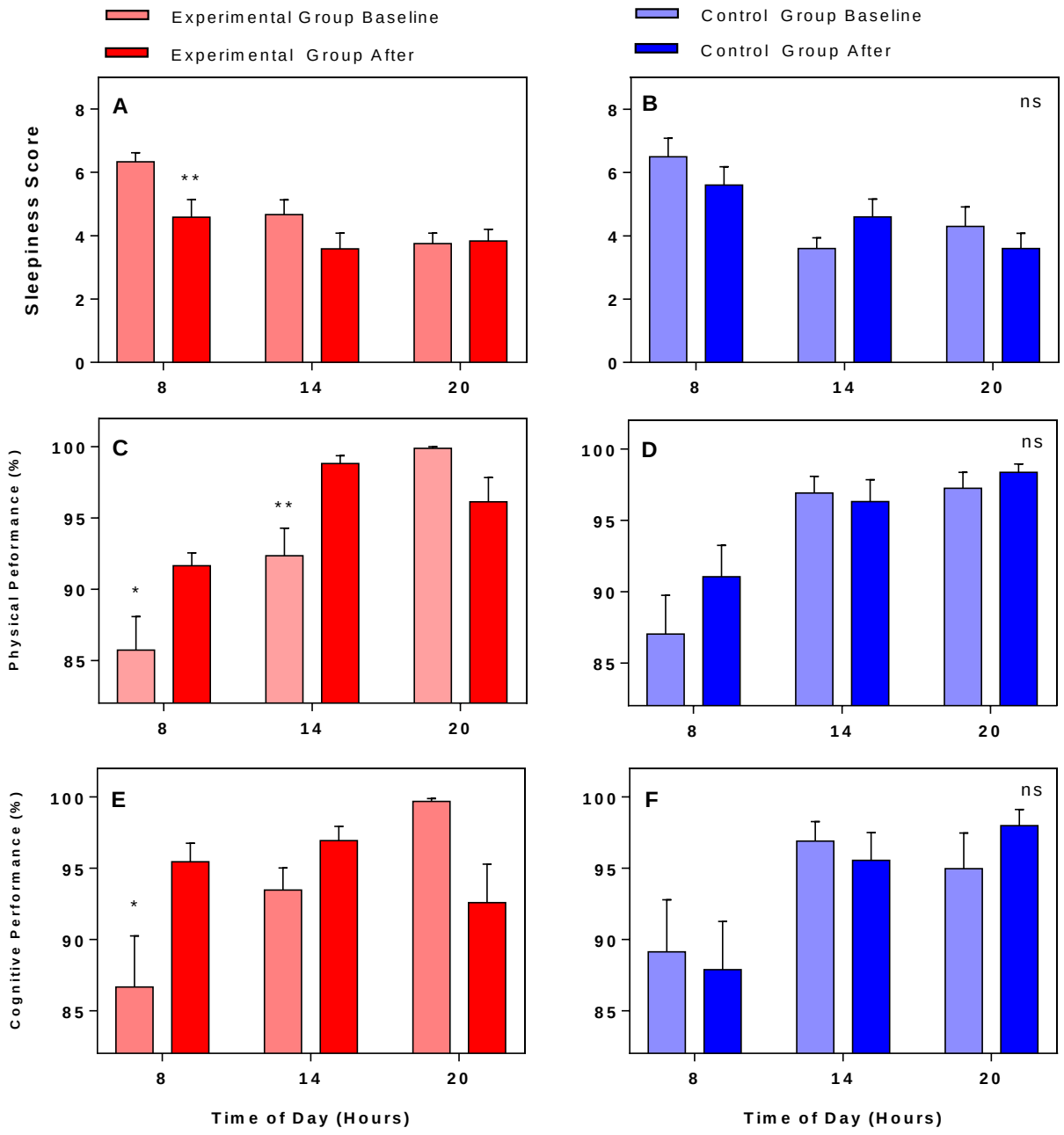


Figure 5.10. Performance and sleepiness data at baseline and after interventions for experimental (red) and control (blue) groups. A&B: KSS, C&D: PVT performance, and E&F: MVC performance. Values are shown as mean ± SEM.

5.4.6.4 Diurnal Performance Profiles

Using second order polynomial regression analysis, peak performance time can be identified from best fit diurnal variation curves (Figure 5.12). KSS score was highest at 08:00 h for the whole group at baseline (6.41 ± 0.30) as well as for both experimental (4.58 ± 0.56) and control groups (5.60 ± 0.58) after interventions (Figure 5.12A). At baseline, physical performance was highest at 20:00 h (98.69 ± 0.57%) which remained for the control group after interventions (98.37 ± 0.60%). In the experimental group peak physical performance occurred at 15:21 h (99.08%) after interventions, which was closest to performance at 14:00 h (98.81 ± 0.55%) (Figure 5.12B). The same was seen for cognitive performance with peak performance occurring at 20:00 h for both whole group at baseline (97.56 ± 1.22%) and control group post intervention (97.99 ± 1.13%), but earlier for the experimental group who achieved 97.11% at 12:30 h, again closet to performance at 14:00 h (96.93 ± 1.01%) (Figure 5.12C).

There was a 14.15% difference between best and worst physical performance in the experimental group at baseline which was reduced to 7.17% in the experimental group after intervention. For cognitive performance the experimental group reduced performance differences from 13.02% at baseline to 4.35% after interventions. An F test for equal variance showed that there was a significant reduction in variation of performance for MVC ($p=0.0024$) and PVT ($p=0.028$) in the experimental group but no changes in the control group.

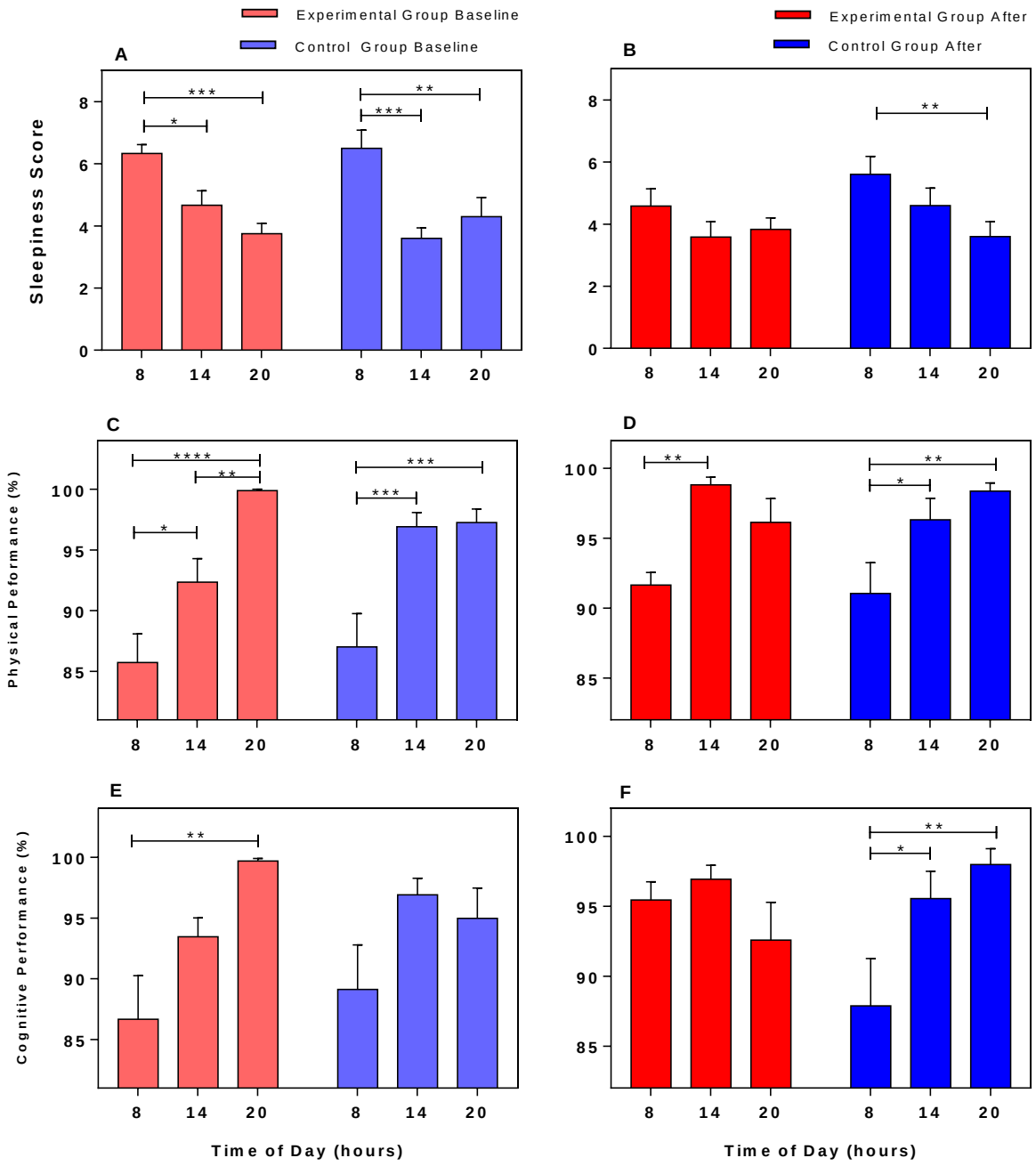


Figure 5.11. Diurnal variations in sleepiness, cognitive and physical performance at baseline and after interventions. for experimental group (red) and control group (blue). A&B: KSS, C&D: PVT performance, and E&F: MVC performance. Results at baseline are shown in A, C and E and results after interventions are shown in B, D and F. Values are shown as mean ± SEM.

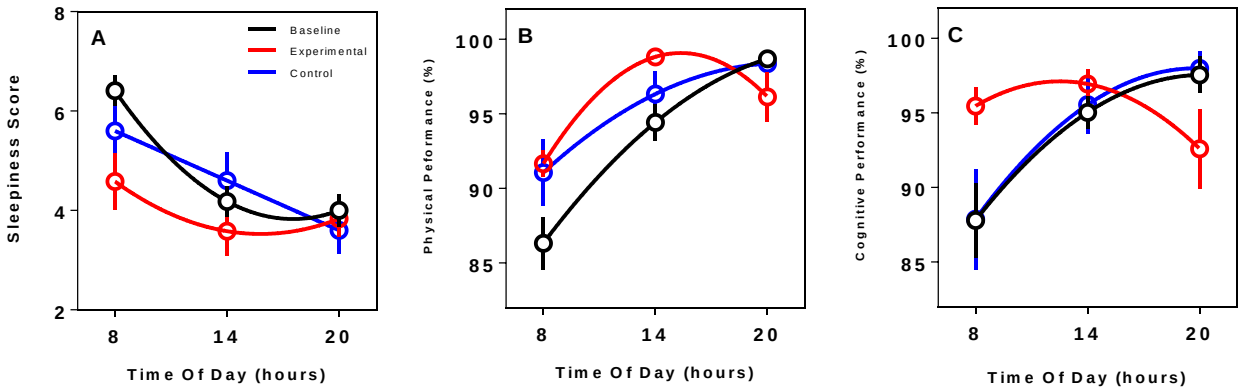


Figure 5.12. Diurnal variation curves for performance profiles and sleepiness. A: KSS score, B: MCV performance and C: PVT performance at baseline (black line) and after interventions for experimental (red line) and control (blue line) groups.

5.4.6.5 Feedback Questionnaire

In the feedback questionnaire individuals were asked to rate a number of parameters from '0: very little' to '10: a great deal'. There was a slight Hawthorne effect observed with the control group rating improvements in sleep quality as 0.80 ± 0.55, mood as 1.40 ± 0.78, productivity as 2.10 ± 1.06 and alertness as 3.1 ± 1.19, but these were not significant compared to a baseline of zero. However, these ratings were significantly higher in the experimental group with an improvement score of 4.25 ± 0.99 for sleep quality, 5.00 ± 1.11 for mood, 6.50 ± 0.75 for productivity and 6.58 ± 0.87 for alertness (Figure 5.13A).

Subjective productivity was rated for morning, afternoon and evening before and after interventions and showed significant diurnal variation at baseline with worst rated productivity in the morning (3.00 ± 0.56 for the experimental group and 4.5 ± 0.82 for the control group), and best in the evening (7.50 ± 0.47 and 5.92 ±0.47 respectively). In the experimental group there was a significant interaction of time and intervention ($F(2.22)=29.00$, $p<0.0001$) as well as a main effect of both time ($F(2,22)=7.79$, $p<0.01$) and intervention ($F(1,11)=5.84$, $p<0.05$). Post hoc tests showed significant time of day

differences from morning to afternoon ($p<0.0001$) and morning to evening ($p<0.0001$) at baseline that were abolished after intervention (Figure 5.13B). After intervention, productivity in the experimental group was significantly higher in the morning (6.92 ± 0.57, $p<0.0001$) and significantly lower in the evening (5.92 ± 0.47, $p<0.05$). No significant differences were recorded for the control group from baseline to after intervention (Figure 5.13C). There was, however, a main effect of time ($F(2,18)=6.46$, $p<0.01$) with significant differences from morning to afternoon ($p<0.01$) and evening ($p<0.0001$) at baseline and after interventions ($p<0.05$ morning to afternoon and $p<0.001$ morning to evening).

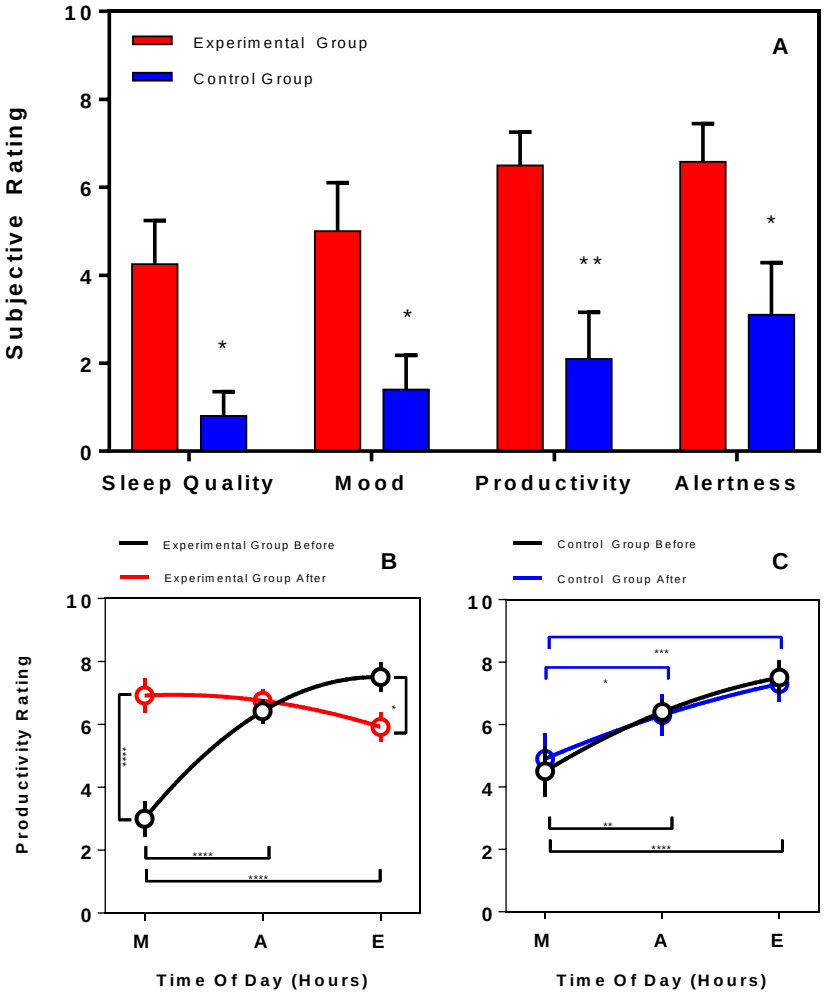


Figure 5.13. Feedback Questionnaire data at baseline and after interventions. A: subjective ratings of sleep quality, mood, productivity and alertness to measure the Hawthorne effect. B&C: diurnal variations in subjective ratings of productivity for morning (M), afternoon (A) and evening (E) before and after interventions in experimental (B) and control (C) groups.

5.5 Discussion

In a society that is under constant pressure to achieve personal best performance, both environmental and biological constraints have been identified as factors affecting health and performance (Organization, 2000). Though both constraints are multifaceted, individual differences have become a key focus in recent years. Here we took a group of LCPs and attempted to phase advance them in a real world setting. The impact of this phase advance on sleep patterns and physiology was compared to a control group. At baseline both groups were comparable in all parameters measured (Table 5.1). MSF_{sc} was highly correlated with DLMO, peak time of cortisol, sleep onset and wake up time, confirming the categorisation of these individuals as LCPs (Table 5.1 and Figure 5.3). Results from the intervention show that a phase advance in a real world setting of around two hours can improve mental well-being and performance by:

1) Decreasing depression anxiety and stress (measured with the DASS).
2) Decreasing mood disturbances (measured with the POMS).
3) Reducing daytime sleepiness in the morning (measured with the KSS).
4) Increasing performance at non-optimal times by an average of 8.8% (cognitive performance) and 6.5% (physical performance).
5) Reducing the variation between best and worst performance over the course of the day by 8.7% (cognitive) and 7.0% (physical), thereby enhancing performance.

5.5.1 Baseline Diurnal Variations in Performance and Sleepiness

At baseline both cognitive (measured with the PVT) and physical (measured with MVC) performance showed a clear diurnal rhythm with worst performance in the morning, increasing throughout the day to best performance in the evening (Figure 5.4). Grip

strength has been reported to peak in the evening (Reilly et al., 2007), with additional studies on muscle strength showing similar results (Callard et al., 2000, Souissi et al., 2010a). Our results found grip strength to be worst in the morning and best in the evening with a 12.37% overall difference between 08:00 h and 20:00 h performance (86.32 ± 1.75 % and 98.69 ± 0.57 % respectively). All times were significantly different from each other with performance in the morning clearly being impaired. This follows a similar pattern to previous studies suggesting peak performance during the evening (Callard et al., 2000). However, studies that have accounted for Circadian Phenotype have uncovered differing patterns of other physical performance measures with ECPs performing better earlier in the day (Facer-Childs and Brandstaetter, 2015a, Rae et al., 2015), which was confirmed in previous Chapters of this thesis.

Diurnal variations in PVT have reported differing outcomes, with some suggesting reaction time is best in the morning (Jarraya et al., 2014a) and others later on in the day (Kline et al., 2010). Other measures of cognitive performance have shown similar results (Drummond et al., 2004). However Blatter and Cajochen (2007) highlight the challenge of performance assessment due to task duration, complexity and difficulty. In the current study, there was a clear diurnal variation in percentage of best reaction time, measured with the PVT, from worst in the morning to best in the evening. Performance at 08:00 h (87.79 ± 2.52 %) was significantly different to performance at both 14:00 h (95.03 ± 1.09 %) and 20:00 h (97.55 ± 1.22) with an overall difference of 9.76% between best and worst (Figure 5.4). This suggests a need to account for individual differences in circadian rhythms when assessing simple cognitive performance measures (Goldstein et al., 2007).

Daytime sleepiness is often at its highest when homeostatic sleep pressure is high and the circadian alerting signal is low, for example when being kept awake into the biological

night (Mitler et al., 1988). However, this study produced conflicting results, with subjective sleepiness at 08:00 h being 6.41 ± 0.30 and decreasing over the day to lowest score (least sleepy) at 20:00 h (4.00 ± 0.33). Interestingly, even at the latest time point measured (20:00 h) individuals were not rating their sleepiness as 'alert, very alert or extremely alert' (values 1-3 on the KSS). This may be due to the fact LCPs have still not reached a peak of alertness and are continuing to rise at 20:00 h, or that the constant social jetlag results in diminished alertness at all times of day measured (Wittmann et al., 2006). This concept would be supported by the different diurnal variations in sleepiness between ECPs and LCPs groups discussed in previous Chapters, with ECPs having lower daytime sleepiness scores between 08:00 h and 20:00 h which are mainly driven by LCPs having significantly lower scores in the morning (Appendix I).

Given that the majority of our society are either at work or school between the hours of 09:00 to 17:00 h, many of these individuals may have impaired performance in the morning (shown here with simple reaction time and grip strength), coupled with high daytime sleepiness. Although KSS was highest in the morning, these individuals do not show any evidence of being acutely sleep deprived during this study due to the similar sleep durations observed previously between ECPs and LCPs. This could suggest that Circadian Phenotype has a greater impact on daytime sleepiness than sleep deprivation, or that LCPs are suffering from more chronic disruptions. This sparked the interest into exploring if these diurnal rhythms can be modified by a phase advance in order to achieve better performance and lower sleepiness at non-optimal times as well as minimise the differences between best and worst performance.

5.5.2 Impact of Interventions on Phase Advance

5.5.2.1 Sleep/Wake Cycles

Wrist actigraphy is often used to measure objective activity and sleep patterns and is currently one of the best ways to monitor individuals non-invasively in their home environment (Ancoli-Israel et al., 2003). PSG is the gold standard of sleep measurements and some variables of actigraphy e.g. sleep efficiency and total sleep time, have been validated against it (de Souza et al., 2003). Actigraphy provides a number of sleep parameters such as sleep onset, wake up, sleep duration, latency and efficiency as well as information about movement during the sleep phase. Along with actigraphy being used to help diagnose CRSDs such as DSPS (Morgenthaler et al., 2007), it has also been used to analyse phase shifts (Sharkey and Eastman, 2002). In this study actigraphy was used to 1) confirm circadian phenotyping at baseline, and 2) explore how sleep parameters are affected by a phase advance.

Actigraphy analysis revealed a significant advance in both sleep onset (02:46 to 01:03 h) and wake up time (10:31 to 08:36 h) after interventions in the experimental group. Remarkably, sleep duration, latency and efficiency all remained similar (Figure 5.5). This suggests that individuals were not simply going to bed earlier but taking longer to fall asleep. If sleep latency had significantly increased and sleep duration significantly decreased, these individuals may have been advancing wake up time but at the detriment of curtailing the sleep phase by still not being able to initiate sleep earlier. Many people use an alarm to get up during the week and compensate for missed sleep on free days (Wittmann et al., 2006). This misalignment of biological and social time (social jetlag) has been linked to health problems such as obesity (Roenneberg et al., 2012), cardiovascular and metabolic risk (Wong et al., 2015) and depression (Levandovski et al., 2011). This is a

phenomenon that regularly occurs in LCPs with an environmental constraint of work/school hours but will not have acutely affected our results as individuals were free to follow preferred schedules. These parameters were also not changed in the control group.

As light is the dominant zeitgeber of the circadian system it has been a central target in the treatment of CSRDs such as DSPS (Rosenthal et al., 1990), and mood disorders e.g. seasonal affective disorder (Rosenthal et al., 1984). It has even been used to promote better sleep in neurodegenerative diseases such as Alzheimer's (Van Someren et al., 1999), and dementia (Mishima et al., 1994). The use of morning light has been used to phase advance the circadian system, whilst evening light has been shown to phase delay the clock (Bolvin et al., 1996, Minors et al., 1991). Although controlled light exposure was not specifically administered in this study, it is likely that the earlier wake up time from 10:31 to 08:36 h would cause a shift in the exposure to natural light in the morning, thereby contributing to a phase advance in the circadian system. Simultaneously, the earlier sleep onset times observed (02:46 to 01:03) would mean a decrease in evening light exposure e.g. from electronic devices, which have been shown to delay DLMO (Santhi et al., 2012) and subsequently delay sleep onset (Chang et al., 2015).

5.5.2.2 Physiology & Behaviour

One of the most reliable and accurate markers of circadian phase is the endogenous melatonin profile (Lewy et al., 1999). DLMO has become a leading technique in circadian research due to its non-invasive nature and convenience of collection prior to sleep (Pandi-Perumal et al., 2007). Together with timed light exposure, exogenous melatonin has been one of the most widely used pharmacological agents in shifting circadian phase. Not only has it been utilised to study the human phase response curve (Lewy et al., 1992, Paul et al.,

2011), but it has also been a target in the treatment of behavioural disorders (Mishima et al., 1994), Alzheimer's (Van Someren et al., 1999) and tolerance of shift work at night (Costello et al., 2014, Folkard et al., 1993). No pharmacological interventions were used in this study specifically to be able to investigate whether purely non-pharmacological means can phase advance the circadian system of LCPs in a real world setting. We found a significant phase advance in DLMO of nearly 2 h (23:56 to 22:07 h). This was coupled with a similar advance in peak timing of cortisol that shifted from 11:19 to 09:06 h (Figure 5.6). Concentrations of both melatonin and cortisol remained at comparable levels. Phase angle, measured as the time between DLMO and sleep onset was also consistent from baseline (2.94 h) to after interventions (2.80 h) suggesting a true phase advance was observed in the experimental group (Figure 5.7).

Timing of food intake has been suggested to have an entraining effect on the circadian system, in particular the peripheral clock found in the liver (Stokkan et al., 2001). We found a significant advance in timing of breakfast (10:33 to 09:25) and observed an increase in the number of days' breakfast was eaten per week although this did not quite reach significance (4.09 to 5.36). Studies have found that a morning carbohydrate rich meal can phase advancing CBT (Krauchi et al., 2002), and that appetite hormones may be regulated by food intake (LeSauter et al., 2009). This research suggests that the earlier breakfast timings viewed here could potentially be contributing to the advance in circadian phase.

In summary, a clear phase advance of around two hours was achieved which allowed the investigation into whether it would impact on mental well-being and diurnal performance profiles.

5.5.3 Impact of Interventions on Mental Well-Being

The association of a delayed sleep phase with reduced mental well-being e.g. depression, has been shown in a number of independent studies. Shirayama et al. (2003) revealed symptoms of depression, nervousness and lack of control in clinically diagnosed patients with DSPS. Others have suggested LCPs have higher rates of depression (Alvaro et al., 2014, Levandovski et al., 2011, Merikanto et al., 2013a). Longitudinal studies have suggested that often sleep issues come before depressive symptoms and have proposed insomnia as a predictor for depression (Baglioni et al., 2011, Chang et al., 1997). Whether it is the depressive symptoms that cause sleep disturbances or vice versa remains largely unknown and proves challenging to detect due to the two being tightly interconnected (Germain and Kupfer, 2008). In addition, different disorders are likely to have differential relationships with sleep and circadian issues e.g. childhood sleep problems have been shown to predict adult anxiety but not depression (Gregory et al., 2005). Nonetheless, targeting sleep and circadian phase has become a focus in the development of novel treatments in neuropsychological disorders such as Alzheimer's (Van Someren et al., 1999), depression (Wehr et al., 1979) and CRSDs (Zhu and Zee, 2012). We found a significant decrease in the DASS, with overall scores reducing from 19.83 ± 3.36 to 11.17 ± 3.07. Interestingly, it was the depression and stress elements of this scale that reduced significantly, with anxiety score not being affected (Figure 5.9). Although anxiety and depression are two separate conditions with different diagnostic criteria, they are often comorbid. However, these results suggest each factor is affected in a different way. Depression specifically has been the target of some studies using phase advances (Wehr et al., 1979), and more research has looked at the relationship between LCPs and depression as opposed to anxiety and stress. However, being able to explore these factors separately would be an important future step within this work. This finding was also seen in the mood disturbances (POMS) with scores

decreasing from 10.33 ± 6.15 to -2.89 ± 4.46. These differences were observed in parameters associated with psychopathology and not subjective ratings of sleep quality (PSQI) or sleepiness (ESS) (Figure 5.8). This finding is intriguing as it suggests psychological factors associated with LCPs, that are not commonly known to individuals unless clinically diagnosed, are being affected by the phase advance more than subjective ratings to do with sleep itself. This could potentially relate to the social constraints creating a 'stigma' around sleep for LCPs (Adan et al., 2012). There could also be methodological reasons for the lack of effect. For example, the sensitivity of subjective questionnaires could be a reason contributing to differences observed, with more granular scales having a greater possibility of detecting small changes.

5.5.3.1 Sleepiness

Daytime sleepiness, measured here using the KSS, is one of the key factors associated with poor performance (Dinges et al., 1997) and higher risk of errors (Dinges, 1995). Increased sleepiness, leading to lapses of concentration and even micro sleeps, has been proposed as a main influence in many of the vehicle related incidents recorded annually (Connor et al., 2001). Being able to reduce daytime sleepiness remains a leading motive in both clinical settings and when considering performance/productivity in the real world (Chong et al., 2006, Phipps-Nelson et al., 2003, Rogers et al., 2001). Here we are able to show that a phase advance of around two hours decreases daytime sleepiness by 1.75 at 08:00 h and 1.08 at 14:00 h. Sleepiness was still at its highest in the morning, although significantly lower than at baseline. This near two point difference in the morning means a change from 'some signs of sleepiness' to 'rather alert' (score of 6 to 4 on the KSS). In addition to the shift in diurnal variation of sleepiness, causing individuals to be least sleepy at 14:00 h

instead of 20:00 h, the phase advance decreased sleepiness as a whole from an average of 4.92 ± 0.74 to 4.00 ± 1.33 (p=0.041, Figure 5.10 & Figure 5.11).

There was loss of significant diurnal variations in KSS score, similarly to what was observed for cognitive and physical performance measures. This could prove useful to those professions that are generally more affected by sleepiness and require high vigilance such as air traffic control, lorry driving and aviation (Borghini et al., 2014).

5.5.4 Impact of Interventions on Performance

Investigations into diurnal variations of both cognitive (Blatter and Cajochen, 2007) and physical (Drust et al., 2005) performance have long been a motivation in sleep and circadian research. Understanding these time of day differences has allowed some studies to shed light on the reason behind the high risk of motor accidents (Lenné et al., 1997), whilst others have examined the effect on performance in athletes. For example, accounting for individual differences in Circadian Phenotypes has shown differing diurnal performance profiles in aerobic performance between ECPs and LCPs (Facer-Childs and Brandstaetter, 2015a, Rae et al., 2015). In line with these suggestions, we now show the potential of manipulating these diurnal variations in LCPs, with a phase advance, to create a profile more comparable to that of ECPs in both cognitive (PVT) and physical (MVC) performance measures (Figure 5.12).

There were significant improvements in cognitive and physical performance at 'non-optimal' times following the phase advance in the experimental group but not in the control group (Figure 5.10 & Figure 5.11). Cognitive performance increased significantly by 8.79% at 08:00 h (86.67 to 95.46 %), showing that morning PVT performance was mostly affected by the phase advance. Physical performance was still lowest in the morning but

had increased significantly by 5.92%. The same was in the afternoon with a significant increase of 6.47%. This morning result may be expected since the phase advance in wake up times from 10:31 to 08:36 is still after the scheduled testing time of 08:00 h. Therefore individuals were still not usually awake at this time and may not be expected to achieve peak performance due to the testing still occurring during the biological night at a 'non optimal' time (Santhi et al., 2013).

There was a significant decrease in the overall percentage variation between best and worst performance over the course of the day. Physical performance difference was reduced by 6.98% ($p=0.0024$) and cognitive performance by 8.67% ($p=0.028$). These changes suggest that a phase advance could minimise the variation of performance over the course of the day. This is supported by the significant time of day differences at baseline dissipating following interventions (Figure 5.11). There were no longer significant differences cognitive (PVT) performance and only between 08:00 h and 14:00 h in physical (MVC) performance. This diminishment in diurnal variation is in line with previous research which showed a much larger range in performance differences for LCPs compared with ECPs (Facer-Childs and Brandstaetter, 2015a, Tamm et al., 2009). Figure 5.12 shows how the diurnal curves of cognitive and physical performance were transformed following interventions, with MVC performance being shifted from 20:00 h to 15:21 h (Figure 5.12B), and PVT performance peaking at 12:30 instead of 20:00 h (Figure 5.12C). These results follow a similar trend in line with the shift in sleep onset, DLMO, peak time for cortisol and wake up times. The experimental group shifted earlier, mirrored by a shift towards earlier peak performance times. This outcome suggests that diurnal performance profiles can be altered and variation between best and worst performance can be significantly reduced with a phase advance.

5.5.5 Feedback Questionnaire

All participants were asked to complete a feedback questionnaire at the end of the study to allow an insight into subjective ratings of sleep quality, mood, productivity and alertness. This was designed specifically to observe any 'Hawthorne effect' arising from being in a research study. There were remarkable similarities in diurnal variation of subjective productivity measured with the feedback questionnaire and performance profiles (Figure 5.13B&C). The experimental group showed a clear shift in diurnal variation of productivity with morning productivity increasing significantly and evening productivity decreasing significantly (Figure 5.13B). The control group remained analogous from baseline to after interventions (Figure 5.13C). The significant time of day differences were also diminished in the experimental group, much like the objective recordings of performance, but remained in the control group. This provides encouraging data to support the relationship between subjective and objective measures, a topic that is so often debated when evaluating workplace performance in the business world (Forth and McNabb, 2008, Van Dongen and Belenky, 2009).

5.5.6 Limitations

Despite the value in using a real world protocol due to its ease of implementation and lack of disruption to individuals' daily lives, it limits the ability to control the environmental and social influences. CR (Mills et al., 1978), and FD protocols (Folkard and Akerstedt, 1989), allow the conclusion of a truly endogenous rhythm through removing external cues. However, this study was not aimed at finding endogenous components to performance but looked at the integrated system as a whole. The combination of endogenous circadian rhythms, sleep homeostasis, environmental cues and social schedules are what affect daily

functioning and diurnal variations in the real world. Therefore, although we cannot conclude the changes we see are strictly attributed to one of these influences, we provide evidence of the ability to phase advance LCPs in a real life setting and the positive outcomes on mental well-being and performance. Another limitation would be the ability to maintain such schedules. If participants were not motivated or willing to continue following such routines it could result in a 'relapse' by reverting back to later schedules. Future studies of longitudinal nature could provide more insight into if these effects can be sustained in the long term.

Here we investigated very simple measures of cognitive (PVT) and physical (MVC) performance and thus we restricted the ability to determine how more complex measures would be affected (Blatter and Cajochen, 2007). Performance itself is multifaceted and cannot be defined by one measure alone, so future work will need to explore how diurnal variations in different tasks are influenced by a phase shift. Nevertheless, these results highlight how a real world phase advance can yield positive outcomes. This is extremely promising for individuals and professions looking to optimise performance. These limitations will be explored further in the final Chapter of this thesis.

5.5.7 Conclusions

Here we show the ability to phase advance LCPs by around two hours using realistic, simple non-pharmacological interventions in the real world. This phase advance had a positive effect on mental well-being. Mood disturbances were reduced, shown by a decrease in moods states score (POMS), as was a reduction in depression and stress score and overall DASS score. This phase advance also impacted on diurnal rhythms of sleepiness, cognitive and physical performance. Being able to shift peak performance could

have immense effects for various different settings within sports and clinically. In the sports world, for example, timings of match and competitions often follow fixed schedules. If athletes were able to shift their biological rhythms and therefore performance peak it could yield huge benefits. We also show that a phase advance reduces the difference between best and worst performance by nearly 7% (physical) and 9% (cognitive), suggesting that the interventions have enhanced performance as a whole.

These findings have the potential to be applied in several different contexts.

1) Novel treatments for mental health in depression and stress could be explored specifically targeting sleep and circadian issues.
2) In the business world, developing strategies to maximise productivity and performance in the work place is a key focus in which this research could be directed.
3) In the sports world, optimising performance to reach the highest level possible is a universal goal for athletes and coaches. Being able to shift diurnal performance could bring about a new angle of added value for athletic performance.
4) These findings could also be of benefit to those individuals in the general population seeking ways to achieve personal best performance.

Despite the need for further research into these areas, this remains an exciting prospect for a society that is under pressure to achieve personal best performance, whilst in constant conflict with biological and environmental constraints.

The experimental Chapters presented in this thesis explored how functional imaging can be used to increase our understanding of human behaviour and performance within the fields of chronobiology and sleep. It has also shown the ability to phase advance LCPs in a real world setting and the positive impact this has on mental well-being and indices of cognitive and physical performance. The final Chapter discussed the main findings and implications of this work, highlighting particular limitations and potential ideas for future work that could be explored.

CHAPTER 6

Thesis Summary and

Concluding Remarks

6.1 Overview

There is little regard for the impact of circadian and sleep disruptions in the modern attitude towards the temporal organisation of society. It is estimated that nearly 70 million individuals in the US alone suffer from some sort of disturbance to the sleep/wake axis which impedes normal functioning and has damaging effects on health and well-being (Altevogt and Colten, 2006). As pressures to reach personal best performance rise, the need to consider the influence of these mechanisms on our health and well-being is becoming increasingly important.

It is well documented that disrupting circadian rhythms results in changes to many physiological processes such as endocrine regulation (Robertson et al., 2013), sleep patterns (Lazar et al., 2013) and CBT (Refinetti and Menaker, 1992). The most common disruptions are jet leg (Sack et al., 2007), seasonal affective disorder (McClung, 2007) and circadian rhythm sleep disorders (CRSDs) such as shift work disorder (Folkard et al., 2005). Night shift work has been established as a probable carcinogen by the World Health Organisation (Costa et al., 2010, Erren et al., 2010), highlighting the adverse outcomes of disruptions to sleep and circadian systems. In 1988, annual costs of over $50 billion were attributed to sleep-related accidents in the US (Leger, 1994), a figure that has no doubt increased considerably over the past three decades. On top of the substantial economic burden these issues hold for public health, there are also extensive indirect costs in the business sector. Sleep disruption is an independent risk factor in occupational accidents (Akerstedt et al., 2002), and loss of productivity, absenteeism along with poor performance costs businesses in the US $150 billion per year (Altevogt and Colten, 2006). Within the UK, the impact of sleep deprivation results in 200,000 lost working days per year, which is estimated to cost the UK economy £40 billion (Hafner et al., 2016). A major factor

influencing these outcomes is a lack of appreciation for individual differences in vulnerability to sleep disruption and circadian misalignment. As such, despite the awareness of the consequences, there is still a long way to go to directly translate research outcomes that could affect change in our rapidly evolving 'round the clock' society.

6.2 Summary of Findings

Much of the research on performance variables has been done without the consideration of contributions from circadian and sleep dependant mechanisms (Goel et al., 2013). What is more, increasing our understanding of the neuronal origins behind these differences can be enhanced using functional imaging techniques.

The first aim of this thesis was to investigate the impact of Circadian Phenotype (ECPs and LCPs) on behaviour and performance. Rs-fMRI was used to explore the intrinsic functional architecture of these groups, and how this relates to cognitive and physical performance measures. The results show clear differences in rs-FC between ECPs and LCPs when exploring the DMN and MN as networks of interest. Time of day differences were also identified in rs-FC of the MN. Furthermore, distinct diurnal curves were revealed in both cognitive and physical performance measures, which could be predicted by rs-FC. Interestingly, when looking at separate Circadian Phenotype groups, it became clear that the majority of time of day differences in performance and sleepiness are driven by significant diurnal variations in LCPs. ECPs show virtually no variation depending on time of day whereas the LCPs seem to be largely impaired during the morning (08:00 h). The same pattern was seen for diurnal variations in rs-FC of the MN, with LCPs having significantly lower FC during the morning. This would be extremely relevant in research using fMRI or investigating sleepiness and performance measures. If studies have not

classified individuals into Circadian Phenotype groups or taken into account the time of day then any differences observed may be due to a large proportion of the sample being LCPs and would therefore not be a true representation of the population as a whole (Facer-Childs and Brandstaetter, 2015b).

The results provide substantial evidence to support the need to consider Circadian Phenotype and time of day when exploring elements of cognitive and physical performance. It is also clear that without this consideration, the interpretation of rs-fMRI data could be misrepresentative in both clinical and research settings.

Secondly, the ability to phase advance LCPs in a real world setting has been shown to have a positive effect on mental well-being and performance. This could hold significant benefits for sectors looking to minimise the adverse outcomes of disruptions e.g. both the private and public sectors of commerce and the sports industry. The following sections will discuss the main findings and how they provide information about performance outcomes. The implications of these results will then be discussed in relation to the fields of sleep/chronobiology, performance and neuroimaging.

6.2.1 Intrinsic Functional Differences between Circadian Phenotypes

The data presented in this thesis establish fundamental differences between ECPs and LCPs in rs-FC when seeding in regions of the DMN and MN. The DMN and MN were explored as networks of interest due to the link to cognitive and physical performance variables gathered. The majority of differences in rs-FC identified higher connectivity in ECPs. It may not seem surprising that the ECP group are associated with positive findings, given that LCPs have been associated with many negative outcomes in terms of health, well-being and performance. Nevertheless, the link between higher/lower FC and performance isn't

always straightforward, as discussed previously. For both DMN seeds, the largest significant cluster identified as higher in ECPs was in the frontal cortex, highlighting strong connectivity between PCC and mPFC but also within the frontal cortex.

Another interesting observation from the data presented in this study is that from both the mPFC seed (Chapter 3) and LM1 seed (Chapter 4) a significant cluster was found in the right insula. The insulae have been proposed as fundamental components of the Salience Network (SN). The SN is linked to dynamic switching between task positive and task negative networks and is thought to play a key role in awareness (Craig, 2009). Increased connectivity between mPFC and rAI has also been associated with longer cumulative sleep durations (Khalsa et al., 2016). This higher inter-network connectivity between both DMN and MN with the SN was seen in ECPs compared to LCPs. This finding could relate to more efficient switching between resting-state and task activation, something which is supported by Menon and Uddin (2010) presenting the right insula as a potential functional hub for salient events. ECPs have also been shown to have enhanced left anterior insula recruitment during tasks (Reske et al., 2015). This idea is reinforced by the fact that ECPs frequently perform better in cognitive and physical performance measures, as shown by the decreased variation in performance in this study.

However, increased FC is not always correlated with positive outcomes, across and between networks. For instance, much ageing research on rs-FC suggests that both increases and decreases in FC can result in reduced specificity of ICNs with age (Ferreira and Busatto, 2013) and are correlated with poorer performance outcomes (Onoda et al., 2012). Although decreased rs-FC is a common theme reported in normal ageing, and has been shown in both the DMN (Damoiseaux et al., 2007), and the MN (Wu et al., 2007), increases in rs-FC have also been found, particularly in the MN (Meier et al., 2012). This

suggests that the relationship of rs-FC to behaviour is complex as higher FC does not necessarily mean better performance. Therefore, interpretations are not straightforward. Not only have increases in rs-FC been linked to reduced performance with ageing but also with mental health issues such as depression (Greicius et al., 2007), and attention deficient hyperactivity disorder (Tian et al., 2006).

Of the three significant clusters found to have higher rs-FC in LCPs, one was within the anterior cingulate cortex. Increased rs-FC of the anterior cingulate cortex has been independently associated with depression (Connolly et al., 2013). Seeing as LCPs are frequently linked to higher rates of depression (Merikanto et al., 2013a), and scored significantly higher than ECPs in the DASS used within this study (Appendix G), this result could prove exciting for future work on the neuronal basis of this association.

6.2.2 Resting State Functional Connectivity and Time of day

This thesis uncovered time of day differences in rs-FC of the MN but not the DMN. These diurnal variations, which are only seen in LCPs, were more pronounced when looking at FC between specific regions of the MN as opposed to average connectivity across all identified clusters. This could be a reason contributing to no time of day differences being identified in the DMN, as the analysis was limited to using average rs-FC values across all regions. However, the fact that no time of day differences were uncovered in rs-FC of the DMN does not necessarily mean that this network is unaffected by time of day. Research using independent component analysis (ICA) has shown the DMN to be highly rhythmic (Blautzik et al., 2013), although this study did not specifically look within Circadian Phenotype groups. Since there were regional differences in the behaviour of FC depending on time of day within the MN, a more detailed examination of FC between specific regions could

uncover diurnal rhythmicity in areas of the DMN. For example the PCC and mPFC have been proposed as key components of the DMN, and their functional relationship has been reinforced by the discovery of anatomical links (Greicius et al., 2009). These specific regions have also been shown to decouple during deep sleep, and recruitment of the frontal cortex has been proposed for maintenance of consciousness (Horovitz et al., 2009). Therefore, the intra-network connectivity between these two regions would be worth exploring further to look at time of day effects. Given the functional role of the DMN, time of day differences between these regions may be expected based on significant diurnal variations in behavioural measures such as sleepiness and indices of cognitive performance.

6.2.3 Phase Advancing Late Circadian Phenotypes

The ability to phase advance LCPs and have a positive effect on mental well-being and performance holds much promise for performance research as well as treatments in clinical settings, although maintaining this in the long term may be difficult. When attempting to manipulate circadian phase there are also a number of constraints that this thesis has highlighted. Study designs which do not include the categorisation into Circadian Phenotypes could affect results and lead to differing conclusions. Secondary effects could occur through manipulating sleep wake cycles, diet and activity which would need to be monitored and accounted for. Although there are many methodological constraints, the implications of circadian misalignment and sleep disruptions bring to light the need to increase our understanding of shifting circadian phase.

An important further step in the analysis of Chapter 5 would be to look in more detail at the activity and light readings gathered from actigraphy. This could provide useful

information into the relative contributions of increased activity versus increased light exposure during the morning following the phase advance schedule. One would expect an increase in light exposure to be the main influence on the circadian system (Roenneberg et al., 2013), although physical activity (Edwards et al., 2009), and food intake (Stephan, 2002), have also been shown to contribute to entrainment. These three factors are most likely interacting at the behavioural level i.e. an individual wakes up earlier causing movement and light exposure to increase, as well an eating breakfast earlier. We found a significant advance in timing of eating breakfast but cannot conclude that this specifically impacted the phase advance without quantifying the relative contributions of light and activity. Using the actigraphy data to determine the amount of light exposure and activity from baseline to after interventions could provide more understanding into the mechanisms behind the observed phase advance and subsequent increase in performance at 'non-optimal' times of day.

6.3 Implications for Sleep & Circadian Research

Investigating sleep and circadian impacts on behaviour and performance comes with a number of methodological issues (Atkinson et al., 2007a). As these two fields are tightly interrelated it is critical to incorporate the interaction when studying either field. Within sleep research, there is a need to consider individual differences in timings and how time of day could influence the results. Likewise, within chronobiological research, controlling and accounting for sleep related effects is necessary to explore circadian mechanisms.

A significant point to make is the fact that this study was carried out within a societally constrained 12 h day (08:00 h to 20:00 h). Compared with ECPs whose natural preferences fit to these timings, LCPs' preferred schedules would be significantly later e.g. 12:00 h to

00:00 h. Is it interesting to note that sleepiness score measured by the KSS, which showed significant diurnal variation in each group driven by a large difference at 08:00 h, was not significantly different between ECPs and LCPs when subjects were not constrained as to what time to take the test during the practice sessions. During the acclimatisation, average KSS score for ECPs was 4.27 ± 0.31 which was not significantly different from 4.65 ± 0.30 in LCPs, shown in Chapter 3. The fact that only 08:00 h KSS score was significantly different in the main study suggests that this is the time mostly affected by acute sleep deprivation in LCPs (Appendix I). Despite being able to follow preferred schedules for this study, and no differences in subjective sleepiness at 14:00 h or 20:00 h, LCPs still seem to be impaired when looking at rs-FC and performance measures at all time points. This may reflect more of the chronic disruptions LCPs experience from persistent circadian misalignment. As discussed by Banks and Dinges (2007), chronic sleep restriction results in neurological and cognitive deficits. However, the authors point out that there are no experimental findings on the effect of chronic sleep restriction using functional imaging. Although this study was not specifically investigating sleep restriction, and our LCPs were not sleep deprived during the course of the study itself, this group tend to be the most affected by sleep restriction. Therefore, the differences in rs-FC uncovered in this thesis could support the idea that persistent sleep disruption and circadian misalignment could result in more chronic changes to intrinsic functional architecture. The finding that LCPs could be mostly affected during a societally constrained day emphasises why Circadian Phenotype and time of day should be taken into account whenever sleep and circadian mechanisms are being investigated.

6.4 Implications for Performance Research

Direct measures of performance are highly relevant to society, but it seems that without taking into consideration the multiple influencing factors, especially the need to group individuals according to their Circadian Phenotype and sleep homeostasis, studies could be missing key results. Therefore, there needs to be an increase in our understanding of how to study these processes, along with the mechanisms contributing to differences in performance.

The distribution of Circadian Phenotypes observed within normal populations comprises around 10% ECPs. However the same is not seen within athlete populations, who tend to have a higher percentage (approximately 50%) of ECPs (Lastella et al., 2016). This emphasises the need to identify LCPs who may be more vulnerable to diurnal variations in performance. In addition, rs-FC has been shown to be higher in athletes (Raichlen et al., 2016), and increases after a period of training (Ma et al., 2011, Taubert et al., 2011, Xiong et al., 2009). Since in this study we show ECPs to have higher rs-FC in the majority of identified regions, one could propose that if the ECP group exercised more regularly than the LCP group it could impact on rs-FC. To prevent this influencing the results, details of exercise were taken into account during screening. When asked the question 'do you exercise regularly' 75 % of the ECPs said yes and 73% of the LCPs answered yes (Appendix G). This suggests that both groups could be comparable in terms of physical activity, and supports the idea that these differences are due to Circadian Phenotype and not specifically the effect of exercise. However, as the question asked did not gather exact details of types of training, and the definition of 'regularly' could be interpreted differently, it does not rule of the possibility that one group could have been more active than the other. This could be

explored further using the actigraphy data to specifically quantify daily activity levels between ECPs and LCPs.

This thesis suggests higher rs-FC in regions of the DMN and MN at rest could enable the brain to be in a state more able to perform a task. However, there is a causality issue with rs-FC, as better performance in tasks could feedback to cause higher FC. As mentioned, increases in rs-FC can be observed after a period of training (Ma et al., 2011, Taubert et al., 2011, Xiong et al., 2009). These studies focus on increases in rs-FC, but do not explicitly link this to an increase in performance. The idea that training can affect rs-FC of ICNs brings to light another interesting question that could be pursued, within the sports world but also in any sector looking to enhance performance. If rs-FC can predict performance outcomes, can it be adapted or strengthen with training over time and result in enhanced performance? If increases in rs-FC with training could show an improvement in performance it would provide more understanding of the neuronal origins of optimising performance. This would also be of interest when phase advancing LCPs to see if rs-FC is affected by the shift earlier and becomes more comparable to that of ECPs.

6.4.1 Performance Applications

The sports world is a sector in which marginal gains are fundamental to success. As such, an interesting area that could be pursued is how this information relates to athletic performance. Athletes are a subset of the population who are searching for ways to improve and enhance performance, with scientific evidence being sought from psychological and physiological research. The participants in this study were not specifically athletes but indices of both cognitive and physical performance were measured which may translate to athletic settings.

With the general view of evening peak performance, suggestions have been made to schedule training for the evening (Drust et al., 2005). However, if the goal is to achieve peak performance in competition, it may be in athletes/coaches best interest to train at specific times of day to try and optimise performance accordingly (Arnett, 2001). Types of training, lengths of training and combinations of training (Faude et al., 2014, Gabbett et al., 2009), as well as injury and injury prevention (McKay et al., 2014), have been reported to be of particular importance. 'Over-training', is the combination of too much training and too little recovery time which can result in physical and psychological impairments of performance (Fry et al., 1991). However, considering time of day with respect to circadian regulation and sleep is rarely a priority. The few studies that have specifically looked at the effect that time of training has on performance have presented some interesting findings. A shift to morning training has been shown to increase morning performance (Chtourou and Souissi, 2012, Sedliak et al., 2008). Another study showed that training in the morning diminishes the diurnal difference in performance whilst training in the evening emphasised it (Souissi et al., 2002). Although exclusive to LCPs, this thesis shows similar results with a decrease in the significant diurnal variations following a phase advance. This could be promising for athletes who need to optimise performance to a certain time of day due to travel and media coverage. If diurnal variations in LCPs were minimised to make them more comparable to ECPs this could result in enhanced performance at previous 'non-optimal' times of day. However, once again, this highlights the need to classify Circadian Phenotype groups to gain a better understanding of performance variations over the course of the day.

6.5 Implications for Neuroimaging Research

Research using functional imaging techniques rarely accounts for the impact of Circadian Phenotype or time of day. However, with increasing research showing that rs-FC is affected by sleep (De Havas et al., 2012, Sämann et al., 2010) and time of day (Jiang et al., 2016, Hodkinson et al., 2014) uses of rs-FC data in clinical or research applications could be misleading.

This thesis has combined both Circadian Phenotype and time of day issues and provided evidence to support the critical need to account for these factors in neuroimaging research. The exclusion of this information is likely to unintentionally mask significant effects when calculating an average across a whole sample. However, future work is required to replicate these results and confirm their reproducibility with different ICNs and more complex cognitive tasks. Despite the DMN being associated with cognitive functioning, it is not directly involved during cognitive tasks. In fact, some have shown that increased DMN connectivity during tasks impairs the ability to perform (Drummond et al., 2005). Although this thesis makes a case for investigating this network, there is a need to explore ICNs that are directly involved with cognitive performance. Perhaps exploring how other networks vary with time of day and Circadian Phenotype could shed some light on the complex mechanisms contributing to cognitive performance outcomes. For example, the DAN could be investigated since it has been shown to differ between Circadian Phenotype groups (Reske et al., 2015).

It becomes slightly simpler when looking at the MN and relationships with an index of physical performance (MVC). The regions of this network identified during rest are directly related to motor function since they are activated when a motor task is performed (Jiang et al., 2004). Interestingly the time of day differences in MVC as a whole group were very

similar to those identified in performance variables such as muscle strength (Callard et al., 2000, Nicolas et al., 2005) and aerobic capacity (Facer-Childs and Brandstaetter, 2015a). In this study, the diurnal variations in MVC were stronger than those in cognitive performance measures, which was reflected in the rs-FC of the MN showing more variation over the course of the day.

6.5.1 Clinical Applications

Rs-FC has been shown to be affected by different pathological states such as Alzheimer's (Wang et al., 2007), depression (Connolly et al., 2013, Gudayol-Ferré et al., 2015), and Parkinson's disease (Luo et al., 2014, Wu et al., 2009). It is also used in clinical and research settings to monitor recovery from stroke (Andrew James et al., 2009, Carter et al., 2012), or as an index of muscle fatigue in healthy subjects (Peltier et al., 2005).

This thesis has revealed significant differences in rs-FC between different Circadian Phenotypes, as well as variations depending on the time of day. These findings could hold significant implications for the use of fMRI data to assess, monitor or support diagnoses in clinical conditions. For example, if an LCP was recovering from a stroke but was scanned in the morning, the rs-FC of the MN could be significantly lower than later on in the day. Without accounting for these factors results could be misinterpreted, leading to an underestimation of a patient's progress in recovery.

A clinical diagnosis of a sleep related disorder has to involve distress or disruption to the individual's daily life. Criteria for a diagnosis of a CRSDs include; a chronic disrupting pattern caused by circadian misalignment, a sleep disturbance and associated impairment that has been on-going for at least 3 months (Sateia, 2014). Therefore, many LCPs may pass

some of the criteria for a CRSD but not seek a diagnosis if there is little negative impact on their life.

LCPs share many symptoms with the clinical diagnosis of DSPS, a CRSD that particularly affects young adults. However, very little research has attempted to investigate the brain structure or function of these clinical populations and compared it to healthy individuals with similar symptoms. As the data in this thesis has concentrated on functional differences, further work could focus on structural differences between ECPs and LCPs. Recent work using DTI has shown that there are white matter differences in LCPs and links it to an accumulation of chronic sleep debt (Rosenberg et al., 2014). Habitual sleep durations and subjective sleep quality have also been shown to predict white matter differences (Khalsa et al., 2017). If structural links were uncovered which could show similarities between LCPs and clinical populations this could provide novel and exciting evidence that LCPs could be a risk factor for developing DSPS. On top of this, new treatments could be explored building on data in this thesis showing that phase advancing LCPs has a positive effect on mental well-being and performance.

6.6 Limitations & Future Work

As with all studies, there are a number of limitations in this research. The following section will aim to cover some of the confounding factors of this study.

6.6.1 Study Design

The purpose of testing at clock times as opposed to internal biological time was to investigate how these two groups behave during the hours of a 'normal working day'

(08:00 h to 20:00 h). This data can therefore be translated into real world settings and hold implications for monitoring performance. The disadvantages of this design are that it cannot separate the number of influences affecting the outcomes, or separate the effects of the circadian system from the sleep homeostat. This becomes a challenge as there is no way of confirming specific mechanisms contributing to the results. Since all participants were following preferred schedules throughout the study and sleep durations were comparable, the study design meant that testing at 08:00 h should have been the only session affected by disruptions in sleep (Appendix G). Consequently, only the LCPs should have been forced to wake up early for the morning session and may have been sleep deprived. This is backed up by a significant difference in KSS score between ECPs and LCPs at 08:00 h but not at 14:00 h or 20:00h (Appendix I). Nonetheless, since the majority of sleep parameters measured e.g. sleep efficiency and duration, were not different between ECPs and LCPs there is no reason to assume that they differ in terms of sleep mechanisms at the 14:00 h or 20:00 h testing session. This is supported by no differences in KSS score at these times (Appendix I). Scheduling the testing sessions based on internal time would have reduced the influence of the sleep homeostat during the 08:00 h session and allowed an investigation into the effect of endogenous influences on the outcomes. However, since the purpose of this study was to investigate these groups in a real world scenario, reducing the sleep dependant mechanisms would minimise the external validity of the results. In addition, if testing had been carried out over a 24 h period it would have allowed an investigation into the circadian variation of rs-FC between Circadian Phenotypes. One could speculate that the LCPs would continue to improve performance measures later into the evening hours as the circadian alerting signal continued to rise until sleep drive increased. Meanwhile, ECPs, whose sleep drive would already be higher than LCPs at this time would become more impaired. This circadian variation would of course be impacted

by the sleep homeostat increasing sleep pressure which would need to be accounted for. This, however, would have been attempting to answer a different question. To explore truly circadian effects, strict lab based protocols e.g. CR or FD, over 24 h or more would be required. These protocols could result in poorer external validity due to the unrealistic settings. In summary, using clock/real time meant that circadian and sleep effects could not be analysed separately and hence we cannot categorically state that either sleep or circadian influences are the dominant aspect in the differences observed. However, it could be argued that the combined approach is more applicable to the real world as behaviour and performance are ultimately impacted by both factors.

6.6.2 Clinical Cut Offs

A summary of all questionnaire, actigraphy and physiological data collected from ECPs and LCPs are given in Appendix G. It would be worth pointing out that LCPs have significantly higher values than ECPs for ESS, DASS and POMS. The average values for LCPs are in the 'higher normal daytime sleepiness range' for the ESS, and the 'normal' range for DASS and POMS. Average PSQI score was 5.05 for LCPs, which is on the clinical cut off (Appendix H). These results may be somewhat anticipated since LCPs have been associated adverse health outcomes, as discussed throughout this thesis. It also strengthens the proposition that LCPs have subclinical symptoms of DSPS which was touched upon in Chapter 5. The difference in mental well-being between ECPs and LCPs was not explicitly looked into and would make for an interesting question in future work. For example, investigating the relationship between mood symptomatology and rs-FC in different Circadian Phenotype groups could provide novel evidence for the neuronal mechanisms linking LCPs to reduced

mental well-being, as well as potential treatments for clinically diagnosed CRSDs through phase advancing.

6.6.3 Genetics

The VNTR polymorphism in the per3 clock gene has been linked to Early and Late type preferences (Archer et al., 2003, Dijk and Archer, 2010). It is important to note that Diurnal Preference and Circadian Phenotype are not always analogous. Diurnal Preference reflects a subjective inclination towards morning or evening, whereas Circadian Phenotype reflects the behaviour of the individual combined with physiological markers. Studies looking at the per3 VNTR polymorphism have shown that individuals with the homozygous (5/5) allele, which is linked to a morning preference, are more vulnerable to sleep loss as their sleep pressure increases at a higher rate during evening hours (Groeger et al., 2008). We did gather information on individual's genotype (Chapter 2, section 2.6.3), but the resulting data showed only one individual that had the homogenous $per3_{(5/5)}$ polymorphism, with the rest being comprised of the heterozygous $per3_{(4/5)}$ and homogenous $per3_{(4/4)}$ (Appendix G). It was expected that the ECPs would have a higher proportion of $per3_{(5/5)}$'s since it is linked to a morning preferences and the LCPs would have more $per3_{(4/4)}$'s (Dijk and Archer, 2010, Pedrazzoli et al., 2010). A potential reason for the lack of $per3_{(5/5)}$'s in the ECP group could be that the majority of research into the per3 polymorphism has linked it to Diurnal Preference, even though some use the phrase 'Chronotype' or 'Circadian Phenotype' which is technically the wrong terminology when reporting MEQ results (Osland et al., 2011). Since Circadian Phenotype represents more of a direct measure of behaviour at the time it may not match an individual's Diurnal Preference and points towards the need for future work.

6.6.4 Neuroimaging

6.6.4.1 Why rs-FC has benefits over task based fMRI

Task based fMRI measures the change in BOLD response as a direct result of an external stimulus, thereby allowing specific activation patterns to be anticipated. Despite this not being possible in resting state studies, rs-fMRI has become a powerful tool to study the brain's functional organisation due to a number of distinct advantages over task based fMRI. Firstly, rs-fMRI does not require individuals to complete any testing. This can be problematic in clinical populations when patient groups do not have the capacity to perform a task. Secondly, activation paradigms are designed to investigate specific domains which does not allow the exploration of multiple networks simultaneously (Carter et al., 2012). This means that, in principle, you can compare rs-FC with multiple tasks performed outside the scanner. Finally, rs-fMRI has been shown to correlate with task based studies to predict behavioural outcomes (Hampson et al., 2006). Therefore, rs-fMRI provides a robust measure to study the functional integrity of the brain in clinical and research settings (Raichle, 2010).

There are some sceptical views about whether rs-FC can be used to infer neuronal activity due to spontaneous BOLD signal being influenced by physiological noise (Birn et al., 2006). Respiratory fluctuations cause increased oxygen in the vascular system of the brain. Many blood vessels lie close to regions of particular networks and some critics suggest this is the reason for seeing ICNs (Wise et al., 2004). However, this concern has been somewhat overcome by ICNs being identified when looking at glucose metabolism through PET scans (Buckner et al., 2008, Raichle et al., 2001), electrical activity (Foster and Parvizi, 2012), as well as changes in rs-FC being shown to correlate with performance and being affected by clinical diagnoses (Broyd et al., 2009). These unwanted effects can also be minimised with

thorough preprocessing steps, although many studies on rs-FC do not account for physiological noise by directly measuring cardiac and respiration rates. Instead GSR or low pass temporal filtering are used to try to remove these artefacts. This study, however, aimed to control for much of the 'non-neuronal' sources by monitoring pulse and respiration rate during scanning which were modelled by RETROICOR and added as nuisance regressors in the subsequent analysis. Due to GSR creating potential bias in rs-fMRI results it was not implemented in the analysis of data gained in this study. The decision not to remove global signal is in line with research suggesting GSR can alter FC and increase the strength of anti-correlations (Murphy et al., 2009, Saad et al., 2012, Saad et al., 2013). As mentioned previously, global signal is made up of multiple factors including physiological noise, WM and CSF signals, movement and scanner variability, all of which were added to the model as nuisance repressors in the hope that the majority of non-neuronal signal had been removed.

6.6.4.2 FMRI Analysis

FMRI data can be analysed in multiple ways, each of which asks a slightly different question. This thesis used seed based FC analysis to provide a simple way to measure rs-FC from a seed in the DMN and MN. This method requires relatively few assumptions about the regions as only the seed is defined. Although a seed based approach limits the analysis to the specific region chosen, it enables changes in FC to be identified over the whole brain. An extension to this analysis, if time had permitted, would have been to use each region of the ICNs as a seed. This would have allowed whole network seed based analysis to be quantified. However, this would have required a technique such as graph theory to summarise the results. The behaviourally relevant differences in FC uncovered in this thesis seem to be specific to certain regions, meaning that averaging or summarising

multiple connections could result in a loss of effects (Bullmore and Sporns, 2009). Other ways to analyse the data e.g. ICA, could have given an insight into the rs-FC of multiple networks and if they vary depending on time of day, as shown by (Blautzik et al., 2013). ROI to ROI analysis is another way the data could have been explored, although defining a seed and a target is more restrictive and spatial re-organisation could have created potential issues with the definition of regions (Goldstone et al., 2016). ROI analysis would also have limited the ability to identify small significant clusters. If the time series from the whole region is averaged, smaller significant regions within this could be missed due to the average signal being used. Using a different type of analysis e.g. ICA, ROI to ROI or graph theory could uncover further effects of rs-FC on performance measures in Circadian Phenotype groups and would be worth exploring in future work. Nonetheless, the reasons for using the approach in this thesis were that it was sensible, realistic and allowed an analysis with relatively few restrictions.

Positive FC is easier to interpret than negative FC and should be less affected by methodological choices such as GSR (Murphy et al., 2009, Saad et al., 2012, Saad et al., 2013). As such, only positive FC was examined in this thesis which did not permit the exploration of anti-correlated networks. This would be an interesting area for further studies as the strength of anti-correlations have been shown to impact on behaviour and be affected by sleep (De Havas et al., 2012). One could hypotheses that as ECPs have increased rs-FC to the majority of regions identified, they could potentially have stronger anti-correlations with ICNs such as the DAN, resulting in an increased ability to switch between networks and therefore produce better performance outcomes. This would be strengthened by ECPs having higher rs-FC to a cluster in the right insula which is part of the SN, discussed previously.

The brain is continuously coordinating, integrating and responding to stimuli over short and long time periods. Although this study explicitly looked at temporal variations in rs-FC over the day and has highlighted the need to account for time of day in future work, there is also a need to investigate fMRI more dynamically over minutes. The majority of rs-fMRI studies have used a static description of FC, averaging a time series over the course of a scan. However, quantifying these changes within this time frame could provide a more detailed representation of FC (Hutchison et al., 2013). Whilst interpretation of dynamic FC remains challenging and comes with methodological issues, it lends promise to a greater understanding of how cognitive performance is affected by temporal fluctuations (Shine et al., 2016).

6.6.5 Generalisability of Performance

The fact that performance is extremely complex means that it cannot be described by one mechanism alone. In addition to the problematic issues caused by task duration, complexity and timing discussed in the introduction, cognitive performance has been documented to be affected by motivation (Roeser et al., 2013) and personality (Preckel et al., 2011, Preckel et al., 2013). Very few studies have been able to measure multiple elements of cognitive performance, bringing to light the need to study combinations of cognitive functions and see how they are affected by time of day (Bennett et al., 2008). Since many of the issues linked to measuring cognitive performance have been discussed in previous sections (Chapter 1 section 1.3), this section will focus specifically on physical performance. Internally physical performance can be measured by physiological markers such as hormones levels (e.g. cortisol, melatonin, and testosterone), CBT, heart rate, respiratory rate and maximum oxygen uptake. External physical performance or athletic performance can be described and monitored through strength, power, aerobic capacity,

anaerobic capacity and specialist skills tailored to certain sports such as accuracy. These varied and complex processes cause the research of 'performance' to be viewed differently by clinicians, psychologists, sports scientists and others. Therefore, instead of tackling the hugely complex task of trying to measure everything, studies have tended to focus on one or two aspects and summarise how those measures of physical performance are affected by time of day in different groups of athletes (Chtourou et al., 2014). This thesis used MVC of isometric grip strength as an index of physical performance for reasons given in previous Chapters. However, it must be acknowledged that this is just one simple index of physical performance and hence cannot be directly linked to other measures, although muscle strength has been shown to correlate with sprint and jump performance (Wisløff et al., 2004). Therefore, the results of Chapters 4 and 5 provide some novel and exciting findings about how MVC is impacted by rs-FC of the MN and how it can be improved with a phase advance in LCPs. Further studies would be needed to explore if these results can be replicated in other elements of performance.

An argument was also made for normalising cognitive and performance measures in Chapters 4 and 5 to reduce the variability between subjects. Converting raw scores to percentages also allowed time of day differences to be quantified in a standardised way. Since scores were relative to the individual, it was evident that there would be one time point that has the lowest average percentage which permitted diurnal variations to be explored. This also allowed the true nature of individual performance changes to be quantified in Chapter 5 as all values were not only relative to each individual but also dependant on each condition. However, using both raw and percentage values in Chapter 4 has emphasised the benefit of using both measures because rs-FC had more predictive power on raw MVC compared to normalised scores. This supports the use of both in future studies.

6.7 Conclusions

The key findings presented in this thesis are:

1. There are differences in rs-FC of the DMN and the MN between ECPs and LCPs.
2. There are differences in rs-FC of the MN depending on time of day.
3. Sleepiness, cognitive and physical performance can be predicted by rs-FC.
4. LCPs can be phase advanced in the real world.
5. A phase advance has a positive impact on aspects of well-being and performance.

These results show that a detailed assessment of Circadian Phenotypes and time of day is critical when investigating rs-FC or performance in research and clinical settings. If there is no knowledge of Circadian Phenotype or the time of day when testing was carried out, the interpretation of fMRI and performance data should be approached with caution. This thesis also shows the presence of diurnal variations in both cognitive and physical performance which can be shifted by a phase advance in a real world setting. This could offer a novel and simple treatment to improve mental well-being and performance in multiple settings. Firstly, it could be of interest to the sports world that is looking to optimise performance. Secondly, it could benefit LCPs in the general population who are suffering from sleep disturbances and have decreased mental well-being. Thirdly, these findings could benefit the business sector as there is a motivation to better understand employees in order to reduce risk, maximise productivity and enhance performance.

The thesis presents the first study of its kind that has combined multiple techniques to examine how Circadian Phenotype and time of day impact on brain function, behaviour and performance in the real world. The results suggest that there may be more endogenous central mechanisms involved in cognitive and physical performance measures, which holds significant implications for both research and clinical work.

Appendices

Appendix A

Example Sleep Diary

Sleep Diary Sheet

Name/ID code **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?							
How quickly do you estimate you fell asleep (in minutes)?							
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 sleep? *(1) Poor; (2) Satisfactory; (3) Average (4) Good; (5) Excellent*							
Did you have any naps during the day? If so, for how long?							
Did you take your actiwatch off? If so what time from and to? *i.e. 10am-10.15am*							

Experiment_____

Appendix B

MORNING SAMPLES - Cortisol Sampling Protocol (yellow labels)

ID Number		Date	……/……/2016
Start time	*(as soon as you wake up)*	Wake up time	……

NB. Please do not eat during the first hour (the first 4 collections). After this hour you may go about your day as usual but please take the sampling kit with you and make sure you store the samples in the freezer as soon as collected.

Instructions for Collection of Saliva Samples

Please find enclosed:

9 YELLOW labelled collection vials in a labelled grip seal bag
1 labelled grip seal bag to put collected samples in
1 saliva sample collection record sheet

Instructions:

*Saliva samples should be collected **at point of first wake up (whilst still in bed)**, every 15 minutes for the first hour and then every 30 minutes from wake up for the next 2 hours. During this period we would ask you to:*

1. Abstain from caffeinated drinks (e.g. tea, coffee, coca cola), alcoholic drinks, drinks containing artificial colouring and food for the period of testing.
2. Refrain from cleaning your teeth, with or without toothpaste, before or during the sampling period.
3. Do not stimulate saliva production by chewing gum or lemons.
4. Upon waking and every 15 minutes (first hour) and then 30 minutes (next 2 hours) collect a saliva sample in the appropriately labelled vial. To do this remove the cap, collect saliva directly into the vial (by spitting into it). **Please try to collect at least 0.5ml volume as marked on example tube** (see ------- fill line)**.** Replace cap and make sure it is secure.
5. Record the time of the saliva collection on the form provided.
6. **It is extremely important that you start sampling AS SOON AS YOU WAKE UP.** After the first 2 samples are taken upon wake up (whilst still in bed) you may go about your daily routine as you wish whilst collecting the samples at specific the time points.
7. **After every 4 samples please place the collection vials in the extra grip seal bags and put in YOUR freezer (-20 °C) until collection by the lead researcher.**

Sample Collection Record Sheet

ID Number

Each of the YELLOW labelled collection tubes you have been given are labelled with the sample number (starting at 1). Please make sure you use each one at the correct time.

Sample Number	Time	Actual Time
1 *(tube labelled 1)*	**Wake up e.g. 0600**	
2 *(tube labelled 2)*	**+ 15mins**	
3 *(tube labelled 3 etc.)*	**+ 30mins**	
4	**+ 45mins**	
5	**+ 1hr**	
6	**+ 1hr 30mins**	
7	**+ 2hrs**	
8	**+ 2hr 30mins**	
9	**+ 3hrs**	

Is there anything you think that could have affected the protocol or your measurements during the sampling period? E.g. late sampling, fell asleep, drank caffeine/smoked, exposed to lots of natural light, ill, cuts in mouth?

Modified protocol curtosy of researchers at the University of Surrey

Appendix C

EVENING SAMPLES - Melatonin Sampling Protocol (white labels)

ID Number | Date ……/……/2016

Start time *(3/4 hours before normal bed time)* | Normal bed time ……

NB. Please try and pick an evening where you can remain indoors seated in a room with dim light (wear sunglasses if you want). You may read/work but do not look at any electronic screens as this can affect your measures (TV, laptop, phone). Make sure you store your samples in the freezer once collected.

Instructions for Collection of Saliva Samples

Please find enclosed:

11 WHITE labelled collection vials in a labelled grip seal bag
1 labelled grip seal bag to put collected samples in
1 saliva sample collection record sheet

Instructions:

Saliva samples should be collected ***every 30 minutes from 4 hours (or at least 3) BEFORE your normal bedtime until one hour AFTER your normal bedtime e.g. If your normal bedtime is 11pm you would start at 7/8pm until 12am.***

During this period we would ask you to:
1. Abstain from caffeinated drinks (e.g. tea, coffee, coca cola) from 17:00 h on the day of collection.

2. Remain **seated indoors** in **dim light** (e.g. a single table lamp on the other side of the room, NO overhead lights, NO electronic screens (computer, laptop, TV), curtains closed).

3. During the sampling period please avoid drinking beverages containing artificial colouring.

4. Refrain from cleaning your teeth, with or without toothpaste, during the sampling period.

5. Do not stimulate saliva production by chewing gum or lemons.

6. **Every 30 minutes** collect a saliva sample in the appropriately labelled vial (starting with sample no. 1 then 2, 3 etc). To do this remove the cap, collect saliva directly into the vial (by spitting into it). Please **try to collect at least 1.5ml volume** as marked on example tube (see -------- fill line). Replace cap and make sure it is secure.

7. Record the time of the saliva collection on the form provided.

8. Should you wish to go to the toilet or make a non-caffeinated drink please do so immediately after collection of a sample and try to be seated again 15 mins before the next sample is due to be collected. Please **wear sunglasses if you leave** the dim lit room.

9. After every 4 samples please place the collection vials in the extra grip seal bags and put in YOUR freezer (-20 °C) until collection by the lead researcher.

Sample Collection Record Sheet

ID Number

Each of the WHITE labelled collection tubes you have been given are labelled with the sample number (starting at 1). Please make sure you use each one at the correct time.

There is a SPARE tube which has an example fill line on (---------), please fill up to this line.

Sample Number	Time	Actual Time
1 *(tube labelled 1)*	**e.g. 2000**	
2 *(tube labelled 2)*	**2030**	
3 *(tube labelled 3 etc.)*	**2100**	
4	**2130**	
5	**2200**	
6	**2230**	
7	**2300**	
8	**2330**	
9	**0000**	
10	**0030**	
11	**0100**	

Is there anything you think that could have affected the protocol or your measurements during the sampling period? E.g. fell asleep, drank caffeine/smoked, exposed to lots of natural light, ill, cuts in mouth?

Modified protocol curtosy of researchers at the University of Surrey

Appendix D

Genotyping Swab Sampling Protocol

By completing this sheet you agree to your data being used for research purposes and consent to the use of human tissue collected by non-invasive buccal mucosa swabs for scientific experiment. They may be sent to the University of Surrey for analysis. All samples will be processed and destroyed within 7 days. All samples will be anonymised and no participant details will be shared.

ID number ……. Date ……/……/……

Signed …….

Instructions for Collection of Buccal Mucosa Swab

Please find enclosed:

1 SARSTEDT sterile swab
1 Individual protocol sheet

Instructions:

1. Label your sterile swab tube with your participant ID number in 'Name' box, date of birth, date of collection and tick M or F
2. Abstain from caffeinated drinks (e.g. tea, coffee, coca cola) and alcoholic drinks and refrain from cleaning your teeth, with or without toothpaste least at least one hour before sampling
3. Thoroughly rinse out your mouth with water twice
4. Remove the swab from the sterile tube
5. Collect tissue by rolling the SARSTEDT sample collection swab firmly on the inside of the cheek, approximately 20 times on each side, making certain to move the brush over the entire cheek.
6. Air dry the swab for 10-15 minutes at room temperature **(please make sure it does NOT touch anything).**
7. Record the date of the swab collection on the form provided.
8. Store buccal mucosa swab in original packaging in the freezer at -20 for collection.

Is there anything you think that could have affected the protocol or your measurements during the sampling period? E.g. cut in mouth, drank caffeine, swab touched something else before storing

Appendix E

Maximum Voluntary Contraction (MVC) Grip Strength Protocol

By completing this sheet you agree to your data being used for research purposes and consent to the use of isometric grip strength using a dynamometer for a scientific study. Data will be held in accordance to the Data Protection Act 1998.

ID Number …… Date ……/……/2016

Instructions for Isometric Grip Strength Test

1. Fill in the front of this form
2. Make sure you are standing with your arms by your side
3. You will be given the device by the researcher
4. Keeping a straight arm down by your side use your **DOMINANT** hand to grip the device as hard as possible
5. Listen carefully to what the research tells you:

"I will count up to three. On three I want you to grip the device as hard as you possibly can for 5 seconds. Ready…..one, two, three……go go go, push hard hard hard, as hard as you can…..and stop there"

6. Repeat three times with at least 2 minutes rest in between

2:30pm

MVC Repeats	Completed?	Score (in KG)
Trial 1		
Trial 2		
Trial 3		

8:30pm

MVC Repeats	Completed?	Score (in KG)
Trial 1		
Trial 2		
Trial 3		

8:30am

MVC Repeats	Completed?	Score (in KG)
Trial 1		
Trial 2		
Trial 3		

Appendix F

Participant Information Pack

Investigating the link between brain structure and function, sleep patterns, genetics, physiology and performance

Principle investigators:

Dr Andrew Bagshaw
University of Birmingham, School of Psychology, Edgbaston B15 2TT
Email:
Telephone:

Elise Facer-Childs
, University of Birmingham, School of Biosciences, Edgbaston B15 2TT
Email:
Telephone:

In this pack you will find:

- Participant information sheets and timelines
- Consent forms
- MRI screening questionnaires + medical history form

- Weekly tick lists and actiwatch logs
- Protocols for saliva sampling
- Sleep related questionnaires for meetings

Participant ID Number:
Group Number:

Study start date:
Study end Date:

Scans Booked for:

If you have any questions before, during or after the study ***please do not hesitate*** *to contact the researchers named above.*

We look forward to your participation.

Appendix G

Summary table of Participant Variables

Summary of demographic, questionnaire, actigraphy and physiological data between ECPs and LCPs. Values are shown as mean ± standard error of the mean (SEM) unless specified. Significance is shown with [a]unpaired two sample t-tests, [b]non-parametric Mann-Whitney or [c]Fisher's exact test. All p values are FDR corrected.

Variable Measured (mean ± SEM)	**ECPs**	**LCPs**	**Significance**
Sample Size	N=16	N=22	n/a
Demographic Variables			
MCTQ Score (hh:mm)	02:24 ± 00:10	06:52 ± 00:17	p<0.0001[a]
Percentage of Males/Females (%)	M: 43.75	M: 31.82	ns[c]
	F: 56.25	F: 68.18	ns[c]
Age (mean ± SD)	24.7 ± 4.6 years	21.3 ± 3.3 years	p=0.028[a]
Height (cm)	171.30 ± 1.97	171.10 ± 2.38	ns [a]
Weight (kg)	66.44 ± 2.78	67.05 ± 2.10	ns [a]
Exercise Regularly (% - yes)	75 %	73 %	n/a
Mental Well-Being Variables			
Pittsburgh Sleep Quality Index (PSQI)	3.50 ± 0.43	5.05 ± 0.52	ns[a]
Epworth Sleepiness Scale (ESS)	4.81 ± 0.77	7.96 ± 0.79	p=0.024 [a]
Depression Anxiety and Stress Scale (DASS)	9.07 ± 1.82	17.24 ± 2.51	p=0.028[a]
Profile of Mood States (POMS)	-6.40 ± 4.02	11.25 ± 4.31	p=0.017[a]
Average days per week eating breakfast	6.06 ± 0.50	4.18 ± 0.52	p=0.035 [a]
Average breakfast time (hh:mm)	07:19 ± 00:13	10:18 ± 00:21	p<0.0001 [a]
Actigraphy Variables and Non Parametric Circadian Rhythm Analysis (NPCRA)			
Bed Time (hh:mm)	22:31 ± 00:09	02:03 ± 00:18	p<0.0001[a]

Appendix H

Get Up Time (hh:mm)	06:32 ± 00:10			10:30 ± 00:18			p<0.0001[b]
Sleep Onset (hh:mm)	22:57 ± 00:10			02:27 ± 00:19			p<0.0001[a]
Wake Up Time (hh:mm)	06:33 ± 0.10			10:13 ± 00:18			p<0.0001[a]
Sleep Duration (hrs)	7.59 ± 0.18			7.70 ± 0.14			ns[a]
Sleep Efficiency (%)	79.29 ± 1.96			77.23 ± 1.14			ns[a]
Sleep Onset Latency (hh:mm)	00:25 ± 00:06			00:25 ± 00:03			ns[b]
Fragmentation Index (FI)	26.97 ± 1.79			33.14 ± 2.36			ns[b]
Inter-daily Stability (IS)	0.42 ± 0.02			0.38 ± 0.02			ns[a]
Intra-daily Variability (IV)	0.92 ± 0.04			0.82 ± 0.04			ns[a]
L5 Onset (hh:mm)	00:18 ± 00:15			03:34 ± 00:19			p<0.0001[a]
M10 Onset (hh:mm)	08:43 ± 00:29			12:39 ± 00:25			p<0.0001[a]
Relative Amplitude (RA)	0.90 ± 0.02			0.82 ± 0.02			p=0.028[b]
Physiological Variables							
Dim Light Melatonin Onset (hh:mm)	20:27 ± 00:16			23:55 ± 00:26			p<0.0001[a]
Phase Angle (hh:mm)	02:28 ± 00:16			02:34 ± 00:18			ns[a]
Peak Melatonin Concentration (pg/nl)	16.54 ± 2.71			23.82 ± 3.10			ns[a]
Peak Time of Melatonin (hh:mm)	22:36 ± 00:20			02:16 ± 00:19			p<0.0001[b]
Cortisol Peak Time (hh:mm)	07:04 ± 00:16			11:13 ± 00:23			p<0.0001[a]
Peak Cortisol Concentration (nmol/l)	20.11 ± 0.92			23.00 ± 2.03			ns[b]
Cortisol Awakening Response (%)	72.04 ± 21.54			112.8 ± 26.91			ns[b]
Area Under the Curve (total time)	97.24 ± 5.55			101.4 ± 8.36			ns[a]
Area Under the Curve (1st hour)	54.74 ± 3.79			63.64 ± 6.10			ns[a]
Genetic Variables							
Period 3 Polymorphism (% of 4/4, 4/5, 5/5)	66	27	7	64	36	0	n/a

Summary tables of Clinical Cut Offs for Questionnaires

Pittsburgh Sleep Quality Index (PSQI) degrees of severity

Category of Sleepiness	PSQI Score
Normal Sleep Quality	0-5
Poor Sleep Quality	5+

Depression, Anxiety and Stress Scale (DASS) degrees of severity

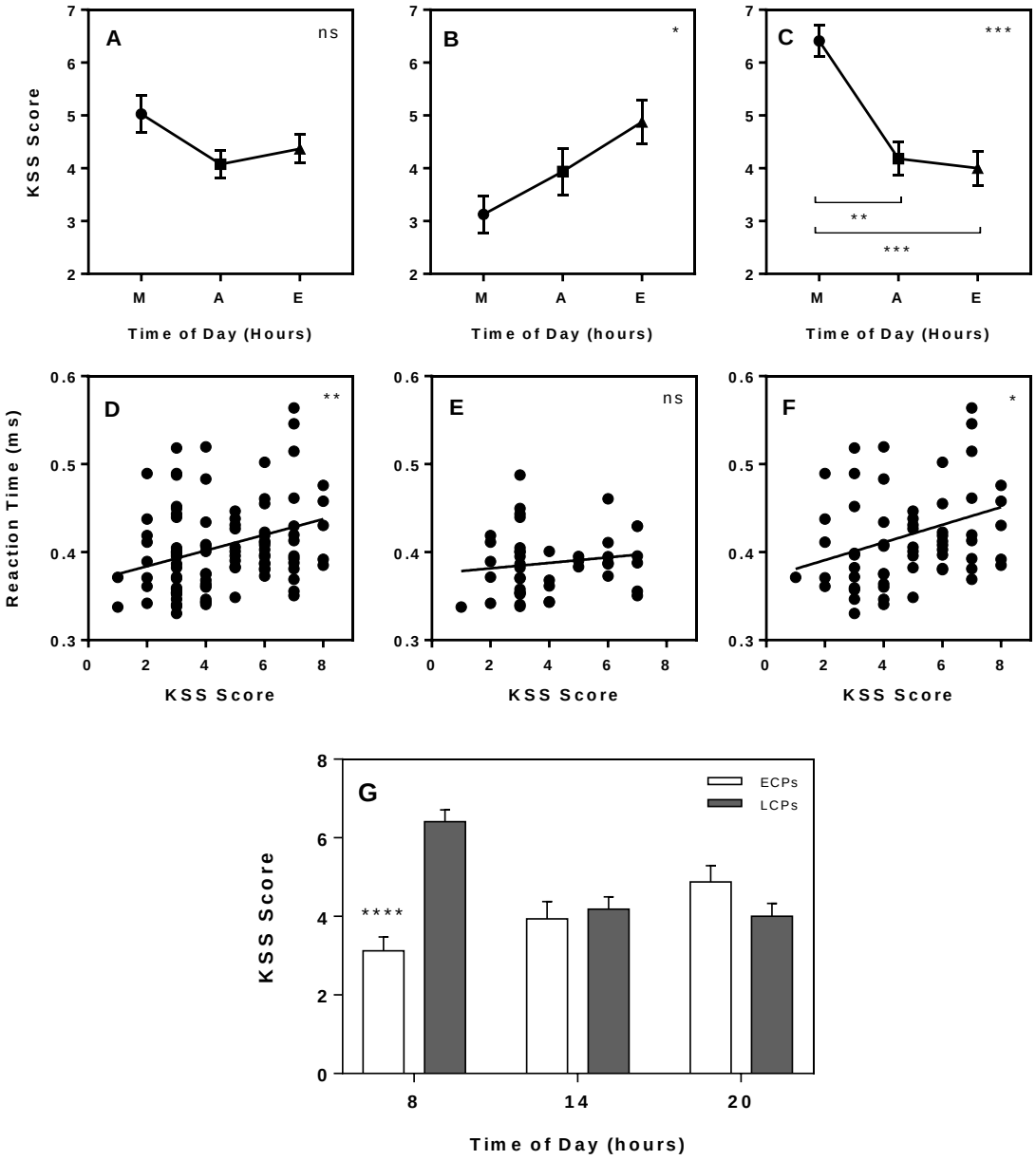
	Depression	Anxiety	Stress
Normal	0-9	0-7	0-14
Mild	10-13	8-9	15-18
Moderate	14-20	10-14	19-25
Severe	21-27	15-19	26-33
Extremely Severe	28+	20+	34+

Epworth Sleepiness Scale (ESS) degrees of severity

Category of Sleepiness	ESS Score
Lower Normal Daytime Sleepiness	0-5
Higher Normal Daytime Sleepiness	6-10
Mild Excessive Daytime Sleepiness	11-12
Moderate Excessive Daytime Sleepiness	13-15
Severe Excessive Daytime Sleepiness	16-24

Appendix I

Relationship Between KSS and PVT in Circadian Phenotypes



Appendix I. KSS score for the whole population (A), ECPs (B) and LCPs (C) at 08:00 h (M - morning), 14:00 h (A - afternoon) and 20:00 h (E - evening). Values show mean ± SEM. D-F show linear regression of KSS against PVT (reaction time, ms) for the whole population (D), ECPs (E), and LCPs (F). (G) Difference in KSS between ECPs and LCPs at 08:00 h, 14:00 h and 20:00 h.

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