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MEASURING A SLEEP/STRESS SWITCH POINT

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PhD

2014

MEASURING A SLEEP/STRESS SWITCH POINT

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A thesis submitted in partial fulfilment of
the requirements of the University of
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Abstract

The Spielman 3P and Cano-Saper models of insomnia focus on the role of stress in the development of insomnia. To date, the impact of both naturalistic stressors and experimental stressors upon sleep have been inconsistent, due to limitations including the varied nature of the stressor, the diverse nature of participants and the lack of standardised experimental protocols for measuring stress and sleep. In addition, previous research has tended not to include an objective marker of stress, and thus cannot confirm that the stressor employed reliably elicits physiological stress. This thesis aimed to examine the effects of stress upon sleep, firstly developing and testing a standard protocol to measure both subjective and objective stress and sleep in the same context. Cortisol, specifically the cortisol awakening response (CAR), was measured as a physiological marker of hypothalamic-pituitary-adrenal (HPA) axis activity and objective sleep was measured using polysomnography. The protocol was used in an experiment to examine the impact of anticipation on sleep and stress, and then tease apart the impact of anticipation and anticipation coupled with demand, on sleep and the CAR. The results of the thesis indicate that anticipation of stress alone is sufficient to disrupt subjective sleep, when teased apart from demand. The results of the thesis also indicate that the CAR is a marker of anticipation and with a potential secondary role as a marker of recovery. Theoretically, the thesis indicates that the precipitating dimension within the Spielman 3P model occurs irrespective of whether the stressor is anticipated or actual.

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Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others. The home sleep and cortisol data within the feasibility study was supplied by Zoe Gotts, however, I analysed the data and interpreted the results. Any ethical clearance for the research has been approved. Approval has been sought and granted by the Faculty of Health and Life Sciences Ethics Committee.

I declare that the word count of this thesis is 57,330 words

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List of Abbreviations

5-HTTLPR:	Serotonin transporter linked promoter region
AASM	American Academy of Sleep Medicine
ACTH	Adrenocorticotropin hormone
A-I-E	Attention-intention-effort
ANOVA	Analysis of variance
AUC	Area under the curve
AUC _G	Area under the curve with respect to ground
AUC _I	Area under the curve with respect to increase
CAR	Cortisol awakening response
CI	Confidence interval
CRH	Corticotropin-releasing hormone
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (Text Revision)
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG	Electromyogram
FIRST	Ford Insomnia Response to Stress Test
HADS	Hospital Anxiety and Depression Scale
HÖ-MEQ	Horne-Östberg Morningness-Eveningness Questionnaire
HPA	Hypothalamic-pituitary-adrenal
HRMTP	High risk model of threat perception
Hz	Hertz

ICSD-2	International Classification of Sleep Disorders, Revised Edition
IGT	Iowa Gambling Task
MANOVA	Multivariate analysis of variance
MEMS	Medication Events Monitoring System
MnInc	Mean increase
MnPO	Median preoptic nucleus
mp-PVH	Medial paraventricular hypothalamic nucleus
MTF	Multi-Tasking Framework
NASA-TLX	National Aeronautics and Space Administration Task Load Index
NICE	National Institute for Health and Care Excellence
nmol/l	Nanomoles per litre
NREM	Non-rapid eye movement
NWAK	Number of awakenings
OR	Odds ratio
PET	Positron emission tomography
PSG	Polysomnography
PSQI	Pittsburgh Sleep Quality Index
PSRS	Perceived Stress Reactivity Scale
PSS	Perceived Stress Scale
PTSD	Post-traumatic stress disorder
PVN	Paraventricular nucleus of the hypothalamus
SAM	Sympathetic-adrenal-medullary
SCN	Suprachiasmatic nucleus

SE	Sleep efficiency
SEM	Standard error of the mean
SOL	Sleep onset latency
STAI	Spielberger State-Trait Anxiety Inventory
SWS	Slow-wave sleep
TIB	Time in bed
TMI	Three Mile Island
TRT	Total recording time
TSST	Trier Social Stress Test
TST	Total sleep time
VAS	Visual analogue scale
VLPOc	Ventrolateral preoptic nucleus core
VLPOex	Extended ventrolateral preoptic nucleus
WASO	Wake after sleep onset

List of publications and abstracts relevant to this thesis

Elder, G. J., Wetherell, M. A., Barclay, N. L., & Ellis, J. G. (2014). The cortisol awakening response – applications and implications for sleep medicine. *Sleep Medicine Reviews, 18* (3), 195 – 204.

Elder, G. J., Ellis, J. G., & Wetherell, M. A. (2012). Associations between stages of sleep and the cortisol awakening response. *SLEEP, 35* (Supplement), A56.

CHAPTER 1.

Sleep, insomnia and stress

1.1. Introduction

The aim of this chapter is to provide an overview of normal sleep, before outlining the relationship between stress and the development of insomnia. This chapter will also provide an overview of the literature regarding the effects of stress upon sleep, including the effects upon both subjective and objective sleep. This chapter will also explore whether there are differences in the relationship between stress responsivity and sleep based upon the nature of the stressor, specifically real-world naturalistic stressors and experimentally-induced stressors.

1.2. Overview of normal sleep and methods of measurement

1.2.1. Normal sleep and polysomnography

‘Normal’ human adult sleep can be divided into two states, comprising non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. These alternate across the night and are defined by different characteristics derived from the electroencephalogram (EEG), electromyogram (EMG) and electrooculogram (EOG: eye movements). When sleep is examined objectively using these simultaneous measures this is known as polysomnography (PSG), which also includes the measurement of heart rate using electrocardiogram (ECG). Objective sleep can be examined in terms of sleep continuity and sleep architecture. Sleep continuity refers to variables including total sleep time (TST), which refers to the number of minutes scored as Stage 1, Stage 2, Stage 3 or rapid eye movement

(REM) sleep, sleep efficiency (SE%), which refers to the total sleep time as a percentage of the total recording time (TRT), ($TST / TRT \times 100 = SE\%$). The TRT refers to the time taken from lights off to lights on. Lights off refers to the time at which the participant is allowed to fall asleep and lights on refers to the end of the recording period (Berry, 2012). A sleep efficiency of 85% or above is considered good (Berry, 2012). Other sleep continuity variables include sleep onset latency (SOL), which refers to the time taken to get to sleep from lights out, the number of awakenings (NWAK) and the duration of wake after sleep onset (WASO) (Keenan & Hirshkowitz, 2011). Sleep architecture refers to the structure of sleep (Berry, 2012) and includes measures such as the percentage of time spent in each sleep stage and the time taken to reach the first expression of each specific stage of sleep (Lauer, Riemann, Wiegand, & Berger, 1991; Spiegelhalder *et al.*, 2012).

1.2.2. *Functions of sleep*

The precise function of sleep is not currently known (Allada & Siegel, 2008) although the importance of sleep is highlighted as sleep, or sleep-like states, are present in all animals (Campbell & Tobler, 1984). The importance of sleep is also highlighted by the fact that studies in rats have indicated that the consequences of total sleep deprivation is death (Rechtschaffen, Bergmann, Everson, Kushida, & Gilliland, 1989). Death occurred after a period of approximately two to three weeks and was accompanied by physical signs including weight loss, a drop in body temperature and the development of ulcers on the tail and paw (Rechtschaffen *et al.*, 1989). Given the importance of sleep, one theory is that sleep has a restorative function, specifically where NREM sleep restores glycogen stores in the brain, which are depleted during wake (Benington & Heller, 1995). A further suggestion is

that sleep has a necessary function related to neural plasticity (Tononi & Cirelli, 2006) or that sleep has a function related to memory, in particular, where slow-wave sleep and REM sleep both optimise memory consolidation (Diekelmann & Born, 2010). Whilst not fully understood, a further possibility is that sleep may have multiple roles, including within energy conservation, learning and memory consolidation, or in the recovery and restoration of cellular processes, which occur during wakefulness (Mignot, 2008).

Sleep is regulated by three processes, including a homeostatic process, a circadian process which is independent of sleep and waking, and an ultradian process occurring within sleep, where sleep alternates between REM and NREM (Borbely, 1982; Borbely & Achermann, 2000; Luyster *et al.*, 2012). Homeostasis refers to the co-ordinated physiological processes which, within an organism, maintain steady states, and sleep homeostasis refers to the aspect of sleep regulation which is dependent upon sleep and wake (Borbely & Achermann, 2000). This is where homeostatic mechanisms act upon any changes from a sleep 'reference level', where sleep deprivation adds to the propensity to sleep and where excess sleep reduces the propensity to sleep (Borbely & Achermann, 2000). The two-process model of sleep regulation is based on the interaction between the homeostatic process (process S) and the circadian process (process C) (Borbely, 1982). Within the model, process S increases during sleep and reduces during wake. The homeostatic sleep pressure increases depending on the amount of time since the last sleep period, where the longer an individual stays awake, the greater the homeostatic pressure to sleep. Short sleep durations also lead to an increase in pressure in order to recover the lost sleep (Borbely & Achermann, 2000; Luyster *et al.*, 2012). It is believed that process C is under the control of a circadian oscillator which is unaffected by the occurrence of

sleep and wake (Borbely, 1982). The process of switching between sleep and wake is under neural control. Saper and colleagues outlined the ‘flip-flop’ model of sleep, describing the sleep/wake switch, where stable wakefulness and sleep results from the inhibition of both wake-promoting and sleep-promoting neurons (Saper, Chou, & Scammell, 2001). Within the flip-flop model, the ventrolateral preoptic nucleus (VLPO) and major arousal systems, and in particular the monoamine systems, inhibit each other and generally avoid an intermediate state between sleep and wake. The antagonism between the VLPO neurons and the monoaminergic cells occurs between large numbers of neurons, meaning that the subsequent sleep/wake transition occurs over a period of minutes in humans, resulting in distinct behavioural and EEG changes (Saper, Fuller, Pedersen, Lu, & Scammell, 2010).

1.2.2.1. Consequences of sleep deprivation and sleep disruption

Sleep disruption and sleep deprivation has a variety of consequences. Ultimately, the main consequence of prolonged sleep deprivation, as shown from animal studies involving rats and dogs, are death (Rechtschaffen *et al.*, 1989). Insufficient sleep has a range of physiological effects which might contribute to health problems including cardiovascular disease and diabetes, which show an association with increased mortality rates (Luyster *et al.*, 2012).

Most knowledge of the effects of sleep deprivation come from studies examining total sleep deprivation, acute sleep deprivation (25-50% of a normal eight hour sleep over a single night) or chronic sleep restriction (50%-75% of a normal eight hour sleep) over several consecutive nights (Faraut, Boudjeltia, Vanhamme, & Kerkhofs, 2012). The results of well-controlled sleep deprivation studies indicate that sleep loss results in a non-specific activation of blood immune

markers and also appears to cause low-level systematic inflammation, as indicated by cytokine and C-reactive protein levels after sleep loss. Importantly, it appears that one night of recovery sleep may not allow the recovery of these markers and that long-term exposure to sleep restriction may result in cumulative negative health results, particularly within night and shift workers (Faraut *et al.*, 2012).

In addition to the physiological effects, sleep deprivation can result in negative effects upon a range of cognitive function, including psychomotor function, executive attention and working memory, although these effects may be moderated by intra-individual differences (Goel, Rao, Durmer, & Dinges, 2009; Van Dongen, Baynard, Maislin, & Dinges, 2004). Sleep deprivation has been shown to result in cognitive impairments comparable to those observed in individuals who had consumed alcohol and were above the legal drink-driving limit (Williamson & Feyer, 2000). Shift work can also result in disruptions to sleep, where shift workers obtain less sleep and also have higher levels of sleepiness than daytime workers, where the two most dominant problems are disturbed sleep and increased levels of fatigue (Åkerstedt, 2003; Wright Jr, Bogan, & Wyatt, 2013). Shift work has been shown to be associated with a range of negative health outcomes, including heart disease, stroke, depression, cancer, obesity, gastrointestinal problems (Wright Jr *et al.*, 2013).

1.2.3. NREM sleep

Historically, NREM sleep was divided into four separate stages, ranging from Stage 1 through to Stage 4 (Rechtschaffen & Kales, 1968) with each stage reflecting distinct properties of the polysomnogram. More recently, updated

American Academy of Sleep Medicine (AASM) guidelines now refer to NREM sleep as N1 (NREM Stage 1) through to N3 (NREM Stage 3) where N3 represents a combination of the Rechtschaffen & Kales (1968) Stages 3 and 4 (Iber, Ancoli-Israel, & Quan, 2007). These stages roughly reflect the depth of sleep. Arousal thresholds, the point at which sleep can be discontinued, are usually at their lowest during Stage 1 sleep and are at their highest during Stage 4 sleep (Carskadon & Dement, 2011).

1.2.4. REM sleep

REM sleep is characterised by a low-voltage, mixed-frequency EEG, muscle atonia and episodic bursts of rapid eye movements. In this stage muscle tone is not visible in the EMG channel and saccadic eye movements occur. Participants awakened from REM sleep typically report detailed, visual dreams and the EEG activity observed in REM sleep is similar to that of wake (Aserinsky & Kleitman, 1953; Carskadon & Dement, 2011; Siegel, 2011).

1.2.5. The structure of sleep during a typical night

A typical sleep episode in the human adult comprises 75% to 80% NREM sleep and 20% to 25% REM sleep, over six to seven cycles. Typical sleep is made up of approximately 2% to 5% of Stage 1 sleep, approximately 45% to 55% of Stage 2 sleep, approximately 3% to 8% of Stage 3 sleep and approximately 10% to 15% of Stage 4 sleep. Less than 5% of the night is comprised of wakefulness (Carskadon & Dement, 2011). Sleep is typically examined in separate sections known as ‘epochs’, which are 30-seconds in duration (Iber *et al.*, 2007; Keenan & Hirshkowitz, 2011).

EEG activity can be separated into frequency bandwidths, where delta activity refers to low frequency waves (< 4 Hertz (Hz)). Theta waves range between 4-7Hz and alpha activity refers to waves in the range of 8Hz to 13Hz and beta waves are low amplitude waves at higher frequencies (Keenan & Hirshkowitz, 2011). A normally-sleeping adult enters sleep through NREM sleep then into REM sleep and then alternates between them, throughout the night, in 90-minute cycles. As such, the first cycle of REM sleep generally occurs 80-90 minutes following the onset of sleep (Carskadon & Dement, 2011).

During the first cycle of sleep in a healthy normal adult, Stage 1 sleep is predominant. This episode lasts between one and seven minutes at the onset of sleep and occurs periodically throughout the night, usually signalling transitions between sleep stages. Stage 1 sleep is characterised by low-voltage, mixed frequency EEG activity, predominantly theta activity in the range of 4 to 7 Hz (Keenan & Hirshkowitz, 2011). Stage 1 sleep contains minimal amounts of slow-wave activity. Slow-wave activity refers to sleep-related waves of delta activity which occur at low frequencies (≤ 4 Hz) and have an amplitude of ≥ 75 millivolts (mV) (Keenan & Hirshkowitz, 2011).

The first episode of Stage 2 sleep, signalling the transition from wake to sleep, generally follows from Stage 1 and lasts for approximately 10 to 25 minutes. This stage is characterised by distinctive EEG patterns including sleep spindles and K-complexes, with low-voltage mixed-frequency background EEG and low amounts of slow-wave activity. Sleep spindles and K-complexes are transient waveform events. A sleep spindle is a short (minimum of .5 seconds) burst of activity, shaped like a spindle, which occur in the 12-14Hz range. K-complexes are sharply contoured, negative waveforms, lasting a minimum of .5 seconds, immediately

followed by a large and typically slower positive waveform (Keenan & Hirshkowitz, 2011). An example of a sleep spindle and K-complex are shown in Figure 1.1.

Stimuli which may awaken someone from Stage 2 sleep, such as calling the name of a person, touching a person or closing a door, will result in an evoked K-complex, but will not result in an awakening if the individual is in a deeper sleep stage

(Carskadon & Dement, 2011; Keenan & Hirshkowitz, 2011). It is generally thought that the function of an evoked K-complex is to protect against internal and external sensations creating sleep disturbances (Bastien, Ladouceur, & Campbell, 2000).

Stage 3 sleep occurs when high-voltage slow-wave activity reaches more than 20% but less than 50% of EEG activity per epoch and Stage 4 occurs when this high-voltage activity comprises more than 50%

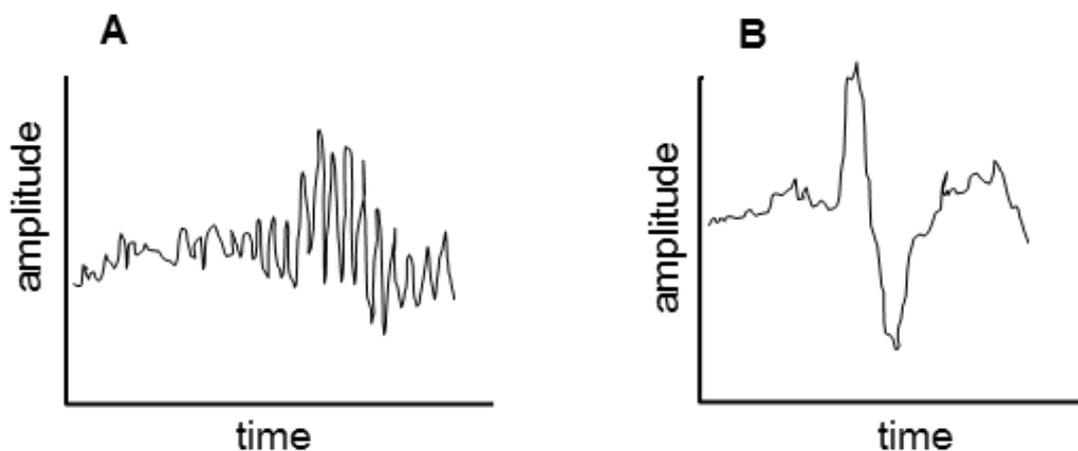


Figure 1.1: Example of an EEG waveform showing (A) a sleep spindle and (B) a K-complex during sleep (Adapted from Berry, 2012, p. 6)

of EEG activity per epoch. This usually lasts 20 to 40 minutes in the first cycle (Carskadon & Dement, 2011; Keenan & Hirshkowitz, 2011). Slow-wave sleep is typically dominant in the first third of the night. After the first third of the night, a

series of body movements usually occur and a brief episode of Stage 3 sleep may occur. This is followed by a brief episode of Stage 2 sleep, lasting five to 10 minutes, which is interrupted by body movements and precedes the first episode of REM sleep (Carskadon & Dement, 2011).

The first cycle of REM sleep typically lasts between one to five minutes. NREM and REM sleep then alternate and periods of REM sleep usually lengthen during the night, dominating in the last third of the night, whilst Stage 3 reduces. The first NREM/REM sleep cycle lasts approximately between 70 to 100 minutes, and the second NREM/REM sleep cycle lasts between approximately 90 to 120 minutes. The total amount of REM sleep obtained over the night is typically between five and 30 minutes (Carskadon & Dement, 2011; Keenan & Hirshkowitz, 2011; Siegel, 2011). Figure 1.2 displays a hypnogram showing the progression of sleep over a typical night in a normally-sleeping, healthy adult.

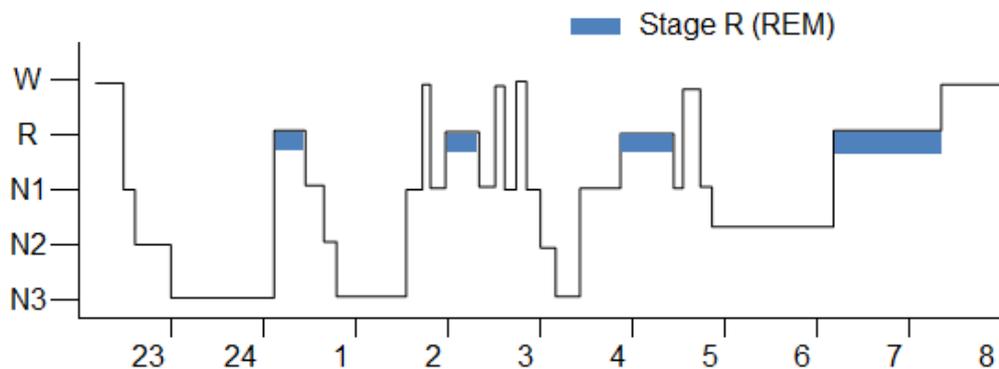


Figure 1.2: A hypnogram displaying normal sleep in a healthy, normally sleeping individual (Adapted from Berry, 2012, p. 80).

1.2.6. Alterations to normal sleep

Stage 1 sleep is associated with a low arousal threshold and an increase in the amount and percentage of Stage 1 sleep is a common indicator of severe sleep disruption (Carskadon & Dement, 2011; Keenan & Hirshkowitz, 2011). A meta-analysis of objective sleep across the lifespan (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004), incorporating a total of 65 studies with normal, healthy participants ($n = 3,577$) ranging in age from 5 to 102 years old, showed that the percentage of REM sleep slightly increased from childhood until the end of adolescence, remaining stable throughout adulthood before declining at the age of 60. Sleep latency increased slightly in accordance with age and the percentage of Stage 1 sleep increased during adulthood. The percentage of Stage 2 sleep increased from childhood onwards until old age. Interestingly, sleep efficiency was the only sleep measure to show a change after 60 years of age, significantly decreasing after this point. However, Vitiello (2006) states that when factors including health burdens, sleep disorders and poor sleep hygiene are accounted for, successfully-aging adults can expect relatively little change in their sleep, with the exception of earlier bed and rise times and a reduced tolerance to circadian phase shifts. Ethnic differences in terms of objective sleep are, however, still poorly understood (Ohayon *et al.*, 2004). Alterations to sleep are observed when sleep is measured in a sleep laboratory for the first time, commonly known as the ‘first-night effect’, where the adaptation to the laboratory results in a reduction in total sleep time, a reduced sleep efficiency, increased REM sleep latency and a reduction in REM sleep alongside increases in both sleep latency and wake after sleep onset (Agnew, Webb, & Williams, 1966; Kim & Dimsdale, 2007; Toussaint *et al.*, 1995).

1.2.7. *Limitations of polysomnography*

Limitations of PSG can include the cost and the invasiveness of the procedure (Blackwell *et al.*, 2008) and despite its accuracy, PSG is unsuitable for the measurement of sleep within insomnia (Littner *et al.*, 2003; Reite, Buysse, Reynolds, & Mendelson, 1995). In addition, PSG is not routinely used in the diagnosis of circadian rhythm sleep disorders resulting from a mismatch between the timing of sleep and the desired timing of sleep (Kushida *et al.*, 2005). Despite these limitations PSG is the current ‘gold standard’ for measuring sleep (Kushida *et al.*, 2001).

1.2.8. *Additional methods of sleep assessment*

In addition to PSG, other methods of sleep assessment are commonly used, including actigraphy and sleep diaries.

1.2.8.1. *Actigraphy*

Actigraphy is a technique where sleep is measured objectively through the use of actiwatches. Actiwatches measure activity using a piezo-electric accelerometer and are typically worn on a non-dominant wrist, although in some occasions leg activity can be monitored (Ancoli-Israel *et al.*, 2003). This records the intensity, amount and duration of movement. The recorded activity is used as a gross indicator of wakefulness or sleep (specifically sleep continuity) and actigraphy is considered to be a reliable measure of sleep and wake activity in healthy adult populations (Ancoli-Israel *et al.*, 2003; Lichstein *et al.*, 2006; Sadeh, 2011). One advantage of actigraphy is that it is a relatively inexpensive method of measuring sleep. An

additional advantage of actigraphy is that it can be used to measure the sleep of individuals who would find it difficult to sleep in a laboratory situation and actigraphy can be used for extended periods of time (Stone & Ancoli-Israel, 2011). However, actigraphy does suffer from some limitations. The accuracy of detecting wakefulness is lower than 60% and can impact upon sleep indices such as total sleep time, sleep efficiency and wake after sleep onset, although extended periods of actigraphy may compensate for this to some extent (Sadeh, 2011).

1.2.8.2. *Sleep diaries*

Sleep diaries are a subjective method of sleep monitoring. These typically require individuals to keep a log of aspects of their sleep that include sleep duration, bed time, wake time, the amount of awakenings and duration of awakenings (Carney *et al.*, 2012). Measures of sleep continuity can be derived from sleep diaries, including TST, SE% (calculated from $TST / \text{time in bed (TIB)} \times 100$), SOL, NWAK and WASO (Berry, 2012). Sleep diaries are a reliable and inexpensive method of obtaining subjective sleep information and are useful in providing general sleep/wake activity information (Berry, 2012; Rogers, Caruso, & Aldrich, 1993). A diary monitoring period of one week is adequate, although up to two weeks may be necessary in the case of treatment studies (Sateia, Doghramji, Hauri, & Morin, 2000).

1.3. Characteristics and diagnosis of insomnia

Individuals with insomnia report impaired daytime functioning and exhibit difficulties in falling asleep, dissatisfaction with the sleep quality or duration and

complain of waking up during the night or too early in the morning (Morin & Benca, 2012; Riemann *et al.*, 2010). The objective assessment of sleep within insomnia, using PSG, can show objective impairments, however these impairments typically display discrepancies with the subjective complaint of poor sleep (Morin & Benca, 2012). The daytime consequences of insomnia also tend to be over-stated, with objective neuropsychological functioning not matching the self-reported daytime deficits (Orff, Drummond, Nowakowski, & Perils, 2007). Two main nosologies are used for diagnosis: the recently-updated Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V) (American Psychiatric Association, 2013) and the American Academy of Sleep Medicine (AASM) International Classification of Sleep Disorders Manual, revised edition (ICSD-2) (American Academy of Sleep Medicine, 2005). The diagnostic criteria for both nosologies (DSM-V and ICSD-2) are listed in Table 1.1 and Table 1.2.

1.3.1. Prevalence of insomnia

Insomnia is a common sleep disorder (Leger & Bayon, 2010) and data assessing prevalence largely comes from point prevalence studies. Morphy and colleagues (Morphy, Dunn, Lewis, Boardman, & Croft, 2007) estimated the prevalence of insomnia in the UK to be 37%, based on the response to four questions assessing trouble falling asleep, waking during the night, trouble staying asleep and waking after the usual amount of sleep feeling unrefreshed. Depending on the criteria applied, the prevalence rate of insomnia can be as high as 50% (Riemann *et al.*, 2010). The use of a more stringent definition of insomnia gives a prevalence rate of approximately 6% (Ohayon, 2002). There are gender differences in the prevalence of insomnia, as the female/male ratio for insomnia symptoms is 1.4 and this ratio

Table 1.1:

DSM-V criteria for Insomnia Disorder (American Psychiatric Association, 2013)

Criterion	Description
A	A predominant complaint of dissatisfaction with sleep quantity or quality, associated with one (or more) of the following symptoms: <ol style="list-style-type: none"> 1. Difficulty initiating sleep (in children, this may manifest as difficulty initiating sleep without caregiver intervention). 2. Difficulty maintaining sleep, characterised by frequent awakenings or problems returning to sleep after awakenings (in children, this may manifest as difficulty returning to sleep without caregiver intervention). 3. Early-morning awakening with inability to return to sleep.
B	The sleep disturbance causes clinically significant distress or impairment in social, occupational, education, academic, behavioural, or other important areas of functioning.
C	The sleep difficulty occurs at least 3 nights per week.
D	The sleep difficulty is present for at least 3 months.
E	The sleep difficulty occurs despite adequate opportunity for sleep.
F	The insomnia is not better explained by and does not occur exclusively during the course of another sleep-wake disorder (e.g. narcolepsy, a breathing-related sleep disorder, a circadian rhythm sleep-wake disorder, a parasomnia).
G	The insomnia is not attributable to the physiological effects of a substance (e.g. a drug of abuse, a medication).
H	Coexisting mental disorders and medical conditions do not adequately explain the pre-dominant complaint of insomnia.
Specify if :	<ul style="list-style-type: none"> - With non-sleep disorder mental comorbidity, including substance use disorders - With other medical comorbidity - With other sleep disorder
Specify if :	<ul style="list-style-type: none"> - Episodic: symptoms last at least 1 month but less than 3 months - Persistent: symptoms last 3 months or longer. - Recurrent: Two (or more) episodes within the space of 1 year
	Note: acute and short-term insomnia (i.e. symptoms lasting less than 3 months but otherwise meeting all criteria with regard to frequency, intensity, distress, and/or impairment) should be coded as another specified insomnia disorder.

Table 1.2:

ICSD-2 criteria for diagnosis of insomnia (American Academy of Sleep Medicine, 2005)

- A. One or more of the following symptoms:
 - Difficulty initiating sleep
 - Difficulty maintaining sleep
 - Waking up too early
 - Non-restorative sleep
 - B. Sleep difficulty occurs despite adequate opportunity for sleep
 - C. At least one of the following daytime symptoms related to the night-time sleep difficulty reported:
 - Fatigue or malaise
 - Attention, concentration or memory impairment
 - Social or vocational dysfunction or poor school performance
 - Mood disturbance or irritability
 - Daytime sleepiness
 - Motivation or energy or initiative reduction
 - Proneness for errors or accidents
 - Tension headaches or gastrointestinal symptoms, or both
 - Concerns or worries about sleep
-

increases to 1.7 when over 45 years of age. Women are also twice more likely than men to be diagnosed with insomnia (Ohayon, 2002). The prevalence of insomnia increases with age, with the prevalence of insomnia reported to be close to 50% in elderly individuals over 65 years of age (Ohayon, 2002).

1.3.2. Comorbidity of insomnia with health problems and disorders

Insomnia is often co-morbid with other illnesses and disorders (Taylor, Lichstein, & Durrence, 2003) including heart disease, high blood pressure, chronic pain (Taylor *et al.*, 2007), cancer (J. R. Davidson, MacLean, Brundage, & Schulze, 2002), anxiety, substance misuse (Roth *et al.*, 2006) and depression (Luc, 2010). Taylor and colleagues (Taylor, Lichstein, Durrence, Reidel, & Bush, 2005) showed that individuals with insomnia were 9.82 times and 17.35 times more likely than

people without insomnia to have clinically significant depression and anxiety, respectively.

Insomnia is in itself a risk factor for depression, as having symptoms of insomnia for more than two weeks resulted in an increased risk of depression during the following one to three years (Riemann & Voderholzer, 2003). Whilst Riemann & Voderholzer (2003) did not specify the extent to which symptoms relate to depression, a meta-analysis by Baglioni and colleagues (Baglioni *et al.*, 2011) showed that insomnia predicted depression with an overall odds ratio (OR) of 2.1 (95% confidence interval (CI): 1.86 – 2.38). Further studies have also observed this comorbidity between insomnia and depression (Buysse *et al.*, 2008) although the relationship can be bi-directional (Jansson-Fröjmark & Lindblom, 2008).

A large-scale Norwegian study ($n = 47,700$) showed a co-morbidity between insomnia symptoms and conditions such as anxiety, depression, conditions with pain such as fibromyalgia, headaches, osteoporosis and musculoskeletal disorders. The strongest associations were shown between insomnia symptoms and mental conditions (OR = 2.66) and pain conditions (OR = 2.75) (Sivertsen, Krokstad, Øverland, & Mykletun, 2009a).

1.3.3. *Costs of insomnia*

Insomnia also has an associated economic and occupational cost. Individuals with insomnia display higher rates of absenteeism from work than good sleepers, being absent from work twice as often as good sleepers (OR = 1.96) (Leger, Massuel, Metlaine, & Group, 2006). Insomnia is also a significant predictor of long-term sick leave from work (Sivertsen, Øverland, Bjorvatn, Mæland, & Mykletun, 2009b). A French study concluded that the annual cost of absenteeism in those with

insomnia is approximately €2,500 per employee, and that this cost is largely burdened by employers (Godet-Cayre *et al.*, 2006).

Whilst the overall cost of insomnia upon society is not known (Leger & Bayon, 2010), studies in various have attempted to estimate the costs of insomnia in relation to the Gross Domestic Profit of various countries. The direct cost of insomnia in France in 1995 was estimated to be 10.2 billion Francs (approximately \$2 billion) (Leger, Levy, & Paillard, 1999) and the total direct costs of insomnia in the United States in 1995 were estimated to be \$13.9 billion (Walsh & Engelhardt, 1999). An additional study estimated the costs of insomnia in the area of Quebec in Canada (Daley, Morin, LeBlanc, Gregoire, & Savard, 2009) at approximately C\$6.6 billion per year. This figure included C\$191 million in medical consultations, C\$16.5 million in prescription medication, C\$1 billion in work absenteeism, and C\$5 billion in the loss of work productivity. This also included alcohol, used as a sleep aid, which cost C\$339.8 million and over-the-counter medication, which cost C\$1.8million. A further study examined the economic burden of insomnia on an individual level and found that in younger adults, the direct and indirect costs were about \$1,250 higher than those without insomnia, and in older adults, direct costs were about \$1,150 higher for those with insomnia (Ozminkowski, Wang, & Walsh, 2007).

Whilst the cost of insomnia to the United Kingdom is unknown, data from the National Institute for Health and Care Excellence (NICE) does highlight the cost of medication prescribed for insomnia in England. A variety of hypnotics referred to as ‘z-hypnotics’, including zaleplon, zolpidem and zopiclone, are often prescribed for insomnia. In the 12-month period from March 2007 to March 2008, a total of 5.2 million prescriptions were dispensed at a cost of £12.3 million. Benzodiazepine

hypnotics are also used to treat insomnia and in the same period 4.7 million of these prescriptions were dispensed, at a cost of £17.9 million (National Institute for Clinical Excellence, 2008). NICE guidelines currently state that these z-hypnotics are appropriate for the management of severe insomnia after considering non-pharmacological measures, but that hypnotics should only be prescribed for a short period of time (National Institute for Clinical Excellence, 2004).

1.4. The role of stress within the development of insomnia

Whilst insomnia is a common sleep disorder, is typically co-morbid with conditions such as depression and has an associated economic cost (Godet-Cayre *et al.*, 2006; Leger & Bayon, 2010; Taylor *et al.*, 2003), the transition from normal sleep to acute insomnia is currently poorly understood (Ellis, Gehrman, Espie, Riemann, & Perlis, 2012). Only one diagnostic system, the ICSD-2 (American Academy of Sleep Medicine, 2005) acknowledges that insomnia may be a direct result of stress. The ICSD-2 states that adjustment insomnia or acute insomnia, which refers to short-term insomnia, occurs in association with an identifiable stressor and is typically short in duration, lasting between a few days and a few weeks. The stressor may include psychological, psychosocial, physical, medical or environmental circumstances and can include events such as interpersonal relationship issues, occupational stress, personal losses, bereavement, a medical diagnosis, visiting or moving to a new location, or changes to the sleeping environment. Events with a positive or a negative valence can act as a stressor in the context of adjustment insomnia (American Academy of Sleep Medicine, 2005). The ICSD-2 states that adjustment insomnia lasts for a maximum of three months and that sleep disturbances are the primary feature. Waking symptoms such as anxiety,

worry, sadness or depression in relation to the stressor may also be apparent, alongside other physical and daytime symptoms.

Previous research has examined the role of stress, in terms of life events, within insomnia. Bastien and colleagues interviewed 345 patients at a sleep disorders clinic and identified precipitating events which were associated with the onset of insomnia. It was shown that 78.3% of participants were able to identify a specific precipitating event. The most common events identified included those related to family, health and work or education events and the majority of these events were negatively valenced (Bastien, Vallieres, & Morin, 2004). Healey and colleagues reported that 23 out of 31 (76.2%) individuals with insomnia reported that their insomnia was subjectively connected to a major event (Healey *et al.*, 1981). Interestingly, each of the remaining eight participants with insomnia (25.8%) reported the occurrence of at least one major life event in their lives at that approximate time point. In addition, those with insomnia experienced a greater amount of stressful life events during the year of insomnia onset when compared to good sleepers (Healey *et al.*, 1981). This finding in terms of the amount of stressful life events was not shown elsewhere, as Morin and colleagues reported that whilst good sleepers and individuals with insomnia reported similar amounts of stressful life events, those with insomnia reported that major negative life events were more intense and that daily stressors had a higher impact (Morin, Rodrigue, & Ivers, 2003). Levels of bedtime arousal and coping style both acted as mediators between daytime stress and night-time sleep. Those with insomnia more frequently relied upon emotion-oriented coping strategies than good sleepers, which focus on reducing the emotional distress resulting from a stressful situation rather than resolving the problem itself. Overall, this suggests that it is the perceived lack of

control over the stressful events, rather than the number of stressful events, which enhance the vulnerability to insomnia (Morin *et al.*, 2003). Taken together, this confirms the role of stressors in insomnia.

Other studies have incorporated objective measures of sleep in order to examine the relationship between sleep and stress within insomnia. Hall and colleagues examined this relationship within healthy adults who met DSM-IV criteria for primary insomnia (Hall *et al.*, 2000). Those who showed an increase in subjective stress burden also showed an associated reduction in delta power and showed a trend towards a higher level of intrusion tendencies. The measure of intrusion tendencies referred to the tendency to experience stress-related intrusive thoughts, measured using a scale in which participants were required to indicate how frequently they experienced symptoms of intrusive thoughts after a stressful event. In a later study Hall and colleagues assessed the relationship between psychological stress and measures of physiological arousal during NREM sleep in those with insomnia. Quantitative EEG showed that an increase in perceived levels of stress was associated with a decrease in delta power and an increase in beta power during NREM sleep (Hall *et al.*, 2007). That said, Riemann (2010) reports that large and well-controlled studies are needed in order to clarify contradictory findings from quantitative EEG studies in insomnia. Overall, stress does show a relationship with insomnia, but it is possible that stress can cause the acute sleep disturbance, presaging the development of insomnia. The following section will briefly outline models of insomnia which specifically describe the circumstances under which acute sleep disturbances occur in response to a stressor (Ellis *et al.*, 2012).

1.5. Models of insomnia with a focus on stress-diathesis

Two theoretical models of insomnia, the Spielman 3P model (Spielman, Caruso, & Glovinsky, 1987; Spielman & Glovinsky, 1991; Spielman, Nunes, & Glovinsky, 1996) and the Cano-Saper rodent model of insomnia (Cano, Mochizuki, & Saper, 2008) have a role of stress as the cause of insomnia. Within both of these models, stress can disrupt sleep by causing an individual to go beyond an insomnia/sleep disturbance threshold or a 'switch point'. This switch point is most clearly identified and incorporated within the Spielman 3P model, although, it is possible that each individual animal within the Cano-Saper model has such an individual switch point. However, this is not identified or described within the Cano-Saper model. This section will provide an overview of each model and will explain the role of the stressor.

1.5.1. *Spielman 3P model of insomnia*

The Spielman 3P model focuses on three factors; *predisposing*, *precipitating* and *perpetuating* factors, describing how normal sleep can lead to acute insomnia and how this can lead to chronic insomnia, by taking an individual over an insomnia threshold or 'switch point' (Spielman *et al.*, 1987; Spielman & Glovinsky, 1991; Spielman *et al.*, 1996). Predisposing factors are biological vulnerabilities which determine the threshold for insomnia. These may factors such as basal metabolic rate, hyper-reactivity to stress, sleep and wakefulness, neurotransmitter alterations, potentially governed by underlying genetic differences between individuals, psychological factors such as vulnerability to depression and anxiety or a tendency to excessively worry or ruminate (Perlis, Shaw, Cano, & Espie, 2011; Spielman & Glovinsky, 1991; Spielman *et al.*, 1996). Social factors may also be an influence, by

having a bed-partner with opposing sleep habits or having undesirable sleeping schedule (Perlis *et al.*, 2011; Spielman & Glovinsky, 1991). Precipitating factors are acute stressors, which can include medical illness and psychiatric illnesses. These factors then interact with the predisposing vulnerability factors resulting in an initial period of sleep disruption (Ellis *et al.*, 2012; Perlis *et al.*, 2011). The final aspect of the model examines perpetuating factors, which are the behaviours which the individual suffering from insomnia adopts in order to either cope with or compensate for the associated sleeplessness/sleepiness. This consists of behavioural and cognitive factors, including excessive time in bed, keeping irregular sleep/wake patterns, the anxiety over the subsequent daytime deficit, the expectation of a bad night and fragmentation of sleep and maladaptive conditioning. In addition, this includes caffeine, hypnotic and alcohol consumption as coping mechanisms. The complaint of insomnia persists even after the stress has ended (Perlis *et al.*, 2011; Spielman & Glovinsky, 1991). Figure 1.3 displays the various stages of the 3P model and the threshold for insomnia.

Spielman & Glovinsky (1991) provide a hypothetical example of the working model in a young man who has a tendency towards eveningness (i.e. exhibiting a preference for evening activities as opposed to morning activities). In the example the stressor is that the young man receives a job promotion that results in a higher level of scrutiny and provokes the development of an anxiety state, which pushes him over the threshold for insomnia. He tries to cope by recouping sleep at the weekend but worries about the impact of his working schedule on sleep loss. By the time the individual seeks treatment for the sleep problems, he has adapted to the increased work demands but still has problems sleeping, reflecting the chronic insomnia stage. The model has been tested to an extent, for example, in the case of

stress-induction studies within good sleepers, however a limitation is that it is difficult to test the model as no specific circumstances or characteristics are provided at each stage of the model (Ellis *et al.*, 2012; Perlis *et al.*, 2011).

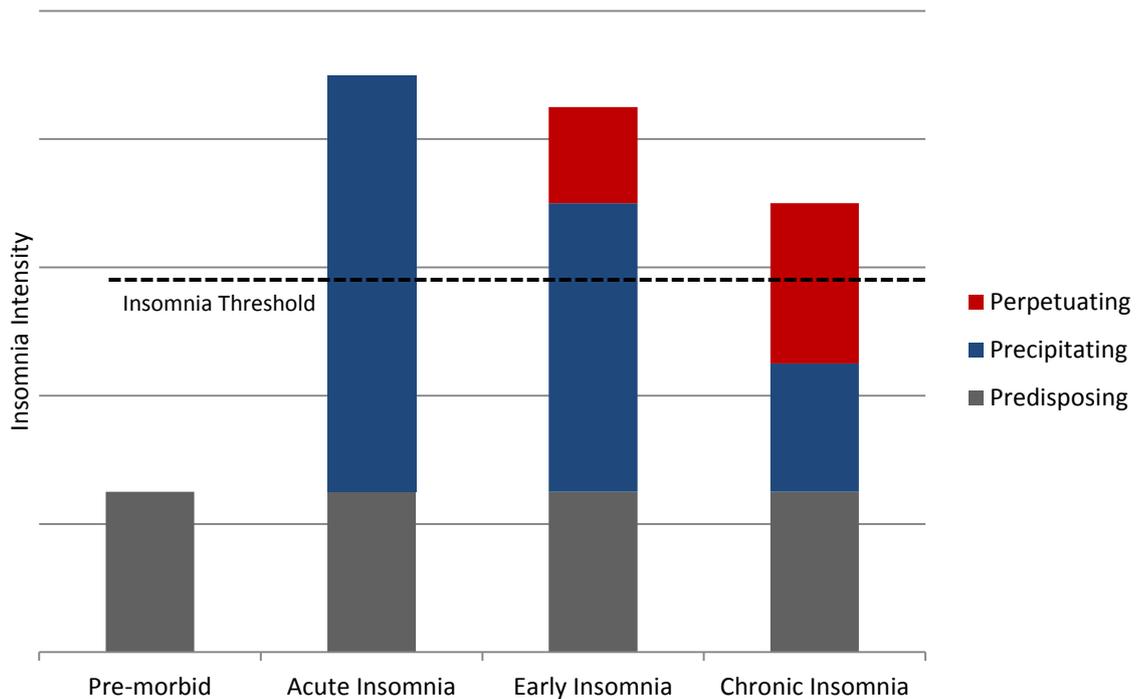


Figure 1.3: Spielman 3P Model (Adapted from Spielman & Glovinsky, 1991, p. 12)

1.5.2. Cano-Saper rodent model of insomnia

The Cano-Saper rodent model of insomnia has offered an insight into the neurobiology of stress-induced insomnia (Cano *et al.*, 2008). Within the Cano-Saper model of insomnia (Figure 1.4) stress was induced by taking a male rat at the peak level of their sleep, and placing it into a dirty cage which had been occupied by another male rat for a period of one week. As rats are extremely territorial and the cage exchange acted as a stressor.

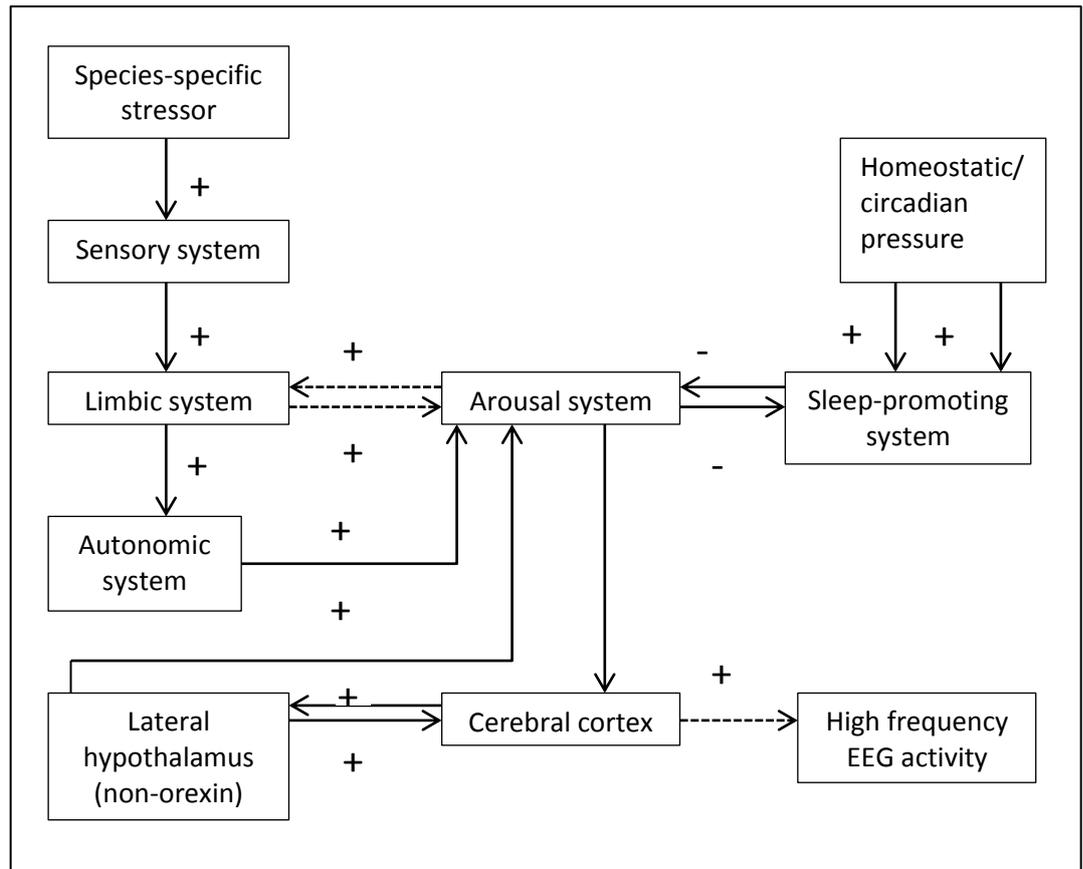


Figure 1.4: Assumed mechanisms involved in Cano-Saper stress-induced insomnia (adapted from Perlis et al., 2011, p. 860. Within the model, + represents activation and - represents inactivation

Exposure to surroundings that have been occupied by another rat, even though the rat is not present, provokes and induces a stress fight-or-flight response through olfactory and visual cues. This includes activation of the autonomic nervous system and of the hypothalamic-pituitary-adrenal (HPA) axis and results in sustained wakefulness (Cano *et al.*, 2008; Perlis *et al.*, 2011). Rats subjected to the cage exchange displayed less sleep than control rats, as expressed through a higher percentage of wake and through a decreased percentage of NREM sleep. This was the case in the first and second hours after the cage exchange, indicating a stress response and five and six hours afterwards, analogous to a period of acute insomnia. The cage exchange rats also displayed almost double the sleep latency than control

rats, showing a mean sleep latency of 58.7 minutes compared to a control group duration of 31.8 minutes. There were no differences in NREM bouts, but cage exchange rats also showed a reduced percentage of REM sleep. Cage exchange rats also showed higher levels of sleep fragmentation, observed through fewer transitions from NREM to REM sleep and a greater amount of transitions to wakefulness.

Neuronal activity in the rats was also examined through measuring the expression of Fos proteins. Increases in Fos levels were observed in the caudal section of the medial paraventricular hypothalamic nucleus (mp-PVH) in the cage exchange rats. The mp-PVH contains corticotropin-releasing hormone (CRH) as well as neurons involved in the control of autonomic function. Overall, Fos activation patterns showed that the cage exchange rats were aroused several hours after the exchange procedure. The cage exchange initially produced acute stress and indicated an increase in both autonomic arousal and in HPA axis function. One interesting finding from this study was that even though cage exchange resulted in sleep disturbances, with these animals sleeping less (25% - 30% in the later phase), increases in Fos levels were observed in neural regions believed to be sleep-promoting. These regions included the ventrolateral preoptic nucleus core (VLPOc), the extended ventrolateral preoptic nucleus (VLPOex) and the median preoptic nucleus (MnPO). Overall this suggested that the stressor evoked the simultaneous activation of sleep systems and arousal systems. Fos activation patterns were also found to be very similar to results from a positron emission tomographic (PET) neuroimaging study conducted in humans with insomnia (Nofzinger *et al.*, 2004), where individuals with insomnia displayed a slightly smaller decrease in relative glucose metabolism than good sleeper controls during the transition from wake to sleep. This was the case in wake-promoting regions of the brain including the

ascending reticular system, hypothalamus, insular cortex, amygdala and hippocampus, as well as in the anterior cingulate and the medial prefrontal cortex. This suggested that the inability to fall asleep as seen in insomnia may be related to the failure of the arousal systems to decline in activity when transitioning from wake to sleep, with the decline in prefrontal cortex activity possibly being a consequence of daytime fatigue.

Therefore, the Cano-Saper animal model of stress-induced acute insomnia fits well with the psychobiological inhibition model of insomnia (Espie, 2002) in that the results suggest that there was an inability to inhibit wakefulness rather than sleep. The cage exchange rats show a unique pattern of activity, as whilst neural activity is the same as that of a sleeping rat, the arousal system and cortex show activity similar to wake (Perlis *et al.*, 2011). Cano and Saper conclude that during insomnia, the VLPO (both the VLPOc and VPLOex) is fully activated due to a combination of homeostatic and circadian pressure, and that the arousal system cannot be turned off due to excitation of the limbic system. Simultaneously, the arousal system is unable to turn off the VLPO due to the input of the homeostatic and circadian systems, with the stronger homeostatic pressure coming from the fact that the stressed rats suffer from partial sleep deprivation (Cano *et al.*, 2008; Perlis *et al.*, 2011). The model involves olfactory signals from the cage exchange being sent to the limbic system, and this activates both the arousal and autonomic systems in addition to non-orexin neurons in the lateral hypothalamus. As a result, the cerebral cortex becomes activated by the input from the arousal system and lateral hypothalamus, generating high-frequency activity during NREM sleep. Co-activation of both sleep and arousal systems would usually be prevented, however the combination of homeostatic and circadian pressure activates the sleep system. The stress results in an activation of the

arousal system and this results in the observed simultaneous neural activation (Cano *et al.*, 2008; Perlis *et al.*, 2011).

A major strength of the Cano-Saper model is that it directly shows the negative effects of a psychosocial stressor, rather than a physical stressor upon sleep variables and upon the neurological markers and thus shows the link between acute stress and sleep (Ellis *et al.*, 2012). As the stressor produced an increase of Fos expression in the mp-PVH, an initiator of the adrenocortical stress response (Cano *et al.*, 2008) a human analogue of the model could be potentially examined wherein the glucocorticoid cortisol, one of the secretory adrenocortical end products of the HPA axis, is measured through blood or saliva sampling (Hucklebridge, Hussain, Evans, & Clow, 2005). However, as this is an animal model it may be difficult to translate and develop this to a human model of provoking and examining acute insomnia in humans (Ellis *et al.*, 2012). One positive aspect of the Cano-Saper model is that it suggests insomnia can be transitional where acute insomnia is either part of the fight-or-flight response to stress or as a consequence, showing that the insomnia is an adaptive response to a perceived or actual threat (Ellis *et al.*, 2012; Perlis *et al.*, 2011).. Whilst the Cano-Saper rodent model identifies useful aspects of the potential neurobiology of acute insomnia, it cannot assess the subjective complaint nor is it relevant to chronic insomnia. Furthermore, not all groups of neurons express Fos in association with action potential activity, which may limit the model when used to examine human neural substrates of acute insomnia (Perlis *et al.*, 2011).

1.6. Models of insomnia with a involvement of stress in sleep maintenance

Whilst, as discussed, the Spielman 3P and Cano-Saper models of insomnia outline the role of stress in causing the insomnia, there are other models of insomnia which have an involvement of stress within sleep maintenance. These models are discussed in the following sections.

1.6.1. Lundh & Broman model of insomnia

Lundh & Broman's (2000) model explains how 'sleep-interfering' and 'sleep-interpreting' processes combine to produce insomnia (Figure 1.5). Sleep-interfering processes refer to those that create physiological, emotional or cognitive arousal. Within the model, physiological and psychological arousal are both potential causes of insomnia and Lundh & Broman suggest that in order to understand the cause of insomnia, the causes of arousal must also be understood. Within the framework of the model, the causes of arousal may result from interactions between a particular event and individual factors. These may include basic arousal levels, 'arousability', shown in response to the arousing event and slow levels of habituation, referring to the return to the basic arousal level following the event. The second part of the model focuses on intra-individual differences in sleep-interpreting processes, referring to the psychological processes involved in each individual's personal definition of insomnia in relation to their current state. This integrative model also takes into account psychological factors where individuals may have a predisposition to both the sleep-interfering processes and the sleep-interpreting processes. These would include having higher basal levels of arousal and arousability, which can include personality traits such as having a high level of emotional sensitivity, being

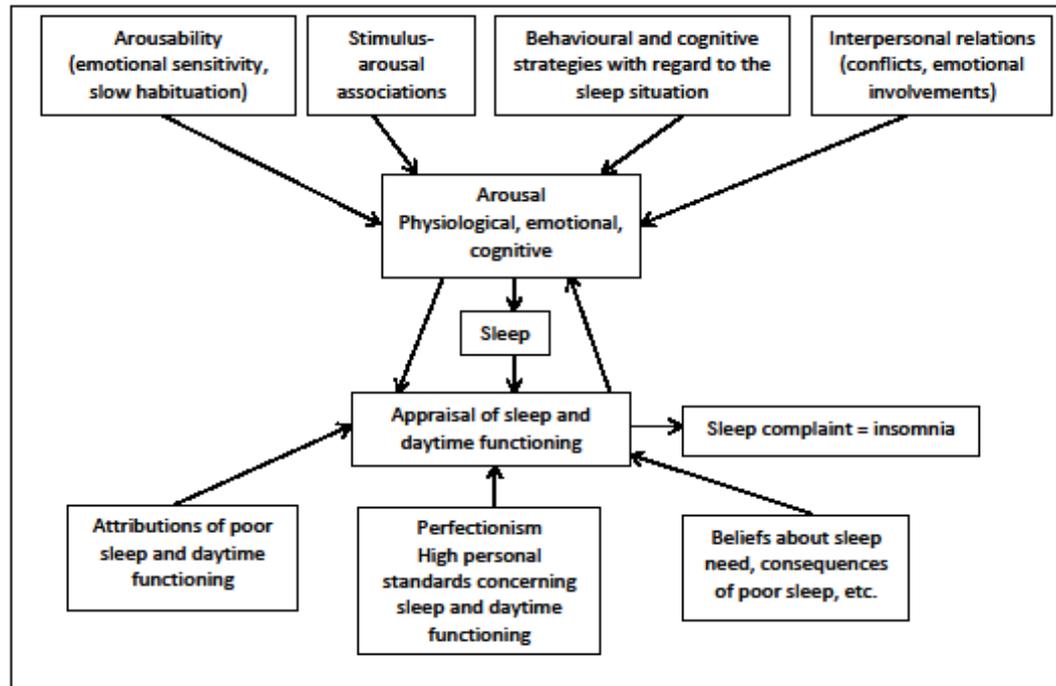


Figure 1.5: Lundh & Broman's Integrative model of insomnia (Adapted from Lundh & Broman, 2000, p. 308)

easily hurt and having a slow recovery from stress. Other psychological variables could include having a personal disposition to worry, experiencing emotional conflicts in personal relationships and emotional involvement in the problems of others. Lundh & Broman (2000) also speculate that the personality trait of perfectionism may also act as a pre-dispositional factor for insomnia, as poor sleep and what counts as impaired daytime functioning are largely subjective experiences. Therefore, the higher the level of perfectionism which an individual has, the lower their threshold level is for perceiving insufficient sleep. Within the framework of the model, perfectionism may also feed into the sleep-interfering processes, such as through worrying about sleeplessness and through predisposing an individual to having a stronger emotional response to a negative life event which may in turn disrupt sleep.

Lundh & Broman's model involves four categories of vulnerability factors for sleep-interfering arousal processes (arousability, stimulus-arousal associations, behavioural and cognitive strategies with regard to the sleep situation and emotional aspects of interpersonal relations) and three categories of vulnerability for sleep-interfering processes (high personal standards, dysfunctional beliefs, and attributions). The fact that this model does take into account intra-individual differences in baseline arousal levels, intra-individual differences in stress responses and intra-individual levels in habituation is a particular strength of the model (Ellis *et al.*, 2012).

Support for the model comes from a study that showed that within normal sleepers, perfectionism was related to the severity of sleeping problems and with an increased level of concern over the negative consequences. In addition, within the same study, a group of insomnia patients showed higher levels of perfectionism than normal sleepers (Lundh, Broman, Hetta, & Saboonchi, 1994). A further study assessed whether perfectionism was related to pre-existing insomnia and future insomnia, at a baseline and at a one-year follow-up time point (Jansson-Fröjmark & Linton, 2007) and an association was shown between perfectionism and insomnia, although this association was weak. Those with insomnia show high levels of neuroticism and display traits related to perfectionism. These include where individuals are over-concerned (lacking in self-confidence and where they have greater doubts about action), however, the role of personality within insomnia is complex and longitudinal studies are needed (van de Laar, Verbeek, Pevernagie, Aldenkamp, & Overeem, 2010). The main drawback of this model is that it does not specifically explain how the sleep-interfering processes and sleep-interpreting processes interact with each other. A further drawback is that the model cannot be

used to determine a causal relationship between these two processes and requires further detail and development (Ellis *et al.*, 2012; Lundh & Broman, 2000).

1.6.2. Espie's psychobiological inhibition model of insomnia

The psychobiological inhibition model of insomnia focuses on the idea that insomnia is a failure of automatic sleep activation and of sleep maintenance (Espie, 2002). In the psychobiological inhibition model, good sleep is the default state. Under normal circumstances both homeostatic and circadian processes are set to have sleep as the default setting rather than insomnia. The psychobiological inhibition model focuses on normalcy rather than on the pathology of insomnia (Espie, Broomfield, MacMahon, Macphee, & Taylor, 2006). The model (Figure 1.6) is framed as being a neurobehavioural, biological model, where good sleep has functional properties of both 'plasticity' and 'automaticity'. Plasticity refers to the capability of the system to react to stressors (situational and personal factors) where

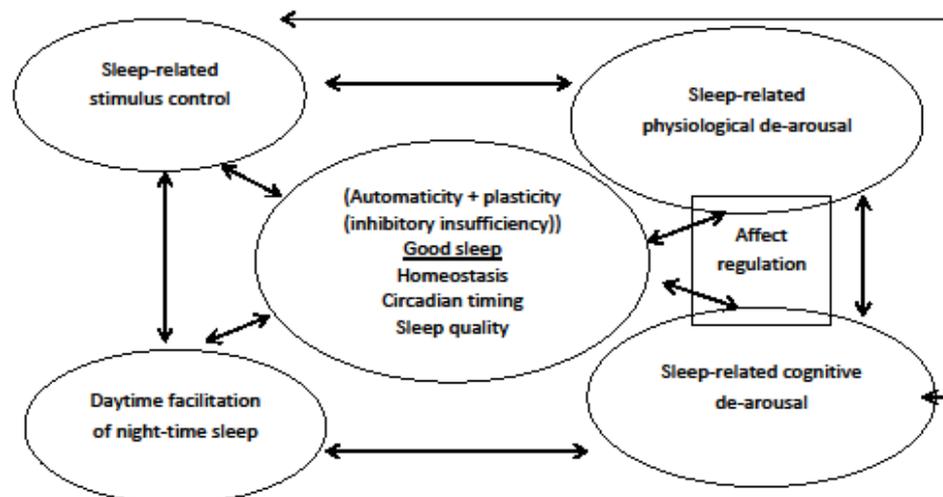


Figure 1.6: Espie psychobiological inhibition model (Adapted from Espie, 2002, p. 227)

the sleep-wake system can tolerate and minimise the effects of night-to-night variability in sleep patterns (Espie, 2002). Automaticity refers to the involuntary nature of a well-adjusted sleep schedule, to habitual associations forming part of a stimulus control paradigm and to the implicit expectations a good sleeper has regarding sleep continuity and quality (Espie, 2002), where this refers to the involuntary regulation of sleep under normal circumstances (Ellis *et al.*, 2012). Automaticity can be disrupted through selective attention to sleep, the explicit intention to sleep and also through the introduction of effort in the sleep process, which Espie terms the ‘attention-intention-effort’ (A-I-E) pathway (Espie *et al.*, 2006).

At the core of the model is an involuntary process of interaction between the sleep homeostat and the circadian timer, associated with the self-perception of good sleep quality. The role of plasticity and automaticity is to defend this core. Espie (2002) notes that both endogenous sleep-related cues such as physical and mental fatigue interact with exogenous, external cues in the environment. Essentially, good sleepers are passive as both these internal and external cues allow conditions for sleep to be set automatically and without effort.

In the model, the defensive properties of the plasticity and automaticity are maintained by four sub-systems that interact with each other, which are a mixture of behavioural, cognitive and biological processes. The subsystems are sleep-stimulus control, physiological de-arousal, cognitive de-arousal, and daytime facilitation. These interactions maintain sleep homeostasis, circadian timing and sleep quality, through action and interaction between these subsystems. A good sleeper is able to accurately interpret both physiological and mental signs of readiness for sleep and the stimulus environment of the bedroom reinforces de-arousal, thus affording the

optimal circumstances for sleep to be initiated. Both the process of physiological and cognitive de-arousal is assumed to occur simultaneously in the model in a parallel manner. The model states that dysregulation would occur as a result of strong negative or positive emotions, which cause arousal.

Whilst the focus of this model is on the inhibition of normal sleep, it does acknowledge insomnia as mediating from the activation of the nervous system, psychological or environmental arousal (Espie, 2002). However, the model does not provide information with regards to the timing and does not discuss circumstances in which an individual would recover from the stress or would continue towards the A-I-E aspect of the model (Ellis *et al.*, 2012). Support for the model comes from the fact that evidence for selective attention to salient cues (i.e. the ‘attentional bias’) is strong enough to suggest it has a casual role in the majority of anxiety disorders and that the experimental evidence is strong when applied to sleep (MacMahon, Broomfield, & Espie, 2006; Marchetti, Biello, Broomfield, Macmahon, & Espie, 2006; Spiegelhalder, Espie, Nissen, & Riemann, 2008; Woods, Marchetti, Biello, & Espie, 2009). However, inconsistencies have been shown in attentional bias studies even when using the same Emotional Stroop Task, although this may be potentially explained by poor sleepers showing an attentional bias towards sleep-related cues and also showing an increased monitoring or avoidance for anxiety-provoking cues (Barclay & Ellis, 2013). Limitations include the fact that most of the model and of the A-I-E pathway component remains to be validated (Perlis *et al.*, 2011).

1.6.3. High risk model of threat perception

A further model of insomnia which acknowledges the role of a stressor is the high risk model of threat perception (HRMTP) which suggests insomnia as a largely

somatic disorder (Perlstrom & Wickramasekera, 1998). In the HRMTP, a perceived threat results in an increase in physiological arousal which inhibits the sleep process (Ellis *et al.*, 2012). In the HRMTP, there are specific psychosocial and psychophysiological risk factors, including a high susceptibility to hypnosis, high levels of neuroticism, repression and the tendency to catastrophise. In the HRMTP, neuroticism interacts with hypnotic susceptibility and levels of catastrophising, in order to predispose a person to have enhanced levels of threat perception for an acute stressor. The predisposing factors also interact with other factors such as major life events, accumulation of daily hassles, and low social support systems and coping skills. This interaction drives, either consciously or unconsciously, psychological and somatic symptoms of insomnia that manifest in sympathetic reactivity.

The interaction between hypnotic ability and the perception of threat results in an increased sympathetic response, and/or increases the sensitivity towards a threat. The main advantage, and also the main limitation of the model, is that the circumstances of the sleep disturbance are extremely specific meaning that other relevant factors may be ignored (Ellis *et al.*, 2012). Some support for the model does come from the finding that those with insomnia display high levels of neuroticism (van de Laar *et al.*, 2010) and a tendency to catastrophise (Harvey & Greenall, 2003), both of which are specific examples within the HRMTP model. That said, it is unclear whether these aspects are the cause or the consequence of the insomnia (van de Laar *et al.*, 2010).

1.6.4. Morin's microanalytic model of insomnia

Morin's microanalytic model of insomnia (Morin, 1993) proposes that maladaptive sleep habits and dysfunctional beliefs perpetuate insomnia (Bélanger,

LeBlanc, & Morin, 2012). Four components of the model (Figure 1.7) feed into the insomnia and relate to each other, including arousal, beliefs and attitudes, maladaptive habits and consequences. The arousal component refers to aspects such as emotional arousal (i.e. fear or sadness), cognitive arousal (i.e. thoughts and images) and physiologic arousal (e.g. pain or muscular tension).

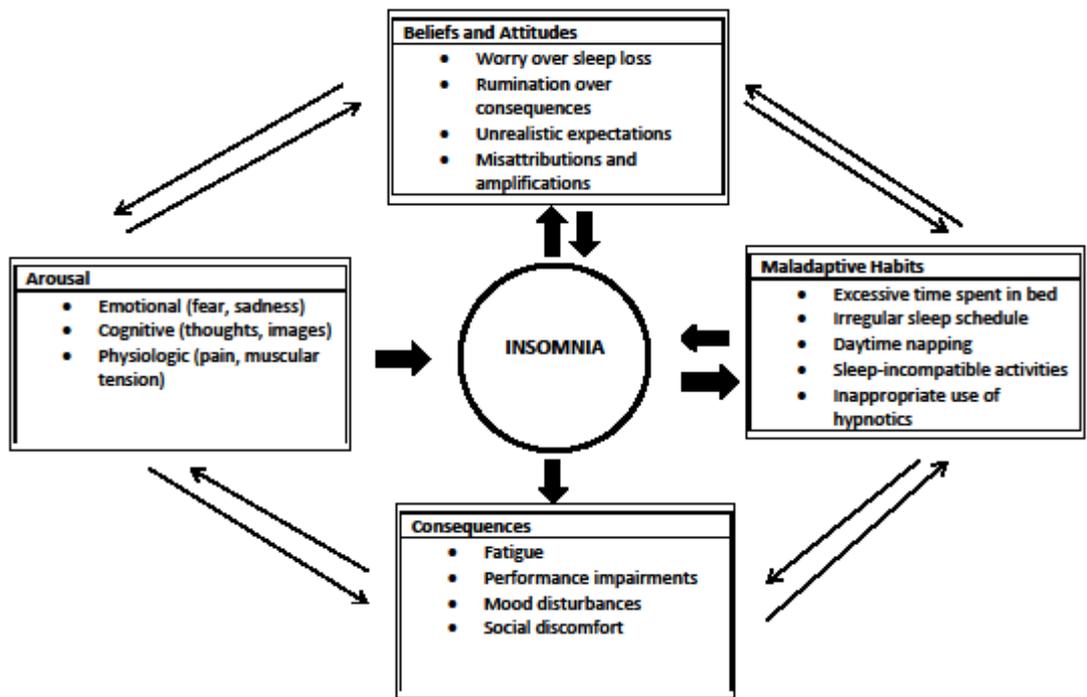


Figure 1.7: Morin's microanalytic model of insomnia.

The beliefs and attitudes component refers to aspects such as the worry over the sleep loss caused by the insomnia and the associated rumination over the consequences of insomnia, unrealistic expectations, and misattributions and amplifications. The maladaptive habits component refers to behaviours such as spending too much time in bed, keeping an irregular sleep schedule, daytime napping and the inappropriate use of hypnotic drugs. The consequences component refers to the consequences of the insomnia, such as fatigue, impaired performance, mood disturbances and social discomfort. One way in which the model has an opportunity

for a stressor to become involved is within the arousal component of the model, as a stressor may cause emotional arousal through fear or sadness; however, the main limitation of the model is that it does not specify the circumstances under which this arousal occurs.

1.6.5. Buysse's neurobiological model of insomnia

Buysse's neurobiological model of insomnia (Buysse, Germain, Hall, Monk, & Nofzinger, 2011) proposes that insomnia is primarily a disorder of sleep-wake regulation, characterised by wake-like activity in neural structures during NREM sleep. This results in simultaneous sleep/wake brain activity patterns that are specific to regions of the brain. The model (Figure 1.8) suggests that during centrally and frontally-defined NREM sleep, those with insomnia show greater levels of brain activity and metabolism in limbic and parietal cortices, the thalamus and hypothalamic-brainstem arousal centres. According to the model, the simultaneous sleep/wake activity may help explain the characteristics of and the effects of treatment within insomnia. Buysse and colleagues give the example that those with insomnia perceive wake or an awareness of the environment despite EEG showing that the individual is obtaining sleep. The model states that even a small increase in the duration of sleep or a decrease in wakefulness with treatment could show an association with a subjective improvement if the treatment caused reductions in waking brain activity. Overall, the model suggests that the psychological and behavioural aspects of insomnia have a neurobiological cause. Support for the model comes from animal studies of insomnia, based on the Cano-Saper model of insomnia which suggests that an acute stressor may lead to biological sleep disturbances.

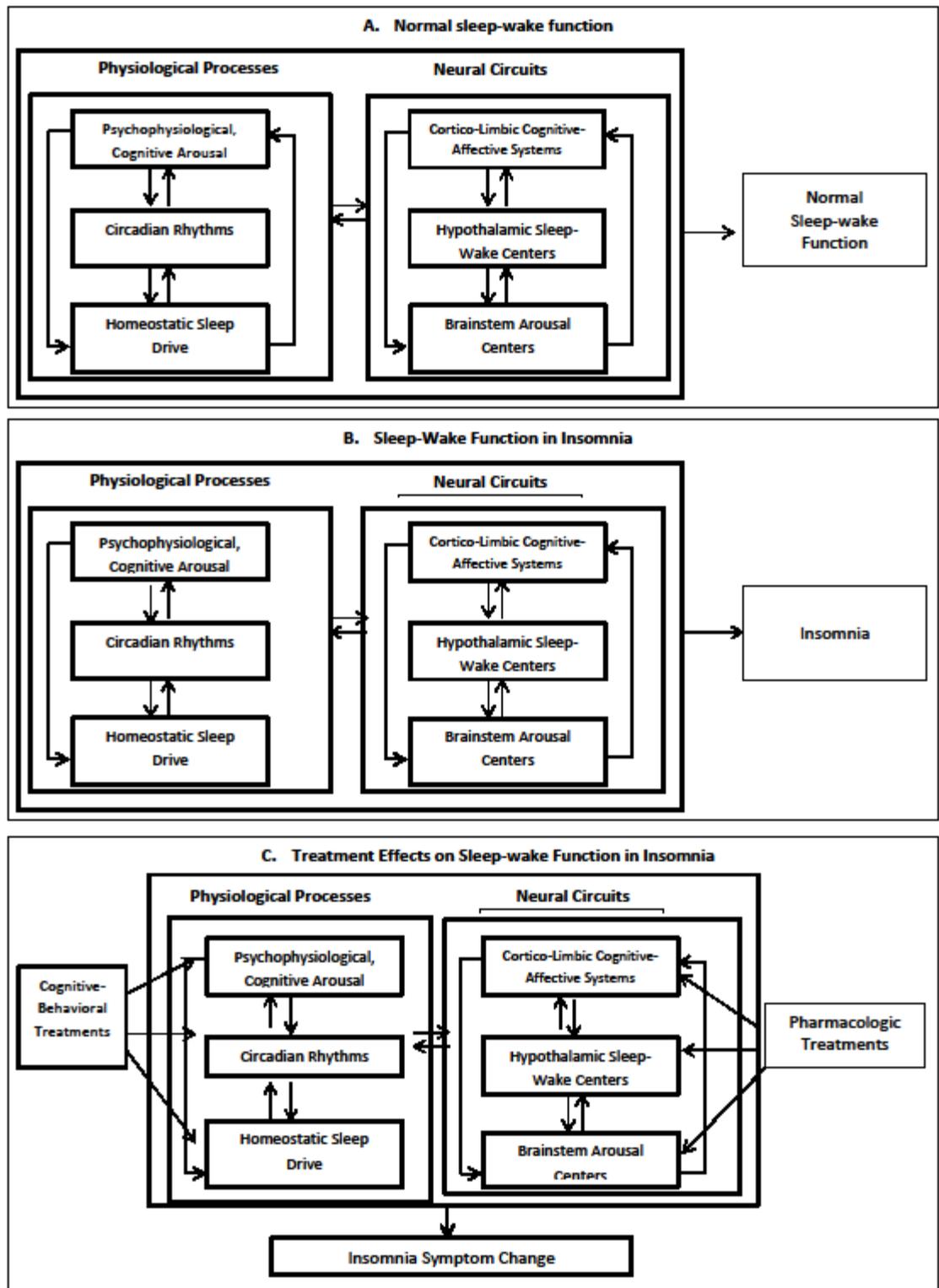


Figure 1.8: Buysse's neurobiological model of insomnia

However, the main limitation of the model is that there is no opportunity in the model for specific stressful event acting as a trigger.

1.6.6. Harvey's cognitive model of insomnia

The starting point of Harvey's cognitive model of insomnia (Harvey, 2002) is an excessive amount of negatively-valenced cognitive activity regarding the amount of sleep obtained and also about the subsequent impact of the sleep disturbances upon health and/or daytime function. This worry and rumination then triggers both autonomic arousal and emotional distress, where the arousal occurs due to sympathetic nervous system activation, resulting in the individual being in an 'anxious' state. The model (Figure 1.9) proposes that as a result of this anxious state, the individual would then preferentially allocate attentional resources in order to monitor for sleep-related threat cues.

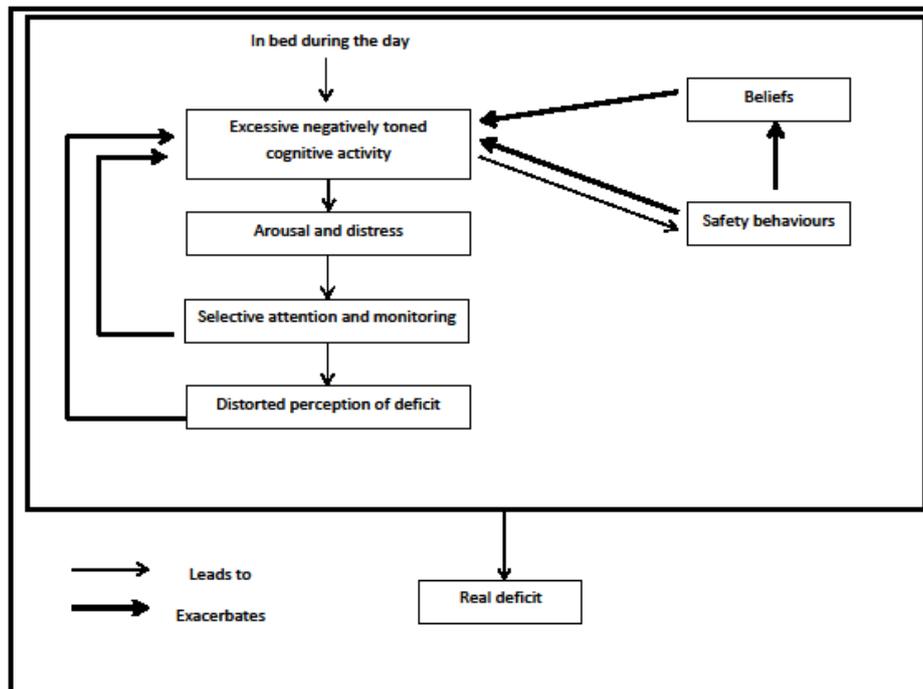


Figure 1.9: Harvey's cognitive model of insomnia

This would occur in terms of monitoring both internal body sensations and external environmental cues for sleep-related threats, for example indicators of sleep loss and a reduced daytime ability to cope or reduced daytime function. The monitoring happens automatically and detection of a sleep-related cue leads to further worry. Due to the selective attention causing the detection of usually unnoticeable cues and the state of increased arousal causing increased body sensations, the chance of detecting a threat is increased. Together these processes lead to the individual overestimating the severity of the deficit in their sleep and in the subsequent daytime functioning. This then fuels further worry and in the context of the model, cognitive activity can be maintained by erroneous beliefs about sleep and worry. These processes continue to exacerbate the negative cognitive activity and the arousal and distress. The excessive and escalating levels of anxiety and worry may actually lead to an actual sleep impairment due to the cognitive activity rather than a deficit in the sleep/wake cycle. Whilst the model does not explicitly acknowledge the role of a stressor, the model does place as much importance upon daytime symptoms as it does night-time symptoms. However, Harvey does acknowledge that precipitants to insomnia may include stressors such as life stress, or an accident, or illness.

1.6.7. Fitchen and Libman cognitive model of insomnia

The Fitchen and Libman model of insomnia (Fichten & Libman, 1991; Libman, Creti, Amsel, Brender, & Fichten, 1997) focuses primarily on insomnia within older adults, although the model does have a role for stressors. The model (Figure 1.10) proposes that predominantly negative cognitive activity, comprising negative, worrying and anxious thoughts alongside self- statements, during periods of nocturnal wake, may act as a mediator of insomnia complaints. The starting point

of the model is the recognition that older people suffer from nocturnal awakenings and the model then proposes that it is the nature of thoughts experienced during nocturnal awakenings which determine whether or not a complaint of insomnia is made. The model suggests that the complaint of insomnia is compounded by negative cognitive activity, including daily event-related concerns and worry, which may include consequences of not obtaining enough sleep (Fichten & Libman, 1991; Libman *et al.*, 1997). Although there is a potential role for negative cognitive activity resulting from a specific stressor, the main limitation is that the model only focuses on insomnia within older adults.

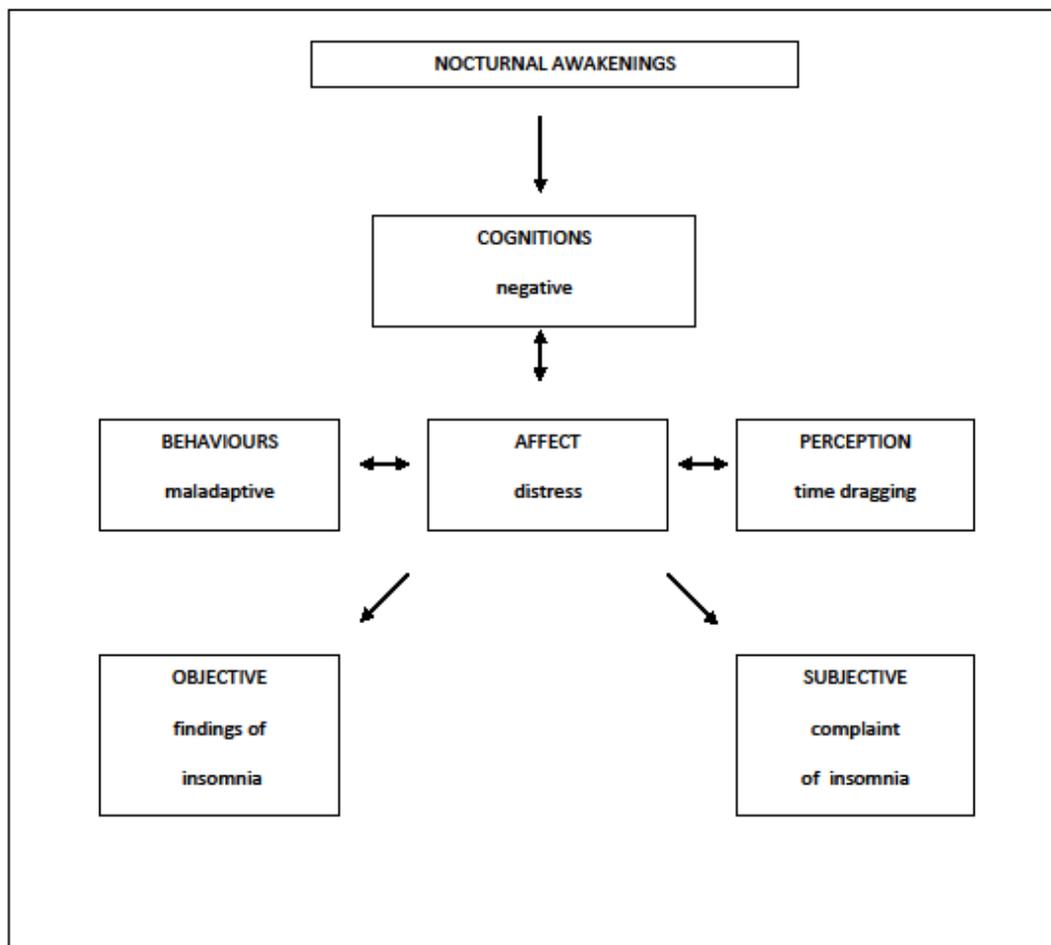


Figure 1.10: Fichten and Libman's cognitive model of insomnia

1.7. The effects of stress upon sleep

The relationship between stress and sleep has been examined using a variety of methods. This has included assessing the effects by using naturalistic stressors and by using experimental stressors. These studies are described in the following sections of the thesis.

1.7.1. Naturalistic stressors

Studies which have examined the effects of naturalistic stressors upon sleep have included events such as natural disasters, war, bereavement and financial strain. Davidson and colleagues (L. M. Davidson, Fleming, & Baum, 1987) assessed subjective sleep in a population of residents from the Three Mile Island (TMI) area, approximately three years after a nuclear incident where subjective sleep was assessed and compared to controls. TMI residents reported more awakenings during the night and reported greater sleep-onset latencies compared to control participants and took longer to return to sleep when awakened compared to controls. Additionally, a greater number of sleep disturbances were related to higher levels of stress. One study followed up on survivors of a ferry disaster, in which survivors and their relatives were referred for assessment of psychological injury, showing that subjective sleep disturbances were one of the most common symptoms (Dooley & Gunn, 1995).

Elsewhere, in a study of state personnel who responded to the September 11 World Trade Center terrorist attacks in 2001 and who were evaluated between 2002 and 2003, subjective sleep problems were one of the most common psychological symptoms, present in 16.4% of individuals (Mauer, Cummings, & Carlson, 2007).

Also following the September 11 attacks, Schuster and colleagues (Schuster *et al.*, 2001) surveyed a nationally representative sample of 560 adults in order to determine their reactions to the incident. In addition, respondents were also asked about their perceptions of how their children reacted to the attacks, providing information on 167 children. The results showed that 11% of adults and 10% of children exhibited sleeping difficulties since the event (as indicated by ‘trouble falling or staying asleep’).

Following the Oklahoma bombings, North and colleagues (North *et al.*, 1999) contacted and assessed a sample of 182 survivors approximately six months after the event, using systematic interviews. Approximately 70% of the survivors assessed reported insomnia and approximately 50% reported suffering nightmares. Natural disasters have also been examined. Following a 1995 earthquake in Japan, a survey of 143 individuals showed that sleep disturbances were the most common symptom, with 63% of respondents reporting a sleep disturbance three weeks after the earthquake (Kato, Asukai, Miyake, Minakawa, & Nishiyama, 1996). One study examined sleep in a sample of 54 adults directly affected by Hurricane Andrew, between six to twelve months following the event (Mellman, David, Kulick-Bell, Hebding, & Nolan, 1995). Participants were asked to detail their sleep before the hurricane and after the hurricane, using the Pittsburgh Sleep Quality Index (PSQI; Buysse, Reynolds III, Monk, Berman, & Kupfer, 1989). Sleep quality was rated to be worse following the hurricane and this was more pronounced in participants with a psychiatric morbidity.

Other studies have examined the impact of war. A study which examined a large sample ($n = 1,045$) of respondents both during the 1991 Gulf War and 30 days following the war (Askenasy & Lewin, 1996) showed that 51% reported suffering

disturbed sleep. It was shown that the war provoked stress in 67.5% of respondents and that 19% of the individuals who reported normal sleep previous to the war were now suffering from insomnia. Seelig and colleagues (Seelig *et al.*, 2010) conducted a longitudinal cohort study in a large sample ($n = 41,225$) of soldiers who were assessed at a baseline period (2001 – 2003) and a follow-up period (2004 - 2006). It was shown that those who were deployed to Iraq or Afghanistan and who were surveyed either during or after the deployment period, showed a shorter subjective sleep duration compared to those who were not deployed, where duration was assessed on the basis of how many hours of sleep participants reported obtaining each night. Those who had experienced deployment, who were either currently deployed or had been deployed in the past, showed increased odds of reporting sleep problems (defined as having trouble falling asleep or staying asleep) than those who were not deployed. It was also shown that combat exposure during the deployment period was an independent predictor of the sleep difficulties.

The effects of more commonplace naturalistic stressors have also been investigated, such as financial strain, health events, bereavement and examinations. Financial strain has been shown to relate to subjective and objective sleep. Hall and colleagues (Hall *et al.*, 2009) reported a relationship between financial strain and subjective sleep quality in a sample of middle-aged women, where greater financial complaints were associated with poorer sleep quality. Financial strain was also related to objective sleep, where greater financial strain was associated with poorer levels of sleep efficiency (Hall *et al.*, 2009). Effects of financial strain have been shown elsewhere in a sample of older adults with a mean age of 74 years (Hall *et al.*, 2008). Sleep was measured using PSG and this study showed that the older adults who reported on-going financial strain to be either “somewhat upsetting” or “very

upsetting” showed a longer sleep latency, greater WASO and lower sleep efficiency compared to those who reported that financial strain to be either absent or “not upsetting”. Lallukka and colleagues (Lallukka, Arber, Rahkonen, & Lahelma, 2010) reported that in a cross-sectional study involving a large sample ($n = 8,960$) of middle-aged participants (aged 40-60 years), childhood economic difficulties were associated with complaints of insomnia amongst both women and men, although a relationship between current economic problems and complaints of insomnia were only observed in women. A separate cohort study assessed Finnish and British public sector workers at baseline and at a follow-up period approximately 5-7 years later (Lallukka *et al.*, 2012). Sleep problems were assessed in the previous four weeks on the basis of difficulties with sleep onset, sleep maintenance, non-restorative sleep at baseline and non-restorative sleep at follow up periods. The results showed that the odds of having a sleep problem were higher in those with persistent economic difficulties, termed as frequent economic difficulties at both the baseline and the follow-up period. Taken together, this suggests that economic difficulties and financial strain, as stressors, have a negative and disruptive influence upon sleep.

The impact of health-related stressors has also been assessed. One study examined women with metastatic breast cancer taking part in a group therapy intervention programme, and found higher baseline levels of life stress to be related to increases in problems both with sleep onset and with daytime sleepiness (Palesh *et al.*, 2007). However, this study has potential problems with temporality as participants took part in the study just over two years (27 months) after receiving a diagnosis and there was no control group. Further, a study used a retrospective sample of 350 patients participating in a cardiovascular disease prevention programme, in which perceived stress over the past month was assessed (Kashani,

Eliasson, & Vernalis, 2012). Overall, increased levels of stress correlated with a poorer sleep quality over the past month, shorter durations of sleep, and higher reported sleepiness and fatigue.

The effects of bereavement on sleep have also been investigated. Objective sleep in a sample of bereaved individuals with major depression was compared to bereaved individuals without depression and healthy control participants, showing reductions in sleep efficiency and in the latency to REM. Bereaved individuals also showed more early morning awakenings, spent a greater percentage of sleep in REM and showed less delta wave generation in the first NREM period compared to controls (Reynolds 3rd *et al.*, 1992).

One study with elderly participants who had experienced spousal bereavement within the last 12 months were followed-up at time intervals of 3, 6, 11, 18 and 23 months. REM density, which refers to the ratio of the number of rapid eye movements per minute of REM sleep, was increased in the bereaved participants as compared to age-matched controls and REM density increased over time, suggesting that REM density may be a correlate of late-life bereavement and a marker of this particular stressor (Reynolds 3rd *et al.*, 1993). However, sleep efficiency, the latency to REM and the ratio of delta sleep were stable, with the exception of slow-wave sleep, over the two-year period. In addition, these were not significantly different between bereaved individuals and control individuals. In addition, since sleep was measured three months after bereavement, it does not exclude the possibility that sleep disturbances may have occurred before the measurement of sleep three months post-bereavement (Reynolds 3rd *et al.*, 1993).

Hall and colleagues (Hall *et al.*, 1997) showed that in a sample of elderly (with a mean age of 65 years) participants with bereavement-related depression, bereavement-related intrusive thoughts and avoidance behaviours were related to measures of objective sleep, where avoidance behaviours referred to attempts made to stop thinking about a stressful event or circumstance (i.e. the bereavement). Subjective (PSQI) and objective measures, in terms of sleep latency, REM latency and sleep maintenance suggested sleep was disrupted in this group. Sleep latency was defined as the time taken to the first 10 minutes of Stage 1 or deeper sleep, interrupted by no more than two minutes of either Stage 1 or wake and sleep maintenance was defined as the percentage of the overnight PSG recording period spent asleep after the onset of sleep. After relevant variables (age, the time since bereavement and the severity of depression) were controlled for, the frequency of intrusive thoughts and avoidance behaviours were associated with a longer sleep latency and a lower delta sleep ratio. Disrupted sleep following bereavement has also been observed in a non-elderly sample of participants, where a greater prevalence of DSM-defined insomnia, on the basis of the DSM-IV text revision (DSM-IV-TR), was observed in students bereaved over the previous two years as compared to those who were not bereaved (Hardison, Neimeyer, & Lichstein, 2005).

Other naturalistic stressors have also been assessed, including educational examinations and work-related psychosocial stress. Vahtera and colleagues (2007) followed a large sample ($n = 19,199$) at baseline and five years later, where sleep disturbances were assessed using a five-point response scale to the single question “how well do you sleep in general?”. The occurrence of stressful life events such as divorce, marital difficulties or the death of a family member was also assessed. Stressful events were predictive of sleep disturbances amongst individuals with no

pre-existing sleep disturbances. Moreover, individuals who had experienced severe events, on the basis of extremely burdensome and stressful events either between zero and six months , or between six months and five years before the measurement of sleep, showed double the odds of disturbed sleep than those who did not experience severe events, even after adjustment for relevant factors. This study also suggested individual factors were involved. The individual liability to anxiety was assessed using a questionnaire assessing general perceptions of stress in daily life and a questionnaire assessing sympathetic nervous system hyperactivity, through indicating the extent to which several statements applied to them. Those who were liable to anxiety were at a higher risk of developing sleep disturbances after the stressful event, but only in the six-month period following the event.

The effects of examinations upon sleep have also been assessed. Holdstock & Verschoor (1974) showed that students showed a reduced total sleep time over a ten-day examination period, measured objectively within a sleep laboratory and compared to an earlier period (two to three months before the exam) or a later period (three months afterwards). No sleep architectural differences were evident with the exception of a greater amount of time spent in Stage 3 sleep during the post-examination period. In a separate study, students were monitored over the course of an exam period using sleep diaries, both one week before the exam and on the morning of an exam (Ellis & Fox, 2004). Whilst levels of perceived stress and sleep catastrophising increased between the time points, there were no differences in sleep efficiency, timing or in the estimated sleep quality. Interestingly, two sleep variables, sleep catastrophising and a later bedtime, were both predictive of perceived stress on the morning of the exam.

The effects of work-related stress have also been shown to affect sleep. Sekine and colleagues (Sekine, Chandola, Martikainen, Marmot, & Kagamimori, 2006) showed that psychosocial stress at work, in addition to aspects such as shift work, working hours, marital status and conflicts between work and family were associated with poor sleep quality in the previous month, assessed using the PSQI, in a large sample ($n = 3,556$) of Japanese civil servants. One self-report study required participants to keep a sleep diary across 42 consecutive days (Åkerstedt *et al.*, 2012). The main predictor of sleep quality was the level of stress and worries at bedtime, with increases in stress and worry corresponding with reduced sleep quality. The study also showed that poor sleep quality was a predictor of stress and worry at bedtime, although the decrease in sleep quality appeared to have a more pronounced effect on stress than on worry. Additionally, the link between daytime stress and poor sleep quality was not significant, which may suggest that the subsequent effects of stress upon sleep are largely due to anticipation of the forthcoming day (Åkerstedt *et al.*, 2012).

Other approaches have assessed the effects of stress upon sleep, where participants have been required to predict low-stress or high-stress weeks in advance of the measurement of sleep. One prospective study (Dahlgren, Kecklund, & Åkerstedt, 2005) followed office workers during a week with high levels of stress and during a week with low levels of stress, where stress levels were predicted by the participants. Sleep quality was measured subjectively by asking participants to rate their sleepiness several times a day and to rate their sleep quality once a day. Sleep was also objectively measured using actigraphy.

There were no differences between high and low stress weeks in terms of the sleep quality reported, the ease of falling asleep or in the level of sleepiness

experienced at awakening. Greater levels of stress or restlessness were shown during a high-stress week than during a low stress week. Greater levels of sleepiness were shown during a high-stress workday than during a high-stress weekend, low stress workday or weekend.

Participants were also required to predict sleep quality for the coming night. Sleep quality was expected to be poorer following a high-stress workday compared to a workday with low levels of stress or a non-workday, irrespective of stress levels. Overall, this indicated that whilst subjective sleep seemed to be relatively unaffected by the change in stress levels during the week, the predicted subjective sleep quality was affected in that individuals expected to have poorer sleep during a week of high-stress. Interestingly, objective sleep was affected, as actigraphy indicated that total sleep time varied between the low-stress and the high-stress weeks, with less total sleep obtained during the high-stress week.

In a separate study, Petersen and colleagues (Petersen, Kecklund, D'Onofrio, Nilsson, & Åkerstedt, 2013) measured objective sleep during a low-stress and high-stress period. In this study a sample of teachers were divided into groups of those who showed either a high sensitivity or a low sensitivity to stress-related sleep disturbances, on the basis of scores obtained on a modified response to stress scale. Participants were asked to identify low-stress and high-stress periods in advance and PSG was used to record sleep in the homes of participants. Irrespective of group differences, a decrease in sleep efficiency was shown during the high-stress period, suggesting an overall effect of stress on sleep. The two groups did differ in their sleep, as the percentage of REM sleep increased in the low-sensitivity group during the high-stress period and the percentage of REM decreased in the high-sensitivity group. Additionally, the high sensitivity group showed an increase in arousals and

transitions between sleep stages during the high-stress condition, with the low sensitivity group showing the opposite pattern in arousals and stage transitions.

Overall, these studies have demonstrated that naturalistic stress does have a disruptive effect upon both subjective and objective sleep; however, the findings are inconsistent. Whilst it is a strength that the effects of serious naturalistic stressors such as war, financial strain and bereavement have been examined, where these represent salient, important and meaningful stressors, there are limitations to this approach. The limitations include the diverse nature of participants, typically with co-morbid conditions and the lack of a suitable control group, the wide variation in the nature of stress, the varied manner in which subjective and objective sleep has been measured and the time lag between the stressful event and the measurement of sleep. The following section describes the effects of experimental studies upon sleep.

1.7.2. Experimental stressors

The effects of stress upon sleep have also been examined experimentally, where a wide range of stressors have been employed, including intelligence tests, cognitive tasks, the stress of sleeping with medical equipment attached to the body and emotional stressors. Koulack and colleagues (1985) examined the effects of stress upon sleep by using intelligence tests. In this study, objective sleep was measured during an adaptation night, a baseline night and during the experimental night where the intelligence tests were completed. Half of the participants experienced an easier version of the tests which could be completed within the time allowed, whilst the other half experienced a harder version of the tests which could not be completed in time. Both groups of participants showed an increased SOL and

decreased REM density during the experimental night, although there were no differences shown in between the two groups of participants. It is possible that the participants who experienced the harder version of the tasks may not have considered these to be more stressful than the group of participants who were able to complete the tests in the allocated time.

The effects of cognitive tasks upon sleep were examined by Wuyts and colleagues, who assessed the effects of 30 minutes of cognitive tasks prior to going to bed (Wuyts *et al.*, 2012a). Participants slept for three nights in a sleep laboratory, with one night spent at home between each night in the laboratory. Sleep obtained during the experimental night, where the tasks were completed, was compared to sleep obtained during a reference night, where tasks were not required to be completed. There were no differences in subjective sleep continuity or quality compared to the reference night, however objective sleep following the tasks showed a longer sleep-onset latency. Differences were also shown in that a higher percentage of high frequency EEG activity was apparent in the first and second episodes of deep sleep following the tasks, indicating that stress can affect high frequency EEG activity.

Other studies have examined the subsequent effects of emotionally-challenging stress, although these studies show large variations in terms of how provocative these stressors could be perceived to be. In one study participants viewed either a psychologically stressful film, comprising a documentary detailing a subincision initiation ceremony, or a neutral film, detailing a London travelogue, before bedtime (Baekeland, Koulack, & Lasky, 1968). Those who watched the stressful film showed greater levels of REM density, however this study did not include an adaptation night and thus it cannot be ruled out that the findings were

influenced by the first-night effect and simply reflected the adaptation to the sleep laboratory environment.

A separate study measured the effects of a negative emotional stressor on objective sleep, where healthy normal sleepers slept in a sleep laboratory for four nights (Vandekerckhove *et al.*, 2011). The first night in the laboratory served as an adaptation night and the second night served as a baseline night, with no manipulation. Following the baseline night, participants were required to complete cognitive tasks during the evening before sleep. Participants were either placed into a neutral or a failure condition. In the neutral condition participants completed the tasks and were given neutral feedback afterwards. In the failure condition participants completed the same range of cognitive tasks, which included a semantic reasoning task that was impossible to complete. Importantly, participants were told that they would be completing a test that reflected both their level of intelligence and was a predictor of their future professional success. Between each task for those in the failure condition a researcher would enter the room and act in an irritated manner. Negative feedback was also given to participants in this condition following the completion of the tasks. In the failure group, EEG was also attached to participants during the task and they were informed that they were moving around too much and causing unobtainable physiological data. When the sleep obtained during the night following the task was compared between the failure and neutral groups, the failure group showed decreases in sleep efficiency, the percentage of REM sleep and TST, whilst they also showed increases in WASO, the total time awake, NWAK, NWAK from REM sleep and in the latency to SWS.

Vein and colleagues (2002) induced stress by asking participants emotionally-provocative questions immediately before sleep. Compared to a baseline

night of sleep following an adaptation night in the laboratory, sleep latency increased and the percentage of slow-wave sleep was increased in the second sleep cycle following the emotional stressful night (Vein *et al.*, 2002).

Other studies have induced stress by requiring participants to sleep with medical equipment attached, such as a catheter or blood pressure cuff. In one study Vitello and colleagues (1996) showed that participants who slept with an intravenous catheter had a reduced total sleep time, sleep efficiency, percentage of REM sleep and percentage of slow-wave sleep compared to the baseline night. They also demonstrated increased sleep latencies, total wake time and the number of awakenings (Vitiello *et al.*, 1996).

Adam (1982) also reported that blood sampling during the night in older adults (aged 53-63 years) resulted in a reduction in total sleep time, sleep efficiency, REM sleep and an increase in wake after sleep onset. Where the effects of blood pressure cuff inflation upon objective sleep were examined, a greater amount of arousals (changes in EEG frequency) and awakenings were shown in the minute of sleep after the cuff inflation compared to the minute of sleep before the inflation. Participants showed some evidence of habituation on a second night of monitoring, as the percentage of arousals was lower following cuff inflation on the second night (Dimsdale, Coy, Ancoli-Israel, Clausen, & Berry, 1993). However, these studies cannot determine whether the effects upon sleep are due to discomfort or due to the fear of disturbing the medical equipment, or whether they are due, as the authors suggest, to psychological anxiety. It is also not known whether these effects continue over multiple nights of monitoring (Kim & Dimsdale, 2007).

One experimental stress study has assessed the effects of parachute jumping upon sleep, where novice and experienced parachute jumpers were studied in a sleep laboratory four nights before a scheduled parachute jump and one night following the jump (Beaumaster, Knowles, & MacLean, 1978). These parachute jumpers were compared to a control group of parachute jumpers who had no scheduled parachute jump during the same period. PSG was recorded for five consecutive nights and all participants were due to complete their jump between the fourth and fifth night of PSG. The effects of anticipation alone were also examined as due to poor weather half of the participants from each group were unable to complete the parachute jump. This showed that there were differences only in the latency to Stage 1 sleep, the latency to Stage 2 sleep and in the percentage of Stage 1 sleep, with experienced jumpers showing an increase in sleep onset latency compared to novice jumpers and novice jumpers showing a decreased amount of Stage 1 sleep compared to experienced jumpers.

Anxiety levels indicated that novice jumpers had higher levels of anxiety compared to experienced jumpers on the night prior to the jump, indicating that the anticipation of the jump provoked stress. In order to examine whether the parachute jump acted as a stressor, PSG data from the third, fourth and fifth nights were compared by group. Sleep the night following the jump showed no differences, suggesting that the parachute jump did not affect sleep. One drawback of this study is that although inexperienced jumpers were included, the study did not use a control group of participants who had never performed a parachute jump. In this case the control group was comprised of experienced jumpers selected at random (who had experience ranging from three to 287 completed parachute jumps). This suggests that

the anticipation of the parachute jump affected sleep but that the demand itself, in terms of the parachute jump, did not.

Aside from the inconsistencies in the nature of the stressor employed within stress studies, it is possible that individual differences in the response to stress may also play a role in the relationship between stress and sleep disturbances. Drake and colleagues assessed sleep during the first night in a sleep laboratory. Participants completed the Ford Insomnia Response to Stress Test (FIRST), which assessed their likelihood of experiencing sleep disturbances in response to a host of common stressful situations. Based on these scores participants were split into two groups (low vulnerability or high vulnerability). Those with a high vulnerability had a lower sleep efficiency and a longer latency to Stage 1 and persistent sleep. These effects remained even after controlling for a history of insomnia symptoms. Insomnia symptoms were more common in those reporting high FIRST scores, with insomnia being reported in a higher proportion of individuals who scored higher on the FIRST (Drake, Richardson, Roehrs, Scofield, & Roth, 2004). A follow-up study showed that healthy sleepers who scored high on the FIRST displayed differences in their reaction to caffeine, in that they displayed an increased latency to persistent sleep, compared to low scorers (Drake, Jefferson, Roehrs, & Roth, 2006). This suggests that the stressor may impact individuals in different ways, resulting in sleep disturbances for one person and not another. In order to examine these potential influences further, a standardised protocol for assessing the effects of sleep and stress is necessary.

Overall, whilst experimental stress does appear to disrupt subjective and objective sleep, the effects of experimental stress upon sleep have been inconsistent. As with studies which have examined the effects of naturalistic stress upon sleep,

this may be due to the wide variety of stressors employed. These have, for example, ranged from intelligence tests (Koulack *et al.*, 1985) and cognitive tasks (Wuyts *et al.*, 2012a) to showing participants psychologically stressful films (Baekeland *et al.*, 1968). The type of task used as a stressor may also affect sleep, potentially accounting for some of the inconsistent findings observed. Kobayashi and colleagues (Kobayashi, Ishikawa, & Arakawa, 1998) asked participants to complete different types of daytime tasks on different days, with four mental tasks (a relaxation task; low-vigilance desk work; high-vigilance desk work; a 600km car driving task) and one physical task. The latency to four separate cycles of REM was measured during the night after each task. The standard deviation of the latencies to the third and fourth REM cycle were significantly larger following the car task than observed following any other task, suggesting that the type of task given may affect objective sleep. This study was limited by the small sample size ($n = 5$) but it does suggest that the type of task may influence sleep.

In order to overcome the inconsistencies shown in previous experimental studies assessing the impact of stress upon sleep, it will be necessary to measure the effects by standardising the manner in which sleep and stress are measured within the same context. Where naturalistic studies of stressors and their effects upon sleep have been examined, such as bereavement, war and natural disasters, these are also associated with demand. For example, in the case of bereavement, the emotional stress of the bereavement will also be associated with practical demands related to the bereavement. This is also the case with natural disasters such as earthquakes or war, as the emotional stress of the event is also associated with a variety of demands related to the event.

Other naturalistic studies are also associated with demand. Where the objective sleep of teachers was measured by Petersen and colleagues during a low-stress and high-stress period, estimated by the teachers in advance, (Petersen *et al.*, 2013) this study did not disentangle the effects of stress from demand, as a high-stress week could have included a week with high levels of demand. The study involving parachute jumpers conducted by Beaumaster and colleagues (Beaumaster *et al.*, 1978) did separate the stress from the demand, requiring participants to complete a parachute jump, where sleep was not affected following the stress of a parachute jump. Interestingly, this study indicated that the anticipation of stress may affect sleep.

The nature of the control group used in demand studies may also be problematic, for example, whilst Beaumaster and colleagues (Beaumaster *et al.*, 1978) examined the effects of a parachute jump between experienced and novice jumpers, in this case the control group included novice jumpers who still had experience of completing parachute jumps. The anticipation of stress may also have affected naturalistic studies as where Petersen and colleagues required teachers to estimate a low-stress and high-stress week in advance, it may have been the case that the teachers were estimating a low-demand and high-demand week in advance (Petersen *et al.*, 2013). A series of naturalistic and experimental studies have explicitly examined the effects of the anticipation of stress upon sleep and these are described in the following section.

1.7.3. *The impact of anticipation upon sleep*

Anticipation has also been shown to affect both subjective and objective sleep, in both naturalistic and experimental stress studies. Torsvall and colleagues examined the effects of on-call duty in a group of Swedish ship engineers. The engineers were asked to complete a sleep diary during a tour of duty, lasting between one and three months, whilst they were aboard ships, measuring sleep quantity and quality (Torsvall, Castenfors, Åkerstedt, & Fröberg, 1987). When the engineers were on watch duty every two to four nights they were allowed to sleep but were awakened by automatic alarms when there was a malfunction in the machinery. Whilst on watch duty engineers exhibited disturbed sleep, showing a reduced total sleep time of more than 90 minutes per watch night. The engineers also reported poorer perceived quality of sleep compared to free nights. Interestingly, it was reported that when the engineers were on watch duty, but where no alarms occurred, the engineers still showed greater difficulties in falling asleep, less sleep and a poorer sleep quality compared to a free night. The engineers also found it more difficult to rise in the morning and reported feeling less refreshed with higher levels of sleepiness following a night on watch with no alarms compared to a free night.

Wright and colleagues (Wright, Schnur, Montgomery, & Bovbjerg, 2010) assessed objective sleep in women the night before breast cancer surgery and observed that a higher number of intrusive thoughts were associated with a lower total sleep duration and a reduced sleep efficiency. However, this study did not have a control group.

The effects of acute demand upon heart rate during sleep were investigated in a study which used a control group of normal sleepers and a 'stress' group (Hall *et al.*, 2004). Those in the stress group were told immediately before lights out that they

would have to give a 15-minute speech upon awakening the following morning, to be rated for both content and quality, with two minutes preparation time for the talk. The heart rate of participants was recorded during the night, and the demand resulted in a decrease in levels of heart-rate variability, a measure of parasympathetic modulation, during both REM and NREM sleep. However, there were no differences between the groups in measures of objective sleep continuity or sleep architecture.

In a further study, Hall and colleagues (Hall, Buysse, Reynolds, Kupfer, & Baum, 1996) instructed healthy young participants that upon awakening the following morning, they would either experience a demand condition of having to give a 15 minute speech which would be evaluated or a control condition of reading magazines. Intrusive thoughts, related to the stress of the task, were shown to interfere with sleep, resulting in an increased sleep latency and an increased number of awakenings during the final two hours of sleep. However, stress-related thoughts showed no relationship with measures of sleep architecture, although these were associated with a lower delta wave generation during the first cycle of sleep. Overall, this indicated that stress-related intrusive thoughts delayed the onset of sleep and disrupted sleep continuity in the second half of the night, with the reductions in delta sleep possibly reflecting the effects of stress.

An additional study, using a similar method of inducing demand, showed there to be a lower REM count during the last (fourth) REM cycle in the demand group compared to the control group (Germain, Buysse, Ombao, Kupfer, & Hall, 2003). REM count was used as a marker of REM density, where this referred to the number of computer-detected rapid eye movements divided by the number of REM minutes. Subjective ratings of stress were shown to mediate the effects of acute stress exposure on the REM count during the final REM period and that these

subjective stress ratings were partially determined by the interaction between exposure to the stressor and the seeking of social support as a method of coping. In addition, changes in state anxiety were shown to predict REM latency and that the changes in state anxiety were partially moderated by interactions between the exposure to stress and seeking social support, and also by the interaction between the stress exposure and avoidance. However, the effects upon objective sleep were indirect and whilst it is likely that participants found the anticipation of the upcoming speech to be uncomfortable, the task was extremely short. As such, participants may only have been preparing for a low-intensity short period of demand and the lack of any direct effects upon sleep are likely to have been due to the task not being demanding enough to disrupt sleep.

Wuyts and colleagues (2012b) investigated the effects of a simulated on-call situation where participants ($n = 16$) spent three nights within a sleep laboratory. The sequence of the nights was counterbalanced and participants had a one night wash-out period between each night. On one night participants were informed, approximately half an hour before going to bed, that they would hear an unspecified sound at randomly determined time intervals during the night. Participants were required to respond by pressing a button three times as quickly as possible otherwise sounds would be played for the rest of the night. However, no sounds were actually played at any time point during the night. Compared to the control night, participants displayed a significantly lower sleep efficiency, a trend towards a greater sleep onset latency and a greater percentage of wake during the simulated on-call night. Analysis of subjective sleep diaries showed trends towards a lower sleep duration during the on-call night, with participants claiming to have slept 27 minutes less during this night and the length of wake after sleep onset periods were significantly longer.

Additionally, there were trends towards a greater percentage of wake after sleep onset and towards a lower sleep efficiency during the on-call night. Whilst this suggests that upcoming demands can disrupt subjective sleep, these were only trends (Wuyts *et al.*, 2012b).

Objective methods of measuring sleep have also been employed to examine the effects of real-world anticipation upon sleep. Torsvall and Åkerstedt (1988) conducted a pilot study, in order to examine the effects of on-call duty on objective sleep and wake, as on-call nights have previously been associated with disturbed sleep and apprehension. In the study, five male ship engineers underwent PSG whilst on-board, during both free nights and on-call nights that involved attending to alarms. On-call nights resulted in a shorter duration of sleep and reduced amounts of REM and slow-wave sleep (SWS), compared to free nights. On-call nights were also associated with increased sleepiness the following day. Reductions in EEG power density, which are a measure of EEG variability and which are sensitive to the effects of sleep loss, were also observed during the first cycle of sleep. This was shown even when no alarms occurred; suggesting that even the anticipation of an interruption can disrupt sleep. However, one limitation of this particular study is the small sample size ($n = 5$). Kecklund and Åkerstedt (2004) used a combination of two studies involving normal sleepers in order to investigate whether the apprehension of the next working day affected objective sleep and subjective sleep quality. One of the studies recorded sleep in the homes of participants and the second study recorded the sleep of participants in a truck berth. Objective sleep was measured using one night of PSG. Subjective measures were also assessed, where participants were required to rate mood and levels of apprehension assessed by how pleasant or difficult they expected their next day to be. Levels of tension, nervousness,

uneasiness and stress, and the expected difficulty in awakening the next morning were also assessed. Subjective measures of sleep quality were obtained upon awakening. The relationship between subjective mood before sleep and objective sleep was examined. Greater levels of apprehension at bedtime were significantly related to a decreased duration of slow-wave sleep. This was also related to an increased percentage of Stage 2 sleep. As the apprehension of the next day showed a high level of co-variation with tension, nervousness, uneasiness and stress, the researchers concluded that the apprehension was related to bedtime state anxiety and to levels of stress.

However, disadvantages of the study include that PSG was only measured on one night, meaning results may have been influenced by the ‘first-night effect’, which may have acted as a stressor in itself. In addition, the combination of studies included shift workers and the measurement environment differed between studies. As the study was correlational in nature it cannot be definitively concluded that the effects upon sleep were due to apprehension or stress. In this case it may simply have been the effects of the apprehension of work activities the following day which affected sleep, rather than a direct stressor.

The anticipation of work stress has also been examined. In one study participants were asked to log their levels of stress at bedtime, and ambulatory PSG recorded sleep parameters over: a) a night before a day with low levels of expected stress; b) a night before a normal work day; and c) a night before a day with high levels of anticipated stress (Åkerstedt, Kecklund, & Axelsson, 2007). The sleep associated with a low-level stress day was compared to that of a high-level stress day. The high stress nights showed reduced sleep efficiency, a higher percentage of

WASO and a longer latency to slow-wave sleep compared to the low-stress nights (Åkerstedt *et al.*, 2007).

Experimental studies have also shown that the potential effects of anticipation upon sleep should be taken into consideration and this presents an opportunity for further research. Wuyts and colleagues required good sleepers to complete 30 minutes of cognitive tasks before going to bed within a sleep laboratory, comparing subjective and objective sleep to a reference night (Wuyts *et al.*, 2012a). Subjective sleep continuity or quality did not change compared to the reference night, although differences in objective sleep were shown through a higher percentage of high frequency EEG activity in the first two episodes of deep sleep after the tasks. However, participants slept in their own home between the reference and the cognitive task night and the order to the reference and task night were counterbalanced. As there was a delay between sleep measured during the reference night and task night in the laboratory, it is possible that the anticipation of forthcoming activities and obligations during the day at home may have influenced the result of this study.

Overall, naturalistic and experimental stressors do appear to disrupt subjective and objective sleep, however the results are inconsistent. These inconsistent findings may be due to the wide variation in the nature of the stress employed. Within naturalistic stress studies, these have included stressors such as natural disasters, war, health events and financial problems; within experimental stress studies these have included stressors such as intelligence tests, cognitive tasks and emotional films. Moreover, both naturalistic and experimental studies have shown that the anticipation of stress can affect subjective and objective sleep although these results are also inconsistent. However, experimental studies which

have assessed the effects of stress upon sleep have typically done so using a variety of demands, whereas naturalistic and experimental studies suggest that anticipation may also affect sleep, albeit with inconsistent results.

Taken together, there is a wide variation in the measurement of stress and sleep. Furthermore, anticipation may affect sleep alongside demand. Therefore, in order to accurately assess the effects of stress upon sleep there is a clear need to standardise a protocol by which stress and sleep can be measured within the same context. As demand and anticipation both appear to affect subjective and objective sleep, this thesis will assess the impact of stress upon sleep by standardising the measurement protocol. This will separate out the effects of anticipation and demand, where subjective and objective sleep will be measured.

1.8. Physiological markers of stress

Whilst experimental studies have assessed the effects of stress upon sleep, these studies have not incorporated the simultaneous measurement of a physiological marker of stress. For this reason it is unclear whether the stressor employed is objectively stressful or whether the chosen stressor is only subjectively stressful. Furthermore, physiological markers of stress are of relevance to insomnia. Bonnet & Arand (2010) state that whilst there is evidence of physiological arousal in various systems during sleep in patients with insomnia, the causal nature of the arousal is unclear. Physiological arousal has been shown in individuals with insomnia in terms of heart rate (Bonnet & Arand, 1998), where increased heart rate was shown in all stages of sleep in those with insomnia. Differences were also shown when the heart rate data were analysed using spectral analysis. Those with insomnia showed

increased low frequency power and decreased high frequency power in all stages of sleep, indicating increased sympathetic nervous system activity within insomnia. Evidence of increased arousal within insomnia has also been shown in other physiological markers. Bonnet and Arand showed that individuals with insomnia displayed an increased metabolic rate, shown through increased oxygen use, compared to control individuals (Bonnet & Arand, 1995).

The hyperarousal concept of insomnia, which assumes that insomnia results from an interaction between psychological and physiological variables, has a potential role for increased cortisol levels (Perlis, Giles, Mendelson, Bootzin, & Wyatt, 1997; Perlis, Pigeon, & Drummond, 2006; Riemann *et al.*, 2010). Similarly, the Cano-Saper animal model of insomnia showed that a psychosocial stressor in the form of a dirty cage exchange resulted in increased HPA axis activity (Cano *et al.*, 2008). Although the use of neuroendocrine research within insomnia is not yet well-established (Riemann *et al.*, 2010), cortisol has previously been examined in individuals with insomnia as a marker of HPA axis activity, in a series of studies.

Vgontzas and colleagues examined urinary levels of cortisol in individuals with insomnia, where sleep was measured objectively and 24-hour cortisol levels showed a positive relationship with the level of WASO, suggesting cortisol was related to the extent of the sleep disturbance (Vgontzas *et al.*, 1998). Other studies have compared the degree of HPA axis activity between those with insomnia and healthy controls. In a later study examining blood levels of cortisol, those with insomnia showed significantly higher levels of cortisol during the evening and early part of the night (between 2100h and 0030h) compared to healthy controls (Vgontzas *et al.*, 2001).

Similarly, Rodenbeck and colleagues (Rodenbeck, Huether, Rüter, & Hajak, 2002) also showed that those with insomnia displayed higher levels of nocturnal cortisol as compared to a healthy control group. Nocturnal awakenings showed a significant relationship with evening cortisol levels in both controls and those with insomnia. Those with insomnia also showed a relationship between the first four hours of nocturnal cortisol and sleep, where TST, SE, the percentages of Stage 1 and slow wave sleep, and the number of sleep cycles were negatively related to total cortisol secretion and where WASO, the latency to slow wave sleep and REM sleep were positively related to total cortisol secretion in the first four hours of the night.

Overall, this potentially reflected altered HPA axis activity within individuals with insomnia. In contrast, Riemann and colleagues did not observe any differences in nocturnal levels between those with insomnia and healthy controls (Riemann *et al.*, 2002).

Studies have also measured cortisol through saliva sampling. Backhaus and colleagues focused on salivary cortisol levels and compared levels of cortisol in the morning between those with insomnia and healthy sleepers (Backhaus, Junghanns, & Hohagen, 2004). Cortisol levels were collected in the home at awakening, 15 minutes later and at bedtime, over a period of seven consecutive days. Individuals with insomnia displayed lower cortisol levels at awakening than healthy controls did.

Awakening cortisol levels showed a negative relationship with the number of nightly awakenings across both groups, as assessed using subjective sleep measures during the seven day sampling period, potentially suggesting a link between sleep and cortisol. Taken together, the links between stress, sleep, cortisol and insomnia suggests that cortisol is an ideal measure of stress to examine alongside sleep

disturbances. Cortisol can be reliably measured in saliva in a non-invasive manner and is representative of plasma cortisol levels (Wetherell *et al.*, 2006). As cortisol can be measured non-invasively, it is an ideal candidate for use as a physiological marker of stress within the thesis.

A range of studies which have examined stress have used cortisol as an outcome measure in relation to psychosocial stress tasks within the laboratory. For example, Kirschbaum and colleagues assessed the effects of psychosocial stress upon cortisol levels using a stressful psychosocial task known as the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993), which has an anticipation period and a test period.

The TSST involves placing participants in a situation where they are instructed to assume the position of a job applicant within an interview. The test comprises a 10-minute anticipation period and a 10-minute test period, in which participants are required to deliver a speech and perform a verbal mathematical subtraction task as quickly as possible. The TSST has been shown to be stressful, in terms of plasma and salivary cortisol levels, and more than 70% of participants showed an increase in cortisol levels of 2.5 nanomoles per litre (nmol/l), representing a two to four-fold increase from baseline cortisol levels (Kirschbaum *et al.*, 1993).

A meta-analysis of 208 cortisol studies (Dickerson & Kemeny, 2004) showed that acute psychological stress resulted in increased cortisol, however, this varied depending on the nature of the stress, where larger reactions were shown in performance tasks with elements of socio-evaluative threat or uncontrollability. Tasks which combined both of these elements provoked the largest increases in cortisol levels. Overall, cortisol is an easy to measure marker of physiological stress,

is relevant to insomnia and is therefore an ideal measure to include within the thesis to confirm that the protocol elicits stress. Furthermore, cortisol has been shown to increase in relation to psychosocial tasks including the TSST and, as such, represents an ideal physiological marker of HPA axis function.

Cortisol secretion shows a marked circadian pattern and sharp increases in cortisol levels are observed immediately upon awakening, known as the cortisol awakening response (CAR). This is an ideal physiological parameter for the study of the effects of stress within the thesis, as this appears to be a reliable marker of the circadian system which is one of the processes involved in sleep-wake regulation. As will be discussed in the following chapter, the CAR may function as a marker of anticipation or as a marker of demand. The following chapter will discuss the potential use and application of the CAR within the thesis as a physiological marker of HPA axis function. A devised standard protocol for the measurement of the CAR within the thesis will also be outlined.

CHAPTER 2.

Cortisol and the cortisol awakening response

2.1. Physiological stress and cortisol

The term ‘stress’ refers to experiences which are considered to be challenging, either emotionally or physiologically (McEwen, 2007). As part of the primary response to stress, the sympathetic nervous system and HPA axis are activated, leading to the ‘fight-or-flight’ response where catecholamines, which increase heart rate and blood pressure, are released into the bloodstream alongside glucocorticoids (Lee & Harley, 2012; McEwen, 1998, 2007). The HPA axis is an endocrine system which allows adjustment and adaptation to the bodily and environmental challenges (Fries, Dettenborn, & Kirschbaum, 2009; Hucklebridge *et al.*, 2005). When the HPA axis is activated, glucocorticoids, including corticosterone and cortisol are released, which redirect energy resources with the overall aim of restoring homeostasis (J. P Herman *et al.*, 2003).

Cortisol is essential to life and has a wide range of physiological effects, acting upon almost every cell within the body (Bamberger, Schulte, & Chrousos, 1996; Kudielka & Kirschbaum, 2005). Cortisol has a central role within metabolism as it mobilises resources to provide energy in response to the increased metabolic demand caused by challenge, may prevent arterial hypotension, and also inhibits the immune system; specifically inhibiting inflammatory and immune reactions, therefore preventing tissue damage. (Bamberger *et al.*, 1996; Kudielka & Kirschbaum, 2005; Tsigos & Chrousos, 2002). Cortisol also regulates other systems, including the sympathetic-adrenal-medullary (SAM) axis and the cardiovascular system (Kudielka & Kirschbaum, 2005).

The perception of an acute threat initiates the secretion of cortisol through a cascade of hormones in the HPA axis. This cascade commences with the release of CRH from the hypothalamus, which in turn triggers the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary gland, terminating with the release of cortisol from the adrenal cortex (Clow, Thorn, Evans, & Hucklebridge, 2004).

Importantly, unlike other indices of HPA axis function, cortisol can be reliably measured in saliva, levels of which are relative to those found in plasma. As such the measurement of cortisol in saliva provides a non-invasive index of HPA axis activity (Wetherell *et al.*, 2006). Although considerable research exists on the causes and correlates of changes in neuroendocrine HPA axis activity, research regarding its role in the stress - sleep relationship is still in its infancy. More specifically, the cortisol awakening response (CAR), which refers to the sharp increase in cortisol levels observed immediately following awakening, provides an ideal parameter for the study of sleep and wakefulness as it is a reliable marker of the circadian system – one of the processes involved in sleep-wake regulation (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010; Kudielka, Hellhammer, & Wüst, 2009; Morris, Aeschbach, & Scheer, 2012; Wetherell *et al.*, 2006).

2.2. Patterns of cortisol secretion

The suprachiasmatic nucleus (SCN), the body's central pacemaker, is responsible for the overall co-ordination of the HPA axis and links between the SCN and paraventricular nucleus of the hypothalamus (PVN) synchronise the time of day with neuroendocrine output (Buijs, van Eden, Goncharuk, & Kalsbeek, 2003).

Interestingly, the SCN pathway regulates the secretion of melatonin (Claustrat, Brun, & Chazot, 2005). The secretion of hormones in the HPA axis are subject to a regulatory negative feedback loop, in that the release of cortisol signals the inhibition of ACTH from the pituitary gland and subsequent inhibition of cortisol. A number of brain regions appear to be involved in the regulation of the HPA axis, including the hypothalamic sub-regions and the thalamus which connects to the PVN (Fries *et al.*, 2009). Other regions indirectly mediate HPA axis activity, including the hippocampus, amygdala and prefrontal cortex (J. P. Herman, Ostrander, Mueller, & Figueiredo, 2005), however the specific influence of these regions and of those which directly innervate the PVN upon the CAR are unknown (Fries *et al.*, 2009).

In addition to its response to acute stressors, cortisol secretion fluctuates according to a marked circadian pattern, as shown in Figure 2.1. Over the course of a typical day, levels of cortisol increase during the hour post-awakening, followed by a steep decline over the next three hours after awakening. This is then followed by a more gradual decline over the remainder of the day, reaching the lowest point during the first half of the sleep period (Fries *et al.*, 2009). During sleep, cortisol levels remain low and then rise again until morning awakening affording the production and regulation of melatonin (Edwards, Evans, Hucklebridge, & Clow, 2001; Fries *et al.*, 2009; Wilhelm, Born, Kudielka, Schlotz, & Wust, 2007).

Diurnal cortisol levels are a useful and important measure of HPA axis activity throughout the day and can provide information regarding the degree of change in cortisol levels from early morning to late evening, which typically show a decline (E. K. Adam & Kumari, 2009). Generally, a steeper diurnal decline is associated with better psychosocial and physical health and flatter slopes have shown an association with a greater number of adverse health outcomes and earlier

mortality (E. K. Adam & Kumari, 2009; Saxbe, 2008). Additionally, the diurnal decline shows a relationship with chronic stress, where chronic stress is associated with a flatter diurnal cortisol slope (G. E. Miller, Chen, & Zhou, 2007). Typically, the cortisol awakening response (detailed in Section 2.3) is not included when the diurnal cortisol slope is calculated (E. K. Adam & Kumari, 2009). Pre-sleep cortisol levels, which are generally measured at bedtime, are also an important measure of HPA axis activity since they are typically assessed as part of the diurnal slope. High levels of cortisol at bedtime can lead to flatter diurnal cortisol slopes (E. K. Adam & Kumari, 2009). However, the assessment of diurnal cortisol can place a high burden on participants and compliance is important (Saxbe, 2008).

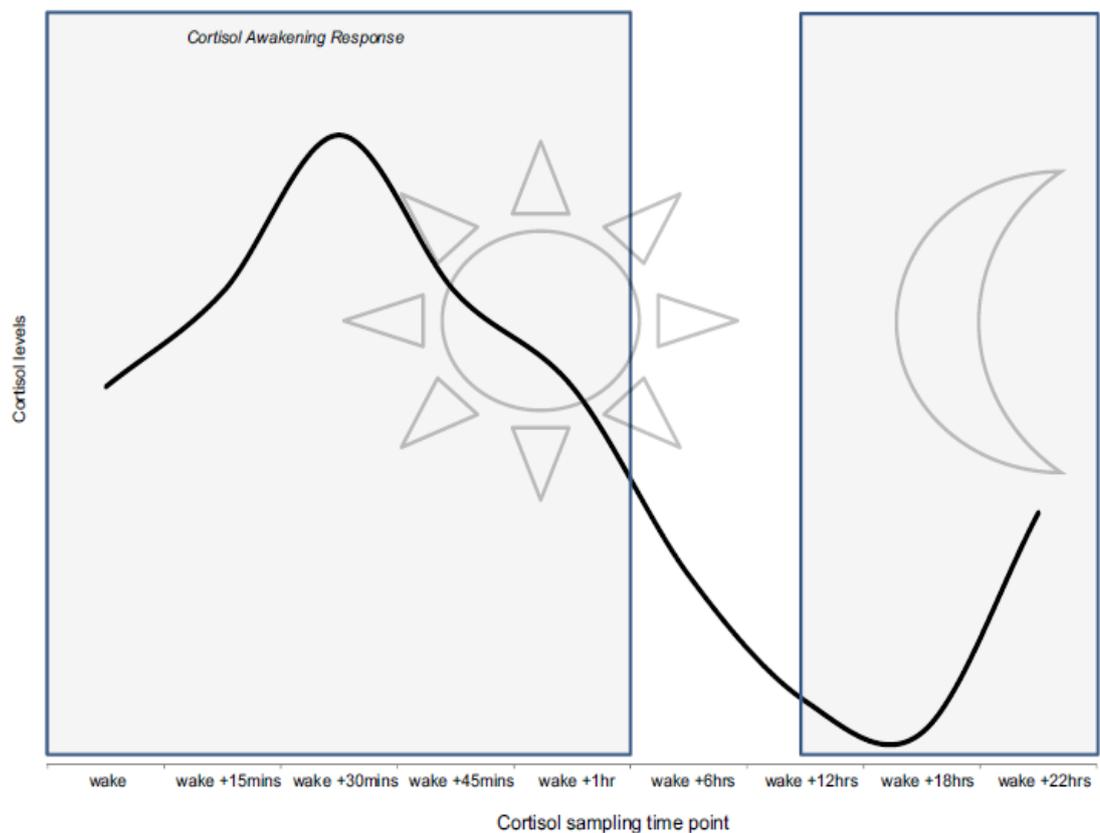


Figure 2.1: Typical cortisol awakening response (CAR) and diurnal cortisol profile over a twenty-four hour period in healthy individuals. The first shaded area represents the cortisol awakening response and the second shaded area represents cortisol levels during sleep.

2.3. The cortisol awakening response

The CAR refers to the sharp increase in cortisol levels observed immediately following awakening (shown as the first shaded area of Figure 2.1). The CAR is a distinct aspect of the diurnal cortisol profile and is considered a genuine response to awakening (Buckley & Schatzberg, 2005; Edwards *et al.*, 2001; Fries *et al.*, 2009; Schmidt-Reinwald *et al.*, 1999; Wilhelm *et al.*, 2007). During the CAR phase, cortisol levels increase by anywhere between 38%–75% compared to awakening levels, peaking approximately 30–45 minutes post-awakening (Clow *et al.*, 2004; Fries *et al.*, 2009). Whilst the CAR is dependent upon the transition from sleep to wake (Wilhelm *et al.*, 2007), it appears to be significantly larger following morning awakenings compared to afternoon awakenings (Griefahn & Robens, 2008; Wilhelm *et al.*, 2007). Interestingly, the CAR has been shown to be absent after an evening nap, suggesting both a role for the circadian rhythm of HPA axis in relation to the CAR, and a potential influence of light during morning awakening (Federenko *et al.*, 2004). The CAR is typical in healthy adults, demonstrable in approximately 73%–77% of individuals, and has been shown to exhibit a moderate to high level of intra-individual stability across two consecutive sampling days ($r = .63$) (Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004; Wüst *et al.*, 2000b). Despite this level of stability, daily fluctuations in the CAR have been associated with a range of psychosocial factors (E. K. Adam, Hawkley, Kudielka, & Cacioppo, 2006; Chida & Steptoe, 2009). Intriguingly, negative CARs, characterised by a decrease in levels of cortisol from awakening to post-awakening, have also been observed (Eek *et al.*, 2006). Although observed, it is possible that negative CARs occur due to methodological errors, including poor adherence to the sampling protocol, rather

than psychosocial influences (Wüst, Federenko, Hellhammer, & Kirschbaum, 2000a).

2.3.1. *Measurement indices of the CAR*

The CAR can be quantified and analysed in several ways. For example, at specified sampling time points (Backhaus *et al.*, 2004; Junghanns, Horbach, Ehrental, Blank, & Backhaus, 2007), or averaged cortisol values over the whole CAR period (Dockray & Steptoe, 2011). Other techniques involve examining the total cortisol secretion over the CAR period, typically expressed as arbitrary units, using the area under the curve (AUC) (Wüst *et al.*, 2000b). This method is, however, only appropriate when three or more saliva samples from the CAR period are available (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). The AUC of the CAR can be calculated in two ways. Firstly, AUC can be examined in relation to zero (area under the curve with respect to ground: AUC_G) which provides a measure of total cortisol output over the entire CAR period (Clow *et al.*, 2004) (in this case the first and last CAR samples have a smaller contribution than other values) (Pruessner *et al.*, 2003). Secondly, AUC_I is calculated with respect to increases between the first CAR sample and all subsequent samples, providing a measure of the total cortisol secretion over the CAR period (Clow *et al.*, 2004; Edwards *et al.*, 2001; Pruessner *et al.*, 2003). The area under the curve with respect to increase (AUC_I) identifies changes in the CAR over time, however, as would be expected, is particularly sensitive to the first awakening sample of the CAR (Clow *et al.*, 2004). The CAR dynamic (i.e. the magnitude of change from awakening) can also be assessed and reported. For example, peak change levels can be assessed by calculating the difference between cortisol levels at awakening (i.e. the first sample

of the CAR) and the peak cortisol levels observed during the post-awakening CAR period (E. K. Adam *et al.*, 2006; Dockray & Steptoe, 2011). Alternatively the mean increase during the CAR period with respect to the first awakening sample (known as the mean increase or MnInc) can be calculated based on the average of all post-awakening samples (Wüst *et al.*, 2000b).

Each measure of the CAR is different and the choice of index should reflect the number of sampling points during the CAR period; however, measures of overall cortisol secretion and the dynamic of the response are typically reported (Clow *et al.*, 2004). In order to provide a comprehensive overview of the CAR in relation to the research question throughout the thesis, several indices will be used within analyses. These indices will include: cortisol levels at each sampling point, awakening values, total secretion during the CAR period (AUC_G) the peak value within the CAR and the dynamic of the response (peak change).

2.4. Potential functions of the CAR

Despite increasing numbers of studies focusing on the CAR, its precise function and purpose remains unclear (Clow *et al.*, 2010). It has been suggested that the CAR has potential roles within the processes of arousal, energy boost, and/or anticipation.

2.4.1. Termination of sleep inertia and arousal

Acute administration of cortisol can reduce feelings of fatigue, thus demonstrating its bioenergetic effects upon psychological functioning (Tops, Van Peer, Wijers, & Korf, 2006). The typical incline of cortisol over the CAR period corresponds to increasing alertness over the period of sleep inertia, with cortisol

levels peaking with the timing of full alertness (Ikeda & Hayashi, 2008). The duration and severity of sleep inertia is, however, dependent on a number of other factors, including sleep duration, stage of sleep prior to awakening, time of day, and sleep deprivation, suggesting that these factors should be considered when assessing the relationship between the CAR, sleep inertia and stress-related sleep loss (Tassi & Muzet, 2000). The proposed relationship between the CAR and sleep inertia therefore suggests that the CAR may help to regain arousal upon awakening. In support, associations between indices of the CAR and arousal have previously been demonstrated. For example, levels of self-reported arousal are positively related to the CAR, and a change in levels of self-reported arousal has been positively associated with changes in MnInc levels (Thorn, Hucklebridge, Esgate, Evans, & Clow, 2004; Thorn, Hucklebridge, Evans, & Clow, 2009). Arousal-related performance has also been associated with the CAR. In one study, responsiveness, as measured by an auditory reaction time task, increased 15 minutes post-awakening in line with post-awakening increases in cortisol initially and continued in a dose-response manner thereafter (Ikeda & Hayashi, 2008). In contrast, lower levels of cortisol have been associated with perceived fatigue in older adults and subjective sleepiness and exhaustion in a sample of academics (E. K. Adam *et al.*, 2006; Dahlgren, Kecklund, Theorell, & Åkerstedt, 2009). Overall, the evidence points to a relationship between the CAR, the end of sleep inertia, and increased arousal.

2.4.2. *Adaptation and anticipation*

There is evidence that the CAR is an adaptive mechanism, either in aiding recovery from prior negative experiences and/or preparing the organism for forthcoming demands (Lovell, Moss, & Wetherell, 2011). In support of the recovery

hypothesis, subjective feelings of loneliness, sadness, threat and lack of control immediately before bed have been associated with a greater CAR the following day in a sample of older adults (E. K. Adam *et al.*, 2006). In the same sample, self-reported fatigue was also associated with awakening cortisol levels but only on the first morning of sampling. The authors conclude that changes in the CAR occur in relation to the level of perceived challenge, demonstrating day-to-day variability (i.e. a state rather than trait adaptation process).

The relationship between the anticipation of forthcoming events and CAR indices has also been examined. CARs, as determined by the mean increase between awakening and 30 minutes post-awakening, have been shown to be approximately three times greater on work days, characterised as more demanding, than non-work days (Kunz-Ebrecht *et al.*, 2004; Thorn, Hucklebridge, Evans, & Clow, 2006).

Similarly, higher levels of cortisol following awakening have been observed on workdays in those who report greater levels of perceived worry and chronic work overload (Schlotz, 2004), and greater MnInc levels have been observed on two consecutive work days as compared to levels during non-work-days (Thorn *et al.*, 2006). Further, Schlotz and colleagues (2004) observed weekday/weekend differences in the CAR measured over six consecutive days, characterised by a steeper rise to peak levels and higher subsequent cortisol levels at each sampling point on weekdays compared to weekends (Schlotz, 2004).

Similar findings have been observed in relation to differing types of background stress. For example, in a sample of newly qualified doctors, greater CARs were observed at the beginning of a clinical rotation, characterised by greater uncertainty, perceived lack of control and greater perceived workload, compared with the end of

a rotation whereby perceived control and certainty had increased (Brant, Wetherell, Lightman, Crown, & Vedhara, 2010). Further evidence for the role of the CAR as an anticipatory mechanism is provided by Rohleder and colleagues (Rohleder, Beulen, Chen, Wolf, & Kirschbaum, 2007) who observed higher awakening cortisol levels on the day of a ballroom dancing competition compared to a practice day, despite similar levels of physical exertion. Competition studies have also shown effects upon the CAR, where cortisol levels were increased 30 minutes following awakening in tennis players and in motorcycle riders on the first day of a competition, compared to a rest day without competition (Filaire, Alix, Ferrand, & Verger, 2009; Filaire, Filaire, & Le Scanff, 2007). Judo athletes have also shown increased cortisol levels at awakening on the morning of regional and inter-regional competition when compared to a resting day three weeks prior to competition (Filaire, Sagnol, Ferrand, Maso, & Lac, 2001).

Finally, days off from work, associated with reduced demand and expectancy, are related to negative CARs, where cortisol levels decrease after awakening (Eek *et al.*, 2006). Together, these studies demonstrate the adaptive nature of the CAR in relation to salient forthcoming challenges. Further, Hellhammer and colleagues (Hellhammer *et al.*, 2007) conclude that the measurement of the CAR on any specific day is subject to the influence of greater situational than trait factors. As such, the CAR should be measured on two or more consecutive days, and work days should be distinguished from non-work days.

In support are findings from a novel methodology involving a single case study over a period of 50 consecutive days (Stalder, Evans, Hucklebridge, & Clow, 2010a). Approximately 22% of the intra-individual variance in total cortisol secretion, over the CAR period, was explained by subjective levels of mood and

daily anticipation of activities and obligations. Together, these findings provide a compelling case for the CAR serving as a potential marker of anticipation.

Furthermore, the role of the hippocampus in HPA axis function and its involvement in the processing of space, time, environmental cues and memory consolidation, provides a potential neurological basis for this proposed preparatory function (Fries *et al.*, 2009; Sweatt, 2004).

2.5. Sleep and the CAR

In order to further understand the links between the CAR and sleep, it is necessary to investigate potential regulatory processes which influence cortisol secretion prior to initiation of the CAR (Clow *et al.*, 2010). In particular, the specific features of sleep including its duration, number and level of awakenings have been investigated in relation to the CAR.

2.5.1. Sleep duration, sleep disturbances and sleepiness

The relationship between sleep duration and the CAR has been examined, using both subjective and objective measures, albeit with mixed findings. Interestingly, the strength and direction of these findings appear to be influenced by measurement timings and CAR reporting indices. Self-reported short sleep duration has been associated with a steeper rise in cortisol from awakening to 30 minutes post-awakening in a large population ($n = 2,751$) of middle-aged adults (Kumari *et al.*, 2009). Moreover, Stalder and colleagues (Stalder, Hucklebridge, Evans, & Clow, 2009) showed a link between self-reported sleep duration and the CAR across 50 measurement days. Awakening cortisol levels (i.e. the first CAR sample) were

positively related to sleep duration and time of awakening, although this was not the case for the AUC_G underscoring the importance of the measurement indices used.

Contrastingly, Zhang and colleagues found no differences in three-day awakening cortisol levels between short and long sleepers (assessed by actigraphy) (Zhang *et al.*, 2011). That said, when assessing actigraphically measured time in bed rather than sleep duration, differences between short and long sleepers in CAR indices did emerge. Lower cortisol levels at awakening were observed in those with a ‘shorter’ time in bed (conceptualised as < seven hours), compared to those with a ‘normal’ or ‘longer’ time in bed (both conceptualised as > seven hours). A systematic review of 23 studies examining the relationship between subjective and objective sleep duration and the CAR confirms this relationship between longer sleep duration and increased cortisol levels at awakening although as the relationship with time in bed was unreported it is difficult to disentangle the relationship between sleep disturbance and the CAR (Garde, Karlson, Hansen, Persson, & Åkerstedt, 2012). However, a negative correlation, albeit small, between self-reported sleep duration and the mean increase during the CAR ($r = -.16$) has also been observed (Wüst *et al.*, 2000b).

Confusingly, other studies have failed to find associations between self-reported as well as actigraphically measured sleep duration and the CAR. For example, one study assessed the relationship between the CAR peak change at awakening, 20 minutes post-awakening and 40 minutes post-awakening, with sleep problems over a three-month period (Hansen *et al.*, 2012). Self-reported sleep duration was not related to the CAR (either in terms of the maximum increase in levels from awakening to either the second or third sample, or in terms of the AUC). Finally, Stalder and colleagues found no relationship between the CAR awakening

sample or AUC_G and sleep duration using actigraphy (Stalder, Evans, Hucklebridge, & Clow, 2010b).

It is conceivable that the relationship between sleep duration and the CAR is mediated by stress as opposed to individual differences in sleep need, which could account for the inconsistency in the findings presented above. However, Dahlgren and colleagues (Dahlgren *et al.*, 2005) examined work stress in relation to subjective and objective measures of sleep and the CAR. Office workers provided cortisol samples at 15 minutes post-awakening during one work day and during one non work-day, once during a working week of high stress and once during a working week of low stress, based on their predicted workload. During the high stress week participants reported more problems falling asleep, and also reported feeling more subjectively sleepy at the end of the day. Furthermore, actigraphy indicated that less sleep was obtained during the high stress week and during work days. However, there were no differences in cortisol levels at 15 minutes post-awakening between the high and low stress weeks, or between a work day and a non-work day.

These studies would have benefited from a more comprehensive assessment of the CAR through additional sampling points; however, these discrepant findings do suggest that the relationship between sleep duration and the CAR is complex. This also raises the possibility that perhaps the association between stress and the CAR may in fact be influenced by sleep disturbances, rather than duration per se. For example, in one study, self-reported sleep disturbances were related to both the maximum increase and the AUC during the CAR period; and frequency of awakening problems and exhaustion upon awakening were associated with the maximum increase (Hansen *et al.*, 2012). That said, as there are few studies examining sleep disturbances in relation to the CAR, further research is needed,

including the objective monitoring of sleep and multiple sampling points in the CAR period, in order to clarify the inter-relationships between the CAR, stress, sleep duration and disturbances.

The relationship between subjective sleepiness and the CAR has also been assessed. A sample of office workers were followed over four consecutive weeks, where participants provided cortisol samples at awakening and 15 minutes post-awakening. After controlling for individual variation, higher levels of subjective sleepiness on awakening and reported levels of exhaustion from the previous day were associated with lower levels of cortisol at awakening. Moreover, high levels of subjective sleepiness and anxiety at awakening were associated with lower levels of cortisol 15 minutes post-awakening (Dahlgren *et al.*, 2009). However, the use of additional sampling points would have allowed for the calculation of a measure of total secretion during the CAR period and provide a more comprehensive overview of the relationship between subjective sleepiness and the CAR.

2.5.2. Effects of nocturnal awakenings, morning awakenings and awakening time

Disturbed sleep through forced nocturnal awakening appears to have little effect upon the CAR in healthy individuals (Dettenborn, Rosenloecher, & Kirschbaum, 2007; Hucklebridge, Clow, Rahman, & Evans, 2000). For example, a single forced nocturnal awakening did not have an appreciable effect upon the CAR compared to a night of normal sleep (Hucklebridge *et al.*, 2000). Moreover, no differences were observed in the CAR in participants who were awoken three times during the night by telephone calls compared to non-interrupted nights (Dettenborn

et al., 2007). That said, as CAR assessments were only taken 15 minutes post-awakening, it is possible that the impact of the awakenings might have occurred later in the CAR period. The mode of awakening also appears not to affect the CAR, as no differences in CAR indices were observed between individuals who woke up naturally or those who used an alarm clock and these findings were mirrored in a single-subject study over multiple days (Stalder *et al.*, 2010a).

However, unexpected awakenings do appear to influence the CAR. In one study participants were informed that they would have a short sleep (awakening at 06:00h), or a long sleep (awakening at 09:00h), before lights out (Born, Hansen, Marshall, Molle, & Fehm, 1999). Greater increases in cortisol, in terms of cortisol concentrations compared before and after awakening, were observed following an unexpected awakening (awakening at 06:00h during an anticipated long sleep) compared to an expected awakening time. However, the time at which post-awakening samples in this study were obtained is unclear, thus limiting the interpretation of these findings. A more comprehensive assessment of the CAR is needed in order to determine the direct effects of expected vs. unexpected awakening upon HPA axis function, and it is possible that any effects upon the CAR are also driven by architectural properties of the prior sleep period.

The time of awakening appears to have an impact upon the CAR. Thorn and colleagues showed that individuals who woke at an earlier time displayed greater AUC and a steeper increase in cortisol during the CAR period compared to those who woke later (Thorn *et al.*, 2006). Another study confirmed this finding in that earlier awakening times (04:54h to 08:03h) were associated with higher awakening cortisol levels. As awakening time is typically linked to sleep duration, this finding may be a potential artefact of sleep duration. That said, two studies have reported

negative associations between awakening time and cortisol after controlling for sleep duration (Edwards *et al.*, 2001; Kudielka & Kirschbaum, 2003). However, no effects of awakening time on dimensions of the CAR have been observed elsewhere (Kunz-Ebrecht *et al.*, 2004; Pruessner *et al.*, 1997).

2.5.3. Relationships with sleep architecture

The relationship between sleep architecture and the CAR is unclear. The duration of REM sleep has been shown to negatively relate to cortisol levels measured immediately at awakening (Junghanns *et al.*, 2007). That said, as this sample were a group of alcohol-dependent inpatients, generalizations to other populations are difficult. One study of caregivers and non-caregivers, demonstrated that overall lower awakening cortisol levels were associated with increased percentages of Stage 1, Stage 3 and REM (Fonareva, Amen, Zajdel, Ellingson, & Oken, 2011). Further, in a sample of veterans with PTSD and a sample of healthy controls, no measures of sleep architecture were related to the CAR across both groups, in terms of the AUC_G (van Liempt *et al.*, 2013). Considering these inconsistencies, especially within such diverse populations, the specific relationship between the CAR and sleep architecture needs to be thoroughly investigated in normal sleepers before being extended to other populations. Additionally, it is difficult to determine whether changes to the HPA axis alter sleep architecture, or whether it is sleep architecture that drives HPA axis activity. It is possible that this relationship is bi-directional, since the administration of cortisol appears to increase slow-wave sleep (SWS) and decrease REM sleep; whilst it has also been shown that the number of episodes of SWS and REM sleep are both related to decreases in

nocturnal cortisol levels (Follenius, Brandenberger, Bandesapt, Libert, & Ehrhart, 1992; Steiger, 2002).

2.6. Changes to sleep

2.6.1. Shift patterns

Shift work results in significant alterations in a range of sleep parameters, all of which have also been associated with modifications to the CAR. In line with the observed relationship between early awakening and an increased CAR, early shift patterns have been associated with greater CARs compared to working late or night shift patterns in nurses (Federenko *et al.*, 2004). Public transport workers also showed a greater CAR on early shifts compared to either day shifts or days off, both in terms of the MnInc and AUC_I. Interestingly, these differences in the CAR were no longer apparent when controlling for perceived levels of stress and perceived sleep quality (Williams, Magid, & Steptoe, 2005). Abrupt changes in shift patterns also appear to influence the CAR, for example, one study assessed the CAR in permanent day-shift and night-shift employees after changing to a schedule of quickly-rotating mornings, evenings and night-shifts (Kudielka, Buchtal, Uhde, & Wüst, 2007). Although the CAR was still observable across the groups, day workers shifted to permanent night-shift displayed more blunted CARs (i.e. a reduced magnitude of response in cortisol levels from awakening to peak). It is difficult to determine whether shift work has a direct effect upon the HPA axis and the CAR, or whether subsequent effects upon the HPA axis are mediated by changes in sleep. Sleep disturbances are commonly observed as a result of shift work, and the level of disturbance resulting from irregular work hours is similar to the level of disturbance

observed in those with insomnia (Åkerstedt, 2003). Further research will be needed to examine this relationship after controlling for the sleep disturbances apparent in shift work.

2.6.2. *Sleep deprivation*

Attempts have been made to assess the effects of both sleep restriction and deprivation on HPA axis function and the diurnal rhythm of cortisol, with mixed results. Following a four-day baseline period (eight hours of sleep per night), an enforced sleep restriction of two hours per night for a period of eight days (six hours of sleep per night) resulted in reduced post-awakening peak cortisol responses (Vgontzas *et al.*, 2004). Contrastingly, Voderholzer and colleagues found no effects of different amounts of sleep restriction (varying from five to nine hours) on the AUC during the CAR period in a group of adolescents (Voderholzer *et al.*). This raises the issue of age-related changes in the CAR which will be considered later. In terms of sleep deprivation, no differences were observed in the absolute cortisol levels of individuals experiencing partial (four hours sleep per night) and total sleep deprivation over a 32-hour period when compared with those maintaining a normal sleep/wake schedule (Leproult, Copinschi, Buxton, & Van Cauter, 1997). Likewise, Cote and colleagues (2013) found no differences in awakening cortisol levels after a normal night of sleep compared to sampling at the same time following a night of sleep deprivation. However, it is possible that any potential effects of sleep deprivation on the CAR may have occurred later in the CAR phase, since only a single awakening sample was collected. Taken together, these studies suggest that sleep restriction and partial or total sleep deprivation have little impact upon cortisol levels during the CAR period. The latter finding is somewhat surprising, given that

the CAR is considered to occur exclusively as a response to awakening. Further research using more a rigorous protocol is therefore required in order to clarify the effects.

2.7. Methodological considerations

The CAR, given its finite sampling period, is particularly sensitive to the influence of a range of methodological factors that should be carefully considered both in interpreting the existing literature and in the design of future studies.

2.7.1. Age, sex differences and menstrual cycle phase

The CAR has been observed in infants as young as two months of age, which coincides with the emergence of circadian rhythmicity (Stalder *et al.*, 2013). More generally, HPA axis activity has been shown to decline across the lifespan, although this is not consistently agreed upon (Hatzinger, Brand, Herzig, & Holsboer-Trachsler, 2011). Two cross-sectional studies observed no differences in CAR indices, including the AUC and mean increase, between children, younger, and older adults (Pruessner *et al.*, 1997; Wüst *et al.*, 2000b). However, other studies suggest the association between the CAR and age is apparent but more complex. For example, one study demonstrated a weak positive relationship between age and cortisol levels immediately after awakening ($r = .20$), in a sample aged between 4–75 years, but a weak negative correlation with the total amount of cortisol secreted during the CAR period ($r = -.20$) such that older individuals show greater cortisol levels on awakening, yet lower levels of secretion during the CAR period (Kudielka & Kirschbaum, 2003). Furthermore, in a sample of older adults aged from 60 - 91

years, increasing age was weakly associated ($r = -.28$) with a reduction in the rise in cortisol levels from awakening to the peak response (Evans *et al.*, 2011). Overall, it would appear that the impact of age upon the CAR, if any, is minimal.

Some studies have reported sex differences in the CAR. For example, during workdays females have been shown to display a greater increase in cortisol levels between awakening and 30 minutes post-awakening, compared to males (Kunz-Ebrecht *et al.*, 2004). Despite this, others have failed to observe any differences in CAR indices between males and females (Kudielka & Kirschbaum, 2003). Overall, the effects of sex appear to be minimal, and explain only between one to three per cent of the variability in levels of cortisol secretion during the CAR (Fries *et al.*, 2009; Pruessner *et al.*, 1997). However, sex differences in the CAR have been observed in the presence of illness. Bengtsson and colleagues (Bengtsson *et al.*, 2010) measured the CAR in healthy controls and individuals with metabolic syndrome. No sex differences were apparent in healthy controls, however in individuals with metabolic syndrome, women displayed a greater relative CAR than men. These apparently contradictory findings in relation to sex differences could be explained by menstrual cycle phase. One study showed that the increase in cortisol levels during the CAR, in terms of the difference between the maximum cortisol value and cortisol levels at awakening, was elevated during ovulation (Wolfram, Bellingrath, & Kudielka, 2011). However, other studies report no effects of cycle phase upon the CAR (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999; Kudielka & Kirschbaum, 2003). Another study investigated the effect of contraceptive use on the CAR and found that the effects were minimal, accounting for approximately four per cent of variability (Pruessner *et al.*, 1997). As such the evidence of sex difference on the CAR appears equivocal at best.

2.7.2. Adherence

Many of the inconsistencies observed in CAR studies, the majority of which are performed in ambulatory settings, may be attributed to methodological issues, specifically pertaining to adherence to sampling protocols. It is possible that reliance upon self-sampling at the required times could lead to difficulties. A failure to comply with correct sampling times can lead to invalid results, or at worst, data that could be misinterpreted. For example, negative CARs, characterised by a decrease in levels of cortisol from awakening during the CAR period are associated with days off from work (Eek *et al.*, 2006). This association may be valid, however, it may also reflect poor adherence to the timing of sampling protocol during the CAR period. That is delays between awakening and the collection of the awakening sample are more frequent in those showing negative CARs (Eek *et al.*, 2006). Adherence is particularly important during the CAR period following the awakening sample. The rate of increase of cortisol levels is typically between 38%-75% in this time, although increases greater than 100% have also been shown, and any deviation from the required sampling times can therefore significantly increase measurement error (Clow *et al.*, 2004).

Techniques can be employed to provide more objective checks on levels of adherence to protocols in ambulatory settings. For example, some studies employ the use of devices such as Medication Events Monitoring System (MEMS) track caps, which time-stamp the opening of saliva collection bottles and therefore log sampling times (Kudielka, Hawkey, Adam, & Cacioppo, 2007). One study which measured the CAR at wake and 30 minutes post-awakening using MEMS observed an adherence rate of 74% (Kudielka, Broderick, & Kirschbaum, 2003). Of the non-compliant participants, the majority (82%) failed to collect two or more samples at

the required time, significantly impacting the subsequent CAR data. Specifically, the net increase in cortisol levels from awakening to 30 minutes post-awakening was more than three times greater in adherent participants. Whilst adherent participants showed a typical increase from awakening to 30 minutes post-awakening, non-adherent participants showed a blunted CAR that was directly related to increasing delays in sampling. As the CAR typically peaks approximately 30 minutes following awakening, any delays in the collection of this first awakening sample will distort the CAR, typically resulting in a flattening of the peak response relative to awakening and blunting the overall CAR (Griefahn & Robens, 2010; Thorn *et al.*, 2006). There is also evidence that self-reported awakening and sampling times are good indicators of adherence and are equally as reliable as electronic measures of compliance (Kraemer *et al.*, 2006; Okun *et al.*, 2010).

Actigraphy has also been used to compare self-reported awakening times with actual awakening times. Self-reported awakening times were accurate, with 92% showing a deviation of less than 15 minutes from the objective awakening times. That said, there was a trend towards reduced CARs in those with differences between objective and subjective awakening times of between five and 15 minutes (DeSantis, Adam, Mendelsohn, & Doane, 2010). Moreover, studies employing the use of polysomnography (PSG) have shown that delays of longer than 15 minutes between the time of awakening and the first awakening sample of the CAR can result in a reduced CAR indices (Okun *et al.*, 2010).

Adherence may also be an issue in terms of sample contamination. It is important that participants have nil by mouth, except water, at least 30 minutes prior to, and during, the CAR sampling period. In addition, participants must avoid smoking or brushing their teeth to avoid sample contamination through abrasion and

vascular leakage (Clow *et al.*, 2004). Rarely have these factors been reportedly assessed or controlled for.

2.7.3. Light

As the SCN is responsible for overall co-ordination of the HPA axis and receives light information from the retina (Buijs *et al.*, 2003) various studies have specifically examined the effects of light upon the CAR. Figueiro and Rea (2012) sampled cortisol at awakening and every 20 minutes thereafter between 06:00h and 07:20h in a sample of sleep-restricted adolescent participants (aged 12-17 years) who had slept for four and a half hours. Participants were either exposed to dim light of less than five lux or short wavelength blue light, immediately after the first awakening sample of the CAR phase. Those exposed to blue light displayed an enhanced CAR and a greater level of cortisol secretion throughout the CAR period, expressed as the AUC_I, compared to dim light-exposed participants.

A separate study also examined the effects of light in sleep-deprived participants. In this case participants experienced a baseline period of dim light of less than 150 lux and were gradually exposed to increasing bright light over a period of three hours, which was then reversed. This occurred either in the morning after 20 hours or 30 hours of constant wakefulness. A rapid elevation in blood cortisol levels was observed, but only when participants were exposed to light in the morning rather than in the afternoon (Leproult, Colecchia, L'Hermitte-Balériaux, & Van Cauter, 2001). Of note, participants were kept fully awake, and so would have been acutely sleep deprived when the light exposure occurred. This may have confounded cortisol production during the CAR period.

Following a normal sleep period, Thorn and colleagues (Thorn *et al.*, 2004) employed the use of a dawn simulation light device, which increased in intensity to levels of approximately 250 lux in the 30 minutes prior to awakening. Both an increased CAR and increased levels of total cortisol output in terms of the AUC_G were observed for the dawn simulation condition compared to normal awakening. Likewise, Scheer and Buijs (Scheer & Buijs, 1999) found that participants exposed to 800 lux of light during the CAR period using light goggles had increased cortisol levels at 20 minutes post-awakening and 40 minutes post-awakening compared to those who remained in darkness. Due to the potentially confounding effects of light, it will be extremely important to control for light during the measurement of the CAR.

2.8. Summary

The CAR is a useful marker of stress and does appear to show a relationship with measures of sleep, albeit with mixed findings. This is particularly apparent with regards to sleep duration and in order to clarify the relationships between the CAR, stress, sleep duration and disturbances a more comprehensive and rigorous measurement of the CAR will be necessary, alongside objective monitoring of sleep. This will also enable the relationships between measures of objective sleep architecture, sleep continuity and the CAR to be examined in more detail.

As the CAR may have a function relating to the anticipation of upcoming demand (Filaire *et al.*, 2009; Filaire *et al.*, 2007; Filaire *et al.*, 2001; Fries *et al.*, 2009; Rohleder *et al.*, 2007; Sweatt, 2004) and/or may function as a mechanism to assist the regaining of arousal upon awakening (Thorn *et al.*, 2004; Thorn *et al.*,

2009), the CAR will be an ideal physiological marker for examining the physiological effects of demand and the anticipation of demand.

However, there are potential factors which may impact upon the accurate measurement of the CAR, including light (Figueiro & Rea, 2012; Scheer & Buijs, 1999; Thorn *et al.*, 2004), unexpected awakenings (Born *et al.*, 1999), the time of awakening (Stalder *et al.*, 2009; Thorn *et al.*, 2006) and the deviation from required sampling times (Clow *et al.*, 2004). As the relationship between these variables and the CAR is complex and comparisons across studies are limited by differing measurement protocols and methods, a standard protocol for the measurement of the CAR will be necessary in order to assess the physiological effects of stress upon the CAR within the context of the thesis. The use of a consistent and controlled measurement protocol will also provide further information regarding the function of the CAR. The following section describes a suggested protocol for measurement of the CAR on the basis of previous studies.

2.9. Suggested standard measurement protocols

In order to assess the relationships between sleep factors and subsequent effects on the CAR, it is recommended that samples are measured immediately upon awakening and 15, 30, 45 and 60 minutes post-waking (Elder, Wetherell, Barclay, & Ellis, 2014). The use of these time points will allow for the assessment of an individual's peak response during the CAR period whilst providing sufficient samples to calculate total cortisol secretion (as measured by the AUC_I and AUC_G) and the mean increase in levels (MnInc) (E. K. Adam *et al.*, 2006; Clow *et al.*, 2004; Dockray & Steptoe, 2011; Pruessner *et al.*, 2003; Wüst *et al.*, 2000b). Despite the

moderate to high levels of intra-individual stability within the CAR, it is recommended that CAR measurements be taken on at least two consecutive days (Hellhammer *et al.*, 2007). The CAR of a single day is largely determined by situational factors and this will help assess and disentangle trait rather than state influences. Furthermore, sampling days should be typical in terms of activities, for example, protocols should account for differences observed in the CAR between work and leisure days (Kunz-Ebrecht *et al.*, 2004; Schlotz, 2004; Thorn *et al.*, 2006). Hansen and colleagues (Hansen, Garde, & Persson, 2008) note that researchers may wish to register and include the time of sampling within the statistical analysis of CAR data, or that samples are obtained at the same time each day in order to control for the diurnal variation in cortisol levels.

2.10. Aim of the thesis

The overall aim of the thesis is to assess the effects of stress upon subjective sleep, objective sleep and the CAR as physiological marker of HPA axis activity. Both the Spielman 3P and Cano-Saper models of insomnia (Spielman *et al.*, 1987; Spielman & Glovinsky, 1991; Spielman *et al.*, 1996) focus on the role of stress in the development of insomnia, however the effects of both naturalistic and experimental stressors, whilst disruptive to subjective and objective sleep, are largely inconsistent.

As there is no standardised method for assessing the effects of stress upon sleep, these inconsistencies may arise from the wide variation in the stressor employed, the diverse nature of the participants and the method employed to measure the effects upon sleep. Furthermore, naturalistic and experimental studies have also shown that the anticipation of stress can disrupt sleep however these results are also

inconsistent, where diverse stressors and measurement protocols have been used. Moreover, studies which have examined the effects of stress upon sleep have tended not to include a physiological marker of stress within the protocol, where it cannot be objectively confirmed that the stressor employed is stressful.

This thesis will therefore assess the effects of stress upon sleep by developing and testing a standardised measurement protocol. The protocol will measure the effects of stress upon subjective sleep, objective sleep and the CAR as an objective marker of HPA axis activity. As the anticipation of stress appears to disrupt sleep, the measurement protocol will tease apart the effects of anticipation. This will be done by measuring the effects of anticipation, or anticipation coupled with subsequent demand, upon subjective sleep, objective sleep and the CAR.

Chapter 3 of the thesis will propose and outline a standardised method for measuring subjective sleep, objective sleep and the CAR. Chapter 4 describes an initial feasibility study conducted in order to examine the feasibility of using the protocol within a home environment and within a laboratory environment, with the aim of informing the location for later studies where the protocol includes a stressor. Chapter 5a and 5b describe the use of this protocol within a laboratory environment, with the aim of examining the interactions between subjective sleep, objective sleep and the CAR. Chapter 6 extends the protocol by examining the effects of stress upon subjective sleep, objective sleep and the CAR, where the anticipation is either not met or is met with subsequent demand. Chapter 7 summarises the results of the thesis and provides a general discussion, where the results are discussed in relation to the Spielman 3P and the Cano-Saper models of insomnia. The following chapter outlines the standardised method for measuring the effects of stress upon sleep in the same context.

CHAPTER 3.

Method

3.1. Introduction

This chapter describes the standardised protocol developed in order to measure the effects of stress upon sleep, where subjective sleep, objective sleep and the CAR were measured. The standardised protocol was used within Study 1, which aimed to assess the suitability of the protocol and examine the relationship between subjective sleep, objective sleep and the CAR. The protocol was also used within Study 2, where the anticipation of stress was either met, or not met, with subsequent demand.

3.2. Participants and recruitment

Healthy, normal sleepers were recruited separately to Study 1 and 2 through emails sent to university staff and students. Potential participants were excluded if they were currently experiencing or if they had experienced any current sleep disorders or problems, on the basis of an initial screening questionnaire (Appendix A). Current smokers were not allowed to participate due to the negative effects of nicotine upon both subjective and objective sleep (Roehrs & Roth, 2011) and also to avoid any potential effects of cortisol sample contamination (Clow *et al.*, 2004). Participants were also excluded if they were over 40 years of age, as HPA axis activity has been shown to decline across the life span (Hatzinger *et al.*, 2011), if they reported a current complaint or a history of depression and/or anxiety, or if they reported the use of medication with the potential to affect HPA axis function. Participants were also unable to take part if they reported trans-meridian travel (i.e. travel across three or more time zones) in the preceding three month period in order

to control for any effects upon sleep, since symptoms of jet lag can persist for weeks after the flight (Drake & Wright Jr, 2011).

3.3. Measures

Participants completed measures of sleep quality, perceived stress in the past month, anxiety and depression, chronotype and vulnerability to stress-related sleep disturbances within Study 1 and Study 2.

3.3.1. Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI; Buysse *et al.*, 1989; Appendix B) is a self-rated questionnaire which assesses sleep disturbances over the past month. The PSQI consists of seven components, measuring subjective sleep quality, sleep latency, sleep duration, sleep efficiency and sleep disturbances, the use of any sleeping medication and the occurrence of any related daytime dysfunction (e.g. “during the past month, how would you rate your sleep quality overall?”). The PSQI gives seven component scores and these are summed to provide an overall score of between 0 and 21. The cut-off point for poor sleepers is a score of five, which provides a sensitivity of 89.6% and a specificity of 86.5% to correctly distinguish between good and poor sleepers. The PSQI is a reliable measure of sleep quality ($\alpha = .83$) (Backhaus, Junghanns, Broocks, Riemann, & Hohagen, 2002).

3.3.2. *Perceived Stress Scale*

The Perceived Stress Scale (PSS; S. Cohen, Kamarck, & Mermelstein, 1983; Appendix C) measures the extent to which life situations are perceived as being stressful and consists of 14 items (e.g. “in the last month, how often have you found that you could not cope with all the things that you had to do?”). These items are rated between zero (never) to four (very often). Total possible scores on the PSS range from zero to 56, with higher scores representing higher levels of perceived stress. The PSS has good reliability ($\alpha =$ approximately .85) (S. Cohen *et al.*, 1983).

3.3.3. *Hospital Anxiety and Depression Scale*

The Hospital Anxiety and Depression Scale (HADS; Zigmond & Snaith, 1983; Appendix D) is a self-reported measure of anxiety and depression. Participants are asked to respond to a series of statements (e.g. “I feel tense or wound up”) with one of four possible responses. Each response is scored from zero to three. The HADS consists of two separate seven-item components, measuring anxiety and depression. The HADS provides a total score of between zero and 21. For both subscales, a score ranging between zero and seven is considered to be normal; eight to 10 is considered to be borderline and 11 or above is considered to be abnormal. A meta-analysis found that the mean reliability of the anxiety component of the HADS to be $\alpha = .83$ and the mean reliability of the HADS depression component to be $\alpha = .82$ (Bjelland, Dahl, Haug, & Neckelmann, 2002).

3.3.4. *Horne-Östberg Morningness-Eveningness Questionnaire*

The Horne-Östberg Morningness-Eveningness Questionnaire (HÖ-MEQ; Horne & Ostberg, 1976; Appendix E) assesses self-reported chronotype, i.e. the preference for morningness or eveningness. The HÖ-MEQ consists of 19 items (e.g. “considering only your own ‘feeling best’ rhythm, at what time would you get up if you were entirely free to plan your day?”), which provide a total score ranging from 16 – 86. Higher scores indicate an earlier chronotype: ‘definitely evening’ (16-30), ‘moderately evening’ (31 - 41), ‘neither type’ (42 - 58), ‘moderately morning’ (59-69) or ‘definitely morning’ (70 – 86). The HÖ-MEQ has a high level of consistency ($\alpha = .82$) (Smith, Reilly, & Midkiff, 1989) and has been shown to negatively relate with bed times, wake times and the nadir for physical activity (Thun *et al.*, 2012). The HÖ-MEQ is a widely used measure and appears to be a good predictor of circadian period or circadian phase (Sack *et al.*, 2007).

3.3.5. *Ford Insomnia Response to Stress Test*

The Ford Insomnia Response to Stress Test (FIRST; Drake *et al.*, 2004; Appendix F) is a measure which assesses stress-related vulnerability to sleep disturbance. The FIRST consists of nine items which present hypothetical situations (e.g. “after a stressful experience during the day”), and individuals are asked how likely they are to experience difficulty sleeping under these situations. Respondents circle an answer on a one (not likely) to four (very likely) scale, with potential scores ranging from nine to 36. The FIRST has a good level of reliability ($\alpha = .83$) (Drake *et al.*, 2004).

3.3.6. *Perceived Stress Reactivity Scale*

As Study 2 of the thesis assessed the effects of stress upon sleep and the CAR, participants in Study 2 completed the Perceived Stress Reactivity Scale (PSRS; Schlotz, Yim, Zoccola, Jansen, & Schulz, 2011; Appendix G). The PSRS is a 23-item questionnaire which assesses perceived stress reactivity in a range of potentially stressful situations. Individuals are presented with a range of situations which they may have experienced in the past and are required to indicate which answer, from three possible responses, most closely matches their own response to that situation. The PSRS provides a total score ranging between zero and 46 which is given by the sum of the five subscales. Higher scores represent higher levels of reactivity to perceived stress.

3.4. Procedure

Participants who expressed an interest in each study completed a brief screening form prior to their enrolment which was returned by email (Appendix A). All participants completed a two-week baseline monitoring period immediately prior to each study, in order to ensure that they had a stable sleep/wake cycle and that they did not display any potential sleep disorders or evidence of circadian rhythm problems. Participants were monitored during this period through the use of sleep and mood diaries and actigraphy. All participants provided written informed consent at the time of enrolment. Study 1 and Study 2 were approved separately by the Northumbria University Faculty of Health and Life Sciences Research Ethics Committee. The protocol is summarised in Figure 3.1.

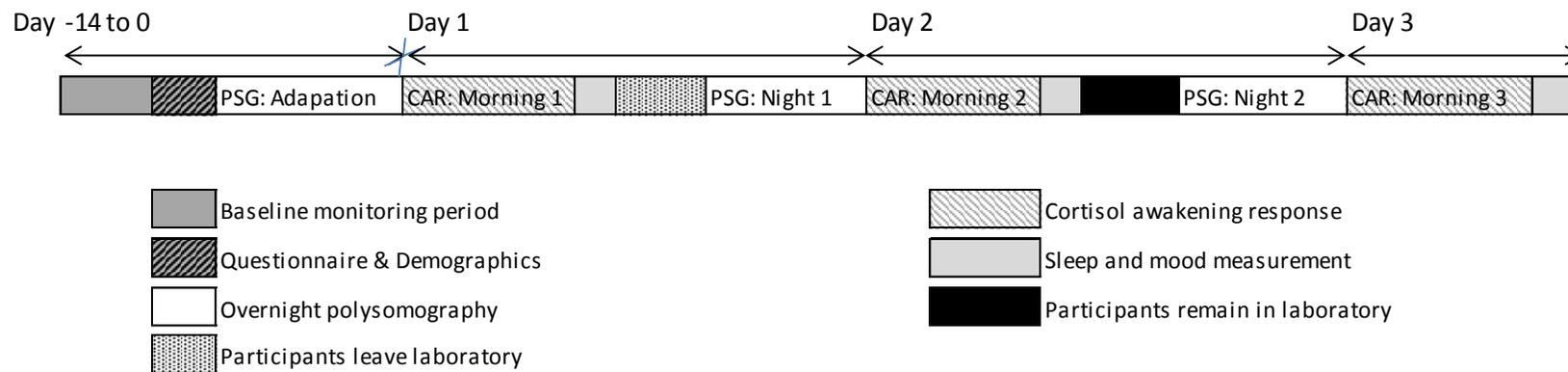


Figure 3.1: Study 1 protocol

3.4.1. Baseline monitoring period

Participants were required to complete a baseline monitoring period in the 14 days immediately prior to participation, where they were monitored using sleep and mood diaries and actigraphy. These are described below.

3.4.1.1. Sleep and mood diaries

Participants were provided with a sleep and mood diary (Appendix H) at the start of the baseline period and were instructed to complete this as soon as possible after awakening each morning. In the sleep diary, participants were required to record their waking time and the time at which they got out of bed each morning. Participants also recorded the time at which they went to bed the night before, and the time at which they turned out the lights to go to sleep the night before. Participants were also required to record how long it took them to fall asleep, how many times they woke up during the night, how long they were awake during the night, and estimated total sleep duration. From this information various sleep continuity parameters were derived. These parameters included total sleep time (TST), the total duration of time in bed (TIB), sleep efficiency (SE%), which refers to the percentage of time asleep from the amount of time spent in bed ($SE\% = TST / TIB \times 100$), wakefulness after sleep onset (WASO) which refers to the total duration of awakenings on each night and sleep-onset latency (SOL), which refers to the time taken to get to sleep from lights out (Buysse, Ancoli-Israel, Edinger, Lichstein, & Morin, 2006; Carney *et al.*, 2012). Participants were also asked to indicate whether or not they used any sleeping pills or other over-the-counter medication during the baseline period. Participants were included within the study if a subjective inspection of their completed baseline sleep diaries indicated no evidence of any irregular sleep-

wake patterns. The sleep diary also contained four questions asking about subjective sleep quality with each question being rated on a zero to four point Likert scale. These four questions were a) “how well do you feel this morning?” b) “how enjoyable was your sleep last night?” c) “how mentally alert were you in bed last night?” and d) “how physically tense were you in bed last night?”

The mood section of the diary presented participants with six statements (“I feel calm”; “I feel tense”; “I am upset”; “I feel relaxed”; “I feel content”; “I feel worried”) and asked them to mark how they felt at that particular moment on an 100mm visual analogue scale (VAS), where 0mm indicated ‘not at all’ for the relevant statement and 100mm indicated ‘very much’. These six statements map onto the six-item short-form version of the state scale of the Spielberger State-Trait Anxiety Inventory (STAI; Marteau & Bekker, 1992) which assesses state anxiety and indicates how an individual feels at that moment. The original short-form version of the STAI requires participants to respond using a Likert scale rated between zero and four. Participants could attain a maximum score of 300 on this version of the short-form STAI, with higher scores reflecting higher levels of state anxiety (positive items were reverse scored as in the original version). Participants were also presented with the statement (“I feel stressed”) to which they were required to respond to in the same manner, marking on a 100mm VAS.

3.4.1.2. Actigraphy

Sleep/wake activity of participants during the baseline period was also monitored using actigraphy. Actiwatches measure activity using a piezo-electric accelerometer. This records the intensity, amount and duration of movement in all

directions, and stores an activity count within the memory of the actiwatch. The recorded activity is used as a gross indicator of wakefulness and sleep. Actigraphy is considered to be a reliable measure of sleep and wake in healthy, adult populations (Ancoli-Israel *et al.*, 2003; Lichstein *et al.*, 2006). Actiwatches (AW4, Cambridge Neurotechnology, United Kingdom) were worn on the non-dominant wrist and activity was recorded in one-minute epochs using a medium sensitivity setting. Actigraphy was used to confirm the relative circadian stability of participants. This was confirmed through subjective inspection of baseline actograms, which provided an indication of individual sleep/wake cycles during the baseline period. Actigraphy data was not used in any further analyses.

3.4.2. Laboratory protocol

Participants arrived at the sleep laboratory between 8.00pm and 9.00pm on the adaptation night. The sleep laboratory (Northumbria Centre for Sleep Research; NCSR) consists of two separate, en-suite bedrooms with a kitchen, laboratory control room and a conference room/lounge, with the appearance of a comfortable home environment. Photographs and a diagram of the NCSR are included in Appendix I.

Participants slept for three consecutive weekday nights (Adaptation, Night 1 and Night 2) in the NCSR and remained in the sleep laboratory for a full day between Night 1 and Night 2. PSG was applied on each night in order to measure sleep). The CAR was measured on consecutive weekday mornings (Morning 1, Morning 2 and Morning 3) following each night of sleep. Saliva samples were obtained immediately upon awakening and at subsequent time intervals of 15

minutes until 60 minutes post-awakening. Participants remained in bed during the CAR collection period in low ultraviolet light of approximately one lux.

Immediately after the final sample lights were turned on and participants were required to complete a sleep and mood diary (identical to the diary completed during the baseline period) whilst still in bed. Participants were then allowed time to shower and dress before a further saliva sample was taken at 120 minutes post-awakening.

Participants were provided with breakfast, were instructed to adhere to their usual daily routine and allowed to leave the laboratory after this point on Morning 1.

Participants returned to the laboratory at the same time (between 8.00pm and 9.00pm) on Night 1 and PSG was applied as before. Participants remained in the sleep laboratory during Day 2. This was done in order to ensure participants remained in a stable, consistent environment with control over factors such as light and daytime activity levels.

Participants were not allowed to exercise in order to minimise potential effects upon daytime cortisol levels or upon sleep (Driver & Taylor, 2000; Kudielka *et al.*, 2009). Participants were allowed to perform activities including reading, watching television or films or use of the internet if completing Study 1 or the anticipation condition of Study 2 (detailed in Chapter 6). Saliva samples were obtained at time intervals of every 60 minutes (Study 1) or every four hours (Study 2) and participants were provided with meals at standard time points (2 hours, 6 hours and 10 hours post-awakening). Participants were allowed snacks if required during the day. Participants were asked to refrain from consuming caffeinated drinks after 6pm before visiting the laboratory on the adaptation night and on Night 1, as caffeine has a half-life of 3.5 to 5 hours and consumption has been shown to result in a delayed sleep onset (Nishino & Mignot, 2011). Participants were also not

allowed to consume caffeinated drinks during the laboratory stay between Night 1 and Morning 2. Participants were fully debriefed and thanked on Morning 3 following the pre-breakfast saliva sample. Participants were paid £150 upon completion of each separate study (Study 1 or Study 2).

3.4.3. Polysomnography

PSG was applied on each night (Adaptation, Night 1 and Night 2). Recording times were scheduled in accordance with habitual sleep/wake patterns from sleep diaries completed during the baseline monitoring period. Recording times matched habitual sleep/wake patterns as closely as possible. PSG included EEG electrodes placed at FP₁, FP₂, F₃, F₄, C₃, C₄, P₃, P₄, O₁, O₂, A₁, A₂ and C_z in accordance with the International 10-20 system (Jasper, 1958). The locations of the electrodes are indicated in Figure 3.2.

EMG measurements were taken from the chin using submental electrodes and EOG measurements were taken 1cm below and lateral to the outer canthus of the left and right eyes. Electrodes were referenced to linked mastoids with a forehead ground electrode placed at FPz. Impedance levels were maintained below 5 k Ω during the recording period. Additional EMG electrodes were placed on the left and right anterior tibialis muscles during the adaptation night in order to ensure that participants did not display any evidence of restless legs or excessive periodic limb movements. As participants were asked to detail any breathing or sleeping problems at the time of enrolment, breathing was not measured on the adaptation night.

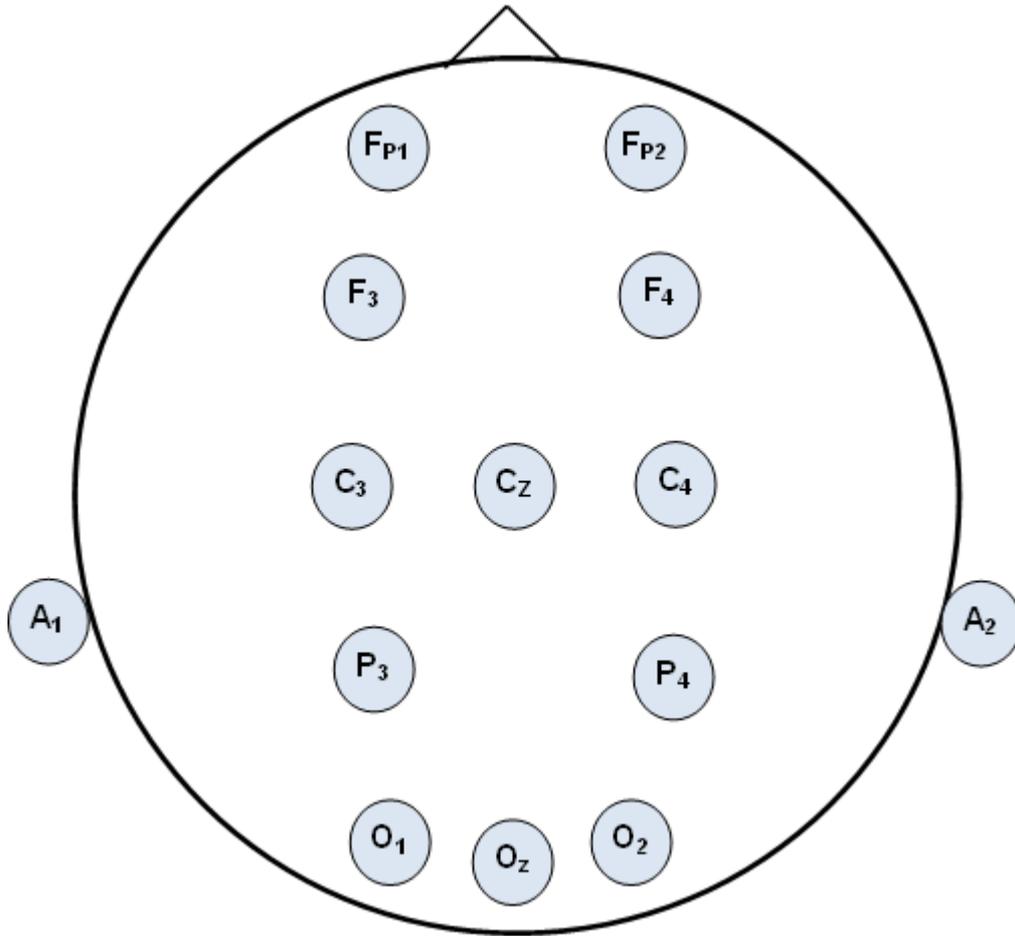


Figure 3.2: EEG electrode locations used in all studies. Fp = frontopolar, F: frontal, C = central, P = parietal, O = occipital. A1 and A2 represent mastoid electrodes. Figure adapted from Berry (2012, p. 66).

PSG was measured using a wireless, lightweight (weighing approximately 220g, including the battery) ambulatory polysomnography system worn by participants (SOMNOscreen, SOMNOmedics GmbH, Randersacker, Germany). EEG/EMG/ECG electrodes were connected to a head box and main interface unit, which was secured using adjustable Velcro straps. As the system was wireless, participants were able to move around the sleep laboratory freely with the PSG unit attached. PSG data was recorded using software (DOMINO, SOMNOmedics GmbH, Randersacker, Germany) at a sampling rate of 128 Hz for all EEG channels and 256Hz for EMG and ECG channels. All recordings were scored in 30-second epochs

in accordance with American Academy of Sleep Medicine guidelines (Iber *et al.*, 2007) by the same external scorer blind to the aims of each study. As PSG data obtained from healthy normal sleepers during the first night in a laboratory is typically altered, known as the ‘first-night effect’ (Agnew *et al.*, 1966), adaptation night data were not scored or used in any further analyses. For example, PSG data obtained during an adaptation night from healthy normal sleepers in a sleep laboratory environment typically shows alterations in REM sleep latency, increased levels of wake, a decrease in total sleep time and a reduction in sleep efficiency (Toussaint *et al.*, 1995).

Table 3.1:

Description of polysomnographically-measured sleep continuity and architecture parameters (based on Keenan & Hirshkowitz (2011))

Measure	Description
Total sleep time (TST)	The number of minutes scored as N1, N2, N3 or REM sleep
Wake time after sleep onset (WASO)	Minutes scored as wake from the first epoch of sleep to lights on.
Sleep efficiency (SE%)	Total sleep time (TST) as a percentage of total recording time (TRT) $((TST / TRT \times 100) = SE\%)$.
Time in N1, N2, N3 and REM (%)	Time scored individually as N1, N2, N3 and REM sleep as a percentage of total sleep time (TST).
REM, N1, N2 or N3 latency (mins)	The elapsed time from lights out to the first epoch of stage REM, N1, N2 and N3 sleep in minutes.
Sleep onset latency (SOL)	The elapsed time from lights out to the first epoch of stage REM, N1, N2 or N3 sleep in minutes.
Number of awakenings	Number of stage wake occurrences.

REM = rapid eye movement sleep, N1 = Stage 1 sleep, N2 = Stage 2 sleep, N3 = Stage 3 sleep.

Analysis of the PSG data examined measures of sleep continuity and of sleep architecture. Sleep continuity variables were total sleep time (TST), time in bed (TIB), sleep efficiency (SE%), wake after sleep onset (WASO), sleep onset latency

(SOL) and the number of awakenings (NWAK). Sleep architecture measures included the percentages of Stage 1 (N1), Stage 2 (N2), Stage 3 (N3) and rapid eye movement (REM) sleep and the latency to these stages (Lauer *et al.*, 1991; Spiegelhalder *et al.*, 2012). An overview and description of these parameters are described in Table 3.1.

3.4.4. Cortisol sampling

Cortisol samples were obtained using Salivettes (Sarstedt, Leicester, UK). These require the participant to chew on an absorbent piece of cotton, before depositing the cotton into a plastic storage tube. All cortisol samples were collected by a researcher and participants were instructed to chew on the Salivette for a period of 1 minute, which was measured using a stopwatch. Cortisol samples were stored in a domestic refrigerator immediately following collection, and samples were frozen at -20°C at the earliest opportunity until assaying. Samples were centrifuged at 3000 rpm for 15 minutes and assays were performed in-house using the luminescence immunoassay method, in accordance with manufacturer instructions (Salimetrics, Newmarket, UK).

3.4.5. Cortisol awakening response (CAR)

The CAR was measured on Morning 1, Morning 2 and Morning 3, through cortisol samples collected from saliva immediately at awakening and at subsequent time intervals of 15 minutes, between awakening and 60 minutes post-awakening (awakening, + 15 minutes, + 30 minutes, + 45 minutes, +60 minutes). CAR sampling occurred on three consecutive weekday mornings as a minimum of two CAR

measurements are needed to achieve reliable trait measures (Hellhammer *et al.*, 2007). Awakening samples were obtained by a researcher waking the participant at the pre-determined awakening time and immediately collecting a saliva sample. Participants were allowed to return to sleep between samples and the researcher left the room between obtaining samples. PSG recording continued during the CAR period. All samples were collected in low-intensity ultraviolet light (approximately one lux). All participants were instructed to remain in bed unless it was absolutely necessary (e.g. to go to the toilet), and were not permitted to eat or drink, with the exception of a small amount of water, in order to avoid sample contamination. All cortisol samples were collected by a researcher and participants were instructed to chew on the Salivette for a period of 1 minute, which was measured using a stopwatch. This ensured consistency and ensured that a sufficient amount of saliva was collected.

Cortisol levels during the CAR period were examined in four different ways. Cortisol levels at each time point of the CAR (measured in nanomoles per litre (nmol/l)), awakening cortisol levels (nmol/l), the peak value, the area under the curve with respect to ground (AUC_G) and the CAR peak change were calculated. The peak value refers to the highest value of the CAR, observed between the sample taken at 15 minutes post-awakening and the sample obtained at 60 minutes post-awakening. The area under the curve with respect to ground (AUC_G) provides a measure of total cortisol secretion over the CAR period, from the first awakening sample until the final CAR sample) (Clow *et al.*, 2004). The AUC_G formula¹ places

¹ AUC_G formula =

$$((T1+T2)/2*15)+((T2+T3)/2*15)+((T3+T4)/2*15)+((T4+T5)/2*15)$$

less emphasis on the first and last CAR samples, since these have a smaller contribution to the area measure than other sample time points (Pruessner *et al.*, 2003). Total cortisol output was expressed in arbitrary units. The peak change value provides a measure of increase during the CAR period and is calculated by subtracting awakening levels of cortisol from the peak cortisol value observed during the remainder of CAR period (between wake + 15 minutes and wake + 60 minutes) (E. K. Adam *et al.*, 2006; Dockray & Steptoe, 2011).

3.5. Feasibility study

Whilst the protocol outlined in this chapter measures sleep and the CAR in a laboratory setting, a feasibility study was conducted with the aim of determining whether the protocol could be employed within a home environment. This is described in the following chapter.

CHAPTER 4.

Feasibility Study

4.1. Introduction

The aim of the feasibility study was to test the suitability of the standard protocol within a home environment compared to a laboratory environment. The feasibility study examined whether it would be possible to introduce stress into the protocol, at a later stage, within a home environment, or whether the protocol was better suited to measurement within a laboratory environment.

4.2. Method

4.2.1. Participants and recruitment

Eight healthy good sleepers (four male and four female, $M_{\text{age}} = 31.91$ years, $SD_{\text{age}} = 4.60$ years) participated in the feasibility study. Four individuals participated in the laboratory study ($M_{\text{age}} = 28.56$ years, $SD_{\text{age}} = 1.17$ years) and four individuals participated in the home study ($M_{\text{age}} = 35.25$ years, $SD_{\text{age}} = 4.27$ years). Participants were recruited using advertisements placed around a university campus and through emails sent to university staff and students. Inclusion and exclusion criteria are described in Section 3.2.

4.3. Procedure

The procedure for the laboratory group was identical to that described in Section 3.4. The home group followed a similar procedure, however participants in the home group were not required to attend the sleep laboratory and underwent

ambulatory PSG recording in their own homes. The home group collected CAR samples in their own homes. Participants in the home group were able to carry out their normal daily activities immediately after the final CAR sample (wake + 60 minutes) each morning and were not restricted in their activities between Day 2 and Night 2.

4.3.1. Baseline monitoring period

The baseline monitoring period was identical to that described in in Section 3.4. Participants completed sleep and mood diaries for two weeks and underwent actigraphy for two weeks prior to participation.

4.3.2. Polysomnography

The procedure for PSG within the laboratory group was identical to that described in Section 3.4.3. For the home group, ambulatory PSG was used to measure sleep in the homes of the participants and PSG was applied to participants by a trained researcher. Electrodes were placed at the same locations and recordings were obtained using the same equipment as previously described (Section 3.4.3). The researcher then left the home of the participants following PSG application, in order to allow them to sleep naturally, before returning during the CAR sampling period in order to remove PSG. Participants were instructed to adhere to their regular sleeping patterns and were responsible for scheduling their own sleep periods. Participants in the home group were instructed to press a button on the ambulatory PSG equipment immediately before lights off and immediately after lights on, which placed a time

marker on the PSG. This was used in order to accurately determine lights off and lights on times for use in further analyses.

4.3.3. Cortisol awakening response sampling

The CAR of the laboratory group was measured as outlined in Section 3.4.5, where samples were collected by a researcher in low-intensity ultraviolet light (approximately one lux) and all samples were timed for 60 seconds. Participants in the home group were required to self-collect saliva samples during the CAR period and were provided with collection instructions (Appendix J). Participants were also required to record the time at which each sample was obtained during the CAR period. Participants were asked not to eat or to brush their teeth during the CAR period in order to avoid sample contamination but were free to get up and leave their bed during the collection period. Participants were asked to ensure that Salivettes were saturated and to estimate a collection time of 60 seconds. Following the collection period participants were instructed to refrigerate their samples until collected by a researcher.

4.3.4. Treatment of results

Due to the small sample size, the age of both groups was compared using a Mann-Whitney test. In order to ensure that the sleep of participants in the home group and laboratory group did not differ, data from the baseline monitoring period were averaged across non-work days. Subjective and objective sleep variables were compared between the two groups using descriptive statistics, due to the small sample size and as the feasibility of the using the standard protocol within a home or

laboratory environment was the primary aim of this study. Objective measures of sleep continuity (TST, SE%, WASO, SOL and NWAK) and sleep architecture (percentages of N1, N2, N3 and REM sleep and the latency to these stages) were summarised as described in described in Section 3.4.3. Objective measures of sleep were averaged across Night 1 and Night 2, for home and laboratory participants, as normal sleepers have previously shown low night-to-night variability in studies where sleep has been measured at home using PSG, even when measured up to four months apart (Quan *et al.*, 2002). Analysis of the CAR focused on cortisol levels at each sampling time point and other parameters (awakening levels, peak value, peak change and AUC_G) were not examined, due to the purpose of the study and the limited sample size. CAR data was unavailable for one participant due to the sample containing too little saliva for assaying and this participant was excluded from any further analyses. Due to the limited sample size, non-parametric analyses were used to compare the CAR between groups. As there is no non-parametric alternative to a mixed ANOVA (Field, 2013), a series of Mann-Whitney *U* tests were used to compare cortisol levels at each time point of the CAR between home and laboratory environments. Separate tests were performed for the CAR on Morning 2 and Morning 3.

4.4. Results

4.4.1. Demographics

The laboratory group were significantly younger than the home group ($U = 1.00, Z = -2.03, p < .05$).

4.4.2. Baseline subjective sleep and sleep quality data

Baseline measures of sleep continuity, collected during the two-week baseline period, were broadly similar between the two groups. Measures of sleep quality were also similar during the baseline period, although participants in the laboratory group reported that they felt better and rated their sleep to be more enjoyable than the home group. This indicated that the home and laboratory groups were well-matched in terms of subjective sleep prior to the assessment of objective sleep and the CAR. This is summarised in Table 4.1.

Table 4.1:

Baseline subjective sleep continuity and quality for home and laboratory group

Variable	Home		Laboratory	
	Mean	<i>SD</i>	Mean	<i>SD</i>
SOL	14.85	9.53	12.50	6.03
NWAK	1.13	.88	1.20	.98
WASO	9.75	12.23	7.59	8.98
TST (mins)	488.25	31.60	478.97	83.32
TIB (mins)	563.04	58.07	531.80	86.51
SE (%)	89.63	6.53	93.35	2.57
How well do you feel? (0 – 4)	2.46	.78	3.07	.66
How enjoyable was your sleep? (0 – 4)	2.58	.69	3.43	.45
How mentally alert were you in bed last night? (0 – 4)	1.22	.17	.81	.56
How physically tense were you in bed last night? (0 – 4)	.84	.28	.59	.40

SOL: sleep onset latency, NWAK: number of awakenings, WASO: wake after sleep onset, TST: total sleep time, TIB: time in bed, SE: sleep efficiency.

4.4.3. Home and laboratory objective sleep comparisons

Sleep continuity was similar between both groups and although home participants showed a higher WASO, this group showed a larger standard deviation compared to the laboratory group. This was also the case for SOL. Measure of sleep architecture showed that the percentage of sleep spent in each stage was largely similar, although the laboratory group showed a slightly longer latency to each stage of sleep. Objective measures of sleep continuity and sleep architecture are summarised in Table 4.2.

Table 4.2:

Summary of objective sleep continuity and architecture measures for home and laboratory participants

	Home		Laboratory	
	Mean	SD	Mean	SD
TST (mins)	444.38	51.63	439.13	29.80
SE (%)	92.78	5.28	92.01	3.96
WASO (mins)	23.56	23.18	16.50	8.87
SOL (mins)	13.63	10.78	21.25	18.40
NWAK	11.75	5.58	14.63	3.20
Time in REM (%)	28.36	3.32	23.56	4.14
Time in N1 (%)	2.96	.86	3.38	.92
Time in N2 (%)	50.03	1.54	53.79	6.85
Time in N3 (%)	18.63	2.28	19.26	6.03
Latency to REM (mins)	70.31	44.41	100.44	46.50
Latency to N1 (mins)	13.63	10.78	21.25	18.40
Latency to N2 (mins)	17.06	11.29	26.63	16.34
Latency to N3 (mins)	31.44	8.60	41.00	16.23

TST = total sleep time, SE(%)= sleep efficiency (%), WASO = wake after sleep onset,

SOL = sleep onset latency, NWAK = number of awakenings

4.4.4. Cortisol awakening response

There were no significant differences in cortisol levels (all p -values $> .05$) between each group on the second or third morning of measurement. Overall, CARs obtained in a laboratory environment were very similar to CARs obtained in a home environment. Whilst the cortisol levels at each sampling point were lower within the laboratory group than the home group, these showed a large standard deviation, indicating a large amount of individual variability in cortisol levels, and were not statistically significant. The home group appeared to show a slightly delayed peak CAR on the final morning compared to the second morning, with cortisol levels peaking 45 minutes following awakening. The laboratory group also showed differences in their CAR between Morning 2 and Morning 3, where cortisol levels showed a secondary rise between the sample obtained at 45 minutes after awakening and the final sample. The CAR is summarised in Figure 4.1.

4.4.5. Feasibility

The standard protocol was well-suited to both a home and a laboratory environment and participants did not report any difficulties with regards to protocol adherence in either environment. Where the protocol was applied to the home environment, the measurement of sleep using ambulatory PSG did not present any problems in terms of feasibility or in terms of data analysis, and there were no objective sleep data lost. One participant within the home group had a missing saliva sample during the CAR period, where the saliva sample did not contain enough saliva to allow subsequent assaying.

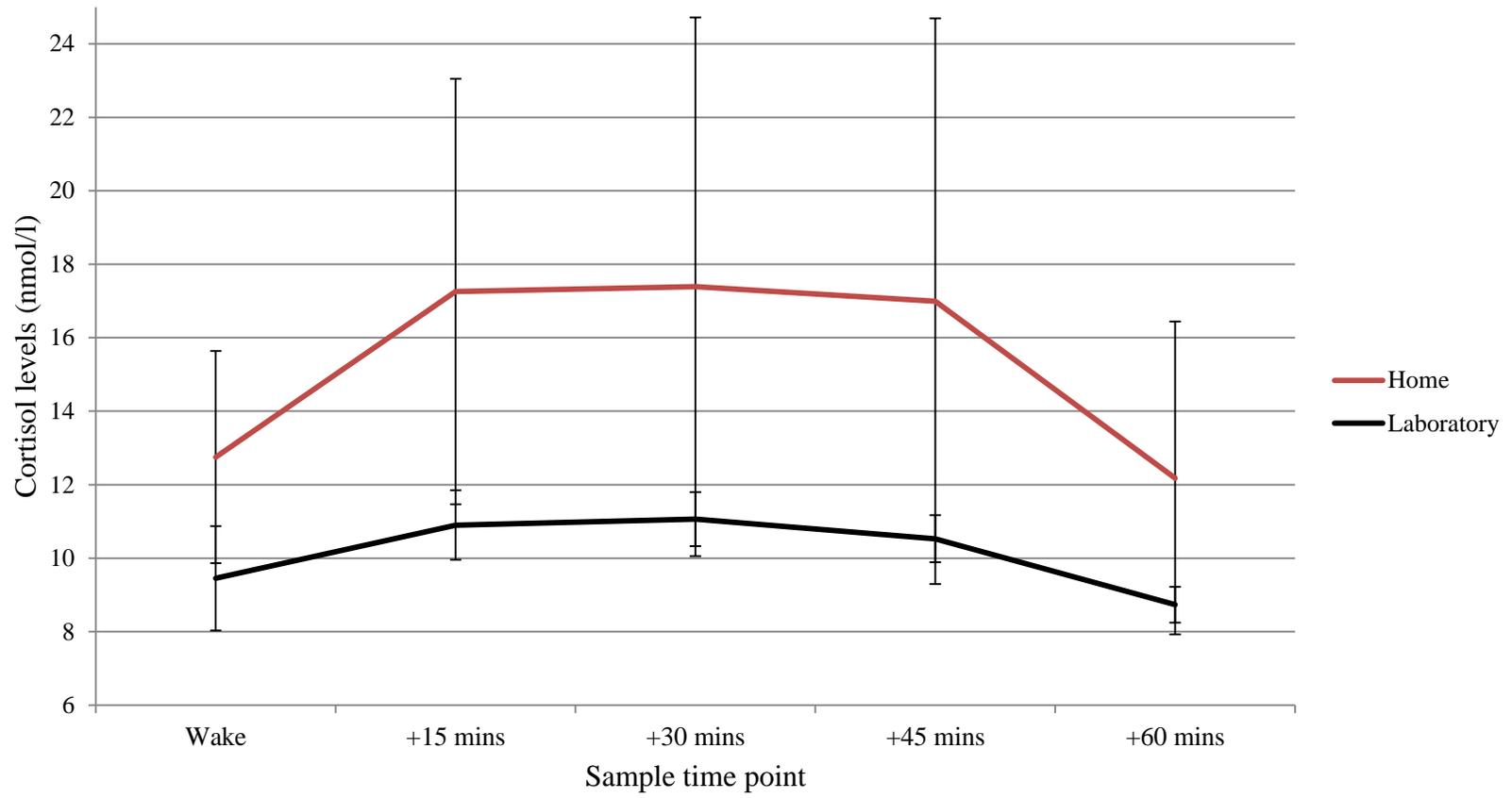


Figure 4.1: Mean (\pm SEM) Morning 2 CAR cortisol levels at each sampling time point for the laboratory or home group.

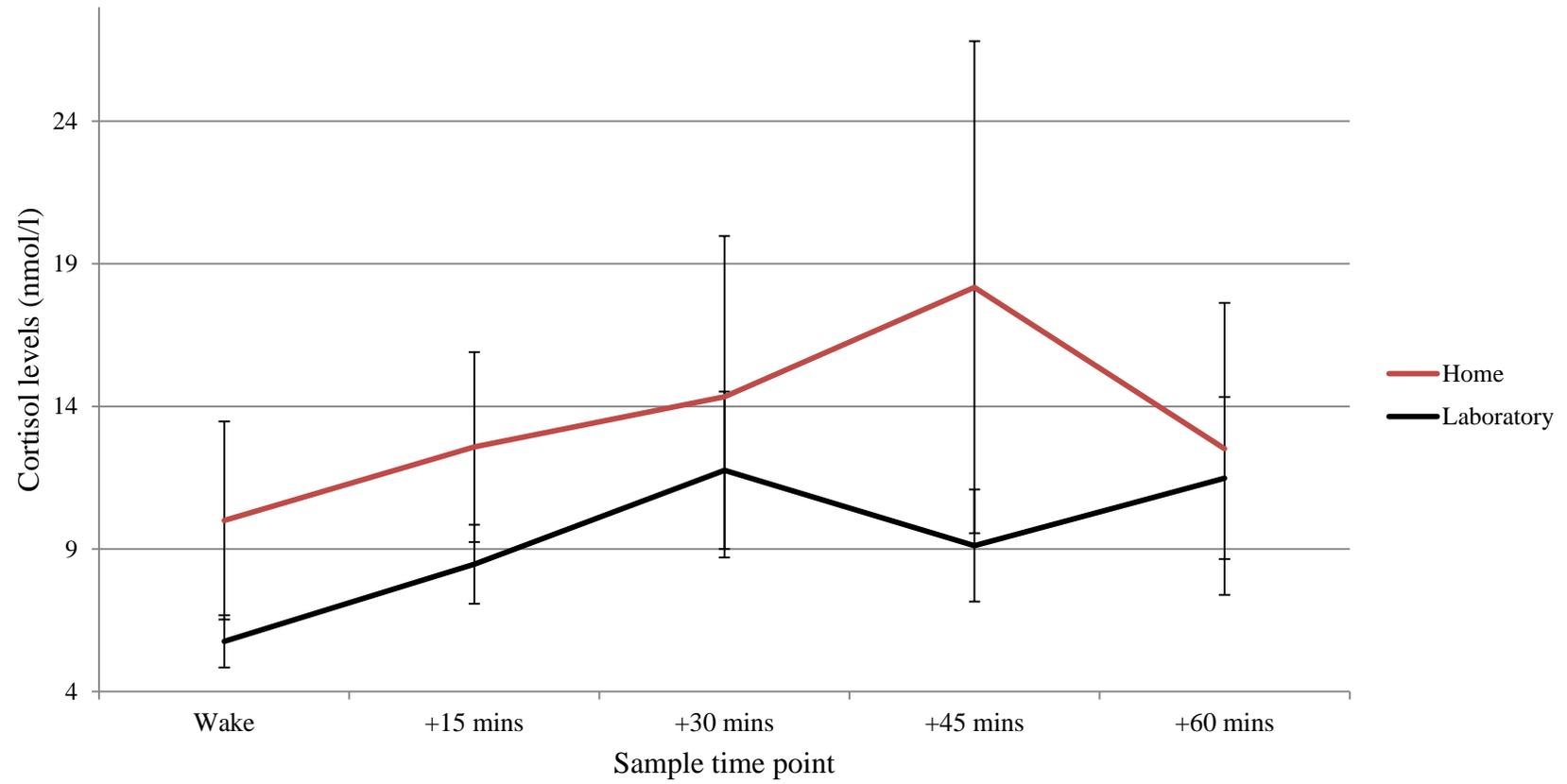


Figure 4.2: Mean (\pm SEM) Morning 3 CAR cortisol levels at each sampling time point for the laboratory and home group.

Table 4.3:

Comparison of Morning 2 cortisol levels (nmol/l) at each time point of the cortisol awakening response between home and laboratory environments

Condition	Awakening		+15 minutes		+30 minutes		+45 minutes		+60 minutes	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Home	12.75	5.00	17.26	10.03	17.39	12.70	16.99	13.33	12.18	7.38
Laboratory	9.45	2.84	10.90	1.90	11.06	1.47	10.53	1.29	8.74	.98

Table 4.4:

Comparison of Morning 3 cortisol levels (nmol/l) at each time point of the cortisol awakening response between home and laboratory environments

Condition	Awakening		+15 minutes		+30 minutes		+45 minutes		+60 minutes	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Home	10.00	6.02	12.58	5.77	14.34	9.76	18.18	14.94	12.51	8.87
Laboratory	5.76	1.84	8.47	2.77	11.76	5.52	9.12	3.93	11.49	5.69

4.5. Discussion

The purpose of the feasibility study was to examine the suitability of the standard protocol within a home or a laboratory environment. Overall, the protocol is suitable for use in either environment. Measures of objective sleep were very similar between the home and laboratory groups, with minor differences where the home group showing a higher WASO and lower SOL compared to the laboratory group. The laboratory group also showed longer latencies to each stage of sleep although the percentage of sleep spent in each stage was similar and each measure was

accompanied by a large standard deviation, potentially reflecting a high amount of individual variability. In terms of feasibility, no nights of sleep were lost in either environment and ambulatory PSG was suitable for recording objective sleep in a home environment.

A home protocol is also suitable for measurement of the CAR. Whilst cortisol levels at each sampling point of the CAR within the laboratory group were lower compared to the home group, indicating that the CAR was lower in the laboratory, the differences between the groups were not significant and cortisol levels were accompanied by a large standard deviation in the home environment. However, one cortisol sample during the CAR period was unable to be included within analyses due to the sample containing too little saliva for analysis. It is possible that as participants in the home condition self-collected saliva samples, this particular sample may not have been collected by the participant as requested.

The effects of light may also account for the subjective differences shown in the CAR between the home and laboratory environments. No restrictions were placed upon the levels of light when the CAR was measured in a home environment; however the CAR was collected in extremely low light levels (approximately one lux) within the laboratory environment. This is a relevant factor as the SCN controls the overall co-ordination of the HPA axis (Buijs *et al.*, 2003) and whilst light has previously been shown to affect the CAR (Figueiro & Rea, 2012; Scheer & Buijs, 1999; Thorn *et al.*, 2004), the precise impact is not currently clear. In order to minimise the influence of light upon the CAR by measuring the CAR in consistently low light levels, it will be advantageous to adopt a laboratory protocol for the measurement of the CAR. An additional consideration is that whilst participants in the home measurement group were instructed to remain in bed wherever possible

during the CAR measurement period, they were not monitored during this point and it is possible that movement influenced the results. A further strength of measuring the CAR within a laboratory environment is that a laboratory environment offers a greater level of control over the activities of participants between Night 1 and Night 2. As the anticipation of forthcoming stress appears to disrupt sleep (Torsvall & Akerstedt, 1988; Torsvall *et al.*, 1987) and as the CAR may also function as a marker of anticipation (Filaire *et al.*, 2009; Filaire *et al.*, 2007; Filaire *et al.*, 2001; Rohleder *et al.*, 2007) it will be advantageous to use the protocol to measure sleep and the CAR within a laboratory situation. This will offer greater levels of control over factors which may affect the CAR, including light and movement.

The following chapter outlines Study 1, where the aim of the study was to use the standardised protocol to examine the relationship between subjective sleep, objective sleep and the CAR, in the absence of stress, within a laboratory environment.

CHAPTER 5a.

Study 1: Subjective sleep and the CAR

5.1. Introduction

The aim of Study 1 was to investigate the relationship between subjective sleep, objective sleep and the CAR, using the standardised protocol within a controlled laboratory environment, in the absence of stress. Overall, the relationship between subjective sleep and the CAR is currently unclear and may differ depending on the measure of the CAR reported. Kumari and colleagues (Kumari *et al.*, 2009) showed that a shorter self-reported sleep duration was associated with a steeper rise in cortisol levels between awakening and 30 minutes post-awakening in middle-aged adults and Stalder and colleagues (Stalder *et al.*, 2009) showed that awakening levels were positively related to both subjective sleep duration and the time of awakening; however total secretion was not related to these sleep variables.

Another study showed a small negative correlation between subjective sleep duration and the mean increase in cortisol levels during the CAR (Wüst *et al.*, 2000b). Hansen and colleagues showed that when the relationship between the CAR peak change and sleep problems were examined over a three-month period, subjective sleep duration showed no relationship to the CAR. This was the case in terms of the increase in cortisol levels from awakening to the second or third sample and also in terms of total secretion during the CAR period. Taken together, the relationship between subjective sleep and the CAR appears to be inconsistent but this may be due to variations in CAR measurement techniques between studies. For instance, Kumari and colleagues (2009) examined the rise in cortisol between awakening and 30 minutes post-awakening in middle-aged adults, showing a shorter

sleep duration was associated with a steeper rise in cortisol. Stalder and colleagues (2009) showed that awakening cortisol levels were positively related to both sleep duration and the time of awakening, although there was no relationship between total secretion in terms of the AUC_G and these measures. A small negative correlation between self-reported sleep duration and the CAR, as measured using the mean increase, has been shown elsewhere (Wüst *et al.*, 2000b). Study 1 overcame these limitations by examining multiple indices of the CAR. A further aim of Study 1 was to examine the relationship between indices of the CAR and mood. Hellhammer *et al.* (2007) determined that a CAR measured on a single day is largely subject to the effects of situational factors rather than trait factors, which implies that there may be a role for mood within the regulation of the CAR or that mood is associated with the CAR in some way. Further, a single case study determined that 22% of the intra-individual variance within total levels of cortisol secreted during the CAR period was explained by subjective levels of mood and the daily anticipation of activities and obligations (Stalder *et al.*, 2010a). An additional purpose of Study 1 was to collect baseline subjective sleep, CAR and cortisol data to allow comparisons with Study 2.

Another aim of Study 1 was to examine the relationship between measures of objective sleep continuity and sleep architecture and the CAR, within healthy, normal good sleepers. A meta-analysis of 23 CAR studies demonstrated that subjective and objective sleep duration was positively related to cortisol levels at awakening (Garde *et al.*, 2012), however, as discussed in Chapter 2, the relationship between sleep architecture and the CAR is unclear. Few studies have a) examined this relationship in a sample of healthy good sleepers and b) within a controlled environment. No relationship was observed between sleep architecture and total

secretion during the CAR period in healthy controls and army veterans with post-traumatic stress disorder (van Liempt *et al.*, 2013). Elsewhere, the duration of REM sleep in alcohol-dependent inpatients has been shown to be negatively associated with awakening cortisol levels (Junghanns *et al.*, 2007). An association between lower awakening cortisol levels and increased percentages of Stage 1, Stage 3 and REM sleep has also been demonstrated in a study of caregivers compared to non-caregivers (Fonareva *et al.*, 2011). However, differences in sleep architecture were shown between these groups, with caregivers showing an increased SOL and percentage of sleep spent in Stage 1, alongside a reduction in the percentage of sleep spent in REM compared to non-caregivers. It is possible that these between-group differences may have driven the relationship between sleep and the CAR rather than the differences between Stage 1, Stage 3 and REM sleep. In addition, these studies typically differ in the time at which CAR samples are obtained and show differences in the reported method of measurement and CAR indices, examining awakening levels (Fonareva *et al.*, 2011; Junghanns *et al.*, 2007) or total secretion (AUC_G) over the CAR period (van Liempt *et al.*, 2013). Due to these variations, it is difficult to determine the relationship between objective sleep and the CAR in good sleepers.

The present study aimed to establish the relationship between objective sleep and various indices of the CAR within healthy, normal sleepers in a standardised sleep laboratory environment, with consistent light levels and objective monitoring of sleep. The relationship between subjective sleep and the CAR is discussed in the present chapter (Chapter 5a) and the relationships between objective sleep and the CAR are discussed in the following chapter (Chapter 5b).

5.2. Method

5.2.1. Participants

A total of 18 normal healthy sleepers, nine male and nine female, ($M_{\text{age}} = 23.46$ years, $SD_{\text{age}} = 3.21$ years) participated in Study 1. All participants were recruited and screened as described in Section 3.2.

5.2.2. Treatment of results

CAR data from three participants were excluded due to saliva samples containing an insufficient volume of saliva for analysis ($n = 2$) and excessively high (>75 nmol/l, Kunz-Ebrecht *et al.*, 2004) cortisol levels ($n = 1$). Cortisol levels during the CAR period were examined for normality using Kolmogorov-Smirnov tests. Since these were non-significant in each case (all values $p > .05$) non-transformed data were used in all analyses. Cortisol samples collected 120 minutes post-awakening ($n = 16$) were analysed separately from the CAR sampling period and data was excluded ($n = 2$) due to saliva samples containing insufficient volume of saliva for analysis ($n = 1$) and excessively high (>75 nmol/l) cortisol levels ($n = 1$). As three participants were excluded from CAR analyses, demographic data for participants with complete CAR data ($n = 15$) were reported.

In order to examine whether there were differences between the baseline period and laboratory period in terms of subjective sleep continuity and quality, these measures were compared using a series of paired-samples *t*-tests. Representative sleep continuity and quality data was calculated from the monitoring period using the average of the second, third, ninth and tenth day of sleep diary data as this corresponded with Night 1 and Night 2 of the laboratory protocol and

therefore provided a representative Night 1 and Night 2 during the baseline period. This was compared to subjective sleep from the second and third night of the laboratory period using a series of paired-samples *t*-tests. Subjective sleep continuity and quality were compared between Morning 2 and Morning 3 using paired-samples *t*-tests. Comparisons between subjective baseline and laboratory levels of anxiety and perceived stress, and differences between Morning 2 and Morning 3 levels of anxiety and perceived stress were examined using paired-samples *t*-tests.

In order to investigate any differences in the overall CAR between each morning and at time point, a 3 (morning) \times 5 (time point) mixed ANOVA was used and Greenhouse-Geisser adjusted degrees of freedom were reported where appropriate. (Kunz-Ebrecht *et al.*, 2004). In order to examine whether additional indices of the CAR (awakening levels, peak value, peak change and total secretion as measured using the AUC_G value) differed between each morning, a series of one-way ANOVAs were conducted on these measures. A one-way ANOVA was conducted on cortisol levels at wake + 120 minutes between Morning 1, Morning 2 and Morning 3 ($n = 16$). ANOVA effect sizes were reported using partial eta squared (η^2_p) values, where η^2_p values of .01, .06 and .14 represent small, medium and large effect sizes respectively (J. Cohen, 1988). Relationships between CAR, sleep and mood variables were examined using a series of Pearson correlations.

5.3. Procedure

All participants provided written informed consent, and the study was approved by the Northumbria University Faculty of Health and Life Sciences Research Ethics Committee. Upon arriving at the laboratory on the adaptation night,

participants were asked to complete a questionnaire booklet containing a range of measures (PSQI, PSS, HADS, HÖ-MEQ and FIRST) and to provide their age as detailed in Section 3.3. The procedure for Study 1 was identical to that described in Section 3.4 and is summarised in Figure 5.1. Participants received a full debrief upon completion of the study on Morning 3 and received £150.

5.3.1. Cortisol awakening response sampling

Laboratory CAR sampling was conducted as described in Section 3.4.5. Cortisol samples were obtained at intervals of every 15 minutes between awakening and one hour after awakening (wake, +15 minutes, +30 minutes, +45 minutes and +60 minutes) in order to obtain cortisol levels at each time point of the CAR and to determine additional indices of the CAR, including awakening levels, the peak change in levels, total secretion (AUC_G) and peak value during the CAR period.

5.3.2. Polysomnography

The procedure for measuring PSG during Study 1 was identical to that described in Section 3.4.3.

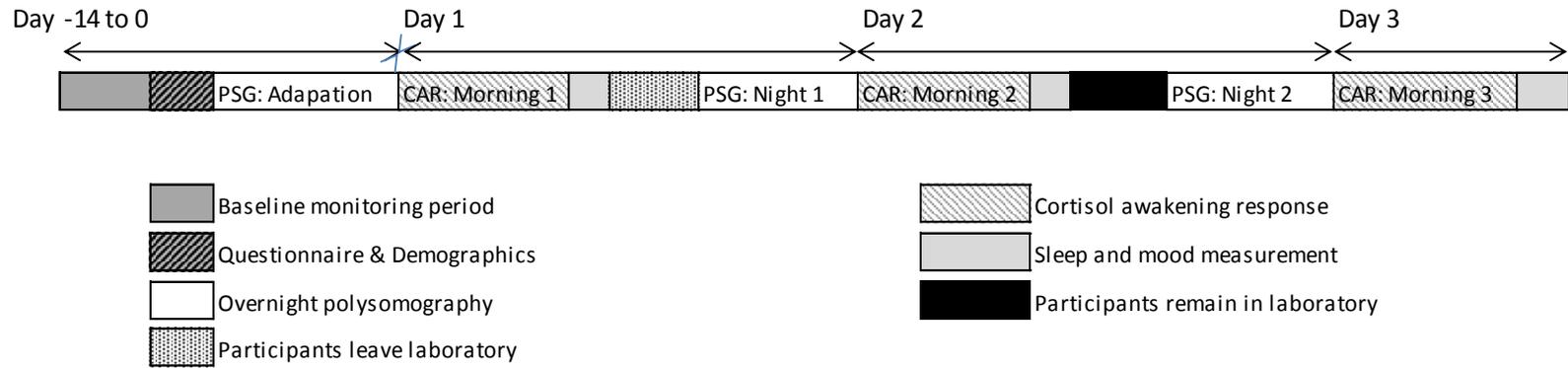


Figure 5.1: Schematic of study protocol for Study 1

Task	wake +2hrs	+3hrs	+4hrs	+5hrs	+6hrs	+7hrs	+8hrs	+9hrs	+10hrs	+11hrs	+12hrs	+13hrs	+14hrs
Saliva sample													
Meal provided													

Figure 5.2: Schedule of participant activities during the full day (Day 2) spent in the laboratory.

5.3.3. *Daytime cortisol sampling*

Cortisol samples were taken at wake + 120 minutes on Morning 1, Morning 2 and Morning 3 in order to observe the diurnal profile following the CAR period.

5.3.4. *Daily activities*

After collection of the wake +120 minutes cortisol sample, breakfast was provided on each morning. Breakfast was standardised for all participants and was consistent across each morning, where participants were allowed a small bowl of cereal, a piece of toast with butter or jam or fruit. Participants were allowed to combine each item (e.g. they were permitted to have chosen both a bowl of cereal and toast) if they wished to do so. Participants were allowed to leave the laboratory on Morning 1 after this point and were instructed to adhere to their usual daily routines before returning on Night 1. Following breakfast on Morning 2, participants remained in the laboratory under observation throughout Day 2. Participants were not allowed to nap or exercise and remained in the meeting room area of the laboratory until Night 2 performing sedentary activities including watching TV, films and reading (Section 3.3). Two standardised meals were provided during Day 2: lunch was provided six hours following awakening, which consisted of a sandwich and crisps, and dinner was provided 10 hours following awakening, which consisted of a microwave ready meal served with salad. Participants provided saliva samples every 60 minutes until lights out on Night 2. Following the provision of breakfast on Morning 3, participants were fully debriefed and were allowed to leave the laboratory.

5.3.5. *Pre-sleep cortisol sampling*

On all three nights, pre-sleep cortisol sampling occurred immediately before lights out, whilst the participant was in bed. Samples were collected in normal bedroom lighting levels of approximately 240 lux. As Study 1 focused on the CAR, daytime cortisol and pre-sleep cortisol levels were not included in any further analyses within Chapter 5.

5.3.6. *Overnight awakenings*

Participants were allowed 30 minutes in order to return to sleep if they awakened at least two hours before their scheduled waking time on the second or third night. If the participant did not return to sleep within this period, a saliva sample was obtained and they were allowed to return to sleep. If a participant woke within two hours of their scheduled wake time, they were allowed five minutes to return to sleep before saliva was sampled. This cut-off point of two hours before awakening was chosen due to the proximity of this time point to the scheduled CAR period, and if the participant did not return to sleep within 15 minutes of this point, the full CAR sampling procedure was followed. This was to minimise the chance of a participant awakening and the resulting CAR being missed or distorted, since the CAR appears to be a genuine response to awakening, although it is unclear whether this only refers to natural awakening (Buckley & Schatzberg, 2005; Edwards *et al.*, 2001; Fries *et al.*, 2009; Wilhelm *et al.*, 2007). This was also considered as the link between the SCN and PVN synchronises the time of day with neuroendocrine output (Buijs *et al.*, 2003), meaning that unplanned early awakenings may have still resulted in a CAR.

5.4. Results

5.4.1. Demographic and questionnaire data

Age and questionnaire data are presented in Table 5.1. PSQI data indicated the participants to be good sleepers (< 5) and HADS anxiety and depression scores were in the normal range (<7).

Table 5.1:

Summary of demographic and questionnaire measures for full sample of participants (n = 18) and participants with complete CAR data (n = 15).

Measure	Mean	SD
Age	23.67	3.49
PSQI	3.80	1.32
ISI	3.00	2.27
PSS	18.87	6.53
HADS Anxiety	5.67	2.64
HADS Depression	1.67	1.40
HO-MEQ	50.57	8.53
FIRST	19.36	4.89

PSQI: Pittsburgh Sleep Quality Index, PSS: Perceived Stress Scale, HADS: Hospital Anxiety and Depression Scale, HO-MEQ: Horne-Östberg Morningness-Eveningness Questionnaire, FIRST: The Ford Insomnia Response to Stress Test

5.4.2. Subjective sleep: baseline vs. laboratory comparison

No significant differences were shown in measures of continuity with the exception of TST, as participants slept for a shorter period of time during the laboratory period compared to the two-week baseline monitoring period ($t(17) = 2.39, p < .05$). When measures of sleep quality were compared between the baseline monitoring and laboratory period, participants reported that in terms of

their sleep quality, they felt slightly less well during the laboratory period ($t(17) = 2.72, p < .05$). The results are summarised in Table 5.2.

Table 5.2:
Baseline and laboratory comparisons of subjective sleep data (n = 18)

Variable	Baseline		Laboratory	
	Mean	SD	Mean	SD
SOL	16.89	7.79	14.86	6.89
NWAK	.97	.76	1.35	1.15
WASO (mins)	8.94	9.03	8.36	8.56
TST (mins)	470.78	50.28	445.06	29.34
TIB (mins)	544.94	67.57	521.81	25.54
SE (%)	88.27	7.22	85.33	4.68
How well do you feel? (0 – 4)	2.69	.64	2.42	.67
How enjoyable was your sleep? (0 – 4)	2.81	.50	2.69	.71
How mentally alert were you in bed last night? (0 – 4)	1.23	.50	1.19	.55
How physically tense were you in bed last night? (0 – 4)	.77	.49	.67	.51

SOL: sleep onset latency, NWAK: number of awakenings, WASO: wake after sleep onset, TST: total sleep time, TIB: time in bed, SE: sleep efficiency.

5.4.3. Subjective sleep: representative baseline vs. laboratory comparison

Whilst few differences were shown between the baseline period and the laboratory study in terms of subjective sleep continuity and sleep quality, the baseline period as a comparison also included weekends and thus may not have been representative of participant daily routines. Participants showed significant reduction in total sleep time ($t(17) = 2.23, p < .05$), sleep efficiency, ($t(17) = 2.11, p = .05$) and in how well they felt ($t(17) = 2.79, p < .05$) within the sleep laboratory. This indicated that there were subtle differences in subjective sleep continuity and quality between the representative Night 1 and Night 2 baseline period and the laboratory period. The results are summarised in Table 5.3.

5.4.4. *Subjective sleep: Morning 2 and Morning 3 laboratory comparisons*

No differences were shown between each morning in either measures of sleep continuity or sleep quality ($p > .05$). The results are summarised in Table 5.4.

5.4.5. *Subjective state anxiety and perceived stress: baseline vs. laboratory comparisons*

Levels of state anxiety ($t(17) = 3.05, p < .01$) and subjective stress ($t(17) = 2.79, p < .05$) were significantly lower during the laboratory period compared to the baseline period. Mean levels of state anxiety and subjective stress during the baseline and laboratory periods are shown in Figures 5.2 and 5.3.

Table 5.3:
Comparisons between baseline Night 1 and Night 2 and laboratory subjective sleep data (n = 18)

Variable	Baseline Night 1 and Night 2		Laboratory	
	Mean	SD	Mean	SD
SOL	14.35	7.20	14.86	6.89
NWAK	.99	.99	1.35	1.15
WASO (mins)	5.65	6.53	8.36	8.56
TST (mins)	478.16	64.70	445.06	29.34
TIB (mins)	552.07	99.19	521.81	25.54
SE (%)	89.43	8.01	85.33	4.68
How well do you feel? (0 – 4)	2.79	.71	2.42	.67
How enjoyable was your sleep? (0 – 4)	2.84	.54	2.69	.71
How mentally alert were you in bed last night? (0 – 4)	1.19	.64	1.19	.55
How physically tense were you in bed last night? (0 – 4)	.64	.54	.67	.51

SOL: sleep onset latency, NWAK: number of awakenings, WASO: wake after sleep onset, TST: total sleep time, TIB: time in bed, SE: sleep efficiency.

Table 5.4:

Night 1 and Night 2 comparison of subjective sleep data (n = 18)

Variable	Night 1		Night 2	
	Mean	SD	Mean	SD
SOL	15.00	6.64	14.72	9.31
NWAK	1.47	1.36	1.22	1.22
WASO (mins)	7.89	7.21	8.83	14.21
TST (mins)	448.89	27.20	441.22	38.06
TIB (mins)	521.67	26.40	521.94	25.39
SE (%)	86.17	5.47	84.50	5.46
How well do you feel? (0 – 4)	2.39	.85	2.44	.62
How enjoyable was your sleep? (0 – 4)	2.72	.75	2.67	.91
How mentally alert were you in bed last night? (0 – 4)	1.22	.65	1.17	.71
How physically tense were you in bed last night? (0 – 4)	.61	.50	.72	.57

SOL: sleep onset latency, NWAK: number of awakenings, WASO:

wake after sleep onset, TST: total sleep time, TIB: time in bed, SE:

sleep efficiency.

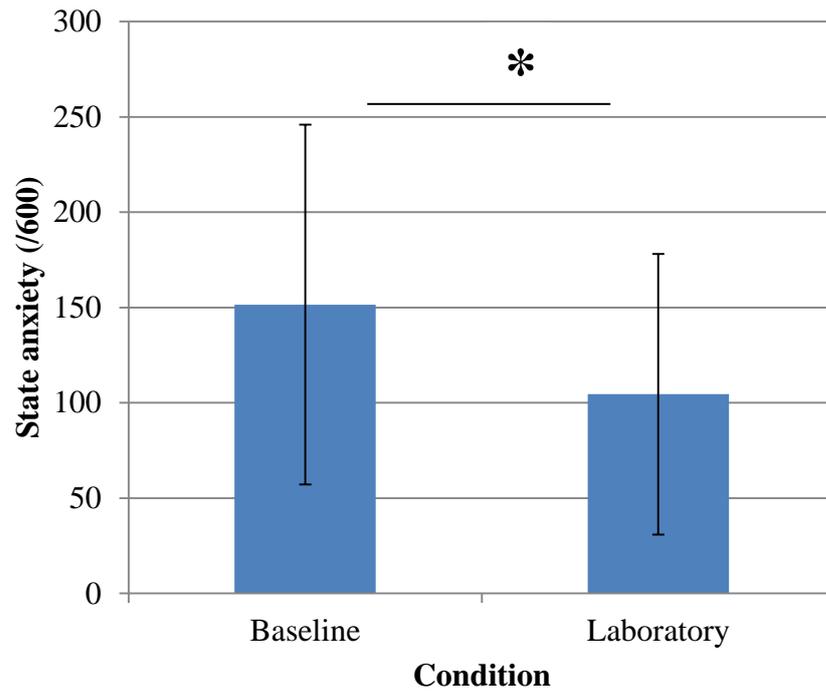


Figure 5.3: Mean (\pm SD) levels of state anxiety during baseline and laboratory periods ($n = 18$) $*p < .05$.

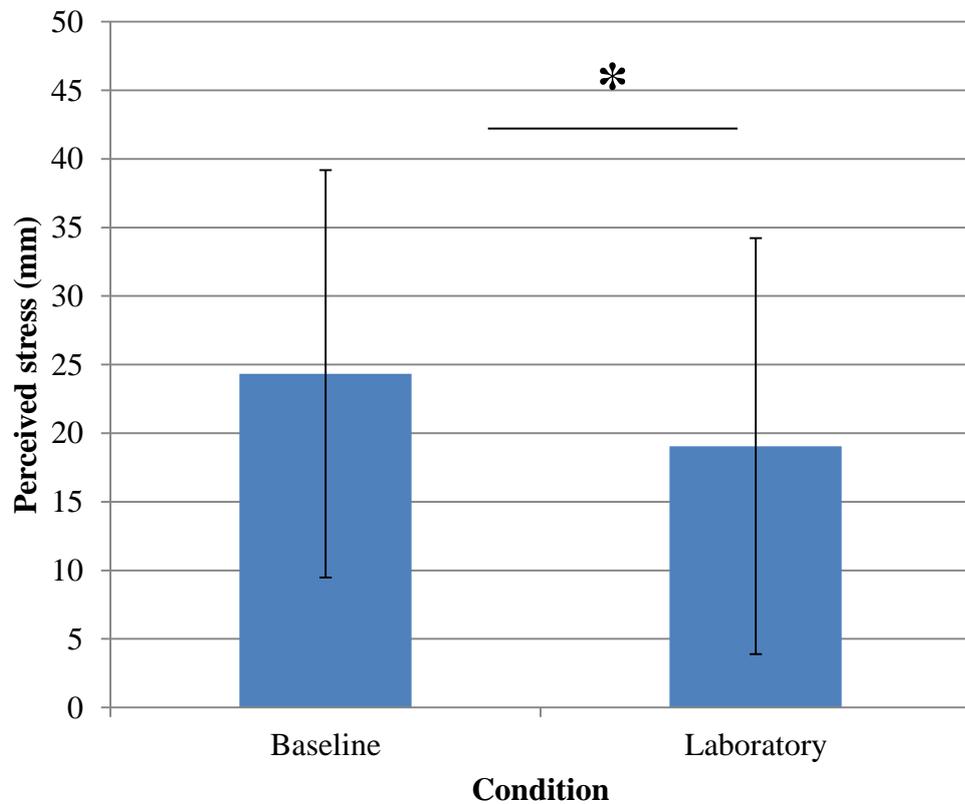


Figure 5.4: Mean (\pm SD) levels of subjective stress during baseline and laboratory periods ($n = 18$) $*p < .05$.

5.4.6. CAR: temporal stability

The CAR was compared across Morning 1, Morning 2 and Morning 3 in order to examine whether the CAR showed a different pattern depending on the morning of measurement. The observed CAR patterns were as expected, with cortisol values peaking 30 minutes post-awakening. There was a significant main effect of time point ($F(2.21, 31.03) = 7.47, p < .01, \eta^2_p = .35$). The main effect of the morning of measurement was not significant ($F(2,28) = .07, p > .05, \eta^2_p = .01$). The morning \times time point interaction ($F(4.26, 59.63) = 2.03, p > .05, \eta^2_p = .13$) was not significant. Overall, the shape of the CAR was not significantly different when compared across each morning. Cortisol levels during the CAR are displayed in Table 5.6 and in Figure 5.6. There were no significant differences observed in awakening cortisol levels ($F(2,44) = 1.26, p > .05, \eta^2_p = .06$), peak change values ($F(2,44) = 3.05, p > .05, \eta^2_p = .13$), peak sample values ($F(2,44) = .16, p > .05, \eta^2_p = .01$) or in AUC_G values by morning ($F(2,44) = .01, p > .05, \eta^2_p = .00$). This indicated that there were no differences in the CAR each morning. Additional indices are summarised in Table 5.6.

5.4.7. Relationship between the CAR and subjective sleep continuity and quality

Cortisol levels at awakening were not associated with subjective sleep continuity or quality. The peak change showed a significant positive relationship with NWAK ($r(15) = .51, p = .05$). The negative relationship between the peak value of the CAR and how enjoyable the sleep of the previous night was rated showed a trend towards significance, ($r(15) = -.51, p = .052$) and the negative relationship between total cortisol secretion and how well participants felt upon awakening

showed a trend towards significance, ($r(15) = -.50, p = .059$). The results are summarised in Table 5.7.

Table 5.5:

Cortisol levels (nmol/l) at each time point of the cortisol awakening response by condition (n = 15)

Morning	Awakening		+15 minutes		+30 minutes		+45 minutes		+60 minutes	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Morning 1	8.96	4.46	9.54	4.36	10.38	4.26	9.69	4.50	8.29	3.56
Morning 2	8.85	4.47	9.10	4.26	10.58	3.62	9.40	3.97	8.66	3.56
Morning 3	6.80	3.61	8.58	3.71	10.47	3.98	10.03	4.33	10.01	4.08

Table 5.6:

Morning 1, Morning 2 and Morning 3 additional indices of the cortisol awakening response (n = 15)

Morning	Peak Change		AUC _G		Peak Value	
	Mean	SD	Mean	SD	Mean	SD
Morning 1	2.58	3.49	573.64	233.03	11.55	4.32
Morning 2	2.32	2.77	567.50	219.04	11.17	3.67
Morning 3	5.22	4.26	562.35	199.78	12.03	4.43

AUC_G: area under the curve with respect to ground

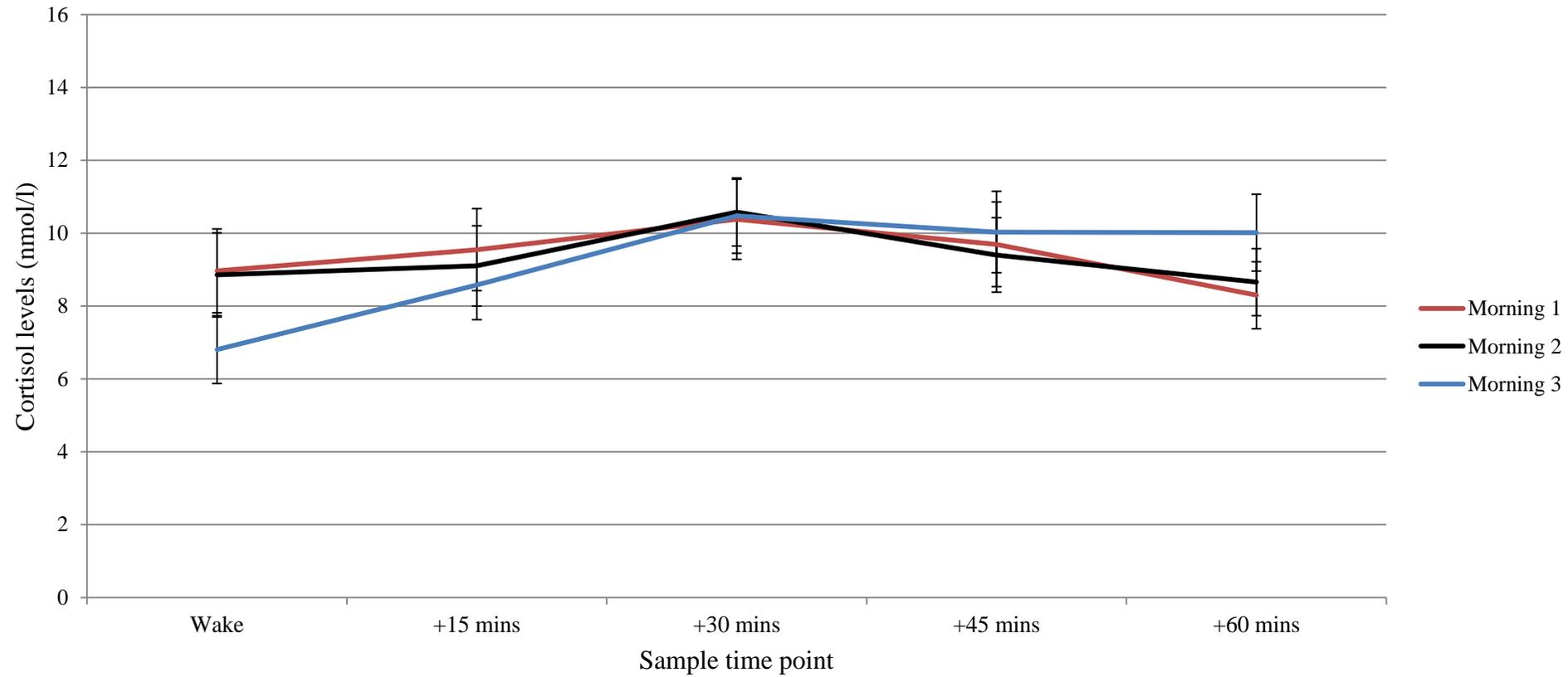


Figure 5.5: Mean (\pm SEM) Morning 1, Morning 2 and Morning 3 CAR cortisol levels ($n = 15$). There were no significant differences between each morning shown in other indices (awakening levels, peak change, peak sample value or AUC_G)

5.4.8. Cortisol: 120 minutes post-awakening samples

Cortisol levels measured at 120-minutes post-awakening were significantly different ($F(2,44) = 3.82, p < .05$) and Bonferroni-corrected *post-hoc* tests showed that cortisol levels were significantly higher on Morning 3 compared to Morning 2 ($p = .029$).

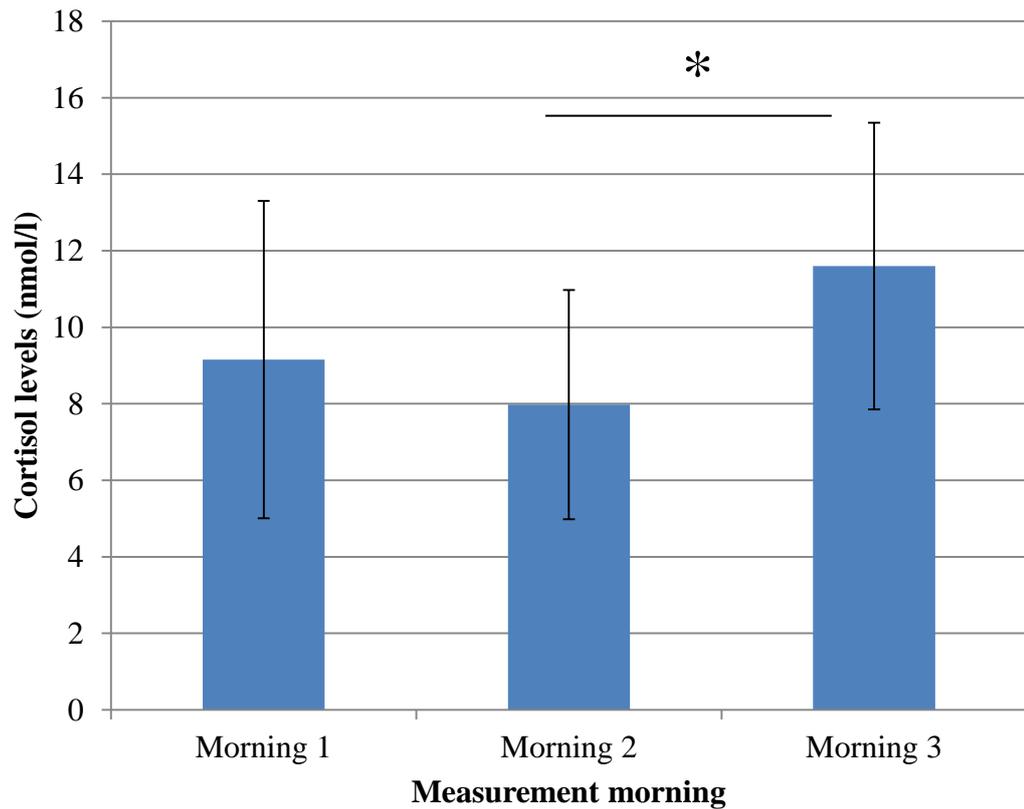


Figure 5.6: Comparison of mean (\pm SEM) cortisol levels at wake +120 minutes ($n = 16$). * $p < .05$.

Table 5.7:

Correlations between subjective sleep continuity and indices (awakening levels, peak change, AUC_G and the peak value) of the cortisol awakening response (n = 15)

	Awakening levels	Peak Change	AUC _G	Peak Value
SOL	-.11	-.02	-.12	-.18
NWAK	-.03	.51*	.32	.22
WASO (mins)	-.01	.01	.00	-.04
TST (mins)	.03	.23	.19	.22
TIB (mins)	.02	.13	.11	.11
SE (%)	.01	.19	.14	.17
How well do you feel? (0 – 4)	-.37	-.18	-.49	-.50
How enjoyable was your sleep? (0 – 4)	-.35	-.23	-.51	-.49
How mentally alert were you in bed last night? (0 – 4)	-.44	.12	-.36	-.44
How physically tense were you in bed last night? (0 – 4)	.40	.11	.47	.48

SOL: sleep onset latency, NWAK: number of awakenings, WASO: wake after sleep onset, TST: total sleep time, TIB: time in bed, SE(%): sleep efficiency. AUC_G: area under the curve with respect to ground.

* $p < .05$

5.4.9. *Relationship between the CAR and average laboratory levels of subjective anxiety and perceived stress*

A significant positive relationship was observed between cortisol levels at awakening and average laboratory levels of perceived stress, ($r(15) = .55, p < .05$) and a significant positive relationship between the peak value of the CAR and average laboratory levels of perceived stress, $r(15) = .52, p < .05$. This is summarised in Table 5.8.

Table 5.8:

Correlations between average laboratory levels of anxiety and perceived stress and indices of the cortisol awakening response (n = 15)

	Awakening	Peak Change	AUC _G	Peak Value
Anxiety	.45	-.20	.32	.37
Perceived stress	.55*	-.08	.50	.52*

AUC_G: area under the curve with respect to ground

* $p < .05$

5.5. Summary of Chapter 5a

Overall, subjective sleep did not differ between Night 1 and Night 2 and the CAR did not differ between the morning of measurement, indicating that both subjective sleep and the CAR are temporally stable within normal sleepers. For the CAR, this was the case for the profile of the CAR and across multiple indices, including awakening levels, the peak change, total cortisol secretion or in the peak value of the CAR. The only association observed between subjective sleep and the CAR was a positive relationship between the increase of the CAR, in terms of the

peak change, and the number of awakenings. The following chapter summarises the relationship between objective sleep and the CAR shown in Study 1 before discussing the results.

CHAPTER 5b.

Study 1: Objective sleep and the CAR

5.6. Introduction

Chapter 5b summarises the relationship between objective sleep and the CAR using the standard protocol in the absence of stress, before discussing the overall findings from Study 1.

5.7. Treatment of results

Analysis of the PSG data examined measures of sleep continuity and of sleep architecture. Sleep continuity variables were total sleep time (TST), sleep efficiency (SE), wake after sleep onset (WASO), sleep onset latency (SOL) and the number of awakenings (NWAK). Sleep architecture measures included the percentages of Stage 1 (N1), Stage 2 (N2), Stage 3 (N3) and rapid eye movement (REM) sleep, and the latency to these stages. As the analysis of the CAR excluded three participants, PSG data from the same three participants were excluded from analyses. PSG data obtained during the adaptation night were excluded from further analyses due to potential first-night effects. The relationships between objective sleep continuity, objective sleep architecture and the CAR were analysed using Pearson correlations. The first morning CAR was excluded from analyses to allow comparisons with PSG data.

5.8. Results

5.8.1. Polysomnography

Objective measures of sleep continuity and sleep architecture are summarised in Table 5.9.

5.8.2. *Relationship between the CAR and objective sleep*

Objective measures of sleep continuity (WASO, SOL, TST, SE% and NWAK) were not significantly associated (all p values $> .05$) with the indices of the CAR. In terms of sleep architecture (% of REM, N1, N2, N3 and the latency to these stages), negative relationships were observed between awakening levels and the percentage of time spent in Stage 2 sleep, $r(15) = -.53, p < .05$, and between the CAR peak change and REM latency, $r(15) = -.60, p < .05$. These are summarised in Table 5.10.

Table 5.9
Summary of objective measures of sleep continuity and sleep architecture (n = 15)

Variable	Mean	SD
TST (mins)	430.12	26.46
SOL (mins)	14.28	11.07
NWAK	13.87	4.89
WASO (mins)	13.12	6.59
SE%	94.02	2.77
Time in REM (%)	22.36	3.20
Time in N1 (%)	3.60	1.20
Time in N2 (%)	53.94	5.15
Time in N3 (%)	20.10	5.02
Latency to REM (mins)	105.87	33.79
Latency to N1 (mins)	14.28	11.07
Latency to N2 (mins)	20.73	12.59
Latency to N3 (mins)	33.73	13.77

TST: total sleep time, SOL: sleep onset latency, NWAK: number of awakenings, WASO: wake after sleep onset, SE: sleep efficiency, REM: rapid eye movement sleep, N1: stage 1 sleep, N2: stage 2 sleep, N3: stage 3 sleep.

Table 5.10:

Correlation coefficients between measures of sleep continuity, sleep architecture and indices of the cortisol awakening response (n = 15)

	WASO	SOL	TST	SE(%)	NWAK	REM (%)	N1 (%)	N2 (%)	N3 (%)	Latency to REM	Latency to N1	Latency to N2	Latency to N3
Awakening levels	-.16	.18	.06	-.05	-.16	.31	.03	-.55*	.37	.04	.18	.25	.14
Peak change	.31	-.30	.16	.12	.21	.01	-.12	.27	-.25	-.60*	-.30	-.32	-.14
AUC _G	-.05	.01	.19	.06	-.07	.33	.02	-.43	.23	-.24	.01	.07	.05

* $p < .05$

WASO: wake after sleep onset, SOL: sleep onset latency, TST: total sleep time, SE (%): sleep efficiency (%), NWAK: number of awakenings, REM: rapid eye movement sleep, N1: stage 1 sleep, N2: stage 2 sleep, N3: stage 3 sleep.

5.9. Discussion

5.9.1. Subjective sleep and the CAR

The main aim of Study 1 was to investigate the relationship between the CAR and subjective sleep continuity within healthy good sleepers in a laboratory environment. Temporal stability was shown in subjective sleep, as there were no differences between Night 1 and Night 2, and also in the CAR. The CAR was stable and did not differ across mornings, in terms of the profile and in terms of awakening levels, peak change, total cortisol secretion and in the peak value of the CAR.

The only significant association observed between subjective sleep and the CAR was a positive relationship between the increase of the CAR, as indicated by the peak change, and NWAK. Where previous studies have examined the relationship between subjective sleep and the CAR, the findings have been mixed. The lack of relationship between subjective sleep duration and the CAR, in any indice of the CAR (awakening cortisol levels, peak change, total secretion and in the peak value) in Study 1 was consistent with a previous study (Hansen *et al.*, 2012). However, studies measuring sleep subjectively using sleep diaries have shown conflicting results. A relationship between the CAR and sleep duration has been reported elsewhere both in terms the rise in the CAR and in the awakening sample (Kumari *et al.*, 2009; Stalder *et al.*, 2009) and also in terms of the mean increase during the CAR period, although the reported correlation was small ($r = -.16$) (Wüst *et al.*, 2000b).

Generally, the strength and direction of the relationship appears to be influenced both by the measurement timings and the reporting indices of the CAR. Therefore, the differences between studies may reflect differences in the

measurement of the CAR between studies. It is a strength of the current study that although sleep was measured subjectively, the CAR was measured in a controlled sleep laboratory environment where the relationship between the sleep and the CAR was not influenced by methodological factors relating to participant adherence.

Associations between the CAR and subjective anxiety and stress were also examined in Study 1, where subjective anxiety and perceived stress were measured. Overall perceived stress at awakening was related to two indices of the CAR, being positively related to both awakening cortisol levels and to the peak of the CAR, potentially indicating a relationship between daily activities and the CAR.

Whilst not part of the CAR, cortisol levels measured at wake +120 minutes were significantly higher on Morning 3 compared to Morning 2. Participants remained in the laboratory between Morning 2 and Morning 3 under controlled conditions and followed the same routine. It is possible that the increased cortisol levels at this point on Morning 3 reflect the anticipation of leaving the sleep laboratory and preparing for activities and obligations upon leaving, but that the anticipation of these upcoming demands was not strong enough to disrupt the CAR.

There appears to be some evidence to suggest that the CAR is a marker of anticipation (Fries *et al.*, 2009). CARs (measured on the basis of the mean increase between awakening and 30 minutes post-awakening) have been shown to be approximately three times greater on work days which were characterised as being more demanding than non-workdays (Kunz-Ebrecht *et al.*, 2004; Thorn *et al.*, 2006). As there were no differences in levels of state anxiety or in perceived stress between Morning 2 and Morning 3, this would suggest that it is not the anticipation of a negative or stressful event/series of demands which has affected cortisol levels at

wake + 120 minutes. Instead this suggests that it is the anticipation of an upcoming demand which has affected cortisol levels and that the valence (i.e. whether it is positive or negative) is unimportant, as indicated by the lack of differences shown in negative states.

A single case study has previously shown that subjective mood and the daily anticipation of activities and obligations (irrespective of valence) influence the CAR, explaining approximately 22% of the intra-individual variance in total cortisol secretion over the CAR period (Stalder *et al.*, 2010a). Furthermore, examples from competition studies have shown that demand can affect the CAR, irrespective of whether the upcoming demand is positive or negative. Rohleder and colleagues (Rohleder *et al.*, 2007) observed that awakening cortisol levels were increased on the day of a ballroom dancing competition compared to a practice day, despite similar levels of physical exertion. Other competition studies have shown elevated cortisol levels at 30 minutes post-awakening in male and female tennis players on the first day of a competitive tennis match, compared to a sample obtained from a rest day during a period without competition (Filaire *et al.*, 2009). Similarly, elevated awakening cortisol levels have been shown in judo athletes on the morning of regional and inter-regional judo competitions compared to a resting day three weeks before competition (Filaire *et al.*, 2001) and elevated +30 minutes levels in motorcycle riders before a qualifying trial and race compared to a resting day two weeks previously (Filaire *et al.*, 2007). Taken together, these studies suggest that the anticipation of upcoming demands can affect the indices of the CAR. That said, in these studies the CAR was not typically measured in a comprehensive manner and the relationship observed between demand and various indices outlines the importance of measuring multiple indices of the CAR in the context. As these

studies suggest a link to the anticipation of forthcoming demands as reflected in different indices of the CAR, it will be important to measure multiple CAR indices. Accordingly, the lack of controlled conditions in previous studies may mean that their results are subject to methodological limitations, as discussed in Chapter 2. For example, the findings may have been influenced by factors including light (Figueiro & Rea, 2012; Scheer & Buijs, 1999; Thorn *et al.*, 2004), unexpected awakenings (Born *et al.*, 1999), the time of awakening (Stalder *et al.*, 2009; Thorn *et al.*, 2006) and the deviation from required sampling times (Clow *et al.*, 2004).

Whilst overall sleep within the laboratory was largely representative when compared to the baseline period, total sleep time and sleep efficiency were reduced during the laboratory period. However, participants reported significantly lower levels of state anxiety and lower levels of perceived stress within the laboratory environment, therefore the subjective differences in sleep may not have occurred as a result of anxiety or perceived stress. The perceived reduction in total sleep time and reduction in sleep efficiency may have occurred due to the enforced sleep/wake schedule, despite the efforts made to schedule the sleep/wake episodes in accordance with each participant's habitual routine. It is possible that the lower levels of state anxiety and perceived stress may have been due to the sleep environment. Subjective sleep did not differ between the second and third morning within the laboratory. The lack of relationship between subjective sleep and indices of the CAR may also have been due to the limited sample size employed within Study 1, reducing the statistical power.

Overall, Study 1 indicated that the only relationship observed between subjective sleep and the CAR was a relationship between perceived NWAK and the dynamic of the CAR. Future studies should explore this relationship using objective

methods of sleep, in order to confirm whether there is an underlying casual mechanism. Few studies have examined the relationship between the CAR and objective measures of sleep architecture or sleep continuity and the CAR in normal, healthy sleepers. Negative associations between the duration of REM sleep and awakening levels of cortisol have been shown in alcohol-dependent inpatients (Junghanns *et al.*, 2007) and lower awakening cortisol levels were associated with percentages of Stage 1, Stage 3 and REM in a study of caregivers and non-caregivers (Fonareva *et al.*, 2011). No associations have been shown between measures of sleep architecture and the CAR in a sample of veterans with PTSD and healthy controls, where the CAR was measured in terms of the AUC_G (van Liempt *et al.*, 2013).

5.9.2. *Objective sleep and the CAR*

A further aim of Study 1 was to examine the relationship between objective measures of sleep and the CAR, in a highly-controlled sleep laboratory environment. Overall, certain measures of sleep architecture were associated with the CAR as lower awakening cortisol levels were associated with a greater percentage of Stage 2 sleep and a greater decrease in the dynamic of the CAR was associated with a greater latency to REM sleep.

The current study also showed there to be no relationship between objective sleep duration and cortisol levels at awakening, in contrast to a meta-analysis which showed that previous subjective and objective sleep duration was positively related to cortisol levels at awakening (Garde *et al.*, 2012). Total cortisol secretion and sleep architecture were unrelated, a pattern shown elsewhere in healthy controls and army veterans (van Liempt *et al.*, 2013).

The current study showed that awakening levels were positively associated to Stage 2 sleep and that the peak change, representing the dynamic of the CAR, was negatively related to REM latency. This was in contrast to the relationship previously shown in caregivers and non-caregivers and in alcohol-dependent inpatients, where lower awakening cortisol levels were associated with increased percentages of Stage 1, Stage 3 and REM sleep (Fonareva *et al.*, 2011) and where the duration of REM sleep was shown to negatively relate to cortisol levels at awakening (Junghanns *et al.*, 2007). These discrepant findings may have been due to the fact that previous studies did not only examine healthy good sleepers, with between-group architectural differences potentially acting as a confound. It is a strength of the current study that only healthy good sleepers were assessed. That said, as Study 1 indicated that different measures of subjective and objective sleep were related to different indices of the CAR, it may be the case that gross EEG is not a sensitive enough measure to accurately examine the relationship between objective sleep and the CAR. One way in which this could be overcome is through the use of more sophisticated techniques such as power spectral analysis, which would provide an insight into the discrepancies between subjective and objective sleep.

An advantage of the current study is that the study had an extremely high level of control over factors known to affect the CAR. Saliva samples were obtained by a researcher, meaning that participants were not relied upon to self-collect saliva samples. This offered advantages over a home-based protocol as sampling times were strictly adhered to and participants complied with sampling instructions. This is of particular importance since deviation from the required sampling time can affect the CAR (Clow *et al.*, 2004). The collection of the awakening sample is particularly important as a delayed awakening sample can distort the CAR, by flattening the peak

and blunting the overall shape of the CAR (Griefahn & Robens, 2010; Thorn *et al.*, 2006).

A further strength is that there was no variation in the time of awakening each morning and that the scheduling of sleep and wake was as closely matched to each participant's habitual schedule as was possible. An advantage of this was that the influence of the awakening time would have been minimised (Stalder *et al.*, 2009; Thorn *et al.*, 2006).

In addition, the laboratory environment offered high levels of control over environmental factors known to affect the CAR. Light has been shown to affect the CAR (Figueiro & Rea, 2012; Scheer & Buijs, 1999; Thorn *et al.*, 2004). As the CAR was sampled in extremely low ultraviolet light conditions, the relationship between objective sleep and the CAR was not influenced by variations in light levels. Furthermore, the laboratory environment ensured that participants were monitored at all times, minimising potential sample contamination (Clow *et al.*, 2004). It is possible that discrepant findings from other studies may have been influenced by these environmental differences between studies.

The results of Study 1 may also provide preliminary evidence that the CAR has a function related to memory consolidation during sleep. The current study showed that lower cortisol levels at awakening were associated with a greater percentage of Stage 2 sleep. As Stage 2 sleep may enhance memory consolidation, either individually or in conjunction with REM and slow-wave sleep (Marshall & Born, 2007), it is possible that the CAR is related to memory consolidation or has a function within consolidation. The current study showed that the dynamic of the CAR was also shown to be related to REM latency, as a greater decrease in the

dynamic was associated with a greater latency to REM sleep. Vandekerckhove & Cluydts (2010) suggest that the effects of sleep upon mood and emotion of the following day are thought to be affected via REM sleep and that there is a relationship between emotional experience and changes in REM sleep. The relationship between the dynamic of the CAR and REM latency may represent the effects of emotional regulation upon the CAR. Another possibility is that this represents emotional memory consolidation, as REM sleep has been speculated to support consolidation of these memories (Diekelmann, Wilhelm, & Born, 2009). It is an advantage of the current study that healthy individuals remained in a highly controlled environment and that the CAR was examined using multiple indices.

These findings suggest that the relationship between sleep architecture and the CAR may affect measurement indices of the CAR in a different manner. Limitations of the present study include the correlational nature of the study, meaning the causal relationships between sleep architecture and the CAR have yet to be clarified and a further limitation is the relatively small sample size. As discussed earlier in Chapter 5, previous studies have demonstrated an association between forthcoming demands and indices of the CAR, in terms of awakening levels (Filaire *et al.*, 2001; Rohleder *et al.*, 2007) and 30 minutes post-awakening (Filaire *et al.*, 2009; Filaire *et al.*, 2007). Furthermore, Study 1 has demonstrated that cortisol levels were greater immediately prior to leaving the laboratory on the final day. It is possible that these increased levels reflect the anticipation of forthcoming demands, following a day in a highly-controlled environment with no external stimuli and the completion of sedentary activities. If indices of the CAR are subject to the influence of forthcoming demand, this can be experimentally manipulated using a similar protocol to Study 1. Study 2 will explicitly include forthcoming demand within the

protocol and examine the subsequent effects upon multiple indices of the CAR. This will overcome the limitations of previous studies where the CAR was not measured in a comprehensive manner, and will allow us to determine whether the CAR is indeed a biological marker of anticipation or demand.

CHAPTER 6.

Study 2

6.1. Introduction

The Spielman 3P model of insomnia (Spielman *et al.*, 1987; Spielman & Glovinsky, 1991; Spielman *et al.*, 1996) and the Cano-Saper animal model of insomnia (Cano *et al.*, 2008) both have a role for stress in the disruption of sleep and in the development of insomnia. Previous research has assessed the effects of naturalistic stressors and have shown that subjective and objective sleep disturbances are commonplace, however, whilst disruptive to sleep, the findings are inconsistent (Askenasy & Lewin, 1996; L. M. Davidson *et al.*, 1987; Dooley & Gunn, 1995; Kato *et al.*, 1996; North *et al.*, 1999; Schuster *et al.*, 2001). The inconsistencies may in part be due to the wide range of stressors employed, including financial strain, health events, bereavement and examinations (Hall *et al.*, 2008; Hall *et al.*, 2009; Hardison *et al.*, 2005; Palesh *et al.*, 2007; Reynolds 3rd *et al.*, 1992; Reynolds 3rd *et al.*, 1993; Wright *et al.*, 2010). In addition to the diverse stressors employed and in the diverse nature of participants, there is a typically a time lag between the stress and the measurement of sleep.

The effects of experimental stress studies have also shown inconsistent effects upon subjective sleep and objective sleep. As with naturalistic stressors, whilst disruptive, these inconsistent findings may be due to the wide variation in the nature of the stress employed, which has included tasks such as intelligence tests, cognitive tasks or where participants have viewed psychologically stressful films (Baekeland *et al.*, 1968; Koulack *et al.*, 1985; Vandekerckhove *et al.*, 2011; Wuyts *et al.*, 2012a). This wide variation in the nature of demand may influence sleep since the type of

task employed may differentially affect sleep (Kobayashi *et al.*, 1998). As well as the wide variation in the nature of the demand, other potential confounding variables include where the stress is experienced during the first night (Baekeland *et al.*, 1968). Stress studies typically do not separate the effects of demand from the stress, with the exception of Beaumaster and colleagues (Beaumaster *et al.*, 1978) which assessed the effects of the anticipation and effects of a parachute jump in novice and experienced parachute jumpers, showing that the anticipation of the jump affected the latencies to Stage 1 and Stage 2 sleep, where experienced jumpers displayed an increased SOL compared to novice jumpers and where novice jumpers showed a decreased amount of Stage 1 sleep. Following the jump, there were no differences in sleep between the two groups indicating that the demand did not affect sleep. A further limitation of studies assessing the effects of stress upon sleep is that they do not typically include a physiological marker of stress, meaning that it is difficult to verify that individuals find the stress paradigms used to be stressful.

Anticipation has been shown to affect subjective and objective sleep, in both naturalistic and experimental studies. Anticipation has been shown to affect the sleep of a group of engineers during watch duty (Torsvall *et al.*, 1987). Whilst on duty, the engineers displayed a sleep loss of more than 90 minutes per night and reported a poorer perceived sleep quality. Engineers also reported greater difficulties in falling asleep, less sleep and poorer sleep quality during watch duty nights, even when no alarms occurred, compared to a free night. Similarly, a study of five male ship engineers on-call showed a shorter duration of sleep and reduced periods of REM and slow wave sleep, compared to free nights. On-call nights also led to reductions in EEG power density during the first cycle of sleep, when no alarms occurred, which suggested the anticipation of upcoming demands disrupted sleep (Torsvall &

Akerstedt, 1988). Since anticipation can affect subjective and objective sleep, the influence of anticipation needs to be taken into account within studies assessing the effects of stress upon sleep. In one study good sleepers completed 30 minutes of cognitive tasks before going to bed within a laboratory environment, where subjective and objective sleep was compared to a reference night (Wuyts *et al.*, 2012a). There were no differences in subjective measures of sleep continuity or quality compared to the reference night, however objective sleep-onset latency was greater following the tasks. Objective differences were also shown in that a higher percentage of high frequency EEG activity was apparent in the first and second episodes of deep sleep following the tasks. Participants spent a night in their own home between the reference and task night, and the order of the reference night and task night was counterbalanced between participants. Due to the delay between the reference night and the task night, it is possible that the anticipation of forthcoming demands may have influenced the results.

Anticipation may also affect the CAR, with evidence coming from studies showing that the mean increase of the CAR is greater on more demanding work days than non-workdays (Kunz-Ebrecht *et al.*, 2004; Thorn *et al.*, 2006). As discussed in Chapter 2, competition studies have shown that demand can affect indices of the CAR, irrespective of the valence of the upcoming demand (Filaire *et al.*, 2009; Filaire *et al.*, 2007; Filaire *et al.*, 2001; Rohleder *et al.*, 2007). In addition, Study 1 showed that cortisol levels were higher at 120 minutes following awakening on Morning 3, immediately prior to participants leaving the sleep laboratory. Although this time point would be viewed as outside that of the typical CAR period (60 minutes), it is possible that the increased cortisol levels observed at this point reflected the anticipation of activities and obligations upon leaving the laboratory.

Subjectively, participants also showed a steeper CAR increase on the Morning 3, again potentially reflecting anticipation.

Participants had remained in the sleep laboratory between the second and third mornings under controlled conditions, with standardised activities and mealtimes. As there were no differences shown in state anxiety or in perceived stress between second and third morning, this suggests that the anticipation of upcoming demand, irrespective of valence, may affect cortisol levels immediately prior to leaving the laboratory. Since anticipation appears to affect both sleep and the CAR, it will be necessary to separate the effects of anticipation and the effects of demand upon sleep. The potential effects of upcoming demand upon sleep and the HPA axis could therefore be appropriately tested through the manipulation of demand and anticipation within a consistent, controlled environment. This will be investigated within Study 2, where participants will believe that they will experience demand at the time of study enrolment. However, not all participants will experience demand. This will ensure that the effects of anticipation alone upon sleep and the CAR can be examined.

Study 2 had two main aims. The first aim was to examine whether anticipation of forthcoming demand affected subjective sleep, objective sleep and indices of the CAR. The second aim was to examine the effects of anticipation, where this was met with subsequent demand, upon the same measures. Evidence from previous studies has suggested that the CAR may function as a marker of anticipation (Filaire *et al.*, 2009; Filaire *et al.*, 2007; Filaire *et al.*, 2001; Rohleder *et al.*, 2007) however this has included studies which have relied upon a single sample obtained during the CAR period (Filaire *et al.*, 2009; Filaire *et al.*, 2007; Filaire *et al.*, 2001). A further limitation of these studies is that they do not measure the CAR

in a controlled environment, meaning that the CAR may be influenced by other factors, including light, differences in environment and adherence to the required time of sampling. Study 2 will employ the protocol previously used in Study 1 to measure sleep and the CAR, as this protocol addressed these limitations by examining the CAR in a comprehensive manner, through multiple saliva samples within a controlled laboratory environment. Study 1 observed elevated levels of cortisol immediately prior to leaving the controlled sleep environment on the final day of the study and it is speculated that these increased levels reflect anticipation of forthcoming demands.

Study 2 will investigate this through experimentally manipulating anticipation of forthcoming demand and exploring the effects of this anticipation upon multiple indices of the CAR (CAR profile, awakening levels of cortisol, peak values, total secretion and the peak change). Study 2 will employ a manipulation where the anticipation and demand are separated. The anticipated demand will either be met, where participants will be required to complete the cognitive tasks which they had been informed of at the time of enrolment, or will not be met, where participants will be informed that they are no longer required to complete the range of cognitive tasks, shortly before they are due to do so. This will allow the effects of anticipation, where the subsequent demand is met, to be compared with the effects of anticipation where the subsequent demand is not met. Study 2 therefore had two research questions:

1. What are the effects of anticipation of forthcoming demand upon subjective sleep, objective sleep and the CAR?
2. What are the effects of anticipation upon these measures when the anticipation is met with subsequent demand?

6.2. Method

6.2.1. *Participants and recruitment*

A total of 22 normal healthy sleepers (11 male and 11 female; $M_{\text{age}} = 23.42$ years, $SD_{\text{age}} = 3.61$ years) participated in Study 2 and all participants were recruited and screened as described in Section 3.2. All participants provided written informed consent, and the study was approved by the Northumbria University Research Ethics Committee. Participants were paid a total of £150 and were provided with a full debrief upon completion of the study.

Participants were allocated to one of two groups: anticipation and demand (AD, $n = 11$), where participants were informed in advance that they were required to complete the tasks described in Section 6.3.1 and completed these on Day 2, or the anticipation alone (AA, $n = 11$) group, where participants were informed in advance of the tasks but were told on Day 2 that they were not required to complete the tasks. Participants were placed in the AD group first in order to minimise the likelihood of participants in the AA group informing other participants of the manipulation. Objective sleep and CAR data from Study 1 participants (nine male and nine female; $M_{\text{age}} = 23.46$ years, $SD_{\text{age}} = 3.21$ years) were used as a control group for comparisons in order to measure the effects of anticipation upon subjective sleep, objective sleep and the CAR, since these participants followed the same protocol where sleep and the CAR were measured in the absence of demand. Night 2 subjective sleep, objective sleep and the Morning 2 CAR were compared between the control group and all participants in Study 2 (i.e. participants who had been informed of forthcoming demand). This was done in order to examine how anticipation affected these variables in participants who were anticipating upcoming demand, compared to individuals (the control group) who were not expecting any upcoming demand.

6.2.2. *Measures*

The measures completed were described in Section 3.3 (PSQI: Pittsburgh Sleep Quality Index, PSS: Perceived Stress Scale, HADS: Hospital Anxiety and Depression Scale, HÖ-MEQ: Horne-Ostberg Morningness Eveningness Questionnaire, PSRS: Perceived Stress Reactivity Scale) and participants completed these measures upon arrival at the laboratory on the adaptation night.

6.2.3. *Polysomnography*

PSG was applied on the adaptation night, Night 1 and Night 2 following the procedure described in Section 3.4.2. PSG obtained from the adaptation night was excluded from all further analyses in order to avoid any potential influence of a first-night effect.

6.2.4. *Pre-sleep mood diaries*

Participants also completed mood diaries on the adaptation night, Night 1 and Night 2, in bed, prior to lights out. The mood diaries were identical to those described in Section 3.4.1.

6.2.5. *Cortisol and cortisol awakening response (CAR) sampling*

Cortisol samples were obtained using Salivettes (Sarstedt, Leicester, UK) as described in Section 3.4.4. All samples were collected by a researcher and participants were instructed to chew on the Salivettes for 60 seconds. Saliva samples were obtained in order to measure the cortisol awakening response, daytime cortisol levels and pre-sleep cortisol levels. The CAR was measured in the same manner as

described in Section 3.4.5, where samples were obtained at time intervals of 15 minutes (awakening, +15, + 30, +45 and +60 minutes).

6.2.6. Subjective sleep and mood

Participants were also required to complete sleep and mood diaries on Morning 1, Morning 2 and Morning 3 which assessed subjective sleep and quality. The diaries were identical to those described in Section 3.4.1.1. Sleep and mood diaries were completed 60 minutes following awakening, whilst participants were still in bed.

6.3. Procedure

6.3.1. Anticipation and demand group

At the time of study enrolment, all participants were informed that they would be required to spend a full day (Day 2) in the sleep laboratory and would be required to perform a range of demanding tasks for the full day. Following enrolment, participants were monitored for two weeks prior to the laboratory stay using sleep and mood diaries (Section 3.4.1.1) and actigraphy (Section 3.4.1.2) in order to ensure relative sleep and circadian stability. Participants arrived at the sleep laboratory between 8.00pm and 9.00pm on the adaptation night. Participants provided questionnaire and demographic information at the time of arrival on Night 1. PSG (Section 3.4.3) was applied on the adaptation night, Night 1 and Night 2 and lights out and lights on times were scheduled in accordance with habitual bed time and

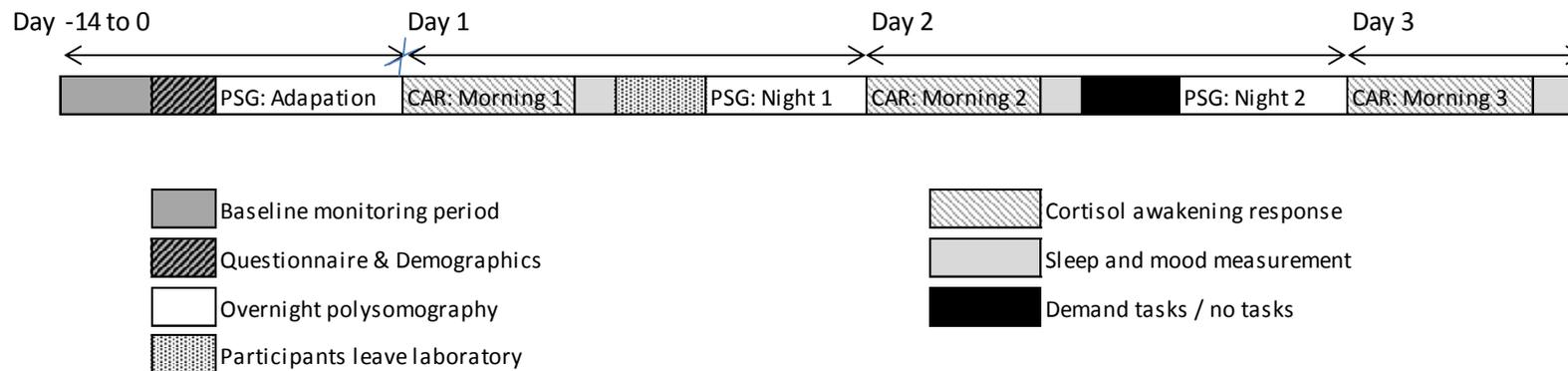


Figure 6.1: Study 2 protocol

Task	wake +2hrs	+3hrs	+4hrs	+5hrs	+6hrs	+7hrs	+8hrs	+9hrs	+10hrs	+11hrs	+12hrs	+13hrs	+14hrs
Saliva sample	■			■			■			■			■
Mood diary		■	■	■		■	■	■		■	■	■	
Meal provided	■				■				■				
NASA-TLX		■	■	■		■	■	■		■	■	■	
MTF		■	■	■		■	■	■		■	■	■	
AES			■										
IGT											■		

MTF = Multi-tasking Framework, AES = Altered Emotional Stroop, IGT = Iowa Gambling Task

Figure 6.2: Schedule of demand tasks (for AD group only) during the assessment period on Day 2

sleep durations from baseline sleep diaries. Participants were required to complete the mood section of the sleep and mood diaries (Section 3.4.1.1) and a saliva sample was obtained prior to lights out each night. Participants were allowed to leave the laboratory two hours following awakening on Morning 1 after being instructed to adhere to their usual routine and returned to the laboratory on Night 1. Immediately before lights out on Night 1, all participants were instructed to read an instruction sheet, delivered by the same researcher (Appendix J) which provided details of the daytime tasks to be completed the following day. Saliva samples were obtained for measuring of the CAR between awakening and 60 minutes post-awakening. Electrodes were removed at this point and participants were required to complete a sleep and mood diary. A saliva sample was obtained at +120 minutes. Participants remained in the sleep laboratory during Day 2 for the full day and were not permitted to leave or exercise at any point.

The anticipation and demand (AD) group refers to the group of participants where the anticipation of forthcoming demand was met through the completion of a range of demanding tasks during Day 2. Participants in the AD condition completed a range of tasks in accordance with the schedule in Figure 6.1, where the tasks were chosen in order to ensure high levels of demand. Participants in the AD completed three different tasks during Day 2. Participants completed a modified version of the Emotional Stroop task (Barclay & Ellis, 2013), the Multi-Tasking Framework (MTF, Purple Research Solutions; Wetherell & Sidgreaves, 2005) and the Iowa Gambling Task (IGT; Bechara, Damasio, Damasio, & Anderson, 1994). Further task information is provided in the following section (Section 6.3.2). A variety of tasks were included in order to ensure that participants were uncertain about the type of task that they would perform next. In order to ensure high levels of motivation

throughout the testing day, participants were informed at the time of enrolment that the individual with the highest score on a randomly-selected task would win an Apple iPad. Participants were allowed to perform activities including reading, watching television/films or use of the internet between tasks.

To allow comparison with control participants from Study 1, additional saliva samples were obtained at wake + 5 hours, wake + 8 hours, wake + 11 hours and wake + 14 hours. Participants were provided with standardised meals during Day 2 (as described in Section 5.3.4). Breakfast was provided 2 hours post-awakening, lunch was provided at six hours post-awakening and dinner was provided 10 hours post-awakening. Participants were provided with additional snacks (e.g. fruit or chocolate) upon request, provided this did not occur 30 minutes before the next saliva sample. Participants received a full debrief at the end of the study on Morning 3.

6.3.2. *Day 2 demand tasks*

Participants within the AD condition completed three different tasks during Day 2, detailed in the following sections. All testing was completed on a PC with a 17" monitor within the participant's bedroom within the sleep laboratory.

6.3.2.1. *Modified Emotional Stroop task*

Participants completed an modified version of the Emotional Stroop task (Barclay & Ellis, 2013) four hours post-awakening. The Emotional Stroop task is a measure of attention to sleep-related stimuli and 20 sleep-related, 20 neutral and 20

nonspecific threat lists were presented randomly in the centre of the computer screen in either red, blue, green or yellow coloured writing. Participants were required to respond by pressing a computer key with a corresponding coloured sticker as quickly as possible. An initial fixation cross was displayed for 500ms before the initial stimulus and for 500ms between each word. The word remained on the screen until a response was recorded. Written instructions were displayed on the screen prior to starting the task and participants initially completed a practice trial of 20 separate neutral words. The modified version of the Emotional Stroop task extended the task to a total of 120 trials and participants were told that the task would contain a rule change at an unspecified point. The first 60 trials of the task followed the protocol described above (i.e. pressing the corresponding computer key to the colour of the word displayed). After 60 trials the rules changed, where the colours of the words changed. Red words were then displayed in orange, yellow words were displayed in black, green words were displayed in brown and blue words were displayed in purple. Participants had a chart of the new rules beside them during the task and were required to respond in the same manner as before (e.g. by pressing the red key when an orange word appeared).

6.3.2.2. *Multi-tasking Framework*

Participants were required to complete the Multi-Tasking Framework (MTF, Purple Research Solutions, UK) at seven time points throughout the day. The MTF consists of eight possible tasks which can be run individually or in combination and up to four tasks can be displayed simultaneously (Wetherell & Sidgreaves, 2005). Tasks can be run at a low, medium or high level of workload intensity. The MTF is representative of everyday demanding situations and has been shown to reliably

elicit workload stress, where increases in physiological and psychological variables (blood pressure, heart rate, perceived workload and mood) have been shown in relation to increases in the workload intensity of the MTF (Wetherell & Carter, in press). The MTF is also a suitable task for repeated use within the same sample of participants (Scholey *et al.*, 2009).

The four tasks used in the current study (Figure 6.3) were the auditory monitoring task, where individuals had to identify a target tone from two auditory tones played at regular intervals, the telephone number task, in which a 10 digit number had to be entered into a telephone keypad, a visual monitoring task, where six bars rose, at different speeds, towards a target line and participants had to click each bar in the order in which they rose. The final task was a number tap task, where a grid of 12 digits ranging from one to nine appeared on the screen and individuals were required to click on the highest digits in each set. Points are awarded for correct responses and points are deducted for incorrect responses or missed responses. A running score was displayed in the middle of the screen. Participants were required to complete all tasks simultaneously and tasks were set at a medium workload intensity, in order to ensure that participants found the tasks demanding but not impossible to complete. Participants were given a demonstration run lasting two minutes and each run of the MTF lasted for 10 minutes. Participants were instructed to perform the MTF to the best of their ability, where they were to be as fast and as accurate as possible. Participants were reminded of their score from the previous run of the MTF immediately prior to the next run. The score obtained during each run of the MTF was displayed on a scoresheet attached to a wall, in sight of the participant.

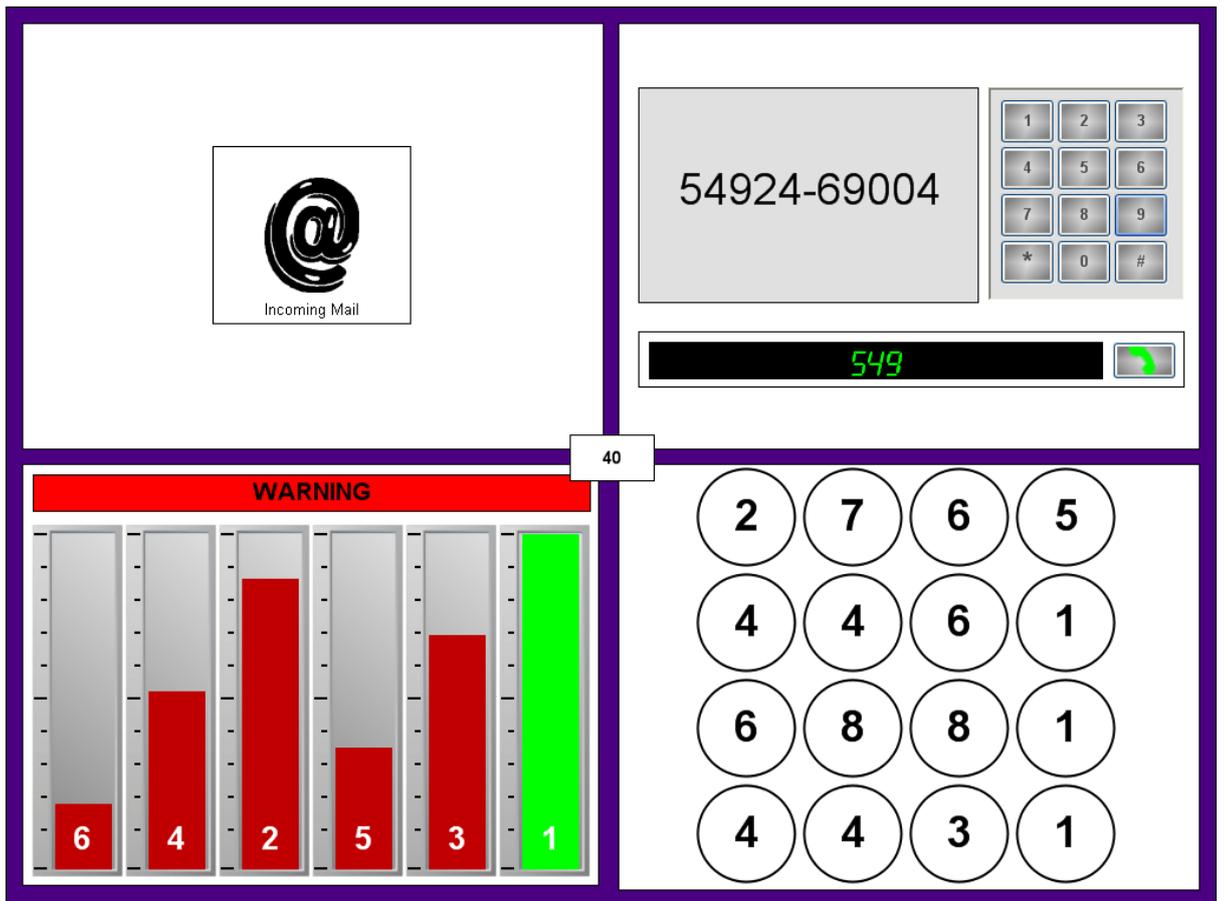


Figure 6.3: Example of the Multi-tasking Framework.

6.3.2.3. Iowa Gambling Task

Participants were required to complete a modified version of the Iowa Gambling Task (IGT; Bechara *et al.*, 1994) 12 hours after awakening, which is a measure of decision-making. Participants were initially presented with a screen providing instructions (Appendix L) which were a simplified version of those provided by Bechara, Tranel & Damasio (2000). Participants were informed that the game involves choosing one cards from any of four decks and that they would receive an amount of points which varies depending on the card chosen. Participants are informed before commencing the task that their main aim in the game is to attain as many points as possible. They are also informed that they are free to decide which

deck of cards they can select a card from and are also informed that they can switch from one deck of cards to another at any point. Participants were presented with a choice of four cards, equal in appearance and size. Following each card selection, a message was displayed indicating whether the participant won or lost points and the corresponding value of each gain/loss. Each deck of cards has a corresponding reward and punishment schedule which the participant is unaware of. Participants are not informed of how many card selections are available in the game and the task ends after 100 card selections. Participants gain 100 points when choosing a card from deck A or deck B and gain 50 points when choosing from deck C or deck D. Although the schedule and magnitude of punishments differ, deck A and deck B incur higher penalties than deck C or deck D. Deck A and deck B both incur net losses of 250 points after 10 trials and deck C and D result in a net gain of 250 points after 10 trials. Choosing from deck A and deck B results in a loss and are disadvantageous decks for the player, whereas deck C and deck D result in a net gain and are advantageous for the player. Normal, healthy participants display a tendency to pick more cards from advantageous decks and fewer cards from disadvantageous decks over the duration of the task (Bechara *et al.*, 1994).

6.3.2.4. *Perceived effort (NASA-TLX)*

Participants in the AD group were also required to report their levels of perceived workload immediately after each task by completing the NASA-Task Load Index (NASA-TLX; Hart & Staveland, 1988). The NASA-TLX (Appendix N) consists of six subscales (measuring mental, physical and temporal demand, effort, performance and frustration). Participants were required to mark on a 100mm VAS anchored with 'low' at one end and 'high' at the other.

6.3.3. *Anticipation alone group*

The anticipation alone (AA) group refers to the group of participants where the anticipation of forthcoming demand prior to Day 2 was not met during Day 2. Participants within the AA group followed the same procedure as the AD group, until immediately following provision of the wake +120 minutes saliva sample on Day 2, participants in the AA group were provided with standardised instructions (Appendix M) which informed them that they were no longer required to complete any tasks. Participants in the AA group spent their day performing the same activities (e.g. reading, watching television/films or using the internet) as the AD group did between the completion of tasks. Additional saliva samples were obtained and standardised meals were provided at identical time points to the AD group, with snacks provided upon request. Participants received a full debrief at the end of the study on Morning 3.

6.3.4. *Mood: state anxiety and perceived stress*

Participants in both the AA and AD groups were required to complete mood diaries measuring state anxiety and subjective stress (as described in Section 3.4.1.1) during Day 2 (Appendix N) Both groups completed these at the same time points during Day 2 (at wake +3hrs, +4hrs, +5hrs, +7hrs, +8hrs, +9hrs, +11hrs, +12hrs, +13hrs) and the AD group completed these following each task.

6.3.5. *Treatment of results*

6.3.5.1. *Baseline, age and demographic data*

No participants showed evidence of sleep or circadian problems from two-week baseline sleep diary data or actigraphy data. Actigraphy data was not used in any further analyses. Scores obtained from tasks completed during Day 2 were not included within any further analyses as the purpose of the tasks was to induce demand. In order to compare age and questionnaire data between the AA and AD groups, these measures were compared using a series of independent-samples *t*-tests.

6.3.5.2. *Night 1 subjective and objective sleep*

In order to compare measures of subjective sleep continuity and mood on Night 1, the AA and AD groups were combined to provide an anticipation group as both the AA and AD groups at this time point would have expected to complete the Day 2 demand tasks during the following day. This was compared to data obtained from the two-week baseline period, excluding non-work days, using a series of paired-samples *t*-tests. In order to compare objective sleep between the anticipation (AA & AD) and control group on Night 1, multivariate analysis of variance (MANOVA) tests were conducted on measures of sleep continuity (TST, SE%, WASO, SOL and NWAK), on measures of sleep architecture (percentages of time spent in REM, N1, N2 and N3 sleep) and on the latency to each stage of sleep (REM, N1, N2 and N3).

6.3.5.3. *Night 1 pre-sleep cortisol levels*

In order to examine the effects of anticipation upon pre-sleep cortisol levels, Night 1 pre-sleep cortisol levels were compared between the combined anticipation (AA & AD) groups and control participants, using an independent-samples *t*-test. One participant was removed from the control group due to excessively high (> 75nmol/l) cortisol levels.

6.3.5.4. *Morning 2 CAR*

CAR data from three participants in the control group were excluded due to saliva samples containing an insufficient volume of saliva for analysis ($n = 2$) and due to excessively high (>75 nmol/l, Kunz-Ebrecht *et al.*, 2004) cortisol levels ($n = 1$). Due to samples containing an insufficient volume of saliva for analysis, CAR data was excluded from some participants from the AD group ($n = 3$) and AA group ($n = 2$). The Morning 2 CAR was compared between anticipation (AD & AA) and control groups using a 2 (condition) \times 5 (time point) mixed ANOVA. Effect sizes were reported alongside ANOVA results, expressed as partial eta squared (η^2_p). Separate independent-samples *t*-tests were conducted to compare additional Morning 2 CAR indices (awakening levels, peak change, AUC_G and peak value) between the AA and control groups.

6.3.5.5. *Night 2 subjective sleep, mood and objective sleep*

Measures of subjective sleep continuity and quality and mood obtained during Night 2 were compared between the AA and AD groups using a series of independent-samples *t*-tests. In order to compare the objective sleep of the AA and

AD group on Night 2, MANOVAs were conducted on measures of sleep continuity (TST, SE%, WASO, SOL and NWAK), on measures of sleep architecture (percentages of time spent in REM, N1, N2 and N3 sleep) and on the latency to each stage of sleep (REM, N1, N2 and N3).

6.3.5.6. *Morning 3 CAR*

The Morning 3 CAR was compared between AA and AD groups using a 2 (condition) \times 5 (time point) mixed ANOVA. Additional indices (awakening levels, peak change, AUC_G and peak values) were compared between AA and AD groups using a series of independent-samples *t*-tests.

6.3.5.7. *Morning 2 and Day 2 perceived stress and state anxiety*

Levels of perceived stress following the CAR period (wake +1 hour) were compared between the AD and AA groups on Morning 2 using independent-samples *t*-tests. This was done in order to examine whether the two groups showed any differences in perceived stress prior to either completing or not completing the demand tasks on Day 2. Perceived levels of stress from the first and last Day 2 mood diaries (completed at wake + 3 hours and at wake + 13 hours) were compared between the AD and AA groups using a 2 (condition) \times 2 (time point) mixed ANOVA in order to examine whether AD participants showed any difference in perceived stress levels following the first task and the final task. Day 2 daytime levels of state anxiety were compared between the AA and AD groups using a 2 (condition) \times 10 (time point: 60 minutes post-awakening (representing the end of the CAR period; wake +1hr) and at each time point during Day 2) mixed ANOVA.

Effect sizes were reported alongside ANOVA results, expressed as partial eta squared (η^2_p). Scores from the NASA-TLX (AD participants only) were not analysed, however a subjective inspection the scores on each subscale indicated the effects of the demanding tasks were consistent throughout Day 2.

6.3.5.8. *Day 2 and Night 2 cortisol: diurnal and pre-sleep levels*

Day 2 diurnal cortisol levels were compared between the AA ,AD and control groups using a 3 (condition) \times 5 (time point: wake + 5hrs, wake + 8hrs, wake + 11hrs, wake + 14hrs, presleep) mixed ANOVA. Effect sizes were reported alongside ANOVA results, expressed as partial eta squared (η^2_p), Day 2 total cortisol secretion was calculated using the AUC_G formula (as discussed in Section 3.4.5) using cortisol levels at wake + 5 hours, wake + 8 hours, wake + 11 hours, wake + 14 hours and presleep cortisol levels. Greenhouse-Geisser corrected degrees of freedom were reported where appropriate. The AUC_G was compared between the AA and AD groups using an independent-samples *t*-test.

6.4. Results

6.4.1. *Demographic and questionnaire comparisons*

The AA and AD group did not show any significant differences (all *p*-values $>.05$) in age, demographics or in any questionnaire measure, as summarised in Table 6.1. In addition, both groups did not show any significant differences in mood (subjective stress or in state anxiety) either during the baseline period (*p* $>.05$), on Morning 2 (*p* $>.05$) or on Morning 3 (*p* $>.05$).

6.4.2. Does anticipation affect sleep, the CAR and pre-sleep cortisol levels?

6.4.2.1. Subjective sleep: Night 1 and baseline comparison

Participants estimated their subjective total sleep time to be significantly reduced ($t(21) = -2.91, p < .01$) during the night before stress in the laboratory (Night 1), compared to their two-week baseline period. Participants also reported significantly higher subjective levels of mental alertness in bed during the night prior to stress in the laboratory ($t(21) = -2.44, p < .05$), indicating that participants felt

Table 6.1

Age and demographic comparisons: AD and AA groups.

Measure	AD		AA	
	Mean	SD	Mean	SD
Age	24.66	4.54	22.19	1.88
PSQI	3.45	2.11	3.27	1.74
PSS	23.82	6.16	22.45	8.19
FIRST	18.82	7.28	18.45	4.87
HADS Anxiety	4.73	2.69	6.09	3.59
HADS Depression	1.91	1.92	2.82	1.99
HÖ -MEQ	48.27	9.68	50.27	10.27
PSRS Total	21.27	9.01	16.45	5.87

PSQI: Pittsburgh Sleep Quality Index, PSS: Perceived Stress Scale, FIRST: First Insomnia Response to Stress Test, HADS: Hospital Anxiety and Depression Scale, HÖ -MEQ: Horne-Östberg Morningness-Eveningness Questionnaire, PSRS: Perceived Stress Reactivity Scale

more mentally alert during the night before the anticipated stressor compared to an equivalent baseline period. Participants also displayed a trend towards displaying a higher NWAK ($t(21) = 2.00, p = .058$) during the night prior to an anticipated stressor, compared to their two-week baseline period. This is summarised in Table 6.2.

Table 6.2
Subjective sleep continuity and quality comparisons: Anticipation (AA & AD) baseline and Night 1

Variable	Anticipation Baseline		Anticipation Night 1	
	Mean	SD	Mean	SD
SOL	19.43	18.14	19.19	16.07
NWAK	1.36	1.07	.87	.76
WASO (mins)	7.73	9.31	5.32	6.25
TST (mins)	457.64	55.99	484.91	62.76
TIB (mins)	534.55	41.83	561.18	75.58
SE (%)	85.56	8.33	86.21	8.46
How well do you feel? (0 – 4)	2.73	.83	2.84	.64
How enjoyable was your sleep? (0 – 4)	2.91	.81	2.89	.53
How mentally alert were you in bed last night? (0 – 4)	.91	.61	1.21	.67
How physically tense were you in bed last night? (0 – 4)	.64	.85	.78	.55

AA: anticipation alone, AD: anticipation and demand, SOL: Sleep onset latency, NWAK: number of awakenings, WASO: wake after sleep onset, TST: total sleep time, TIB: time in bed, SE (%): sleep efficiency

6.4.2.2. *Objective sleep: Night 1 anticipation (AA & AD) and control comparison*

The anticipation of the upcoming demand did not affect objective sleep on Night 1, as indicated by no significant effect of condition upon sleep continuity, upon sleep architecture or upon the latency to each stage of sleep (all p -values > .05). This is summarised in Table 6.3.

Table 6.3
Objective sleep: Anticipation and Control Night 1 Comparisons

Variable	Anticipation (AA&AD)		Control	
	Mean	<i>SD</i>	Mean	<i>SD</i>
TST (mins)	452.80	<i>41.21</i>	434.78	<i>23.44</i>
SE (%)	95.96	<i>1.89</i>	94.71	<i>2.19</i>
WASO	8.68	<i>4.31</i>	12.08	<i>7.27</i>
SOL	10.25	<i>7.21</i>	12.28	<i>9.47</i>
NWAK	12.09	<i>4.95</i>	13.78	<i>5.78</i>
Time in REM (%)	24.07	<i>6.15</i>	22.59	<i>4.17</i>
Time in N1 (%)	3.30	<i>1.85</i>	3.38	<i>1.41</i>
Time in N2 (%)	51.06	<i>8.33</i>	54.45	<i>5.76</i>
Time in N3 (%)	21.58	<i>5.86</i>	19.58	<i>5.12</i>
Latency to REM (mins)	95.80	<i>36.67</i>	109.75	<i>39.92</i>
Latency to N1 (mins)	10.25	<i>7.21</i>	12.28	<i>9.47</i>
Latency to N2 (mins)	16.41	<i>12.42</i>	17.19	<i>11.08</i>
Latency to N3 (mins)	27.68	<i>12.82</i>	29.50	<i>12.63</i>

AA: anticipation alone, AD: anticipation and demand, TST: total sleep time, SE (%): sleep efficiency, WASO: wake after sleep onset, SOL: Sleep onset latency, NWAK: number of awakenings, REM: rapid eye movement, N1: stage 1, N2: stage 2, N3: stage 3.

6.4.2.3. *Pre-sleep cortisol levels: Night 1 anticipation (AA & AD) and control comparison*

Night 1 pre-sleep cortisol levels were compared between anticipation (AA & AD) and control groups. The differences between pre-sleep cortisol levels for the anticipation ($M = 1.69$ nmol/l; $SD = 1.99$ nmol/l) and the control ($M = 1.45$ nmol/l; $SD = .62$ nmol/l) groups was not significant ($t(37) = -.46, p > .05$), indicating that there were no differences in pre-sleep cortisol levels between individuals who were anticipating upcoming demand and control participants who were not.

6.4.2.4. *CAR: Morning 2*

The Morning 2 CAR was compared between anticipation (AA & AD) and control groups. There was a significant main effect of time point ($F(2.92, 84.80) = 6.64, p < .001, \eta^2_p = .19$) reflecting the typical changes in cortisol levels during the CAR period. The main effect of condition was not significant ($F(1,29) = .83, p > .05, \eta^2_p = .03$). The time point \times condition interaction was significant ($F(2.92, 84.80) = 2.91, p < .05, \eta^2_p = .09$) indicating that the anticipation group displayed a steeper CAR and displayed higher levels of cortisol throughout the CAR period, compared to the control group (Figure 6.4). Follow-up tests were conducted on each time point of the CAR using Bonferroni corrections for multiple comparisons ($.05/5 = .01$) however no comparisons reached significance at the corrected level. The CAR is shown in Table 6.4 and Figure 6.4. Analysis of additional CAR indices (awakening cortisol levels, peak change, AUC_G and peak value) showed that there was a significant effect of the condition on peak change ($t(24.60) = -2.09, p < .05$), where the anticipation group showed a greater peak change on Morning 2 compared to the

Table 6.4

Cortisol levels at each time point of the CAR: Morning 2 Anticipation (AA & AD) and Control comparisons

Variable	Awakening		+15 minutes		+30 minutes		+45 minutes		+60 minutes	
	Mean	<i>SD</i>	Mean	<i>SD</i>	Mean	<i>SD</i>	Mean	<i>SD</i>	Mean	<i>SD</i>
Anticipation (AA/AD)	8.23	4.45	10.76	5.76	12.44	5.13	11.64	4.67	12.12	5.47
Control	8.85	4.47	9.10	4.26	10.58	3.62	9.40	3.97	8.66	3.56

AA: anticipation alone, AD: anticipation and demand

Table 6.5

Additional indices of the CAR: Morning 2 Anticipation (AA & AD) and Control comparisons

Group	Awakening		Peak Change		AUC _G		Peak Value	
	Mean	<i>SD</i>	Mean	<i>SD</i>	Mean	<i>SD</i>	Mean	<i>SD</i>
Anticipation (AA/AD)	8.23	4.45	5.73	5.35	675.19	274.51	13.96	6.04
Control	8.85	4.47	2.32	2.77	567.50	219.04	11.17	3.67

AA: anticipation alone, AD: anticipation and demand , AUC_G: area under the curve with respect to ground

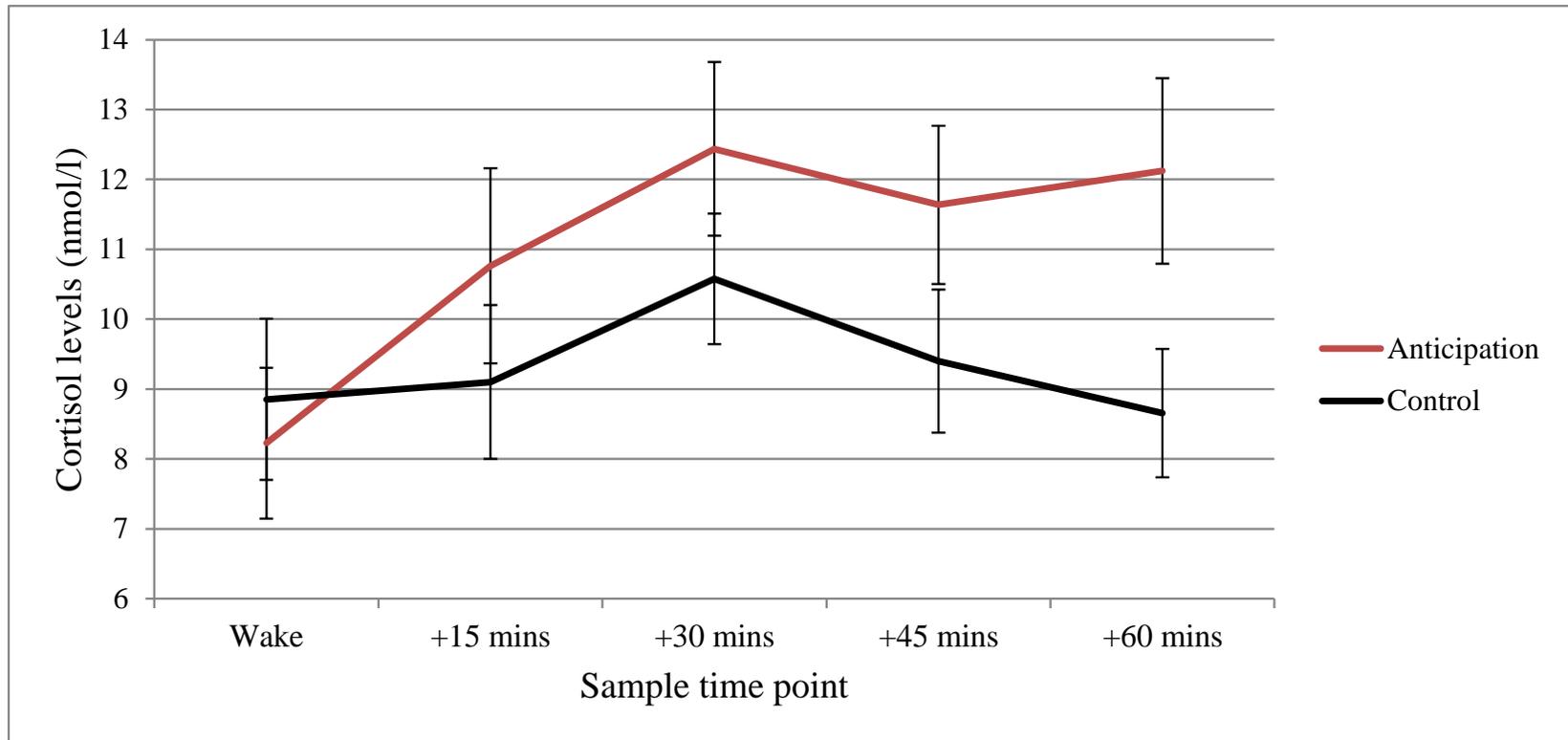


Figure 6.4: Mean (\pm SEM) cortisol levels at each time point of the CAR: for Morning 2 Anticipation (AA&AD) and Control groups. Significant effect of condition observed upon peak change, where anticipation group showed a greater peak change than the Control group. There were no significant effects shown upon awakening levels, AUC_G or peak value.

control group. There was no significant effect of condition on awakening levels, total secretion (AUC_G) or on the peak value of the CAR ($p > .05$). Additional indices are summarised in Table 6.5.

6.4.3. Does anticipation met with demand affect daytime mood and cortisol levels?

Day 2 levels of state anxiety, perceived levels of stress, daytime cortisol levels were compared between AA and AD groups.

6.4.3.1. Morning 2 perceived stress and state anxiety

Following the CAR period on Morning 2, mood diaries indicated that participants in the AD and AA conditions did not show any differences in perceived stress ($t(19) = 1.11, p > .05$) or in state anxiety ($t(19) = 1.42, p > .05$) prior to the AA group being informed that they were not required to complete any demand tasks on Day 2.

6.4.3.2. Day 2 perceived stress

The condition \times time point interaction was not significant ($F(1,20) = .22, p > .05, \eta^2_p = .01$) and neither the main effects of time point ($F(1,20) = 3.73, p > .05, \eta^2_p = .16$) or condition ($F(1,20) = .59, p > .05, \eta^2_p = .03$) were significant. This indicated that participants in the AD condition did not consider Day 2 to be more stressful than the AA group.

6.4.3.3. Day 2 state anxiety

There was a significant main effect of time point ($F(4.43, 88.59) = 3.04, p < .05, \eta^2_p = .13$) and a significant main effect of condition ($F(1,20) = 4.66, p < .05, \eta^2_p = .19$). The time point \times condition interaction was significant ($F(4.43, 88.59) = 2.71, p < .05, \eta^2_p = .12$) with a medium effect size, which indicated that the AA and AD groups showed different levels of state anxiety during Day 2. Follow-up tests were conducted and were adjusted for multiple comparisons ($.05/10 = .005$). The AD group showed significantly higher levels of state anxiety at wake + 3 hours ($p = .003$), which occurred after the AD group completed the first task. Daytime levels of state anxiety are summarised in Figure 6.5.

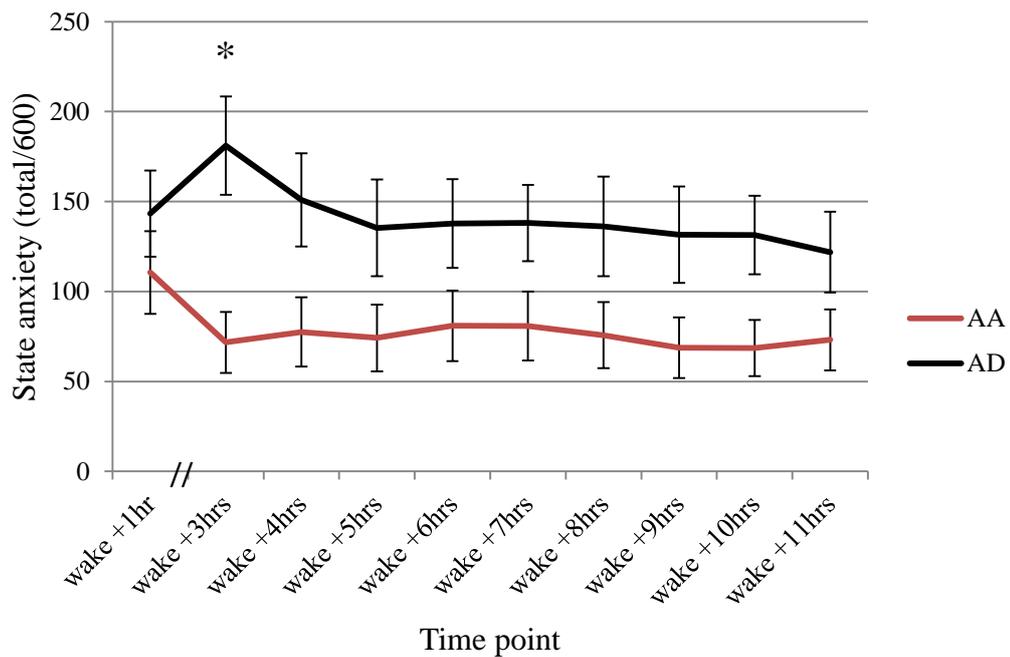


Figure 6.5: Mean (\pm SEM) Day 2 AA and AD state anxiety levels (* $p < .05$)

6.4.3.4. Day 2 diurnal cortisol levels

Day 2 diurnal (daytime and presleep) levels were compared between the AA, AD and control groups. The main effect of time point was significant ($F(2,02, 70.74) = 3.15, p < .05, \eta^2_p = .08$) reflecting the decline in diurnal cortisol levels throughout the day. The main effect of condition was significant ($F(2,35) = 3.28, p = .05, \eta^2_p = .16$, however Bonferroni-adjusted follow-up tests were not significant, indicating that overall diurnal cortisol levels were not significantly different between all groups irrespective of time point. The time point \times condition interaction was not significant ($F(4,04,70.74) = .98, p > .05, \eta^2_p = .05$, indicating that the diurnal decline in cortisol levels at specific time points did not differ between those who anticipated demand, whether or not this was met, and the control participants who did not anticipate or experience demand. This is shown in Figure 6.6. There were no significant differences in total cortisol secretion, in terms of the AUC_G , between AA ($M = 4010.76, SD = 2119.09$ arbitrary units), AD ($M = 3623.25, SD = 1075.68$ arbitrary units) and control ($M = 3000.09, SD = 897.03$ arbitrary units) groups, ($F(2,36) = 1.88, p > .05, \eta^2_p = .09$).

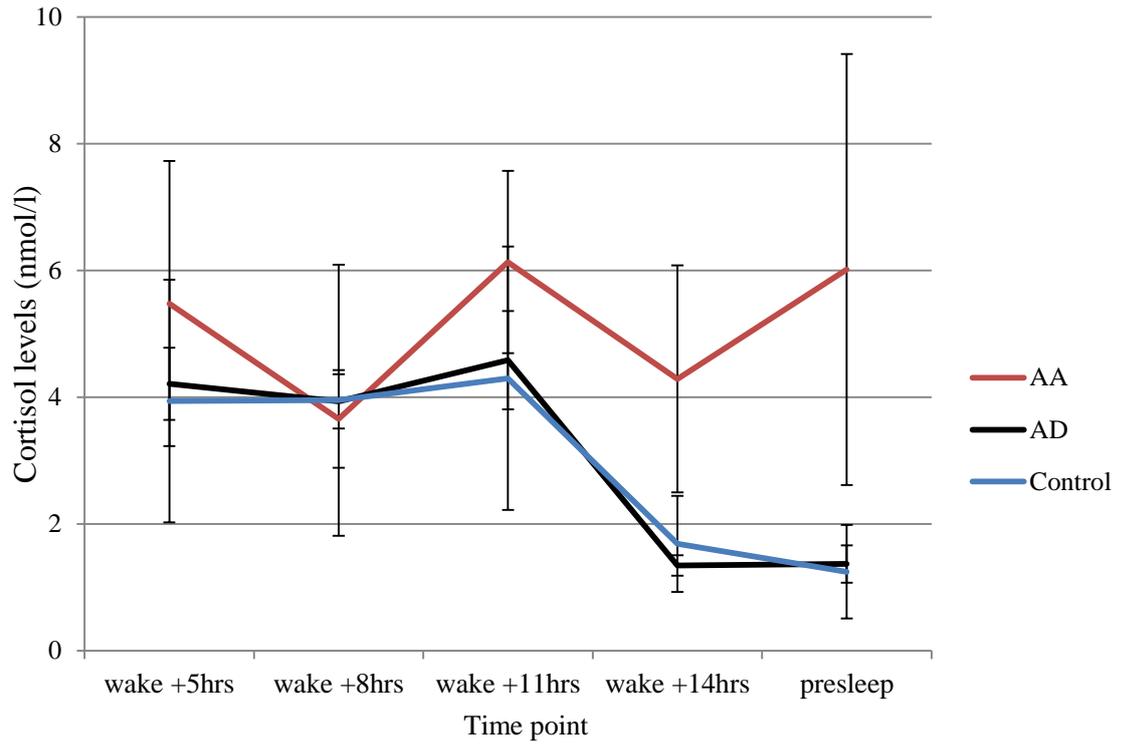


Figure 6.6: Mean (\pm SEM) Day 2 AA and AD cortisol levels.

Table 6.6

Cortisol levels at each time point of Day 2: AA and AD comparisons

Variable	Wake + 5hrs		Wake +8hrs		Wake +11hrs		Wake +14hrs		Pre-sleep	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AA	5.48	7.46	3.66	2.55	6.13	4.77	4.29	5.94	6.01	11.28
AD	4.21	1.89	3.93	1.42	4.58	2.57	1.34	0.53	1.37	0.98

AA: anticipation alone, AD: anticipation and demand

6.4.4. *Does anticipation alone, or anticipation and demand, affect sleep and the CAR?*

The effects of anticipation and anticipation, where this was met with demand, and the subsequent effects of the manipulation upon subjective sleep and objective sleep the night following the manipulation were also measured. The subsequent effects of the manipulation upon the CAR of the following morning were also measured. This was examined by comparing Night 2 subjective and objective sleep, and the Morning 3 CAR, between the AA and AD groups.

6.4.4.1. *Subjective sleep: Night 2*

The AA and AD groups showed no significant differences on any measure of subjective sleep continuity or quality on Night 2, following the manipulation (all p -values $> .05$). The difference between the AA and AD groups in terms of SOL showed a trend towards significance ($t(12.02) = 2.05, p = .063$). Participants who had experienced anticipation where this was not met with subsequent demand (AA group) took approximately twice as long to get to sleep as participants who had experienced anticipation where this was met with demand (AD group) This is summarised in Table 6.7.

6.4.4.2. *Objective sleep: Night 2*

The results showed that there was no effect of condition on objective sleep continuity, sleep architecture or upon the latencies to each stage of sleep on Night 2 (all p -values $> .05$). This indicated that participants who had experienced

anticipation alone, did not show any differences in objective sleep when compared to participants who had experienced anticipation where this was met with subsequent demand. Night 2 measures of objective sleep continuity, sleep architecture and the latency to each stage of sleep are summarised in Table 6.8.

Table 6.7

Subjective sleep continuity and quality: Night 2 AA and AD comparisons

Variable	AA Night 2		AD Night 2	
	Mean	SD	Mean	SD
SOL	21.36	16.45	10.68	5.25
NWAK	1.00	.89	1.18	1.15
WASO (mins)	5.82	6.66	3.36	5.84
TST (mins)	455.82	60.45	470.27	40.04
TIB (mins)	532.27	42.80	539.55	44.52
SE (%)	85.44	7.70	87.20	3.27
How well do you feel? (0 – 4)	2.82	.75	2.82	.75
How enjoyable was your sleep? (0 – 4)	3.00	.63	3.09	1.04
How mentally alert were you in bed last night? (0 – 4)	.73	.47	.91	.94
How physically tense were you in bed last night? (0 – 4)	.64	.67	.64	.67

AA: anticipation alone, AD: anticipation and demand, SOL: Sleep onset latency, NWAK: number of awakenings, WASO: wake after sleep onset, TST: total sleep time, TIB: time in bed, SE (%): sleep efficiency

Table 6.8

Objective sleep: AA & AD Night 2 Comparisons

Variable	AA		AD	
	Mean	SD	Mean	SD
TST (mins)	456.86	40.48	443.00	39.48
SE (%)	96.21	2.13	94.55	2.74
WASO	9.55	9.17	12.45	10.22
SOL	8.27	5.40	13.23	10.25
NWAK	10.91	4.44	12.73	4.34
Time in REM (%)	24.08	7.33	23.86	6.32
Time in N1 (%)	2.62	1.46	3.74	1.76
Time in N2 (%)	51.19	7.03	51.48	9.13
Time in N3 (%)	22.10	5.67	20.90	6.66
Latency to REM (mins)	95.00	46.38	102.27	57.93
Latency to N1 (mins)	8.27	5.40	13.23	10.25
Latency to N2 (mins)	13.68	7.24	21.14	9.88
Latency to N3 (mins)	26.23	9.96	33.95	14.00

AA: anticipation alone, AD: anticipation and demand, TST: total sleep time, SE (%): sleep efficiency, WASO: wake after sleep onset, SOL: Sleep onset latency, NWAK: number of awakenings, REM: rapid eye movement, N1: stage 1, N2: stage 2, N3: stage 3.

6.4.4.3. CAR: Morning 3

Results of the comparison between the AA and the AD groups on the Morning 3 CAR showed that there was a main effect of time point ($F(2.52, 37.74) = 9.43, p < .001, \eta^2_p = .39$) reflecting the typical changes in cortisol during the CAR period. The main effect of condition was not significant ($F(1,15) = .39, p > .05, \eta^2_p = .03$). The time point \times condition was not significant ($F(1,15) = .39, p > .05, \eta^2_p = .03$) indicating that participants who had experienced anticipation alone did not show a different CAR profile to those who had experienced anticipation where this was met with subsequent demand. Visually, both the AA and AD groups showed a similar profile until 30 minutes post-awakening, with the AA group showing sustained levels of cortisol and a steeper decline compared to the AD group. The cortisol profile between both groups was similar until 30 minutes post-awakening, where the AA group showed sustained levels of cortisol and a steeper rate of decline than the AD group. The Morning 3 CAR is shown in Table 6.9 and Figure 6.7. There were no significant differences between the AA and AD groups on any additional CAR indices during Morning 3 (awakening cortisol levels, peak change, AUC_G and peak value). Additional indices are summarised in Table 6.10.

Table 6.9:

Cortisol levels at each time point of the CAR: Morning 3 AA & AD comparisons

Variable	Awakening		+15 minutes		+30 minutes		+45 minutes		+60 minutes	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AA	6.41	3.04	9.16	3.83	13.33	7.03	11.39	5.65	10.57	6.50
AD	7.00	3.79	8.60	4.49	14.37	10.24	14.17	8.49	14.90	7.62

AA: anticipation alone, AD: anticipation and demand

Table 6.10:

Additional CAR indices: Morning 3 Anticipation & Control comparisons

Group	Awakening		Peak Change		AUC _G		Peak Value	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AA	6.41	3.04	8.54	6.70	635.44	258.62	14.94	7.59
AD	7.00	3.79	10.13	8.47	721.30	396.51	17.12	10.32

AA: anticipation alone, AD: anticipation and demand, AUC_G: area under the curve with respect to ground

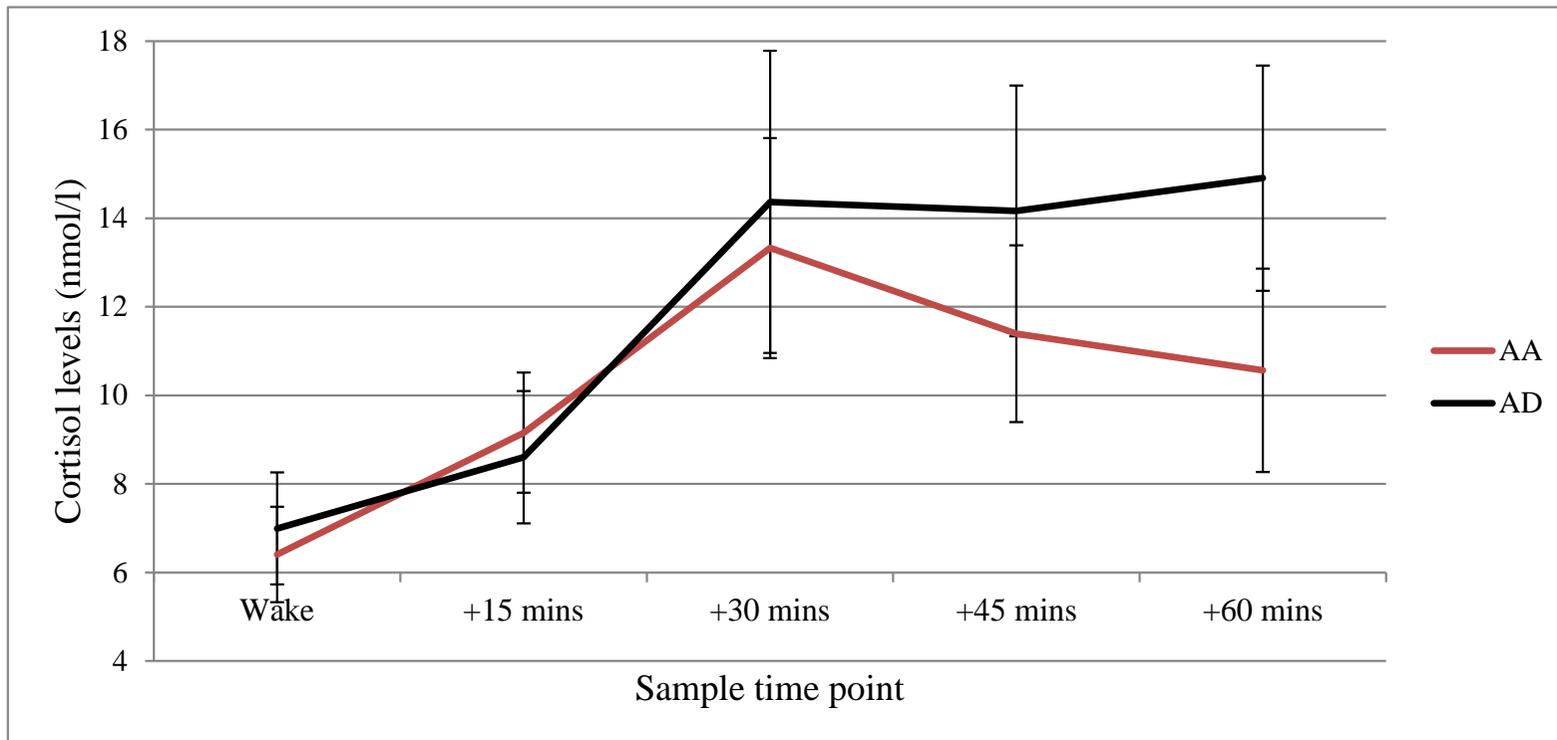


Figure 6.7: Mean (\pm SEM) cortisol levels at each time point of the CAR: Morning 3 AA & AD groups. There was no significant effect of condition upon awakening cortisol levels, peak change, AUC_G or the peak value.

6.5. Discussion

The aim of Study 2 had two aims, where the first aim was to investigate whether the anticipation of forthcoming demand affected subjective sleep, objective sleep and multiple indices of the CAR (the CAR profile, awakening levels of cortisol, peak values, total secretion and the peak change). The second aim was to examine the effects of anticipation where this was subsequently met with demand, upon these measures. This was done through an experimental manipulation where anticipation and demand were separated. Participants were informed that they were required to complete a full day of cognitive tasks at the time of enrolment and were informed on the day of testing that they were no longer required to do so (the AA group of participants) or completed these tasks as expected (the AD group of participants). This allowed the effects of anticipation, where this was met with demand, to be compared with the effects of anticipation, where this was not met with subsequent demand, upon subjective sleep, objective sleep and the CAR.

6.5.1. *Does anticipation affect sleep and the CAR?*

The anticipation of demand affected subjective sleep, where there was a reduction in total sleep time on Night 1 compared to their two-week baseline period immediately before participation (Table 6.2). This was not due to a reduction in TIB due to being in the laboratory, since TIB was not significantly different between the baseline period and the laboratory stay. Importantly, the anticipation of demand led to higher levels of mental alertness during the night prior to the anticipated stressor, indicating that the manipulation was effective. The anticipation of demand did not affect objective sleep (Table 6.3) in terms of sleep continuity, sleep architecture or in the latency to each stage of sleep. The findings of the current study correspond with

earlier studies which have displayed that the anticipation of forthcoming demands can disrupt subjective sleep, as Torsvall and colleagues (1987) showed that engineers on watch duty but where no alarms occurred showed greater subjective difficulties in falling asleep, obtained less sleep and rated their sleep quality as being poorer in comparison to a free night. Similarly, Wuyts and colleagues (Wuyts *et al.*, 2012b) showed that participants in a simulated on-call situation showed a lower subjective sleep duration compared to a reference night. The results of Study 2 with regards to objective sleep differ in comparison to earlier studies which have shown that anticipation also affects objective sleep. Torsvall and Akerstedt (1988) showed that on-call nights on board a ship led to a shorter duration of objective sleep and reduced amounts of REM and slow wave sleep as compared to a free night. However, these studies required participants to believe that they would experience sleep disruption during the night and differs from the current study as participants were not expecting to be awoken during the night.

The results of Study 2 also showed that anticipation can affect the CAR, potentially indicating that the CAR is a marker of anticipation. This was shown in the CAR measured on Morning 2, as participants who believed that they would experience a full day of demanding cognitive tasks showed a steeper CAR, with higher levels of cortisol throughout the CAR period, compared to a control group who were not expecting any demand, with a medium effect size ($\eta^2_p = .09$; Table 6.4, Figure 6.4). Those who were expecting demand also showed a steeper peak change in the CAR compared to the control group who were not expecting demand (Table 6.5). This builds on preliminary evidence from competition studies that the CAR is a marker of anticipation, in terms of awakening levels (Filaire *et al.*, 2001; Rohleder *et al.*, 2007) and cortisol levels 30 minutes post-awakening (Filaire *et al.*, 2009; Filaire

et al., 2007) where the CAR was measured in a less comprehensive manner and where the CAR was not measured in a controlled environment. The findings of Study 2 also build upon the findings of Study 1. Within Study 1, the standard protocol was used in the absence of stress, showing that cortisol levels were highest immediately before leaving the sleep laboratory on the final morning, potentially reflecting the effects of anticipation of forthcoming demands outside of the laboratory. Interestingly, CAR profiles within both groups appeared to show a large amount of individual variability, reflected in the large associated standard deviations. This was despite the CAR being measured in a laboratory environment with extremely high levels of control over factors known to affect the CAR, including light, which potentially indicates that individuals may vary in their response to anticipation (i.e. some individuals show a greater CAR than others do when anticipating upcoming demands). Overall, this provides strong evidence that the CAR, measured in a comprehensive and controlled manner, is a marker of anticipation.

6.5.2. *Does anticipation alone, or anticipation and demand, affect sleep and the CAR?*

The experience of anticipation and demand, in terms of completing the full day of demanding tasks within the laboratory, did not affect subjective sleep during the subsequent night (Night 2; Table 6.7). Whilst any differences were not statistically significant, individuals in the AD group, who expected and completed the range of demanding tasks, showed double the subjective sleep-onset latency than the AA group (21.4 minutes compared to 10.7 minutes), who would have been expecting tasks but did not complete them. However, this difference was not statistically

significant. The AD group also showed a large amount of variability with regards to SOL. This was also the case for WASO, TST and SE. Although there were no statistically significant differences in terms of objective sleep between the AA and the AD group during Night 2 (Table 6.8), interestingly, the AA group displayed a greater sleep efficiency, less WASO, a lower SOL and fewer NWAK than the AD group. In addition, the latencies to each stage of sleep appeared to be reduced within the AA group compared to the AD group. This may reflect individual differences in the response to stress during Day 2 within the AD group. Whilst not statistically significant, this pattern of high individual variability was not displayed within the cortisol levels of the AD group during Day 2 (Figure 6.6), as the AA group appeared to show a higher level of variability in cortisol levels. Overall, this suggests that there were individual differences in the response to the unexpected manipulation and there may have been individual differences in the subsequent effects upon subjective and objective sleep.

The results of Study 2 differ from other studies which have used demand as a stressor. Wuyts and colleagues (Wuyts *et al.*, 2012a) showed that 30 minutes of cognitive tasks before bed showed there to be no differences in subjective sleep continuity or quality compared to a reference night, however objectively there was a longer SOL following the demand. However, participants spent a night in their own homes between the reference and task night meaning that any potential effects of anticipation and/or demand during the time spent outside of the laboratory may have influenced the results. Koulack and colleagues (1985) showed that demand prior to sleep, in the form of either easy or hard intelligence tasks, led to an increase in objective SOL and a decrease in REM density compared to a control night. However, there were no between-group differences in this study and it may have

been the case that participants completing hard tasks did not consider these to be harder than the group completing easy tasks.

The CAR on Morning 3 did not show any statistically significant differences between the AA and AD groups, either in terms of cortisol levels or in any of the additional indices (awakening levels, peak value, total cortisol secretion over the CAR period, or peak change; Table 6.9 & Table 6.10). The cortisol profile between both groups was similar until 30 minutes post-awakening, where the AA group showed sustained levels of cortisol and a steeper rate of decline than the AD group (Figure 6.7). Whilst this was not statistically significant and the effect size was small-to-medium ($\eta^2_p = .03$), the AD group displayed mean cortisol levels which were approximately 1 nmol/l greater than the AA group at 30 minutes post-awakening, approximately 2.5 nmol/l greater at 45 minutes post-awakening and approximately 4.5 nmol/l greater at 60 minutes post-awakening. Thorn and colleagues (2006), investigating weekend and weekday differences in the CAR, observed a highly significant ($p = .001$) interaction between the time point of sampling and condition with similar differences in cortisol levels during the CAR in a larger sample of participants ($n = 48$). Cortisol levels were similar at awakening, and levels at each sampling point were higher at subsequent intervals (15, 30 and 45 minutes post-awakening) with similar differences in cortisol levels at each time point (approximately 1 nmol/l at wake +15 minutes, 2 nmol/l at wake + 30 minute and approximately 3 nmol/l higher at wake +45 minutes) to the present study. Whilst this must be interpreted with caution, it provides preliminary evidence that the CAR may also serve as a marker of recovery from previous demand, as well as a marker of forthcoming demand. Adam and colleagues (E. K. Adam *et al.*, 2006) showed that subjective loneliness, sadness, threat and a lack of control immediately before bed

was associated with a greater CAR during the following day in a sample of older adults.

6.5.3. *Study strengths and limitations*

The major strength of the current study is that the effects of stress upon sleep were measured through the use of a standard protocol. This enabled the effects of anticipation upon to be teased apart from the effects of anticipation where this was met with subsequent demand. Furthermore, this study also included a physiological marker of stress by examining the CAR as a marker of HPA function. A particular strength is with regards to the collection of the CAR, since this was performed with an extremely high level of control over factors including light, where the CAR was collected in ultraviolet light. A further strength is with regards to the nature of the demanding tasks used within the thesis, as the IGT and modified Stroop task were included to induce uncertainty and the MTF was included at multiple time points. The MTF is representative of everyday situations, has been shown to reliably elicit physiological and psychological stress and is suitable for the repeated testing of participants (Wetherell & Carter, in press).

Potential limitations of the study include the relatively small sample size, the homogenous age range of the participants, the short length of the protocol and the fact that participants followed their habitual sleep/wake schedule. However, these limitations are not likely to have affected the results of the thesis and offer opportunities for future research. These limitations are discussed in more detail in the following chapter.

6.5.4. *Summary*

Study 2 indicated that the anticipation of forthcoming demand, affected subjective sleep but not objective sleep. This was shown as participants who expected to complete a full day of demanding cognitive tasks showed a reduced subjective total sleep time during the night before the demand (Night 1), compared to a baseline period prior to the laboratory stay. Anticipation did not affect objective sleep during the night before demand. In addition, the CAR, a marker of HPA axis activity, was affected by anticipation of forthcoming demands. This was shown as participants who expected to complete demanding tasks showed a steeper CAR, with higher levels of cortisol during the CAR period, compared to a control group. In addition, the peak change was steeper within participants who were expecting demand. No differences were shown in objective sleep. Participants who experienced anticipation, where the demand was met, displayed higher levels of anxiety immediately after the first cognitive task compared to participants who experienced anticipation where this was not met with subsequent demand. This indicated that the experimental manipulation was effective and provoked anxiety.

Sleep and the CAR were examined on the night of sleep following the manipulation and there were no effects shown upon subjective sleep or objective sleep. There was a trend towards an increased SOL in those who had experienced anticipation and demand; taking approximately twice as long to fall asleep than those who experienced demand. The CAR measured on the morning following the manipulation, showed that whilst the differences were not statistically significant, individuals who had experienced anticipation met by demand appeared to show a steeper, sustained CAR compared to those who had experienced anticipation only. This potentially indicates that the CAR may have a secondary adaptive role.

Overall, the results indicate that the anticipation of a stressor, whether this is met, or not met by subsequent demand, disrupts subjective sleep but not objective sleep.

Additionally, this is reflected in the CAR, with Study 2 providing evidence that the CAR is a marker of anticipation with a possible secondary role in the adaptation to demand.

CHAPTER 7.

General discussion

7.1. Overview

This chapter will provide an overall summary of the thesis and will explain the findings in relation to the Spielman 3P and Cano-Saper models of insomnia, which focus on the role of stress in the development of insomnia. This chapter will outline that the precipitant factor within the 3P model can be separated into anticipation and demand, before summarising the associated implications for practice and policy. This chapter will also explain the additional contributions of the thesis to the CAR literature before summarising general strengths and limitations. Potential future directions are suggested, in particular focusing on the influence of predisposing factors within the context of the 3P model. These would determine how these factors mediate the effects of anticipation upon sleep and may include personality variables and the influence of genetics.

7.2. Thesis summary

The overall aim of the thesis was to examine the effects of stress upon sleep, where subjective and objective sleep was measured, teasing apart the effects of anticipation, where this was either met, or not met, with subsequent demand. Previous studies which have examined the effects of stress upon sleep have done so using naturalistic stressors such as earthquakes or war, or using experimental stressors. However, the results of the effects of naturalistic stressors upon sleep have been shown to be inconsistent, due to effects such as the wide variation in the nature

of the stress, the diverse nature of participants and the time lag between the stressor and the measurement of sleep. The effects of experimental stressors upon sleep have also been shown to be inconsistent. A further limitation of the experimental stressors is that typically they have not included a physiological marker of stress within the protocol, meaning that the effectiveness of the stressor cannot be objectively verified.

Overall, these inconsistent findings may be due to the differences in the measurement of sleep and stress. These inconsistent findings necessitated the development of a standardised measurement protocol for the measurement of sleep and stress in the same context where a physiological marker of stress was included. Cortisol was included as a marker of HPA axis function, focusing on the CAR, where cortisol levels increase sharply upon awakening. Cortisol was included a marker due to the relevance of HPA axis activity to insomnia and due to its use within stress studies and the CAR was included due to a) the close link between the CAR and sleep/wake; and b) as the function of the CAR was speculated to be related to anticipation, or demand, with both potential functions being relevant to the main objective of the thesis. A standardised protocol for measuring stress and sleep in the same context was developed in the thesis and an initial feasibility study measured sleep and the CAR in a home or a laboratory environment using the protocol.

The aim of the feasibility study was to determine whether or not the protocol could feasibly be used within a home environment, where sleep was measured over three consecutive weekday nights and the CAR over three subsequent weekday mornings. Whilst there were minimal differences in sleep and the CAR between the two measurement locations, the laboratory protocol offered a higher level of control over participant activities between the second and third nights of sleep and

measurement of the CAR on the second and third mornings. In addition, the choice of a laboratory environment afforded greater control over other factors known to affect the CAR, including light, since the CAR was measured in extremely low levels of light within the laboratory environment. This minimised the SCN input to the HPA axis and subsequent effects upon the CAR.

Study 1 examined the relationship between subjective sleep, objective sleep and the CAR using the protocol in the absence of stress, showing a positive relationship between subjective NWAK and the peak change of the CAR. Importantly, subjective sleep, objective sleep and the CAR were shown to be temporally stable and a further important finding of Study 1 was that cortisol levels were highest on the final morning of the study, immediately before leaving the laboratory at wake + 120 minutes. As all participants remained in a highly-controlled laboratory environment between the second and third mornings, it is possible that this either reflected the anticipation of obligations and activities upon leaving the laboratory, or that this indicated a reaction to previous activities.

Study 2 used the standard protocol to assess the impact of stress upon subjective sleep, objective sleep and the CAR. Within Study 2, all participants expected that they would experience a full day of demanding tasks during Day 2 within the laboratory, thus allowing the effects of the anticipation of stress upon sleep and the CAR to be measured. Study 2 also assessed the effects of anticipation and demand upon these measures, as whilst all participants expected stress, only half of the participants completed the tasks.

The results of Study 2 showed that anticipation alone affected subjective sleep but not objective sleep, where participants displayed a lower subjective total sleep

time compared to a two-week normative baseline period but not objective sleep. The CAR was also affected by anticipation, where anticipation led to greater cortisol levels throughout the CAR and a steeper peak change compared to control participants who had followed an identical protocol in the absence of stress. Anticipation and demand did not affect either subjective sleep or objective sleep and whilst anticipation and demand did not lead to any statistically significant changes within the CAR, the alterations shown to the CAR were meaningful. Overall, the most important contribution of the thesis to the sleep and stress literature is that the thesis showed that the anticipation alone of stress can disrupt sleep, when teased apart from anticipation and demand.

As outlined in Chapter 1, two theoretical models of insomnia, the Spielman 3P model (Spielman *et al.*, 1987; Spielman & Glovinsky, 1991; Spielman *et al.*, 1996) and the Cano-Saper animal model of insomnia (Cano *et al.*, 2008) outline how stress can lead to poor sleep and the development of insomnia. The Spielman 3P model of insomnia has a role for stress as precipitating factors, which can include acute stressors such as medical or psychiatric illnesses, or in the example provided by Spielman and colleagues, where a young man receives a job promotion (Spielman *et al.*, 1987; Spielman & Glovinsky, 1991; Spielman *et al.*, 1996), interacting with predisposing vulnerability factors and resulting in a period of sleep disruption.

The Cano-Saper rodent model of insomnia also has a role for stress (Cano *et al.*, 2008). Within the Cano-Saper model of insomnia, stress was induced by placing a male rat at the peak level of sleep and placing it into a dirty cage which had previously been occupied by a male rat for a period of one week, with the rat showing sleep disturbances and HPA axis activation.

Previous research has assessed the impact of natural stressors, such as natural disasters and wars, showing that sleep disturbances are common following such events (Askenasy & Lewin, 1996; L. M. Davidson *et al.*, 1987; Dooley & Gunn, 1995; Kato *et al.*, 1996; North *et al.*, 1999; Schuster *et al.*, 2001). The disruptive effects have been shown across multiple measures of subjective and objective sleep continuity. For example, a study which examined participants during and 30 days after the Gulf War indicated that 51% of participants reported disturbed sleep and that 19% of participants who reported normal sleep before the war developed insomnia (Askenasy & Lewin, 1996).

However, these studies have typically examined the effects upon subjective sleep and have used cross-sectional study designs. Major limitations of this approach include the fact that the stressful event is associated with a range of associated and upcoming demands and also that there is a time lag between the stressful event and the assessment of sleep. Whilst the studies have examined a relationship between stress and insomnia, they do not account for the initiation of the period of poor sleep and examine how this influences the development of insomnia. For example, an earthquake is likely to affect various aspects of an individual's life (e.g. their home and their job) with a range of associated demands (e.g. finding or repairing a home) and upcoming demands (e.g. having to find new employment).

Other studies examined the effects of more common naturalistic stressors upon sleep (both objective and subjective), including financial strain, health events including illness or the anticipation of surgery, bereavement, and examinations (Hall *et al.*, 2008; Hall *et al.*, 2009; Hardison *et al.*, 2005; Palesh *et al.*, 2007; Reynolds 3rd *et al.*, 1992; Reynolds 3rd *et al.*, 1993; Wright *et al.*, 2010). The findings from these studies have also been inconsistent but have indicated that the stressors cause

disruptions to subjective and objective sleep. For example, one study indicated that older adults who reported that they found on-going financial strain to be either “somewhat upsetting” or “very upsetting” showed objective sleep differences compared to older adults who did not report on-going financial strain or rated it as being “not upsetting”, showing a longer SOL, greater WASO and lower sleep efficiency (Hall *et al.*, 2008). However, limitations of these studies include; the lack of adequate control groups, the cross-sectional design and the diverse nature of participants included in the studies (typically with co-morbid conditions). Due to the nature of the stress, these would have had both anticipation and demand associated with the stress and in order to accurately assess the impact of stress upon sleep, it was necessary to separate the anticipation from the demand. For example, in the case of an illness, the illness has both direct and indirect disruptive effects upon sleep. Potential direct effects may occur from the illness itself and from associated medication, and indirect effects may come from the overall stress arising from the situation and from the anticipation of financial loss. Whilst the effects of naturalistic stressors upon sleep typically lead to sleep disturbances, the effects upon subjective and objective sleep problems are inconsistent and in the outcome measures used.

Other limitations of naturalistic studies have included the wide variation in the nature of the stress and in the diverse nature of participants. The effects of experimental stressors upon subjective sleep and objective sleep have been examined and have been shown to be disruptive, leading to effects including an increase in objective SOL and decrease in REM density following intelligence tasks (Koulack *et al.*, 1985) and increases in objective SOL following cognitive tasks (Wuyts *et al.*, 2012a). As with naturalistic stressors, there have been a wide variation in the nature of stress employed, including tasks such as intelligence tests or watching

psychologically stressful films (Baekeland *et al.*, 1968; Koulack *et al.*, 1985; Vandekerckhove *et al.*, 2011; Wuyts *et al.*, 2012a).

Experimental studies also have other limitations, including potential confounds from the stress being experienced during the first night of a laboratory protocol (Baekeland *et al.*, 1968). These studies are also limited by the fact that they do not tend to include a physiological marker of stress, meaning that it cannot objectively be shown whether the stress paradigm is physiologically stressful or not. In addition, anticipation has also been shown to affect subjective and objective sleep, in both naturalistic and experimental studies, with inconsistent findings (Torsvall & Akerstedt, 1988; Torsvall *et al.*, 1987).

Overall, naturalistic and experimental studies of stress showed that whilst stress disrupted subjective and objective sleep, the overall findings were inconsistent. The inconsistent findings are potentially due to factors such as the wide variation in stressors and in the diverse nature of study participants. Additionally, other studies showed that anticipation was also shown to affect subjective and objective sleep. The thesis therefore developed a standard protocol for examining the effects of stress upon sleep, where the protocol teased apart the effects of anticipation from anticipation where this was met with subsequent demand. Within the protocol, all participants experienced anticipation; however, not all participants experienced demand. Previous research which has examined the relationship between stress and sleep has not included a physiological marker of stress, meaning that it is not possible to determine whether or not individuals found the stressor to be objectively stressful. For this reason, the stress hormone cortisol, which is the end product of the HPA axis (Hucklebridge *et al.*, 2005) was included within the thesis as a physiological marker of stress. Increases of cortisol are typically observed following

an acute psychosocial stressor (Dickerson & Kemeny, 2004; Kirschbaum *et al.*, 1993)

The HPA axis is of importance within insomnia since cortisol is a method by which physiological hyperarousal can be measured, with cortisol being involved within hyperarousal models of insomnia (Riemann *et al.*, 2010). The Cano-Saper model of insomnia showed that hyperarousal as measured through HPA axis activation occurred following a stressor. Within the model, a stressor was employed in the form of a cage exchange where male rats were placed into another cage which had been occupied by another male rat, resulting in a stress response due to the territorial nature of the rat and where the stress resulted in sleep disturbances and HPA axis activation (Cano *et al.*, 2008).

It had previously been shown that 24-hour cortisol levels within a group of individuals with insomnia were positively related to the level of WASO, suggesting a relationship with sleep disturbance (Vgontzas *et al.*, 1998). It had also been shown that those with insomnia displayed higher levels of cortisol in the evening and early part of the night (Vgontzas *et al.*, 2001) and higher nocturnal levels of cortisol compared to a control group of normal sleepers (Rodenbeck *et al.*, 2002). However a separate study showed that there were no differences in nocturnal cortisol levels between those with insomnia and healthy controls (Riemann *et al.*, 2002). Other studies had focused on cortisol levels at awakening, potentially accounting for these differences, where Backhaus and colleagues measured salivary cortisol levels, at awakening, 15 minutes post-awakening and at bedtime over a period of seven consecutive days (Backhaus *et al.*, 2004). Those with insomnia showed lower cortisol levels at awakening than controls and awakening cortisol levels showed a negative relationship with nightly awakenings.

The thesis measured the CAR as a marker of HPA axis activity, referring to the increase in cortisol levels observed upon awakening. The CAR was chosen as an ideal physiological marker given the close relationship of cortisol secretion with the circadian system (Morris *et al.*, 2012). The CAR was also included as a physiological marker of HPA axis function as the potential functions of the CAR were very closely related to the main objective of the thesis. These potential functions may include acting as a marker of anticipation (Filaire *et al.*, 2009; Filaire *et al.*, 2007; Filaire *et al.*, 2001; Rohleder *et al.*, 2007) or as a marker of recovery (E. K. Adam *et al.*, 2006). However, as summarised in Chapter 2, the CAR is affected by a range of methodological factors including; light (Figueiro & Rea, 2012; Scheer & Buijs, 1999; Thorn *et al.*, 2004), unexpected awakenings (Born *et al.*, 1999), the time of awakening (Stalder *et al.*, 2009; Thorn *et al.*, 2006) and the deviation from required sampling times (Clow *et al.*, 2004).

The protocol developed within the thesis standardised the measurement of sleep and the CAR. This was important as despite the close links between sleep and the CAR, the relationship between sleep and the CAR was shown to be inconsistent, potentially due to methodological factors. A meta-analysis of 23 CAR studies had previously shown that sleep duration was positively related to awakening cortisol levels during the CAR (Garde *et al.*, 2012). However, very few studies had examined the relationship between objective sleep and the CAR in a sample of good sleepers or within a controlled environment. In addition, different measures of the CAR had been included where the relationship was examined.

For example, it was shown that there was no relationship between measures of sleep architecture and total cortisol secretion during the CAR period in healthy controls or army veterans with PTSD (van Liempt *et al.*, 2013). REM sleep was

shown to negatively relate to awakening cortisol levels within alcohol-dependent inpatients (Junghanns *et al.*, 2007) and awakening cortisol levels and increased percentages of Stage 1, Stage 3 and REM sleep in caregivers compared to non-caregivers. However in the study of caregivers both groups showed differences in their objective sleep, with an increased total percentage of Stage 1 and decreased REM sleep compared to non-caregivers (Fonareva *et al.*, 2011).

In order to provide a standardised way by which the relationship between stress and sleep could be examined within the thesis, Chapter 3 outlined a common method for measuring subjective sleep, objective sleep and the CAR, where the CAR was measured using multiple indices. Following the development of the standard protocol, a feasibility study conducted within Chapter 4 investigated whether the standard protocol could feasibly be used within a home environment, or whether a laboratory environment was potentially more suitable. The feasibility study measured objective sleep and the CAR in healthy good sleepers, where they were assessed within either a home environment or in a laboratory environment, with increased levels of control over bed and awakening times, monitoring of sleep, collection of the CAR and consistency in environment and daily activities. One cortisol sample was missing during the CAR period within a home environment compared to the laboratory environment, potentially as a result of adherence to sample collection instructions.

The measurement of objective sleep, through ambulatory PSG, was well-tolerated and did not result in any missing data. Measures of objective sleep continuity and sleep architecture were similar between the two groups, with some subtle differences shown in WASO, SOL and in the latency to each stage. The CAR profile was similar when measured within a home and laboratory environment,

however cortisol levels at each time point appeared to be lower when measured within a laboratory environment, with lower standard deviations in cortisol levels at each time point. This was potentially due to the influence of extraneous factors during the CAR period, including variations in light levels and the inability to control awakening times and movement within a home environment. Whilst the results of the feasibility study indicated that the protocol was suitable either for use within a home or laboratory environment, in order to maximise the level of control over the protocol, Study 1 was conducted within a laboratory environment. This was of particular relevance to light, since light can potentially affect the CAR and saliva samples were collected in very low ultraviolet light in the laboratory environment.

Study 1 tested the protocol in order to measure sleep and the CAR, and to observe the relationship between these two measures in normal sleepers. A total of 18 healthy good sleepers participated for three consecutive nights, where the CAR was measured on three consecutive weekday mornings following each night of sleep. Between the second and third morning of the protocol, participants remained in the laboratory environment and were allowed to perform activities including reading, computer work and watching films but were not allowed to leave the laboratory or exercise at any point. Chapter 5a and 5b assessed the relationship between subjective sleep, objective sleep and the CAR, where the CAR was measured comprehensively through multiple indices (cortisol levels at each sampling point, total cortisol secretion during the CAR period, peak change and peak cortisol levels). Subjective sleep remained constant between the second and third night of measurement. The CAR did not show any differences between three mornings of measurement, either in terms of the cortisol levels at each time point of the CAR or in any of the indices measured, indicating that the CAR is temporally stable within

normal sleepers in a laboratory environment. A positive relationship was observed between the number of subjective awakenings and the peak change of the CAR. Chapter 5 also investigated the relationship between mood and the CAR. Average levels of perceived stress at awakening were related to two indices of the CAR, where perceived stress showed a positive relationship with cortisol levels at awakening and with the peak value of the CAR. Since Study 1 used the protocol to measure sleep and the CAR in the absence of stress, this provided a first indication that the anticipation of upcoming demands may be associated with the CAR.

The relationship between objective sleep and the CAR was also examined in Study 1, which showed that that certain measures of objective sleep architecture were associated with the CAR. Lower awakening cortisol levels were associated a greater percentage of Stage 2 sleep and a greater decrease in the peak change of the CAR was associated with a greater latency to REM sleep. Study 1 showed that outside the CAR period, cortisol levels were greatest immediately prior to leaving the laboratory on the final morning, in addition to a subjectively steeper and more sustained CAR on Morning 3.

Participants had remained in the sleep laboratory between the second and third mornings under controlled conditions, with standardised activities and mealtimes, and displayed shown no differences in state anxiety or perceived stress between the second and third mornings. Due to the temporal stability of sleep and mood, this suggested that the anticipation of upcoming activities may have influenced cortisol levels.

The controlled nature of Study 1 suggested that this may have been, as one possibility of many, due to the anticipation of forthcoming demands upon leaving the

laboratory. As such, this suggested that the CAR may be influenced by forthcoming demands. Previous studies had demonstrated an association between forthcoming demands and indices of the CAR, in terms of elevated awakening levels (Filaire *et al.*, 2001; Rohleder *et al.*, 2007) and elevated cortisol levels at 30 minutes post-awakening (Filaire *et al.*, 2009; Filaire *et al.*, 2007). Whilst these studies have suggested that the CAR may function as a marker of anticipation, it is also a possibility that the CAR may function as a marker of demand and Study 2 explored this possibility further.

Study 2 used the same protocol to examine the relationship between stress and sleep, where anticipation, and the combination of anticipation and demand, were teased apart. Specifically, the effects of teasing anticipation and anticipation where this was met with subsequent demand, and their impact upon subjective sleep, objective sleep and the CAR were measured. Healthy participants followed the same protocol as employed in Study 1; however, participants were informed at the time of enrolment that they would experience one full day of demanding tasks within the sleep laboratory. Whilst all participants were informed of the upcoming tasks, only half of the participants completed the tasks, as the participants who were not required to do so were informed of this change on the morning of testing. This ensured that whilst all participants anticipated the stressor, only half of the participants within Study 2 experienced anticipation and demand.

The anticipation and demand condition included three cognitive tasks, completed at specific time points throughout the day. The MTF (Wetherell & Sidgreaves, 2005) was completed at multiple time points during the day. The other two demand tasks within Study 2, which employed a modified version of the Emotional Stroop (Barclay & Ellis, 2013) and of the Iowa Gambling Task (Bechara

et al., 1994; Bechara *et al.*, 2000) were chosen because they induce uncertainty since the rules of the tasks change. Overall, the anticipation of stress affected subjective sleep, as indicated by the reduction in total sleep time on Night 1 compared to the baseline period. This was not due to a reduction in TIB since TIB was not significantly different between the baseline period and the laboratory stay. That said, there were no between-group differences in either subjective or objective measures of sleep on Night 2, following the full day of tasks (i.e. those who had experienced anticipation and demand) compared to those who had experienced just the anticipation.

This suggests that the addition of demand to anticipation had limited impact. Whilst there were no statistically significant differences in any subjective or objective sleep measure on Night 2, participants in the anticipation met with demand group showed a subjective SOL that was double the length of participants who did not complete the tasks. This SOL had a high associated standard deviation which suggested a wide variation in the individual SOL. The AA group also showed a greater sleep efficiency, less WASO, a lower SOL and fewer NWAK than the AD, alongside reduced latencies to each stage of sleep. This may reflect individual differences in the response to stress.

The anticipation of upcoming stress affected one dimension of the CAR, as participants in Study 2 showed a steeper peak change in cortisol levels during the CAR period compared to individuals who were not expecting any stress (using the equivalent data from those in Study 1). No between-group differences were observed in total cortisol secretion, awakening cortisol levels or peak values. The anticipation group showed a subjectively larger CAR profile than a control group and although this was not statistically significant this also suggested that the CAR may have a role

within anticipation. Taken together these findings suggest that the CAR may function as a marker of anticipation (Filaire *et al.*, 2009; Filaire *et al.*, 2007; Filaire *et al.*, 2001; Rohleder *et al.*, 2007).

The CAR, as measured on Morning 3 of the study, suggested that there were no differences between those who had experienced demanding tasks and those who did not in any dimension of the CAR. However, whilst there were no statistically significant differences in cortisol levels during the CAR period, the CAR profile was elevated within those who completed the tasks, which suggested that the CAR may also function as a marker of recovery.

Overall, Study 2 suggested that the anticipation of stress disrupts subjective sleep and that the CAR appears to function as a marker of anticipation, potentially with a secondary ‘recovery’ role. Overall, the results of the thesis indicate that the anticipation alone of an upcoming stressor is sufficient to affect subjective sleep. In addition, the anticipation of stress also resulted in the activation of the HPA axis, as reflected in the increased peak change of the CAR. The following section discusses the overall findings of the thesis in relation to models of insomnia that explicitly outline the role of a stressor in the development of insomnia.

7.3. Thesis findings in relation to models of insomnia with a focus on stress-diathesis

The overall findings of the thesis show greater levels of support for the Spielman 3P model of insomnia (Spielman *et al.*, 1987; Spielman & Glovinsky, 1991; Spielman *et al.*, 1996). This is explained further in the following section.

7.3.1. *Cano-Saper rodent model of insomnia*

The Cano-Saper animal model of insomnia (Cano *et al.*, 2008) is a model where stress is induced by placing a male rat, at the peak phase of their sleep, into a dirty cage which had been occupied by another male rat for a period of one week. Due to the territorial nature of rats, this cage exchange acted as a stressor. Rats who experienced the cage exchange displayed a higher percentage of wake and a decreased percentage of NREM sleep, both in the initial two-hour period following the stressor and in a second two-hour period measured five and six hours following the stressor, analogous to a period of acute insomnia. Cage exchange rats also took twice as long to get to sleep than control rats and showed a reduced percentage of REM sleep. This was the case in the first and second hours after the cage exchange, indicating a stress response and five and six hours afterwards, analogous to a period of acute insomnia. The cage exchange rats also displayed almost double the sleep latency than control rats, showing a mean sleep latency of 58.7 minutes compared to a control group duration of 31.8 minutes. There were no differences in NREM bouts, but cage exchange rats also showed a reduced percentage of REM sleep. Cage exchange rats also showed higher levels of sleep fragmentation, observed through fewer transitions from NREM to REM sleep and a greater amount of transitions to wakefulness. The study also examined neuronal activity by measuring the expression of Fos proteins, showing an increase in Fos levels in the caudal mp-PVH, an area which contains CRH. Importantly, this indicated that the cage exchange procedure affected objective sleep and increased HPA axis activity.

In Study 2 of the thesis, the anticipation of stress did have an effect upon the HPA axis, as reflected in the CAR peak change measured on the second morning.

This indicated that the anticipation of the stressor led to an increased CAR and also a steeper peak change in cortisol levels during the CAR period. Conversely, the anticipation of stress did not affect objective sleep. However, the assumed mechanisms within the Cano-Saper model of insomnia are where the stressor impacts upon high-frequency EEG activity (Perlis *et al.*, 2011) relating to gross changes in sleep EEG. Although the effects of stress upon high-frequency EEG were not assessed in Study 2 we expected at least some gross EEG changes to be apparent. One explanation for the disparity in findings rests on the fact of extrapolating from animal to human models. Furthermore, the thesis showed that anticipation of stress disrupted subjective sleep, whereas the Cano-Saper model cannot take subjective sleep disturbances or the element of anticipation reported into account. Based on the findings of the cage-exchange study, Cano and colleagues believe that the effects of the stressor reflect an inability to inhibit wakefulness instead of an inability to sleep (Cano *et al.*, 2008).

However, within Study 2 the effects of anticipation did not affect objective sleep and only affected subjective total sleep time, where anticipation led to a reduced total sleep time compared to a two-week habitual baseline period. This instead indicates that the anticipation of stress does not lead to an inability to inhibit wakefulness but instead potentially indicates that stress causes a perceived inability to sleep. That said, this discrepancy may arise from the extrapolation of the animal model to humans. The ability to translate this animal model to humans may be improved by employing neuroimaging methods in order to examine regionally-specific brain activity in relation to the anticipation of an upcoming stressor. Additionally, quantitative EEG could be employed in order to examine whether

high-frequency activity is affected by the anticipation of stress in humans, employing the protocol used within Study 2.

7.3.2. *Spielman 3P model of insomnia*

The Spielman 3P model of insomnia (Spielman *et al.*, 1987; Spielman & Glovinsky, 1991; Spielman *et al.*, 1996) frames insomnia in terms of 3Ps: predisposing factors, precipitating factors and perpetuating factors. Within the context of the 3P model, Study 2 acted as a precipitating event by introducing stress, where the effects of anticipation alone affected subjective sleep and also affected the CAR. As there appeared to be a high variability in the individual response to the anticipation, as shown in the CAR and in cortisol levels throughout the full day (Day 2) spent within the laboratory, further work will be needed in order to understand how predisposing factors, such as the underlying genetic differences or psychological factors, such as the tendency to excessively worry to ruminate (Perlis *et al.*, 2011; Spielman & Glovinsky, 1991; Spielman *et al.*, 1996), can influence the extent to which anticipation can affect sleep. Additionally, the findings of the thesis offer a greater level of support for the Spielman 3P model than the Cano-Saper model. The results of the thesis are compatible with the sleep/stress switch point aspect of the model, where the anticipation of the upcoming stressor can result in an individual going beyond their threshold, or ‘sleep/stress switch point’, in terms of the insomnia. The most important finding from the thesis, in relation to the Spielman 3P model, is that the results of Study 2 suggest that the precipitating factor of the model can also include the anticipation of stress rather than the stressful experience itself. As the Spielman 3P model currently frames the precipitating factor as being a

stressor, in terms of a stressful event, rather than the anticipation of stress, the following section adapts the 3P model to reflect the findings of the thesis.

7.4. Proposed adaptation of Spielman 3P model

The proposed adaptation of the Spielman 3P model, based on the findings of the thesis, suggests that the precipitating factor can be either the anticipation of stress or the experience of stress. This was shown in Study 2 as the anticipation of stress, where participants believed that they would experience a series of demanding tasks at multiple time points during the following day, disrupted subjective sleep. This stress also resulted in HPA axis activation where the CAR peak change was steeper compared to a control group who followed the same protocol in the absence of stress. Within the adapted 3P model, the anticipation of stress, whether this is positive or negative in nature and whether this is accompanied by the resulting demand or not, can be considered to be a precipitating factor, where this is accompanied by HPA axis activation.. The anticipation of stress then interacts with the predisposing factors and pushes the individual above the insomnia threshold (i.e. the sleep/stress switch point) at the acute insomnia stage of the model. The perpetuating factors are identical to the original model, where the individual employs the behaviours described in an attempt to lessen the impact of the precipitant factor.

The hypothetical example provided by Spielman & Glovinsky (1991), where a young man receives a job promotion, can also be framed in terms of the thesis findings. In the example provided by Spielman & Glovinsky (1991) state that the stress of the young man receiving a job promotion (the precipitating event) results in a higher level of scrutiny, provoking the development of an anxiety state and pushing

the individual over the threshold for insomnia. The individual tries to cope by recouping the lost sleep at the weekend (perpetuating factors). As the thesis has shown that it is the anticipation of the precipitant factor which can disrupt sleep, the anticipation of the job promotion and the anticipation of the increased scrutiny result in the precipitating factor, which is the response to the anticipated stress. This interacts with the predisposing factors and pushes the young man over the insomnia threshold. The demand of the new job further adds to the poor sleep. At the next stage of the model, the anticipation of the stress has subsided and the young man attempts to compensate with the perpetuating factors as in the original model.

7.4.1. Associated implications for practice

The findings of the thesis have implications in terms of practice. Within the context of the adapted 3P model, it is the anticipation of stress which interacts with the predisposing factors which pushes the individual above the insomnia threshold potentially resulting in the secondary physiological response in terms of the CAR. The results from the current thesis also indicate that it is the anticipation of a forthcoming stressful event rather than the frequency or the valence of a stressful event, which is important in evoking the physiological response shown in the CAR. Previous research has examined life events within insomnia. Healey and colleagues (Healey *et al.*, 1981) reported that during the year in which they developed a sleep problem, individuals reported that they had experienced a greater number of stressful life events in comparison to good sleepers and also reported a greater number of negative events. In a separate study, Bastien and colleagues (Bastien *et al.*, 2004) reported that where individuals with insomnia reported a precipitating event associated with the onset of the sleep disturbance, 65% of these were negative. The

findings of the thesis indicate that the anticipation of stress, and not just the stressful event itself or the frequency or greater accumulation of stressful events, which results in a sleep disturbance. It is possible that by attempting to reduce the disruptive effect of anticipation, this will prevent the individual from reaching the threshold for insomnia.

It is possible that therapeutic methods might be employed to reduce the impact of the response in those 'at risk' of sleep disturbances in order to reduce the impact of anticipation and reduce the risk of the individual reaching the insomnia threshold. This potentially shifts the focus away from the treatment of the insomnia to the prevention of insomnia, focusing on primary prevention rather than secondary treatment. Such an approach may complement existing non-pharmacological treatments for insomnia which incorporate stress management techniques, specifically cognitive-behavioural therapy for insomnia (CBT-I). CBT-I consists of behavioural components, cognitive components and recommendations which integrate both components, focussing on sleep education (Manber *et al.*, 2011). Methods for lessening the impact of anticipation may include stress reduction strategies or coping strategies. As participants within Study 2 felt significantly more mentally alert in bed during the night before they believed they would complete a range of demanding tasks (i.e. when anticipating the stressor within Study 2), it is possible that such strategies may be effective at this point. Harvey & Payne (Harvey & Payne, 2002) tested the effects of a pre-sleep imagery distraction task within a group of individuals with insomnia, in which they were instructed to imagine an interesting, engaging, pleasant and relaxing situation. The individuals who were given the imagery distraction task showed a shorter subjective SOL than the group without instructions. It is possible that such a strategy may reduce the impact of

anticipation within normal sleepers and it should be examined whether this results in subsequent effects upon the CAR during the following morning. Additionally, such an approach could aim to reduce catastrophising, as individuals with insomnia show the tendency to catastrophise (Harvey & Greenall, 2003). As the anticipation of stress also affected the CAR, where the anticipation of stress led to greater cortisol levels over the CAR period and a steeper peak change in the CAR compared to a two-week baseline period, future research should examine whether the CAR reflects the degree of sleep disturbance associated with the anticipation of stress. Combined with the potential treatment options described above, future work should determine whether these indices of the CAR can be used as a marker of therapeutic efficacy.

7.4.2. Associated implications for policy

The thesis findings also have implications with regards to policy. As the results show that the anticipation of stress can disrupt sleep, the focus of insomnia should turn to prevention, using methods such as coping strategies or distraction techniques, in addition to the secondary treatment of the insomnia disorder. Aiming to prevent the insomnia occurring in the first place through treatments designed to lessen anticipation may be cost-effective, given that the cost of prescribing z-hypnotics (zaleplon, zolpidem and zopiclone) and benzodiazepine hypnotic drugs cost approximately £12 million and £18 million over the 12-month period from March 2007 to March 2008 (National Institute for Clinical Excellence, 2008).

The prevention of insomnia may also be cost-effective if this was able to reduce the associated economic impact, since individuals with insomnia show double the rate of absenteeism from work compared to good sleepers (Leger *et al.*, 2006), that

insomnia is a predictor of long-term sick leave (Sivertsen *et al.*, 2009b) and that the costs of insomnia are typically burdened by the employer (Godet-Cayre *et al.*, 2006).

The results of the thesis may also have implications with regards to policy regarding populations such as caregivers. As anticipation can affect subjective sleep, it is possible that where a caregiver anticipates a stressful event as a result of a policy change, they may exhibit disrupted sleep. It may be the case that caregivers have a greater susceptibility to anticipation, compared to a non-caregiver, due to chronic stress exhibited with caregiving, meaning that they would be more likely to cross the insomnia threshold. Caregivers for children with autism or attention deficit hyperactivity disorder have been shown to exhibit greater levels of psychological distress compared to non-caregiver parent controls (Lovell, Moss, & Wetherell, 2012) and elderly spousal caregivers of individuals with dementia have shown increased levels of psychological distress, in turn related to HPA axis activity, compared to controls (Vedhara *et al.*, 1999). Therefore, in order to reduce the disruptive effects of anticipation of a policy change upon sleep, if shown to be effective, the primary prevention strategies outlined in the previous section should be disseminated to these populations.

7.5. Additional contributions to the CAR literature

The current thesis also contributes to the CAR literature. The protocol outlined for measurement of the CAR in the thesis has the potential be used within other populations within a sleep medicine framework in order to examine HPA axis function, including within individuals with insomnia (Elder *et al.*, 2014). A further

contribution of the thesis to the CAR literature is regarding the relationship between the CAR and sleep. The secretion of cortisol follows the circadian rhythm (Morris *et al.*, 2012), pointing towards a potential role of sleep, however where the relationship between subjective sleep and the CAR had been assessed, the findings have been inconsistent. The results of the thesis add to the CAR literature in two ways, a) by informing the relationship between the CAR and sleep, measured subjectively and objectively, in a highly-controlled manner and b) by confirming that the CAR is a primarily a marker of anticipation, with a potential secondary role as a marker of demand. Study 1 showed that the subjective number of awakenings and that certain measures of objective sleep architecture were associated with the CAR, with lower awakening cortisol levels showing an association with a greater percentage of Stage 2 sleep and where the peak change showed an association with a greater latency to REM sleep. These results potentially indicated that the CAR may have an involvement within memory consolidation, since Stage 2 sleep may enhance memory consolidation either individually or in conjunction with REM and SWS (Marshall & Born, 2007) or that it may specifically support emotional memory consolidation (Diekelmann *et al.*, 2009). A further possibility is that relationship may have represented the effects of emotional regulation upon the CAR (Vandekerckhove & Cluydts, 2010).

Study 2 provided strong evidence that the CAR is a marker of anticipation, reflecting the anticipation of forthcoming demands. This was shown within Study 2 as participants who expected to complete a full day of demanding tasks, experiencing anticipation, displayed a steeper CAR with higher levels of cortisol during the CAR period, compared to a control group, within the context of a standardised protocol with extremely high levels over the CAR. This adds to

previous preliminary evidence showing that the CAR is a marker of anticipation , in terms of awakening levels (Filaire *et al.*, 2001; Rohleder *et al.*, 2007) and cortisol levels 30 minutes post-awakening (Filaire *et al.*, 2009; Filaire *et al.*, 2007). The results of the thesis also indicate that the CAR may have a secondary adaptive role regarding demand, as the CAR measured on the morning after the experimental manipulation within Study 2 indicated that individuals who had experienced anticipation where this was met by demand showed a subjectively steeper and more sustained CAR compared to participants who had experienced anticipation only. This indicated that the CAR may have a secondary role as a marker of adaptation and builds on other work showing that the CAR may have a role in recovery, where subjective feelings of loneliness, sadness, threat and a lack of control prior to bedtime, in older adults, were associated with a greater CAR displayed on the following morning (E. K. Adam *et al.*, 2006). As discussed in the previous section, if the CAR can objectively indicate the extent to which sleep is disrupted by the anticipation of stress, then this has implications for practice, since the CAR could potentially be used as a marker of therapeutic efficacy.

7.6. General strengths and limitations of the thesis

The major strength of the thesis is that the effects of stress upon sleep were measured using a standard protocol, specifically developed for this purpose. Specifically, the protocol enabled the effects of anticipation upon sleep to be teased apart from the effects of anticipation where this was met by subsequent demand. In addition, the thesis included a physiological marker of stress, through the inclusion of cortisol as a marker of HPA axis function. The development and use of the protocol is a particular strength of the thesis, since this has specifically allowed the

effects of anticipation where this has not been met with demand to be teased apart from the effects of anticipation where this has been met with demand.

The inclusion of the CAR as a marker of HPA axis activity is a specific strength of the thesis as previous stress studies have tended not to include a physiological marker of stress, meaning that it cannot be confirmed that the stressor reliably elicits stress. A further strength is in the nature of the demanding tasks used within Study 2, which were shown to reliably elicit stress.

In addition to the IGT and modified Stroop tasks, which were designed to induce uncertainty, participants completed the MTF at seven points throughout the day. The MTF is a task which is representative of everyday demanding situations and has been shown to elicit stress with corresponding physiological and psychological responses (Wetherell & Carter, in press). Due to the development of the standard protocol, the use of this protocol in the measurement of stress upon sleep resulted in two important findings. The first important finding was that it is the anticipation of a stressor alone which can disrupt subjective sleep and the second important finding is with regards to the CAR, providing strong evidence that this functions as a marker of anticipation.

There are potential general limitations within the thesis, however these are not likely to have impacted the overall findings and in some cases are major strengths of the design. One potential limitation is within the homogenous nature of participants included within Study 1 and Study 2, since participants were generally young adults drawn from a university staff and/or student population. However, this potential limitation is actually a particular strength of the thesis. Ellis and colleagues (Ellis, Perlis, Bastien, Gardani, & Espie, 2014) have previously demonstrated that

the mean age of onset of acute insomnia occurs at approximately 33 years of age. As the population included within Study 1 and Study 2 were relatively young, with a mean participant age of approximately 23 years within Study 1 and Study 2, it is therefore an advantage that participants were assessed in each study prior to the onset of any sleep disturbances.

Whilst future research could examine potential differential effects of age upon the anticipation of stress, potentially within children or older adults, this was beyond the scope of the thesis. A further potential limitation is with regards to power. Whilst the sample size employed within Study 2 was relatively small, significant findings were observed regarding the effects of anticipation upon subjective sleep and it is possible that an increased sample size may have increased the observed effects.

A further potential limitation is that any night-to-night variability in sleep and the CAR was unable to be taken into consideration within Study 1, which assessed the relationship between sleep and the CAR in the absence of stress, or within Study 2, which assessed the effects of anticipation, where this was either met or not met with subsequent demand, upon sleep and the CAR. However, there is currently no standard protocol for measuring sleep and the CAR other than the protocol outlined within the thesis and Study 1 showed that subjective sleep, objective sleep and the CAR were temporally stable between nights. Therefore, this is unlikely to have adversely impacted upon the results of the thesis. One potential solution would be to extend the protocol in order to examine the night-to-night variability within sleep and the CAR over multiple consecutive nights and mornings and indeed Wohlegemuth and colleagues (Wohlgemuth, Edinger, Fins, & Sullivan, 1999) suggest that up to fourteen consecutive nights of PSG might be necessary in order to

obtain stable objective sleep data. In the case of the CAR, Hellhammer and colleagues suggest that the CAR should be collected over six consecutive days are needed to reduce the impact of trait measures (Hellhammer *et al.*, 2007). However this was in the case of home CAR measurements and the laboratory environment employed within Study 2 afforded an extremely high level of control over factors known to affect the CAR, including light. Additionally, for practical reasons it was not feasible to extend the protocol employed within the thesis beyond the current period. Whilst this limitation does offer an opportunity for future research, it is not likely to have impacted upon the results of the thesis.

A final potential limitation is that within Study 1 and Study 2, participants were allowed to sleep in the laboratory in accordance with their routine sleep/wake schedule and were not instructed to adhere to a pre-determined awakening time. This could be considered a limitation as this more accurately models a non-work day scenario where individuals are relatively free to follow their own sleep/wake schedule, rather than a work day where sleep/wake schedules are socially determined (i.e. by work). However, in the absence of a standard protocol for measuring sleep, stress and the CAR within the same context, and in the absence of a prior understanding of how sleep and the CAR relate to each other within the context of a relatively free sleep/wake schedule, it was beyond the scope of the thesis to examine this. This was also not possible due to practical reasons. Future studies could use the protocol employed within the thesis in order to examine if a pre-determined sleep/wake schedule increases the impact of anticipation upon sleep and the CAR.

7.7. Future directions

7.7.1. *Timing of stress*

One way in which the thesis could be extended could be to vary the timing of the stressor. Whilst the thesis showed that the anticipation of stress affected subjective sleep and HPA axis activity in terms of the CAR, participants anticipated stress at a specific time point in advance (i.e. on Day 2 of the study). As the results of the thesis have shown that the anticipation of a stressor at a specific time can disrupt sleep and the CAR, one potential future direction would be to employ the standard protocol and extend this over a period of multiple days. This would allow the protocol to examine whether the effects of anticipation of a stressor at an unspecified time point are greater than the effects of anticipation at a pre-determined time point and to examine the subsequent effects upon the CAR.

7.7.2. *Predisposing factors*

The main future direction for research is to examine individual predictors of the extent to which the anticipation of stress disrupts sleep. Research examining individual predictors has, to date, the response to sleep disturbances, but has not specifically examined how these affect the anticipation of stress. Drake and colleagues examined the response to stress, where participants completed the FIRST questionnaire, assessing the likelihood of experiencing sleep disturbances in response to stressful situations. Participants were split into a low and high vulnerability group on the basis of the FIRST scores and the high vulnerability group showed objective sleep disruptions to the first night spent within a sleep laboratory, even after controlling for a history of insomnia symptoms. Those with high FIRST scores reported insomnia to be more common (Drake *et al.*, 2004). Where Drake and colleagues examined how these factors can influence the response to stress and

suggest that there are individual factors influencing the response, it will be necessary to examine how these factors affect the impact of anticipation.

In the context of the original 3P model of insomnia (Spielman *et al.*, 1987; Spielman & Glovinsky, 1991; Spielman *et al.*, 1996), Study 2 examined the impact of the precipitating event in by examining the effects of stress upon sleep. In the context of the 3P model, this suggests instead that the anticipation of stress affects sleep, interacts with predisposing factors and subsequently pushes the individual over the insomnia threshold. It is possible that there are individual variations in the degree to which anticipation disturbs sleep, potentially driven by predisposing factors.

One future direction would be to investigate the extent to which personality variables moderate the effects of the anticipation upon subjective sleep and the CAR. It has previously been shown that within a study of normal sleepers, perfectionism showed a relationship with the perceived severity of sleeping problems and also with higher levels of concern regarding the negative consequences of the sleeping problem (Lundh *et al.*, 1994). A group of insomnia patients displayed higher levels of perfectionism than normal sleepers (Lundh *et al.*, 1994) and associations, albeit weak, have been shown in another study (Jansson-Fröjmark & Linton, 2007). One approach would be to examine how individuals with high or low levels of perfectionism and assess the approach of the anticipation of a stressor upon subjective sleep, objective sleep and HPA axis activity. Similarly, other relevant personality traits which may be investigated could include neuroticism (van de Laar *et al.*, 2010).

Another approach would be to examine potential genetic influences and examine how these interact with the anticipation of stress. Within adults, twin genetic studies have shown that insomnia does appear to be heritable (Barclay & Gregory, 2013). Similarly, there is also scope for genetic factors being a predisposition towards insomnia within the Spielman 3P model (Spielman *et al.*, 1987; Spielman & Glovinsky, 1991; Spielman *et al.*, 1996). Genetic influences are also relevant to cortisol, used in the thesis as a marker of HPA axis activation, since a meta-analysis of 1,686 study participants showed that individuals who were homozygous carriers for the serotonin transporter linked promoter region (5-HTTLPR) gene short allele displayed increased levels of salivary cortisol secretion in response to psychosocial stress paradigms than individuals with either short/long or long/long genotypes (R. Miller, Wankerl, Stalder, Kirschbaum, & Alexander, 2013). Within the context of the hyperarousal model of insomnia, Riemann and colleagues (Riemann *et al.*, 2010) speculate that the vulnerability towards insomnia may be inherited genetically. One stress study has examined the relationship between 5-HTTLPR, sleep and stress in a sample of dementia caregivers and non-caregiver control participants (Brummett *et al.*, 2007). Dementia caregivers rated their sleep quality as being poorer than controls, Whilst there was no direct effect of the 5-HTTLPR genotype on sleep quality, there was an interaction between genetics, sleep and stress, where dementia caregivers who had the short allele of the 5-HTTLPR showed the poorest sleep quality compared to controls. Taken together, the influence of genetics should be explored in relation to the effects of stress upon sleep and the CAR and this represents one promising approach for future research.

7.8. Conclusions

This thesis has examined the effects of stress upon subjective sleep, objective sleep and the CAR. This was done through the development of a standard protocol where the effects of anticipation upon sleep and the CAR, where this was either met by subsequent demand or not met by subsequent demand, were measured. Overall, the anticipation of stress can disrupt very specific aspects of sleep and the HPA axis functioning. As a consequence it is proposed that the Spielman 3P model frames the anticipation of stress as an additional precipitating factor. The thesis has also shown that the CAR primarily appears to function as a marker of anticipation with a potential secondary role as a marker of demand. The findings of the thesis have implications for practice, since interventions should primarily target potential disruptive effects of anticipation, with secondary treatment upon the resulting sleep disturbance, using methods such as coping strategies. The findings of the thesis also have implications for policy and are relevant to groups such as caregivers, since the anticipation of forthcoming event may result in a sleep disturbance. The findings of the thesis suggest that one future direction is to examine the influence of predisposing factors upon the anticipation of stress. These predisposing factors include variables such as neuroticism, perfectionism and genetic variables, including the effects of the 5HTTLPR gene. By understanding how the predisposing factors relate to the disruptive effects of anticipation, this will further the understanding of the sleep/stress switch point.

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Appendix A

Example screening questionnaire used in the feasibility study, Study 1 and Study 2



Screening Questionnaire

Date: _____

Name of potential participant: _____

Please answer the following questions so that it can be determined whether or not you meet the criteria to take part in this study.

I. SLEEP DIFFICULTIES

Have you ever had a sleep problem: Yes No (If no go to section 2)

If yes, Is this an ongoing problem at the moment? Yes No

How long have you had the sleep problem or how long did it last? _____ Years
_____ Months

What is the nature of your sleep problem?

Getting off to sleep / Staying Asleep / Waking too early / Feeling unrefreshed on waking / Sleeping at odd times / Excessive daytime sleepiness

2. OTHER SLEEP/WAKE DISORDERS

Have you travelled over three time zones in the last six months? Yes No

Have you or your spouse/bed partner ever noticed one of the following in the last month:

A. RESTLESS LEGS: Crawling or aching feelings in the legs (calf) and inability to keep legs still;

Yes No

B. PERIODIC LEG MOVEMENTS: Leg twitches or jerks during the night;

Yes No

- C. APNOEA: Snoring, pauses in breathing at night, short of breath, choking at night; morning headaches, chest pain, dry mouth;
 Yes No
- D. NARCOLEPSY: Sleep attacks, sleep paralysis, hyp. hall., cataplexy;
 Yes No
- E. GASTRO-ESOPHAGEAL REFLUX: Sour taste in mouth, heart burn; reflux;
 Yes No
- F. PARASOMNIAS: Nightmares, night terrors, sleep walking, sleep talking;
 Yes No
- G. SHIFT WORK DISORDER: Rotating work patterns
 Yes No

3. NATURE OF SLEEP/WAKE DIFFICULTY

How much sleep do you think you need to function during the day ___ Hours
 ___ Minutes

How much sleep do you get on a typical weekday ___ Hours ___ Minutes

How much sleep do you get on a typical weekend ___ Hours ___ Minutes

4. GENERAL HEALTH

Do you consider yourself to be in good health? Yes No

Do you suffer from an ongoing illness (e.g. Asthma or Diabetes?) Yes No

If yes, could you specify?

Are you currently on any medication? Yes No

-If yes, what, and at what

dose _____

Have you suffered a head injury? Yes No

Do you have a history or current complaint of depression or anxiety Yes No

5. DEMOGRAPHICS

Address: _____

Post code: _____ E-mail-
 address: _____

Telephone: _____ Mobile
phone: _____

Preferable time for future contact (check one): Morning__ Afternoon __
Evening __

Date of birth: _____ Age: _____ Gender: Male__
Female__

I agree to be contacted in the future from the Northumbria Centre for Sleep Research?
__Yes __No

-What would be your preferred medium to receive the questionnaires, sleep diaries
and more information regarding this study? __Online (internet access)
__Hard copies

Now please e-mail this questionnaire back to greg.elder@northumbria.ac.uk so that
your eligibility to take part in the study can be assessed. Once you have done this the
researcher will be in touch with you shortly. Please send any questions to the same e-
mail address.

Thank you for your time!

Appendix B

Pittsburgh Sleep Quality Index

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

Please answer *all* the questions.

1. During the past month, when have you usually gone to bed at night?
USUAL BED TIME: _____
(i. e. 10pm or 22.00)

2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?

NUMBER OF MINUTES: _____

2b. How long have you usually been awake during the night?

NUMBER OF MINUTES: _____

3. During the past month, when have you usually got up in the morning?

USUAL GETTING UP TIME: _____

4. During the past month, how many hours of *actual* sleep did you get at night? This may be different to the number of hours you spend in bed.

HOURS OF SLEEP PER NIGHT: _____

4b. How many nights per week do you usually have difficulties sleeping?

NUMBER OF NIGHTS PER WEEK: _____

5. During the past month, how often have you had trouble sleeping because you:

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
(a) Cannot get to sleep within 30 minutes				
(b) Wake up in the middle of the night or early morning				
(c) Have to get up and use the				

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
(e) Cough or snore loudly				
(f) Feel too cold				
(g) Feel too hot				
(h) Had bad dreams				
(i) Have pain bathroom				

reason(s), please describe: _____

How often during the past month have you had trouble sleeping because of this?

- Not during the past month
- Less than once a week
- Once or twice a week
- Three or more times a week

6. During the past month, how would you rate your sleep quality overall?

- Very good
- Fairly good
- Fairly bad
- Very Bad

7. During the past month, how often have you taken medicine (prescribed or 'over the counter') to help you sleep?

- Not during the past month
- Less than once a week
- Once or twice a week
- Three or more times a week

8. During the past month, how often have you had trouble staying awake while driving, eating meals or engaging in social activity?

- Not during the past month
- Less than once a week
- Once or twice a week
- Three or more times a week

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

- No problem at all
- Only a very slight problem
- Somewhat of a problem
- A very big problem

10. Do you have a bed partner or room-mate?

- No bedroom partner or room-mate
- Partner/ room-mate in other room
- Partner in same room, but not same bed
- Partner in same bed

If you have a room-mate or bed partner, ask him/ her how often in the past month you have had:

(a) Loud snoring

- Not during the past month
- Less than once a week
- Once or twice a week
- Three or more times a week

(b) Long pauses between breaths while asleep

- Not during the past month
- Less than once a week
- Once or twice a week
- Three or more times a week

(c) Legs twitching or jerking while you sleep

- Not during the past month
- Less than once a week
- Once or twice a week
- Three or more times a week

(d) Episodes of disorientation or confusion during sleep

- Not during the past month
- Less than once a week
- Once or twice a week
- Three or more times a week

(e) Other restlessness while you sleep; please describe

- Not during the past month
- Less than once a week
- Once or twice a week
- Three or more times a week

Appendix C

Perceived Stress Scale

(K) The questions in this scale ask you about your feelings and thoughts during the **last month**. In each case, you will be asked to indicate how often you felt or thought in a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer each one as a separate question. That is, don't try to count up the number of times you felt a particular way, but rather indicate the number that seems like a reasonable estimate. Please circle the most appropriate response.

	1.Never	2.Almost Never	3.Some Times	4.Fairly Often	5.Very Often
In the last month, how often have you been upset because of something that happened unexpectedly?	1	2	3	4	5
In the last month, how often have you felt that you were unable to control the important things in your life?	1	2	3	4	5
In the last month, how often have you felt nervous and stressed?	1	2	3	4	5
In the last month, how often have you dealt with irritating life hassles?	1	2	3	4	5
In the last month, how often have you felt that you were effectively coping with important changes that were occurring in your life?	1	2	3	4	5
In the last month, how often have you felt confident about your ability to handle your personal problems?	1	2	3	4	5
In the last month, how often have you felt that things were going your way?	1	2	3	4	5
In the last month, how often have you found that you could not cope with all the things you had to do?	1	2	3	4	5
In the last month, how often have you been able to control irritations in your life?	1	2	3	4	5
In the last month, how often have you felt that you were on top of things?	1	2	3	4	5
In the last month, how often have you been angered because of things that happened that were outside of your control?	1	2	3	4	5
In the last month, how often have you found yourself thinking about things that you have to accomplish?	1	2	3	4	5
In the last month, how often have you been able to control the way you spend your time?	1	2	3	4	5
In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	1	2	3	4	5

Appendix D

Hospital Anxiety and Depression Scale

(J) Read each item and tick the box opposite the reply which comes closest to how you have been feeling in the past week. Don't take too long over your replies: your immediate reaction to each item will probably be more accurate than a long thought-out response. *Tick only one box in each section.*

- | | |
|---|---|
| <p>1. I feel tense or "wound up"</p> <p>Most of the time. <input type="checkbox"/></p> <p>A lot of the time. <input type="checkbox"/></p> <p>Time to Time, Occasionally <input type="checkbox"/></p> <p>Not at all <input type="checkbox"/></p> | <p>8. I feel as if I am slowed down</p> <p>Nearly all the time <input type="checkbox"/></p> <p>Very often <input type="checkbox"/></p> <p>Sometimes <input type="checkbox"/></p> <p>Not at all <input type="checkbox"/></p> |
| <p>2. I still enjoy the things I used to enjoy</p> <p>Definitely as much. <input type="checkbox"/></p> <p>Not quite as much. <input type="checkbox"/></p> <p>Only a little. <input type="checkbox"/></p> <p>Hardly at all <input type="checkbox"/></p> | <p>9. I get a sort of frightened feeling like butterflies in the stomach</p> <p>Not at all <input type="checkbox"/></p> <p>Occasionally <input type="checkbox"/></p> <p>Quite often <input type="checkbox"/></p> <p>Very often <input type="checkbox"/></p> |
| <p>3. I get a sort of frightened feeling as if something awful is about to happen</p> <p>Very definitely and quite badly . . . <input type="checkbox"/></p> <p>Yes, but not too badly <input type="checkbox"/></p> <p>Time to Time, Occasionally <input type="checkbox"/></p> <p>Not at all <input type="checkbox"/></p> | <p>10. I have lost interest in my appearance</p> <p>Definitely <input type="checkbox"/></p> <p>I don't take so much care as I should <input type="checkbox"/></p> <p>I may not take quite as much care . . <input type="checkbox"/></p> <p>I take just as much care as ever . . . <input type="checkbox"/></p> |
| <p>4. I can laugh and see the funny side of things</p> <p>As much as I always could <input type="checkbox"/></p> <p>Not quite so much now <input type="checkbox"/></p> <p>Definitely not so much now <input type="checkbox"/></p> <p>Not at all <input type="checkbox"/></p> | <p>11. I feel restless as if I have to be on the move</p> <p>Very much indeed <input type="checkbox"/></p> <p>Quite a lot. <input type="checkbox"/></p> <p>Not very much. <input type="checkbox"/></p> <p>Not at all <input type="checkbox"/></p> |
| <p>5. Worrying thoughts go through my mind</p> <p>A great deal of the time <input type="checkbox"/></p> <p>A lot of the time <input type="checkbox"/></p> <p>From time to time but not too often <input type="checkbox"/></p> <p>Only occasionally <input type="checkbox"/></p> | <p>12. I look forward with enjoyment to things</p> <p>As much as ever I did <input type="checkbox"/></p> <p>Rather less than I used to <input type="checkbox"/></p> <p>Definitely less than I used to <input type="checkbox"/></p> <p>Hardly at all <input type="checkbox"/></p> |
| <p>6. I feel cheerful</p> <p>Not at all <input type="checkbox"/></p> <p>Not often <input type="checkbox"/></p> <p>Sometimes <input type="checkbox"/></p> <p>Most of the time <input type="checkbox"/></p> | <p>13. I get sudden feeling of panic</p> <p>Very often indeed <input type="checkbox"/></p> <p>Quite often <input type="checkbox"/></p> <p>Not very often <input type="checkbox"/></p> <p>Not at all <input type="checkbox"/></p> |
| <p>7. I can sit at ease and feel relaxed</p> <p>Definitely <input type="checkbox"/></p> <p>Usually <input type="checkbox"/></p> <p>Not Often <input type="checkbox"/></p> <p>Not at all <input type="checkbox"/></p> | <p>14. I can enjoy a good book or radio or TV programme</p> <p>Often <input type="checkbox"/></p> <p>Sometimes <input type="checkbox"/></p> <p>Not Often. <input type="checkbox"/></p> <p>Very seldom <input type="checkbox"/></p> |

Appendix E

Horne-Östberg Morningness-Eveningness Questionnaire

(L)

- a) Please read each question very carefully before answering.
- b) Answer all questions.
- c) Each question should be answered independently of others. Do **NOT** go back and check your answers.
- d) Please select **ONE** answer only and respond by checking [x] the most appropriate answer.
- e) Please answer each question as honestly as possible. Both your answers and results will be kept in strict confidence.

1. Considering your own feelings, at what time would you get up if you were entirely free to plan your day?

Time:

2. Considering only your own feelings, at what time would you go to bed if you were entirely free to plan your day?

Time:

3. If there is a specific time you have to get up in the morning, to what extent are you dependent on being woken up by an alarm clock?

- a. Not at all dependent []
- b. Slightly dependent []
- c. Fairly dependent []
- d. Very dependent []

4. Assuming adequate environmental conditions, how easy do you find getting up in the morning?

- a. Not at all easy []
- b. Slightly easy []
- c. Fairly easy []
- d. Very easy []

5. How alert do you feel during the first half hour after having woken in the morning?

- a. Not at all alert []
- b. Slightly alert []
- c. Fairly alert []
- d. Very alert []

6. How is your appetite during the first half hour after having woken in the morning?

- a. Not at all good []
- b. Slightly good []
- c. Fairly good []
- d. Very good []

7. During the first half hour after having woken in the morning, how tired do you feel?

- a. Very tired []
- b. Slightly tired []
- c. Fairly refreshed []
- d. Very refreshed []

8. When you have no commitments the next day, at what time do you go to bed compared to your usual bedtime?

- a. Seldom or never later []
- b. Less than one hour later []
- c. 1-2 hours later []
- d. More than 2 hours later []

9. You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him is between 0700 and 0800h. Bearing in mind nothing else but your own inclinations, how do you think you would perform?

- a. Would be on good form []
- b. Would be on reasonable form []
- c. Would find it difficult []
- d. Would find it very difficult []

10. At what time in the evening do you feel tired and in need of sleep?

Time:

11. You wish to be at your peak for a test which you know is going to be mentally exhausting and lasting for two hours. You are entirely free to plan your day, when would you do this task?

- a. 0800 – 1000 []
- b. 1100 – 1300 []
- c. 1500 – 1700 []
- d. 1900 – 2100 []

12. If you went to bed at 2300h at what level of tiredness would you be?

- a. Not at all tired []
- b. A little tired []
- c. Fairly tired []
- d. Very tired []

13. For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Will you:

- a. Wake up at the usual time and not go back to sleep []
- b. Wake up at the usual time and doze []
- c. Wake up at the usual time and go back to sleep []
- d. Wake up later than usual []

14. One night you have to remain awake between 0400 and 0600h. You have no commitments the next day. Which suits you best:

- a. Not to go to bed until 0600h []
- b. Nap before 0400h and sleep after 0600h []
- c. Sleep before 0400h and nap after 0600h []
- d. Sleep before 0400h and remain awake after 0600h []

15. You have to do two hours physical work. Which hours would you prefer to do it between:

- a. 0800 – 1000 []
- b. 1100 – 1300 []
- c. 1500 – 1700 []
- d. 1900 – 2100 []

16. You have decided to engage in some physical exercise. A friend suggests that you do this between 2200 and 2300h twice a week. How do you think you would perform:

- a. Would be on good form []
- b. Would be on reasonable form []
- c. Would find it difficult []
- d. Would find it very difficult []

17. Suppose that you can choose your own work hours, but had to work five hours in the day. Which five consecutive hours would you choose:

Hours:

18. At what time of day do you feel your best?

Time:

19. One hears of “morning” and “evening” types. Which do you consider yourself to be?

- a. Morning type []
- b. More morning than evening []
- c. More evening than morning []
- d. Evening type []

Appendix F

Ford Insomnia Response to Stress Test

(M) When you experience the following situations, how likely is it for you to have difficulty sleeping? Please circle an answer even if you have not experienced these situations recently.

Before an important meeting the next day

Not likely Somewhat likely Moderately likely Very likely

After a stressful experience during the day

Not likely Somewhat likely Moderately likely Very likely

After a stressful experience in the evening

Not likely Somewhat likely Moderately likely Very likely

After getting bad news during the day

Not likely Somewhat likely Moderately likely Very likely

After watching a frightening movie or TV show

Not likely Somewhat likely Moderately likely Very likely

After having a bad day at work

Not likely Somewhat likely Moderately likely Very likely

After an argument

Not likely Somewhat likely Moderately likely Very likely

Before having to speak in public

Not likely Somewhat likely Moderately likely Very likely

Before going on holiday the next day

Not likely Somewhat likely Moderately likely Very likely

Appendix G

Perceived Stress Reactivity Scale

(N) This questionnaire asks about your reactions to situations which you may have experienced in the past. Three answers are suggested. Please indicate the answer that most closely describes your own reaction in general. Please don't skip any item, even if it may be hard to find the best answer.

1. When tasks and duties build up to the extent that they are hard to manage...
 - I am generally untroubled
 - I usually feel a little uneasy
 - I normally get quite nervous

2. When I want to relax after a hard day at work...
 - This is usually quite difficult for me
 - I usually succeed
 - I generally have no problem at all

3. When I have conflicts with others that may not be immediately resolves...
 - I generally shrug it off
 - It usually affects me a little
 - It usually affects me a lot

4. When I make a mistake...
 - In general, I remain confident
 - I sometimes feel unsure about my abilities
 - I often have doubts about my abilities

5. When I'm wrongly criticised by others...
 - I am normally annoyed for a long time
 - I am annoyed for just a short time
 - In general, I am hardly annoyed at all

6. When I argue with other people...
 - I usually calm down quickly
 - I usually stay upset for some time
 - It usually takes me a long time until I calm down

7. When I have little time for a job to be done...
 - I usually stay calm
 - I usually feel uneasy
 - I usually get quite agitated

- 8. When I make a mistake...**
- I am normally annoyed for a long time
 - I am normally annoyed for a while
 - I generally get over it easily
- 9. When I am unsure what to do or say in a social situation...**
- I generally stay cool
 - I often feel warm
 - I often begin to sweat
- 10. When I have spare time after working hard...**
- It often is difficult for me to unwind and relax
 - I usually need some time to unwind properly
 - I am usually able to unwind effectively and forget about the problems of the day
- 11. When I am criticised by others...**
- Important arguments usually come to my mind when it is too late to still make my point
 - I often have difficulty finding a good reply
 - I usually think of a reply to defend myself
- 12. When something does not go the way I expected...**
- I usually stay calm
 - I often get uneasy
 - I usually get very agitated
- 13. When I do not attain a goal...**
- I usually remain annoyed for a long time
 - I am usually disappointed, but recover soon
 - In general, I am hardly concerned at all
- 14. When others criticise me...**
- I generally don't lose confidence at all
 - I generally lose a little confidence
 - I generally feel very unconfident
- 15. When I fail at something...**
- I usually find it hard to accept
 - I usually accept it to some degree
 - In general, I hardly think about it

- 16.** When there are too many demands on me at the same time...
- I generally stay calm and do one thing after the other
 - I usually get uneasy
 - Usually, even minor interruptions irritate me
- 17.** When others say something incorrect about me...
- I usually get quite upset
 - I normally get a little bit upset
 - In general, I shrug it off
- 18.** When I fail at a task...
- I usually feel very uncomfortable
 - I usually feel somewhat uncomfortable
 - In general, I don't mind
- 19.** When I argue with others...
- I usually get very upset
 - I usually get a little bit upset
 - I usually don't get upset
- 20.** When I am under stress...
- I usually can't enjoy my leisure time at all
 - I usually have difficulty enjoying my leisure time
 - I usually enjoy my leisure time
- 21.** When tasks and duties accumulate to the extent that they are hard to cope with...
- My sleep is unaffected
 - My sleep is slightly disturbed
 - My sleep is very disturbed
- 22.** When I have to speak in front of other people...
- I often get very nervous
 - I often get somewhat nervous
 - In general, I stay calm
- 23.** When I have many tasks and duties to fulfil...
- In general, I stay calm
 - I usually get impatient
 - I often get irritable

Appendix H

Example subjective sleep diary



SLEEP & MOOD DIARY

Instructions:

This diary is designed to provide a record of how you feel and your experience of sleep. Each page relates to your mood and sleep experiences for a particular day. Please complete one page each morning, soon after you wake up. Take a few minutes to do this, trying to be as accurate as you can. We are interested in how you feel at the time you complete the diary. It is your best estimate that we are looking for, but try not to get into the habit of clockwatching at night.

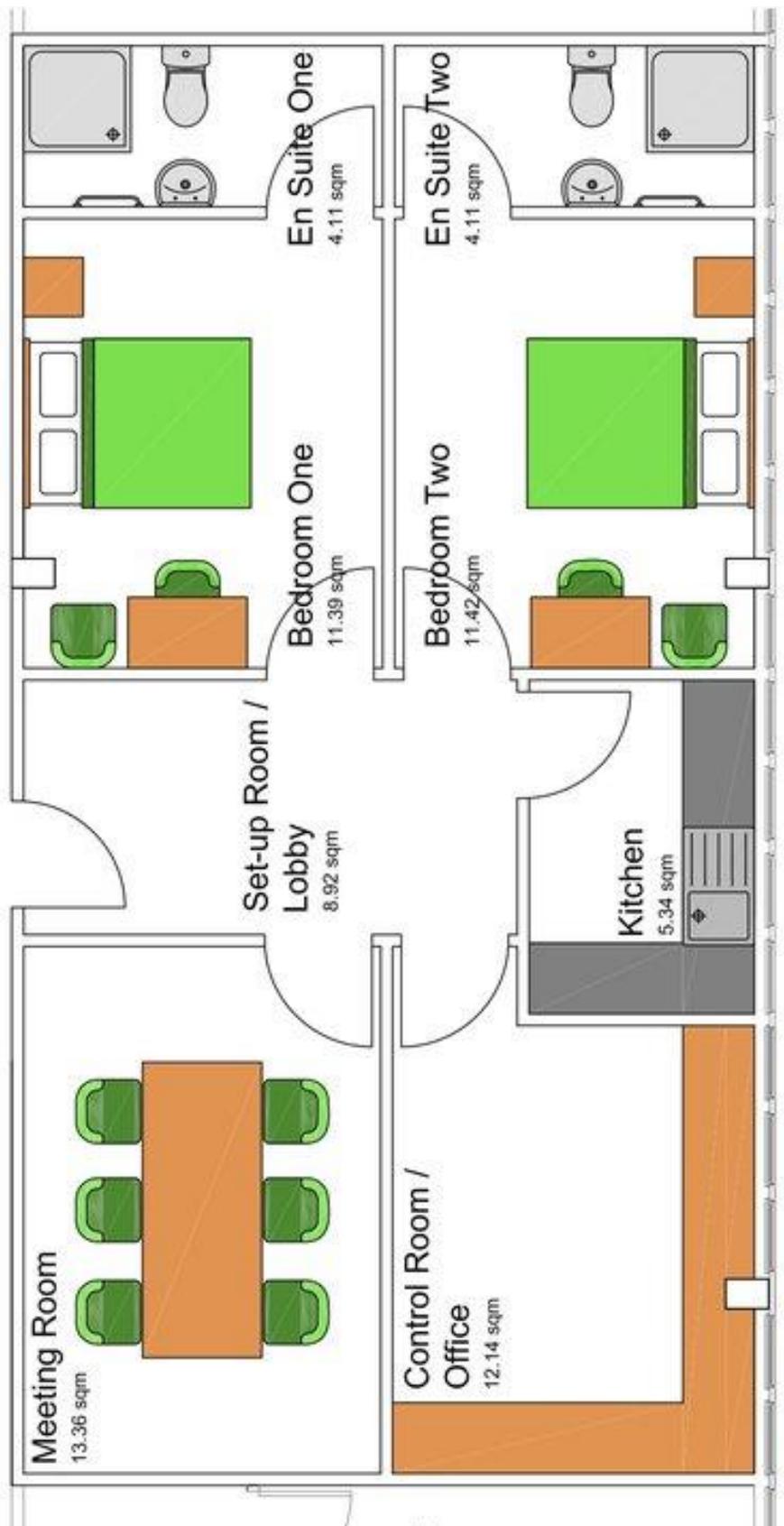
Participant Number: _____

Study title: _____

Date: _____

Appendix I

Diagram of the Northumbria Centre for Sleep Research



Appendix J

Feasibility study – home group saliva sample collection instructions

You will go to bed at your usual bedtime, and you will wake at the time you would usually wake up on a morning. You will be asked to provide saliva samples immediately upon awakening and 15, 30, 45 and 60 minutes after this, including one sample early afternoon (before eating lunch) and one before sleeping. This will be done by chewing on a dental cotton swab for approximately a minute. For the first hour after you wake, you will not be allowed to eat or brush your teeth as this can interfere with the measurements we are taking. You will be allowed to drink water. You will be required to keep all saliva samples in a household fridge until the researcher collects them.

Appendix K

Study 2 written instructions given to all participants prior to sleep on Night 1.

“On your second day in the sleep lab, you will be required to complete a range of mentally demanding computerised tests at regular intervals throughout the day and provide four saliva samples. Your performance on each task will be carefully monitored and it is very important that you perform as well as you possibly can on these tasks. Each task will take between 10 and 20 minutes to complete. These tasks will be completed from the moment you wake until you go to bed. We will measure your mood before each task, and we will also monitor the amount of effort that you are putting into each task.

Your tests will include a measure of attention, where you have to press the coloured key on the computer keyboard corresponding with the letter of the word you are shown. It is important that you do this as quickly as you possibly can.

You will also have to complete a combination of four tasks where you have to perform several tasks at once. You will have to complete this as quickly and accurately as possible, and you will also need to try to achieve the highest score you possibly can. In this task you will be able to see your score, and you should do your very best to beat your score each time as you will complete this particular task at various points during the day. It is very important that you make sure that you are performing all of the tasks to the best of your ability.

The final task which you will complete is a decision-making card task, and again your job is to work out what the rules are, and to achieve as high a score as you possibly can. You will have the chance to win an iPad if you achieve the highest score on a randomly-selected task.”

Appendix L

Iowa Gambling Task instructions

“You will be presented with 4 decks of cards and will be asked to choose from one at a time. You can choose from any deck and change between decks as you see fit. Each time you select a card, the computer will inform you of a change in your score. The object of the task is to gain as much money as possible. Some decks will help you to achieve this goal better than others. Try and make your decisions as if you were using your own money. After you select a card, you must wait 6 seconds to select another. Press the SPACE key to begin.”

Appendix M

Study 2 written instructions given to participants in the anticipation alone (AA) condition

“You will no longer have to complete the demanding tasks today, and you are free to perform other activities in the laboratory. You will still be eligible to win the iPad, as everyone who participates in this study will be placed into a random draw irrespective of whether or not they have performed the tasks. The details of the study you are taking part in are extremely important and crucial to the research questions being addressed. For this reason, we kindly ask that you do not discuss the details of the study with others beyond the fact that you are taking part in a sleep study where your sleep is monitored in the laboratory. If it appears that details of the study have been discussed we reserve the right to remove your entry into iPad prize.”

Appendix N

Study 2 Day 2 mood diary

Day 2 Mood Diary 1

(GE_S2_P__T1)

Read each statement then mark on the line at the most appropriate point to indicate
how you feel right now, at this moment

I feel calm* _____
not at all very much

I feel tense* _____
not at all very much

I am upset* _____
not at all very much

I feel relaxed* _____
not at all very much

I feel content* _____
not at all very much

I feel worried* _____
not at all very much

I feel stressed _____
not at all very much

Appendix O

NASA-TLX

Please mark each line at the point which matches your experience of the test you have just completed.

A) **MENTAL DEMAND** – How much mental demand and perceptual activity was required (thinking, deciding, calculating, remembering, looking etc)? Was your task easy or demanding, simple or complex?

Low _____ High

B) **PHYSICAL DEMAND** – How much physical activity was required (pulling, turning, controlling activating etc)? Was your task easy or demanding, slow or brisk, slack or strenuous, restful or laborious?

Low _____ High

C) **TEMPORAL DEMAND** – How much time pressure did you feel due to the rate of the task? Was the pace slow and leisurely or rapid and frantic?

Low _____ High

D) **EFFORT** – How hard did you have to work, mentally and physically, to achieve your level of performance?

Low _____ High

E) **PERFORMANCE** – How successful do you think you were in performing the tests? How satisfied were you with your performance ?

Poor _____ Good

F) **FRUSTRATION** – How insecure, discouraged, irritated, stressed and annoyed versus secure, gratified, content, relaxed and complacent did you feel?

Low _____ High

Appendix P

Feasibility study (laboratory participants) and Study 1 participant information sheet

PARTICIPANT INFORMATION.

TITLE OF PROJECT: Measuring the cortisol awakening response in good sleepers within a controlled sleep environment

Participant ID Number:

Principal Investigator: Greg Elder

Investigator contact details:

Northumbria Centre for Sleep Research, NB408 Northumberland Building,

Northumbria University NE1 8ST

Email: greg.elder@northumbria.ac.uk

This project is funded by: Northumbria University

Number of participant points / payment:0 / £150

INFORMATION TO POTENTIAL PARTICIPANTS

1. What is the purpose of the project?

The purpose of the project is to investigate levels of a specific hormone (cortisol) in healthy, good sleepers both upon awakening and throughout the course of the day, within a sleep laboratory.

2. Why have I been selected to take part?

You are a healthy, non-smoking individual, aged between 18-40 who has indicated that you would like to take part.

3. What will I have to do?

In the two weeks before visiting the sleep laboratory, you will complete a sleep diary providing details including the time you went to bed, how long it took you to get to sleep, and what time you woke up. In this period you will also wear an actiwatch (a small wrist-watch like device worn like a watch) which will measure your movements. If you are female you will be asked basic questions regarding your menstrual cycle and oral contraceptive use as these can both affect the hormone we are measuring. Please try as best as you can to keep the time you go to bed and get up relatively stable during this two-week period.

Your participation in the study will last three days in total, and you will stay overnight in the sleep laboratory during the first night, having electrodes applied to your scalp and body to measure various aspects of your sleep. The electrodes are for measurement purposes only and cannot themselves generate any electrical signals. The equipment that will be attached to you is specially designed to be worn whilst you sleep, and is hard-wearing. Two research staff will remain in the sleep research unit at all times and will monitor you sleeping via bedroom CCTV which will record audio and video for your safety. You will go to bed at your usual bedtime, and you will then be woken up at the usual time you awake based on the information you provided, and will be asked to provide saliva samples immediately upon awakening and 15, 30, 45 and 60 minutes after this. This will be done by chewing on a dental cotton swab for approximately a minute. For the first hour after you awake, you will not be allowed to eat or brush your teeth as this can interfere with the measurements we are taking. You will be allowed to drink water.

You will then be free to leave the sleep laboratory whilst still wearing the actiwatch, and you

will be asked to return to the sleep laboratory with the actiwatch the following evening at 8pm. You will stay a second night in the sleep laboratory, will again be woken at your usual awakening time, and will be asked to provide saliva samples immediately upon awakening and 15, 30, 45 and 60 minutes later in the same manner as before. You will then provide saliva samples every hour in the same way until it is your usual bedtime, and you will not be able to take anything by mouth for 30 minutes before each sample. During the day you will be free to perform activities such as reading books and watching films within the sleep laboratory, but you will not be permitted to leave the laboratory.

You will then spend a final night in the sleep laboratory in the same way as before. and will again be woken at your usual awakening time in order provide a saliva sample immediately. You will then again provide samples 15, 30, 45 and 60 minutes after you awake, and you will then be free to leave the sleep laboratory.

4. What are the exclusion criteria (i.e. are there any reasons why I should not take part)?

You will be unable to take part in the study if you are currently a shift worker, as this may mean you have an unstable sleep/wake cycle. You also cannot take part if you are suffering from any sleep disorders (e.g. jet lag or insomnia), if you have any uncontrolled illnesses, or if you suffer from any disorders including depression or hormonal disorders. You cannot take part in this study if you currently smoke, as this can affect the measurements we are taking. You cannot take part if you are under 18 or are over 40 years of age. We request that you do not participate in any other research studies whilst taking part in the current study, including within the two-week period where you wear the actiwatch and fill out the sleep diary, as this may result in your exclusion from the study. We would request that you provide us with full details of any other study if you believe this may be the case.

5. Will my participation involve any physical discomfort?

The sleep laboratory is designed to be a comfortable environment similar to a home environment. Whilst you are unlikely to suffer any physical discomfort, the application of electrode conducting gel may cause minor irritation. You will be able to withdraw from the study at any time by informing the researcher.

6. Will my participation involve any psychological discomfort or embarrassment?

Your participation is unlikely to involve any psychological discomfort or embarrassment. It is possible that you may find your first night in the sleep laboratory to be unusual.

7. Will I have to provide any bodily samples (i.e. blood, saliva)?

You will have to provide saliva samples throughout the study as described earlier, and this will be carried out by appropriately-trained staff in accordance with risk-assessed procedures.

8. How will confidentiality be assured?

All data provided will be completely anonymous, and will not be linked to you in any manner. You will be allocated a participant code that will always be used to identify any data that you provide. Your name or other personal details will not be associated with your data, for example the consent form that you sign will be kept separate from your data.

Only the research team will have access to any identifiable information; paper records will be stored in a locked filing cabinet and electronic information will be stored on a password-protected computer. This will be kept separate from any data and will be treated in accordance with the Data Protection Act.

9. Who will have access to the information that I provide?

Any information and data gathered during this research study will only be available to the research team identified in the information sheet. Should the research be presented or published in any form, then that information will be generalized (i.e. your personal information or data will not be identifiable). Only the research team will have access to any identifiable information; paper records will be stored in a locked filing cabinet and electronic information will be stored on a password-protected computer. This will be kept separate from

any data and will be treated in accordance with the Data Protection Act.

10. How will my information be stored / used in the future?

All information and data gathered during this research will be stored in line with the Data Protection Act. During that time the data may be used by members of the research team only for purposes appropriate to the research question, but at no point will your personal information or data be revealed. Insurance companies and employers will not be given any individual's information, samples, or test results, and nor will we allow access to the police, security services, social services, relatives or lawyers, unless forced to do so by the courts.

11. Has this investigation received appropriate ethical clearance?

Yes, the study and its protocol has received full ethical approval from the Northumbria University School of Life Sciences Ethics Committee.

12. Will I receive any financial rewards / travel expenses for taking part?

Yes. You will receive £150 payable upon full completion of the study for your time. If you withdraw before the end of the study you will receive £25 payment upon completion of the first night, or £75 after the second night.

13. How can I withdraw from the project?

During the study itself, if you do decide that you do not wish to take any further part then please inform one of the research team as soon as possible, and they will facilitate your withdrawal and discuss with you how you would like your data to be treated in the future. After you have completed the research you can still withdraw your data by contacting one of the research team (their contact details are provided in section 14).

If, for any reason, you wish to withdraw your data please contact the investigator within a month of your participation. After this date, it may not be possible to withdraw your individual data as the results may already have been published. As all data are anonymised, your individual data will not be identifiable in any way.

14. If I require further information who should I contact and how?

If you require further information regarding the study, would like to withdraw your data, or to make a complaint you can either contact the principal investigator (Greg Elder - greg.elder@northumbria.ac.uk) or Dr. Jason Ellis (jason.ellis@northumbria.ac.uk)

Appendix Q

Example participant consent forms for feasibility study (laboratory participants) and Study 1

INFORMED CONSENT FORM

Project Title: Measuring the cortisol awakening response in good sleepers within a controlled sleep environment

Principal Investigator: Greg Elder

Participant Number: _____

*please tick
where applicable*

I have read and understood the Participant Information Sheet (*Version 1.2, Feb 2011*)

I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.

I understand I am free to withdraw from the study at any time, without having to give a reason for withdrawing, and without prejudice.

I agree to take part in this study.

I would like to receive feedback on the overall results of the study at the email address given below. I understand that I will not receive individual feedback on my own performance.

Email address.....

Signature of participant..... Date.....

(NAME IN BLOCK LETTERS).....

Signature of Parent / Guardian in the case of a minor

.....

Signature of researcher..... Date.....

(NAME IN BLOCK LETTERS).....

FOR USE WHEN VIDEO/TAPE RECORDINGS WILL BE TAKEN

Project title: Measuring the cortisol awakening response in good sleepers within a controlled sleep environment

Principal Investigator: Greg Elder

Participant Number: _____

I hereby confirm that I give consent for the following recordings to be made:

Recording	Purpose	Consent
Video of bodily movements during sleep	To assess any undiagnosed sleep problems and to monitor your safety overnight	
Sound recordings	To assess any snoring events that occur overnight and to monitor your safety	

Clause A: I understand that other members of the Northumbria Centre for Sleep Research may be exposed to the recording(s) and be asked to provide judgements. The outcome of such judgements will not be conveyed to me unless it is discovered I have a sleep problem that is detrimental to my health. My name or other personal information will never be associated with the recording(s).

Tick the box to indicate your consent to Clause A

Signature of participant..... Date.....

Signature of Parent / Guardian in the case of a minor
 Date.....

Signature of researcher..... Date.....

FOR USE WHEN TISSUE IS BEING REMOVED AND STORED

Project Title: Measuring the cortisol awakening response in good sleepers within a controlled sleep environment

Principal Investigator: Greg Elder

Participant Number: _____

I agree that the following tissue or other bodily material may be taken and used for the study:

Tissue/Bodily material	Purpose	Removal Method
<i>e.g. saliva</i>	<i>e.g. for cortisol analysis</i>	<i>e.g. via Salicaps</i>
Saliva	For cortisol analysis	Saliva collection tubes

I understand that if the material is required for use in any other way than that explained to me, then my consent to this will be specifically sought. I understand that I will not receive specific feedback from any assessment conducted on my samples, but should any kind of abnormality be discovered then the investigator will contact me.

I understand that the University may store this tissue in a Licensed Tissue Bank only for the duration of the study, it will then be destroyed.

Method of disposal:

- Clinical Waste
- Other

If other please specify.....

I consent to the University distributing this tissue to partners in this research study, outside of the University, for further testing (please tick the box if you agree).

Signature of participant..... Date.....

Signature of researcher..... Date.....

Appendix R

Example participant debrief sheet for feasibility study (laboratory participants) and Study 1

PARTICIPANT DEBRIEF

TITLE OF PROJECT: Measuring the cortisol awakening response in good sleepers within a controlled sleep environment

Principal Investigator: Greg Elder

Investigator contact details:

Northumbria Centre for Sleep Research, NB408 Northumberland Building,

Northumbria University NE1 8ST

Email: greg.elder@northumbria.ac.uk

Participant Identification Number: _____

1. What was the purpose of the project?

The purpose of the project was to investigate the pattern of the levels of a specific stress hormone (cortisol) both when you woke up, and over the course of a day. This particular hormone can be detected in saliva. The patterns of this hormone have not been examined in healthy people who have been staying in a sleep laboratory, and the purpose of the project was to develop a way of investigating this hormone so we can test and help develop treatments for individuals with sleep problems.

2. How will I find out about the results?

You can be notified of the results of the study after completion, via email or post, if you provide the researcher with these details. The researcher will email or post a general summary of results will be emailed or posted to you approximately 12 weeks following the completion of the study.

3. Will I receive any individual feedback?

You can receive a general summary of the research findings. This is standard research practice and is to protect your anonymity. You can request to see the information we have however we cannot provide feedback regarding this.

4. What will happen to the information I have provided?

You be allocated a participant number that will always be used to identify any data that you provide, and your name or other personal details will not be associated with your data. The information you have provided will then be combined with the information of other volunteers in order to provide meaningful results. You will not be identified at any point and all data will be completely anonymous.

5. How will the results be disseminated?

It is expected that the results of the study will be disseminated through presentations and publications.

6. Have I been deceived in any way during the project?

No, you have not been deceived in any way during this project.

7. If I change my mind and wish to withdraw the information I have provided, how do I do this?

If you wish to withdraw the information you have provided, please contact the principal investigator at the above postal or email address. All information will then be destroyed.

If you have any concerns or worries concerning the way in which this research has been conducted, or if you have requested, but did not receive feedback from the principal investigator concerning the general outcomes of the study within a few weeks after the study has concluded, then please contact Chair of the School Ethics Committee, Dr Nick Neave via email at nick.neave@northumbria.ac.uk.

Appendix S

Example participant information sheet: Study 2

Version 1.0

May 2012

PARTICIPANT INFORMATION.

TITLE OF PROJECT: Measuring the effects of cognitive demand upon sleep and cortisol.

Participant ID Number:

Principal Investigator: Greg Elder

Investigator contact details:

Northumbria Centre for Sleep Research,
NB408 Northumberland Building,
Northumbria University NE1 8ST
Email: greg.elder@northumbria.ac.uk

This project is funded by: Northumbria University
Number of participant points / payment: 0 / £150

INFORMATION TO POTENTIAL PARTICIPANTS

1. What is the purpose of the project?

The purpose of the project is to investigate the effects of mentally demanding tasks upon sleep, and levels of a specific hormone called cortisol, which plays a role in regulating sleep and wake periods. The study will be conducted in healthy, good sleepers within a sleep laboratory.

2. Why have I been selected to take part?

You are a healthy, non-smoking, individual, aged between 18-40 with a stable sleep-wake cycle, who has indicated that you would like to take part.

3. What will I have to do?

In the two weeks before visiting the sleep laboratory, you will complete a sleep diary providing details including the time you went to bed, how long it took you to get to sleep, and the time at which you woke up. In this period you will also wear an actiwatch (a small device which measures movement, worn like a watch) which will measure your movements. Please try as best as you can to keep the time which you go to bed and the time at which you get up relatively stable during this two-week period.

Your participation in the study will last three days in total, and you will sleep for three nights (Nights 1 – 3) and stay for one full day (from Morning 2 until Night 3) in the sleep laboratory. Each night you will have electrodes applied to your scalp and body to measure various aspects of your sleep. The electrodes are for measurement purposes only and cannot themselves generate any electrical signals, and the equipment that will be attached to you is specially designed to be worn whilst you sleep. Two research staff will remain in the sleep research laboratory at all times and will monitor you sleeping via bedroom CCTV which will record audio

and video for your safety. You will also be asked to complete a booklet of questionnaires on Night 1. If you are female, you will be asked basic questions regarding your menstrual cycle and oral contraceptive use as these can both affect the hormone we are measuring. You will be asked to again wear an actiwatch from your arrival at the sleep laboratory on Night 1, until you complete the study on Morning 3.

You will go to bed at your usual bedtime each night after providing us with a saliva sample, which will be done by chewing on a dental cotton swab for approximately a minute. You will not be allowed to eat or brush your teeth for a period of one hour before each sample when you are in the laboratory as this can interfere with the measurements we are taking. Each morning, you will be woken up at a specified time based on your usual sleeping patterns, and will be asked to provide saliva samples immediately, and 15, 30, 45, 60 and 120 minutes after this. For the first hour after you wake, you will remain in bed in low light and will be allowed to nap between each sample if you wish. During this time, You will be allowed to drink a small amount of water following the first sample if required. You will also be asked to complete a sleep and mood diary before you get out of bed. Once you have completed the sleep and mood diary we will remove any equipment you are wearing and will allow you time to get ready, before we take the final saliva sample (120 minutes after your wake time)

On Morning 1 (the first morning you wake up in the lab) you will be free to leave the sleep laboratory whilst wearing an actiwatch, and you will be asked to return to the sleep laboratory with the actiwatch later that day for your second night.

On Morning 2, you will provide saliva samples in the same manner as on Morning 1. Following this, you will remain in the sleep laboratory for a full day and will be required to complete a range of mentally demanding computerised tests throughout this day. These tests will include measures of attention, decision-making and multi-tasking, and will be explained in full on the second morning. **Your performance will be monitored and it is very important that you perform as well as you possibly can on these tasks.** These tasks will last between 5 and 20 minutes depending on the task which you complete, and you will complete these at hourly intervals until late evening/night-time. We will also measure your mood before and after each task, and we will also ask you the amount of effort you put into each task. You will also be required to provide additional saliva samples at several time points on this day.

On Morning 3, you will provide saliva samples in the same way as on the previous two mornings. You will then receive a full debrief and you will then be free to leave the sleep laboratory.

Please note that you will not be allowed to have any electrical equipment in your bedroom with you at any time during your stay, as this may interfere with our equipment.

4. What are the exclusion criteria (i.e. are there any reasons why I should not take part)?

You will be unable to take part in the study if you are currently a shift worker, as this may mean you have an unstable sleep/wake cycle. You also cannot take part if you are suffering from any sleep disorders (e.g. jet lag or insomnia), if you have taken a trans-meridian flight within the previous three months, if you have any uncontrolled illnesses, or if you suffer from any disorders including depression or hormonal disorders. **You cannot take part in this study if you currently smoke**, as this can affect the measurements we are taking. You cannot take part if you are under 18 or

are over 40 years of age, and you may be unable to take part in this study if you have previously taken part in a study within the Northumbria Centre for Sleep Research.

5. Will my participation involve any physical discomfort?

The sleep laboratory is designed to be a comfortable environment very similar to your home environment. Whilst you are unlikely to suffer any physical discomfort, the application of electrode conducting gel may cause minor irritation. You will be able to withdraw from the study at any time by informing the researcher.

6. Will my participation involve any psychological discomfort or embarrassment?

Your participation is unlikely to involve any psychological discomfort or embarrassment. It is possible that you may find your first night in the sleep laboratory to be unusual.

7. Will I have to provide any bodily samples (i.e. blood, saliva)?

You will have to provide saliva samples throughout the study as described earlier, and this will be carried out by appropriately-trained staff in accordance with risk-assessed procedures.

8. How will confidentiality be assured?

All data provided will be completely anonymous, and will not be linked to you in any manner. You will be allocated a participant code that will always be used to identify any data that you provide. Your name or other personal details will not be associated with your data, for example the consent form that you sign will be kept separate from your data.

Only the research team will have access to any identifiable information; paper records will be stored in a locked filing cabinet and electronic information will be stored on a password-protected computer. This will be kept separate from any data and will be treated in accordance with the Data Protection Act.

9. Who will have access to the information that I provide?

Any information and data gathered during this research study will only be available to the research team identified in the information sheet. Should the research be presented or published in any form, then that information will be generalized (i.e. your personal information or data will not be identifiable). Only the research team will have access to any identifiable information; paper records will be stored in a locked filing cabinet and electronic information will be stored on a password-protected computer. This will be kept separate from any data and will be treated in accordance with the Data Protection Act.

10. How will my information be stored / used in the future?

All information and data gathered during this research will be stored in line with the Data Protection Act. During that time the data may be used by members of the research team only for purposes appropriate to the research question, but at no point will your personal information or data be revealed. Insurance companies and employers will not be given any individual's information, samples, or test results, and nor will we allow access to the police, security services, social services, relatives or lawyers, unless forced to do so by the courts.

11. Has this investigation received appropriate ethical clearance?

Yes, the study and its protocol have received full ethical approval from the

12. Will I receive any financial rewards / travel expenses for taking part?

Yes. You will receive £150 payable upon full completion of the study for your time. If you withdraw before the end of the study you will receive £25 payment upon completion of the first night, or £75 after the second night. Additionally, you will have the chance to win an iPad if you achieve the highest score on a randomly-selected task, taken from the set of cognitive tasks you will be required to complete following your second morning in the sleep laboratory.

13. How can I withdraw from the project?

During the study itself, if you do decide that you do not wish to take any further part then please inform one of the research team as soon as possible, and they will facilitate your withdrawal and discuss with you how you would like your data to be treated in the future. After you have completed the research you can still withdraw your data by contacting one of the research team (their contact details are provided in section 14).

If, for any reason, you wish to withdraw your data please contact the investigator within a month of your participation. After this date, it may not be possible to withdraw your individual data as the results may already have been published. As all data is anonymous, your individual data will not be identifiable in any way.

14. If I require further information who should I contact and how?

If you require further information regarding the study, would like to withdraw your data, or to make a complaint you can either contact the principal investigator (Greg Elder - greg.elder@northumbria.ac.uk) or Dr. Jason Ellis (jason.ellis@northumbria.ac.uk)

Appendix T

Participant consent forms: Study 2

INFORMED CONSENT FORM

Project Title: Measuring the effects of cognitive demand upon sleep and cortisol.

Principal Investigator: Greg Elder

Participant Number: _____

*please tick
where applicable*

- I have read and understood the Participant Information Sheet (*Version 1.0, May 2012*)
- I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.
- I understand I am free to withdraw from the study at any time, without having to give a reason for withdrawing, and without prejudice.
- I agree to take part in this study.
- I would like to receive feedback on the overall results of the study at the email address given below. I understand that I will not receive individual feedback on my own performance.

Email address.....

Signature of participant.....	Date.....
(NAME IN BLOCK LETTERS).....	
Signature of Parent / Guardian in the case of a minor	
.....	

Signature of researcher.....	Date.....
(NAME IN BLOCK LETTERS).....	

FOR USE WHEN VIDEO/TAPE RECORDINGS WILL BE TAKEN

Project title: Measuring the effects of cognitive demand upon sleep and cortisol.

Principal Investigator: Greg Elder

Participant Number: _____

I hereby confirm that I give consent for the following recordings to be made:

Recording	Purpose	Consent
Video of bodily movements during sleep	To assess any undiagnosed sleep problems and to monitor your safety overnight	
Sound recordings	To assess any snoring events that occur overnight and to monitor your safety	

Clause A: I understand that other members of the Northumbria Centre for Sleep Research may be exposed to the recording(s) and be asked to provide judgements. The outcome of such judgements will not be conveyed to me unless it is discovered I have a sleep problem that is detrimental to my health. My name or other personal information will never be associated with the recording(s).

Tick the box to indicate your consent to Clause A

Signature of participant..... Date.....

Signature of Parent / Guardian in the case of a minor
 Date.....

Signature of researcher..... Date.....

FOR USE WHEN TISSUE IS BEING REMOVED AND STORED

Project Title: Measuring the effects of cognitive demand upon sleep and cortisol.

Principal Investigator: Greg Elder

Participant Number: _____

I agree that the following tissue or other bodily material may be taken and used for the study:

Tissue/Bodily material	Purpose	Removal Method
<i>e.g. saliva</i>	<i>e.g. for cortisol analysis</i>	<i>e.g. via Salicaps</i>
Saliva	For cortisol analysis	Salivettes

I understand that if the material is required for use in any other way than that explained to me, then my consent to this will be specifically sought. I understand that I will not receive specific feedback from any assessment conducted on my samples, but should any kind of abnormality be discovered then the investigator will contact me.

I understand that the University may store this tissue in a Licensed Tissue Bank only for the duration of the study, it will then be destroyed.

Method of disposal:

Clinical Waste

Other

If other please specify.....

I consent to the University distributing this tissue to partners in this research study, outside of the University, for further testing (please tick the box if you agree).

Signature of participant..... Date.....

Signature of researcher..... Date.....

ACTIWATCH SIGN OUT / ACTIWATCH RETURN SHEET

Project title: Measuring the effects of cognitive demand upon sleep and cortisol.

Principal Investigator: Greg Elder

Participant Number:

Actiwatch serial number:

Participant contact number:

I understand that I am responsible for the actiwatch at all times and may have to replace it if it is lost or damaged whilst in my possession.



Actiwatch signed out:

Signature of participant: Date.....

Signature of researcher:..... Date.....

Actiwatch returned:

Signature of participant:
Date.....

Signature of researcher:..... Date.....

Driving Disclaimer.

Dear Participant,

Due to the nature of the research we request that for your safety you do not drive or operate heavy machinery after leaving the Northumbria Centre for Sleep Research. As you have slept in an environment that is not your usual, please be aware that although you may feel fine, there is a risk you could become drowsy.

Due to this we advise you to either get public transport to your next destination, and if this is not suitable we will be able to arrange a Taxi for you. Therefore the Northumbria Centre for Sleep Research associates will accept no responsibility should you choose to drive or do an activity that may pose a safety risk if your performance is impaired by drowsiness.

Thank You

Northumbria Centre for Sleep Research.

Participant name: _____

I have read and understood the above information: Signed:

_____ Date ____/____/____

Appendix U

Participant debrief form – Study 2

PARTICIPANT DEBRIEF

TITLE OF PROJECT: Measuring the effects of cognitive demand upon sleep and cortisol.

Principal Investigator: Greg Elder

Investigator contact details:
Northumbria Centre for Sleep Research,
NB408 Northumberland Building,
Northumbria University NE1 8ST
Email: greg.elder@northumbria.ac.uk

Participant Identification Number: _____

1. What was the purpose of the project?

The purpose of the project was to investigate how both the experience of cognitive demand and the anticipation of forthcoming demand can affect both sleep and the levels of cortisol, a stress hormone which can be detected in the saliva samples you provided.

Half of the participants in this study will have completed the cognitive tasks described to you in the start of the study, allowing us to examine how the effects of realistic cognitive demands can affect your sleep and cortisol. The other half of participants who took part in this study will have been told on the day of the cognitive testing that they were no longer required to complete any of the tests. This will allow us to examine sleep and cortisol in those who anticipated completing the tasks, but did not actually do any of them.

All the sleep and cortisol measurements from this study will be compared to those from an earlier study in which people slept normally, allowing us to investigate how the expectation of demands may affect sleep quality.

2. How will I find out about the results?

Following completion of the study, the researcher will provide participants with a detailed explanation of the study and an explanation of the results. This will be sent to you by email approximately 12 weeks following the completion of the study.

3. Will I receive any individual feedback?

Individual feedback can be provided upon request, but this may not include any interpretation of scores.

4. What will happen to the information I have provided?

You are allocated a participant number that will always be used to identify any data that you provide, and your name or other personal details will not be associated with your data. The information you have provided will then be combined with the information of other volunteers in order to provide meaningful results. You will not be identified at any point and all data will be completely anonymous.

5. How will the results be disseminated?

It is expected that the results of the study will be disseminated through presentations and publications.

6. Have I been deceived in any way during the project?

Yes. As explained in section 1, only half of the participants in this study will have completed the cognitive tasks which you were told you would have to complete in the study, and the remaining half will have been told on the day that they were not required to complete any of the tasks. This allows us to examine the effects of cognitive demand caused by completing the computerised tasks, and the anticipation of the demand by believing that you were going to complete the tasks, have upon your sleep and on your cortisol levels.

If you completed the cognitive testing, you will have been informed that your performance would be closely monitored and you would have the chance to win an iPad if you achieved the highest score on a randomly-selected cognitive task. This is not the case as your performance will not be examined, but it was necessary for us to make sure that you were highly motivated throughout the day. If you did not complete the cognitive testing, you will have had these reasons explained to you at the start of your full day.

To ensure fairness, **all participants who took part in this study will be placed into a prize draw for an iPad**, which will be drawn randomly by an independent party when the study is complete.

7. If I change my mind and wish to withdraw the information I have provided, how do I do this?

If you wish to withdraw the information you have provided, please contact the principal investigator at the above postal or email address. All information will then be destroyed.

If you have any concerns or worries concerning the way in which this research has been conducted, or if you have requested, but did not receive feedback from the principal investigator concerning the general outcomes of the study within a few weeks after the study has concluded, then please contact Chair of the School Ethics Committee, Dr Nick Neave via email at nick.neave@northumbria.ac.uk.