Sleep and sleep timing in relation to light and emotional processing

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Abstract

Sleep is a complex process: the timing of sleep is regulated by two systems (the sleep homeostat and the circadian clock) and there are many potential functions of sleep. The aim of this thesis was to investigate: the impact of light on the regulation of sleep timing (study 1) and the role of sleep in emotional processing (study 2).

Study 1 used natural variations in environmental light levels at different geographical locations, to examine the influence of daily light irradiance on sleep timing and chronotype using the Munich chronotype questionnaire (MCTQ). 6443 students were included in this study from six universities from the northern and southern hemispheres. Students in southern hemisphere cities had earlier sleep timings than those in the northern cities. Daily irradiance was higher in the southern hemisphere cities. The amount of time spent outside, age and sex, but not daily irradiance, influenced sleep timings.

Study 2 explored the impact of an analogue traumatic event (trauma film) in students who were either sleep deprived or not sleep deprived on intrusive memories ("flashbacks"), sleep physiology and the impact of an increased risk of a mood disorder on this relationship. In this study the sleep deprived participants (n=19) reported fewer intrusive memories to the trauma film than those not sleep deprived (n=22). A change in sleep physiology was observed in the first sleep period following the trauma film, which was more pronounced in the sleep deprived group: increased levels of arousal, REM density and activity in the occipital region. Only three participants at-risk of a mood disorder completed study 2: their data are presented as case studies.

In conclusion this research has demonstrated that differences in sleep timings exist between cities in the southern and northern hemispheres and has confirmed that many factors can influence sleep timing. It has also been demonstrated that following a highly emotional event not sleeping may have a beneficial effect, which has implications for the treatment of people after trauma.

Glossary

The explanation for abbreviations and terminology used in this thesis.

Acrophase: peak of biological (e.g. circadian) rhythm

Actiwatch: device used to measure wrist movement

Activity VAS: Visual Analogue Scale used to assess time spent doing visual-spatial and verbal activities

aMT6: 6-hydroxymelatonin sulphate: marker for melatonin

Analogue traumatic event: trauma film

ASD: Acute Stress Disorder

Bonferroni correction: statistical correction for multiple testing: 0.05/alpha, where alpha is the number of tests

CAR: Cortisol Awakening Response

Chronotype: individual variation in the timing (phase) of the circadian clock

Circadian clock: internal timing keeping system

Circadian rhythm: biological rhythm that is driven by the circadian clock to around 24 hours.

Dissociation: a state of detachment from time, space and the self

Diurnal mood: change in mood over the course of a day

DSS: Dissociative State Scale

EEG: electroencephalography

Emotional bias: tendency to view object or situation in an either positive or negative light

Emotional processing: a collection of processes that enable an emotional event to be perceived, understood, remembered and responded to

Entrainment: synchronisation of the circadian clock with the external environment

EPQ: Eysenck Personality Questionnaire

Explicit memory: memory relating to the facts and details of an event

Familial risk: risk associated with genetic predisposition inherited from parents

FH: Family History

fMRI: functional Magnetic Resonance Imaging.

GHQ: General Health Questionnaire

HPA axis: Hypothalamic-Pituitary-Adrenal axis

HQ: Health Questionnaire

IES: Impact of Event Scale

Implicit memory: memory relating to emotions and sensations experienced to event

Intrusive memories/ intrusions: autobiographical memories that are involuntary recalled

Irradiance: power of electromagnetic radiation per unit area (W/m^2)

L5: five hours of least activity

M10: ten hours of most activity

MCTQ: Munich ChronoType Questionnaire

MDQ: Mood Disorder Questionnaire

Mental imagery: the perception of sensory information without the input of external stimuli

MEQ: Morningness-Eveningness Questionnaire

MINI: Mini International Neuropsychological Interview

Mood disorder: range of conditions including depression and bipolar disorder

Mood VAS: Visual Analogue Scale for mood

mPFC: medial PreFrontal Cortex

MSFsc: Mid-Sleep point of Free days, Sleep Corrected

nFH: no Family History

NREM sleep: Non Rapid Eye Movement sleep

PANAS: Positive And Negative Affect Scale

Period: length of one full cycle of a biological rhythm

Peritraumatic: occurring during or immediately following a traumatic event

POMS: Profile Of Mood State

Post-partum: after pregnancy

Pre-partum: during pregnancy

PSG: polysomnography

PSQI: Pittsburgh Sleep Quality Index

PTSD: Post-Traumatic Stress Disorder

RAT: Remote Associates Task

REM density: frequency of rapid eye movements during REM sleep

REM sleep: Rapid Eye Movement sleep

SCN: SupraChiasmatic Nucleus

SJL: Social Jet Lag

Sleep homeostat: increased sleep need with continued wake

STAI: State Trait Anxiety Index

Stressor: Stressful event created in study two using the RAT

SWA: Slow Wave Activity

SWS: Slow Wave Sleep

Trauma film: method used to create analogue traumatic event in study two.

YLDs: Years Lived with Disability

Zeitgeber: time giver: environmental signal for entrainment of the circadian clock

1. Background

Sleep is a complex behaviour that requires definition. Although periods of inactivity are observed in most forms of life including plants and unicellular organisms (Siegel 2011), 'true' sleep has only been recorded in less than 50 vertebrates including humans (Siegel 2008). This 'true' sleep is defined as a reversible state of immobility with reduced responsiveness to external sensory input. Moreover, sleep is homeostatically regulated, meaning that the loss of sleep is compensated for in the following sleep period (Siegel 2008). It is this form of sleep ('true sleep') that will be considered in this thesis. The regulation of sleep involves the interaction of multiple brain regions and neurophysiological, and neurochemical, pathways, which control the timing, duration and structure of sleep. The function of sleep however is less well understood. At present there are a number of theories for the function of sleep including cellular restoration, energy conversation and memory consolidation (Lockley and Foster 2012, Mignot 2008). This thesis focuses on two aspects of sleep: i) the impact of light on the regulation of sleep timing and ii) the role of sleep in the regulation of emotional processing. **Figure 1.1** illustrates these aspects of sleep. The aim of this chapter is to provide: 1) a brief overview of sleep, 2) a discussion of the involvement of the circadian clock and light in the regulation of sleep timing, 3) an introduction to the role of sleep in emotional processing, with particular emphasis on intrusive memories and 4) to detail the possible impact of mood disorders on the relationship between sleep and emotional processing.

1.1. Sleep

Sleep is a highly structured process: a progression through five stages, starting with the four stages of non-rapid eye movement (NREM) sleep (stage 1, 2, 3 and 4) and followed by rapid eye movement (REM) sleep. This progression usually cycles over a 90-120 minute period, four to five times a night (**figure 1.2**) (Lockley and Foster 2012).

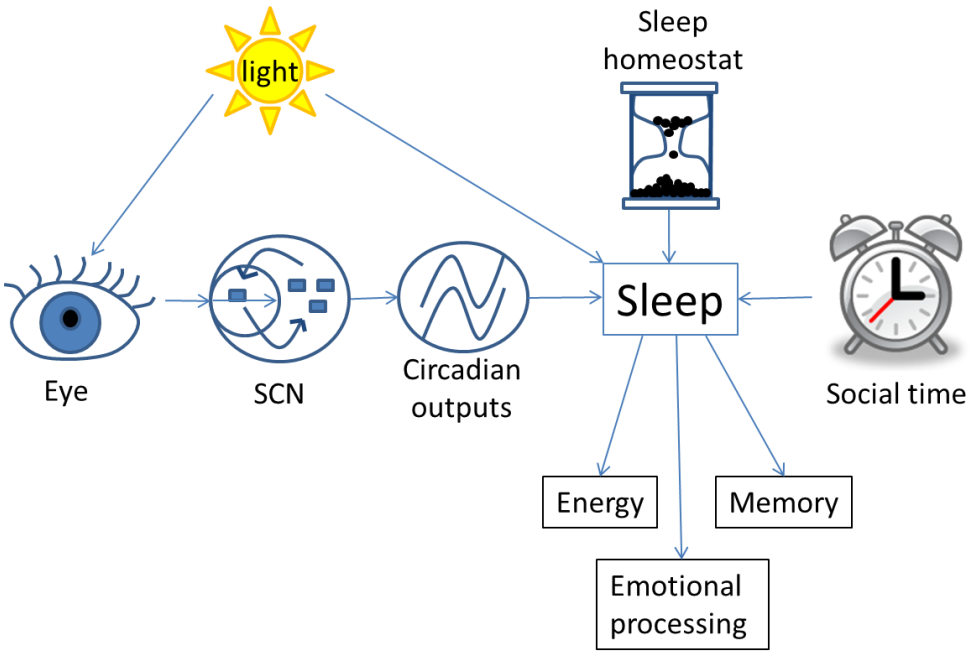


Figure 1.1 Sleep regulation and function: a representation of how the factors involved in the regulation of sleep timing and the functions of sleep that are relevant to this thesis are integrated. SCN: Suprachiasmatic nucleus.

During the night the composition of these cycles shifts from being dominated by slow wave sleep (SWS: stages 3 and 4 of NREM sleep) at the beginning of the night, towards a greater proportion of REM sleep in the latter half of the night. REM sleep is characterised by not only rapid eye movements, but also temporary motor paralysis which prevents dreams that predominately occur during REM sleep being acted out (Hobson 2009). In terms of brain activity, REM sleep is the most active sleep state and the most wake-like sleep state (Hobson 2009). NREM sleep by contrast is the most restorative sleep state and is associated with the quality of sleep, where by the more SWS the better subjective sleep quality is reported to be (Akerstedt et al. 1997). NREM sleep is characterised by slow wave activity (delta waves) that increases with increasing sleep depth: at least 20% of a sleep epoch must be slow wave activity to be classified as slow wave sleep (Dijk 2009).

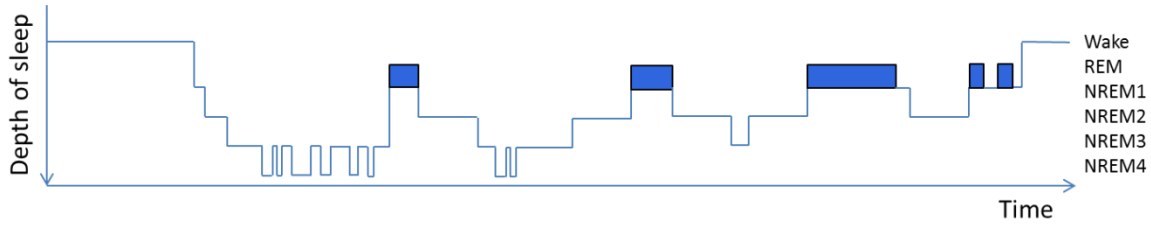


Figure 1.2 Sleep hypnogram. A representation of a typical sleep period for a healthy adult. The sleep stages are seen to progress over the course of the sleep period, where more slow wave sleep (NREM 3 and 4) is seen at the beginning of the night, and more REM sleep at the end of the night. NREM1=stage 1 sleep, NREM2 = stage 2 sleep, NREM3 = stage 3 sleep, NREM4 = stage 4 sleep.

These slow waves are synchronised oscillations of brain activity that spread from a definite point (usually in the prefrontal or orbitofrontal regions) through the cortex (Massimini et al. 2004), and are thought to connect different networks in the cortical region of the brain (Dijk 2009).

The precise structure of sleep is highly variable between individuals (Tucker et al. 2007). However, within a healthy individual sleep structure tends to be highly stable, and specific wave forms recorded using electroencephalography (EEG) show trait-like characteristics. Sleep spindles, are short bursts of high frequency waves in the 12-14 Hz range found in stage 2 NREM sleep. Despite showing different characteristics between individuals sleep spindles are highly consistent within an individual (De Gennaro and Ferrara 2003). A highly stable profile of EEG events within the 8-16 Hz frequency range has also been shown to be unique to an individual. This 'EEG fingerprint' is seen within NREM sleep and allows discrimination between individuals in 92% of comparisons (De Gennaro et al. 2005). Recently De Gennaro and colleagues (De Gennaro et al. 2008) have shown that this trait is highly heritable (96%) from a study of monozygotic and dizygotic twins. In fact this 'EEG fingerprint' has been shown to be one of the most heritable human traits (De Gennaro et al. 2008).

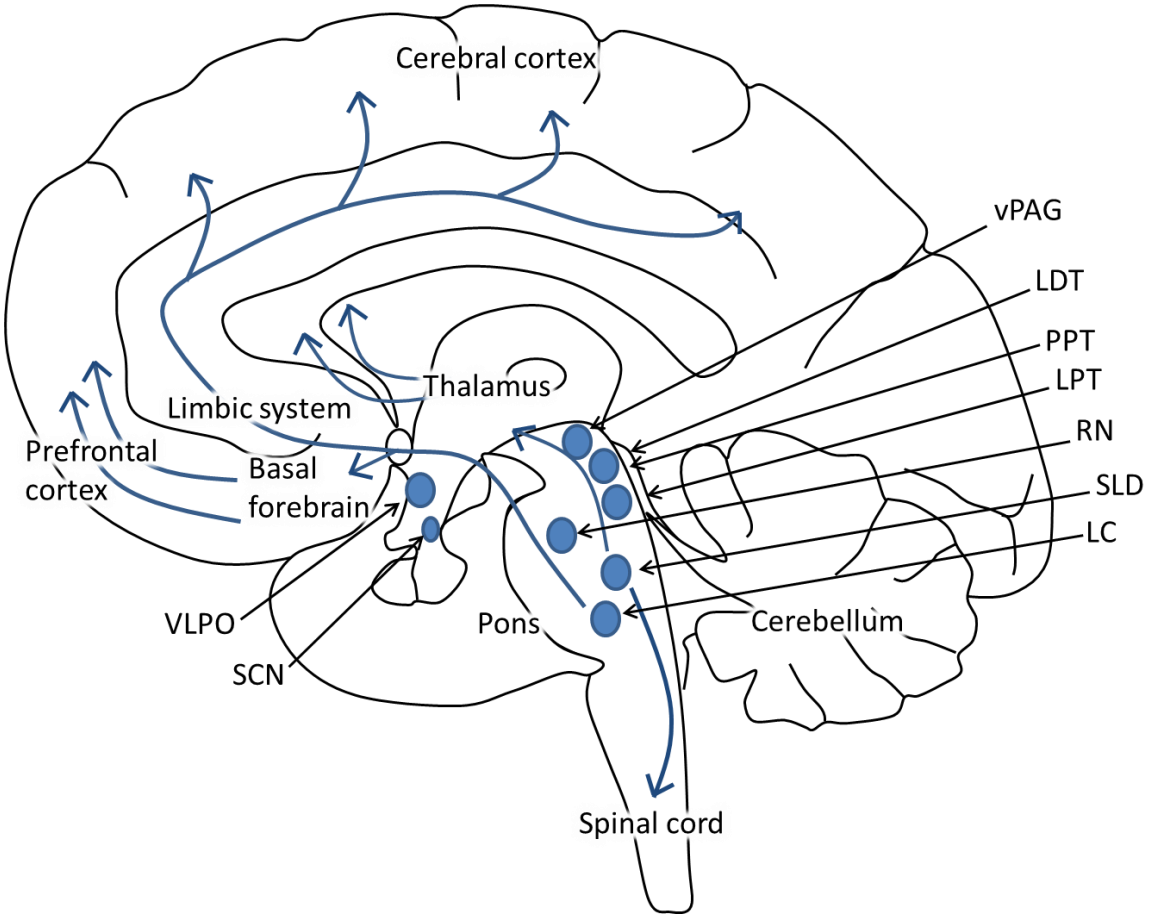


Figure 1.3 Brain regions involved in the sleep-wake cycle. This is a representation of some of the brain regions involved in sleep-wake cycle. The arrows depict the arousal pathway (Datta and Maclean 2007). Abbreviations of the brain regions: SCN = supachiasmatic nucleus; VLPO = ventrolateral preoptic nucleus; vPAG = vental periaqueductal grey; LDT = laterodorsal tegmental nuclei; PPT = pedunculopontine tegmental nucleus; LPT = lateral pontine tegmentum; RN = raphe nucleus; SLD = sublaterodorsal nucleus; LC = locus coeruleus. Adapted from (Lockley and Foster 2012).

The induction of sleep is controlled by multiple neurophysiological and neurochemical pathways. However, sleep cannot be considered in isolation but rather in the context of the balance between sleep and wake. **Figure 1.3** shows some of the main brain areas involved in the regulation of the sleep-wake cycle.

Wakefulness is sustained by the arousal system which is centred on projections from the brain stem, hypothalamus and basal forebrain via the thalamus to the cerebral cortex (Datta and Maclean 2007, McCarley 2007). The progression of wake to NREM sleep involves the inhibition of

the arousal system via GABAergic and galaninergic signalling from the ventrolateral preoptic nucleus (Datta and Maclean 2007, McCarley 2007). The subsequent transition from NREM to REM sleep involves the cessation of monoamingeric signalling and an increase in cholingeric signalling (Datta and Maclean 2007, McCarley 2007). Regulation of this transition has been proposed to be under the control of a 'flip-flop' switch, where reciprocal inhibitory GABAergic signalling between REM-on and REM-off areas of the mesopontine tegmentum within the brainstem results in REM sleep being either on or off (Lu et al. 2006).

1.2. Sleep timing

The current model for the regulation of sleep involves the sleep homeostat (process S) and the circadian clock (process C) (Borbely 1982, Borbély 2009, Borbely and Achermann 1999). These two systems interact to control the timing, duration and structure of sleep. The model is represented in **figure 1.4**. The circadian clock regulates the timing of arousal levels, which in a healthy adult are highest during the day and lowest at night. In addition, the sleep homeostat increases sleep pressure, i.e. the need for sleep, with continued wakefulness. Hence the alignment of these two systems; low levels of arousal and high levels of sleep pressure, creates an environment conducive for sleep, which normally occurs in the late evening.

The sleep homeostat has an intuitive effect on sleep regulation: the longer spent awake the higher sleep pressure and the more likely sleep will occur. Once asleep sleep pressure gradually dissipates the longer spent asleep. To date, the best candidate for the mechanism underlying the sleep homeostat involves adenosine. Both adenosine and its agonists increase sleep and enhance slow wave activity, while adenosine antagonists, including caffeine, increase time spent awake (Basheer et al. 2004). Moreover adenosine accumulates during the day, as it is realised when energy is used i.e. the breakdown of adenosine triphosphate (ATP) (Benington and Heller 1995, Benington et al. 1995).

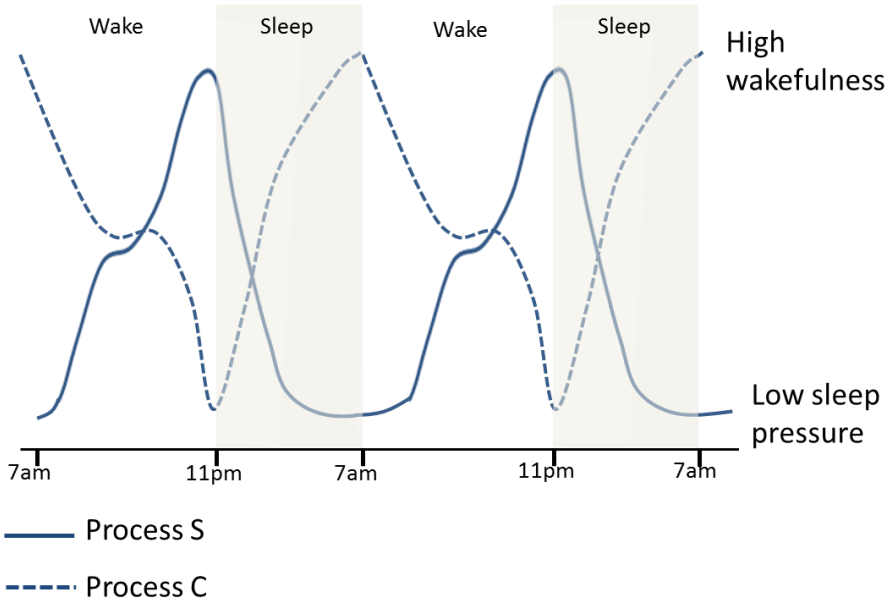


Figure 1.4 Two process model for the regulation of sleep timing. The two processes interact with the circadian clock (process C) causing in a decrease in arousal during wake which dissipates during sleep, and the sleep homeostat (process S) producing an increase in the need for sleep during continued wake which reduces with sleep. Adapted from Katharina Wulff (Personal communication).

Therefore it is possible that the increasing levels of adenosine during wake act as a signal for sleep onset. In contrast, the circadian clock is a much more complicated system and is involved in more than just the regulation of sleep timing.

1.2.1. The circadian clock

The circadian clock is an internal time keeping system that runs with a cycle of about 24 hours (circa diem – about a day). This system allows not only our internal system to run at the same pace as the daily environmental changes, but also to anticipate these changes to ensure the most appropriate behaviours occur at the most appropriate times of day. The clock keeps time via an auto-regulatory series of feedback loops controlling the transcription of a number of core 'clock' genes. The full cycle of which runs to around 24 hours. Although a circadian clock has been found in some form in all eukaryotes and some prokaryotes (Foster et al. 2005), there are differences in the genes involved and the precise feedback loops that operate. The remainder of this thesis will

focus on the circadian clock of mammals. Within mammals, including humans, the highly conserved core clock genes are focused on the regulation of the *Per* and *Cry* genes by the transcriptional regulators BMAL1 and CLOCK (Shearman et al. 2000). This highly conserved group of genes produce a molecular cycle of gene expression, protein synthesis, and protein degradation that takes around 24 hours to complete. This molecular clock is found in most cell types, which within an organ synchronise to ensure the coordination of the tissue. These peripheral clocks are in turn synchronised by the central master clock to keep the whole body running to the same time (Reppert and Weaver 2002). The master clock is situated in the suprachiasmatic nucleus (*SCN*), a small region (approximately 20,000 neurons) of the anterior hypothalamus (Ralph et al. 1990).

The circadian clock drives the cyclical change of many processes including blood pressure, core body temperature, metabolism, hormonal synthesis and release, and even the timing of the cell cycle (Foster et al. 2005). More complex processes are also influenced by the clock including mood and cognition as well as the sleep-wake cycle (Monk et al. 1997, Bjerner et al. 1955). Importantly these processes retain their cyclical change under constant conditions, indicating that they are being driven by the internal clock, rather than the environmental factors that the circadian clock is synchronised to. The majority of biological rhythms are circadian i.e. cycle over 24 hours, however, there are also processes that cycle over shorter (ultradian) or longer (circannual: yearly) periods, for example REM sleep (Czeisler et al. 1980) and reproduction in some birds (Gwinner 2003) respectively. Although the circadian clock drives these biological rhythms they can of course be influenced by other factors. For example, although the sleep-wake cycle is driven by the circadian clock, social and work commitments can greatly influence when someone goes to bed. The effect of these factors must be taken in to account when measuring a circadian rhythm: in this example work and social commitments must be removed to determine the underlying circadian influence on the sleep wake cycle. In humans there are two experimental designs used to separate the influence of these external factors. Firstly a constant routine can be

used, where the participants are kept in a unchanging situation in terms of light, temperature, body position and calorific intake thereby removing the cyclical change of these external factors (Wirz-Justice 2007). Alternatively a forced desynchrony protocol can be used which involves the separation of the circadian clock from the external factors, by making the environmental period either much longer or shorter than the clock, e.g. 20 or 28 hours (Wirz-Justice 2007). These protocols set the 'gold standard' for the measurement of human circadian rhythms. However, these techniques are intensive for both the participants and researchers, therefore more often robust circadian rhythms are measured under normal conditions to give an approximation of the core circadian clock. Both core body temperature and melatonin release provide a robust rhythm and a good marker for the circadian clock (Wirz-Justice 2007, Lewy et al. 1999). Both these rhythms are also influenced by external factors e.g. exercise and light respectively, however these can both be easily manipulated to assess the underlying rhythm.

Although the circadian clock is very good at keeping time to a 24 hour cycle, environmental conditions also change day by day. To keep up with these changes the clock uses a number of environmental signals (zeitgebers – time givers) to modify the phase of the internal clock so that it matches the external phase. This process is known as entrainment. The most important zeitgeber, particularly for humans, is light. Not only does it provide daily information, with light intensity and wavelength changing over the day, but also seasonal information as day length changes over the course of the year. The only exception for the latter is at the equator where day length is relatively stable over the year, changing by only a few minutes. Light does however still provide daily information at the equator.

To detect these changes in light the circadian system uses photosensitive cells, which in mammals and humans are found in the eye (Foster 1998). In addition to the photosensitive cells used for vision (rods and cones) an additional cell type is involved in the perception of light (Freedman et al. 1999, Lucas et al. 1999). These cells are photosensitive retinal ganglion cells and contain the

photopigment melanopsin (Berson et al. 2002, Sekaran et al. 2003). Melanopsin is a vitamin A derived molecule that is maximally sensitive to blue light with a wavelength of around 480nm (Lucas et al. 2001, Thapan et al. 2001, Hankins et al. 2008, Zaidi et al. 2007). Although the visual photoreceptors (rods and cones) are thought to play a role in light perception required for entrainment of the clock, photosensitive retinal ganglion cells are sufficient for this role: animals lacking both rods and cones, but with functioning photosensitive retinal ganglion cells, are still able to entrain to a light/dark cycle despite being visually blind (Freedman et al. 1999). Moreover humans who are blind, but still have their eyes exhibit strong circadian rhythms compared with those who have physically lost both eyes (Lockley et al. 1997, Skene et al. 1999, Zaidi et al. 2007). In summary the circadian clock provides a robust time keeping system that can remain synchronised to the external environment to enable the changing conditions to be anticipated ensuring that bodily functions occur at the most appropriate time of day.

In humans the core molecular clock does not run exactly to 24 hours and it runs at slightly different lengths in different individuals. In humans, under laboratory conditions, the length (period) of the clock has been reported to range from 23.47-24.64 hours (Carskadon et al. 1999, Czeisler et al. 1999, Wright et al. 2001, Koorengevel et al. 2002). This difference in period length results in variations in the timing of both core processes and behaviour, not least the sleep/wake cycle, giving rise to a subdivision of individuals into early, late and intermediate types. These subdivisions are known as chronotypes, where colloquially early types are known as larks, while late types are known as owls. The timing of sleep is the most easily recognised behaviour influenced by chronotype: early types prefer to go to bed early and wake up early compared with late types, who prefer to go to bed late and wake up late. Sleep timings can be used as a marker of chronotype, as long as external factors such as work and social commitments are taken into account. Moreover chronotype can be used as an indicator of sleep timings: individuals that have a late chronotype are likely to go to bed and wake up late. Other behaviours are also influenced by chronotype such as mood and cognitive performance, for example early types tend to have

higher levels of positive affect than late types (Biss and Hasher 2012) and perform better in the morning compared with late types (Clarisse et al. 2010) .

A number of factors influence chronotype, including age, sex and genetic background. In terms of age, chronotype is fairly dynamic within an individual. During adolescence an individual tends to have a later and later chronotype, which is observed as teenagers go to bed later and later. The progression tends to reach its peak in early adulthood (19-21 years old) which is a time of life that individuals tend to have their latest bed times. From then on chronotype becomes earlier again, with individuals tending to go to bed earlier and earlier (Duffy and Czeisler 2002, Dijk et al. 2000, Roenneberg et al. 2003). Sex differences have also been reported, although not consistently, with men tending to have a later chronotype than women (Randler and Diaz-Morales 2007, Roenneberg et al. 2004, Adan and Natale 2002). However this difference between men and women is not observed at all ages, perhaps explaining some of the inconsistencies in the literature (Roenneberg et al. 2007a). Unsurprisingly, considering the robust conservation of the core genes that make up the molecular clock, there are many genetic polymorphisms associated with different chronotypes (Archer et al. 2003, Toh et al. 2001, Archer et al. 2010), which means that the same chronotype often runs in families.

Geographical location also influences chronotype, whereby the further east (within the same time zone) the earlier sleep timings on days free from social or work commitments, hence chronotype is also likely to be earlier (Roenneberg et al. 2007b). Since light is the most important zeitgeber for the entrainment of the human circadian clock, it is thought that the effect of geographical location is due to the different timings and intensity of light experienced at the different geographical locations. Although to date this has never been directly tested in the field, there is some evidence, which will be disused below, that different aspects of light can influence the circadian clock above and beyond entrainment to the light/dark cycle.

1.2.1.1. Chronotype, sleep timing and light

The timing, duration, intensity and wavelength of light alter how light affects the circadian clock (Duffy and Czeisler 2009). The human circadian clock is maximally sensitive to light during the biological night (Czeisler et al. 1989, Jewett et al. 1997). Light presented in the late biological night i.e. early in the morning, causes a phase advance: shifts the clock to an earlier time. If presented in the early biological night i.e. in the evening, light causes a phase delay: shifts the clock to a later time. This in turn alters the clock's outputs, for example in terms of sleep timings: a phase advance results in going to bed earlier and a phase delay results in going to bed later. The extent to which light advances or delays the clock at different times of the night and day gives rise to a phase response curve. The human phase response curve is reproduced in **figure 1.5** from Khalsa and colleagues (Khalsa et al. 2003). Humans were found to be sensitive to light throughout the biological day, therefore lacking a 'dead zone', a period when no phase advances or delays are observed (Jewett et al. 1997).

In humans the intensity of light has been found to have differing effects when given at the same time of day. In a series of experiments conducted by Duffy, Czeisler and colleagues (Duffy and Czeisler 2009), high light levels (180-9500lux) presented early in the biological day resulted in a phase advance of the clock while dim light (12 lux) or darkness resulted in phase delays. This indicates that it is not only the time of day at which light is given that is important for entraining the circadian clock but also the intensity of that light. However, in these studies light was presented over a relatively short period of time (5-6.5 hours), and the participants were kept in dim light or darkness for an extensive period preceding the light stimulus. Furthermore these studies only focused on the short-term influence of these light exposure periods on circadian physiology. Hence extrapolation of these finding to the effect of environmental light levels on chronotype is difficult.

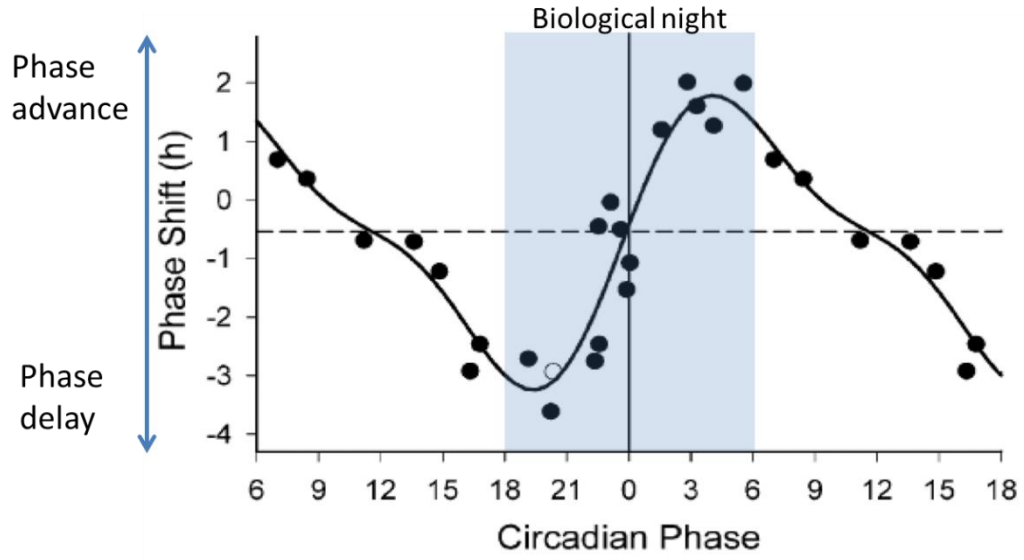


Figure 1.5 The human phase response curve. The phase shifts of the circadian clock due to 6.5 hour periods of bright light, determined by assaying plasma melatonin concentrations (Khalsa et al. 2003). Phase delays are represented as a negative phase shift. Figure adapted from Khalsa and colleagues (Khalsa et al. 2003).

In terms of naturalistic studies very few studies have been conducted to date. Over the course of one week, Martinez-Nicolas and colleagues (Martinez-Nicolas et al. 2011) measured body temperature at the wrist, light exposure and sleep in a group of university students. A correlation was reported between higher levels of light exposure in the morning and a phase advance of the circadian rhythm of body temperature, which gives an indication of the effect of light on the circadian clock in the short-term. The impact of light on chronotype, rather than short-term circadian plasticity, has been addressed in a large scale survey of chronotype conducted by Ronnenberg and Merrow (Roenneberg and Merrow 2007) using the Munich chronotype questionnaire (MCTQ). **Box 1.1** provides an explanation for the use of the MCTQ as a measure of chronotype. In the study, over 40,000 individuals were used to correlate the midpoint of sleep on free days corrected for sleep debt (MSFsc) with daily light exposure.

Box 1.1: Munich Chronotype Questionnaire

The Munich ChronoType Questionnaire (MCTQ) is a well validated questionnaire for the assessment of chronoytpe based on sleep timings. It calculates the sleep midpoint from self-reported bed times and get up times. Importantly the sleep midpoint is calculated for free days, i.e. days where sleep timings are not influenced by work or social commitments, and corrected for oversleep that occurs as a result of accumulated sleep dept on work days (MSFsc : Mid Sleep point of Free days Sleep Corrected). The sleep midpoint has been validated against sleep logs (Roenneberg et al. 2003), actigraphic, melatonin and cortisol rhythms (unpublished data from (Roenneberg et al. 2007a) and positively correlated to the Horne-Östberg's Morningness-Eveningness Questionnaire (MEQ) ($r > 0.7$, (Zavada et al. 2005), which in turn has been validated against temperature, melatonin and cortisol rhythms (Horne and Ostberg 1976, Duffy et al. 1999).

Sleep debt is the accumulation of lost sleep that can occur usually on work days as a result of work and social commitments, for example having to wake up earlier than preferred, to get to work. MSFsc was found to be earlier with increasing time spent outside, which was found to plateau at around two hours of daily light exposure (Roenneberg and Merrow 2007). However, this study only looked at hours of light exposure not the light intensity received. To date the only evidence for light intensity having a long term effect on circadian output comes from birds. Starlings show a seasonal change in testis size to prevent unnecessary energy expenditure outside the breeding season. Although driven by photoperiod (i.e. the length of day light), this same change in testis size can be produced by changes in light intensity (Bentley et al. 1998). This demonstrates that a long term circadian output can be driven by light intensity, although to date only in birds.

In summary the effect of light on the circadian clock is due to more than just its presence or absence i.e. the timing of the light/dark cycle. Light intensity and duration provide additional information for the entrainment of the circadian clock. To date, only the duration of light exposure has been shown to have a long term effect on the circadian clock in humans.

1.3. Sleep function

The function of sleep unlike the regulation of sleep is still not fully understood. Sleep is a very vulnerable state, with the loss of most sensory perception, and therefore must have provided a strong selection pressure to have remained throughout evolution. However, there is much debate as to whether sleep is the maladaptive by-product of a, as yet undiscovered, vital function that necessitates the rendering of the body into an unconscious state or whether it is a process that results in many advantageous outcomes such as conservation of energy, and avoidance of predators (Lockley and Foster 2012, Siegel 2011). There are three functions of sleep that have received the most attention: cellular restoration; energy conservation and memory consolidation. These three functions of sleep have been reviewed extensively (e.g.(Lockley and Foster 2012, Mignot 2008). Briefly, the most compelling evidence for the restoration of cellular components is due to sleep dependent expression of genes involved in biosynthesis, transport and the maintenance of vesicles (Mackiewicz et al. 2007). In terms of energy conservation, NREM sleep is less metabolically costly than wake, although REM sleep is as active as wake (Nofzinger 2006). The resulting effect is that sleep does provide a less costly state that can be used to save energy at appropriate times of the day for example when food availability is low or predators are active.

The third function, the role of sleep in memory consolidation, is highly relevant to this thesis. Sleep enhances both declarative or explicit memory (i.e. conscious recall of facts or events), and non-declarative memory which includes procedural memory (i.e. how to do something) and implicit memory, which is the unconscious recognition of emotional and physiological responses to the event: for example on being shown a picture of a face and being told this person is good, the explicit memory is of the face while the implicit memory is that the person is good (Fox 2008, Walker and Stickgold 2004). Memory consolidation involves the reactivation of memories causing them to be strengthened. These memories are then integrated with pre-existing memories by removing invariant or irrelevant features and transferred to long-term memory storage (Born and Wilhelm 2012). This process is largely driven by slow wave activity: slow oscillations originating

from the neocortex synchronise neural activity in brain areas that are thought to be involved in memory consolidation including the hippocampus and thalamus (Born and Wilhelm 2012). At the cellular level this process is likely to be under the control of synaptic homeostasis (Tononi and Cirelli 2006). The synaptic homeostasis theory states that during conscious experience, i.e. during wake, synapses are made and strengthen but, owing to limited energy resources and space within the brain, the capacity for this process is restricted. During sleep a redistribution of total synaptic strength is thought to occur, perhaps selectively enhancing pathways activated by preceding waking experience, resulting in renewed capacity for synaptic formation (Cirelli and Tononi 2000, Knott et al. 2002, Eyre et al. 2003, Cirelli et al. 2004, Donlea et al. 2009, Gilestro et al. 2009). Although the underlying mechanisms have yet to be fully elucidated, there is increasingly convincing evidence implicating slow wave sleep (Huber et al. 2006, Huber et al. 2008, Tononi 2009).

1.3.1. Sleep and emotional processing

Sleep is important for the regulation of mood. On a superficial level sleep, or more often the lack of it, can drastically change our mood with people experiencing a worsening of a range of self-reported moods including depression, anger, frustration, tension and anxiety (Lieberman et al. 2005, Dinges et al. 1997, Scott et al. 2006, Orton and Gruzelier 1989, Caldwell et al. 2004, James and Gregg 2004, Sagaspe et al. 2006, Van Dongen et al. 2003). What is less well understood is the relationship between sleep and emotional processing.

Emotional processing is a general term that encompasses a range of processes, including: emotional perception; contextualisation based on past experiences; emotional regulation; emotion production and expression, i.e. the production of emotional reactions; and finally emotional memories (**figure 1.6**) (Fox 2008).

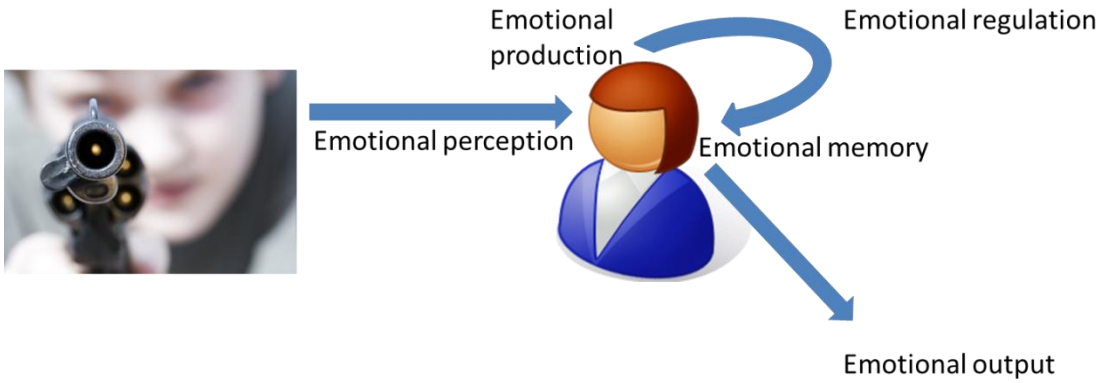


Figure 1.6 Emotional processing. Diagram represented the different aspects of emotional processing.

Sleep has been shown to affect many of these aspects such as the recognition of emotions (emotional perception) and emotional reactions: the lack of sleep causes a blunting in the recognition of angry and happy facial expressions (van der Helm et al. 2010) and intensifies negative responses to adverse events while positive reactions to goal enhancing events are blunted (Zohar et al. 2005). Hence the lack of sleep results in poorer emotional processing by: i) reducing the ability to perceive emotions, ii) causing enhanced reactions to negative events, but at the same time iii) reducing the reaction to positive events.

The aspect of emotional processing that has been given the most attention is that of emotional memory. As previously discussed sleep is important for memory consolidation, and this is enhanced for emotional events compared with neutral events (Walker and van der Helm 2009, Hu et al. 2006); and is specifically dependent on REM sleep (Cartwright et al. 1975, Greenberg et al. 1983, Wagner et al. 2001, Nishida et al. 2009, Walker and van der Helm 2009, Hu et al. 2006). Moreover, sleep deprivation has been shown to impair the encoding of emotionally positive and neutral words compared to negative words. Demonstrating that, individuals who have been sleep deprived encode more negative memories than positive or neutral memories (Unpublished data reported in (Walker and van der Helm 2009)).

To date the focus of this field of research has been on explicit memories that are consciously recalled. There is, however, another type of memory that unconsciously reactivates known as intrusive memory.

1.3.1.1. Intrusive memories

Intrusive memories or intrusions are autobiographical memories that are involuntarily recalled and appear to spontaneously reactivate into a person's consciousness i.e. the type of memory that just pops into one's head without thinking about it or "flashbacks". An example of an intrusive memory could be suddenly seeing an image of the beach that you went to on holiday last year. Intrusive memories more often involve mental imagery, which can be the images and sounds experienced during the event but can actually involve any of the senses (Kosslyn et al. 2001). Intrusive memories can also involve verbal thoughts for example " the sea was really blue", in conjunction with a mental image or on its own. Mental images, compared with verbal thoughts are more often associated with past emotional events and also induce a stronger emotional reaction when experienced (Holmes and Mathews 2010).

Intrusive memories are often triggered by a relevant cue, for example a bucket and spade could reactivate the memory of a holiday on the beach, however they can also spontaneously enter a person's consciousness (Mace 2004). On average healthy adults are thought to have up to five intrusive memories a day, of which between 80 and 90% are triggered by a relevant cue such as an object, smell, feeling or activity (Kvavilashvili and Mandler 2004, Berntsen 1996, Berntsen and Hall 2004, Mace 2004). These intrusions generally do not present as a problem as they tend to be related to positive rather than negative events (Berntsen 1996, Berntsen and Hall 2004).

Intrusive memories can also be recurrently experienced i.e. the same intrusive memory can be experienced repeatedly. The presence of this type of intrusive memory is the classical hallmark of post-traumatic stress disorder (PTSD), where intrusions or flashbacks to a traumatic event are vividly re-experienced. PTSD is a condition that can develop after an extremely traumatic event

and the full diagnostic criteria are outlined in **box 1.2**. Briefly PTSD is characterised by symptoms of re-experiencing the event which includes intrusive memories, avoidance of reminders of the event (for example not getting in a car following a car accident) and finally hyperarousal (American Psychological Association 2000). Importantly these symptoms must be experienced for at least one month following the trauma to be classified as PTSD. Intrusive memories are also seen within other mental health problems including depression, where around 85% of depressed individuals experience intrusive memories (Kuyken and Brewin 1994). Recurrent intrusive memories have also been documented in a non-clinical population (Berntsen and Rubin 2008) where they are experienced for positive as well as negative events.

To date very little is known about why intrusive memories occur to some events and why this type of memory can become highly distressing and frequent for some people following trauma. Moreover very little research had been conducted on intrusive memories experienced by a non-clinical population. The current theories surrounding intrusive memories in relation to PTSD, conceptualise intrusive memories as 'faulty information processing' (Brewin and Holmes 2003) and postulate that the formation of intrusive memories is due to a shift in the way an event is processed or laid down: from focusing on the meaning and context of the event towards the sensory impressions (Brewin and Holmes 2003). This results in memories that not only have inappropriate contextual information but also no coherent time code, which means that when experiencing an intrusive memory it can feel like the original event (Brewin et al. 1996a, Ehlers and Clark 2000). However, the fact that recurrent intrusive memories are also experienced in the general population, and to positive as well as to negative events, suggests that these memories may not be 'faulty' but merely a different type of memory, the significance of which is not yet understood (Berntsen and Rubin 2008).

Box 1.2: *DSM-IV-TR* Criteria for PTSD

A. The person has been exposed to a traumatic event in which both of the following were present:

- the person experienced, witnessed, or was confronted with an event or events that involved actual or threatened death or serious injury, or a threat to the physical integrity of self or others.
- the person's response involved intense fear, helplessness, or horror.

B. The traumatic event is persistently reexperienced in one (or more) of the following ways:

- recurrent and intrusive distressing recollections of the event, including images, thoughts, or perceptions
- recurrent distressing dreams of the event
- acting or feeling as if the traumatic event were recurring (includes a sense of reliving the experience, illusions, hallucinations, and dissociative flashback episodes, including those that occur on awakening or when intoxicated)
- intense psychological distress at exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event
- physiological reactivity on exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event

C. Persistent avoidance of stimuli associated with the trauma and numbing of general responsiveness (not present before the trauma), as indicated by three (or more) of the following:

- efforts to avoid thoughts, feelings, or conversations associated with the trauma
- efforts to avoid activities, places, or people that arouse recollections of the trauma
- inability to recall an important aspect of the trauma
- markedly diminished interest or participation in significant activities
- feeling of detachment or estrangement from others
- restricted range of affect (e.g., unable to have loving feelings)
- sense of a foreshortened future (e.g., does not expect to have a career, marriage, children, or a normal life span)

D. Persistent symptoms of increased arousal (not present before the trauma), as indicated by two (or more) of the following:

- difficulty falling or staying asleep
- irritability or outbursts of anger
- difficulty concentrating
- hyper vigilance
- exaggerated startle response

E. Duration of the disturbance (symptoms in Criteria B, C, and D) is more than 1 month.

F. The disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.

Regardless of the underlying mechanism, intrusive memories to a trauma can be highly distressing and disruptive to a person's life. Thus there is a great need for further research into these memories. One direction which is being taken with this research is to investigate intrusive memories in a laboratory setting. Since is it clearly unethical to subject people to real life trauma, analogue traumatic events are created using the trauma film paradigm. The trauma film paradigm has been reviewed extensively by Holmes and Bourne (Holmes and Bourne 2008). Essentially it consists of showing subjects a series of film clips that contain scenes of a distressing or traumatic nature, for example a car crash or suicide. The subjects can then be assessed before and after the analogue trauma for any number of behavioural parameters such as mood or cognitive processing. Moreover, in this setting researchers are able to manipulate the situation in which the subjects experience the analogue trauma. Using this technique Holmes and colleagues (Stuart et al. 2006, Holmes et al. 2004) have begun to unravel the mechanism underlying the frequency of intrusive memories. Healthy individuals were required to complete either a visual-spatial task, a verbal task, or no task, while watching a trauma film. The subjects who completed the visual-spatial task reported fewer intrusive memories to the trauma film than the subjects who had completed no task, while the subjects who completed the verbal task reported more intrusions than both the no task and visual-spatial task groups (Stuart et al. 2006, Holmes et al. 2004). Therefore, it appears that activation of the visual-spatial processing pathway reduces the frequency of intrusions while activation of the verbal processing pathway increases the frequency. Furthermore the same response has been found when the tasks were completed either 30 minutes or four hours after the analogue traumatic event (Holmes et al. 2010, Holmes et al. 2009).

1.3.1.2. Sleep and intrusive memories

Unlike consciously recalled memories, very little is known about intrusive memories and sleep. Research in both humans and animals suggests that sedation after trauma can lead to an increase in the incidence of PTSD, which since PTSD is characterised by intrusive memories, suggests that

sedation following a trauma may promote the generation of intrusive memories. In humans benzodiazepine (a sedative) treatment following a trauma resulted in 9 out of 13 patients meeting diagnostic criteria for PTSD six months after the trauma compared with 3 out of 13 controls pair-matched for levels of PTSD symptomology one week after the trauma (Gelpin et al. 1996). A similar finding was also reported by Mellman and colleagues (Mellman et al. 2002). In a randomized placebo controlled study, 6 out of the 11 trauma survivors prescribed benzodiazepine treatment following the trauma went on to develop PTSD compared to 3 out of the 11 placebo controls (Mellman et al. 2002). In rats, the animals treated with the benzodiazepine, alprazolam, immediately after exposure to stress showed increased PTSD like behaviour patterns and increased freezing in response to a cue of the original stressor (Matar et al. 2009). As a result of this the British National Institute for Health and Clinical Excellence (NICE: National Collaborating Centre for Mental Health 2005) and the American Department of Veteran Affairs and Department of Defence (VA/DoD: The Management of Post-Traumatic Stress Working Group 2010) recommends against the use of benzodiazepines in acutely traumatised people. Despite this it is still common practice to use benzodiazepine treatment following trauma (Zohar et al. 2011). Indicating a great need for further research to understand the interaction between sleep, sedation and intrusive memories.

1.3.2. Sleep deprivation

As considered above, there is mounting evidence for a role of sleep in the regulation of mood and emotional processing and much of the research investigating this relationship has involved studying the effect of sleep deprivation. Sleep deprivation is a stressful and often boring situation. In an experimental setting, individuals are often sleep deprived in isolation and in carefully controlled experiments in the absence of caffeine and other stimulants as well as under constant environmental conditions such as light and temperature. Within real life situations individuals can control certain aspects to make sleep deprivation easier such as consuming caffeine. However it still usually occurs in a stressful or boring situation such as night shifts where either

more responsibility is placed on individuals due to a limited work force, or conversely less stimulating work is being conducted. This raises the possibility that changes, especially to mood are the result of the situation rather than the lack of sleep. Although this is always a possibility when assessing changes in subjective mood, objective assessment of mood and emotions have also been conducted using brain imaging techniques. The limbic system is a set of brain structures including the hippocampus, amygdala and anterior thalamic nuclei located at the inner edge of the cortex that is involved in the regulation of mood and emotions (Crossman and Neary 2005). Functional magnetic resonance imaging (fMRI) has shown a 60% greater activation of the amygdala to negative picture stimuli in sleep deprived individuals compared with non-sleep deprived controls (Yoo et al. 2007). Moreover the sleep deprived individuals showed weaker connectivity between the amygdala and the medial-prefrontal cortex (mPFC), an area of the brain thought to provide inhibitory top-down control on amygdala function regulating emotional responses (Davidson 2002, Sotres-Bayon et al. 2004). Thus, the effect of sleep deprivation on mood and emotional processing is more than a consequence of the stress of being deprived of sleep. Moreover, it has been proposed that sleep is needed to reset the connectivity between the mPFC and amygdala to allow proper control and function of this system ensuring appropriate emotional responses (Walker and van der Helm 2009). Thus, it appears that sleep itself is important for the regulation of mood and emotional processing.

The effects of sleep deprivation and chronic sleep restriction are much further reaching than on just mood and emotional processing. A vast array of deleterious effects have been reported, such as; i) increased blood pressure, ii) increased evening cortisol levels, iii) increased ghrelin levels, iv) decreased leptin levels, which is thought to promote an increase in hunger and appetite, v) cognitive impairment and vi) diminished mood (e.g. (Spiegel et al. 2004, Leproult et al. 1997, Banks and Dinges 2007). Moreover, many health conditions have been associated with chronic sleep restriction including obesity, diabetes, hypertension and cardiovascular disease (Knutson and Van Cauter 2008, King et al. 2008, Cappuccio et al. 2007, Knutson et al. 2009). As a

consequence sleep deprivation and chronic sleep restriction are generally considered detrimental to health. Nevertheless there is increasing evidence that sleep deprivation in certain situations may be beneficial. In some clinically depressed individuals sleep deprivation ameliorates mood, i.e. it has an anti-depressant action (Wirz-Justice and Van den Hoofdakker 1999). This phenomenon is very short lived as it usually only improves mood while the patient remains awake: following sleep the depressed mood returns. However, the effect can be prolonged when used in combination with other therapies such as medication (Wirz-Justice and Van den Hoofdakker 1999). Sleep deprivation has also been found to extinguish the fear response. Kuriyama and colleagues (Kuriyama et al. 2010) showed healthy participants a series of aversive and neutral motor vehicle accident films, which all involved similar street scenes and either ended with a traffic accident or no accident. Recognition tasks were carried out immediately after encoding (i.e. watching the film), and then three and ten days later. In the initial recognition task all participants showed high fear responses to both the aversive and neutral films. By day 3 those who had been sleep deprived showed an extinction of the physiological as well as self-reported fear responses to the neutral films. Suggesting that the unnecessary fear responses (to the neutral situations) were eliminated leaving only those directed to the real threat situations. Hence sleep deprivation may be beneficial in more aversive situations such as when clinically depressed or threatened. From an evolutionary perceptive this makes sense. Before the onset of artificial lights and our 24/7 society, a period of total sleep deprivation would generally only result from a highly traumatic or emotional event such as a battle, a hunt or possibly a natural disaster. During these periods people would have had to remain awake to survive the situation. By staying awake after the trauma this may have also aided the processing of that event either by continued wake or prevention of sleep. In the long term, this could then result in a better response to the traumatic event, for example fewer intrusive memories. This possibly beneficial role of sleep deprivation in emotional processing after trauma has not been previously recognised and it is only now becoming apparent, from the observed consequences of sedating people following

trauma i.e. an increase in PTSD, that a lack of sleep in these circumstances may allow as yet ill understood beneficial emotional processing to occur.

In summary although the true function of sleep remains elusive, it is clear that there are many benefits of sleep, whether it is through the conversation and restoration of energy or the consolidation of the day's activities. It is also becoming increasingly apparent that sleep is involved in the regulation of mood and many aspects of emotional processing, in particular emotional memory. What is less well understood is the impact of sleep on intrusive memories. Moreover, the interaction between sleep and emotional processing has so far only been considered in healthy individuals. In patients with clinical depression, sleep deprivation can act as an antidepressant (Wirz-Justice and Van den Hoofdakker 1999). However the relationship between sleep and emotional processing in people with mood disorders, including depression, is poorly understood.

1.4. Mood disorders

Mood disorders encompass a range of conditions including unipolar depression and bipolar disorder. These disorders can be hugely disruptive to a person, often have their origins in young adult life, particularly bipolar disorder (Weissman et al. 1996), and can become lifelong conditions. The lifetime prevalence of bipolar disorder is in the order of 1%, while depression of all types is found in about 10% of the population (National Collaborating Centre for Mental Health 2004, National Collaborating Centre for Mental Health 2006). This burden is also seen on a global scale as depression is currently the leading cause of disability as measured by years lived with disability (YLDs) for both men and women, and was classified as the third leading contributor to the global burden of disease in 2004 (World Health Organization 2004). Despite its lower prevalence, bipolar disorder is also within the top ten causes of disability: seventh for men and eighth for women as assessed by YLDs.

Mood disorders are primarily characterised by a disturbance of an individual's mood. The type and severity of the mood disturbance experienced determines the classification of the disorders. **Box 1.3** details the different conditions. Depression is characterised largely by a decrease in mood, while in bipolar disorder there is also an elevation of mood during mania and hypomania. How these states arise is the concern of much research. Both unipolar depression and bipolar disorder are associated with maladaptive emotional processing strategies: rumination (Nolen-Hoeksema et al. 2009) and suppression (Campbell-Sills et al. 2006) in depression and enhanced positive emotional reactivity during bipolar mania (Gruber et al. 2008). Emotional biases in information processing also exist in both conditions, with a negative emotional bias seen in depression (e.g. (Rubinow and Post 1992, Surguladze et al. 2004) and a positive emotional bias in bipolar mania (Murphy et al. 1999). An emotional bias is a tendency to see a situation in a more positive or negative way than expected. For example, after leaving a message for a friend who does not then call back, a neutral bias could that they are just busy, a positive bias may be that they are doing something very exciting and will tell you all about it later, while a negative bias would be that your friend hates you and doesn't want to talk to you. In mood disorders, these emotional biases are in fact thought to be a trait phenomenon as they are also seen within euthymic bipolar patients, i.e. patents between depressive or manic episodes (Roiser et al. 2009). Abnormal emotional processing is also seen in patients whose depression has remitted. These individuals were better at recognising emotions than healthy controls and currently depressed patients, which is thought to contribute to vulnerability to relapse (Anderson et al. 2011).

Box 1.3: Classification of mood disorders

Unipolar depression is characterised by major depressive episodes where depressive symptoms last at least two weeks. Recurrence of these episodes is classified as major depressive disorder (MDD). Bipolar disorder involves the presence of elevated mood episodes as well as often depression. The severity of the elevated mood states subdivides the classification of bipolar disorder, whereby mania is experienced in bipolar I disorder (BDI) and the less severe hypomania is experienced in bipolar II disorder (BDII). Within bipolar disorder the cycling between mood states varies in frequency. On average an individual may experience up to three episodes of altered mood within 12 months. However there are cases where the frequency is much higher, to the extreme of daily cycling (Papadimitriou et al. 2005). This is known as rapid cycling bipolar disorder and is seen in both BDI and BDII. Mixed episodes are also seen, whereby an individual can experience symptoms of both mania and depression at the same time.

Although mood disturbance is the primary symptom that denotes the classification of mood disorders, disruptions are also seen in other systems, including sleep (Armitage 2007), circadian rhythms (Souetre et al. 1989, Drennan et al. 1991, Benedetti et al. 2007, Echizenya et al. 2012, Claustrat et al. 1984), and the hypothalamic-pituitary-adrenal (HPA) stress axis (Bhagwagar et al. 2005, Deuschle et al. 1997, Vreeburg et al. 2009, Burke et al. 2005). Disruption to sleep has long been associated with mood disorders, and in fact is a symptom of both depressive and manic episodes. The sleep disruptions seen within mood disorders are detailed in **table 1.1**. These sleep disruptions are most severely seen within episodes of altered mood but are also seen between episodes to the extent that individuals can use these changes in sleep pattern to predict their own relapse into an altered mood state (Bauer et al. 2006, Bauer et al. 2008).Although traditionally the disruptions seen in these systems were thought to be either a consequence of the condition or a side-effect of the medication, it is becoming increasingly clear that these disruptions are fundamental aspects of the disorders and may even be a causal factor for the development of the disorders. The evidence for this comes from research on individuals who have an increased risk of developing a mood disorder.

	Sleep architecture
Healthy	Sleep latency of about 10-15 minutes, 1st REM period after about 90 minutes after sleep onset, short first REM period (1-5 minutes), progressively lengthens, NREM/REM cycle takes about 80-120 minutes, 2-5% of total sleep time is spent awake (Armitage 2007).
Depression	Hypersomnia (increased daytime sleepiness and sleep duration), prolonged sleep latency, bouts of intermittent wakefulness, increased stage one sleep, decreased SWS, shortened latency to first REM period, increased REM density, early morning awakenings (Armitage 2007).
Bipolar disorder: depression	Hypersomnia, increased daytime napping, reduced or increased REM latency, greater REM fragmentation, trend towards more sleep spindles, prolonged SWS latency and increased REM density (Harvey 2008).
Bipolar disorder: mania	Reduced need for sleep, shorter REM latency, increased REM activity and increased REM density (Harvey 2008, Hudson et al. 1988, Hudson et al. 1992).

Table 1.1 Sleep architecture.

In these individuals similar disruptions are seen in sleep (Rao et al. 2009, Friess et al. 2008) and mood (Chan et al. 2007, Brotman et al. 2008).

1.4.1. High risk individuals

Mood disorders are known to run in families, indicating an underlying genetic element to the development of these conditions. Unsurprisingly this is not a simple case of genetic predisposition, but involves multiple genes within multiple pathways. Moreover there are also environmental factors that have been associated with an increased risk of developing a mood disorder, ranging from trait personality characteristics, in particular neuroticism (Kendler et al. 1993, Clayton et al. 1994) to social interactions (Rosenquist et al. 2011). Of these, mood lability (the ups and downs of mood) has been found to be a particularly strong predictor for the development of both bipolar disorder and depression (Angst et al. 2003). Furthermore gene/environment interactions have been established, with the family environment created by individuals diagnosed with a mood disorder adding to the risk passed on to their offspring (Chang et al. 2001, Adrian and Hammen 1993, Ostiguy et al. 2009). Despite the range of risk factors, the

genetic risk remains the most well established, with a heritability of around 40% for depression in women and slightly lower for men (20-30%) (Kendler et al. 2001, Bierut et al. 1999, Kendler et al. 2006) and around 70% for bipolar spectrum disorders for males and females (Edvardsen et al. 2008). In light of this the majority of research in to early warning signs for the onset of a mood disorder has been carried out in individuals with a relative, usually first degree, with a diagnosed mood disorder. In this thesis a familial history for a mood disorder was used to investigate the relationship between sleep and emotional processing in individuals with an increased risk for the development of a mood disorder.

1.4.1.1. Sleep, emotional processing and intrusive memories in individuals at-risk of mood disorders

Healthy individuals with a positive family history of either depression or bipolar disorder consistently show a shorter REM latency (time taken to enter REM sleep) and a higher REM density (amount of eye movements in REM sleep) compared to individuals with no elevated risk (Rao et al. 2009, Friess et al. 2008), both of which have been observed in depression and bipolar disorder (see **table 1.1**). Furthermore, in an otherwise healthy population, insomnia has been found to increase the risk of the subsequent onset of depression (Breslau et al. 1996, Buysse et al. 2008). Together this indicates that sleep disruptions may be a causal factor for the development of mood disorders.

In terms of emotional processing, emotional biases are seen in high risk individuals as well as in individuals with a mood disorder. In high risk individuals, a decrease in positive and an increase in negative processing for a variety of tasks is seen, for example highly neurotic individuals were quicker to identify negative versus positive emotions than controls (Chan et al. 2007). A similar deficiency is also seen in children with familial risk for bipolar disorder as they reported more errors in labelling emotions than controls (Brotman et al. 2008).

Intrusive memories are experienced by people with depression and bipolar disorder. In depression intrusions are experienced by around 85% of patients (Kuyken and Brewin 1994) and generally involve memories of past negative life events as well as thoughts and mental imagery relating to death, illness and injury of family and friends (Brewin et al. 1996b). These intrusions are frequently dealt with using avoidance strategies (Newby and Moulds 2011a, Kuyken and Brewin 1999) and play a role in the persistence of depression (Brewin et al. 1999). Moreover increased avoidance and intrusion-related distress is experienced in recovered depressed individuals although to a lesser extent than in currently depressed individuals (Newby and Moulds 2011a, Newby and Moulds 2011b, Spenceley and Jerrom 1997). Although less well documented intrusive memories are also increased in all states of bipolar disorder (euthymic, depression and mania), and have similar content to those seen in unipolar depression (Gregory et al. 2010, Tzemou and Birchwood 2007). During mania, mental imagery can actually be perceived as positive, though generally it embodies unrealistic goals that can lead to risky behaviour (Holmes and Mathews 2010). However, experimental research investigating intrusive memories is problematic in individuals with a mood disorder, as it is unethical to subject a patient with a mental health problem to any type of trauma even if an analogue one. Moreover research with patients is often confounded by the various medications that the patients are taking to manage their condition, whereas individuals at high risk of a mood disorder, but otherwise healthy, are normally drug naïve. To date only one study has investigated intrusive memories in high risk individuals. In this study Malik and colleagues (Malik et al. Manuscript) found that individuals with a behavioural high risk for a mood disorder (higher mania-like symptomology as assessed by the mood disorder questionnaire compared with controls) experienced more intrusive memories to an analogue traumatic event (trauma film) than controls.

In summary mood disorders encompass a range of conditions that show sleep, circadian, mood and emotional processing disruptions. Moreover these disruptions can also be seen to some extent in individuals who are healthy but have an elevated risk of developing either depression or

bipolar disorder. However what has yet to be investigated is the relationship between sleep and emotional processing.

1.5. Summary and aims for thesis

Sleep is a highly complex process involving multiple interacting systems and networks. Sleep timing is controlled by the interaction of the sleep homeostat and the circadian clock, and the latter is in itself a complex system. Individual variations in the circadian clock give rise to chronotypes and differences in preferred sleep timings. The circadian clock uses light to remain synchronised to the external environment. However the effect of light on the circadian clock is due to more than just its presence or absence i.e. the timing of the light/dark cycle. Light intensity and duration provide additional information for the entrainment of the circadian clock. To date in humans, only the duration of light exposure has been shown to have a long term effect on the circadian clock: sleep timing and chronotype are earlier with more light exposure. In response to this, the first question this thesis aimed to answer was: **do environmental light levels influence sleep timing and chronotype?** Therefore study one was designed to investigate the impact of light intensity on sleep timings and chronotype in a longitudinal naturalistic setting, exploiting different light intensities in different geographical locations and times of the year.

In terms of emotional processing it is becoming increasingly apparent that sleep is important for the recognition of emotions, emotional reactions and conscious emotional memory. What is less well understood is the impact of sleep on spontaneous emotional memories, i.e. intrusive memories. Hence the second study aimed to answer the question: **what is the relationship between sleep and intrusive memory frequency?** Specifically the objective of the study was to investigate the effect of sleep versus sleep deprivation on the frequency of intrusive memories generated by an analogue traumatic event. To achieve this healthy participants were either sleep deprived or allowed to sleep as normal following exposure to an analogue traumatic event, a trauma film. Participants were assessed at baseline for good sleep, circadian profile, physical and

mental health and throughout the experiment the participant's mood and sleep were assessed by a variety of behavioural and physiological measures. This study also allowed an examination of the possible beneficial effects of sleep deprivation following trauma. Furthermore the same study enabled an additional question to be addressed: **what is the effect of an analogue traumatic event on sleep physiology?** The answer to this was explored using polysomnographic recordings made before and after the analogue traumatic event.

Finally the investigation into sleep and emotional processing was extended into those individuals with an elevated risk of developing a mood disorder. Sleep, the circadian clock and emotional processing are all disrupted in individuals at-risk of a mood disorder. However what is not known is the relationship between sleep and emotional processing. Hence the second study was extended to address the question: **is the relationship between sleep and emotional processing different in people at-risk of a mood disorder?** In addition to this, this experiment could help further characterise this population which could provide potential biomarkers for the recognition of individuals with a high risk of developing a mood disorder and also identify potential targets for the prevention and/or treatment of mood disorders.

1.6. Overview of chapters

Chapter 2: Methods and materials

The studies presented in this thesis are the first of their kind to be conducted in our department. Hence the undertaking of these studies involved new techniques and protocols to be established. This chapter discusses how these experiments were designed as well as providing an exhaustive list of all the measures used within the experiments with details of how and when they were used.

Chapter 3: Sleep timing, chronotype and light

This chapter describes study one which was designed to address the question: **do environmental light levels influence sleep timing and chronotype?** This study investigated the effect of different environmental light levels due to geographical location and season, on sleep timing and chronotype. Six universities from around the world are included in the study, though it was designed and analysed in this department. Seasonal variation is also investigated between spring and autumn.

Chapter 4: Sleep, sleep deprivation and intrusive memories

The behavioural and psychology measures addressing mood and emotional processing (intrusive memories) in relation to sleep from study two are reported in this chapter, with the aim of addressing the question: **what is the relationship between sleep and intrusive memory frequency?** More specifically the chapter details the results of an experiment looking at the effect of sleep and sleep deprivation on the frequency of intrusive memories generated by an analogue traumatic event. The analogue traumatic event used was a series of film clips. Furthermore this experiment investigated the effect of sleep and sleep deprivation on an additional stressful situation.

Chapter 5: Analogue trauma and sleep physiology

Using the same protocol as used in Chapter 4, this chapter reports on analysed polysomnographic recordings made before and after the analogue traumatic event to answer the question: **what is the effect of an analogue traumatic event on sleep physiology?** The results explore the impact of an analogue traumatic event on sleep and the correlation between sleep characteristics and intrusive memories.

Chapter 6: High risk of mood disorders, sleep and intrusive memories

The experiment for this study follows on from Chapters 4 and 5, using the same protocol but with individuals with a familial risk of a mood disorder. This allows the final question of this thesis to be addressed: **is the relationship between sleep and emotional processing different in people at-risk for a mood disorder?** This is a preliminary study so the results are reported as three case studies.

Chapter 7: Concluding remarks

The aim of this chapter is to assess whether each of the research questions detailed above have been answered by this thesis. Moreover the implications of the findings of this thesis will be discussed along with suggestions for future research.

2. Methods and materials

This thesis consists of two studies. Study one was an investigation into the effect of environmental light intensity on sleep timing and chronotype. Study two explored three aspects of the relationship between sleep and emotional processing: the psychological response, the physiological effect on sleep and the impact of elevated risk for a mood disorder. These studies required the establishment and development of new methods and techniques. The aim of this chapter is to discuss these study protocols in terms of how the methods, measures and data analyses were developed, as well as to provide a separate explanation of all the methods and techniques used. All the questionnaires and scales used in this study are provided in appendix VI. The precise protocol used for each study is detailed in the relevant data chapter. Finally both studies were carried out in a healthy student population aged 18-25 years of age, the benefits and drawbacks of this population are discussed.

2.1. Student population

The advantage of studying a young adult population is that they are less likely to have underlying medical conditions and be taking medication, which reduces the risk of confounding by these factors. However, for individuals in this age range it can be a period of great change, both physiologically and psychologically. Chronotype, which influences the preference for sleep timing, is influenced by age. In early adulthood (early 20's) people tend to show their latest chronotype, which means that this is the period in their lives when they tend to prefer to go to bed and wake up at the latest time of day (Roenneberg et al. 2007a). This period is also a period of stressful life events such as relationships, leaving home, and for students the pressure of exams and university life. Because of the importance of stressful life events in the development of many mental health conditions, not least depression (Mazure 1998), it is not surprising that three quarters of lifetime mental health conditions begin by the mid-20s (Kessler et al. 2007). For this thesis a student population was chosen to limit the possible cofounding effects of poor health and drug use. This population also provided a substantial recruitment pool of individuals living within a homogenous

environment, to ensure the participants were experiencing similar life events. Finally in light of the increased risk for the development of a mental health condition within this age group, participants recruited for study two, investigating sleep and emotional processing, were required to meet a very stringent set of criteria to ensure no current or past mental health problems.

2.2. Development of methods

The two studies in this thesis used different methodologies and techniques. The rationale behind each study, along with a discussion of the methods and techniques used, will be presented for each study separately.

2.2.1. Study one: effect of light on sleep timing and chronotype

Interest into the impact of the intensity of light exposure on chronotype arose from observations concerning lecture timings in Australia compared to the UK. In Perth, Australia not only were the lecture times much earlier than Oxford, but that they were well attended and the students did not complain about the early start. This combined with the much higher light levels experienced in Perth, as compared with Oxford, led to the suggestion that the amount of light the students were exposed to might underlie their ability to attend early lectures. Light is known to effect sleep timing: the timing, intensity and duration of light exposure all influence the entrainment of the circadian clock in humans (Duffy and Czeisler 2009, Martinez-Nicolas et al. 2011). However, to date in humans, only the duration of light exposure has been shown to have a long term effect on the circadian clock: sleep timing and chronotype are earlier with more light exposure (Roenneberg and Merrow 2007). Therefore, the primary aim of this study was to investigate the impact of light intensity on chronotype in a longitudinal naturalistic setting, by determining chronotype and light levels from different geographical locations.

Six universities were recruited to join the study from around the world: University of Oxford, UK; University of Groningen, Netherlands; Ludwig Maximilians University, Munich, Germany; University of Western Australia, Perth, Australia; Monash Univeristy, Melbourne, Australia;

Univeristy of Auckland, New Zealand. In additon a seasonal influence was also investigated: two months were chosen, May and October, in which sleep timings and light levels were recorded. This enabled a comparison of spring with autumn: May in the northern hemisphere and October in the southern hemisphere for spring, and October in the northern hemisphere and May in the southern hemisphere for autumn. These months were also chosen as they were the only ones when all the universities were in term time. A representaion of the study protocol is given in **figure 2.1**.

Chronotype was assessed by the Munich Chronotype Questionnaire (MCTQ). The MCTQ enables the sleep midpoint to be derived from self-reported bed times and get up times. Importantly the sleep midpoint is calculated for free days, i.e. days where sleep timings are not influenced by work or social commitments, and corrected for oversleep that occurs as a result of accumulated sleep dept on work days (MSFsc : Mid Sleep point of Free days Sleep Corrected, details are provided in section 2.5.1 (p.52). Validation of the MCTQ is discussed in **box 1.1** in Chapter 1 section 1.2.1.1, p.11).The online version of the MCTQ was used making it easier to contact a large number of students.

The questionnaire was accessed via a website (https://www.bioinfo.mpg.de/mctq/core_work_life/core/introduction.jsp). The questionnaire is available in four languages (English, German, Dutch and French), and for the study the questionnaire was used in the native language of each country.

Light levels were assessed by a number of measures. The amount of time participants spent outside (light exposure) was determined from the MCTQ. The irradiance (W/m^2: power of electromagnetic radiation per unit area) for each city was also obtained for each day for the study collection period i.e. May and October 2010. The daily irradiance was obtained from a number of different sources which are detailed in section 2.5.1, p.52.

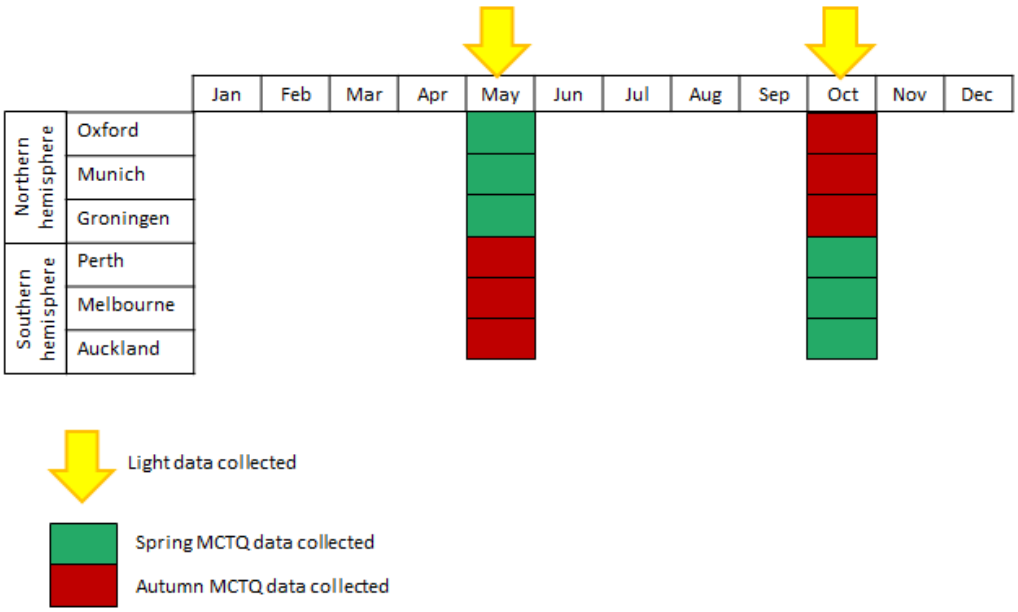


Figure 2.1 Study one protocol.

This study was designed and set up at the University of Oxford. Each collaborating university was contacted about the study and invited to take part. Although collaborators provided input into the study design, the final protocol was defined at the University of Oxford. Each university was allowed to use the MTCQ data collected at their site, but only the full MTCQ data set and light data were obtained and analysed for this thesis.

2.2.1.1. Participant recruitment

Students were recruited from each university by the local research team. Students at five of the universities (University of Groningen, Netherlands; Ludwig Maximilians University, Munich, Germany; University of Western Australia, Perth, Australia; Monash Univeristy, Melbourne, Australia; Univeristy of Auckland, New Zealand) were contacted via email that was sent out to the whole student body. Unfortunately the University of Oxford does not allow students to be mass emailed about research projects, therefore at this university students were recruited via posters and emails sent out by willing university departments and colleges. Students were sent an information sheet detailing the purpose of the study and directing them to the MCTQ website if they wished to complete the study. On the website an additional information page gave

instructions on how to complete the questionnaire. Informed consent by participants was assumed by the fact that they continued to the questionnaire and submitted the completed questionnaire.

Participants received information regarding their chronotype on completion of the questionnaire. Further incentives, were also provided by some universities, dependent on university policy, for example here at the University of Oxford, all participants were placed in a prize draw for £50 for both collection periods.

2.2.2. Ethics

Ethical approval for this study was obtained from the local ethics committee for each university involved in the study. For the University of Oxford ethical approval was sought from, and given by, the Central University Research Ethics Committee (CUREC). The study was assigned the code: MSD/IDREC/C1/2009/103.

2.2.3. Study two: investigation in to the relationship between sleep and emotional processing

Sleep, mood and emotional processing are highly complex and often subjective experiences, which makes testing these systems difficult. When faced with such complex systems experiments are designed principally in one of two ways: 1) highly controlled experiments that try to account for all confounding variables, for example constant routine or desynchronisation protocol, see section 1.2.1 (p.6) for details (Wirz-Justice 2007), or 2) naturalistic experiments that observe the systems in question under the normal daily routines of the participants in the study. Both of these types of experiments have their merits and drawbacks. The aim of this study was to try to combine these two methods, creating an experiment that controls for as many confounding factors as possible without removing the naturalistic setting completely. The reason for this was that this study aimed to address the relationship between sleep, mood and emotional processing but in a situation that would be more applicable to a real life setting. Therefore the experimental manipulation of sleep and emotional processing was undertaken in a laboratory setting, while

baseline assessments, sleep and the primary psychological outcome (intrusive memories) were observed largely in the home environment under the participants' normal routine. An overview of the study is illustrated in **figure 2.2**. In brief, participants were: i) exposed to an analogue traumatic event to trigger the generation of intrusive memories, ii) then either sleep deprived or allowed to sleep as normal, and iii) exposed to an additional stressor following the analogue traumatic event. The participants for the study were carefully selected to create as homogenous population as possible. Each aspect of the study is described below and includes a description of protocol refinement undertaken during the study.

2.2.4. Experiment

Group assignment: the participants were assigned to either the sleep deprived group or the non-sleep deprived group using a minimisation randomisation procedure. Minimisation is a procedure that ensures balance between study groups when low study numbers are used (Altman and Bland 2005, Scott et al. 2002). Participants are initially randomised to groups and additional participants are then allocated to groups to ensure equal numbers and sex ratio. Participants were assigned to a group at the beginning of the study.

Analogue traumatic event: It is unethical to create a real life traumatic event to generate intrusive memories; hence an analogue traumatic event was used. The analogue traumatic event was created using a series of film clips that included depressing and traumatic content. This film was based on a film created by Lang and colleagues (Lang et al. 2009) with an additional film clip (from Martin Scorsese's The Big Shave, 1967) added to increase the intensity of the response to the film. This additional film clip was added after seven (four sleep deprived and three non-sleep deprived) participants had already completed the study. Initially the study was designed to include a neutral film to control for the trauma film. However, unfortunately no such film existed at the time when this study started.

		Baseline	Experiment									
		Day1 - 13	Day14				Day15				Day16		Day17-20
			Day		Night		Day		Night		Day	Night	
Actigraphy				Study film				Stressor					
Cortisol		XX											
Melatonin		X											
Sleep deprivation													
Polysomnography		XX			X*				X**				
Baseline questionnaires***		X											
Experimental questionnaires	mood VAS		X		X		X		X				
	STAI		X		X		X		X				
	DSS		X		X								
	Film follow up				X								
	PANAS				X		X						
	Activity VAS						X						
	Intrusion dairy												
	IES						X						
	RAT follow up								X				

* Non-sleep deprived participants only
** Sleep deprived particiapnts only
*** Baseline questionnaires: EPQ, GHQ, HQ, MINI, MDQ, MEQ, PSQI, POMS

Figure 2.2 Study two protocol

Moreover several attempts to create a neutral film were unsuccessful, due to the difficulty in producing completely neutral material of high quality and without being boring to watch. Despite this in the initial stages of the study the participants were under the impression that there were two films: a trauma film and neutral film. However, during this stage of the study the participants were never informed which film they would be watching hence this should not have affected their perception of the film. The participants were informed that the film they would watch may contain scenes that they may find distressing and that they were free to stop watching it at any point. Once the possibility of a neutral film was removed from the study, the participants were still not informed of the content of the film, only that it contained scenes they may find distressing. Therefore all the participants in the study had the same expectation as to the nature of the film. All the participants watched the trauma film in the Sleep/Circadian Neuroscience Sleep Laboratory at the John Radcliffe Hospital in Oxford.

Sleep deprivation: Following the analogue traumatic event the participants in the sleep deprived group remained in the Sleep/Circadian Neuroscience Sleep Laboratory. In total the participants remained awake for 36 hours: 12 hours before the trauma film i.e. daytime and 24 hours following the trauma film. Originally the sleep deprived participants were allowed to go home the following morning i.e. after approximately 12 hours in the sleep lab, and required to keep themselves wake until the following evening. However, after the first four participants it was decided that they should remain in the sleep lab for the entire sleep deprivation period following the trauma film i.e. 24 hours. This was to ensure that not only were the participants not sleeping, or consuming caffeine, but also to control the environment the participants were exposed to in terms of light, and emotional stimulation. Within the sleep lab a number of factors were controlled to try to create as standardised environment as possible. To prevent interference with the trauma film and to maintain a stable emotional experience the participants were not allowed to watch TV or DVD's, or listen to music. Light levels were very carefully controlled as light can influence mood and alertness (Cajochen et al. 2000). A light level of between 165-276 lux, depending on position within the room, was maintained for the whole time the participants were in the sleep lab, apart from when the participants watched the trauma film when the lights were turned off. The participants were not allowed to use a computer, as a laptop screen not only emits light but this tends to be blue-enriched, which is the wavelength of light that the circadian clock is maximally sensitive to (Zaidi et al. 2007). Cajochen and colleagues (Cajochen et al. 2000) produced a dose response curve showing a sigmoidal relationship between increasing light levels and alertness levels. The light levels within the sleep lab were kept at 165-276 lux, which is in the middle of this sigmoidal distribution. Although it was acknowledged that this light level would influence mood and alertness, so would any light level. Therefore, it was decided to choose a light level that would if anything improve mood and make it easier to stay awake. This is more akin to a natural situation when someone is trying to keep themselves awake, potentially making the results of this study more applicable to real life situations. Throughout the sleep deprivation

period the participants were constantly monitored by either myself or a research assistant, to ensure that the participants did not sleep and complied with the regulations, for example did not use a computer. Research assistants were medical students from the University of Oxford. They were trained to remove EEG electrodes, re-apply electrodes and how to monitor the participants.

Non-sleep deprived group: The non-sleep deprived participants underwent exactly the same protocol as the sleep deprived participants; expect that following the trauma film they went home. That evening the non-sleep deprived participants were instructed not to watch TV or DVD's or listen to music to try to make the environment following the trauma film as similar as possible to the environment experienced by the sleep deprived participants.

Stressor: In order to enhance the emotional experience created by the traumatic film, the participants were exposed to an additional stressor. The stressor was undertaken the morning following the trauma film and sleep deprived night enabling the effect of sleep deprivation on the response to a stressor to be assessed. The stressor used was the failure version of the remote associates task (RAT) (McFarlin and Blascovich 1984). The RAT is a cognitive task that the participants were led to believe was easy to perform, but was in fact difficult, and was designed to make the participants fail: they were told that the average score obtained on the task was much higher than the actual average score achieved on the task. Therefore the participants are lead to believe that they had performed below average. The stressor was conducted in the sleep lab.

Questionnaires and scales: The response to the experiment was measured with a number of questionnaires and scales. The full details of how these questionnaires and scales were used are detailed in section 2.5.2.1 (p.54). Mood was assessed immediately before and immediately after the trauma film and stressor using the mood visual analogue scale (mood VAS) (Monk 1989) and state-trait anxiety inventory (STAI) (Spielberger et al. 1983). The participants' mood and level of sleepiness were assessed every two hours throughout the experiment with the mood VAS and

Karolinska sleepiness scale (KSS) (Kaida et al. 2006) respectively. The participants' dissociative state was assessed immediately before and after watching the trauma film using the dissociative state scale (DSS) (Bremner et al. 1998). This was because dissociation has been found to alter emotional processing (Ozer et al. 2003). To evaluate each event follow up questionnaires were used after the trauma film and stressor. After the trauma film the participants were asked to rate how familiar they were with the clips used in the trauma film and also how personally relevant they found the content. After the stressor the participants rated how difficult they found the task and how they rated their performance.

Intrusive memories were assessed using two measures. Firstly the participants reported all the intrusive memories they experienced in an intrusion diary for one week after the trauma film. Only intrusions that were recorded after the experimental manipulation i.e. from the first day after the trauma film after either a night of sleep, or after 12 hours of sleep deprivation, were included in the analysis. This was to allow comparisons between the groups. The second measure used was the impact of event scale (Horowitz et al. 1979), a clinical scale assessing the three subdivisions of post-traumatic stress disorder symptomology: intrusions, avoidance and hyperarousal.

Sleep: Polysomnographic (PSG) recordings were conducted to determine the effect of the experiment on sleep physiology. All the participants had an adaption and baseline recording. The adaption night was to get the participants used to wearing the PSG equipment so that the baseline night was as true a representation of the participants sleep as possible. The non-sleep deprived participants had their sleep recorded the night immediately following the trauma film while the sleep deprived participants were recorded during the sleep deprivation period and on the first night of sleep following sleep deprivation i.e. the recovery night. PSG recordings during the sleep deprivation period were undertaken to ensure the participants did not sleep, however the participants were also monitored by either myself or a research assistant to prevent sleeping.

Reducing confounding factors: In order to reduce the potential cofounding effects of caffeine and alcohol, the participants were asked to abstain from both of these before the experiment. Initially the idea of participants maintaining their normal caffeine intake was contemplated, to avoid the withdrawal effects of caffeine. However, James and Rogers (James and Rogers 2005) report that self-administration of an individual's normal daily level is difficult. Hence it was decided to instruct participants to stop consuming caffeine altogether. Hence the participants were asked to abstain from caffeine and alcohol three days before the experiment. Participants were also tested for drugs of abuse during the experiment. Drugs tested were: cannabinoids; cocaine including crack cocaine; amphetamines including methylamphetamine, ecstasy, pseudoephedine and ephedrine; benzodiazepines; barbiturates; and opiates including heroine, codeine, dihydrocodeine and pholcodeine.

Activity monitoring: During the study (after 12 participants: six sleep deprived and six non-sleep deprived) two additional measures were introduced, which were to look at the activities the participants undertook after watching the trauma film: activity VAS and activity log. The reason for the introduction of these measures was due to the fact that one participant reported a very high level of intrusive memories and that this participant spent the entire sleep deprivation period reading, something most participants did not do. Since it is known that verbal activities undertaken either during or up to four hours after experience an analogue traumatic event cause an increase in intrusion frequency, while visual spatial activities do the opposite (Holmes et al. 2004), what the participants did after watching the film may impact on the intrusion frequency.

2.2.5. Baseline assessment

Before the experiment the participants were characterised in terms of their mood, personality and health. A circadian screen was undertaken to assess the participants' circadian rhythms, and the cortisol awakening response was assessed in all the participants. The cortisol awakening

response is thought to be a potential biomarker of psychological and physical health (Fries et al. 2009). PSG recordings of the participants' sleep were also made at baseline as discussed above.

Mood, personality and health: The participants' trait mood, personality, and personal and family health were assessed by the profile of mood state (POMS) (McNair et al. 1992), Eysenck personality questionnaire (EPQ) (Eysenck and Eysenck 1964) and the health questionnaire (HQ) (Spitzer et al. 1999) respectively. How the participants' mood changed over the day (diurnal mood) was assessed using the mood VAS. Participants completed the scale every 2 hours from 8am to 10pm for two days before the experiment began. The scales were completed at fixed time points, rather than being aligned to the participants' individual sleep timings i.e. at 10 am rather than two hours after waking, etc. The advantage of aligning the time points to the participants' sleep timings is that the questionnaire would be completed during the same circadian phase rather than clock time. However, in this study participants were excluded with extreme sleep timings, therefore all the participants were deemed to have a similar circadian phase. Therefore fix time points were chosen to allow direct comparisons between the participants. The mood VAS scales used to assess diurnal mood were also used to assess mood fluctuation: how much mood changes over the day. For details of how mood fluctuation was calculated see mood VAS in section 2.5.2.1 (p.54).

Circadian screen: Extreme morning and evening types were excluded using the morningness-eveningness questionnaire (MEQ) (Horne and Ostberg 1976). The participants' rest-activity cycle was recorded using an actiwatch (for full details see section 2.5.2.2. p.58) that was worn for 10-14 days before the experiment, enabling long term monitoring for their rest-activity pattern which was used as an approximation for the sleep-wake cycle. Although the sleep-wake cycle is driven by the circadian clock it is also influenced by other factors such as work and social commitments (Borbely 1982, Roenneberg et al. 2003). Therefore a more robust rhythm was also measured: the melatonin rhythm is a good marker of the circadian clock (Wirz-Justice 2007, Lewy

et al. 1999) and can be measured by analysis of its metabolite (6-hydroxymelatonin sulphate: aMT6s) which is found in the urine. Urine samples were collected over a 48 hour period once within the study to enable two full circadian cycles of melatonin levels to be determined (the full details are provided in section 2.5.2.2 p.58).

Cortisol awakening response: Cortisol is one of the key outputs of the hypothalamic-pituitary-adrenal (HPA) axis stress response (de Kloet et al. 2005). Although cortisol release shows a robust circadian rhythm, the cortisol awakening response (CAR) is distinct from the circadian modulation and is in fact thought to be directly related to the sleep-wake transition (Wilhelm et al. 2007). The CAR is the increase in cortisol in the first half hour immediately after waking (Fries et al. 2009). Abnormalities to the CAR have been associated with many physiological and psychiatric conditions including cardiovascular disease, chronic pain and depression (Fries et al. 2009). Therefore, the CAR can be used as a marker for health status. In this study the CAR was assessed by analysis of salivary cortisol levels at four time points after waking: immediately after waking, then 15, 30 and 45 minutes after waking (full details in section 2.5.2.2 p.58).

2.2.6. Eligibility screening

The key systems investigated in this study (sleep, mood and emotional processing) are highly variable between individuals. Therefore a homologous population as possible was selected to reduce variability as much as possible. Firstly all the participants included in the study were university students and so living in a similar environment: living independently from their parents, with the responsibility of caring for themselves, plus living with the added pressure of studying at university. The full inclusion and exclusion criteria are detailed in **table 2.1**. Briefly the participants were required: i) to be aged 18-25 years of age, ii) have no personal or family history of any psychological or psychiatric disorders, iii) no history of drug or alcohol abuse, iv) to be non-smokers, v) to have good sleep quality and vi) to not have an extreme diurnal preference. A

difference set of criteria were applicable for at-risk participants, which are detailed in Chapter 6 (section 6.2.1 p.177).

Eligibility for the study was assessed at two stages. First, upon contact with a potential participant, initial screening was undertaken ensuring basic eligibility such as age, location etc., all of which were confirmed at the study interview (for full details see **table 2.1**). Second, a study interview was conducted to inform the participant about the study, to obtain informed consent for the study and to complete the final eligibility screening. During the study interview the Mini International Neuropsychological Interview (MINI) (Sheehan et al. 1998) was conducted and the participants were required to complete a number of questionnaires: the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al. 1989), Morningness-eveningness Questionnaire (MEQ), Mood Disorder Questionnaire (MDQ) (Hirschfeld et al. 2000) and General Health Questionnaire (GHQ) (Hankins 2008a, Hankins 2008b).

Three of the exclusion criteria used in the study, were added during the course of the study. The first was to exclude participants who went to bed regularly later than 2am. This, in addition to the MEQ, ensured that the selected participants were not extreme late types (which are more likely than extreme morning types in people of this age range (Roenneberg et al. 2003)). Secondly participants were excluded if they had a history of fainting in situations that may be induced by the trauma film. For example, if fainting had ever occurred due to the sight of blood, since the trauma film contained scenes of blood. This was introduced as several participants had fainted in another study using similar film footage undertaken by collaborators (Emily Holmes and colleagues). Additional measures were also introduced in the study protocol to minimise the risk of fainting as discussed below. Finally participants were excluded if they regularly consumed high levels of caffeine (more than 300mg per day). This was to ensure that the three day abstinence from caffeine during the experiment was a sufficient wash out period.

Participants <u>without</u> family history of mood disorders	**Participants <u>with</u> family history of mood disorders**	**Level of screening**
18-25 years old	18-25 years old	Initial
Student	Student	Initial
Live in oxford	Live in oxford	Initial
Non smoker	Non smoker	Initial
Not been in another study that uses film footage	Not been in another study that uses film footage	Initial
Not travelled through a time zone in the last two weeks	Not travelled through a time zone in the last two weeks	Initial
Low caffeine consumption (below 300mg/day)	Low caffeine consumption (below 300mg/day)	Initial
Get 6-8 hours of sleep per night	Get 6-8 hours of sleep per night	Initial
Don't go to bed after 2am regularly	Don't go to bed after 2am regularly	Initial
No history of fainting	No history of fainting	Initial
Not pregnant (women only)	Not pregnant (women only)	Initial
No personal history of psychiatric or psychological disorders	No personal history of psychiatric or psychological disorders excluding hypomanic episodes	Study interview: negative screen on MINI, MDQ and GHQ
No family history of psychiatric or psychological disorders	Must have at least one first degree relative with a diagnosed mood disorder	Study interview: negative screen on MINI
No personal or family history of drug or substance abuse	No personal history of drug or substance abuse	Study interview: negative screen on MINI
Good sleep quality	N/A	Study interview: score of 5 or less on PSQI
Intermediate or moderately morning/evening diurnal preference	N/A	Study interview: MSQ score of 52-69

Table 2.1 Inclusion and exclusion criteria

Initially the Mood Disorder Questionnaire (MDQ) was used as a screening tool and participants were only included with a low score (0-2). The MDQ is described in full in section 2.5.2.1 (p.54). Briefly, the MDQ can be used as a screening tool for bipolar disorder or manic symptomology. The questionnaire consists of three questions and a positive screen for bipolar disorder requires all three questions to meet certain criteria. As long as a positive screen is not obtained on the scale as a whole, the first question can be used in isolation to look at manic symptomology. This question lists 13 symptoms of mania. An individual reporting having experienced up to two symptoms from this list, has previous been used as a low vulnerability marker for the

development of a mood disorder (Rock et al. 2010). Hence in this study a score of 0-2 was initially required, as an additional screen to ensure the participants had a low risk of developing a mood disorder. However, further investigation into the use of this criterion as a vulnerability marker for mood disorders revealed little evidence supporting this claim, therefore this exclusion criteria was modified to allow any score on the MDQ as long as a positive screen was not obtained.

Training for the administration of the Mini International Neuropsychological Interview (MINI) was provided by Professor Guy Goodwin from the Department of Psychiatry, University of Oxford.

2.2.7. Participant recruitment

Participants were recruited via a number of sources: poster advertisement displayed within University of Oxford and Oxford Brookes University premises; online advertisement on websites for the Nuffield Laboratory of Ophthalmology (NLO), the University of Oxford and the Department of Psychiatry; online advertisement on the free online newspaper "The Daily Info"; a stall at the University of Oxford's fresher's fair; and also by word of mouth. Potential participants were sent a copy of the study information sheet, and if interested they were initially screened for eligibility for the study via email. After passing the initial screen, they were invited to attend a study interview. During the study interview the study was explained in detail and any questions or concerns addressed. Written informed consent was obtained by the principal researcher (Kate Porcheret) before the final eligibility screening was undertaken. Full details of the initial eligibility screening and study interview are described in section 2.2.6 (p. 46).

2.2.8. Ethics

Ethical approval for this study was sort and obtained from the NHS National Research Ethics Service (NRES). The original application was sent to the Milton Keynes ethics committee which later was taken over by the Berkshire ethics committee. The study was assigned the REC number: 9/H0603/2. NHS trust approval was sought and obtained from the Oxford Radcliffe Hospitals NHS Trust R&D and was assigned the R&D number: 5866. Throughout the course of the study six

amendments were made to the original application to cover the changes made to the protocol, all of which received both NRES and R&D approval. Additional ethical approval for the recruitment of students from Oxford Brookes University was sought and obtained from the Oxford Brookes University Research Ethics Committee. A timeline of the ethical approval processes is detailed in **table 2.2**.

2.3. Development of measures

During study 2 the mood VAS was modified to improve its sensitivity. Initially the scale ranged from 'not at all' to 'extremely' and included 12 moods: sad, calm, horrified, happy, fearful, hopeless, angry, alert, sleepy, awkward and tense. However, it was found that the full range of the scale wasn't used so the scale was modified so the high end anchor was changed to 'very' to make the scale more sensitive. In addition four more moods were added (restless, irritable, racing thoughts and anxious) to assess moods affected by sleep deprivation and trauma (American Psychologyical Association 2000, Spiegel et al. 2004, Leproult et al. 1997, Banks and Dinges 2007). These changes were made after the first four participants (all sleep deprived) had completed the study.

2.4. Development of analysis

Several changes were made to data analysis in study 2 in terms of i) which measures were included in the final analysis and ii) the scoring of the PSG recordings.

2.4.1. Exclusion of measures

In the final analysis, the Karolinska sleepiness scale (KSS) and mood VAS scales that were completed every two hours during the experiment (not including those used to assess diurnal mood) were not used, as they did not enable a comparison between the sleep deprived and non-sleep deprived groups, as they were completed throughout the sleep deprivation period by the sleep deprived group, while the non-sleep deprived participants were sleeping.

Ethics application submitted:	16th December 2008
Ethics committee meeting:	28th January 2009
Ethics approval received subject to amendments:	25th February 2009
Approval of amended protocol:	1st June 2009

Table 2.2 Ethical approval timeline

The activity log was not used in the final analysis as it was also not comparable between the groups as it was completed over different time periods i.e. the evening before going to sleep for the non-sleep deprived group and the entire night for the sleep deprived group. Finally, although intrusive memories can involve mental images and verbal thoughts, only those involving mental images were included in the final analysis. This was because mental images are more associated with past emotional events and induce a stronger emotional reaction (Holmes and Mathews 2010). Also verbal thoughts specifically generated by the experiment i.e. those related to the trauma film, were more difficult to differentiate from other thoughts.

2.4.2. PSG scoring

PSG recordings can be scored by one of two methods: the original Rechstschaffen and Kales method (Rechstschaffen and Kales 1968) or the new American Academy of Sleep Medicine (AASM) method (Iber et al. 2007). Initially the PSG recordings were scored according the ASSM criteria. However, due to the montage used for the PSG recordings the scoring method was changed to the Rechstschaffen and Kales criteria. The reason for this was that the electroencephalographic (EEG) channels (the channels recording brain activity on the scalp) were referenced to the midline central derivation (Cz) rather than to the mastoids (A1 and A2). The amplitude of the EEG wave forms is determined by the distance between the recording electrode and the reference electrode. The amplitude of the waveforms is important for scoring of slow wave activity since delta waves have to have amplitude of 75uV. In the AASM criteria, the EEG is

scored from one central channel (C3 or C4) and the EEG channels are assumed to be referenced to A1 or A2. Since the distance between C3 and A2 is longer than to Cz as used in this study, the amplitude of the waveforms was lower than expected. However, in the Rechtschaffen and Kales scoring, delta waves are scored from the frontal channel (Fz) which has a more similar distance to Cz as the C3 to A2 distance. Therefore, the PSG recordings were rescored using the Rechtschaffen and Kale criteria.

2.5. Methods and measures

The methods used in these studies cover a wide range of techniques studying both physiological and psychological parameters. The vast majority of the measures used were questionnaires and scales, most of which are well validated and have been used in previous research. Those not used in previous research were designed specifically for this study. The methods and measures used are listed alphabetically for each study separately.

2.5.1. Study one

Light data: Two measures of light were used: light exposure and light dose. Light exposure is a measure of the number of hours spent outside. It was calculated based on the self-reported number of hours spent outside for free days and work days on the MTCQ. The weighted average of the number of hours reported for free days and work days was calculated using the number of work days also reported on the MTCQ. If no work days were given, five work days were assigned for that participant. The equation for light exposure: ((light exposure on work days *number of work days) + (light exposure of free days * (7-number of work days)))/7.

Light dose is a measure of the light intensity received during the period of light exposure. An individual's light dose was calculated for the day they completed the MTCQ, to create an individual reading for each participant to create a continuous data set to enable statistical analysis to be performed. The daily irradiance (a measure of light per unit area) of that day was divided by the hours of daylight on that day and then multiplied by the number of hours spent

outside on average (light exposure). The equation for light dose: (daily irradiance/hours of daylight)*light exposure. The hours of daylight for each day of the collection periods for each city were calculated using the world clock (http://www.timeanddate.com/worldclock).Daily irradiance for the collection periods (May and October 2010) were obtained from three sources. The data for Oxford, Groningen and Munich were provided by Dr Lucien Wald (Ecole des Mines de Paris). These data were obtained from Meteosat satellite images and converted into data maps of solar radiation using the Heliosat method by Dr Wald (www.helioclim.org).The data for Perth and Melbourne were obtained from the Australian Bureau of Meteorology. These data were derived from satellite imagery processed by the Bureau of Meteorology from the Geostationary Meteorological satellites GMS-4, GMS5 and MTSAT-1T of the Japan Meteorological Agency and the USA's National Oceanic and Atmospheric Administration (NOAA) GOES-9 satellite (information provided by the Australian Bureau of Meteorology). Finally the data for Auckland were obtained from the New Zealand Metrological office. The data were provided from ground station readings (ground station: Auckland 1962).

Munich chronotype questionnaire (MTCQ): The online version of the MCTQ (Roenneberg et al. 2003) was used in the native language of the country of each university. The MCTQ consists of six questions concerning sleep timings. The most important aspect of the questionnaire is that these questions are asked for both work days and free days. Free days are those which involve no work or social commitments. There are also questions concerning work timings, light exposure and participant demographics. The primary outcome of the MCTQ is the Mid Sleep Point on free days (MSF). This parameter is calculated as the midpoint between sleep start and sleep end. The MSF was corrected for oversleep on free days (MSFsc): oversleep occurs as a result of sleep debt, as described by Ronnenberg and colleagues (Roenneberg et al. 2004). Equation for MSFsc: MSF – (SDf – (((nWD*SDw)+(7-nWD)*SDf)*7) where SDf is the sleep duration on free days, SDw is the sleep duration on work days and nWD is the number of work days. This correction was carried out

using the number of work days given in the questionnaire. In cases when the number of work days was not specified, five work days were assigned.

2.5.2. Study two

2.5.2.1. Self-report measures

Activity visual analogue scale (activity VAS): The activity VAS was specifically designed for this study. It was used to rate the amount of time spent doing visual-spatial or verbal activities in the first 12 hours post trauma film. Visual-spatial activities were defined as "using your imagination e.g. jigsaw, cards, board games" and verbal activities were defined as "talking to other people, reading, writing". Each type of activity was rated by marking a 100 millimetre line from 0% to 100% of the participants' time, giving a score from 0 to 100.

Dissociative state scale (DSS): The 19 item version of the DSS (Bremner et al. 1998) was used to assess the dissociative state of the participants immediately before and after watching the trauma film. To assess the level of dissociation the participants had to rate how much they felt from 'not at all' to 'considerably' for a list of questions including 'do things seem to be moving in slow motion?' and 'do you space out, or in some other way, lose track of what is going on?' The score for each question was summated to give a score from 0 to 57.

Eysenck personality questionnaire (EPQ): The 90 question version of the EPQ (Eysenck and Eysenck 1964) was used to define trait personality characteristics. The scale consists of yes/no questions assessing four personality characteristics: extroversion, neuroticism, psychoticism and lie, with a possible maximum score of 21, 23, 25 and 21 respectively.

General health questionnaire (GHQ): The 12 item short version of the general health questionnaire (Hankins 2008a, Hankins 2008b) was used as baseline assessment of depressive symptomology. Originally devised as a screening tool for identifying minor psychiatric conditions, the questions concern symptoms of a depressive episode. The questionnaire asks participants to

rate if they have experienced, and to what extent, 12 symptoms of depression within the last two weeks. The GHQ was scored using the Likert method, giving an outcome score range of 0-36 and a threshold of 11/12 for an indication of mental illness (Goldberg et al. 1997).

Health questionnaire (HQ): The HQ was an adapted and abridged version of the Patient Health Questionnaire (Spitzer et al. 1999) and used to record demographics and to screen for any medical conditions and other factors that can affect circadian and sleep behaviour, e.g. night shifts, jet lag, etc.

Impact of event scale (IES): The revised 22 item version of the IES (Horowitz et al. 1979) was used to evaluate post-traumatic stress disorder symptomology in relation to watching the trauma film. Each item on the IES was rated for how distressing it had been found from 'not at all' to 'extremely'. The items on the scale were divided into those relating to the three symptom types: intrusions, avoidance and hyperarousal. Both intrusions and avoidance had a range of scores from 0 to 32, while hyperarousal had a score from 0 to 24. The scale was amended to make it suitable for a sleep deprived population by replacing 'sleep' with 'restful state' for items 2 and 15. The scale was completed 12 hours after watching the trauma film: 12 hours into the sleep deprivation period and following a night of sleep for the non-sleep deprived participants.

Intrusion diary: The intrusion diary required participants to report their intrusive emotional memories of the film for each of the six days following the experimental manipulation i.e. from the morning after sleep deprivation. Intrusions were defined as spontaneously occurring memories of the trauma film (Holmes et al. 2009). The intrusion diary required the participants to report all the intrusive memories experienced each day and time of day. They also had to indicate if the intrusive memory involved mental images, verbal thoughts or both, although only those involving mental images were used in the final analysis. Finally the participants had to detail the content of each intrusive memory, so that it could be verified as matching a scene in the trauma film. The instructions given about completion of the diary were standardised and required the

participants to provide feedback to ensure complete understanding. For the precise instructions given see the study protocol (appendix V). Recorded intrusive memories were only included in the analysis if they could be related back to the trauma film i.e. involved a specific scene or theme from the film.

Mini international neuropsychological interview (MINI): The MINI (Sheehan et al. 1998) is a structured interview that is based on DSM-IV and ICD-10 criteria for psychological and psychiatric conditions and was used as a screen for the participants' psychiatric and psychological health. Participants that screened positively for any mental health subscale were excluded. A positive screen is dependent on the participant answering questions that suggest they may have a mental health problem. See appendix VI for a copy of the MINI.

Mood disorder questionnaire (MDQ): The original version of the MDQ (Hirschfeld et al. 2000) was used as a baseline assessment of manic symptomology. Originally devised as a screening tool for bipolar disorder, it was used in this study as a measure of manic symptomology. The questionnaire consists of three questions: the first asking whether any of 13 symptoms have been experienced, the second asking, if one or more symptom had been experienced, if they had ever been experienced at the same time and finally asking to what extend these symptoms impact on the participant, from 'not at all' to 'serious problem'. A positive screen requires all of the following: i) seven or more symptoms to have been experienced, ii) that they have occurred at the same time and iii) for the impact of these symptoms to be either a moderate or serious problem. Participants were excluded with a positive screen.

Mood visual analogue scale (mood VAS): A mood VAS consisting of 15 moods was used for assessment of current mood, based on a scale originally devised by Monk (Monk 1989). Each mood was rated by marking a 100 millimetre line from 'not at all' to 'very', giving a score between 0 and 100. The moods included were: sad, calm, horrified, happy, fearful, hopeless, angry, alert, sleepy, awkward, tense, restless, irritable, racing thoughts and anxious.

The mood VAS was used for assessment of diurnal mood. Diurnal mood ratings were assayed over two days during the study. The participants were asked to complete the mood VAS every two hours from 8am to 10pm. The data were binned into two hour epochs 8am to 10am, 12pm to 2pm, 4pm to 6pm and 8pm to 10pm. The final epoch (8pm to 10pm) on the second collection day was removed from analysis as this time point occurred after the trauma film. The same scales were also used to assess mood fluctuation i.e. how much mood changed over the day. This was calculated based on the variation in the scores given for each mood. The standard deviation of the mean was calculated for each mood, participant, and for the whole collection period (two days). The mean standard deviation for all the moods was then calculated for each participant. A high value indicates more mood fluctuation as there is more variation in the scores given on the mood VAS.

The mood VAS was also used to assess the impact of the trauma film and stressor on mood, for which the scale was completed immediately before and after watching the trauma film and completing the stressor task. For this assessment the mood VAS was used to create a positive and negative affect score: Positive affect = (happy + calm)/ 2; Negative affect = (sad + horrified + fearful + hopeless)/ 4.

Morningness, eveningness questionnaire (MEQ): The MEQ (Horne and Ostberg 1976) was used as a measure of diurnal preference (an approximation of chronotype). The questionnaire consists of 19 items asking a range of questions relating to the preferred timings of sleep, and physical and cognitive activities, resulting in a score between 30 and 86. Participants were classified as extreme or moderate morning or evening types or neither type based on the scoring classification for a student population: extreme morning type = 70-86; moderately morning type = 59-69; neither type = 42-58; moderately evening type = 31-41, extreme evening type 16-30 (Horne and Ostberg 1976). Extreme morning or evening types were excluded.

Pittsburgh sleep quality index (PSQI): The PSQI (Buysse et al. 1989) is a measure of sleep quality. The questionnaire consists of 10 items relating to the participants' own perception of their sleep habits and quality. The items were used to calculate the global sleep quality score which has a range of 0- 21. Participants were excluded with a score of 6 or more as a score of 5 or below is considered to indicate good sleep quality.

Positive and negative affect scale (PANAS): The original short version of the PANAS (Watson et al. 1988) was used to assess the effect of the trauma film on mood. The scale consists of 10 positive and 10 negative affect scales, to be rated from 'not at all' to 'extremely', giving a range of scores from 10 to 50 for both positive and negative affect.

Profile of mood state (POMS): The long version of the POMS (McNair et al. 1992) was used to define trait mood characteristics. The scale consists of 65 items used to assess six mood states: tension-anxiety, depression-dejection, anger-hostility, vigour-activity, fatigue and confusion, with a maximum score of 36, 60, 48, 32, 23 and 24 respectively. A total mood disturbance score was calculated: (tension-anxiety + depression-dejection + anger-hostility + fatigue + confusion) - vigour-activity (Markus and Firk 2009). The total mood disturbance was only calculated for the participants without a family history for a mood disorder.

State-trait anxiety inventory (STAI): The STAI (Spielberger et al. 1983) was used to assess anxiety levels pre- and post-watching the trauma film. The scale consists of 20 statements concerning states of mind, which were rated from 'not at all' to 'very much so'. The scale had a range of scores from 0-80.

2.5.2.2. Objective measures

Actigraphy: The actiwatch with light sensor (AWL) from CamNtech Ltd., Cambridge (http://www.camntech.com/) was used within the study. The participants were asked to wear the watch on their non-dominant wrist, and to keep it uncovered as much as possible. The

participants kept a sleep and activity diary while wearing the watch to aid sleep analysis of the actigraphy data. The participants were told to record when they went to bed, when they woke up, when they took the watch off, and also when they undertook high levels of activity (such as cycling, jogging) and low levels of activity (such as reading, watching TV). The watches used were rotated between the two groups (sleep deprived and non-sleep deprived) to minimise the effect of differences between the activity and light sensors of the different watches. The actigraphy data was analysed with version 7 of the actiwatch activity and sleep analysis software (CamNtech Ltd., Cambridge). The actigraphic data was sampled in one minute epochs. Recordings were adjusted for daylight saving time if applicable by adding or removing an hour of data during the sleep period of the relevant night in autumn and spring respectively. Periods when the watch had been removed for more than half an hour and less than three hours were edited by replacing the missing data with the average activity count for that day. For analysis of 'sleep' parameters the bed times and get up times were assigned using the information recorded in the sleep and activity diary. The software was then used to calculate the parameters relating to the sleep period as shown in **table 2.3** and have previously been defined by Wulff and colleagues (Wulff et al. 2012). Days were excluded from analysis if the watch had been taken off for a period of three hours or longer. The sleep parameters for the days included were averaged for each 'week', where a 'week' had a minimum of four days. The 'weeks' were then averaged for the whole study. Weighted averages were performed for 'weeks' that contained unequal numbers of days. Nonparametric circadian rhythm analysis was also performed using version 7 of the activity and sleep analysis software (CamNTech Ltd., Cambridge). This analysis involves an average 24 hour profile to be calculated for each week, by overlaying each day midnight to midnight (Wulff et al. 2012). Again days with periods of more than three hours without data were excluded. A minimum of two consecutive days were used for the analysis and weighted averages were performed to create 'week' and study averages.

Sleep parameter	Calculation
Sleep start	First 10 minute period of immobility (no activity) following bed time with no more than 1 epoch of movement (CamNTech 2007)
Sleep end	Consecutive six minute period of activity before get up time below a threshold (activity score of 6) with no more than two epochs above this threshold (CamNTech 2007)
Total sleep time	Time from sleep start to sleep end, including periods of 'wake'.
Actual wake time	Time spent awake during sleep period (from sleep start to sleep end). An epoch is defined as awake with a total activity score of 40 or more.
Actual wake (%)	Percentage of wake relevant to total sleep time
Sleep efficiency (%)	Percentage of sleep relevant to total sleep time
Sleep latency	Time from bed time to sleep start
Wake bouts	Number of periods of wake
Mean sleep bout time	Mean length of time spent in continuous sleep during total sleep period
Mean wake bout time	Mean length of time spent in continuous wake during total sleep period
Fragmentation index	The number of one minute immobility periods as a percentage of the total sleep time

Table 2.3 Sleep parameters

The circadian parameters are defined in **table 2.4**, unless stated these parameters have been previously defined by Wulff and colleagues (Wulff et al. 2012).

Polysomnography (PSG): Two polysomnographic systems were used: Somnomedic PSG 10-20 (Somnomedics, Randersacker, Germay: http://www.somnomedics.eu/) and the Vitaport VP3 (TEMEC Instruments B.V, Kerkrade, The Netherlands: http://www.temec.com/portal/index.php). Both systems were recorded with the same electrode montage: six 10-20 electroencephalogram channels (Pfz, Fz, C3, C4, Pz, Oz) and two electro-oculogram (EOG) channels (left and right) and a ground channel on the forehead, all referenced to Cz.

Circadian parameter	Calculation
Interdaily stability	Ratio between the variance of the average 24 hour pattern around the mean and overall variance (Van Someren et al. 1997)
Intradaily variability	Ratio of the mean squares of the difference between successive hours and mean squares around the grand mean (Van Someren et al. 1997)
Rest phase (L5) onset	Time of onset of the five hours of least activity
Rest phase (L5) activity level	Mean activity score for the L5 period
Active phase (M10) onset	Time of onset of the 10 hours of most activity
Active phase (M10) activity level	Mean activity score for the M10 period
Relative amplitude	Ratio between M10 and L5 (Van Someren et al. 1997)

Table 2.4 Circadian parameters

Two electromyogram (EMG) channels (submental left and right), where also used, referenced to the chin or Cz, for the Somnomedics and Vitaport devices respectively. The EOG and EMG channels had a sampling rate of 256 Hz, and the EEG channels a sampling rate of 128 Hz.

The data were scored for 30 second epochs according to the American Academy of Sleep Medicine (AASM) criteria (Iber et al. 2007), with the exception that NREM stages 3 and 4 were scored separately according to the Rechstschaffen and Kales (Rechstschaffen and Kales 1968) criteria: a stage 3 epoch has 20-50% delta waves (0-2Hz and amplitude greater than 75μV) and stage 4 epoch has more than 50% delta waves. During scoring notch or low pass filters were applied to the channels to remove high frequency noise to enable classification of the epoch, but not applied to the channels for spectral frequency analysis. Data analysis is discussed in the methods section of Chapter 5 (section 5.2.1, p.132).

Salivary cortisol: Morning saliva samples were collected for assessment of the cortisol awakening response. The levels of free cortisol were assayed in the morning saliva samples provided by the participants for two days during the study. The assaying of the samples was performed by Stockgrad LTD. A radioimmunoassay of cortisol using ^{125}I cortisol was performed (see appendix VII

for the full protocol). The collection was performed by the participants in their home environment, under a normal routine. Participants were instructed to start the saliva collection as soon as they had woken up and then 15, 30 and 45 minutes after waking. They were asked to remain lying in bed throughout the collection period, as well as not to eat or drink, brush their teeth or smoke and not to consume alcohol the night before.

Urine melatonin: Urine samples were collected over a 48 hour period for the assessment of a metabolite of melatonin (6-hydroxymelatonin sulphate: aMT6s). The assaying of the samples was performed by Stockgrad LTD. A radioimmunoassay of aMT6s using AN^{123} as a labelled tracer was used for this analysis (Aldhous and Arendt 1988). The full protocol for how this was performed is in appendix VII. The collection was performed by the participants in their home environment, under the participants' normal routine. Participants were instructed on how to collect the urine, measure the volume and to aliquot two samples for analysis. To analyse the circadian rhythm of the aMT6s data a best fit was applied to the data using a non-linear regression model. Analysis from the raw data was less accurate due to limited data points, large time intervals between the data points and aberrant data points that can distort the picture. Therefore a non-linear regression model was appropriate for this data. From this analysis the period, amplitude, acrophase and mesor of the rhythm was determined. **Figure 2.3** illustrates these features. The period is the length of time to complete one cycle, the amplitude is the size of the rhythm from the mean value (mesor) to its peak. Finally the acrophase is the time of the rhythm's peak, while the nadir is the time of the rhythm's minimum. The model applied to the data was $MT=c+(Amp \times \cos(2\pi \times ((t-tc)/T)))$, where MT is the aMT6s secretion rate (ng/h), t is time, c is the mesor, Amp is amplitude, tc is the phase angle and T is period (Wulff et al. 2012). The model was fitted using a program written for the solver add-in for Microsoft Excel courtesy of Roelof Hut. With this program the model was fitted to the data: a fixed period was used and iterations of the phase, model constants and amplitude where performed to get the best fit. The period was changed in subsequent analysis if the data could not be fitted.

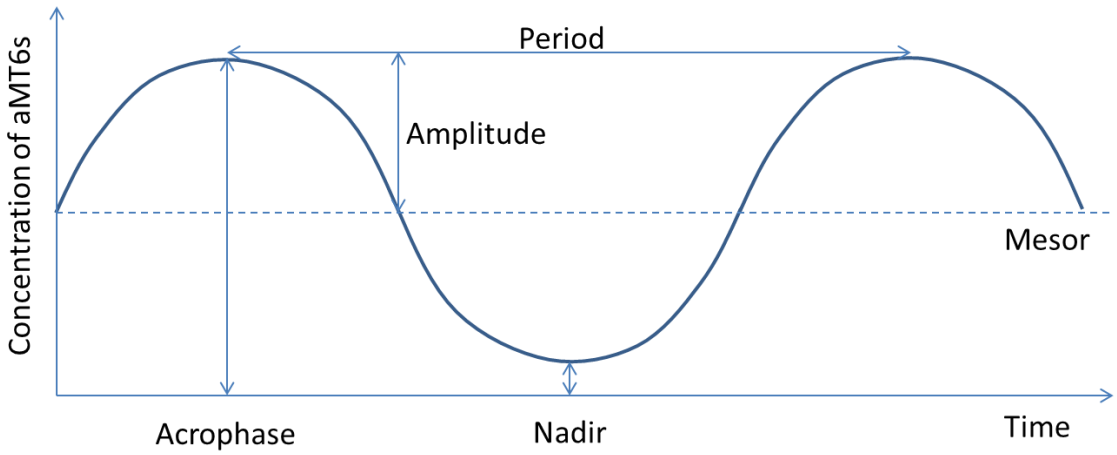


Figure 2.3 Circadain rhythm parameters

2.5.2.3. Study events

Trauma film: The trauma film consisted of a 15:01 minute compilation of a series of depressing and traumatic clips compiled by Lang and colleagues (Lang et al. 2009), with the addition of a clip from Martin Scorsese's The Big Shave (1967). The film contained six clips from films and television adverts, depicting scenes such as a funeral, suicide and bullying. For the full details of each clip see **table 2.5**. Before viewing the film the participants were blind to the content of the film, although they were made aware that they may find some scenes distressing. They were instructed to imagine they were there at the scene watching the events unfold in front of their eyes, to give the film their full attention, not to close their eyes or look away. However, they were told they were free to stop the trauma film at any time. The film was shown on a laptop computer with a 15-inch screen. The participants watched the film alone, while the researcher remained outside. The lights were switched off to increase concentration on the film and the volume was set to full volume using the laptop speakers. After watching the trauma film, the participants were asked to rate how familiar they were with the film clips used in the trauma film and how personally relevant they found them. Both questions were rated from 'not at all' to 'extremely' on an ordinal scale from 0 to 10.

Clip	Reference	Length	Scene description
1	Shawshank redemption	04:42 minutes	Release of bird from window, leaving prison, sitting on a bus, walking down the street/ nearly hit by a car, inside home/ bedsit, working in supermarket, feeding birds in park, in bed at night, packing bag, writing "brooks was here", suicide by hanging.
2	Big shave	01:22 minutes	Man shaving and cutting himself, blood running down face and into sink, running water mixed with blood
3	Advert	36 seconds	Teenagers coming out of school, texting on phone, walking along street, crossing road without looking, boy hit by van, funeral, carrying coffin.
4	Dead poet's society	5:24 minutes	Boy wearing crown of twigs, walking around home at night naked, parents sleeping, getting gun out of desk, parents woken up, looking for son, father finding son shot, mother and father crying. Waking up boy in bed at night, snowy fields, boys walking in snow upset.
5	Advert	1 minute	Girl in bedroom, teacher in classroom, mother in kitchen, girl in library, family in park, girl in canteen, mother and daughter all indicating that a child is being bullied
6	Unknown	2:07 minutes	Girl 1 walking in empty flat, girl 2 sleeping on mattress. Girl 1 sees girl 2 jump from window. See girl 2 on floor.

Table 2.5 Trauma film clips

Stressor: The failure version of the Remote Associates Task (RAT) (McFarlin and Blascovich 1984) was used as a stressor. The RAT is a computerised task run on Eprime, computer software for the design of psychological experiments (http://www.pstnet.com/eprime.cfm). The task was designed to be a difficult cognitive task. However, the participants are told that the task was a simple cognitive task. The participants were read standardised instructions regarding the RAT (see the study protocol: appendix V) (Moberly and Watkins 2006), some of which appeared on the

computer screen. The participants were shown three words on the screen and asked to find the fourth unreported word. They were given two practice trials, then 10 experimental trials. 30 seconds were allowed for each experimental trial and the trial changed automatically. Upon completion the participants were informed that the average score for university students is seven, which is higher than the real average, before being handed their scored answer sheet. Following the task the participants were asked to rate how difficult they found the task from 'very low' to 'very high' and their performance from 'very poor' to 'very good', with both scales having a range of 1-9. Upon completion of the follow up questionnaires, the participants were informed of the true nature of the task.

Sleep deprivation: During the sleep deprivation period, the use of computers, TV, DVD's or music was not allowed but participants could play board games, read, study and talk to the researchers. They were allowed to walk around, but not leave the room. All the participants were continually monitored by staff trained to prevent napping and to ensure compliance and wellbeing. Participants were allowed water ad libitum, and had a choice of snacks every two hours (a sandwich or a piece of fruit). In the morning the participants were allowed a shower. Illumination of the room was kept to between 165-276 lux using artificial fluorescent lights for the entire sleep deprivation period.

3. Sleep timing, chronotype and light

3.1. Introduction

This chapter presents the data from study one which was designed to address the question: **do environmental light levels influence sleep timing and chronotype?** This study had two key hypotheses: 1) that sleep timing and chronotype would be earlier in the southern hemisphere cities studied, and 2) that the earlier sleep timings in the southern hemisphere would be the result of higher environmental light levels in these cities as a result of geographical location.

To summarise the key points of Chapter 1, sleep timing is controlled by the sleep homeostat and the circadian clock (Borbely 1982, Borbély 2009, Borbely and Achermann 1999). Individual variations in the period length of the circadian clock results in, among other things, differences in preferred sleep timings, giving rise to subdivisions of behaviour known as chronotypes: early, late or intermediate. Sleep timings can be used as a marker of chronotype, as long as external factors such as work and social commitments are taken into account. Moreover chronotype can be used as an indicator of sleep timings: individuals that have a late chronotype are likely to go to bed and wake up late.

In terms of the effect of light on chronotype: the timing, intensity and duration of light exposure all influence the entrainment of the circadian clock in humans (Duffy and Czeisler 2009). However, to date in humans, only the duration of light exposure has been shown to have a long term effect on the circadian clock, as sleep timings and chronotype are earlier (i.e. people go to bed earlier and wake up earlier) in individuals that spend more time outside (Roenneberg and Merrow 2007). Therefore the primary aim of this study was to investigate the impact of light intensity on sleep timings and chronotype in a longitudinal naturalistic setting. Different natural light intensities were studied in different geographical locations and at different times of the year.

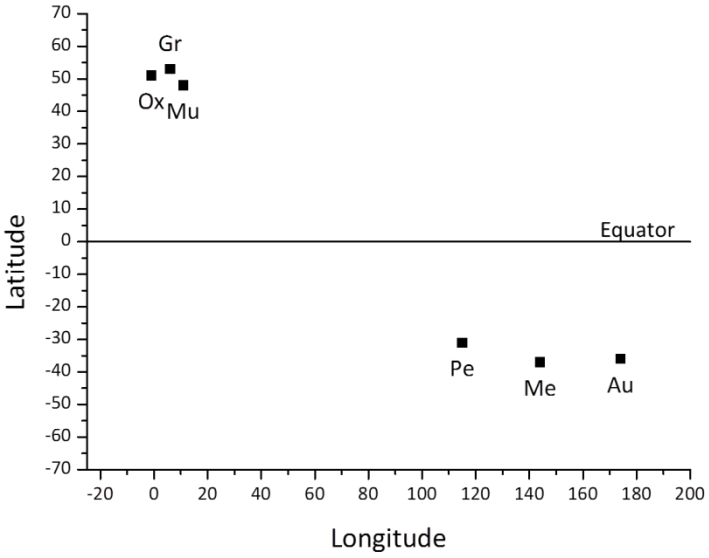


Figure 3.1 Geographical coordinates of participating cities. Latitude and longitude coordinates of Oxford (Ox), Groningen (Gr), Munich (Mu), Perth (Pe), Melbourne (Me) and Auckland (Au).

Chronotype was assessed using the Munich chronotype questionnaire (MCTQ), as described in section 2.5.1 (p. 52) in six cities (Oxford, Munich, Groningen, Perth, Melbourne and Auckland) and two seasons (spring and autumn). The six cities are distributed over the southern and northern hemisphere, and the geographical coordinates are illustrated in **figure 3.1**. These cities were chosen primarily due to the anticipated differences in environmental light levels as a result of geographical location i.e. distance from equator (latitude). Additionally these cities limited the potential cofounding effects of cultural and socioeconomic factors, since they are all of a similar socioeconomic status and cultural background. Since age is also known to affect sleep timings and chronotype a narrow age range of 18-25 years was chosen.

The hypothesis that higher natural light intensities would result in earlier sleep timings and chronotype, was based on the assumption that individuals in this study would be exposed to light throughout the day rather than just receiving light later in the day. As discussed in Chapter 1 (section 1.2.1.1 p.11), the timing of light causes opposing effects (phase advance or delay) on the

clock depending on when it is given: early in the day results in a phase advance, late in the day a phase delay (Czeisler et al. 1989, Jewett et al. 1997, Khalsa et al. 2003). Therefore receiving more light at the end of the day would result in later sleep timing and chronotype.

The impact of social and work commitments on sleep timings can result in a sleep/wake cycle that is out of synchrony with the internal circadian clock. This discrepancy has been coined social jet lag (SJL) as the situation is akin to jet lag produced from traveling across time zones (Wittmann et al. 2006). A high social jet lag indicates a high discrepancy, for example two hours of SJL is like travelling from London to Athens and back every weekend. This discrepancy is often worse for late chronotypes as they tend to get less sleep during work days due to having to wake up for work earlier than their circadian clock dictates (Roenneberg et al. 2007a). It is becoming increasingly apparent that SJL can have deleterious effects on human health as it has been associated with higher stimulant consumption, poorer sleep quality, smoking (Wittmann et al. 2006) and obesity (Roenneberg et al. 2012).

In addition to investigating the relationship between light, sleep timing and chronotype, this study tests a number of other hypotheses concerning chronotype: the influence of latitude and longitude on chronotype; the seasonal impact on chronotype; and whether a seasonal change is due to the tracking of dusk or dawn.

3.1.1. Latitude and longitude

Within the German population, longitude has been associated with chronotype: the further east people live the earlier chronotype (Roenneberg et al. 2004). The same association was also found within German adolescents studying in German schools in 16 different countries around the world, but only when assessed within the same time zone (Randler 2008). The influence of latitude i.e. distance from the equator has yet to be investigated. The closer to the equator the more stable and higher light levels become, due to its position in relation to the sun. Therefore, it was hypothesised that the closer to the equator the earlier chronotype would be.

3.1.2. Season

In terms of the seasonal effect, chronotype (assessed using the mid-sleep point) becomes progressively earlier from winter to spring, with increasing day length, but becomes relatively stable over the summer months (Kantermann et al. 2007). The change in chronotype over the year has been linked to the fluctuations in the timing of dawn (Kantermann et al. 2007). In this study, as participants were studied at differing locations, the impact of latitude needs to be considered. The closer to the equator the less seasonal environmental variations are observed, not least due to less change in day length over the year. Hence it was hypothesised that the closer to the equator the less seasonal variation would be observed in chronotype.

3.1.3. Dawn and dusk

In rodents it has been shown that dusk and dawn are the strongest zeitgebers for entrainment (Roenneberg and Foster 1997). In humans, although chronotype has been linked to the timing of dawn (Kantermann et al. 2007), the influence of dusk has not been investigated. In this study the impact of both dawn and dusk were assessed to determine the underlying influence on human chronotype.

In summary it was hypothesised that the students in the southern hemisphere cities studied would have earlier sleep timings and chronotype, due to higher environmental light levels and being closer to the equator. The students in the southern hemisphere cities studied were also expected to have less seasonal variation as they are closer to the equator. Finally it was hypothesised that for all the students sleep timing and chronotype would be more linked to dawn than dusk.

3.2. Methods

A detailed description of all the methods and measures used in this study can be found in chapter 2 (section 2.5.1. p.52). Below is the protocol used for the study along with the details for additional analysis performed.

3.2.1. Study design

Students aged 17-26 years old were recruited from six different universities from around the world: University of Oxford, UK; University of Groningen, Netherlands; Ludwig Maximilians University, Munich, Germany; University of Western Australia, Perth, Australia; Monash Univeristy, Melbourne, Australia; Univeristy of Auckland, New Zealand. The age range of students included in the study was expanded from 18-25 year olds to include 17 and 26 year old, to increase the number of participants included in the study. Students were asked to completed the online version of the Munich Chronotype Questionnaire (MCTQ) twice: in May and October 2010. Light data was collected during these periods from a number of sources as detailed in section 2.5.1 (p.52).

In total 13,299 individuals completed the MTCQ online world-wide. Over half the participants were excluded from the analysis as they failed to meet the required criteria (see section 3.2.5 p.72). Of the remaining participants, 489 completed the questionnaire during both collection periods (May and October), hence only one time point was included in the analysis. A final 6443 responses were included in the analysis.

The primary outcome measures of this study were sleep timing characterised by the midpoint of sleep on free days (MSF) and light intensity represented by the amount of time spent outside as well as light intensity. These measures are described in full below. Other factors known to influence sleep timing and chronotype including age, sex, use of an alarm clock, and shift work, were also assessed by the MCTQ. These factors were not controlled for between the cities, but either resulted in exclusion from the study (use of alarm clock of free days and shift work) or used as covariates in the statistical comparisons between the cities.

3.2.2. Mid sleep point

The MCTQ was used to obtain the mid sleep point of free days, a measure of chronotype (see section 2.5.1. p.52 for details). In this study a number of manipulations to the mid sleep point (MSFsc: Mid Sleep point on Free days, Sleep Corrected) were made.

3.2.2.1. Correcting for time zone and daylight saving

By definition the local clock time remains the same throughout a time zone. However, local clock time in relation to sun time differs according to longitude within the time zone. Daylight saving adjustment of local clock time causes a further discrepancy between local clock time and sun time. Despite these discrepancies between clock time and sun time, in this study MSFsc was not corrected for daylight saving or longitudinal position in time zone. It is the actual local clock time which is the relevant measure of interest here, as it is local clock time that people used to measure the time of day.

3.2.2.2. MSFsc relative to sunrise and sunset

The timings of sunrise and sunset vary over the year. To determine if chronotype was more closely linked to sunrise (dawn) or sunset (dusk), sleep midpoint was calculated relative to the time of sunrise or sunset on the day the MCTQ was completed: MSFsc relative to sunrise (MSFsc_SR) = MFSsc – time of sunrise; MSFsc relative to sunset (MSFsc_SS) = MSFsc – time of sunset.

3.2.3. Social jet lag

Social jet lag (SJL) is the discrepancy between the sleep-wake cycle that is influenced by social and work commitments and the internal circadian clock. Therefore SJL is calculated as the difference between these two cycles i.e. the work day cycle and that of free days. The mid-sleep point of work and free days was used as a measure of the sleep-wake cycle. SJL = MSF – MSW; MSF = Mid-Sleep point of Free days, MSW = Mid-Sleep point of Work days (Wittmann et al. 2006).

3.2.4. Light levels

Two measures of the light levels experienced by the participants were used: light exposure and light dose. Light exposure is a measure of the number of hours spent outside, while light dose is a measure of the light intensity received during the light exposure. For full details on how these measures were calculated see section 2.5.1 (p.52).

3.2.5. Data sorting

Participants who had completed the questionnaire during both collection periods, spring and autumn, were identified using name and email address. These participants were only included once either in spring or autumn. Individuals were also excluded if they were outside the age range; did not indicate they were currently living in any of the cities of interest; completed the questionnaire outside May or October 2010; or had undertaken shift work at any point. Individuals were excluded if they indicated they used an alarm clock on free days, as this would mean these days were not true 'free' days. To enable comparisons between the hemispheres the months of data collection were assigned to season: May in northern hemisphere and October in southern hemisphere assigned to spring; October in nothern hemisphere and May in southern hemisphere to autumn.

3.2.6. Statistical analysis

Statistical analysis was performed using SPSS (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp). All data satisfied tests for normal distribution. Univariate analysis of variance (one way ANOVA) was performed to test for the effect of city. Univariate analysis of covariance (ANCOVA) analysis was used to enable the introduction of covariant factors. Two way ANOVA and ANCOVA analysis was performed to enable interactions to be investigated. Finally Bonferroni post hoc analysis was applied to enable between city comparisons.

3.3. Results

3.3.1. Demographics

Table 3.1 presents the demographic data for these individuals. Using a one way ANOVA a significant effect of city was found for all parameters apart from consumption of sleep medication.

3.3.2. Mid sleep point on free days

MSFsc relative to local clock time was found to be earlier in the southern hemisphere cities than in the northern hemisphere cities (**Figure 3.2a**). Groningen was found to have the latest MSFsc and Auckland the earliest. Overall the cities were ranked (in order of decreasing MSFsc, averaged for spring and autumn): Groningen and Oxford; Munich; Melbourne; Perth; Auckland. Two way ANOVA analysis of city and season revealed a significant effect of city ($F=25.66$, $p\leq0.001$) but no season effect ($F=0.57$, $p=0.45$) or city/season interaction ($F=1.88$, $p=0.10$). Post hoc group analysis (**figure 3.2b**) revealed that apart from Munich and Melbourne a significant difference was found between the northern and southern hemisphere cities.

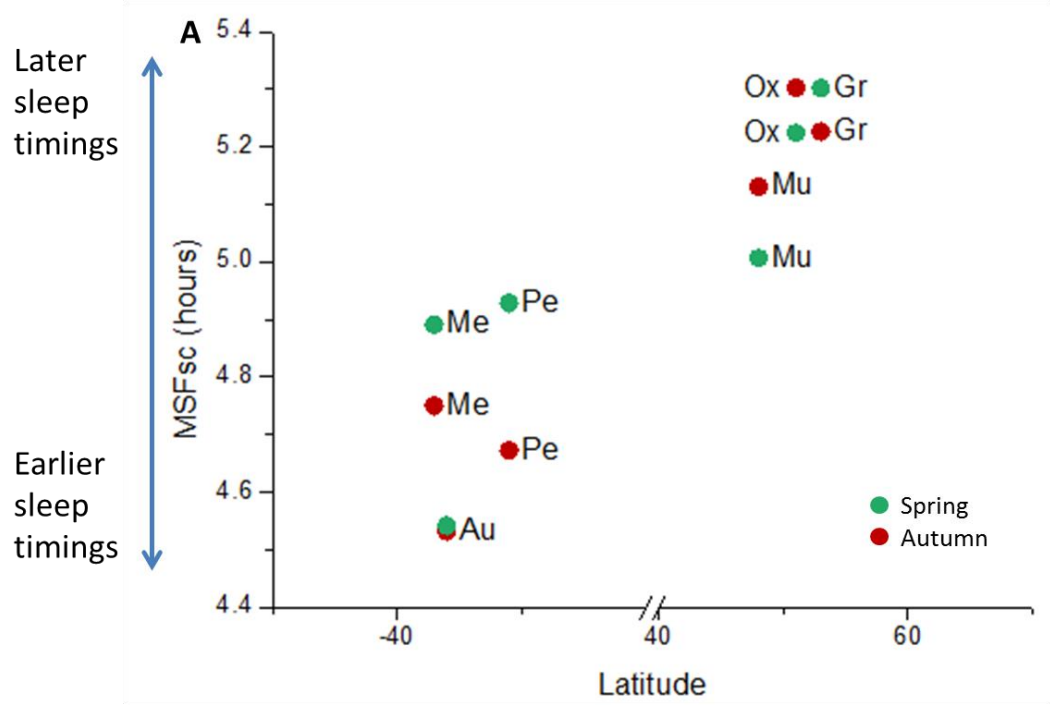
Both age and sex influence chronotype. To ensure the group differences in age and sex were not underlying the city effect on MSFsc, ANCOVA analysis was performed with age and sex as covariates. Both age and sex were found to have an effect on MSFsc (Age: $F=45.64$, $p\leq0.001$, Sex: $F=382.61$, $p\leq0.001$). However this did not remove the city effect, indicating that although age and sex do influence MSFsc, so does which city the participants were from.

3.3.3. MSFsc, age and sex

Further analysis was performed to assess the relationship between age, sex and MSFsc. Within the whole population MSFsc became progressively later with age until 21 years, following which MSFsc decreased (**figure 3.3**).

	Oxford n=302		Groningen n=3050		Munich n=1919		Perth n=342		Melbourne n=368		Auckland n=460		ANOVA F value	ANOVA p value	
Age (Mean:SD)	21.1	*2.1*	21.5	*2.1*	22.5	*1.9*	19.0	*1.4*	20.5	*1.9*	20.0	*2.0*	273.4	0.00	*
Sex (Females:%)	161.0	*53.3*	1996.0	*65.4*	1401.0	*73.0*	202.0	*59.1*	271.0	*73.6*	314.0	*68.3*	15.7	0.00	*
Regular work (Yes:%)	202	*66.9*	1533	*50.3*	1505	*78.4*	230	*67.3*	266	*72.3*	336	*73.0*	96.0	0.00	*
Number of work days (Mean:SD)	5.11	*.54*	4.49	*1.12*	4.40	*1.27*	4.60	*1.03*	4.71	*1.06*	4.97	*.77*	37.9	0.00	*
Smoker (Yes:%)	26.0	*9.0*	510.0	*17.0*	378.0	*20.0*	11.0	*3.0*	22.0	*6.0*	19.0	*4.0*	29.8	0.00	*
Consumes: Alcohol (Yes:%)	238	*79.0*	2488	*82.0*	1336	*70.0*	182	*53.0*	197	*54.0*	203	*44.0*	98.0	0.00	*
Caffeine (Yes:%)	254	*84.0*	2609	*86.0*	1590	*83.0*	245	*72.0*	297	*81.0*	328	*71.0*	11.2	0.00	*
Sleep meds (Yes:%)	11	*3.6*	61	*2.0*	41	*2.1*	7	*2.0*	10	*2.7*	11	*2.4*	0.8	0.57	

Table 3.1 Demographic data with statistical analysis. The mean and count data for each city is presented along with either the standard deviation (SD) or percentage (%) of group total. Where applicable count data (regular workers, smoker, alcohol, caffeine and sleep meds) refers to the number of participants that do that particular parameter e.g. consume caffeine. Univariate analysis of variance (ANOVA) was performed for each parameter separately with city as the grouping factor. * denotes a statistically significant group difference.



B

	Oxford	Groningen	Munich	Perth	Melbourne	Auckland
Oxford		1.000	.289	.000 *	.002 *	.000 *
Groninge	1.000		.000 *	.000 *	.000 *	.000 *
Munich	.289	.000 *		.000 *	.100	.000 *
Perth	.000 *	.000 *	.000 *		1.000	.996
Melbourı	.002 *	.000 *	.100	1.000		.003 *
Auckland	.000 *	.000 *	.000 *	.996	.003 *	

Figure 3.2 MSFsc relative to local clock time. A: MSFsc plotted relative to city latitude for both collection periods: spring (green closed circles) and autumn (red closed circles). The 'X' axis (latitude) is broken to allow better comparison between northern and southern hemisphere cities. B: Table shows the p values for post hoc group comparisons of ANOVA analysis. * denotes a statistically significant group difference.

Hence MSFsc was at its latest at 21 years. Assessment of the hemispheres separately, showed the same profile in the southern hemisphere cities with a peak in MSFsc at around 21 years. However, in the northern hemisphere MSFsc appears to peak slightly earlier at 19 years. In terms of sex differences, MSFsc was found to be significantly higher in males than females in all cities apart from Oxford, where no difference was seen (**figure 3.4**).

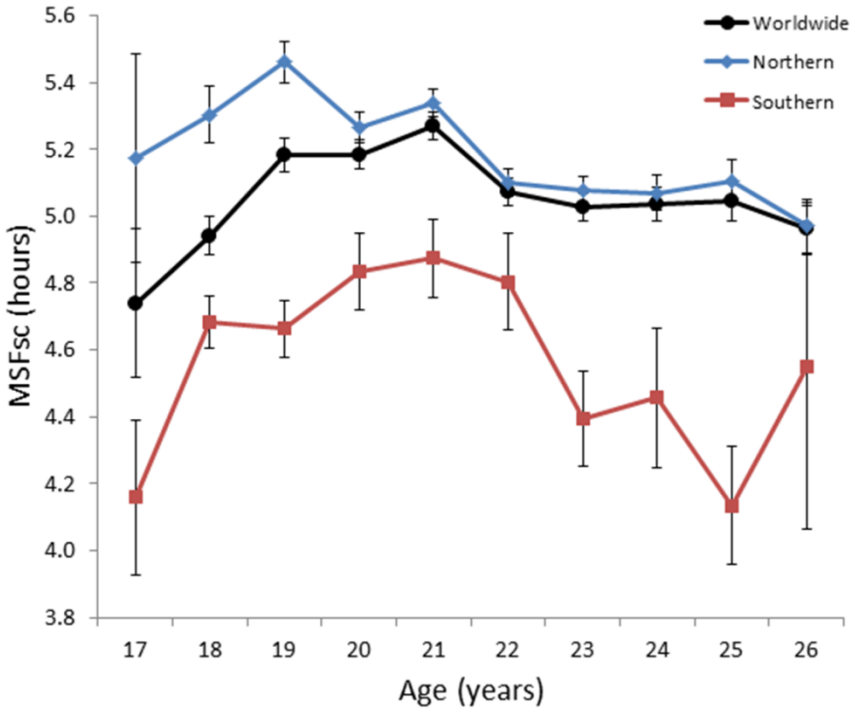


Figure 3.3 MSFsc and age. The mean MSFsc for each age is presented for the whole population (black circles), northern hemisphere cities (blue diamonds) and southern hemisphere cities (red squares). The error bars represent the standard error of the mean.

3.3.4. Light exposure and light dose

Figure 3.5 shows the average light exposure (**figure 3.5a**) and light dose (**figure 3.5b**) for each city in both spring and autumn. A two way ANOVA for group and season was performed for both light exposure and light dose. Light exposure was found to have a group effect ($F=7.11$, $p\leq0.001$) but no season effect ($F=3.24$, $p=0.07$) or city/season interaction ($F=0.72$, $p=0.61$). Based on the average light exposure for spring and autumn the cities were ranked with increasing light exposure as follows: Oxford; Melbourne; Groningen and Auckland; Munich; Perth. Post hoc group analysis is shown in **figure 3.5c**. Light dose on the other hand was found to have a group effect ($F=123.92$, $p\leq0.001$), season effect ($F=428.37$, $p\leq0.001$) and city/season interaction ($F=26.03$, $p\leq0.001$). In terms of increasing light dose in spring the cities were ranked: Munich; Oxford; Groningen; Melbourne; Auckland; Perth. Whereas in autumn they were ranked: Oxford; Groningen; Munich; Auckland; Melbourne; Perth.

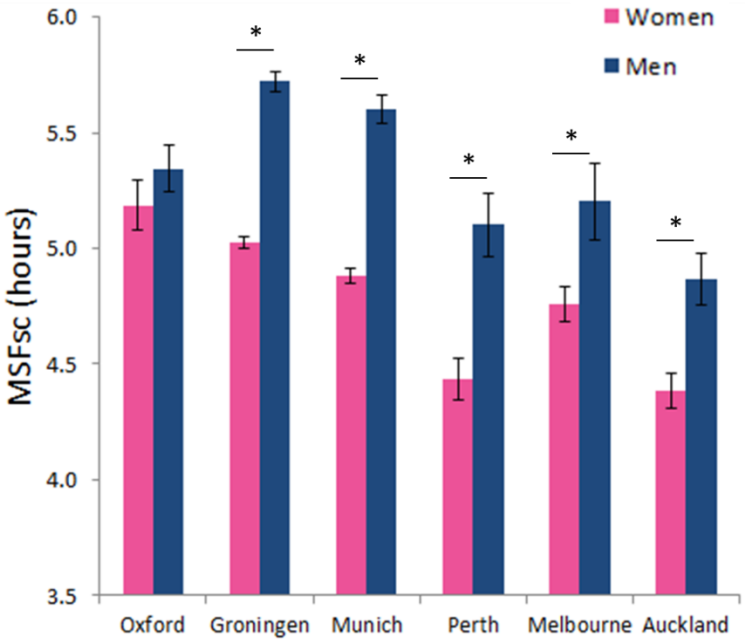
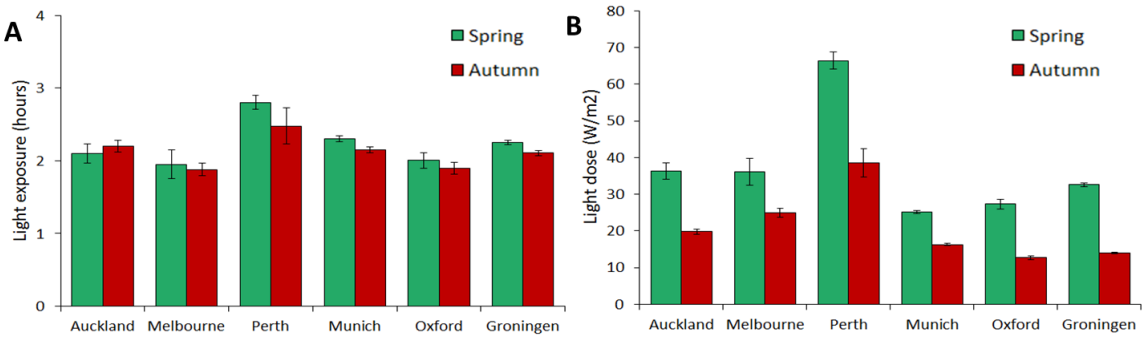


Figure 3.4 MSFsc and sex. The mean MSFsc is presented for men (blue) and women (pink) for each city. Error bars represent the standard error of the mean. * ≤ 0.01.

Despite some rearrangement in the order of increasing light dose from spring to autumn, the southern hemisphere cities were consistently higher than the northern cities, with Perth showing the highest levels in both spring and autumn. Post hoc group analysis for both spring and autumn is shown in **figure 3.5d**.

3.3.5. Impact of light on chronotype

Figure 3.6 presents MSFsc plotted in relation to its position within the dark period i.e. the time from sunset to sunrise. A clear increase in the dark period is seen from spring to autumn for all cities. The position of MSFsc relative to dawn also changes over the year, whereby MSFsc is closer to dawn in spring than in autumn. Furthermore, very little difference in the timing of dawn is seen between the southern and northern hemisphere cities. Indicating, that the timing of dawn cannot be the most influential factor on the timing of MSFsc.



C

	Oxford	Groningen	Munich	Perth	Melbourne	Auckland
Oxford		.054	.008 *	.000 *	1.000	.267
Groningen	.054		1.000	.000 *	.001 *	1.000
Munich	.008 *	1.000		.000 *	.000 *	1.000
Perth	.000 *	.000 *	.000 *		.000 *	.000 *
Melbourne	1.000	.001 *	.000 *	.000 *		.026 *
Auckland	.267	1.000	1.000	.000 *	.026 *	

D

Spring	Oxford	Groningen	Munich	Perth	Melbourne	Auckland
Oxford		.042 *	1.000	.000 *	.233	.009 *
Groningen	.042 *		.000 *	.000 *	1.000	1.000
Munich	1.000	.000 *		.000 *	.013 *	.000 *
Perth	.000 *	.000 *	.000 *		.000 *	.000 *
Melbourne	.233	1.000	.013 *	.000 *		1.000
Auckland	.009 *	1.000	.000 *	.000 *	1.000	

Autumn	Oxford	Groningen	Munich	Perth	Melbourne	Auckland
Oxford		1.000	.006 *	.000 *	.000 *	.000 *
Groningen	1.000		.000 *	.000 *	.000 *	.000 *
Munich	.006 *	.000 *		.000 *	.000 *	.000 *
Perth	.000 *	.000 *	.000 *		.000 *	.000 *
Melbourne	.000 *	.000 *	.000 *	.000 *		.000 *
Auckland	.000 *	.000 *	.000 *	.000 *	.000 *	

Figure 3.5 Light exposure and light dose. The mean light exposure (A) and light dose (B) is presented for each city for spring (green) and autumn (red). Error bars represent the standard error of the mean. The p values for post hoc group comparisons for ANOVA analysis are also shown for the combined seasonal data for light exposure (C) and separate seasons for light dose (D). * denotes a statistically significant group difference.

In terms of light levels during the day, despite light dose being found to differ across cities and seasons, no effect of light dose was found for MSFsc (**table 3.2**). Light exposure however was found to have an effect. Suggesting, that it is not the intensity of light but the duration that has the most impact on chronotype. Since there was no seasonal effect, the average MSFsc for both collection periods was binned into 30 minute epochs of light exposure.

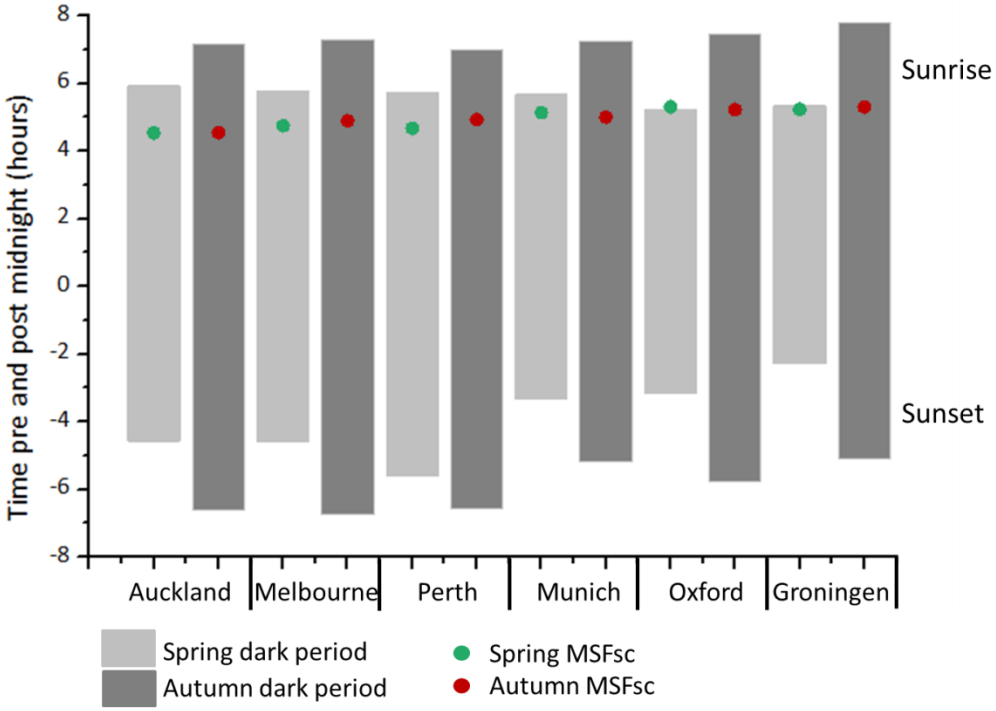


Figure 3.6 MSFsc and dark period. The mean time of the MSFsc for each city and both seasons (spring = green, autumn = red) is shown within the timing of the dark period i.e. the time from sunset to sunrise (spring = light grey, autumn = dark grey). The times of MSFsc and the dark period are plotted in hours before and after midnight. The cities are ordered in increasing distance from the equator from Auckland to Groningen. The MSFsc data presented in this figure is the same as presented in figure 3.2.

Although for both the northern and southern hemispheres an increase in light exposure appeared to be associated with a decrease in MSFsc (**figure 3.7**), this relationship however only trended towards being significant for the northern hemisphere (Pearson correlation for the northern hemisphere: -0.38, p= 0.07; southern hemisphere: -0.04, p=0.40).

3.3.6. Chronotype tracking to dusk or dawn?

To determine whether humans are more linked to dusk or dawn, MSFsc was calculated with reference to sunrise (MSFsc_SR) and sunset (MSFsc_SS) (**figure 3.8a** and **3.8b** respectively). Using a two way ANOVA; a city effect, season effect and city/season interaction was found for both MSFsc_SR and MSFsc_SS (**figure 3.8d**).

	F value	P value
City	37.554	.000 *
Age	40.238	.000 *
Sex	365.822	.000 *
Light dose	1.553	.213
Light exposure	4.386	.036 *

Table 3.2 MSFsc and light. One way ANCOVA for MSFsc and city, with age, sex, light dose and light exposure as covariates. * denotes a statistically significant effect within the model.

The seasonal difference, in terms of percentage change was found to be higher for MSFsc_SR than MSFsc_SS (**figure 3.8c**). Suggesting that chronotype is more linked to dawn than dusk in humans.

3.3.7. Social jet lag

With the exception of Munich, social jet lag (SJL) was found to be higher in the southern hemisphere cities (**figure 3.9a**). ANOVA analysis revealed a city ($F=22.27$, $p\leq0.001$), but no seasonal effect ($F=3.15$, $p=0.08$). Post hoc analysis of city interactions are shown in **figure 3.9b**, indicating that only a few city interactions were significantly different. A seasonal difference for social jet lag was found for Perth ($F=4.85$,$p=0.03$) and Groningen ($F=9.34$, $p\leq0.001$), where in both cases social jet lag was higher in the autumn than spring. Further analysis revealed a negative correlation for both the northern and southern hemisphere cities between social jet lag and sleep duration on work days (**figure 3.10a**): the higher the social jet lag the shorter the sleep duration on work days (northern: Pearson correlation=-0.98, $p\leq0.001$; southern: Pearson correlation=-0.95, $p\leq0.001$). To correlate social jet lag with sleep duration on work days the average sleep duration on work days for every 30 minutes of social jet lag was calculated. ANOVA analysis also found an effect of age and sex, but no interaction, with social jet lag (age: $F=5.50$, $p\leq0.001$; sex: $F=20.49$, $p\leq0.001$; interaction: $F=0.82$, $p=0.46$).

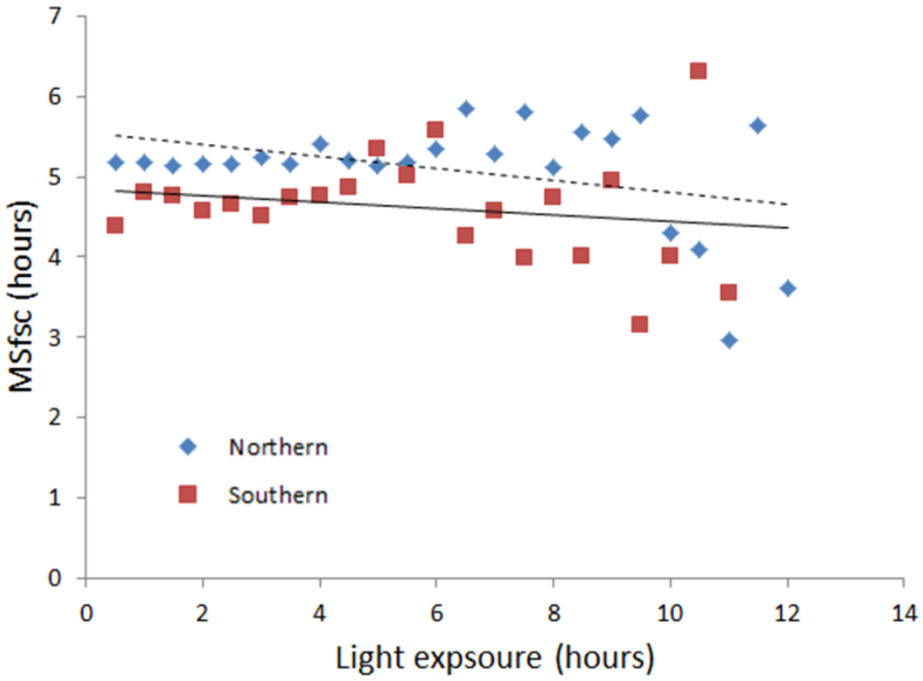
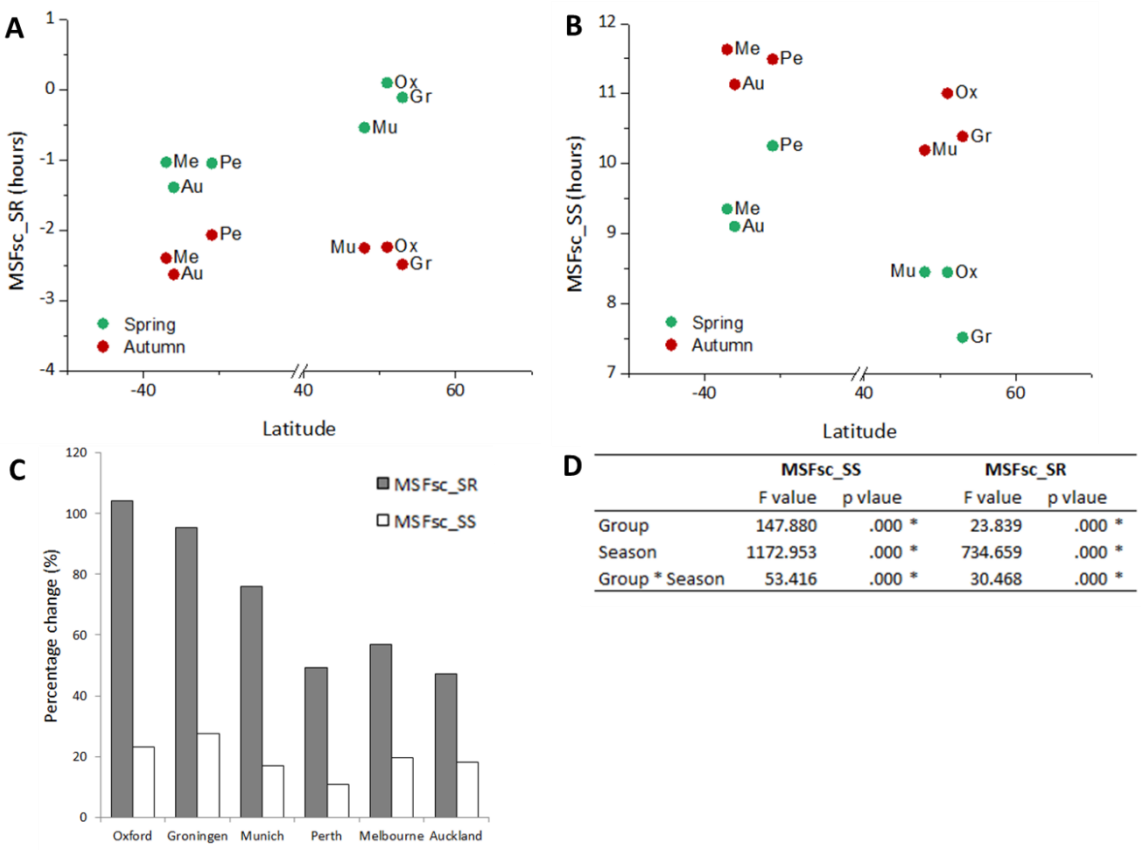


Figure 3.7 MSFsc and light exposure. The mean MSFsc for the northern hemisphere cities (blue diamonds) and southern hemisphere cities (red squares), combined for both seasons, were binned into 30 minute epochs of light exposure. A liner line of best fit was applied for both the northern (dashed line) and southern cities (solid line).

Post hoc analysis of age revealed that 18, 19 and 21 year olds had more social jet lag than 23 and 24 year olds (**figure 3.10b**). In terms of sex, men showed more social jet lag than women (**figure 3.10c**).

3.4. Discussion

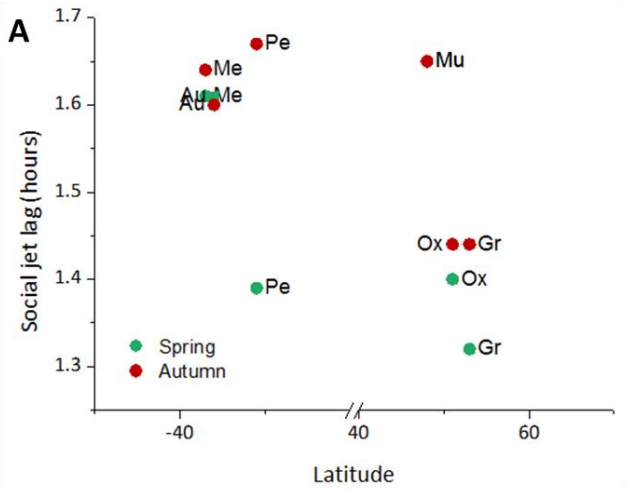
This study has demonstrated that sleep midpoint was earlier in students living in the southern hemisphere cities assessed in this study compared with those in the northern hemisphere. As expected, light levels were found to be higher in the southern cities than northern, particularly in autumn. In terms of the effect of light on the timing of sleep midpoint, only light exposure was found to have an influence, whereby the longer the light exposure the earlier sleep midpoint, for both the northern and southern cities. However, this relationship only showed a trend towards being significant within the northern hemisphere cities.



	MSFsc_SS		MSFsc_SR	
	F value	p vlaue	F value	p vlaue
Group	147.880	.000 *	23.839	.000 *
Season	1172.953	.000 *	734.659	.000 *
Group * Season	53.416	.000 *	30.468	.000 *

Figure3.8 MSFsc relative to sunrise and sunset. The sleep midpoint relative to sunrise (MSFsc_SR: A) and sunset (MSFsc_SS: B) were plotted for each city for spring (green) and autumn (red).The percentage change (C) in MSFsc_SR (grey) and MSF_SS (white) from spring to autumn for each city. Two way ANOVA analysis of city and season (D). * denotes a statistically significant group difference.

Although sleep midpoint was not found to be seasonal when relative to local time, when adjusted for sunrise and sunset, sleep midpoint was found to be seasonal. An effect that was more pronounced in the northern hemisphere cities than southern. The fact that a greater seasonal change was observed with sleep midpoint relative to dawn than dusk in this study, suggests that humans are more linked to dawn rather than dusk. Finally levels of social jet lag were found to be relatively similar between the students in the northern and southern hemisphere cities, with only Groningen, Munich and Auckland, and Munich and Perth showing a significant difference in spring and Munich, Melbourne and Auckland showing a significant difference in autumn.



B

Spring	Oxford	Groningen	Munich	Perth	Melbourne	Auckland
Oxford		1.000	.057	1.000	1.000	1.000
Groningen	1.000		.000 *	1.000	.874	.024 *
Munich	.057	.000 *		.001 *	1.000	1.000
Perth	1.000	1.000	.001 *		1.000	.503
Melbourne	1.000	.874	1.000	1.000		1.000
Auckland	1.000	.024 *	1.000	.503	1.000	

Autumn	Oxford	Groningen	Munich	Perth	Melbourne	Auckland
Oxford		1.000	.130	1.000	.433	.924
Groningen	1.000		.000 *	.578	.004 *	.021 *
Munich	.130	.000 *		1.000	1.000	1.000
Perth	1.000	.578	1.000		1.000	1.000
Melbourne	.433	.004 *	1.000	1.000		1.000
Auckland	.924	.021 *	1.000	1.000	1.000	

Figure 3.9 Social jet lag. The mean social jet lag (A) for each city for spring (green) and autumn (red). The p values from post hoc group comparisons for ANOVA analysis are also shown for the separate seasons (B). * denotes a statistically significant group difference.

However social jet lag was found to be strongly associated with sleep duration on work days: more social jet lag shorter work day sleep duration.

3.4.1. Why is sleep midpoint earlier in the southern hemisphere cities?

The primary hypothesis for this study was that the phase of the sleep midpoint would be earlier with increasing light intensity independent of exposure duration. The data did not confirm this, as light dose (an individual's level of irradiance based on the time spent outside) had no effect on the phase of sleep midpoint. Nevertheless in accordance with previous findings (Roenneberg and Merrow 2007), light duration (time spent outside) was found to influence the phase of the sleep midpoint.

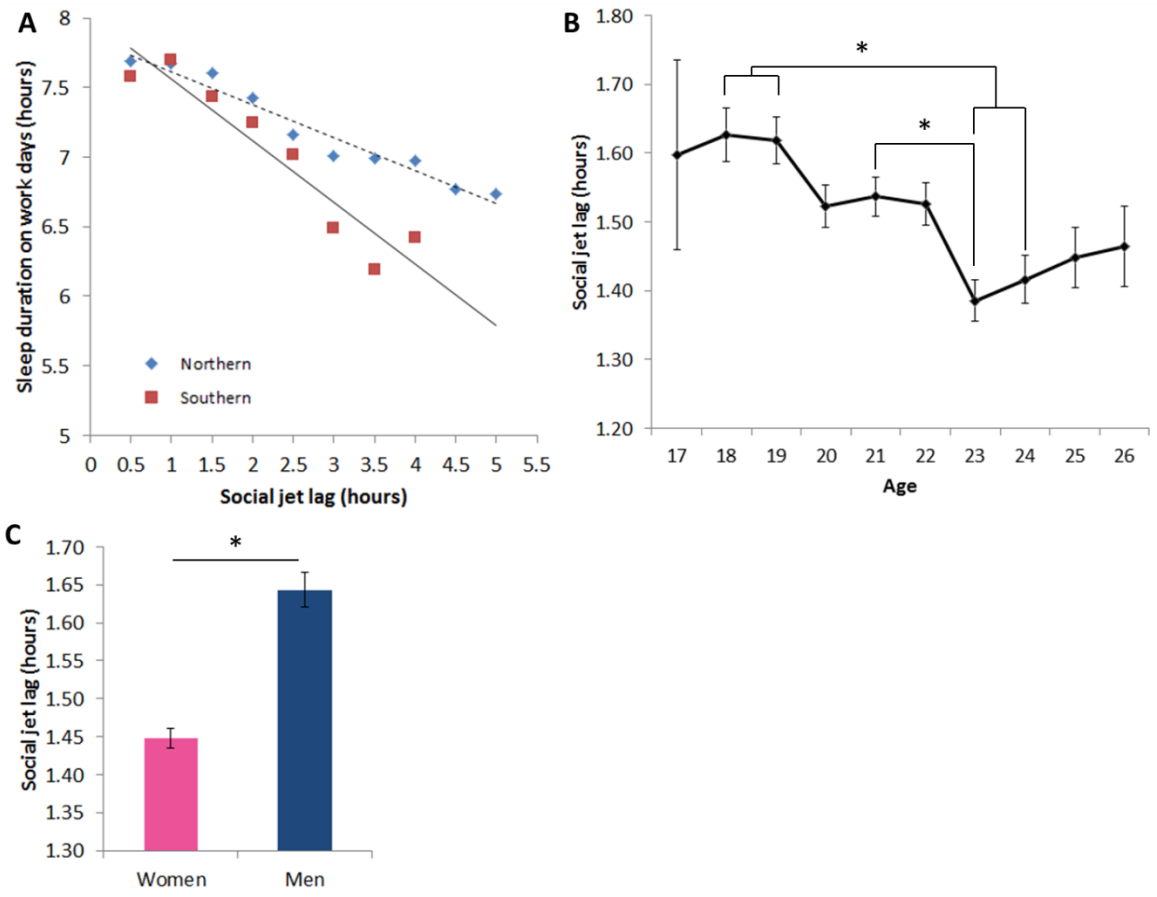


Figure 3.10 Social jet lag in relation to sleep duration, age and sex. The average sleep duration on work days was binned into 30 minute epochs of social jet lag are plotted for the northern (blue diamonds) and southern (red squares) cities (A). Also the average social jet lag is plotted for each age (B) and sex (C) for the whole population. * ≤ 0.05.

Although previously, increased light exposure has been correlated with an earlier phase of the sleep midpoint (Roenneberg and Merrow 2007), this relationship was only found to be trending towards significance in the northern hemisphere cities in this study. Therefore it is unlikely that the time spent outside in daylight can be the sole explanation for the differences seen in the timing of sleep midpoint. Moreover, light exposure was not found to be consistently longer for the southern hemisphere cities, with Melbourne having the second shortest hours of light exposure and Munich having the second longest. Hence there must be additional factors that influence sleep midpoint that are different between these cities.

Age and sex contribute to chronotype, and both age and sex differed between the cities and were found to have an effect on sleep midpoint in this population. In terms of age, the participants in the northern hemisphere cities were on average older than those in the southern cities (one way ANOVA age and hemisphere: $F = 889.34$, $p \leq 0.001$). However, within the age range studied here, sleep midpoint varies: from age 19 it becomes increasingly late, until age 21, after which it becomes increasingly early. The 'peak lateness' of sleep midpoint in this study (21 years of age) was consistent with previous research, where the latest sleep midpoint was found to be 19.5 years of age in women and 21 years in men (Roenneberg et al. 2007a). Considering the cities individually, it fits that participants in Oxford and Groningen have the latest sleep midpoint as participants in both cities had an average age of 21 years. However, Perth had the youngest population (an average of 19 years old) but did not have the earliest sleep midpoint. Indicating that age cannot solely describe the differences seen between the cities. Again in keeping with previous research chronotype was found to be later in men in all cities with the exception of Oxford (Roenneberg et al. 2007a). However, the proportion of males was not consistently higher in the cities that show the later sleep midpoints, which suggest that sex also cannot explain the differences seen alone. Moreover although age and sex both had an effect on sleep mid-point they did not remove the effect of city in the multifactorial analysis, which implies that neither age nor sex can solely explain the variance between the cities.

Another possibility is the relationship between latitude and sleep midpoint. In terms of increasing distance from the equator the cities are ranked: Perth; Auckland; Melbourne; Munich; Oxford; Groningen and in terms of chronotype the cities are ranked from earliest to latest sleep midpoint: Auckland; Perth; Melbourne; Munich; Oxford; Groningen. Hence the two rankings show a high level of similarity with only Perth and Auckland switching places. A possible explanation for this is the angle between the sun and the horizon, which not only changes over the day but also with latitude. The angle between the sun and horizon changes the amount of atmosphere the sunlight has to pass through, changing the spectral composition of the light (Frederick et al. 1989).

However, light actually becomes more enriched in shorter wavelength light i.e. blue light the smaller the angle between the sun and the horizon, which occurs at dusk and dawn (Roenneberg and Foster 1997, Thorne et al. 2009). The circadian system is maximally sensitive to blue light (Zaidi et al. 2007) which is why dusk and dawn tend to be the strongest zeitegbers (Roenneberg and Foster 1997). However, the duration of dusk and dawn becomes shorter the closer to the equator, suggesting that actually there is less blue light the closer one gets to the equator, although this has not been tested in the field. Therefore a later sleep midpoint would actually be expected the closer one gets to the equator. Hence the distance from the equator cannot explain the variation in sleep timings of the different cities.

Finally there could be social factors. Social and work commitments influence sleep-wake timing. They also influence light exposure since artificial lights can be used after the sun has set, which in turn could impact on the circadian clock. Furthermore it is possible that social factors could be entrainment factors in their own right. Social interactions can affect emotions and hence levels of arousal, and thus have the potential to influence the circadian clock (Mistlberger and Skene 2005). However, due to the impact of social factors on the light/dark schedule it is difficult to disentangle direct influence on the circadian clock and those acting via light. Studies using very dim light levels have attempted to disentangle this interaction. However, as critiqued by Mistlberger and Skene (Mistlberger and Skene 2004) these studies have produced little convincing evidence for social interactions influencing the clock, especially since all of these studies do involve some light, even if at very low levels. To overcome this, the impact of social factors were investigated in people who have physically lost both eyes. These individuals consequently have no visual or circadian perception of light, and show free running circadian rhythms i.e. not entrained, despite having strong social time cues (Lockley et al. 1997, Skene et al. 1999). Therefore, social factors do not appear to be entraining factors in their own right, and hence it is highly unlikely that social factors can explain the difference in sleep midpoint. In

summary sleep midpoint is influenced by a number of factors, including light exposure, age and sex, which are likely to interact to affect sleep timing.

3.4.2. Seasonal variation

No seasonal variation was seen for sleep midpoint relative to local time. Although sleep midpoint has been shown to change over the winter months (becoming progressively later with decreasing day length), over summer it is relatively stable (Kantermann et al. 2007). Since the collection periods within this study were either side of summer (May and October) it is not surprising that there is no difference. However, the sleep midpoint relative to both sunrise and sunset varied with season. The reason for this discrepancy is due to the fact that the timings of sunrise and sunset change over the year. Hence the difference in time between the sleep midpoint and the time of sunrise or sunset also changes. For example, with a sleep midpoint of 4.25am the difference between sleep midpoint relative to sunrise in May when sunrise is 5.20am is 0.95 hours, whereas in October when sunrise is 7.50am it is 3.25 hours. So the sleep midpoint is closer to sunrise in the spring than in autumn because sunrise is earlier in the spring. Moreover, the change in sleep midpoint from spring to autumn was found to be greater for sleep midpoint relative to sunrise, indicating that sunrise has more of an impact on chronotype than sunset. This supports the hypothesis that dawn is a stronger zeitgeber for the human circadian clock than dusk. Furthermore, in this study it was observed that this seasonal affect was more pronounced for the cities in the northern hemisphere compared with those in the southern hemisphere. This is likely to be due to the greater change in the timings of sunrise and sunset over the year in those cities in the northern hemisphere in this study as they are situated at a higher latitude i.e. they are situated further away from the equator.

3.4.3. Social jet lag

Very little difference was found in terms of social jet lag within this study. Although the participants within the southern hemisphere cities and Munich appeared to have a higher level of

social jet lag, only Groningen was found to have significant less social jet lag than Munich, Melbourne and Auckland. Previously Wittmann and colleagues (Wittmann et al. 2006) have shown that individuals with a later sleep midpoint have more social jet lag: ranging from less than 1 hour of social jet lag for individuals with a sleep midpoint of earlier than 4am, to more than 3 hours of social jet lag for those individuals with a sleep midpoint of later than 8am. In this study social jet lag was found to range from 1.4 to 1.7 hours which is consistent for levels seen for individuals with sleep midpoints between 4.5 and 5.4 (Wittmann et al. 2006). In terms of the real world effect of this social jet lag, participants in the study were experiencing a discrepancy of around one and a half to two hours between their internal clock and their work and social commitments. In terms of dysfunction this would be similar, in the worst case scenario, to the dysfunction caused by the jet lag of travelling to a time zone with a two hour time difference.

Social jet lag by definition is the misalignment of the sleep-wake cycle and the circadian clock due to work and social commitments (Wittmann et al. 2006). Social jet lag is associated with chronotype: the later the sleep mid-point the more prone to social jet lag, which is due to late types not being able to compensate for early get up times on work day by going to bed earlier the night before (Wittmann et al. 2006). This study has demonstrated a strong correlation between sleep duration and social jet lag, suggesting that individuals with more social jet lag do have shorter sleep durations on work days, a finding that has not previously been published.

Interestingly both Perth and Groningen also showed a seasonal variation, with social jet lag being greater in autumn than spring. The effect of season on social jet lag has never been investigated before and it is unclear why these changes were seen.

3.5. Study limitations and future work

There are a number of potential limitations of this study. Daylight saving occurs in five out of the six cities studied. Daylight saving changes local clock times, and hence the discrepancy between local clock time and sun time. Despite this sleep midpoint was not corrected for daylight saving

because in this study it is the actual local clock time which is the relevant measure of interest, as it is local clock time that people used to measure the time of day. As a result of different recruitment methods used by the different universities, unequal sample sizes were obtained for the collection periods and cities. This may have affected the statistical power of some of the analysis and introduce type II error (Tabachnick and Fidell 2007), meaning that differences may not have been seen. Hence this work needs to be repeated with larger and more equal study groups to fully establish the impact of light on sleep timing and chronotype. Also with regards to the recruitment methodology, very few participants completed the questionnaire during both collection periods. If more students had completed the questionnaire in both seasons intra-individual analysis on the effect of season on sleep midpoint would have been possible, removing the cofounding effects of individual differences. Finally the measures of daily irradiance obtained were limited to the cities as a whole rather than individuals. Although a much more intensive investigation, a smaller study could be conducted to look at individual light exposure and irradiance levels over the year and the association with sleep midpoint. Furthermore the collection of more information regarding light exposure, such as time of day spent outside and use of artificial lights, may provide useful insights into the relationship between the duration of light exposure and sleep midpoint.

3.6. Key findings

- Sleep midpoint (MSFsc) was earlier in southern compared to northern hemisphere cities studied
- Daily irradiance was higher in the southern compared to northern hemisphere cities, particularly in autumn
- Age, sex and light exposure (duration of time spent outside), but not light dose (light intensity) were found to influence sleep midpoint
- Sleep midpoint was found to be more related to dawn than dusk

- Social jet lag was strongly associated with sleep duration on work days: more social jet lag shorter sleep duration

3.7. Conclusions

The aim of this study was to investigate the impact of different natural light intensities due to geographical location on sleep timing and chronotype. Although sleep timing was found to change with geographical location, this difference cannot be explained in this study by differences in light intensity. However light exposure (the amount of time spent outside), age and sex were all found to affect sleep timing. Therefore this study adds to previous research, demonstrating that although sleep timing is regulated by the circadian clock and the sleep homeostat, a number of other factors can also influence when people sleep. The implication of this is that the sleep-wake cycle can become misaligned from the circadian clock. The consequence of this social jet lag is sleep loss, which has also been demonstrated in this study.

4. Sleep, sleep deprivation and intrusive memories

4.1. Introduction

The behavioural and psychological measures addressing sleep and emotional processing (intrusive memories) from study two are reported in this chapter, with the aim of addressing the question: **what is the relationship between sleep and intrusive memory frequency?** In this study the effect of sleep deprivation on the frequency of intrusive memories, compared to normal sleep, was investigated. It was hypothesised that fewer intrusive memoires would be experienced following sleep deprivation than sleep.

As discussed in Chapter 1 (section 1.3.1.2. p.20), very little is known about the relationship between sleep and intrusive memories. Clinical evidence suggests that sedation following trauma may cause an increase in the incidence of post-traumatic stress disorder (PTSD), which is characterised by the presence of intrusive memories (Gelpin et al. 1996, Mellman et al. 2002). Therefore, the aim of this study was to explore the formation of unconsciously reactivated intrusive memories after exposure to an analogue traumatic event (a trauma film) and subsequent sleep or sleep deprivation. Based on the clinical evidence and the importance of sleep for memory consolidation (Walker and Stickgold 2004, Born and Wilhelm 2012), it was hypothesised that the prevention of memory consolidation due to sleep deprivation would reduce the number of intrusive memories generated to the trauma film. Based on this hypothesis it is possible that sleep deprivation may be beneficial following trauma. Considering the detrimental effects of sleep deprivation including cognitive impairment, diminished mood and association with long term poor health (Spiegel et al. 2004, Leproult et al. 1997, Banks and Dinges 2007, Knutson and Van Cauter 2008, King et al. 2008, Cappuccio et al. 2007, Knutson et al. 2009), a beneficial effect of sleep deprivation appears to be inconsistent with this literature. However there is evidence that in certain situations, most likely highly emotional situations sleep deprivation can be beneficial (as reviewed in Chapter 1: 1.3.2. p.21).

In addition, these experiments investigated the effect of sleep deprivation on the response to a stressor. To date only one other study has considered the response to stressors during sleep deprivation. Using a battery of cognitive tasks designed to induce high and low levels of stress Minkel and colleagues (Minkel et al. 2012) found that although the sleep deprived participants became more stressed during low stress tasks than non-sleep deprived controls, no difference in the level of reaction was seen for the high stress tasks. Minkel and colleagues (Minkel et al. 2012) postulate that sleep loss may lower the threshold at which an individual experiences an event as stressful. Within this study the failure version of the remote associates task (RAT) was used as a stressor. The RAT is described in full in Chapter 2 (section 2.5.2.3 p.63). Briefly the RAT is a cognitive task with a sham average score of 7 out of 10 that the participants believe to be very easy and correlated to creative intelligence. In reality the task is quite difficult and on average only a score of 1 out of 10 is achieved (Brown and Dutton 1995).

4.2. Methods

The methods and techniques used to produce the results of this chapter are discussed in detail in Chapter 2 (section 2.5.2. p.54). Within this chapter the precise protocol used is outlined below. The study protocol used within the study can be found in appendix V. This aspect of the study focuses on the effect of sleep deprivation on intrusive memories. Since both sleep and mood, and emotion, are complicated systems the study was designed to include as homologous a population as possible. A baseline assessment of their mood, personality and circadian profiles was conducted under the participants' normal routine. Finally the experimental manipulation was conducted in a laboratory setting to remove as many confounding factors as possible without removing a naturalistic setting completely.

4.2.1. Protocol

The study, for each participant, lasted 16-20 days in total depending on starting date, since the experimental manipulation was carried out over a weekend to minimise disruption to the

participants' university schedules. The protocol involved a study interview, baseline assessment and finally the experimental manipulation. An outline of the experimental design is presented in **figure 4.1**. For full details of all the methods and measures used see Chapter 2 (section 2.5.2. p. 54).

4.2.1.1. Study interview

All participants were required to undertake and pass a study interview before beginning the study. See Chapter 2 (section 2.2.6. p.46).

4.2.1.2. Baseline assessment

Assessment of mood, personality, mental health, as well as general personal and family health was conducted at baseline using a number of questionnaires: morningness-eveningeness questionnaire (MEQ), Pittsburgh sleep quality index (PSQI), Eysenck personality questionnaire (EPQ), profile of mood state (POMS), general health questionnaire (GHQ), mood disorder questionnaires (MDQ) and health questionnaire (HQ). The participants' circadian profiles were monitored: sleep-wake patterns were recorded actigraphically over a period of 10-14 days (AWL; CamNtech Ltd., Cambridge, UK), and mood and melatonin profiles were established over a 48h period. The participants' diurnal mood was assessed using the mood VAS. Participants completed the scale every two hours over two days, and the data was binned into four hour periods. The final time period for the second day was removed from the analysis as in many cases this was conducted during the experimental manipulation. Three participants failed to wear the actiwatch for a sufficient period of time to allow analysis, so were excluded from the actigraphy analysis (two sleep deprived participants, one non-sleep deprived participant). Morning cortisol samples were also collected at baseline.

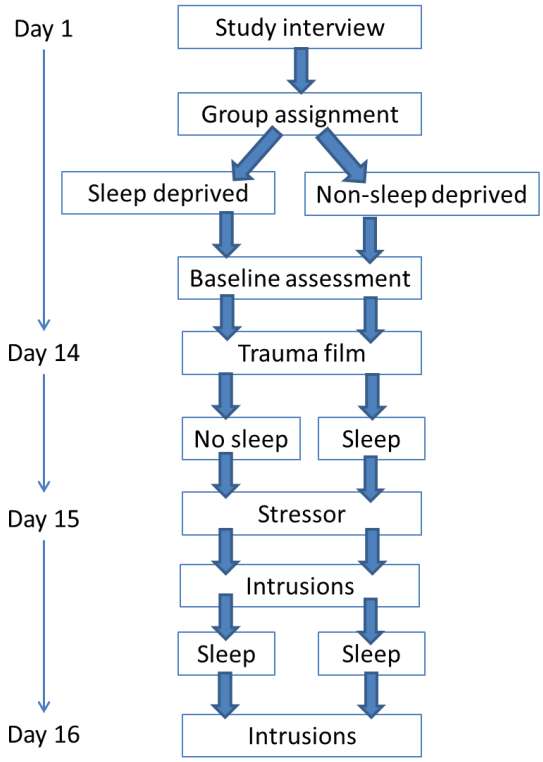


Figure 4.1 Overview of study protocol featuring the main events and differences between the sleep deprived and non-sleep deprived groups.

4.2.1.3. Experimental manipulation

Following the baseline assessment, the participants underwent the experimental manipulation: exposure to an analogue traumatic event, a trauma film, followed by either a period of sleep deprivation or normal sleep. Participants were assigned groups using a minimisation randomisation procedure, upon completion of the study interview. Minimisation is a procedure that ensures balance between study groups when low study numbers are used (Altman and Bland 2005, Scott et al. 2002). Participants are initially randomised to groups and additional participants are then allocated to groups.

On the day of the experimental manipulation, the participants reported to the Sleep/Circadian Neuroscience Laboratory at the John Radcliffe Hospital in Oxford between 7 and 9pm. They were shown the trauma film and completed self-reported scales before and after viewing the film: mood visual analogue scale (VAS), state trait anxiety index (STAI), dissociative state scale (DSS). An additional questionnaire was used to assess response to the film: film follow up questionnaire.

The non-sleep deprived participants were sent home after completing the measures and instructed not to watch any television or listen to any music before going to bed. The sleep deprived participants remained in the sleep laboratory for the night and until 19:00 the following day, after which they were sent home by taxi to sleep. On the morning following the manipulation (12 hours post film), the sleep deprived and non-sleep deprived participants were subjected to a stressor: the remote associates task (RAT). The participants completed two mood scales pre and post the stressor: mood VAS and STAI. Following the RAT the participants also completed the RAT follow up questionnaire. The impact of sleep deprivation on mood compared with sleep was assessed using the positive and negative affect scale (PANAS), participants completed the scale around one hour post film and before undertaking the stressor. Finally, intrusive memories were assessed by the impact of event scale (IES) and also via a self-report diary, which was completed for the following six days.

During the sleep deprivation period, the use of computers, TV, DVD's or music was not allowed but participants could play board games, read, study and talk to the researchers. They were allowed to walk around, but not leave the room. All the participants were continually monitored by staff trained to prevent napping and to ensure compliance and wellbeing. Participants were allowed water ad libitum, and had a choice of snacks every two hours (a sandwich or a piece of fruit). In the morning the participants were allowed a shower. Illumination of the room was kept to between 165-276 lux using artificial fluorescent light for the entire sleep deprivation period.

For three days prior and throughout the experiment none of the participants were allowed to consume any caffeine or alcohol, and were screened for drugs of abuse. None of the participants were allowed to nap during the day before the trauma film and their sleep-wake behaviour was monitored actigraphically for the duration of the experiment.

4.2.2. Data analysis

The actigraphy data were analysed with version 7 of the actiwatch activity and sleep analysis software for Windows (CamNtech Ltd., Cambridge, UK). All statistical analysis was performed using R version 2.12.0 (2010-10-15) (Copyright (C) 2010 The R Foundation for Statistical Computing). All data failed to satisfy checks for normal distribution, therefore statistical tests not assuming a normal distribution were used. Categorical data was tested using Chi-squared or simulated chi-squared in situations of inadequate sample size. The Wilcoxon rank sum test was applied to continuous data, unless the data contained ties, in which case binomial regression was used. Binomial distributed repeated measure analysis was applied to all scales completed pre and post the trauma film and stressor. Finally, as the intrusion data was count data, with a low chance of occurring but with many opportunities to do so (six days), Poisson distributed regression was used. Not all the participants completed all of the measures adequately; therefore some participants were excluded from some of the analysis. The number of participants from each group included in the analysis is shown in appendix III.

4.2.3. Grouping factors

Along with analysis of the participants according to whether they were in the sleep deprived or non-sleep deprived group, the data were also grouped for a number of other factors to assess the impact on intrusive memory frequency. These factors were: past trauma, emotional reaction to the trauma film, behavioural risk for mood disorder and state dissociation.

4.2.3.1. Past trauma

A past real life trauma was defined as “an event that included actual or threatened death or serious injury to you or someone else”, in accordance with DSM-IV criteria for PTSD. As a part of the MINI the participants were asked whether they had ever experienced a traumatic event. Participants were grouped in to those that had experienced a past trauma (trauma) and those that had not (no trauma).

4.2.3.2. Emotional reaction to trauma film

The level of emotion experienced during a trauma has been implicated as a risk factor for the development of PTSD (Ozer et al. 2003). The individual emotional reaction to the trauma film was calculated based on the change in mood score as a result of watching the film. The moods included were those used to create positive and negative affect scores (happy, calm, sad, horrified, fearful, and hopeless). Each participant was ranked according to the degree of change for each mood. The participants were then grouped according to the average ranking of all the moods, into above (high responders) or below average (low responders).

4.2.3.3. Mood disorder questionnaire (MDQ) score

The MDQ was originally devised as a screening tool for bipolar disorder (Hirschfeld et al. 2000), but it has also been used as a tool to assess risk for the development of a mood disorder (Rock et al. 2010). The screen includes 13 questions relating to manic symptoms. As a screening tool seven or more symptoms need to have been experienced, along with these symptoms occurring at the same time and causing a problem, for a positive screen. In previous studies a score of 7 or more has been used to indicate a higher level of risk compared with a low (0-2) MDQ score (Rock et al. 2010). Within this study, only one participant reported having experienced over seven symptoms (though they were not reported to cause a problem, so did not result in a positive screen). Hence the previously used definition of high MDQ was not suitable within this study. Hence the data was split according to a score of 1 or more i.e. the presence of any symptoms on the MDQ (MDQ) and no symptoms reported (no MDQ).

4.2.3.4. State dissociation

The level of dissociation during a traumatic event is thought to play a role in the development of PTSD (Ozer et al. 2003). Hence the participants were grouped into those who experienced no change in the level of dissociation or even a decrease after watching the trauma film (no dissociation) and those who reported an increase (dissociation).

4.3. Results

4.3.1. Recuritment

Seventy-eight participants were recruited from online advertisements and posters displayed around the University of Oxford and Oxford Brookes Campuses. Twenty-one participants failed to meet the inclusion criteria. A further fifteen participants dropped out without completing the study. As described in Chapter 2 (section 2.2.6. p.46), the eligibility criteria were changed during the course of the study. Nine participants were recruited into the study before the criteria changed, however retrospective analysis of the participants revealed that all of these participants satisfied these additional criteria. Fourty-two particicpants completed the study (sleep deprived = 20; non sleep deprived = 22). One participant was excluded from the study for being an outlier for the primary outcome measure, intrusive memories.

4.3.2. Baseline assessment

Table 4.1 presents the groups' demographic, psychological, circadian and sleep characteristics at baseline. As a result of the eligibility critera all participants had a good sleep quality (less than 6 on the PSQI) and did not have an extreme dirunal preference (MEQ). The groups were similar in age, sex, weekly alcohol and daily caffeine consumption, and trait mood (POMS), and psychiatic (GHQ and MDQ) charateristics. In terms of personalility, one subdividon of the EPQ, psychoticism, was found to be significantly higher within the non-sleep deprived group. The participants were 27% white British, 22% white non-British, 22% Chinese, 5% mixed white and Asian, 5% Indian, 2% other Asian background, and 2% African. The remaining 15% did not specify ethnic origin. A Chi-squared simulation revealed no difference in the proportion of ethnic backgrounds between the groups (Pearson statistic = 5.68, p=0.99).

	Sleep deprived (n=19)		Non sleep deprived (n=22)		Test value	P value
Age (Mean: SD)[a]	21.5	*1.7*	21.6	*2.2*	4.74	*.996*
Sex: Females (count: %)[b]	13	*68.4*	15	*68.2*	.000	*.987*
Alcohol (Mean: SD)[c]	3.8	*3.6*	2.8	*4.1*	261	*.174*
Caffeine (Mean: SD)[c]	111.3	*98.3*	122.9	*96.2*	195	*.714*
Morningness-eveningness questionnaire (Mean: SD)[d]	51.6	*9.8*	54.4	*9.4*	1.48	*.139*
Mood disorder questionnaire (Mean: SD)[d]	1.3	*2.2*	2.0	*1.8*	1.79	*.073*
General health questionnaire (Mean: SD)[d]	7.5	*2.2*	9.0	*2.8*	1.93	*.054*
Pittsburgh Sleep Quality Index (Mean: SD)[d]	2.9	*1.2*	2.9	*1.2*	*-.169*	*.866*
Eysenck Personality Questionnaire (Mean: SD)						
Neuroticism[d]	5.4	*3.3*	6.0	*3.1*	.891	*.373*
Psychoticism[d]	2.1	*2.0*	3.5	*1.9*	2.96	***.003***
Lie[d]	9.4	*4.0*	8.7	*4.0*	-.976	*.329*
Extraversion[d]	14.6	*4.3*	14.2	*3.7*	-.595	*.552*
Profile of Mood State: total mood disturbance (Mean: SD)[c]	6.7	*15.3*	8.2	*16.2*	*181*	*.626*
Actigraphy (Mean: SD)						
Sleep start[c]	00:22:53	*00:14:49*	00:26:05	*00:12:04*	158	*.715*
Sleep end[c]	08:18	*00:14*	08:16	*00:11*	171	*1.00*
Total sleep time[c]	07:55	*00:09*	07:49	*00:08*	181	*.749*
Sleep efficiency (%) [c]	85.6	*1.0*	86.3	*1.2*	139	*.357*
Sleep latency[c]	00:06:52	*00:01:16*	00:06:01	*00:00:36*	198	*.264*
Rest phase (L5) onset[c]	01:40:28	*00:15:59*	01:39:39	*00:12:46*	144	*1.00*
Active phase M10 onset[c]	10:55	*00:27*	10:49	*00:15*	159	*.627*
Relative amplitude[c]	.9	*.0*	.9	*.0*	169	*.403*

	test value	p value after BC
a = Chi squared simulation	Pearsons statisitc	0.05
b = Chi squared: pearson	Pearsons statisitc	0.05
c = Wilcoxon rank sum test	W value	0.0045
d = binomialy distributed GLM	z value	0.006

Table 4.1 Demographic data. The demographic data along with the psychological and actigraphic assesment of the rest/activity cycle are presented for the sleep deprived and non-sleep deprived participants, along with the statisitcal group comparisons. The statistical test used is showed in the key below along with the significance level after Bonferoni correction.

4.3.2.1. Circadian rhythms

The participant's circadian rhythms were assesed using a number of measures. The MEQ was used as an intial screen of dirunal perference, whereby extreme morning and evening types were excluded. No difference in MEQ score was found between groups (**table 4.1**).The participants' rest-activity pattern was recorded using the AW7 actiwatch over a period of 10-14 days. Actigraphic monitoring confirmed a good sleep history before the experiment in all participants with an average time in bed of 7.9 hours (standard deviation = 0.03). No difference was seen in any parameter of the rest-activity cycle between the groups (**table 4.1**).

Finally the participants' melatonin rhythms were assessed via the urinary melatonin metabolite 6-hydroxymelatonin sulphate (aMT6). Each participant's data was fitted to a non-linear regression model (for details see Chapter 2, section 2.5.2.2. p.58). All the individual data are in appendix II. Two participants (one sleep deprived and two non-sleep deprived) were excluded from this analysis due to human error in sample collection. Of the remaining 39 participants, the model was fitted for 37 participants: 34 participants had a period of 24 hours, two participants had a longer period (24.3 and 25.6 hours) and one participant had a shorter period (23.7 hours). For the final two participants it was not possible to fit the data to the model. However, both of these participants showed a stable rhythm over the two collection days, but with a much shorter rhythm than the expected 24 hours and with a peak of melatonin during the day (participants SD29 and SD38, data in appendix II). The stability of these rhythms suggests that these are a true representation of the underlying biological rhythms. Indicating that these two individuals appear to have an almost ultradian rhythm which is independent of light exposure since the period is very short and the peak of aMT6 is during the day. To fully understand the biological rhythms within these participants a further examination would be needed, hence for the further analysis of the melatonin rhythms these two participants were excluded.

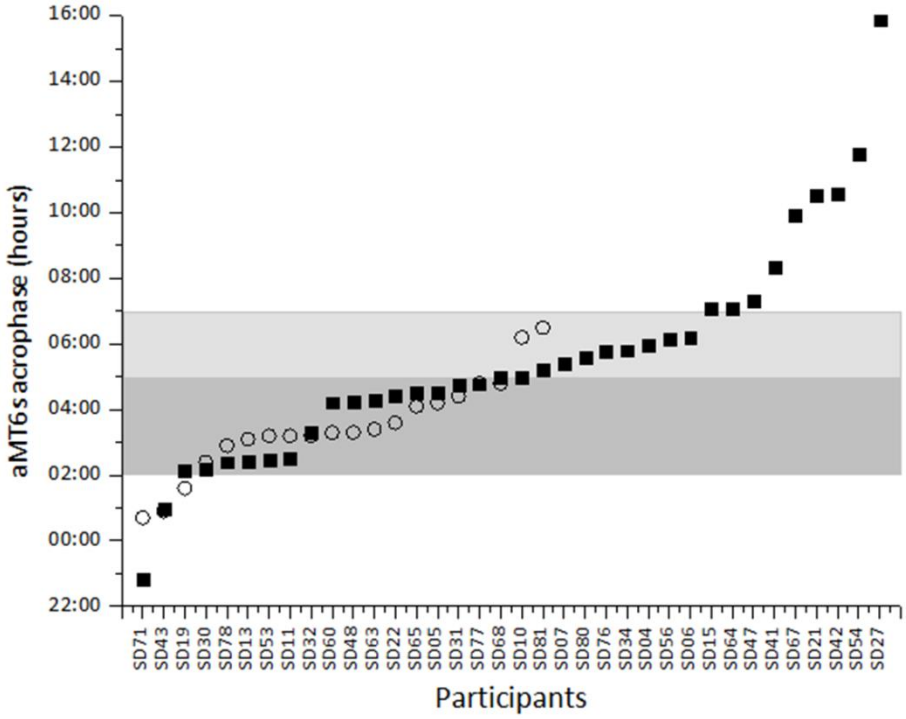


Figure 4.2 aMT6s acrophase. The aMT6s acrophase for each participant (black squares) is presented along side control data (open circles) provided by Benita Middleton. The dark grey area represents the normal range for aMT6s acrophase while the light grey area represents the slightly dealyed range. Two of the five participants (SD67 and SD42) with a very delayed acrophase this due a flat rhythm flat making it difficult to determine the true peak (acrophase). However the remaining participants (SD21, SD54 and SD 27) did appear to have a very delayed rhythm.

Of the participants where the model was successfully fitted the sleep deprived participants (n=17) had an average period of 24 hours (standard deviation = 0.11) and the non-sleep deprived participants (n=20) had an average period of 24.08 (standard deviation = 0.36).

In terms of the phase of the aMT6 rhythm the acrophase (the time of the rhythm's peak, see figure 2.3 p.63) was calculated from the data fitted by the model. The acrophase of each participant is shown in **figure 4.2**. The aMT6 acrophase ranges from 22.84 hours to 15.88 hours, with an average of 5.33 hours (standard deviation = 3.37). Five participants (SD67, SD21, SD42, SD54 and SD27) showed a very delayed acrophase. For two of these participants (SD67 and SD42) this was due to limited time points making the rhythm very flat and therefore difficult to

determine the true peak (acrophase). However the remaining participants based on this one collection period did appear to have a very delayed rhythm.

Group comparisons revealed an average acrophase of 6.23 hours (standard deviation = 3.39) for the sleep deprived participants and 4.56 hours (standard deviation = 3.23) for the non-sleep deprived. T-test analysis found no significant difference between the groups ($F=0.01$, $p = 0.93$). As a result of the wide range in the timing of the aMT6 acrophase, the groups were further subdivided into those that are within the normal range of the aMT6 acrophase (2-5am), slightly delayed (5-7am), delayed (later than 7am) and advanced (earlier than 2am). ANOVA analysis found a significant effect of time of phase groups (normal, slightly delayed, delayed, advanced), but no interaction between time of phase and experimental group (sleep deprived, non-sleep deprived) was found. Indicating that although some participants show a delayed aMT6 rhythm compared to what is normally expected, there was no difference between the experimental groups.

4.3.2.2. Diurnal mood

The participants rated 15 moods on a visual analogue scale (**figure 4.3**). The only moods to show a clear circadain rhythm were 'sleepy' and 'alert', which showed complementary profiles: the peak time for alertness coincided with the lowest sleepiness levels. Since robust circadian rhythms of mood have previously only been demonstrated in extreme chronotypes, the abscene of a rhythm within the majoirty of the moods recorded is not surprising, considering that there were no extreme chronotypes in this study.

Intra- and inter-individual mood fluctuations were investigated across all the moods. **Figure 4.4** shows the mean standard deviation for all the moods for each participant. A range of values were found, suggesting that there is some variation in the amount of mood fluctuation experienced between the participants. Futhermore a even distribution of levels of mood fluctuations were seen between the groups.

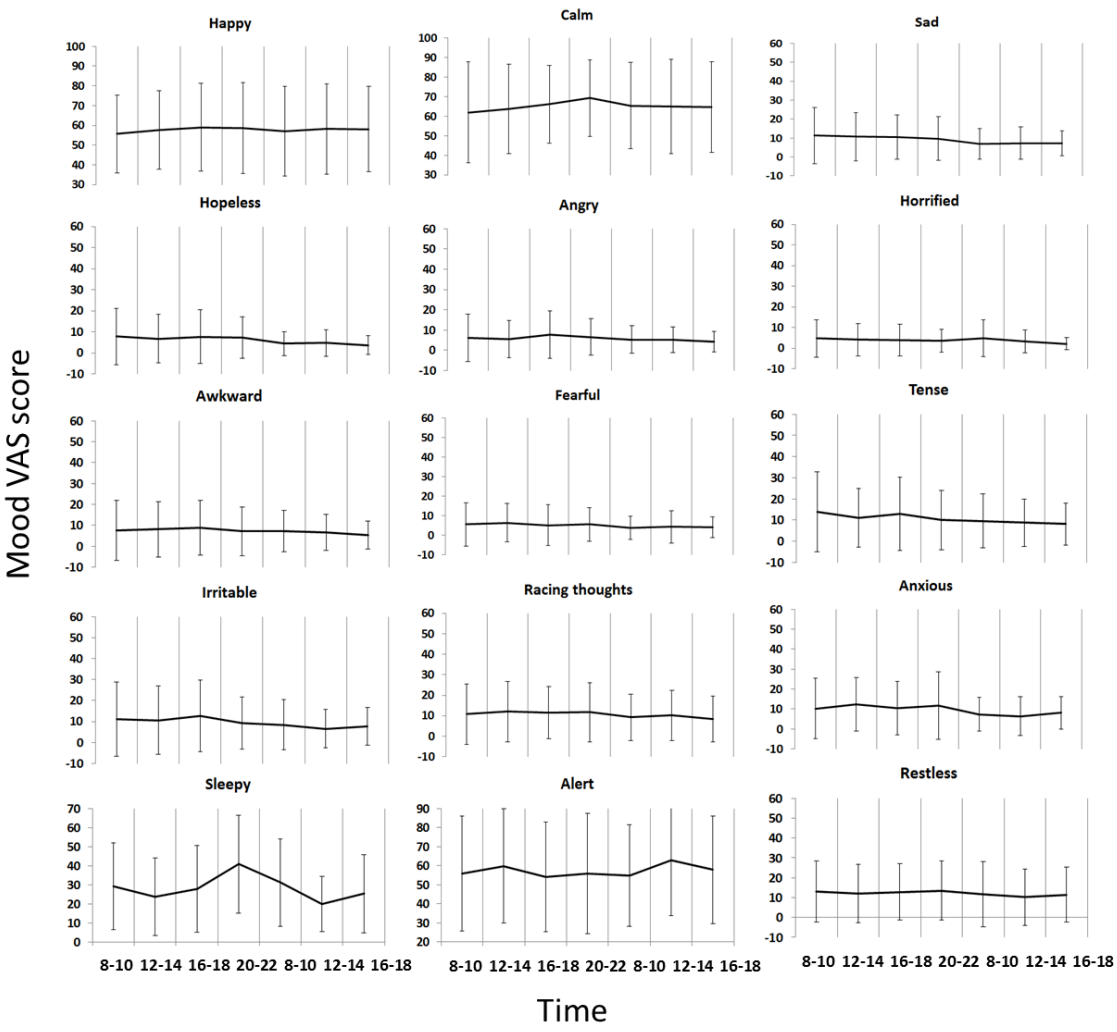


Figure 4.3 Diurnal mood. The mean score for each mood is given in four hour bins over two days. Error bars represent the standard deviation of the mean.

4.3.2.3. Cortisol awakening response

Salivary cortisol levels immediately after waking were analysed on two days for the sleep deprived (n=17) and the non-sleep deprived (n=22) participants. Two sleep deprived participants failed to provide samples. Box plots of the data are presented in **figure 4.5**. Cortisol levels were found to increase over time from waking. Three time points (0 and 20 minutes for day 1 and 0 minutes for day 2) were transformed to ensure normal disruption of all data. A two way (group and time) repeated measure analysis revealed an effect of time ($F=44.42$, $p\leq0.001$) but no group/time interaction ($F=0.98$, $p=0.46$). This indicates that over time cortisol levels showed a significant increase to the same extent for both groups.

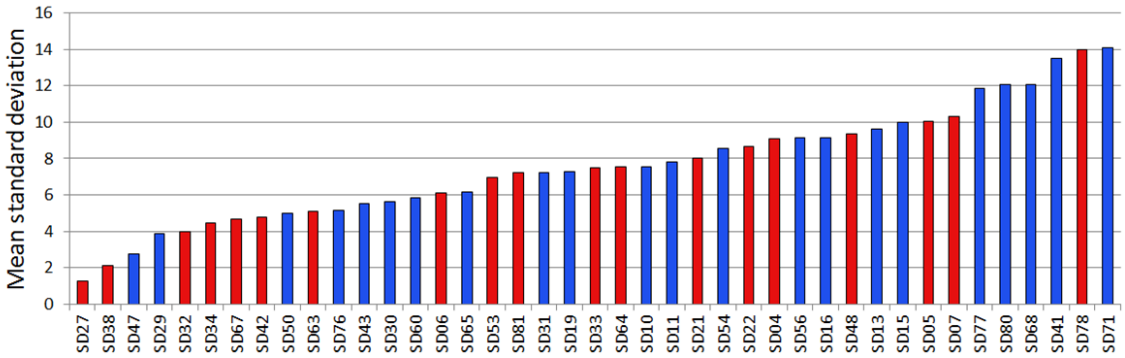


Figure 4.4 Mood fluctuation. The mean stardard deviation of the combined moods is presented for the sleep deprived (blue) and non-sleep deprived (red) participants.

To investigate stability of the cortisol profile between collection days a three way (group, time and day) repeated measure analysis was performed. Consistent with previous analysis an effect of time ($F=128.01$, $p\leq0.001$) but no group/time interaction ($F=0.92$, $p=0.44$) was found. Furthermore, a significant time/day interaction ($F=56.67$, $p\leq0.001$) was found but no time/group/day interaction ($F=1.26$, $p=0.3$). Thus a difference between the collection days did exist indicting some flexibility in the production of cortisol. However, this flexibility was not different between the groups.

4.3.3. Experimental manipulation

4.3.3.1. Response to study film

An immediate change in both positive and negative affect was seen post trauma film (**figure 4.6a**), with a decrease in positive affect and an increase in negative affect. A two way (group and time) binomially distributed repeated measure analysis showed a significant group/time interaction for both positive and negative affect (**table 4.2**: VAS film positive affect and film negative affect respectively). In addition, an increase in dissociation was seen as a result of the trauma film ($z=9.18$, $p\leq0.001$), which was similar for both groups ($z=-0.61$, $p=0.54$) (**figure 4.6b**).

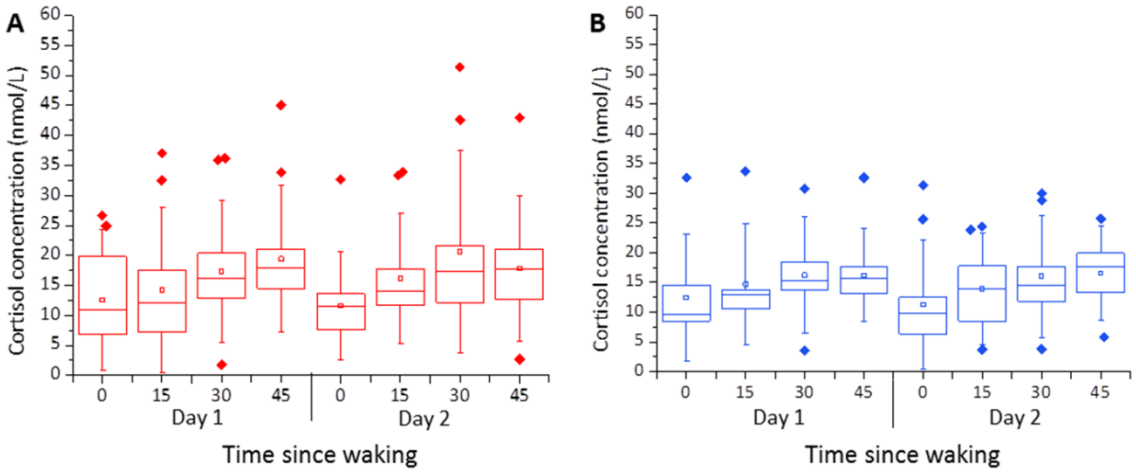


Figure 4.5 Cortisol awakening response. The concentration of cortisol at 0, 15, 30 and 45 minutes after waking is shown for both collection days for the non-sleep deprived (A) and sleep deprived (B) participants. The mean cortisol concentration (open squares) is presented along with the 25, 50 and 75 percentiles (box), standard deviation (whiskers) and outliers (diamonds).

After watching the trauma film the participants were asked to rate how personally relevant they found the content of the film and how familiar they were with the clips used (**figure 4.6c**). Binomial regression revealed no significant difference between the groups in terms of relevance (z=0.016, p=0.987) or familiarity (z=-0.374, p=0.709).

4.3.3.2. Response to stressor

All participants performed below the sham high average score on the RAT. However interestingly the sleep deprived participants actually performed better on average than the non-sleep deprived participants (**figure 4.7a**, binomial regression: Group z=-2.56, p=0.01). Despite finding the task equally difficult, as assessed by the follow up questionnaire, the sleep deprived participants rated their performance as better than the non-sleep deprived participants (**figure 4.7b**, binomial regression: Difficulty; z=0.34, p=0.735, Performance; z=-2.15, p=0.032). Furthermore the sleep deprived participants showed less of an emotional reaction to the RAT in terms of mood, with a smaller increase in negative affect and a smaller decrease in positive affect (**figure 4.7c**).

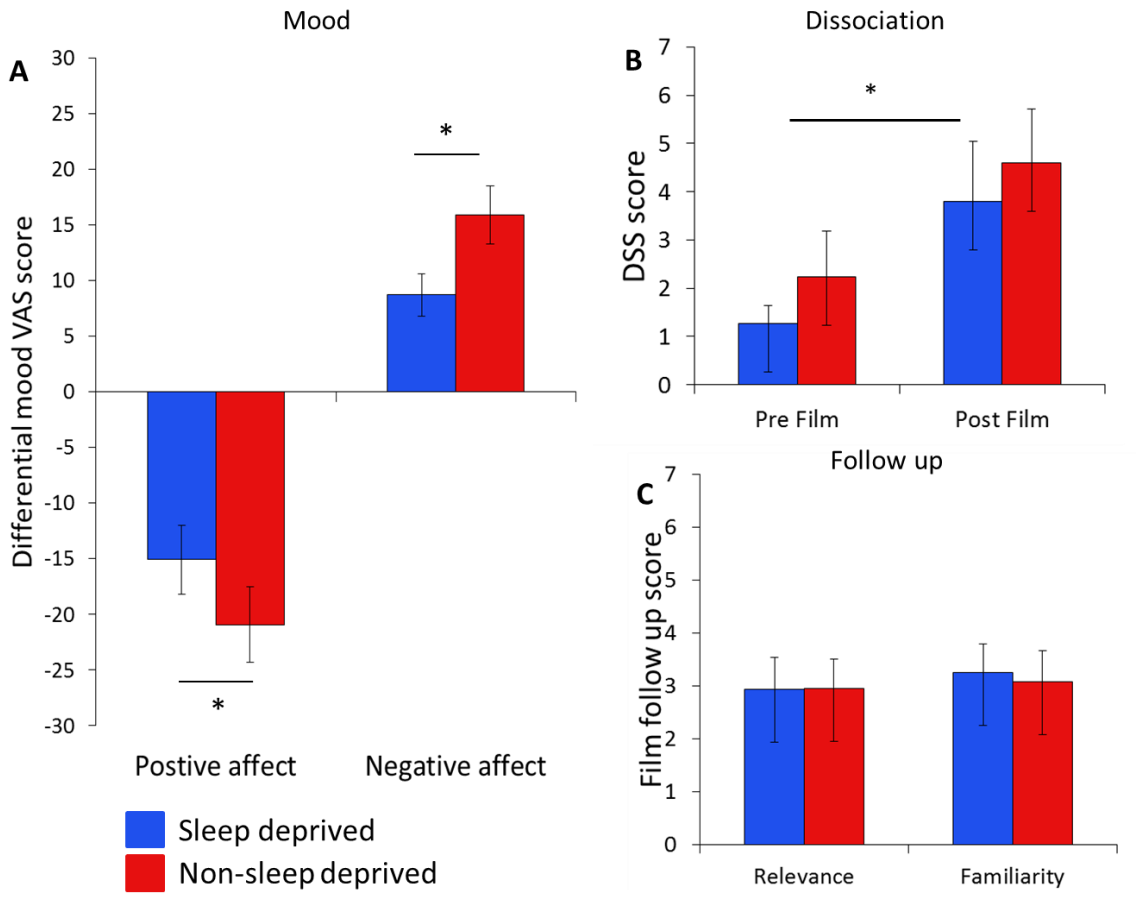


Figure 4.6 Response to trauma film. The change in positive and negative affect (A), and dissociation (B) after watching the trauma film, along with the relevance and familiarity with the content of the trauma film (C) is shown for the sleep deprived (blue) and non-sleep deprived (red) participants. Error bars represent the standard error of the mean. * ≤ 0.01.

Both of which showed a group/time interaction with binomial regression (**table 4.2**: VAS RAT positive affect and RAT negative affect).

4.3.3.3. Effect of sleep deprivation

Over the course of the experimental manipulation, a number of scales were repeated enabling a longitudinal assessment of the effect of sleep deprivation on mood (**figure 4.8**). As expected the sleep deprived participants reported a decrease in alertness (**figure 4.8a**) and an increase in sleepiness (**figure 4.8b**) over the course of the manipulation. A group/time interaction was found for both alertness and sleepiness (**table 4.2**: VAS experiment alertness and experiment sleepiness respectively).

		Group main effect		Time main effect		Interaction effect	
		z value	p vlaue	z value	p vlaue	z value	p vlaue
STAI	Anxiety	-0.16	0.87	7.05	0.00 **	-3.12	0.00 **
VAS	Experiment alertness	1.70	0.09	-17.96	0.00 **	16.04	0.00 **
	Experiment sleepiness	-0.75	0.46	35.92	0.00 **	-31.18	0.00 **
	Film positive affect	-0.66	0.51	-2.54	0.01 *	-2.44	0.01 *
	Film negative affect	0.99	0.32	2.67	0.01 *	2.20	0.03 *
	RAT positve affect	4.15	0.00 **	-0.82	0.41	-4.90	0.00 **
	RAT negative affect	-1.74	0.08	-0.12	0.91	3.35	0.00 **
	Experiment postive affect	-0.87	0.38	-10.64	0.00 **	7.49	0.00 **
	Experiment negative affect	1.67	0.10	2.45	0.01 *	-1.54	0.12
PANAS	Postive affect	0.74	0.46	-9.56	0.00 **	9.32	0.00 **
	Negative affect	1.22	0.22	0.97	0.33	-1.22	0.22

Table 4.2 Binomially distributed repeated measure analysis. The group, time and group/time interaction for all the binomially distributed analysis performed are presented. * ≤ 0.05, ** ≤0.001.

Deconstruction of this analysis revealed that in fact the sleep deprived participants had lower alertness levels and higher sleepiness levels throughout the experimental manipulation (apart from post film). However, these levels became much more pronounced during sleep deprivation. A group/time interaction was also found for anxiety levels (**table 4.2**: STAI anxiety). The anxiety levels of the sleep deprived participants were found to continually increase throughout the experimental manipulation, while those not sleep deprived reverted to baseline levels after a night of sleep and before the stressor (**figure 4.8c**). However, the non-sleep deprived participants did reach a similar level of anxiety after the stressor compared with the sleep deprived participants. Hence, the non-sleep deprived participants actually experienced a greater increase in anxiety levels, since they started out at a lower level. Interestingly in terms of positive and negative affect, only positive affect was found to have a group/time interaction for both the PANAS (**figure 4.8c** and **table 4.2**: PANAS positive and negative affect) and the mood VAS (**figure 4.8d** and **table 4.2**: VAS experiment positive affect and experiment negative affect).

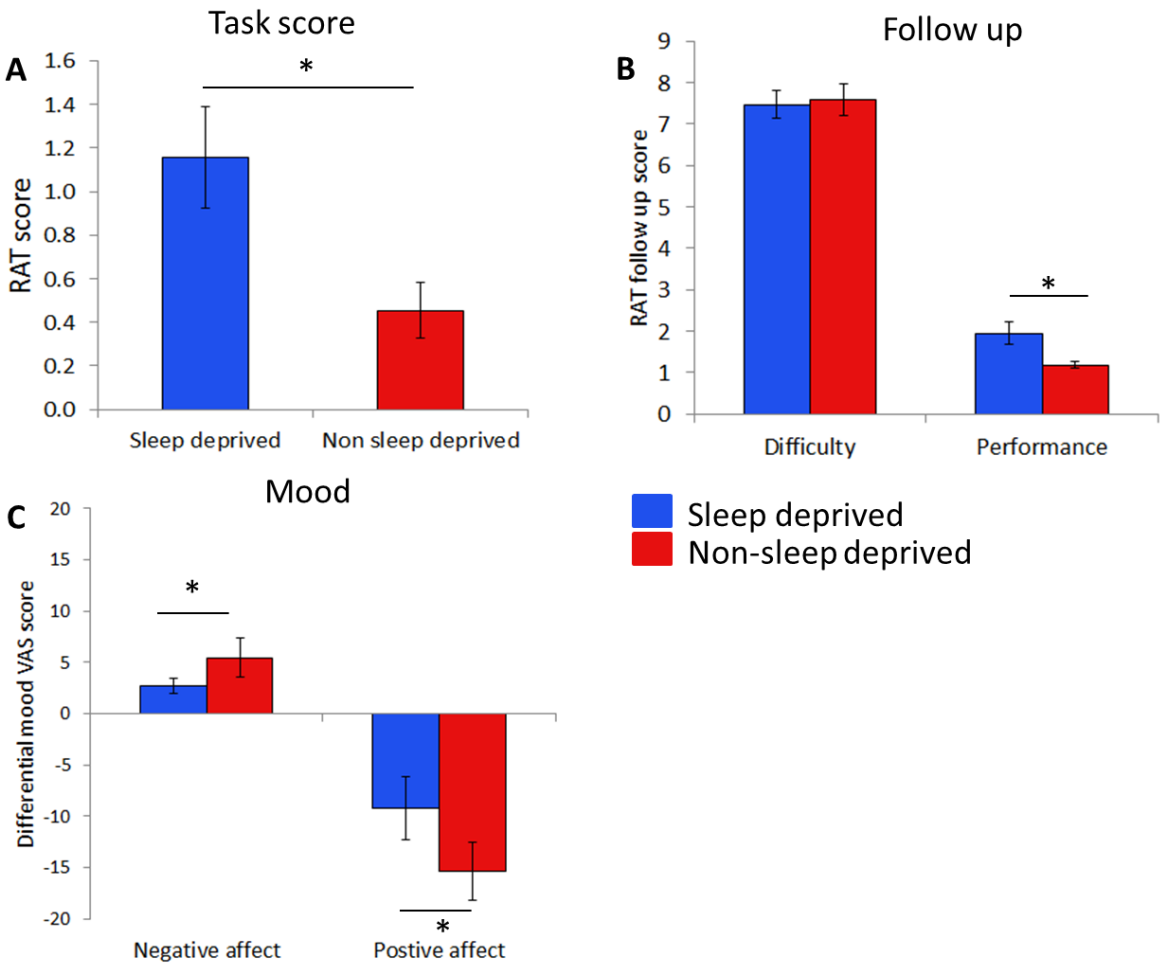


Figure 4.7 Response to stressor. The mean score on the RAT (A), self-rated difficulty and performance of the RAT (B) and change in positive and negative affect after the RAT (C) are illustrated for the sleep deprived (blue) and non-sleep deprived (red) participants. Error bars represent the standard error of the mean. *≤ 0.05.

During the study the non-sleep deprived participants showed an increase in positive affect following a night of sleep, while the sleep deprived participants showed no change from the level experienced immediately post film (mood VAS), and actually a decrease from levels reported around an hour after watching the study film (PANAS).

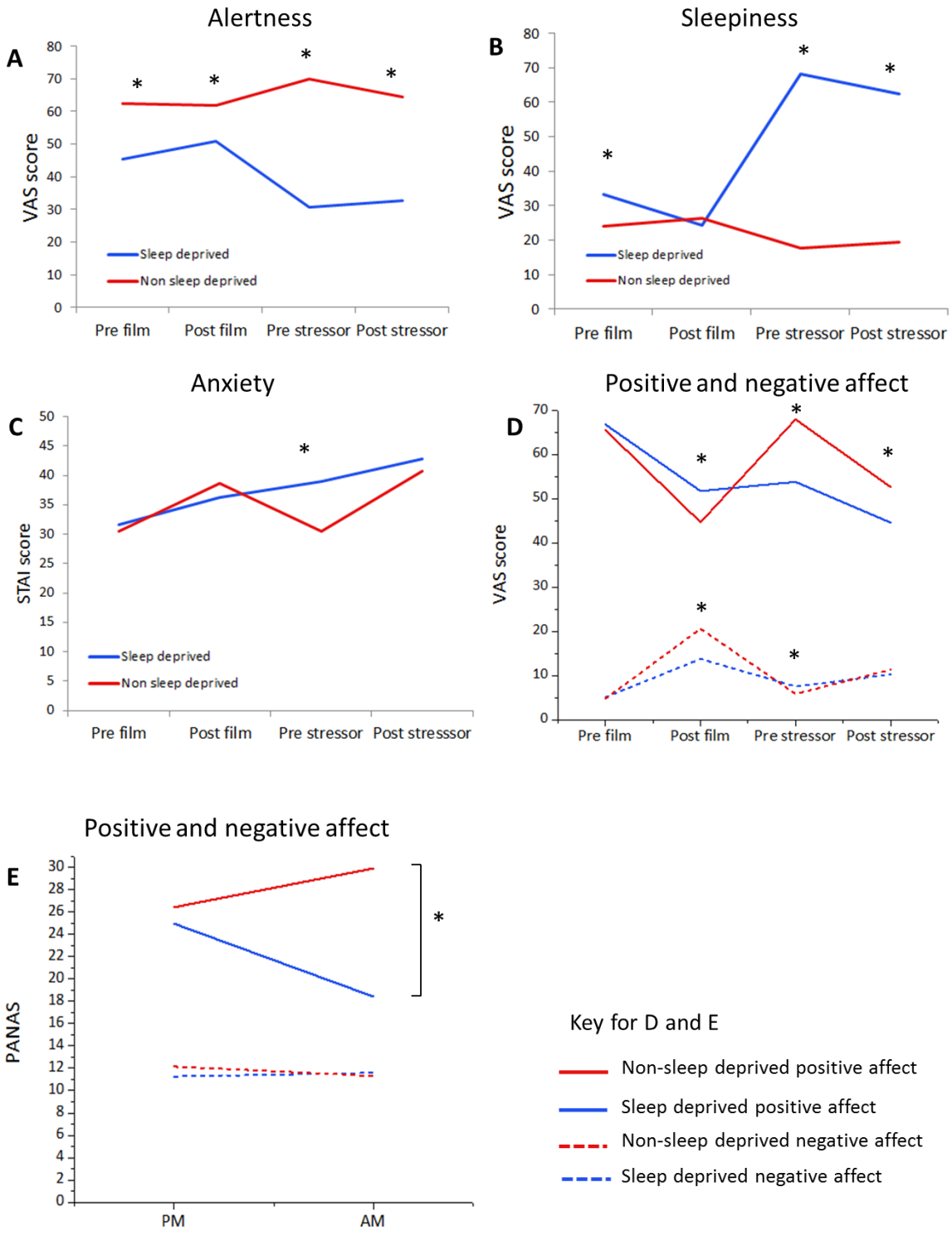


Figure 4.8 Effect of sleep deprivation on mood. The change in alertness (A), sleepiness (B), anxiety (C) and positive (solid line) and negative affect (broken line) as assessed by the mood VAS (D) and PANAS (E) are shown for the sleep deprived (blue) and non-sleep deprived (red) participants. * ≤ 0.01.

4.3.3.4. Visual-spatial and verbal activities

The results from the activity visual analogue scale (activity VAS) showed that the sleep deprived participants spent more time doing visual-spatial type activities than the non-sleep deprived (binomially distributed GLM: z value = -10.54, p≤0.001), while the non-sleep deprived spent more time doing verbal activities (binomially distributed GLM: z value = 4.81, p≤0.001) (**figure 4.9**).

4.3.3.5. Intrusive memories

Over the six days following the experimental manipulation the sleep deprived participants reported fewer intrusive memories than the non-sleep deprived (z=3.94, p≤0.001) (**figure 4.10a**). Day by day analysis (**figure 4.10b**) revealed that the majority of intrusive memories were reported in the first two days and gradually decreased in frequency over the following days. Bearing in mind that the sleep deprived participants did not received any recovery sleep until the end of day 1, the sleep deprived participants reported fewer intrusive memories on both day 1 (z=2.19, p=0.03) and day 2 (z=2.09, p=0.04). Hence, not only do the sleep deprived participants report fewer intrusions while being sleep deprived, but this was maintained following a night of recovery sleep. Of the intrusive memories included in the analysis 93% were matched to a specific scene from the trauma film. The remaining 7% were relevant to a theme from the film i.e. death.

The sleep deprived participants also reported a lower global score on the impact of event scale (IES) the morning following the manipulation (sleep deprived: mean=8.13, standard deviation =5.31; non-sleep deprived: mean=11.52, standard deviation =6.64; binomially distributed GLM (group): z=3.24, p≤0.001), indicating lower PTSD symptomology. Which turned out to be solely within the intrusion subscale (binomially distributed GLM: Group z=4.44, p≤0.001), indicating that the lower frequency of intrusions experienced by the sleep deprived participants was not due to avoiding thinking about the trauma film or a difference in the level of hyperarousal which can also affect intrusion frequency (**figure 4.10c**).

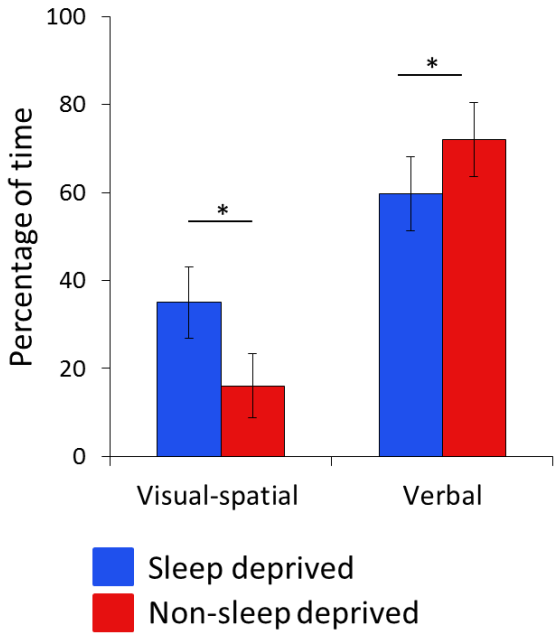


Figure 4.9 Activities. The percentage of time spent doing visual-spatial and verbal activities are shown for the sleep deprived (blue) and non-sleep deprived (red) particpants. Error bars represent the standard error of the mean. * ≤0.001.

4.3.3.6. Additional predictors of intrusion frequency

Throughout the study, the two groups were found to show variation in certain measures: the non-sleep deprived participants recorded a greater change in affect as a result of the trauma film and stressor as well as reporting spending less time doing visual spatial activities and more time doing verbal activities. The groups also differed in one baseline parameter, psychoticism. Hence to ensure these factors were not underlying the group differences seen within intrusion frequency, a Poisson distributed general linear model was fitted with: group; psychoticism; film negative affect; film positive affect; RAT positive affect; RAT negative affect; visual-spatial activities; and verbal activities as predictors. Only group ($z=2.57$, $p=0.01$) and visual spatial activity ($z=2.58$, $p=0.01$) were found to have an effect, with group showing the main effect (odds ratio: group=1.86, visual-spatial activity=1.01).

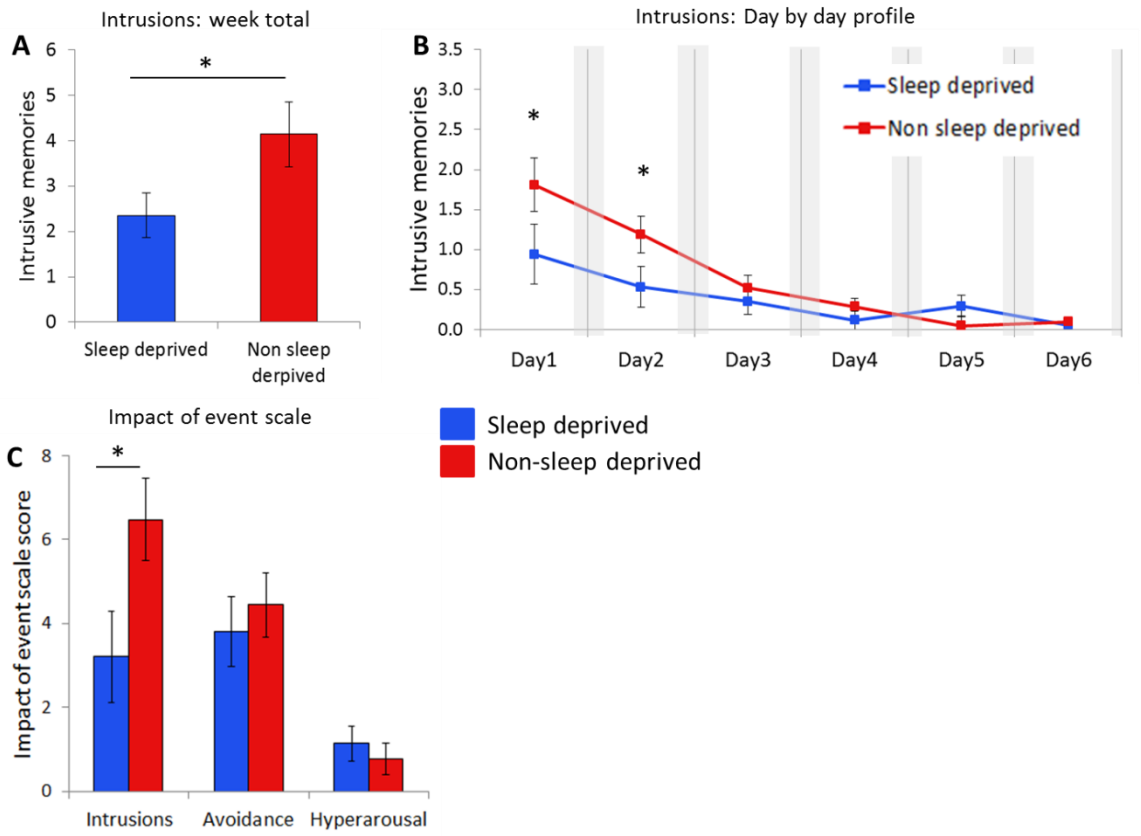


Figure 4.10 Intrusive memories. The mean total number of intrusive memories reported over the six days following the trauma film (A), the day by day profile of intrusion frequency (B) and the impact of event scale scores (C) for the sleep deprived (blue) and non-sleep deprived participants (red). Error bars represent the standard error of the mean. * ≤ 0.05.

Further analysis revealed no correlation between the time spent doing visual spatial activities and intrusion frequency for the sleep deprived participants (r=-0.35, p=2.89). However, a positive relationship was found for the non-sleep deprived participants (r=0.61 p=0.02), indicating that the more time spent doing visual-spatial activities resulted in a more intrusions being experienced.

In addition to sleep deprivation, four additional factors were hypothesised to potentially have an impact on the frequency of intrusive memories: past experience of a real life traumatic event; emotional response to the trauma film; MDQ score; and change in dissociation while watching the trauma film (see section 4.2.3. p.96 for details).

	Additional factors grouping		Sleep deprived/ non-sleep deprived		Interaction	
	z value	p vlaue	z value	p vlaue	z value	p vlaue
Trauma	0.57	0.57	3.06	0.00 **	-0.85	0.40
Emotional response	-0.18	0.86	1.78	0.08	0.26	0.79
MDQ	2.15	0.03 *	2.33	0.02 *	-1.53	0.13
Dissociation	-3.74	0.00 **	-1.44	0.15	4.14	0.00 **

Table 4.3 Poisson distributed general liner models. The effect of the additional grouping factors (trauma, emotional response, MDQ and dissociation), sleep versus sleep deprivation and the interaction on intrusion frequency. *≤0.05, ** ≤0.001.

Each factor was used as an additional grouping factor along with the original sleep/sleep deprivation grouping within separate Poisson distributed general linear models (**table 4.3**). Neither previous experience of real life trauma (**figure 4.11a**), nor the magnitude of the emotional response to the trauma film (**figure 4.11b**) were found to impact intrusion frequency. Conversely, MDQ score was found to impact on intrusion frequency: participants who reported no symptoms (no MDQ) experienced fewer intrusions than those who reported at least one symptom (**figure 4.11c**). Interestingly, dissociative state change was found to not only have a main effect on intrusion frequency, but also an interaction with the original study groups (sleep/sleep deprived) was found. Within the sleep deprived participants an increase in dissociation resulted in fewer intrusive memories being reported, whereas in the non-sleep deprived participants the opposite was the case, with more intrusions being reported with an increase in dissociation (**figure 4.11d**).

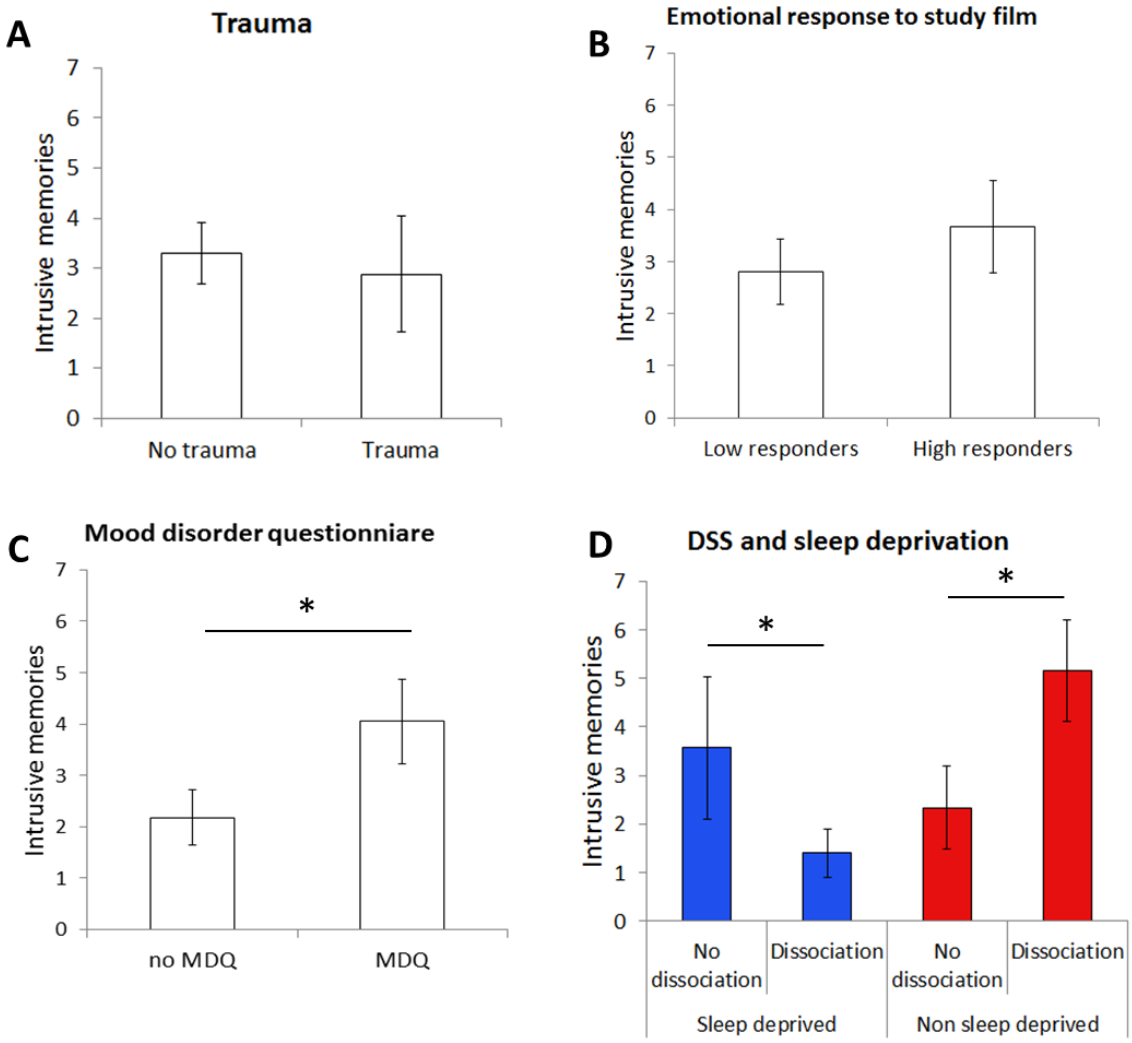


Figure 4.11 Predictors of intrusion frequency. The mean intrusive memories for past trauma experience (A), emotional response to the trauma film (B), mood disorder questionnaire (MDQ) score (C) and dissociation (D). Error bars represent standard error of the mean. * ≤ 0.05.

4.4. Discussion

The aim of this study was to investigate the relationship between sleep and intrusive memories. The data showed that a period of sleep deprivation after the traumatic event resulted in fewer intrusions being reported as compared with a night of sleep. These results were demonstrated by two independent measures: the intrusion diary and the impact of event scale (IES). The majority of intrusions were experienced in the first two days after the experimental manipulation. Furthermore, the frequency of intrusions did not increase following recovery sleep for the sleep

deprived participants, suggesting that extended wakefulness contributes to a sustained response in intrusive memory generation. Mood disorder questionnaire (MDQ) score and dissociative state were also found to impact upon intrusion frequency.

4.4.1. Intrusion frequency

This study demonstrated a very striking relationship between sleep and intrusive memories, whereby extended wakefulness resulted in the generation of fewer intrusions than a period of sleep. To date no other study has directly investigated this relationship. Comparisons can however be drawn with research investigations into posttraumatic stress disorder (PTSD). PTSD is characterised by intrusive memories to a past traumatic event, thus this condition presents a real life, though more extreme, example of the experimental manipulation conducted within this study i.e. the investigation of intrusion frequency following trauma. Clinical research has demonstrated that sedation following trauma may lead to an increase in the incidence of PTSD. In trauma survival patients, benzodiazepine treatment resulted in 9 out of 13 patients meeting diagnostic criteria for PTSD six months after the trauma compared with 3 out of 13 controls (Gelpin et al. 1996). A similar finding was also reported by Mellman and colleagues (Mellman et al. 2002). In a randomized placebo controlled study, 6 out of the 11 trauma survivors prescribed benzodiazepine treatment following the trauma went on to develop PTSD compared to 3 out of the 11 placebo controls (Mellman et al. 2002). Animal research has also demonstrated an association between sedation following trauma and PTSD. In rats, the animals treated with the benzodiazepine, alprazolam, immediately after exposure to stress showed increased PTSD like behaviour patterns and increased freezing in response to a cue of the original stressor (Matar et al. 2009).These results must however be taken tentatively in the context of supporting the research presented in this thesis. Sedation from benzodiazepine treatment is a different state to natural sleep, with changes to REM and NREM sleep being reported: increased latency to REM sleep and higher percentage of REM sleep (Roth et al. 1981) and increased sleep spindles and decreased delta (brain activity seen in slow wave sleep) amplitude (Johnson et al. 1983). Also

benzodiazepines have been found to have anterograde amnesic effects, meaning learning and memory of new information is impaired (Curran 1986). However, this amnesic action may be drug and species specific as Melendez and colleagues (Melendez et al. 2005) found no effect of Zolpidem or Triazolam (two commonly prescribed benzodiazepines) on sleep induced memory consolidation in humans. Despite these limitations the parallels between sedation following real life trauma and sleep deprivation following analogue trauma are intriguing and demand more research into the role of sleep in the generation of intrusive memories following trauma.

4.4.2. Intrusion frequency: Sleep or sleep-deprivation dependent?

It is not possible to determine from this study alone whether a period of sleep results in more intrusive memories being experienced, or sleep-deprivation results in less intrusive memories. However, the frequency of intrusions experienced by the non-sleep deprived participants is comparable with those reported by previous studies that did not involve sleep deprivation: within this study the non-sleep deprived participants reported an average of 4.14 intrusions (standard deviation ± 3.29), while between 3.1 and 6.7 intrusions over one week have been reported in similar studies (Holmes et al. 2004). Meanwhile the average frequency of intrusions for the sleep deprived participants (mean = 2.35, standard deviation = 2.03) in our study lies below this expected range, which suggests that sleep deprivation is causing a decrease in intrusion frequency.

There are several possible explanations for this observation. First, the disruption of memory consolidation by sleep deprivation could reduce the ability to remember the trauma film, resulting in fewer intrusive memories being experienced. Previous studies have found that explicit memory of an analogue traumatic event does not influence the frequency of intrusive memories (Holmes et al. 2004, Nixon et al. 2007). Although using a similar intrusion generation paradigm, these studies did not involve sleep deprivation, and therefore are only testing individual differences in memory recall. Within this study a large impact on memory consolidation has been

imposed, indicating that disruption to memory recall of the traumatic event may still be influencing the frequency of intrusive memories within this study. Moreover since sleep deprivation would also disrupt the redistribution of total synaptic strength in accordance with the synaptic homeostasis hypothesis (Tononi and Cirelli 2006), recovery sleep does not have the same effect as sleep immediately succeeding the traumatic event. Hence, providing a possible explanation as to why no increase in the frequency of intrusive memories was seen following recovery sleep in the sleep deprived participants.

Recently Kuriyama and colleagues (Kuriyama et al. 2010) have demonstrated that implicit but not explicit memory to a highly emotional event was disrupted following sleep deprivation. Explicit memory is related to the details and facts of the event, while implicit memory is the unconscious recognition of emotional and physiological responses to the event: for example on being shown a picture of a face and being told this person is good, the explicit memory is of the face while the implicit memory is that the person is good (Fox 2008). In their study Kuriyama and colleagues (Kuriyama et al. 2010) showed healthy participants a series of aversive and neutral motor vehicle accident films, which all involved similar street scenes and either ended with a traffic accident or no accident. Recognition tasks for explicit and implicit memory of the films content were carried out immediately after encoding, and then 3 and 10 days later. In the initial recognition task all participants showed high fear responses (implicit memory) to both the aversive and neutral films. By day 3 those that had been sleep deprived showed an extinction of the physiological and self-reported fear responses to the neutral films, while the recognition of the events was not different to the non-sleep deprived participants. Therefore the sleep deprived participants' implicit memory of the films had been reduced while the explicit memory was not affected. In another study sleep has been shown to preferentially preserve memory of emotional objects within a scene over the neutral background details (Payne et al. 2008), leading to the postulation that sleep uncouples the emotional aspects of an event from the neutral details and preferentially consolidated the emotional aspects over the neutral (Payne and Kensinger 2011). This therefore

suggests that the implicit memory of the event may be preferentially enhanced by sleep and therefore without sleep this enhancement does not occur and implicit memory is weakened. This hypothesis has recently been tested by Baran and colleagues (Baran et al. 2012) as the recognition (explicit memory) and emotional reaction (implicit memory) to negative pictures was assessed following a period of sleep or daytime wake in healthy adults. Following sleep the emotional reaction was preserved, while it was reduced after wake. Although comparing a period of daytime wake with night sleep, rather than sleep with sleep deprivation this study does support the hypothesis that sleep preserves implicit memory. In this study the recognition of the negative pictures was also improved following sleep compared with wake, which is in contrast to Kuriyama and colleagues (Kuriyama et al. 2010) who found no difference in explicit memory. This inconsistency could be due to different experimental designs as the studies involved different emotional stimuli (pictures versus films) and comparisons with sleep (sleep and wake versus sleep and sleep deprivation). In relation to the data presented in this thesis, the reduced number of intrusive memories following sleep deprivation could be due to diminished implicit memory of the trauma film. Since intrusive memories are thought to arise due to a shift in the processing of an event from focusing on the meaning and context of the event towards the sensory impressions (Brewin and Holmes 2003), this hypothesis presents a strong possibility for the mechanism underling the frequency of intrusive memories following sleep deprivation.

Second, visual-spatial and verbal tasks have opposing effects on intrusion frequency (Holmes et al. 2004), and it is possible that the types of activities the participants were conducting while being sleep deprived underlie the difference in intrusion frequency. Within this study the sleep deprived participants spent more time doing visual-spatial type activities, but less time doing verbal type activities. Multifactorial analysis revealed that only the amount of time spent doing visual spatial type activities, had an effect on intrusion frequency. Interestingly, a positive correlation between visual-spatial type activities and intrusion frequency was found, but only for the non-sleep deprived participants: more time spent doing visual-spatial activities resulted in

more intrusions. This is inconsistent with previous research which indicates that visual-spatial tasks causes a decrease in intrusion frequency (Stuart et al. 2006, Holmes et al. 2004, Holmes et al. 2009, Holmes et al. 2010). This discrepancy is likely to be because of methodological differences: in this study the participants rated the time spent doing different types of activities, rather than being given a specific task to complete such as the computer game Tetris (Holmes et al. 2010). Hence a direct comparison between the types of activities undertaken by Holmes and colleagues (Stuart et al. 2006, Holmes et al. 2004, Holmes et al. 2009, Holmes et al. 2010) and those in this study is not possible as these activities could activate the visual-spatial pathway to different extents. Furthermore, this thesis study relies on the participants to determine whether the activities they performed were visual-spatial or verbal and how much time they spent doing them. Also, in this study the participants rated the time spent doing activities over the 12 hours since watching the trauma film, whereas previous studies used one task, up to a maximum of four hours following the analogue traumatic event (Stuart et al. 2006, Holmes et al. 2004, Holmes et al. 2009, Holmes et al. 2010). Furthermore, although the amount of time doing visual-spatial activities does impact on the frequency of intrusions in this study, this is not likely to be the sole explanation as group (sleep deprived, non-sleep deprived) was still found to have the main effect in the regression analysis performed.

A third possibility is that sleep deprivation itself may impact differentially on the visual-spatial or verbal information processing pathways. Although sleep deprivation has been associated with an impairment of many areas of cognitive function (eg, (Orton and Gruzelier 1989, Pilcher and Huffcutt 1996, Dinges et al. 1997, Lieberman et al. 2005), there is no evidence for the differential impact on visual spatial or verbal information processing. Hence further work is needed to explore this possibility.

4.4.3. Behavioural risk for mood disorder

Participants who reported experiencing at least one symptom for mania on the mood disorder questionnaire (MDQ), experienced more intrusions than those who had not experienced any symptoms. In previous studies a score of seven or more on the MDQ has been used to indicate an increased risk for the development of a mood disorder (Rock et al. 2010). Hence, from this study it cannot be concluded that an increased risk of developing a mood disorder is associated with more intrusions being experienced. Malik and colleagues (Malik et al. Manuscript submitted) however have reported that individuals with a high MDQ score (≥ 7) experience more intrusive memories to a traumatic event than those with a low MDQ score (≤ 6). Thus, the results from this study extend this finding to suggest that individuals who have experienced at least one manic symptom may have an increased susceptibility for intrusive memories.

4.4.4. Dissociative state

Dissociation is a state of detachment from time, space and the self (Wing Lun 2008). Dissociation during or immediately following a traumatic event (peritraumatic dissociation) is thought to be a predictive factor for the development of PTSD symptomology, which includes intrusive memories (Ozer et al. 2003). Within this study an interaction was found between dissociative state and sleep state (sleep deprived or fully rested). For the fully rested participants (non-sleep deprived) more intrusions were reported when an increase in the level of dissociation occurred while watching the trauma film. This is consistent with the finding that dissociation is associated with the development of PTSD. However, for the sleep deprived participants the opposite relationship was found, when more intrusions were reported when no change or a decrease in dissociation was experienced. This suggests that the most protective situation against intrusion frequency appears to be sleep deprivation following increased peritraumatic dissociation. Although at first this may seem counterintuitive, peritraumatic dissociation is thought to prevent the proper processing of sensory information (Wing Lun 2008). Therefore, in parallel with the impairment of

memory consolidation by sleep deprivation, there may be an additive effect of dissociation on the cognitive processing of the traumatic event, and hence the generation of intrusive memories.

4.4.5. Stressor

The remote associates task (RAT) in this study was used to create a stressful situation in order to determine the effect of sleep deprivation on the response to a stressor. Surprisingly the sleep deprived participants were less affected by the stressor than the non-sleep deprived participants. The sleep deprived participants also performed better on the task. However, they rated the difficulty of the task to the same level as the non-sleep deprived, suggesting that performance on the task does not account for the smaller reaction to the task. Moreover, all participants scored well below the sham average of seven correct answers for the task, meaning that all the participants were led to believe that they had performed below average. To date only one other study has looked at the response to stressors during sleep deprivation. Using a battery of cognitive tasks designed to induce high and low levels of stress Minkel and colleagues (Minkel et al. 2012) found that although the sleep deprived participants became more stressed during low stress tasks than non-sleep deprived controls, no difference in the level of reaction was seen for high stress tasks. Minkel and colleagues (Minkel et al. 2012) postulate that sleep loss may lower the threshold at which an individual experiences an event as stressful. An additional hypothesis is raised by the findings reported in this thesis. Since the sleep deprived participants show less of a response to the stressor it could be that these participants have an excuse for their poor performance i.e. they are sleep deprived and are therefore less able to complete the task. Previous studies however, have found the opposite to be true: chronically sleep restricted medical residents showed an enhanced reaction to negative events (Zohar et al. 2005). However, these reactions were to real life events while working in a hospital, compared with a cognitive task in a laboratory as in this study. Hence, more work is needed to establish the effect of sleep deprivation on varying degrees of stressful events and under different environmental conditions. It remains to be fully determine how sleep deprivation, and in more real world conditions chronic

sleep restriction, affects how people respond to stressful events, whether different types of stressful events evoke different responses and what the underlying mechanisms for these reactions are.

4.4.6. A beneficial role of sleep deprivation?

A beneficial role of sleep deprivation is postulated by this research as sleep deprivation results in fewer intrusive memories compared with sleep. Initially this seems counterintuitive considering the detrimental effects of sleep deprivation including cognitive impairment, diminished mood and association with long term poor health (Spiegel et al. 2004, Leproult et al. 1997, Banks and Dinges 2007, Knutson and Van Cauter 2008, King et al. 2008, Cappuccio et al. 2007, Knutson et al. 2009). However there is additional research to suggest that sleep deprivation may not always be bad for us. Recently Kuriyama and colleagues (Kuriyama et al. 2010) have demonstrated that sleep deprivation can extinguish fear responses. Following sleep deprivation fear response relating to neutral film clips were diminished compared with the non-sleep deprived participants, while the fear responses to the aversive film clips did not. This suggests that the responses to the neutral situations were eliminated while those to the real threat situation remained. Sleep deprivation also has an antidepressant action. In some cases of clinical depression, sleep deprivation causes an immediate elevation of mood (Wirz-Justice and Van den Hoofdakker 1999). However, this response is limited to the sleep deprivation period, following sleep the depressed mood returns, unless sleep deprivation is used in combination with other therapies such as light treatment, phase advancing of the sleep schedule and pharmacological interventions (Wirz-Justice and Van den Hoofdakker 1999).

These beneficial effects of sleep deprivation all occur in highly emotional situations: following trauma (Kuriyama et al. 2010), or in clinical depression (Wirz-Justice and Van den Hoofdakker 1999). Thus it is possible that it is only in such situations of high emotional stress that sleep deprivation may be beneficial. From an evolutionary standpoint this makes sense. Before our 24/7 society, a period of total sleep deprivation would most likely have occurred during or

following a highly traumatic or emotional event such as a battle, hunt or possibly a natural disaster. During these periods people would have had to remain awake to survive the situation. A consequence of not sleeping may also be that this aided the processing of the event either by continued wake or prevention of sleep. In the long term, this could then result in a better response to the traumatic event, for example fewer intrusive memories. This possible beneficial role of sleep deprivation in emotional processing after trauma is only beginning to become apparent, not only from studies exploring emotional processing and sleep but also from the association between sedation following trauma and PTSD.

4.4.7. Potential clinical relevance

Sleep deprivation following trauma has a clinical application for the treatment of individuals following real life trauma. Analogue trauma was used in this study to simulate real life trauma. Although it was successful in generating intrusive memories, analogue trauma is still only an approximation of real life trauma. To fully establish the potential clinical relevance of sleep deprivation following trauma, research using real life trauma must be conducted. It is unethical to subject people to real life trauma therefore alternative approaches must be taken to explore this relationship. One approach would be to observe sleep behaviour of real life trauma survivors to determine whether people, do or do not, sleep following trauma. If self-imposed sleep deprivation following trauma is observed, it could be investigated whether people who don't sleep following trauma have a better long-term prognosis i.e. less trauma related symptoms such as intrusive memories, and less chance of developing PTSD. To date only one study into real life trauma has reported sleep behaviour immediately following trauma. During the recent Tohoku-Pacific Ocean earthquake and tsunami, Japan March 2011, Mizuno and colleagues (Mizuno et al. 2011) accidentally collected actigraphy recordings of seven people during the tsunami, who were taking part in an unrelated study. The participants were elderly residents of Sendai, which was directly hit by the earthquake and tsunami. On the first night following the disaster all the participants did sleep, although sleep disturbances were reported. Therefore this study does not

indicate self-imposed sleep deprivation following the trauma. It is difficult to draw conclusions from this research as not only did it only involve seven people, but also it involves a type of trauma that results in lower incidents of PTSD than other types of trauma (Breslau et al. 1998). Moreover these individuals were not being investigated in terms of their response to trauma so the psychological response to the trauma was not studied and no follow up was conducted to determine whether these individuals went on to develop trauma related symptomology or PTSD. To fully address the role of sleep after real life trauma larger studies are needed that investigate a range of different trauma types including those known to induce higher levels of PTSD such as serious accidents, rape and serious physical assault (Breslau et al. 1998).

4.4.8. Study limitations and improvements

There are a number of potential limitations of this study, those limitations that concern the design of the study are detailed in table 4.4, along with any effect each limitation may have had on the results of this study and ways in which each limitation could be resolved for future studies. Firstly the sample size reduced the statistical power for the study. To create as homogenous population as possible the eligibility criteria were very stringent for this study, therefore making recruitment of suitable participants more difficult. An extension of this study, with a larger group of participants, would enable stronger conclusions to be drawn. Secondly memory recall of the trauma film was not tested. As previously discussed (section 4.4.2 p. 116) sleep affects implicit and explicit memory, and sleep deprivation may impair implicit memory and underlie the frequency of intrusive memories. Therefore an assessment of both implicit and explicit memory to the trauma film following sleep or sleep deprivation is required to fully elucidate the mechanism underling the influence of sleep deprivation of intrusive memory frequency. Thirdly in this study the non-sleep deprived participants did not remain in the sleep lab during the experiment, but returned home to sleep. Therefore the participants did not remain in the same environment for the entire study. It is possible that this may have altered the non-sleep deprived participants' reaction to the study. Finally the study protocol was refined during testing, meaning

that some participants underwent a slightly different protocol than others. The details of this are described in Chapter 2 (section 2.2.3. p.38). The changes made to the protocol affected three aspects of the study i) eligibility into the study ii) the mood VAS and iii) the location of the sleep deprivation. In terms of the changes to eligibility all the participants who completed the study before the changes were made were found retrospectively to fulfil these additional criteria. Therefore this change in the eligibility criteria would not have affected the outcome of this study. The mood VAS was altered during the study to be more sensitive. Only four participants used the original unaltered scale and comparison with the participants that completed the altered scale reveal no differences in the average scores. Finally the first four sleep deprived participants were sleep deprived in the sleep lab for the first 12 hours following the trauma film (i.e. during the night), but they went home for the remaining 12 hours. The participants were instructed not to sleep, or consume caffeine, however as they were not being monitored it is not possible to exclude the possibility that this did not happen. Therefore for these participants the effect of the sleep deprivation may have been weakened. If this was the case the expected bias would be for these participants to experience more intrusive memories since the participants that slept reported more intrusive memories than those who were sleep deprived. Therefore if anything, this modification to the protocol would have weakened the difference seen between the groups.

Limitation of study design	Possible effect on results	Resolution of limitation
Eligibility criteria: participants with bed times later than 2am were excluded; participants with a history of fainting were excluded; participants that consumed high levels of caffeine (more than 300mg) excluded.	No effect, as all criteria were met by participants even though not an exclusion criteria on inclusion into the study.	Despite no effect on the results expected, it would always be better to have the same inclusion and exclusion criteria for all participants in a study.
Timing of when participants notified of study group: all the participants knew which group they were in at the start of the study.	Alteration to emotional reaction to the study especially the trauma film as the emotional responses may also have been in relation to having to stay awake. In terms of intrusive memories, this may have caused the trauma film to have less of an impact as the participants were not concentrating on the film, but the following sleep deprivation and therefore could possibly reduce the number of intrusive memories experienced. Conversely the heightened emotional state due to the impending sleep deprivation may have meant that the trauma film had more of an impact and therefore the participants would have been expected to report more intrusions.	Not informing the participants which study group they are in until after the trauma film.
Change of mood VAS scale: scale made to be more sensitive after first four participants	The original mood VAS may have picked up less change in mood during the study. However comparisons between those that used the original and revised scale showed no difference.	Although no effect is expected on the results, it is always better to ensure the participants complete the same scales in a study.
Sleep deprived participants remaining in the sleep lab following the trauma film, while the non-sleep deprived went home	The processing of the trauma film may have been different depending on whether the participants remained or not. For example the sleep deprived participants may have been reminded of the trauma film more than the non-sleep deprived. However if this was the case then it would be expected that the sleep deprived participants would report more intrusive memories, which is not	Ensuring all the participants experience the same environment following the trauma film i.e. all stay in sleep lab or all go home.

	the case. However this difference may have acted to weaken the difference seen between the groups.	
Change in location of sleep deprivation: the first four sleep deprived participants went home after 12 hours of sleep deprivation in the sleep lab, and where required to remain awake until the following evening.	These four participants may have had a weakened sleep deprivation experience as they could have slept (not observed from actimeteric recordings), and/or consumed caffeine or other stimulants. Since the sleep group reported more intrusive memories, weakened sleep deprivation could result in more intrusions being experienced than full sleep deprivation. Therefore this could again have weakened the difference seen between the two groups.	Ensure all participants experience the same degree of sleep deprivation.
Collection period of intrusive memories: participants were informed about intrusion diary immediately after the trauma film even though only the intrusive memories reported from the following morning were included in the analysis. Therefore the sleep deprived participants have a longer period reporting intrusive memories.	Despite not being included in the analysis, having a longer period awake immediately after the film may have resulted in the sleep deprived participants having more time to understand the intrusion diary, recognise intrusions and also having intrusions may trigger more intrusions. Therefore the sleep deprived participants would be expected to report more intrusions, which again could have weakened the difference seen between the groups.	Instructing the participants about intrusive memories the morning following the trauma film.

Table 4.4 Study limitations. Each limitation for the study is outlined along with any possible effect each limitation may have on the results of this study, and what could be done to resolve this limitation for future studies.

4.5. Key findings

- Sleep deprived participants reported fewer intrusive memories following an analogue traumatic event compared with participants that had slept
- The amount of time spent doing visual-spatial activities influenced intrusive memory frequency but not over the effect of sleep or sleep deprivation

- Participants who have experienced at least one symptom of mania reported more intrusive memories than those who have not experienced any
- Sleep deprived participants who showed an increase in dissociation following the trauma film reported fewer intrusions than non-sleep deprived participants who did not show a change in dissociation
- Sleep deprived participants responded less to a stressor than non-sleep deprived participants

4.6. Conclusion

This study has demonstrated that a period of sleep deprivation following an analogue traumatic event results in fewer intrusive memories of that event being experienced. This adds to the limited body of knowledge suggesting that in some situations of high emotional distress, sleep deprivation may be beneficial. The clinical implications of this are somewhat tentative but a real need exists to explore this phenomenon further.

5. Analogue trauma and sleep physiology

5.1. Introduction

This chapter presents the analysis of polysomnographic recordings made before and after the analogue traumatic event to answer the question: **what is the effect of an analogue traumatic event on sleep physiology?** Furthermore, differences in sleep physiology were investigated in relation to emotional processing, to address the question: **what, if any, sleep characteristics are predicative of intrusive memory frequency?** The protocol for obtaining these recordings is described in Chapter 4 (section 4.2.1. p.92). In terms of the effect of an analogue traumatic event on sleep physiology it was hypothesised that REM sleep would be preferentially affected by exposure to the trauma film. In relation to the frequency of intrusive memories it was hypothesised that sleep physiology related to memory consolidation such as slow wave oscillations would be associated with the frequency of intrusions.

REM sleep has been associated with emotional processing; however research into this relationship has not always produced consistent results. Although dreaming can occur in REM and NREM sleep, dreaming in REM sleep tends to be more vivid and emotionally loaded than in NREM sleep (Nielsen 2000). Emotional events, such as aversive films (Baekeland et al. 1968, Goodenough et al. 1975) and cognitive failure tasks (Vandekerckhove et al. 2011) have been reported to affect REM sleep, however not only are the changes to REM sleep inconsistent with both decreases in the amount of REM sleep (Vandekerckhove et al. 2011) and increases in REM density (Baekeland et al. 1968) reported, but also some studies have reported no alterations to REM sleep following an emotional daytime event (Lauer et al. 1987, Goodenough et al. 1975).

The relationship between REM sleep and emotional memory is better defined. REM sleep deprivation studies (Cartwright et al. 1975); early (SWS sleep enriched) versus late night (REM sleep enriched) comparisons (Wagner et al. 2001, Wagner et al. 2006) and REM/NREM contributions to naps (Nishida et al. 2009) have all demonstrated that REM sleep enhances

emotional memory. REM sleep has also been implicated in the modulation of the following day's emotional reactivity. REM sleep has been found to enhance emotional reactions to stimuli presented the following day, suggestion that instead of ameliorating emotional reactions, REM sleep actually makes them stronger (Wagner et al. 2002, Lara-Carrasco et al. 2009). Late night sleep deprivation (Wagner et al. 2002) and REM sleep deprivation (Lara-Carrasco et al. 2009) have demonstrated that REM sleep enhances ratings and arousal scores to negative pictures respectively. Recently, van der Helm and colleagues (van der Helm et al. 2011) have found that decreased emotional reactively is correlated to the level of gamma (30-40Hz) activity in REM sleep, where the lower the level of gamma activity the greater the decrease in emotional reactivity. Therefore, this study suggests, in contrast to Wagner (Wagner et al. 2002) and Lara-Carrasco and colleagues (Lara-Carrasco et al. 2009), that REM sleep is involved in the reduction in subjective emotional responses. However van der Helm and colleagues (van der Helm et al. 2011) only assessed emotional reactivity in relation to REM sleep, and since the participants were allowed a full night of sleep, including NREM sleep, it is possible that NREM sleep may also have been involved in the modulation of emotional reactions.

Other aspects of sleep have also been associated with emotional processing. Emotionally stressful events, both real-life (Akerstedt et al. 2007) and experimentally induced (Vandekerckhove et al. 2011), have been associated with lower sleep efficiency, more time spent awake during the sleep period, longer slow wave sleep latency and reduced total sleep time (Vandekerckhove et al. 2011, Akerstedt et al. 2007, Akerstedt 2006). In addition, slow wave sleep has been hypothesised to be involved in the consolidation of emotional memory (Cai 1991, Cai 1995). As discussed in Chapter 1 of this thesis, slow wave sleep is thought to be a key process underlying explicit memory consolidation. In terms of emotional memory, slow wave sleep has been associated with enhanced positive rating of pictures (Wagner et al. 2002) and increased habituation to negative stimuli compared to REM sleep, meaning that the response to negative stimuli are diminished more quickly (Pace-Schott et al. 2011). Slow wave sleep has also been implicated in the

consolidation of temporal information for emotional memories (Groch et al. 2011). These studies not only suggest that slow wave sleep may have a role in emotional memory consolidation, but also that it results in a positive shift in emotional reactivity while REM sleep appears to cause a negative shift.

As well as sleep states (e.g., NREM, REM sleep) specific frequency bands of electroencephalography (EEG) recorded brain activity, have been associated with emotional processing. As stated above, gamma activity (30-40Hz) in REM sleep has been correlated with emotional reactivity: the less gamma activity the more emotion reactivity is decreased (van der Helm et al. 2011). Also in REM sleep, theta activity (4-7Hz), in the prefrontal region has been associated with emotional memory consolidation: the more theta the better the memory enhancement (Nishida et al. 2009).

5.2. Methods

Polysomnographic (PSG) recordings were made for all the participants who completed study two (sleep deprived = 20; non sleep deprived = 22). For each participant two nights of sleep were recorded for analysis: the night before the experiment (baseline night) and the first night of sleep following the experiment (retest night). One participant was excluded from the analysis as she was an outlier for the primary outcome measure, intrusive memories. Due to technical difficulties seven nights of individual sleep recordings were not suitable to be included in the analysis: three baseline nights (sleep deprived = 2; non sleep deprived = 1) and four retest nights (sleep deprived = 2; non-sleep deprived = 2). In total 77 nights were analysed: 39 baseline nights (sleep deprived = 18; non-sleep deprived = 21) and 38 retest nights (sleep deprived = 18; non-sleep deprived = 20). All the recordings were scored, and artefacts and rapid eye movements annotated manually by myself. The raw sleep parameters (detailed below section 5.2.1 p.132) were calculated by Alpar Lazar, and I performed the analysis of the data.

5.2.1. Analysis

For each PSG recording a range of variables were calculated relating to sleep structure, sleep stages, REM density and spectral frequency. Each variable is detailed below along with the method used to calculate it. Three types of comparisons were conducted with the sleep physiology data. Firstly baseline comparisons were conducted between the two groups (sleep deprived and non-sleep deprived). Secondly, within group comparisons in non-sleep deprived participants were made to assess the effect of the trauma film on sleep physiology. Finally, within group comparisons in the sleep deprived participants were made to evaluate the effect of sleep deprivation and the trauma film on sleep physiology. In addition, direct and indirect associations between sleep physiology and intrusive memories were assessed.

5.2.2. Sleep variables

For each PSG recording the time the participants were ready for sleep i.e. when they switched the lights off and when they got out of bed (lights on) were defined using self-reported times provided by the participants. In cases where the times provided did not correlate with the recordings lights off was defined as the beginning of the last continuous period of quite wake (i.e. wake without lots of movement) before sleep onset and lights on was the beginning of the first continuous period of active wake (i.e. wake with movement) following sleep end. Sleep onset was defined as the first epoch of any sleep stage (NREM: stage 1, 2, 3, 4 or REM) after lights off. Sleep end was defined at the last epoch of sleep before lights on.

5.2.2.1. Sleep structure

Total sleep time (minutes): sleep period in minutes from sleep onset to sleep end.

Sleep latency (minutes): length of time from lights off to first 5 minutes of persistent sleep, which is a period of any sleep stage (NREM: stage 1, 2, 3, 4 or REM) without wake.

Wake after sleep onset: total amount of time spent awake during the sleep period (sleep onset to sleep end). Calculated as absolute time (minutes) and as a percentage of total sleep time (%).

Sleep efficiency (percentage): amount of sleep (NREM: stage 1, 2, 3, 4 or REM) as a percentage of the total sleep time.

SWS latency (minutes): length of time from sleep onset to the first epoch of SWS sleep (stage 3 or 4).

REM latency (minutes): length of time from sleep onset to the first epoch of REM sleep stage.

5.2.2.2. Sleep stages

For each sleep stage (Stage 1, Stage 2, Stage 3, Stage 4 and REM) as well as wake and movement the absolute amount of time and percentage of total sleep time were calculated for the whole night. For each recording the total sleep period (lights off to lights on) was divided into eight equal intervals to allow analysis of the progression of sleep over the night.

5.2.2.3. REM density

Rapid eye movements were manually scored. A rapid eye movement was defined as being faster than 1Hz, having a simultaneous opposite movement (deflection) in both eye channels (EOG1 and EOG2) and any amplitude above the background noise (Khalsa et al. 2002). Only active deflections were included i.e. not those deflections that are the passive return to baseline (Hong et al. 1997). Finally, there must have been at least 200ms between each eye movement (Marzano et al. 2011). The REM density was determined by calculating the number of 4 second REM sleep periods that contained at least one eye movement, and expressed as a percentage of all 4 second REM sleep periods (Battaglia et al. 1999). The REM density was also calculated for each interval of the total sleep period.

5.2.2.4. Spectral frequencies

In order to calculate the spectral frequencies for the sleep recordings, any artefacts were first annotated and excluded from the analysis (Lázár et al. 2010). The spectral frequencies were calculated for each EEG channel: Fpz, Fz, C3, C4, Pz and Oz. C3 and C4 were averaged to create a

central derivation. The spectral frequencies were binned into seven categories, where the average power spectral density for each frequency band was calculated. The frequency bands used were: delta (0.5 to 4 Hz), theta (4.5 to 7.5 Hz), alpha (8 to 10.5 Hz), sigma (11 to 15.5 Hz), beta1 (16 to 20.5 Hz), beta2 (21 to 25.5 Hz) and gamma (26 to 32 Hz). The activity of each frequency band was calculated for REM and NREM sleep for the whole night, each interval of the total sleep time and also for each topographical region. NREM sleep was calculated by summating stage 2, stage 3 and stage 4 sleep. Stage 1 sleep was not included as this sleep stage can be interpreted at quite wake. The spectral frequencies were calculated by Alpar Lazar using the Welch's periodogram method using 4 seconds long non-overlapping epochs each linearly detrended. A 1024 point Hanning window was applied to minimize leakage. The calculations were done in the Python programming language (Python, 2009: http://www.python.org) using the SciPy (http://scipy.org) and Matplotlib (http://matplotlib.sourceforge.net) packages (Lázár et al. 2010).

5.2.2.5. Sleep physiology and intrusive memories

Direct and indirect associations were assessed between sleep physiology and intrusive memories. Direct associations were calculated for each sleep parameter for the whole sleep period. As well as the experimental grouping (sleep deprived, non-sleep deprived), the participants were grouped according to the median split for each sleep parameter for the baseline and retest nights as well as the change between the baseline and retest nights. Indirect associations were assessed by evaluating differences in sleep physiology between the sleep deprived and non-sleep deprived participants, as these groups report different frequencies of intrusive memories: sleep deprived fewer than non-sleep deprived (see Chapter 4 section 4.3.3.5. p.110 for details).

5.2.3. Statistical analysis

Statistical analysis was performed using SPSS version 19 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp). The data were tested for normal

distribution and the appropriate tests were used accordingly. Comparisons between the groups at baseline were performed using the Mann Whitney test. For within group comparisons for the sleep deprived and non-sleep deprived groups, repeated measure analysis was performed using the Wilcoxon signed ranks test. Multivariate analysis was undertaken using a general linear model and repeated measure analysis was used to assess the change over time and position on the scalp between the groups for the spectral frequency data. Both multivariate analysis and repeated measure analysis were used on data that did not satisfy checks for normal distribution, however non-parametric equivalents are not available, therefore Bonferroni corrections (α/ number of tests) were applied to ensure a more stringent acceptance of significance to counteract the weaken accuracy of these tests. Finally, Poisson distributed regression was used to assess the direct associations between sleep physiology and intrusive memories. This analysis was used as the intrusion data was count data, with a low chance of occurring but with many opportunities to do so (six days). Bonferroni corrections were also applied to analysis where increased type I error (false positives) may be applicable and therefore reducing this error that occurs because of multiple testing.

5.3. Results

Representative hypnograms for the baseline and retest nights for a sleep deprived and non-sleep deprived participant are shown in **figure 5.1**. Each sleep period cycles through NREM and REM sleep, with more NREM sleep at the beginning of the night and more REM sleep at the end. Each sleep period shows a similar duration with the exception of the retest night for the sleep deprived participants, which shows the anticipated increase in sleep duration.

5.3.1. Sleep at baseline

At baseline the two groups did not differ in terms of total sleep time, sleep latency or sleep efficiency (**figure 5.2 and table 5.1**).

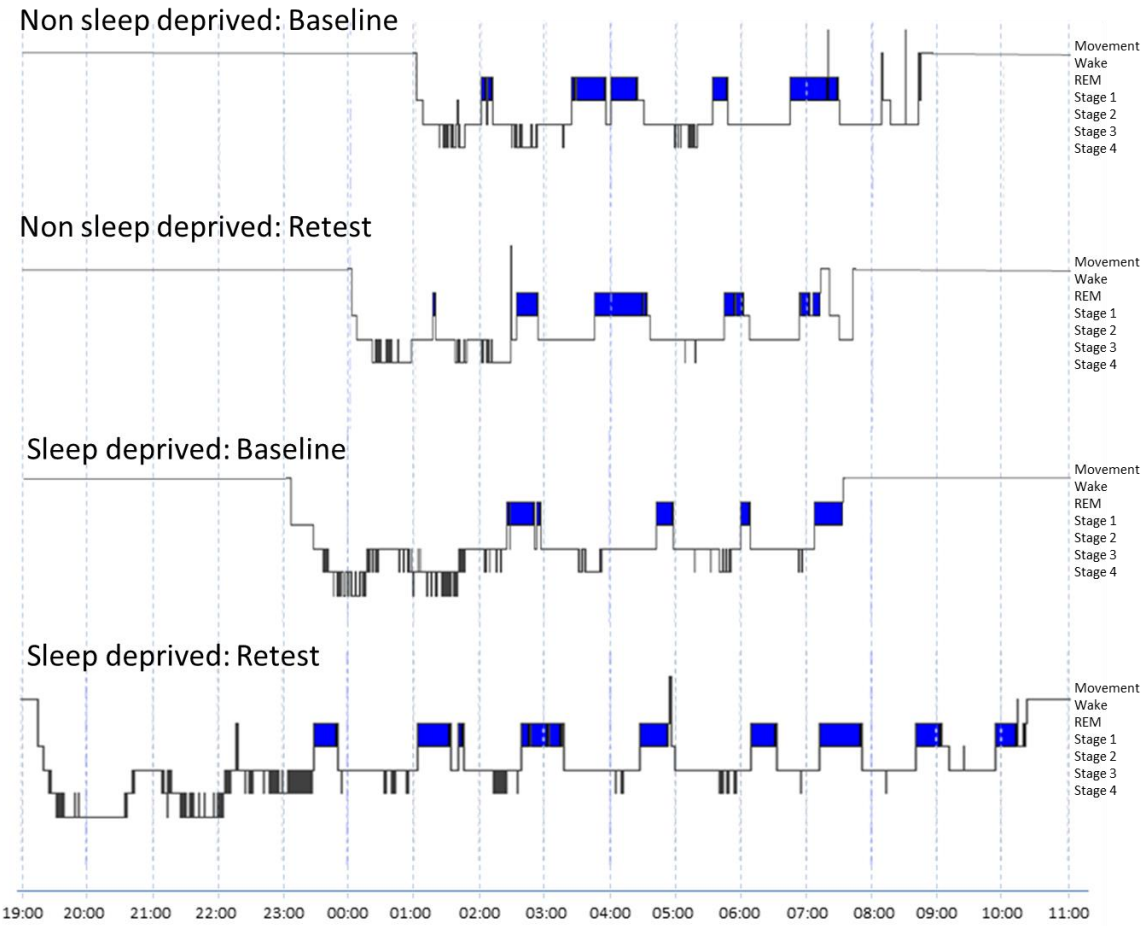


Figure 5.1. Hypnograms. A representative hypnogram from one sleep deprived and one non-sleep deprived participant are shown for the baseline and retest nights. The baseline and retest hypnograms presented are from the same individual.

The groups also showed the same amount of time spent awake during the sleep period (absolute and percentage of TST), and the same latency to the first REM period (REM latency). The sleep deprived group did however show a significantly longer latency to slow wave sleep (SWS latency). However, following correction for multiple statistical testing (Bonferroni correction) this result was no longer significant.

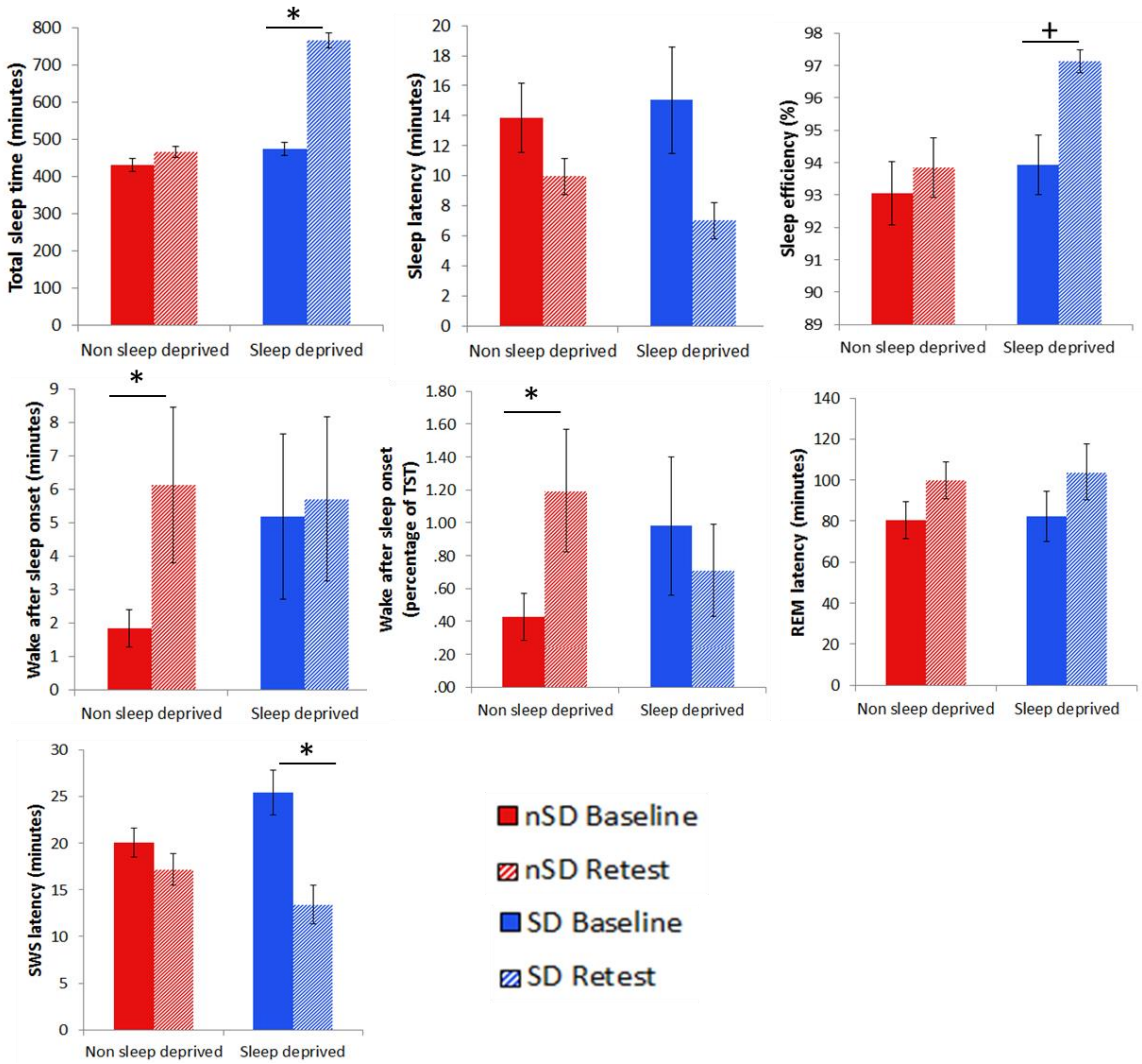


Figure 5.2. Sleep parameters. The average for the sleep deprived (blue) and non-sleep deprived (red) participants for the baseline (solid colour) and retest (striped) nights are shown for all the sleep parameters. * denotes a significant result following Bonferroni correction; + denotes a significant result before, but not following Bonferroni correction. Error bars represent standard error of mean.

5.3.1.1. Sleep stages

No significant difference was found between the groups at baseline in terms of the absolute (**figure 5.3a** and **table 5.2**) and percentage (**figure 5.3b** and **table 5.2**) of time spent in each sleep stage (NREM: stages 1. 2, 3, 4 and REM), time spent awake or movement over the whole night. Over the course of the night the participants showed the expected progression of sleep stages, with more slow wave sleep (stages 3 and 4) at the beginning of the night and more REM sleep at the end of the night, which included both the absolute (**figure 5.4**) and percentage of time (**figure 5.5**).

	Baseline[a]		Effect of trauma film[b]		Effect of trauma film and sleep deprivation[b]	
	Z score	*p vlaue*	Z score	*p vlaue*	Z score	*p vlaue*
Total sleep time	-1.26	*.21*	-1.27	*.20*	-3.52	*.00* *
Sleep latency	-.03	*.98*	-1.20	*.23*	-1.90	*.06*
Wake after sleep onset						
absolute time	-.21	*.84*	-2.90	*.00* *	-.80	*.42*
percentage of TST	-.28	*.79*	-3.25	*.00* *	-.24	*.81*
Sleep efficiency	-.40	*.71*	-.50	*.61*	-2.64	*.01* +
SWS latency	-1.94	*.05*	-1.70	*.09*	-2.95	*.00* *
REM latency	-1.57	*.12*	-.12	*.90*	-.10	*.92*

Table 5.1. Sleep parameter statistical comparisons. The statistical results ([a] Mann Whitney and [b] Wilcoxon signed ranks test) for comparisons between sleep deprived and non-sleep deprivied participants at baseline (baseline) and between baseline and retest nights for non-sleep deprived (effect of trauma film), and sleep deprived (effect of trauma film and sleep deprivation). Signigicance level following Bonferroni correction: 0.05/7=0.007. * denotes a significnat result following Bonferroni correction. + denotes a significnat result before but not after Bonferroni correction.

The sleep deprived participants had significantly more stage 2 (absolute time) than the non-sleep deprived at baseline in interval 7 (**table 5.3**). However, this result was no longer significant following Bonferroni correction, suggesting that it is likely to be the result of multiple testing.

5.3.1.2. REM density

Over the whole baseline night there was no difference in REM density between the two groups (**figure 5.6**: Mann Whitney: Z = -1.70, p= 0.09). REM density increased for both groups over the course of the night (**figure 5.7**). Repeated measure analysis revealed an effect of time but no time/group interaction (time: F=13.76, p≤0.001; time/group: F=1.16, p=0.36). However, individual analysis of each interval revealed that the non-sleep deprived participants had a higher REM density than the sleep deprived participants in interval 4. This result was no longer significant following Bonferroni correction (**table 5.4**).

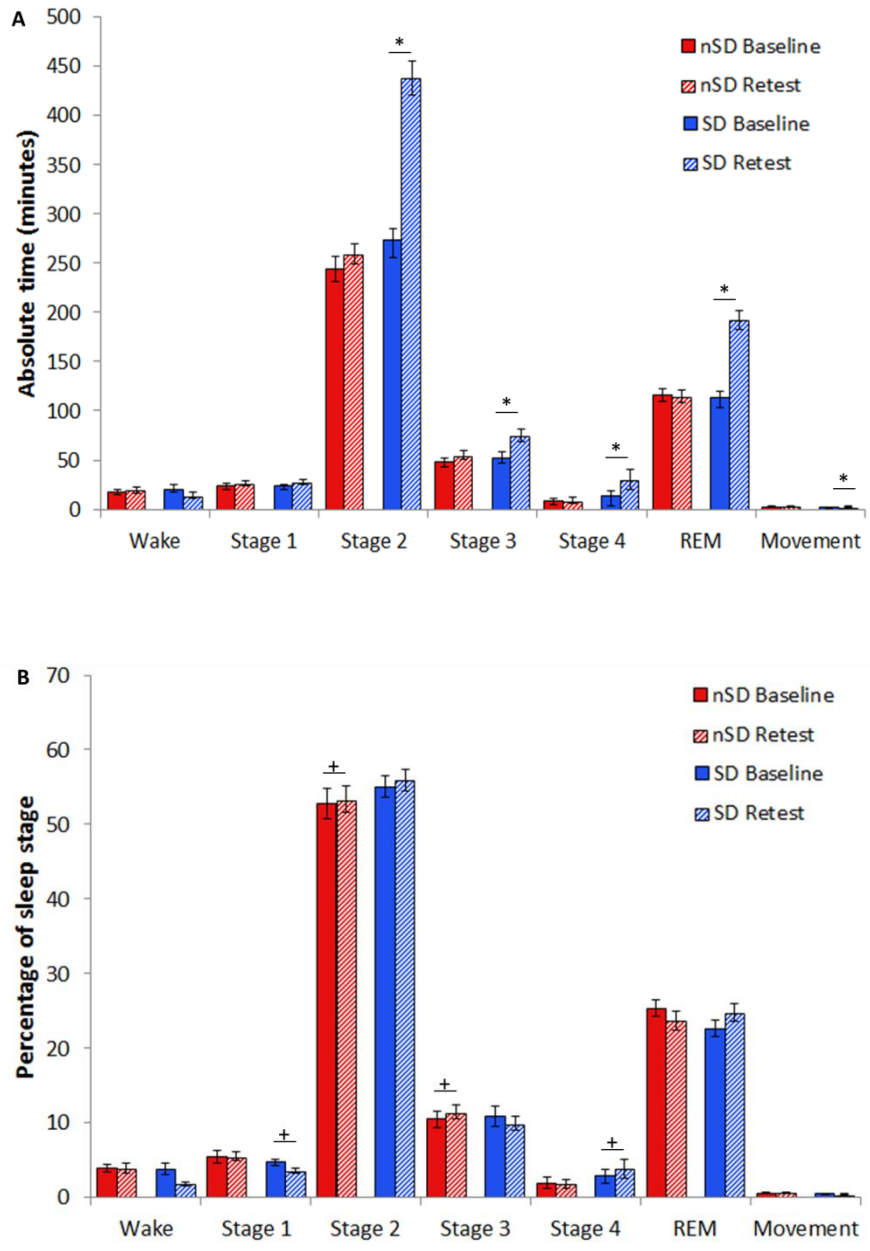


Figure 5.3. Sleep stages. The average absolute (A) and percentage of the total sleep time (B) amount of each sleep stage are presented for the sleep deprived (blue) and non-sleep deprived (red) for the baseline (solid colour) and retest (striped) nights. * denotes a significant result following Bonferroni correction; + denotes a significant result before, but not following Bonferroni correction. Error bars represent standard error of mean.

5.3.1.3. Spectral frequencies

For all the PSG recordings the frequency bands (delta, theta, alpha, sigma, beta1, beta2 and gamma) were analysed separately for REM and NREM sleep. At baseline, differences in the power spectral density for several of the frequency bands were seen (**figure 5.8** and **table 5.5**).

	Baseline[a]				Effect of trauma film[b]				Effect of trauma film and sleep deprivation[b]			
	Absolute		Percentage		Absolute		Percentage		Absolute		Percentage	
	Z score	*p value*	Z score	*p value*	Z score	*p value*	Z score	*p value*	Z score	*p value*	Z score	*p value*
Wake	-.19	.86	-.43	*.68*	-.28	*.78*	-.96	*.34*	-.47	*.64*	-1.91	*.06*
Stage 1	-.65	.52	-.07	*.95*	-.97	*.33*	-1.20	*.23*	-1.14	.26	-2.59	*.01* +
Stage 2	-1.37	.18	-.60	*.56*	-1.04	*.30*	-1.96	*.05* +	-3.57	*.00* *	-.62	*.53*
Stage 3	-.12	.91	-.25	*.82*	-1.77	*.08*	-2.10	*.04* +	-3.17	*.00* *	-.05	*.96*
Stage 4	-1.08	*.31*	-.90	*.40*	-.67	*.50*	-.41	*.68*	-6.58	*.00* *	-2.61	*.01* +
REM	-.28	.79	-1.75	*.08*	-.43	*.66*	-1.09	*.27*	-3.48	*.00* *	-.88	*.38*
Movement	-.13	*.91*	-.34	*.75*	-.41	*.68*	-1.01	*.31*	-6.58	*.00* *	-.74	*.46*

Table 5.2. Sleep stage statistical comparisons. The statistical results ([a] Mann Whitney and [b] Wilcoxon signed ranks test) for comparisons between sleep deprived and non-sleep deprivied participants at baseline (baseline) and between baseline and retest nights for non-sleep deprived (effect of trauma film), and sleep deprived (effect of trauma film and sleep deprivation). Significance level following Bonferroni correction: 0.05/7=0.007. * denotes a significant result following Bonferroni correction. + denotes a significant result before but not after Bonferroni correction.

Following Bonferroni correction the non-sleep deprived participants showed a higher theta activity and higher delta activity in REM sleep. The sleep deprived participants showed a higher beta2 and gamma activity in both NREM and REM sleep. Repeated measure analysis was performed for each frequency band over the course of the night. The activity of each frequency band was found to change over the night for both NREM (**figure 5.9** and **table 5.6**) and REM sleep (**figure 5.10** and **table 5.6**). An interaction between time and group was also found for beta1, beta2 and gamma for NREM and REM sleep, as well as theta for NREM sleep and alpha for REM.

Topographical analysis was also performed for each frequency band. Repeated measure analysis revealed that there was an effect of position on the scalp (channel) for each frequency band for NREM (**figure 5.11** and **table 5.7**) and REM sleep (**figure 5.12** and **table 5.7**). A group/channel interaction was found for delta, theta and sigma for NREM sleep.

5.3.2. Effect of trauma film on sleep physiology

The effect of the trauma film on sleep was assessed by a comparison of the baseline and retest nights in the non-sleep deprived group.

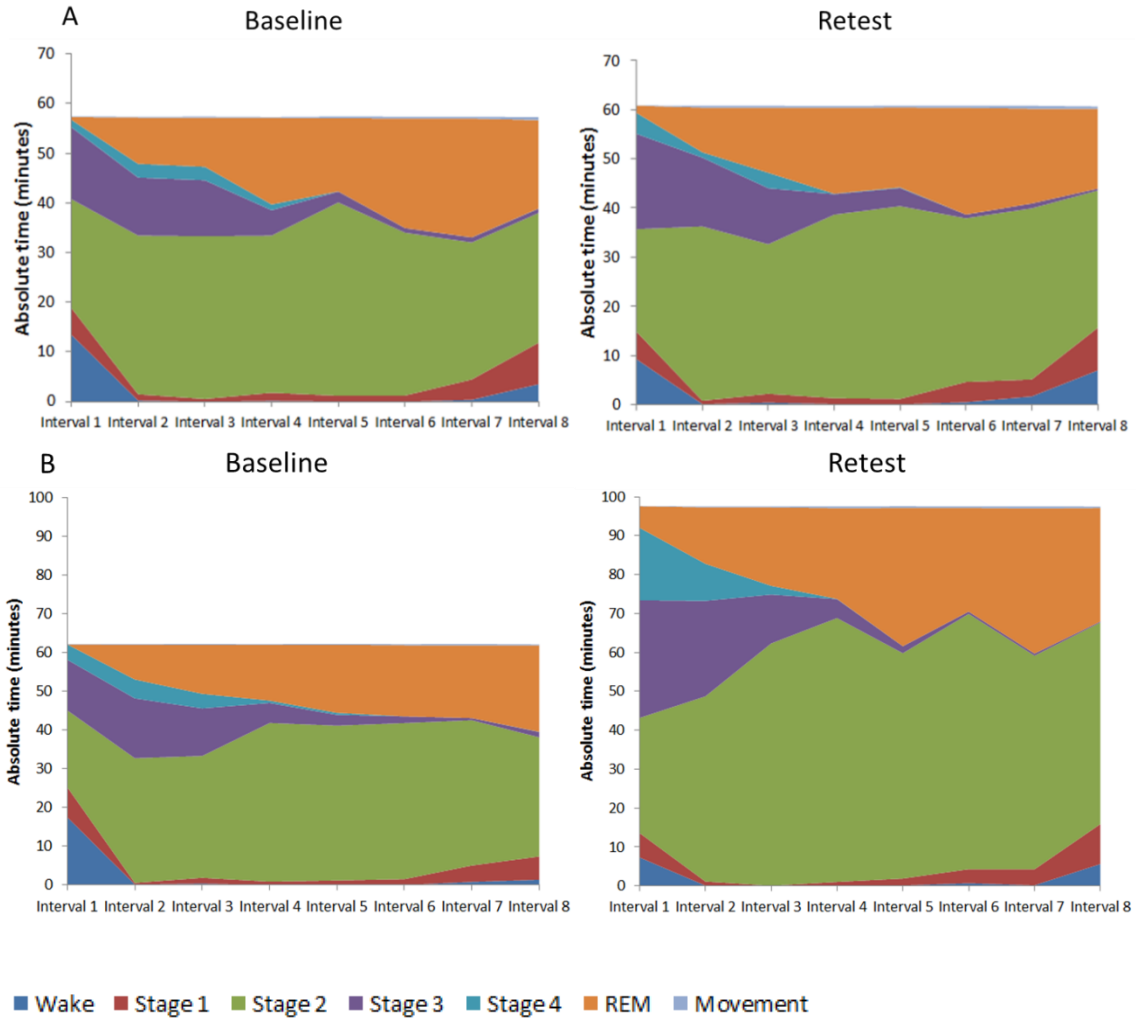


Figure 5.4. Progression of absolute amounts of sleep stages. The average absolute amount of each sleep stage for each interval (wake=blue, stage 1=red, stage 2=green, stage 3=purple, stage 4= light blue, REM=orange, movement=pale blue) for the non-sleep deprived (A) and sleep deprived (B) for baseline and retest nights.

No difference was found in the total sleep time, sleep latency, sleep efficiency, slow wave sleep latency or REM latency from baseline to retest (**figure 5.2** and **table 5.1**). The retest night showed a higher absolute and percentage of time spent awake during the sleep period, indicating that the participants spent more time awake following the film than at baseline.

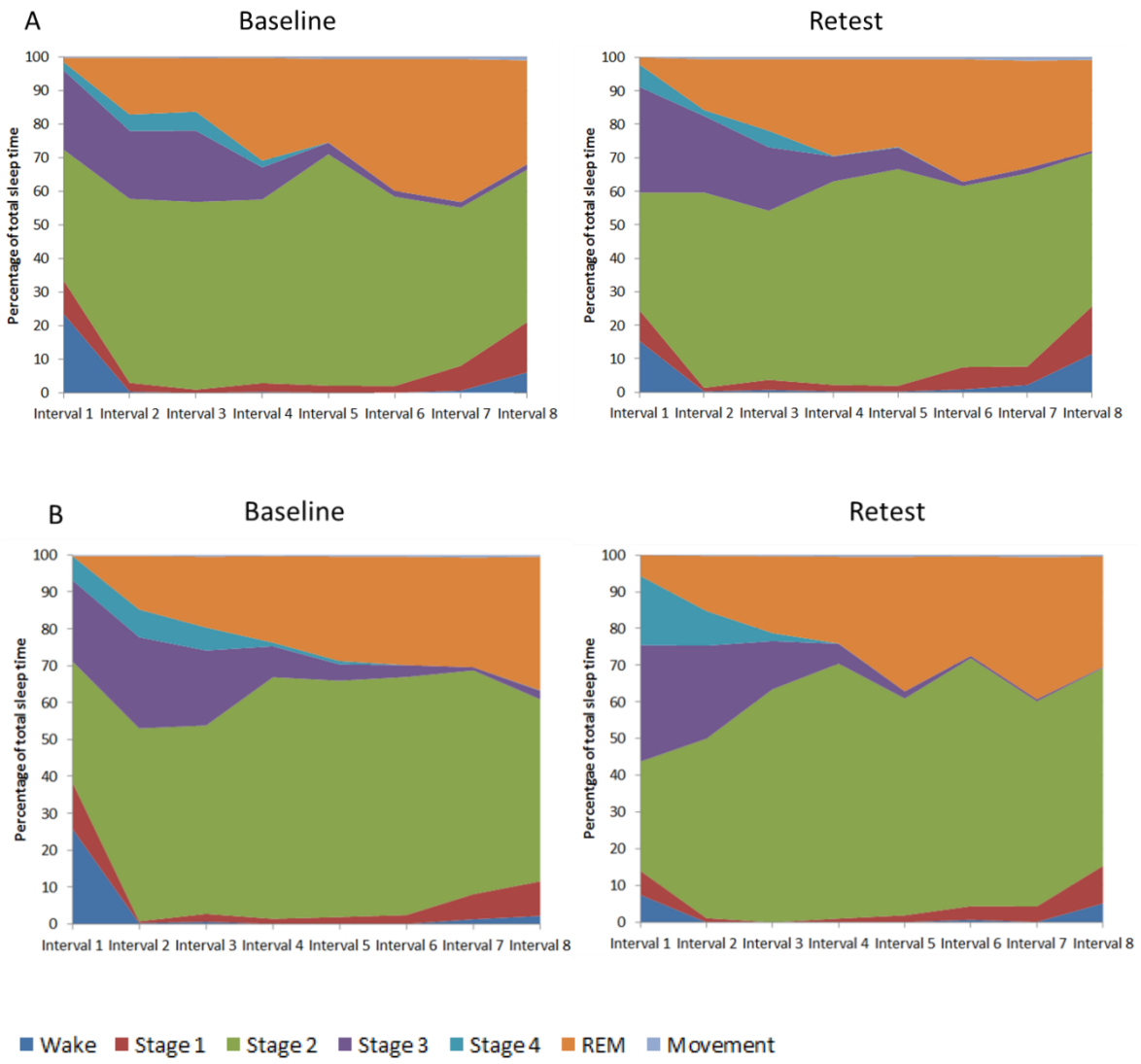


Figure 5.5. Progression of percentage of sleep. The average percentage of each sleep stage of the total sleep time for each interval (wake=blue, stage 1=red, stage 2=green, stage 3=purple, stage 4= light blue, REM=orange, movement=pale blue) for the non-sleep deprived (A) and sleep deprived (B) for baseline and retest nights.

5.3.2.1. Sleep stages

Over the whole night no difference was found in the absolute time spent in each sleep stage between the baseline and post film nights (**figure 5.3a** and **table 5.2**). However, a higher percentage of stage 2 and stage 3 sleep was found in the retest night, although this does not remain following Bonferroni correction (**figure 5.3b** and **table 5.2**).

		Baseline				Effect of film				Effect of film and sleep deprivation			
		Absolute		Percentage		Absolute		Percentage		Absolute		Percentage	
		F value	*p value*	F value	*p value*	F value	*p value*	F value	*p value*	F value	*p value*	F value	*p value*
Wake	Interval 1	.75	*.39*	.12	*.74*	2.66	*.11*	3.24	*.08*	5.14	***.03***	10.59	***.00*** *
	Interval 2	.14	*.71*	.19	*.67*	.19	*.67*	.13	*.72*	.10	*.75*	.29	*.60*
	Interval 3	1.75	*.19*	1.86	*.18*	2.69	*.11*	3.40	*.07*	1.41	*.24*	1.50	*.23*
	Interval 4	1.52	*.23*	1.59	*.22*	.00	*1.00*	.00	*.97*	3.43	*.07*	3.42	*.07*
	Interval 5	.81	*.38*	.81	*.38*	.55	*.46*	.61	*.44*	1.52	*.23*	1.59	*.22*
	Interval 6	.	.	.	.	3.68	*.06*	4.43	***.04*** +	2.22	*.15*	2.25	*.14*
	Interval 7	.83	*.37*	.69	*.41*	.76	*.39*	.68	*.41*	1.48	*.23*	2.05	*.16*
	Interval 8	3.08	*.09*	3.54	*.07*	1.82	*.19*	1.55	*.22*	2.18	*.15*	1.30	*.26*
Stage 1	Interval 1	2.76	*.11*	1.04	*.31*	.04	*.83*	.16	*.69*	.90	*.35*	6.71	***.01*** +
	Interval 2	1.41	*.24*	2.02	*.16*	.69	*.41*	1.19	*.28*	1.55	*.22*	1.12	*.30*
	Interval 3	1.36	*.25*	1.04	*.31*	2.43	*.13*	2.26	*.14*	2.98	*.09*	3.17	*.08*
	Interval 4	.86	*.36*	.89	*.35*	.27	*.61*	.26	*.61*	.04	*.85*	.24	*.63*
	Interval 5	.00	*.97*	.03	*.87*	.01	*.93*	.09	*.77*	.82	*.37*	.00	*.99*
	Interval 6	.11	*.74*	.08	*.77*	5.84	***.02*** +	6.23	***.02*** +	3.29	*.08*	.80	*.38*
	Interval 7	.01	*.92*	.07	*.79*	.23	*.63*	.71	*.40*	.01	*.91*	2.65	*.11*
	Interval 8	1.09	*.30*	2.01	*.17*	.02	*.89*	.03	*.85*	2.61	*.12*	.08	*.78*
Stage 2	Interval 1	.45	*.51*	1.17	*.29*	.12	*.73*	.45	*.50*	3.67	*.06*	.32	*.58*
	Interval 2	.00	*.95*	.17	*.68*	.79	*.38*	.37	*.55*	9.95	***.00*** *	.36	*.55*
	Interval 3	.10	*.75*	.51	*.48*	.31	*.58*	.56	*.46*	36.36	***.00*** *	4.34	***.05*** +
	Interval 4	3.64	*.06*	2.13	*.15*	1.93	*.17*	1.00	*.32*	25.80	***.00*** *	.36	*.55*
	Interval 5	.06	*.81*	.52	*.47*	.00	*.95*	.36	*.55*	14.39	***.00*** *	.94	*.34*
	Interval 6	2.33	*.14*	1.14	*.29*	.01	*.92*	.11	*.74*	28.52	***.00*** *	.21	*.65*
	Interval 7	4.69	***.04*** +	2.96	*.09*	3.13	*.08*	2.21	*.15*	14.34	***.00*** *	.81	*.38*
	Interval 8	1.23	*.28*	.34	*.57*	.17	*.68*	.00	*.96*	28.69	***.00*** *	.71	*.40*
Stage 3	Interval 1	.18	*.67*	.10	*.75*	2.36	*.13*	2.61	*.11*	15.78	***.00*** *	3.19	*.08*
	Interval 2	2.05	*.16*	1.05	*.31*	.83	*.37*	.38	*.54*	7.94	***.01*** *	.02	*.88*
	Interval 3	.14	*.71*	.03	*.87*	.00	*.96*	.22	*.64*	.01	*.93*	2.34	*.14*
	Interval 4	.00	*.99*	.10	*.76*	.24	*.63*	.33	*.57*	.02	*.90*	.73	*.40*
	Interval 5	.15	*.70*	.16	*.69*	1.06	*.31*	1.50	*.23*	.33	*.57*	.95	*.34*
	Interval 6	.87	*.36*	.79	*.38*	.09	*.76*	.21	*.65*	1.93	*.17*	2.72	*.11*
	Interval 7	.31	*.58*	.33	*.57*	.00	*.99*	.00	*.97*	.00	*1.00*	.09	*.77*
	Interval 8	.15	*.70*	.10	*.75*	.73	*.40*	.98	*.33*	.78	*.38*	.91	*.35*
Stage 4	Interval 1	2.52	*.12*	2.48	*.12*	2.97	.09	2.34	*.13*	5.71	***.02*** +	3.67	*.06*
	Interval 2	.90	*.35*	.59	*.45*	.31	.58	.62	*.43*	1.31	*.26*	.19	*.67*
	Interval 3	.26	*.61*	.02	*.89*	.85	.36	.50	*.48*	.43	*.52*	1.51	*.23*
	Interval 4	.37	*.55*	.39	*.54*	.04	.83	.16	*.69*	1.71	*.20*	1.80	*.19*
	Interval 5	1.13	*.29*	1.05	*.31*	.09	.76	.03	*.86*	1.00	*.32*	1.00	*.32*
	Interval 6	.	.	.	.	.00	.95	.06	*.81*	.	.	.	.
	Interval 7	.81	*.38*	.81	*.38*	.58	.45	.93	*.34*	.	.	.	.
	Interval 8	.35	*.56*	.29	*.59*	.09	.76	.18	*.68*	1.00	*.32*	1.00	*.32*
REM	Interval 1	1.69	*.20*	1.53	*.22*	.69	*.41*	.26	*.61*	8.44	***.01*** *	9.14	***.00*** *
	Interval 2	.01	*.91*	.21	*.65*	.01	*.93*	.12	*.73*	2.73	*.11*	.01	*.91*
	Interval 3	.64	*.43*	.42	*.52*	1.19	*.28*	1.24	*.27*	4.11	***.05*** +	.12	*.73*
	Interval 4	.66	*.42*	1.14	*.29*	.00	*1.00*	.07	*.79*	5.22	***.03*** +	.00	*.97*
	Interval 5	.48	*.49*	.28	*.60*	.13	*.72*	.04	*.85*	16.75	***.00*** *	1.84	*.18*
	Interval 6	.71	*.41*	1.64	*.21*	.01	*.93*	.15	*.70*	3.54	*.07*	.14	*.71*
	Interval 7	1.69	*.20*	3.16	*.08*	1.41	*.24*	2.15	*.15*	25.51	***.00*** *	3.27	*.08*
	Interval 8	1.46	*.23*	.68	*.41*	.19	*.67*	.34	*.56*	2.89	*.10*	1.23	*.28*
Movement	Interval 1	.00	*.97*	.00	*.97*	2.58	*.12*	3.07	*.09*	1.16	*.29*	1.99	*.17*
	Interval 2	.00	*.97*	.01	*.92*	1.27	*.27*	1.09	*.30*	.28	*.60*	.01	*.92*
	Interval 3	.00	*.98*	.02	*.88*	.41	*.53*	.34	*.56*	.02	*.88*	.29	*.59*
	Interval 4	.19	*.67*	.05	*.83*	2.16	*.15*	1.40	*.24*	2.12	*.15*	.44	*.51*
	Interval 5	.48	*.49*	.66	*.42*	.00	*.96*	.03	*.86*	1.36	*.25*	.10	*.75*
	Interval 6	.30	*.59*	.37	*.55*	.02	*.88*	.02	*.88*	.33	*.57*	.10	*.76*
	Interval 7	.02	*.88*	.03	*.86*	1.23	*.27*	1.21	*.28*	.45	*.51*	.09	*.77*
	Interval 8	3.56	*.07*	3.83	*.06*	.42	*.52*	.36	*.55*	.43	*.52*	.11	*.74*

Table 5.3. Sleep stage statistical comparisons for each interval. Mutlivariate analysis for comparisons between sleep deprived and non-sleep deprivied participants at baseline (baseline) and between baseline and retest nights for non-sleep deprived (effect of trauma film), and sleep deprived (effect of trauma film and sleep deprivation) for each interval. Significance level following Bonferroni correction: 0.05/8=0.00625. * denotes a significant result following Bonferroni correction. + denotes a significant result before but not after Bonferroni correction.

In terms of the progression of sleep stages (**figure 5.4**, **figure 5.5** and **table 5.3**) there was more stage 1 sleep in the retest night compared to baseline towards the end of the night in interval 6, a result that did not remain following Bonferroni correction (baseline = 1.21 minutes, retest = 4.12 minutes). A higher percentage of stage one and wake, in relation to the total sleep time, was also observed for the sixth interval (baseline: wake = 0.00 %, stage 1 = 2.00 %; retest: wake = 0.82%, stage 1 = 6.68%). This result did not remain significant following Bonferroni correction.

5.3.2.2. REM density

No change in REM density was found between the baseline and retest nights (**figure 5.6**: Wilcoxon: Z = -1.10, p= 0.28). As with the baseline night, an increase in REM density was seen over the night following the trauma film (**figure 5.7**). Repeated measure analysis revealed an effect of time, but no interaction with the recording night was found (time: F=18.43, p≤0.001; time/night: F=0.83, p=0.57). Moreover, no difference was found for any of the intervals (**table 5.4**).

5.3.2.3. Spectral frequencies

For the night as a whole no change in any frequency bands were found between the baseline and retest night (**figure 5.8** and **table 5.5**). All frequency bands were found to change with time for NREM (**figure 5.9** and **table 5.6**) and REM sleep (**figure 5.10** and **table 5.6**) with repeated measure analysis. A time/ night interaction was revealed for beta2 and gamma for NREM and REM sleep, and theta, alpha and beta1 for REM sleep only. Post hoc analysis was performed for each frequency band that showed a time/night interaction: no significant difference after Bonferroni correction was found for any interval between baseline and retest nights for REM or NREM sleep (see appendix IV for data). Position on the scalp (channel) was found to have an effect for all frequency bands in both REM and NREM sleep (**figure 5.11**, **figure 5.12** and **table 5.7**). No channel/night interactions were found for any of the frequency bands for REM sleep, although sigma activity was found to have a channel/night interaction in NREM sleep.

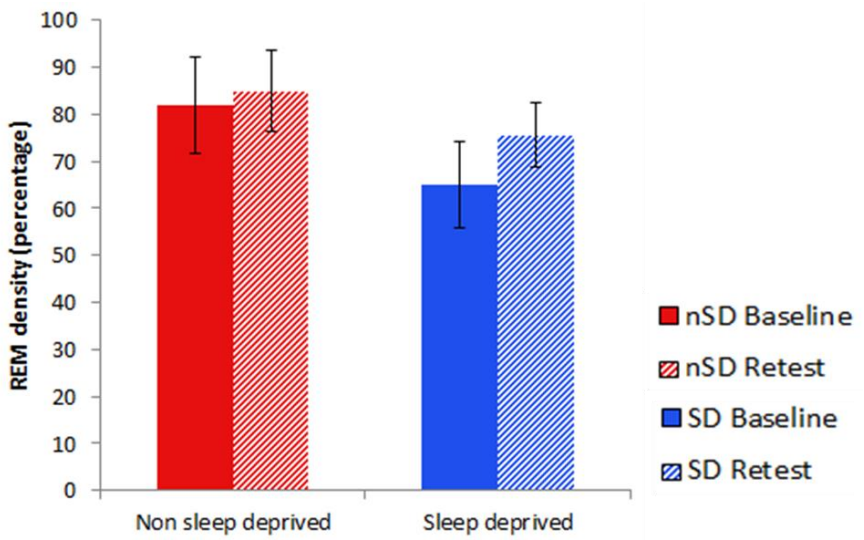


Figure 5.6. REM density for whole night. The average REM density for the whole sleep period for the sleep deprived (blue) and non-sleep deprived (red) for baseline (solid colour) and retest (striped) nights. Error bars represent standard error of mean.

Post hoc analysis revealed no difference between baseline and retest for each channel for sigma activity (see appendix IV for data).

5.3.3. Effect of trauma film and sleep deprivation on sleep physiology

As expected the total sleep time was longer on the retest (recovery) night compared with the baseline night for the sleep deprived participants (**figure 5.2** and **table 5.1**). An increase in sleep efficiency (a result not significant after Bonferroni correction) and a decrease in slow wave sleep latency were also recorded for the retest night.

5.3.3.1. Sleep stages

Following the period of sleep deprivation a rebound in NREM and REM sleep was seen: a higher absolute amount of stage 2, stage 3, stage 4 and REM sleep was seen on the retest night compared with baseline (**figure 5.3a** and **table 5.2**). An increase in movement time was also observed on the retest night. Despite this only stage 1 and stage 4 showed a change in proportion to the total sleep time: a decrease in stage 1 and increase in stage 4 on the recovery night, neither of which remained significant following Bonferroni correction (**figure 5.3b** and **table 5.2**).

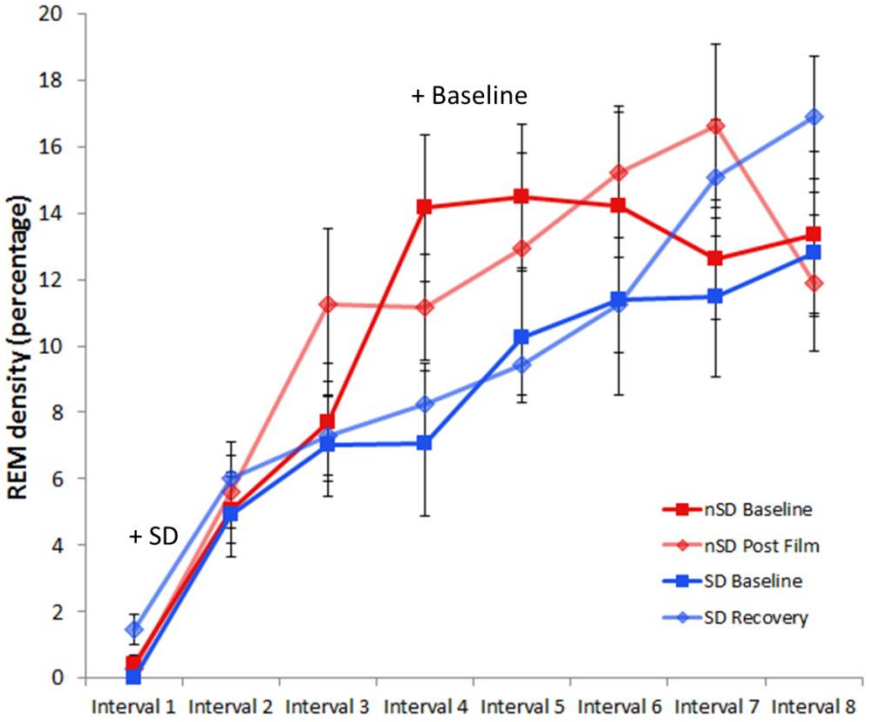


Figure 5.7. Progression of REM density. The average REM density for each interval over the course of the night, for the sleep deprived (blue) and non-sleep deprived (red) for baseline (solid squares) and retest (pale diamonds) nights. + denotes a result that is not significant following Bonferroni correction. Error bars represent standard error of mean.

This profile was maintained in the time course analysis, where an increase in the amount of stage 2 sleep was seen in intervals 2 to 8, stage 3 in intervals 1 and 2, stage 4 in interval 1 and REM sleep in intervals 1, 3, 4, 5 and 7 and a decrease in the amount of time spent awake in interval 1 (**figure 5.4** and **table 5.3**). Following Bonferroni correction the increase in stage 4 sleep and REM sleep in intervals 3 and 4 and the decrease in wake did not remain significant.

In terms of the amounts of sleep as a percentage of the total sleep period, the majority of the changes from baseline to retest night were observed in the first interval. In this interval a decrease in the percentage of wake and stage 1 was seen, while the percentage of REM sleep increased. Following Bonferroni correction the decrease in stage 1 sleep was no longer significant. An increase in stage 2 sleep was also observed in interval 3, which again was not significant following Bonferroni correction.

	Baseline		Effect of film		Effect of film and sleep deprivation	
	Z score	*p value*	Z score	*p value*	Z score	*p value*
Interval 1	-1.24	*.63*	-.58	*.56*	-2.98	*.02* +
Interval 2	-.12	*.91*	-.44	*.66*	-.72	*.48*
Interval 3	-.02	*.99*	-1.05	*.29*	-.19	*.85*
Interval 4	-2.67	*.01* +	-.97	*.33*	-.91	*.37*
Interval 5	-1.27	*.21*	-.97	*.33*	-.40	*.71*
Interval 6	-.69	*.50*	-.72	*.47*	-.23	*.82*
Interval 7	-.55	*.59*	-1.08	*.28*	-1.40	*.16*
Interval 8	-.15	*.88*	-.27	*.78*	-1.23	*.23*

Table 5.4. REM density statistical comparisons for each interval. The statistical results (Mann Whitney) for comparisons between sleep deprived and non-sleep deprivied participants at baseline (baseline) and between baseline and retest nights for non-sleep deprived (effect of film), and sleep deprived (effect of film and sleep deprived) for each interval. Significance level following Bonferroni correction: 0.05/8=0.00625. * denotes a significant result following Bonferroni correction. + denotes a significant result before but not after Bonferroni correction.

5.3.3.2. REM density

No change in REM density was observed on the recovery night as compared with baseline (**figure 5.6**: Wilcoxon: Z = -0.82, p= 0.41). An increase in REM density was seen over the course of the night following the trauma film (**figure 5.7**). Repeated measure analysis revealed an effect of time, but no interaction with the recording night (time: F=24.8, p≤0.001; time/night: F=0.76, p=0.62). Despite this a higher REM density was found for the first interval but this did not remain following Bonferroni correction (**table 5.4**).

5.3.3.3. Spectral frequencies

Over the whole night no differences were seen between the spectral frequency bands in NREM sleep. However, following Bonferroni correction beta2 had a lower activity on the recovery night in REM sleep (**figure 5.8** and **table 5.5**). Repeated measure analysis of the spectral frequencies throughout the night revealed a time effect for all frequencies for REM and NREM sleep and time/night interaction for all frequencies in NREM sleep expect sigma (**figure 5.9** and **table 5.6**) and REM sleep expect theta and beta2 (**figure 5.10** and **table 5.6**). Post hoc analysis was performed for each frequency band that showed a time/night interaction.

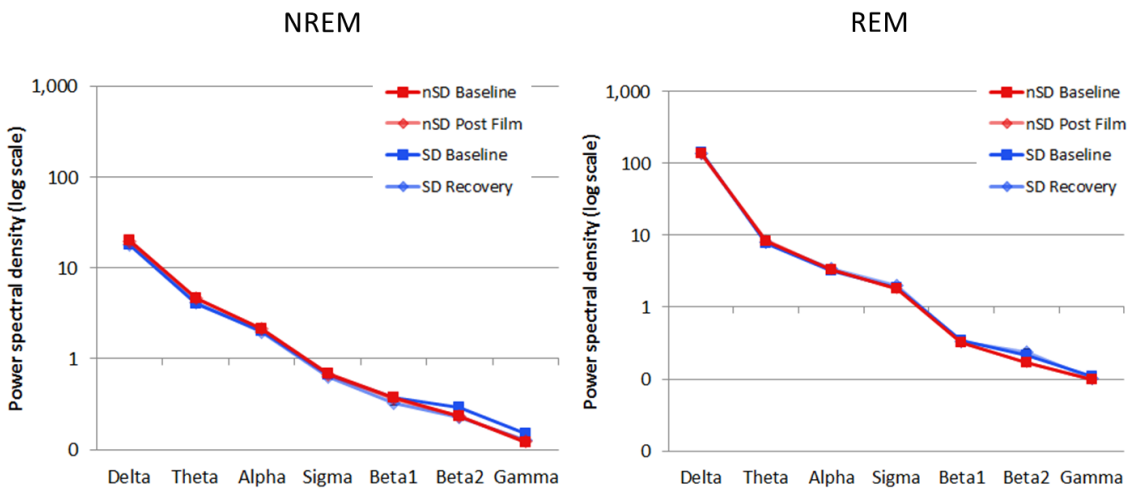


Figure 5.8. Spectral frequency bands for NREM and REM sleep. The average activity for each frequency band for NREM and REM sleep for the whole night, for the sleep deprived (blue) and non-sleep deprived (red) for baseline (solid colour) and retest (pale colour) nights. Error bars are represented for the standard error of mean: in this figure the error bars are unobservable.

No significant difference after Bonferroni correction was found for any interval between baseline and retest nights for NREM sleep. For REM sleep lower gamma activity was found in the second half of the recovery night (intervals 6, 7 and 8) (for data see appendix IV).

Finally topographical repeated measure analysis showed a position effect for all frequencies for both NREM (**figure 5.11** and **table 5.7**) and REM sleep (**figure 5.12** and **table 5.7**). A position/group interaction was found for all frequency bands except beta 2 and gamma in NREM sleep, while only beta2 showed an interaction in REM sleep. For NREM sleep post hoc analysis revealed that in the occipital region delta (no longer significant following Bonferroni correction), theta and sigma had lower activity on the recovery night while alpha and beta 1 had higher activity. Beta 1 also had lower activity on the recovery night in the parietal area, which did not remain following Bonferroni correction. In REM sleep, lower beta 2 activity was seen on the recovery night in the pre frontal, frontal and parietal regions. This was no longer significant following Bonferroni correction.

	Baseline				Effect of trauma film				Effect of trauma film and sleep deprivation			
	NREM		REM		NREM		REM		NREM		REM	
	F score	*p value*	F score	*p value*	F score	*p value*	F score	*p value*	F score	*p value*	F score	*p value*
Delta	.27	*.61*	8.95	*.00* *	.87	*.35*	.55	*.46*	.12	*.73*	.00	*.99*
Theta	5.99	*.01* +	7.96	*.00* *	.95	*.33*	.15	*.70*	1.62	*.20*	.06	*.81*
Alpha	.74	*.39*	.66	*.42*	.47	*.49*	.05	*.83*	2.58	*.11*	.32	*.57*
Sigma	2.06	*.15*	.85	*.36*	.06	*.81*	.40	*.53*	2.80	*.09*	.49	*.49*
Beta1	2.68	*.10*	.12	*.73*	.06	*.81*	.41	*.52*	.29	*.59*	4.34	*.04* +
Beta2	10.98	*.00* *	11.16	*.00* *	.13	*.72*	.34	*.56*	1.27	*.26*	10.06	*.00* *
Gamma	14.12	*.00* *	16.79	*.00* *	.04	*.85*	.35	*.55*	1.81	*.18*	5.09	*.02* *

Table 5.5. Spectral frequency statistical comparisons. The mulitvariate anlaysis for comparisons between sleep deprived and non-sleep deprivied participants at baseline (baseline) and repeated measure analysis between baseline and retest nights for non-sleep deprived (effect of film), and sleep deprived (effect of film and sleep deprived) for each interval. Significance level following Bonferroni correction: 0.05/7=0.007. * denotes a significant result following Bonferroni correction. + denotes a significant result before but not after Bonferroni correction.

5.3.4. Sleep parameters and intrusive memories: direct associations

Intrusive memories, as described in Chapter 1 (section 1.3.1.1. p.17), are spontaneously reactivating memories of emotional events. Chapter 4 (p.91) reported that sleep deprived participants experienced fewer intrusive memories than non-sleep deprived. Since this finding was the primary outcome from this study, the association between the sleep parameters calculated in this chapter and the frequency of intrusive memories was investigated.

5.3.4.1. Sleep structure

Table 5.8 shows the statistical results for the effect of experimental group (sleep deprived or non-sleep deprived), the sleep parameter group (median split: low verses high or change between nights: increase verses decrease) and the interaction between the two. Four parameters were found to have a significant interaction between experimental and sleep parameter groups on intrusive memory frequency: sleep latency, REM latency, wake during sleep period and sleep efficiency (**figure 5.13**).

Sleep latency: On the baseline night participants with shorter sleep latency in the sleep deprived group reported more intrusions than those with a longer sleep latency (Poisson distributed generalised liner model (P glm) B=1.65, p≤0.001).

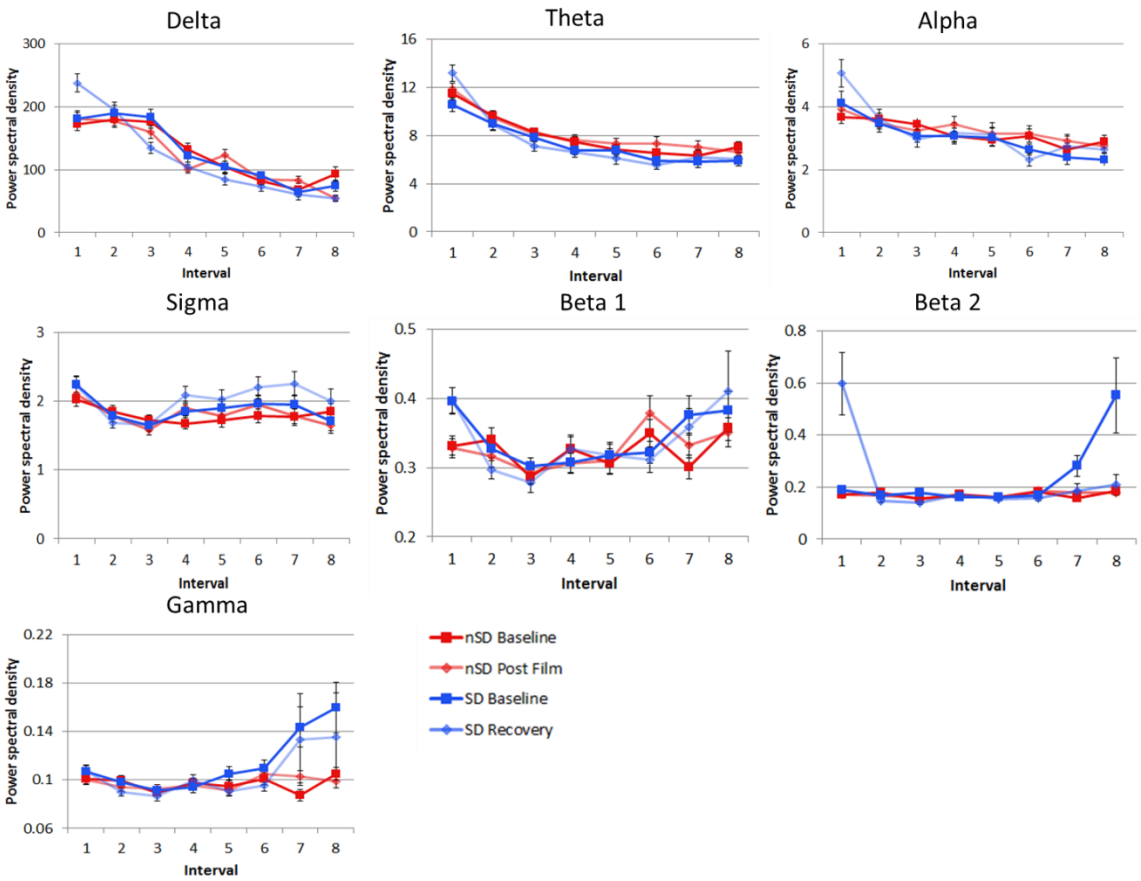


Figure 5.9. Time course of each spectral frequency band for NREM sleep. The average activity for each frequency band for NREM for each interval, for the sleep deprived (blue) and non-sleep deprived (red) for baseline (solid colour) and retest (pale colour) nights. Error bars represent the standard error of mean.

On the retest night the same was also true, where shorter sleep latency was associated with more intrusions for the sleep deprived participants (P glm: B=1.71, p≤0.001). However, the non-sleep deprived participants showed the opposite relationship on the retest night: those with a longer sleep latency had more intrusive memories (P glm: B=-0.70, p=0.01, not significant following Bonferroni correction). Moreover, for the sleep deprived participants those who showed an increase in sleep latency from baseline to retest, had more intrusive memories (P glm: B=-1.01, p≤0.001).

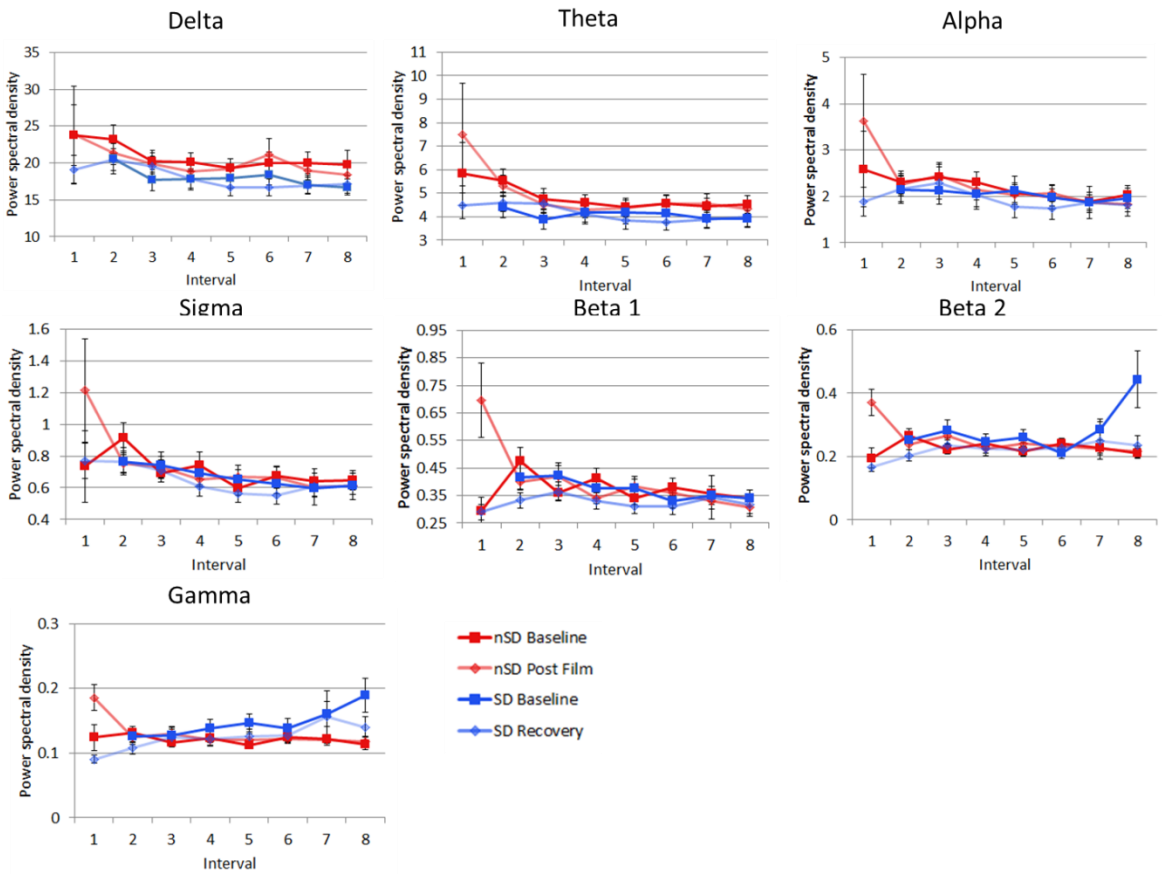


Figure 5.10. Time course of each spectral frequency band for REM sleep. The average activity for each frequency band for REM for each interval, for the sleep deprived (blue) and non-sleep deprived (red) for baseline (solid colour) and retest (pale colour) nights. Error bars represent the standard error of mean.

REM latency: a similar profile was seen for REM latency where the sleep deprived participants had more intrusive memories with a shorter REM latency at both baseline (P glm: B=0.91, p=0.01, not significant following Bonferroni correction) and retest (P glm: B=1.13, p≤0.001), while the non-sleep deprived participants (P glm: B=-0.58, p=0.01, not significant following Bonferroni correction) had more intrusions with a longer REM latency on the retest night. Although an interaction was found for the change in REM latency between the two nights, neither the sleep deprived (P glm: B=-0.60, p=0.06) nor non-sleep deprived participants (P glm: B=0.42, p=0.10) showed a significant difference in intrusive memory frequency.

			Delta		Theta		Alpha		Sigma		Beta1		Beta2		Gamma	
			F value	*p value*	F value	*p value*	F value	*p value*	F value	*p value*	F value	*p value*	F value	*p value*	F value	*p value*
Baseline	REM	Time	9.98	*.00* *	12.36	*.00* *	4.61	*.00* *	8.06	*.00* *	10.13	*.00* *	7.88	*.00* *	8.49	*.00* *
		Time*Group	1.82	*.10*	1.87	*.10*	2.83	*.01* *	2.57	*.02* +	5.79	*.00* *	7.78	*.00* *	6.92	*.00* *
	NREM	Time	26.01	*.00* *	36.31	*.00* *	23.41	*.00* *	21.85	*.00* *	25.12	*.00* *	19.65	*.00* *	17.47	*.00* *
		Time*Group	2.02	*.06*	2.52	*.02* *	1.58	*.15*	2.63	*.01* +	4.85	*.00* *	9.68	*.00* *	5.29	*.00* *
Effect of film	REM	Time	5.48	*.00* *	7.05	*.00* *	5.16	*.00* *	8.15	*.00* *	8.27	*.00* *	5.84	*.00* *	3.30	*.01* *
		Time*Night	1.20	*.32*	4.10	*.00* *	1.89	*.10*	4.79	*.00* *	3.63	*.00* *	4.63	*.00* *	5.24	*.00* *
	NREM	Time	35.18	*.00* *	36.89	*.00* *	27.11	*.00* *	26.33	*.00* *	39.97	*.00* *	28.75	*.00* *	25.05	*.00* *
		Time*Night	.93	*.48*	1.03	*.41*	.67	*.69*	2.00	*.06*	3.61	*.00* +	4.71	*.00* *	3.23	*.00* *
Effect of film and sleep deprivati	REM	Time	8.63	*.00* *	7.25	*.00* *	3.19	*.01* *	10.02	*.00* *	14.81	*.00* *	6.67	*.00* *	6.57	*.00* *
		Time*Night	3.70	*.00* *	2.32	*.04* +	3.28	*.01* *	5.21	*.00* *	4.73	*.00* *	2.80	*.02* +	6.51	*.00* *
	NREM	Time	26.33	*.00* *	37.99	*.00* *	25.85	*.00* *	11.27	*.00* *	20.83	*.00* *	12.73	*.00* *	14.67	*.00* *
		Time*Night	6.65	*.00* *	3.32	*.00* *	2.85	*.01* *	2.59	*.01* +	4.69	*.00* *	7.43	*.00* *	5.20	*.00* *

Table 5.6. Time course spectral frequency statistical comparisons. Repeated measure analysis for comparisons between sleep deprived and non-sleep deprivied participants at baseline (baseline) and between baseline and retest nights for non-sleep deprived (effect of film), and sleep deprived (effect of film and sleep deprivation) for time and time/group and time/night interaction. The first interval was not included in the analysis of REM sleep as not enough participants had REM sleep in this period to allow comparisons between all the intervals. Significance level following Bonferroni correction: 0.05/6=0.0083. * denotes a significant result following Bonferroni correction. + denotes a significant result before but not after Bonferroni correction.

Wake during sleep period: To allow comparisons between the groups despite different sleep durations only the percentage of wake for the total sleep time was used. The sleep deprived participants showed an interaction: the less wake during the sleep period the more intrusive memories (P glm: B=0.89, p=0.01, not significant following Bonferroni correction). There was no interaction for the non-sleep deprived group.

Sleep efficiency: The sleep deprived participants showed a significant effect. The participants that had a decrease in sleep efficiency from the baseline to retest night had more intrusions than those that had an increase in sleep efficiency (P glm: B=0.95, p≤0.001). There was no effect in the non-sleep deprived group.

5.3.4.2. Sleep stages

The statistical analysis of the experimental group (sleep deprived and non-sleep deprived), sleep parameter grouping and the interaction between these two groups are shown in **table 5.9**. Five sleep stages showed an interaction: stage 1, stage 2, stage 4, wake and movement (**figure 5.14**).

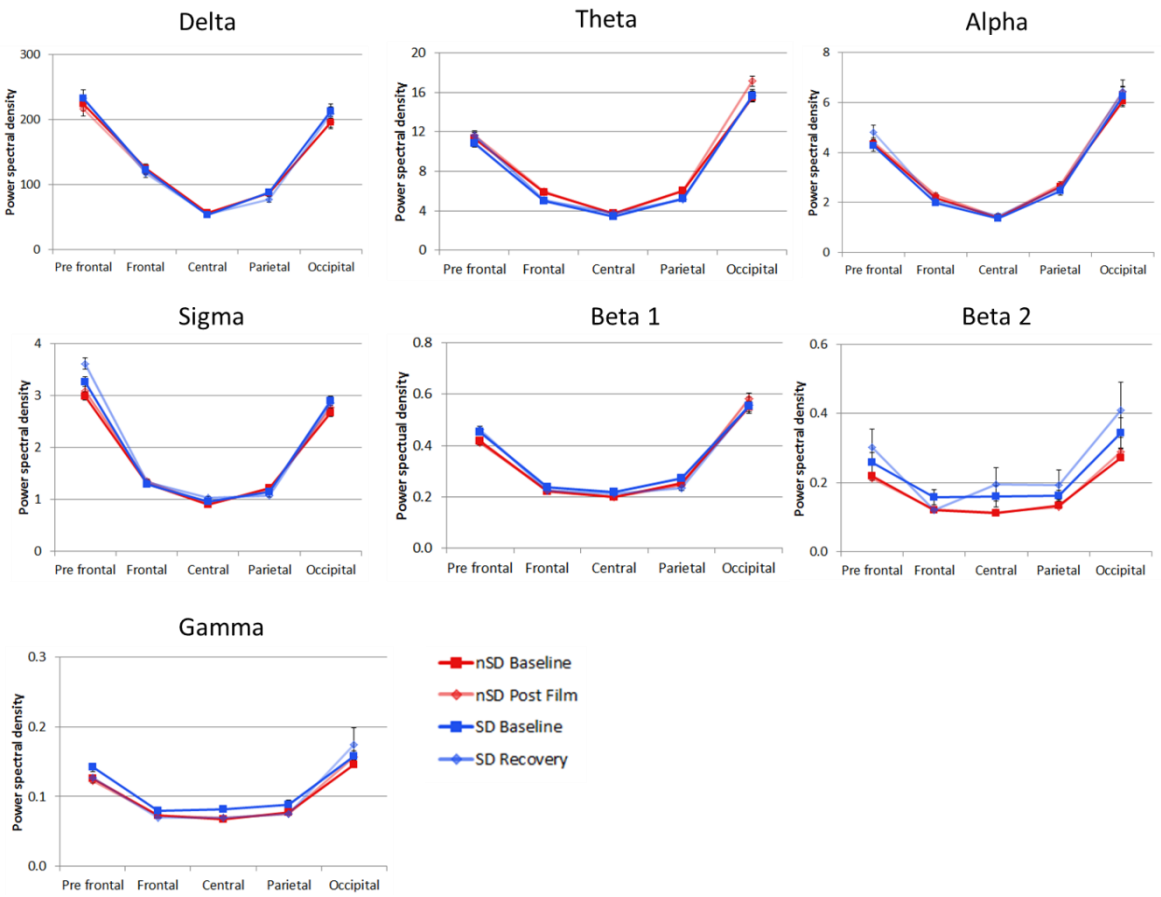


Figure 5.11. Topographical analysis of each spectral frequency band for NREM sleep. The average activity for each frequency band for NREM sleep for each scalp position, for the sleep deprived (blue) and non-sleep deprived (red) for baseline (solid colour) and retest (pale colour) nights. Error bars represent the standard error of mean.

For the sleep deprived participants an increase in stage 1 sleep from baseline to retest was associated with more intrusive memories (P glm: B=-1.49, p≤0.001). Although an interaction was seen for the amount of stage 2 sleep at baseline no significant difference was found for either group (sleep deprived: P glm: B=0.60, p=0.06; non-sleep deprived: P glm: B=-0.43, p=0.06). A significant difference was found in the amount of stage 4 sleep the sleep deprived participants had on the retest night: those with a high amount of stage 4 sleep had more intrusive memories (P glm: B=-1.25, p≤0.001). In terms of the amount of wake, a signficant effect for the sleep deprived participants was found at baseline: the more time spent awake during the sleep period the fewer the intrusions (P glm: B=1.39, p≤0.001), and an increase in the amount of wake from baseline to retest the more intrusions (P glm: B=-1.61, p≤0.001).

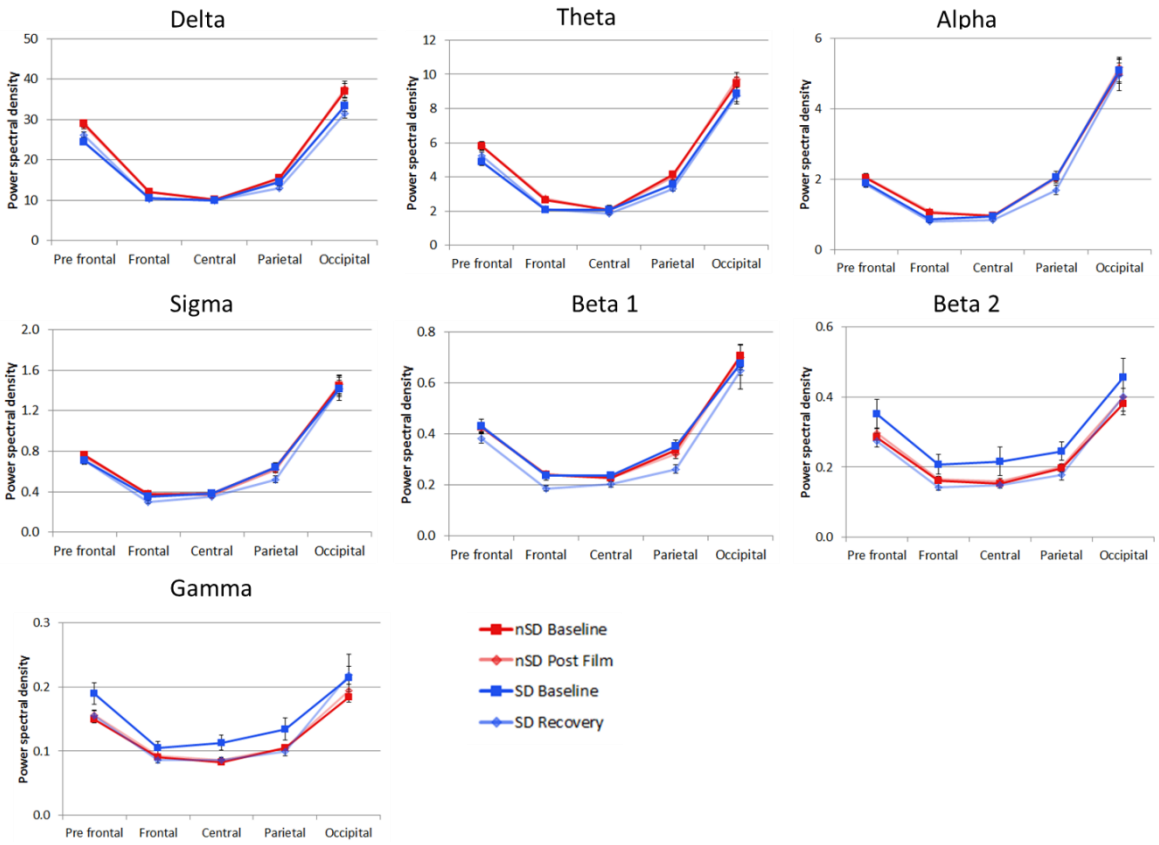


Figure 5.12. Topographical analysis of each spectral frequency band for REM sleep. The average activity for each frequency band for REM sleep for each scalp position, for the sleep deprived (blue) and non-sleep deprived (red) for baseline (solid colour) and retest (pale colour) nights. Error bars represent the standard error of mean.

A decrease in the amount of movement from baseline to retest was assocaited with more intrusions for the sleep deprived participants (P glm: B=1.30, p≤0.001).

5.3.4.3. REM density

The only associations found between REM density and intrusive memory frequency, were found in the sleep deprived participants (**figure 5.15**). At baseline those who had lower REM density had more intrusive memories (P glm: B=0.95, p=0.02, not significant following Bonferroni correction). Moreover, the participants who showed an increase in REM density between baseline and retest had more intrusions (P glm: B=-1.33, p=0.01).

			Delta		Theta		Alpha		Sigma		Beta1		Beta2		Gamma	
			F value	*p value*	F value	*p value*	F value	*p value*	F value	*p value*	F value	*p value*	F value	*p value*	F value	*p value*
Baseline	REM	Channel	181.56	*.00* *	170.66	*.00* *	96.12	*.00* *	90.12	*.00* *	111.97	*.00* *	110.03	*.00* *	75.39	*.00* *
		Channel*Group	3.29	*.01* +	3.63	*.01* +	3.35	*.01* +	2.73	*.03* +	1.03	*.39*	1.77	*.14*	2.30	*.06*
	NREM	Channel	226.33	*.00* *	559.42	*.00* *	262.59	*.00* *	592.28	*.00* *	596.29	*.00* *	354.72	*.00* *	339.75	*.00* *
		Channel*Group	6.53	*.00* *	8.85	*.00* *	3.49	*.01* +	12.13	*.00* *	2.61	*.04* +	3.17	*.01* +	1.19	*.31*
Effect of film	REM	Channel	522.12	*.00* *	503.60	*.00* *	287.78	*.00* *	205.84	*.00* *	197.14	*.00* *	245.96	*.00* *	284.75	*.00* *
		Channel*Night	.75	*.56*	.20	*.94*	1.12	*.35*	.79	*.54*	.48	*.75*	.68	*.61*	1.46	*.22*
	NREM	Channel	268.65	*.00* *	706.02	*.00* *	401.24	*.00* *	538.58	*.00* *	599.44	*.00* *	497.26	*.00* *	541.72	*.00* *
		Channel*Night	3.21	*.01* +	.92	*.45*	.45	*.77*	5.41	*.00* *	2.12	*.08*	2.54	*.04* +	2.55	*.04* +
Effect of film and sleep deprivati	REM	Channel	574.23	*.00* *	243.69	*.00* *	171.63	*.00* *	139.90	*.00* *	104.84	*.00* *	73.57	*.00* *	70.71	*.00* *
		Channel*Night	1.63	*.17*	1.85	*.12*	.93	*.45*	2.24	*.07*	4.88	*.00* *	1.59	*.18*	.99	*.42*
	NREM	Channel	145.25	*.00* *	299.33	*.00* *	91.85	*.00* *	420.41	*.00* *	524.90	*.00* *	183.33	*.00* *	314.30	*.00* *
		Channel*Night	5.65	*.00* *	7.01	*.00* *	6.24	*.00* *	6.90	*.00* *	5.07	*.00* *	1.54	*.19*	2.40	*.05* +

Table 5.7. Topographical spectral frequency statistical comparisons. Repeated measure analysis for comparisons between sleep deprived and non-sleep deprivied participants at baseline (baseline) and between baseline and retest nights for non-sleep deprived (effect of film), and sleep deprived (effect of film and sleep deprived) for channel and channel/group or channel/night interaction. The first interval was not included in the analysis of REM sleep as not enough participants had REM sleep in this period to allow comparisons between all the intervals. Significance level following Bonferroni correction: 0.05/6=0.0083. * denotes a significant result following Bonferroni correction. + denotes a significant result before but not after Bonferroni correction.

5.3.4.4. Spectral frequencies

Four of the seven spectral frequency bands showed a significant interaction between the experimental (sleep deprived and non-sleep deprived) and sleep parameter groups (**table 5.10**): sigma, gamma, alpha and beta1 (**figure 5.16**). The difference in sigma was seen at baseline in the non-sleep deprived participants: those who had a high sigma power had more intrusions (P glm: B=-0.60, p=0.01, a trend following Bonferroni correction). At baseline high gamma power was associated with more intrusions for the sleep deprived participants (P glm: B=-0.83, p=0.01, a trend following Bonferroni correction). The sleep deprived participants showed a difference in alpha power on the retest night: a higher alpha power was associated with more intrusions (P glm: B=-0.80, p=0.01, a trend following Bonferroni correction). Finally although an interaction was seen in the change in beta1 power from baseline to retest, neither group (sleep deprived or non-sleep deprived) showed a significant difference (sleep deprived: P glm: B=0.36, p=0.25; non-sleep deprived: P glm: B=-0.44, p=0.06).

		Experimental group		Sleep parameter group		Interaction	
		B value	p value	B value	p value	B value	p value
Sleep latency	Baseline	-1.58	.00	.02	.94	1.63	.00
	Retest	-1.92	.00	-.70	.01	2.42	.00
	Change	-.03	.90	-.20	.39	-.82	.04
Wake after sleep	Baseline	-1.16	.00	-.30	.19	1.19	.00
onset percentage	Retest	-.04	.86	-.33	.17	-.69	.09
of TST	Change	-.21	.33	-.46	.18	-.30	.55
Sleep efficiency	Baseline	-.20	.45	.17	.45	-.44	.26
	Retest	-.24	.38	.25	.31	-.12	.78
	Change	-.62	.02	.14	.54	.81	.04
SWS latency	Baseline	.03	.91	.76	.00	-.65	.11
	Retest	-.25	.39	-.16	.52	-.08	.85
	Change	.01	.98	-.41	.07	-.40	.32
REM latency	Baseline	-1.09	.00	-.37	.11	1.27	.00
	Retest	-1.30	.00	-.58	.01	1.71	.00
	Change	.13	.63	.42	.10	-1.02	.01

Table 5.8. Sleep parameters and intrusive memories. A Poisson distributed generlised linear model was used to determine the effect for experimental group (sleep deprived and non-sleep deprived), sleep parameter grouping and interaction on intrusive memory frequency for each sleep parameter. Significance level following Bonferroni correction: 0.05/18=0.00278.

5.3.5. Sleep parameters and intrusive memories: indirect associations

In addition to investigating direct associations between intrusive memory frequency and sleep physiology, indirect associations were also explored. The sleep parameters that were found to change from baseline to retest night for either group were investigated for both groups. Since the sleep deprived and non-sleep deprived groups showed a difference in intrusive memory frequency, any differences in sleep parameters between the groups may elucidate the underlying mechanism for intrusion frequency. In this analysis only parameters that are not affected by the length of the sleep period were considered, to allow comparisons between the groups. **Table 5.11** shows the results of this analysis. The sleep deprived participants were found to have a lower percentage of wake and stage 1 sleep on the retest night as well as a decrease in both stages from baseline to retest compared to the non-sleep deprived participants, who showed an increase.

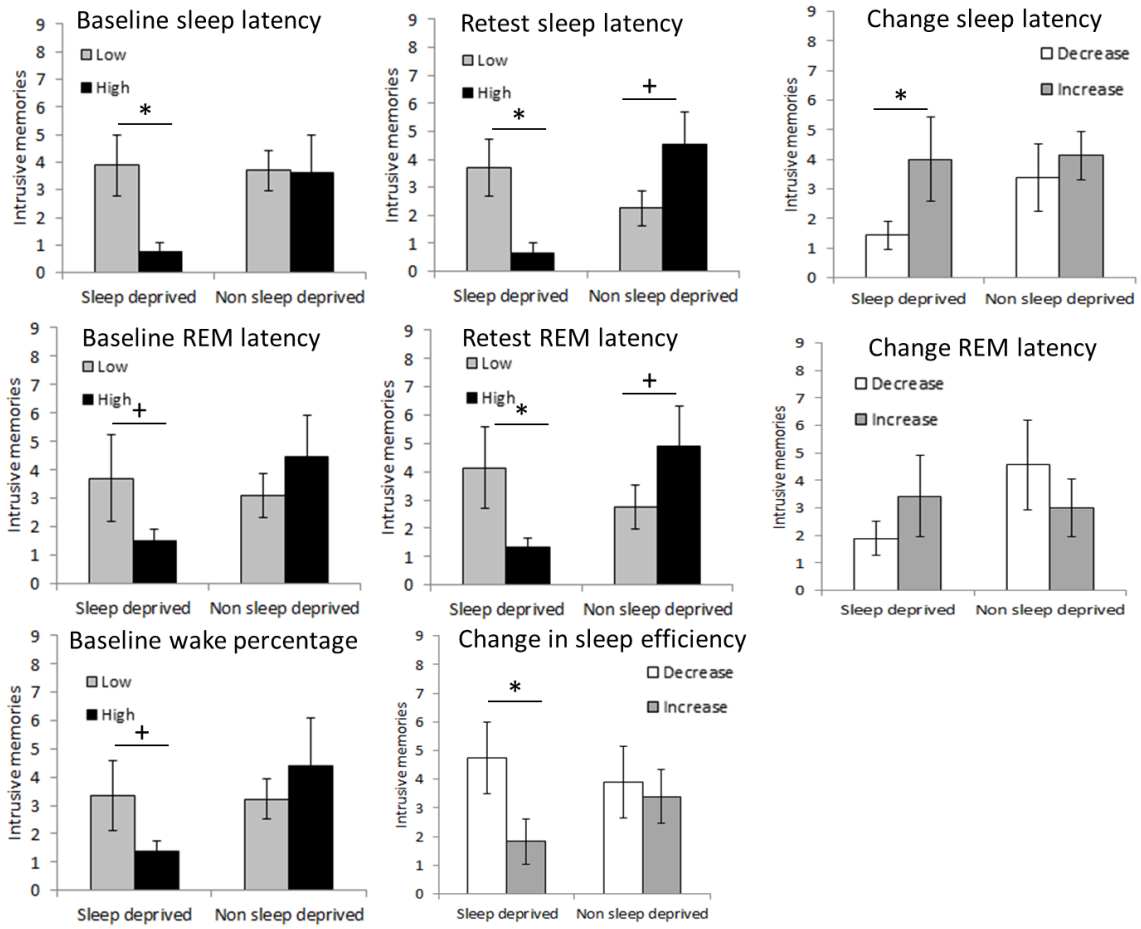


Figure 5.13. Sleep parameters and intrusive memories. Graphical representations of the significant interactions between sleep parameter and group (sleep deprived and non-sleep deprived). The average number of intrusive memories are presented for low versus high (grey and black) and increase versus decreased from baseline to retest night (white and grey) for each sleep parameter for both the sleep deprived and non-sleep deprived participants. * denotes a significant result following Bonferroni correction; + denotes a significant result before, but not following Bonferroni correction. Error bars represent standard error of mean.

Only the percentage of wake and stage 1 sleep remained as a trend following Bonferroni correction. The sleep deprived participants also showed a lower SWS latency, a larger decrease in SWS latency between the baseline and retest nights and higher sleep efficiency on the retest night. After Bonferroni correction, a trend for higher sleep efficiency remained.

		Experimental group		Sleep stage group		Interaction	
		B value	p value	B value	p value	B value	p value
Movement	Baseline	-.26	.28	-.23	.32	-.66	.12
	Retest	-.36	.20	-.03	.91	-.18	.63
	Change	-.23	.35	-1.42	.00	1.53	.00
REM	Baseline	-.36	.13	-.59	.02	-.18	.66
	Retest	-.34	.24	.06	.78	-.22	.57
	Change	-.10	.68	-.64	.02	.36	.35
Stage 1	Baseline	-.30	.34	.65	.01	-.15	.71
	Retest	-.19	.63	.51	.03	-.63	.17
	Change	-.10	.68	.51	.05	-1.39	.00
Stage 2	Baseline	-1.00	.00	-.43	.06	1.03	.01
	Retest	-.77	.01	.09	.71	.61	.12
	Change	.38	.09	-.55	.04	.12	.76
Stage 3	Baseline	-.54	.06	-.19	.40	.18	.64
	Retest	-.27	.30	-.86	.00	.13	.76
	Change	.11	.64	-.68	.02	.32	.43
Stage 4	Baseline	-.32	.20	-.17	.46	-.60	.16
	Retest	-.17	.46	-.39	.09	-.85	.05
	Change	.25	.32	-.45	.04	.03	.95
Wake	Baseline	-1.44	.00	-.18	.43	1.57	.00
	Retest	-.90	.02	-.41	.13	.82	.10
	Change	.33	.15	.42	.09	-1.94	.00

Table 5.9. Sleep stage and intrusive memories. A Poisson distributed generlised linear model was used to determine the effect for experimental group (sleep deprived and non-sleep deprived), sleep parameter grouping and interaction on intrusive memory frequency for each sleep stage. Significance level following Bonferroni correction: 0.05/21=0.00238.

In terms of brain oscillations, the sleep deprived compared with the non-sleep deprived participants were found to have a larger increase in gamma activity from the baseline to retest in the second half of the night (interval 6) and a lower level of beta2 activity in the parietal region on the retest night. The sleep deprived participants also showed lower levels of theta and beta1 activity, and a trend for lower alpha activity in the occipital region on the retest night, as well as a decrease in beta2 activity in the frontal region from baseline to retest, compared with no change for the non-sleep deprived participants. However, following Bonferroni correction, none of these results remained significant.

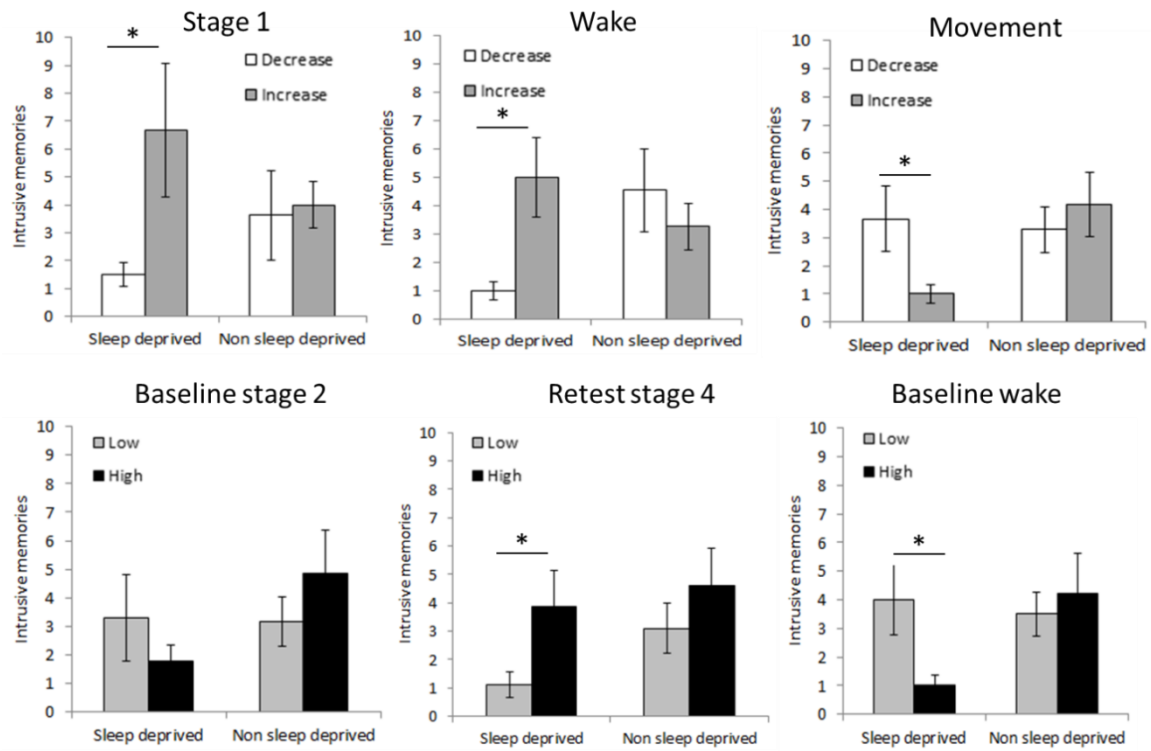
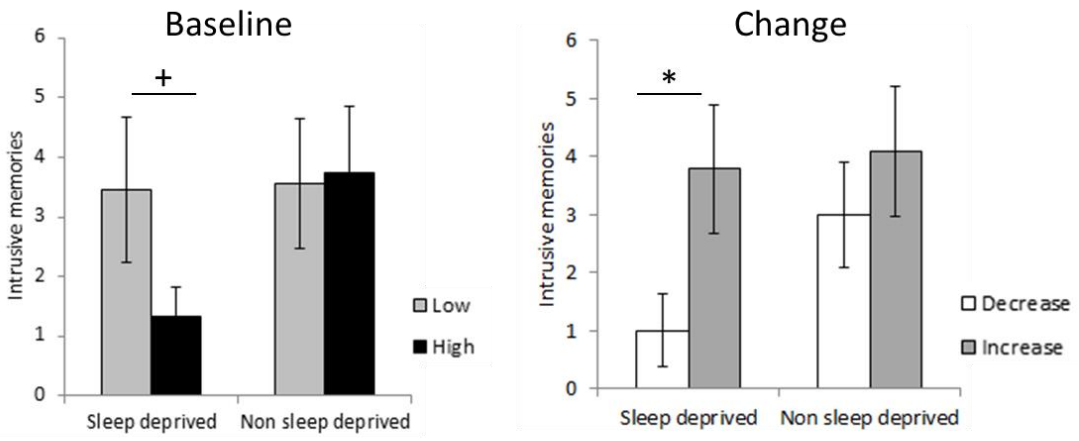


Figure 5.14. Sleep stages and intrusive memories. Graphical representations of the significant interactions between sleep stage and group (sleep deprived and non-sleep deprived). The average number of intrusive memories are presented for low versus high (grey and black) and increase versus decreased from baseline to retest night (white and grey) level of each sleep stage for both the sleep deprived and non-sleep deprived participants. * denotes a significant result following Bonferroni correction; + denotes a significant result before, but not following Bonferroni correction. Error bars represent standard error of mean.

5.4. Discussion

The aim of this investigation was to determine the effect of an analogue traumatic event (trauma film) on sleep physiology. It complements the investigation into the effect of sleep deprivation on emotional processing of a traumatic event (detailed in Chapter 4, p.91). Four different analyses were performed: 1) comparison between the two groups of sleep physiology at baseline, 2) effect of the trauma film on sleep physiology for the non-sleep deprived participants, 3) effect of the trauma film and sleep deprivation on sleep physiology for the sleep deprived participants and 4) associations between sleep physiology and the frequency of intrusive memories.



	Group		REM density group		Interaction	
	B value	p value	B value	p value	B value	p value
Baseline	-1.03	.01	-.05	.82	1.00	.03
Retest	-.38	.18	-.16	.49	.10	.81
change	-.08	.73	-.31	.21	-1.02	.06

Figure 5.15. REM density and intrusive memories. Graphical representations (A) and statistical results from a Poisson distributed generlised linear model (B) of the significant interactions between REM density and group (sleep deprived and non-sleep deprived). The average number of intrusive memories are presented for low versus high (grey and black) and increase versus decreased from baseline to retest night (white and grey) level of each sleep stage for both the sleep deprived and non-sleep deprived participants. * denotes a significant result following Bonferroni correction; + denotes a significant result before, but not following Bonferroni correction. Error bars represent standard error of mean.

5.4.1. Baseline sleep

At baseline no significant differences, after correction for multiple testing (Bonferroni correction), were found for any of the sleep parameters relating to sleep structure, sleep stage or REM density between the sleep deprived and non-sleep deprived participants. Furthermore, the participants showed on average good sleep profiles, with an average total sleep time of 7.5 hours (standard deviation=1.24 hours) and an average sleep efficiency of 93.44% (standard deviation=4.16%). In terms of spectral frequencies some differences were seen between the groups in various frequency bands in both REM and NREM sleep over the whole night as well as over the course of the night and also brain regions.

		Experimental group		Frequency group		Interaction	
		B value	p value	B value	p value	B value	p value
Delta	Baseline	-.49	.03	-.65	.01	-.26	.55
	Retest	-.30	.21	-.44	.06	-.58	.19
	Change	-.19	.46	.28	.22	-.47	.23
Theta	Baseline	-.36	.14	-.34	.14	-.22	.57
	Retest	-.12	.62	.02	.94	-.68	.09
	Change	-.41	.14	.33	.15	-.03	.94
Alpha	Baseline	-.28	.24	-.65	.01	-.25	.53
	Retest	-.02	.93	.03	.91	-.83	.04
	Change	-.37	.14	.12	.59	-.06	.88
Sigma	Baseline	-.84	.00	-.60	.01	.81	.04
	Retest	-.33	.17	-.28	.22	-.48	.28
	Change	-.14	.56	.17	.46	-.69	.09
Beta1	Baseline	-.52	.05	-.12	.59	.09	.81
	Retest	-.49	.06	-.34	.14	.19	.62
	Change	-.78	.00	-.44	.06	.80	.04
Beta2	Baseline	-.32	.26	.21	.38	-.24	.54
	Retest	-.03	.91	.39	.09	-.69	.07
	Change	-.69	.01	-.44	.06	.60	.12
Gamma	Baseline	.21	.49	.27	.27	-1.09	.01
	Retest	-.31	.24	-.14	.54	-.16	.68
	Change	-.38	.10	.07	.76	-.02	.96

Table 5.10. Spectral frequencies and intrusive memories. A Poisson distributed generlised linear model was used to determine the effect of experimental group (sleep deprived and non-sleep deprived), spectral frequency grouping and interaction on intrusive memory frequency for each sleep stage. Significance level following Bonferroni correction: 0.05/21=0.00238.

As discussed in Chapter 1 (section 1.1. p.1), although sleep characteristics are highly conserved within an individual, particularly EEG events (De Gennaro and Ferrara 2003, De Gennaro et al. 2005, De Gennaro et al. 2008), they are variable between individuals (Tucker et al. 2007). Hence, the differences in the spectral frequencies at baseline are perhaps due to the two groups (sleep deprived and non-sleep deprived) containing different individuals. The subsequent analyses performed (described below) were within group comparisons, meaning that the same individuals were assessed on both nights (baseline and retest) removing any influence of individual difference in sleep characteristics.

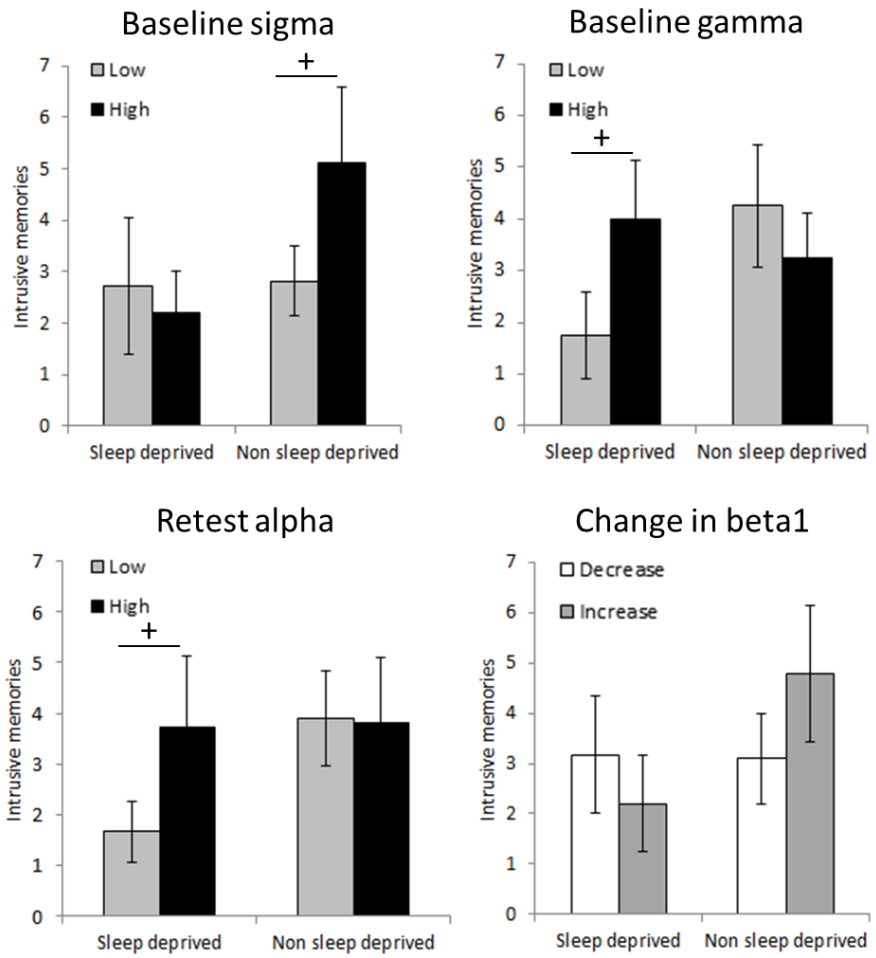


Figure 5.16. Spectral frequency and intrusive memories. Graphical representations of the significant interactions between spectral frequency and group (sleep deprived and non-sleep deprived). The average number of intrusive memories are presented for low versus high (grey and black) and increased versus decreased from baseline to retest night (white and grey) for each sleep stage for both the sleep deprived and non-sleep deprived participants. * denotes a significant result following Bonferroni correction; + denotes a significant result before, but not following Bonferroni correction. Error bars represent standard error of mean.

5.4.2. Effect of trauma film on sleep physiology in non-sleep deprived participants

On the night following the trauma film the participants spent more time awake during the sleep period than on the baseline night. This suggests that the participants exhibited a higher level of arousal following the trauma film. This is consistent with other studies that have reported increased wake during the sleep period following high daytime emotional stress (Vandekerckhove et al. 2011, Akerstedt et al. 2007). However, in contrast to this study, sleep efficiency, total sleep time and SWS latency have also been reported to be affected by daytime emotional experiences (Vandekerckhove et al. 2011, Akerstedt et al. 2007).

		Non-sleep deprived		Sleep deprived				
		Mean	*SD*	Mean	*SD*	test value	*p value*	
WASO	Retest	1.19	*1.70*	.71	*1.15*	-.51	*.62*	a
	Change	.77	*1.29*	-.06	*2.12*	-1.69	*.10*	b
Wake %	Retest	4.22	*3.25*	1.75	*1.29*	-2.73	***.01***	b
	Change	.50	*2.83*	-1.78	*3.21*	-2.05	*.04*	b
Stage 1 %	Retest	5.76	*2.71*	3.58	*1.40*	-2.66	***.01***	b
	Change	.81	*3.18*	-1.15	*1.55*	-1.99	*.05*	b
Stage 4 %	Retest	1.94	*3.29*	3.81	*5.21*	-.95	*.35*	b
	Change	.04	*1.51*	1.47	*2.38*	-1.77	*.08*	b
REM density	Retest	85.00	*40.04*	75.59	*28.79*	-.69	*.50*	b
	Change	6.95	*37.75*	3.07	*22.80*	-.34	*.75*	b
SWS latency	Retest	17.17	*7.84*	13.41	*8.50*	-2.22	*.03*	b
	Change	-2.88	*9.23*	-11.06	*12.94*	-2.27	*.02*	b
Sleep efficiency	Retest	93.84	*4.20*	97.13	*1.48*	-2.8	***0.004***	b
	Change	.79	*4.98*	3.25	*4.06*	-1.46	*.15*	b
Beta 2 in REM	Retest	.23	*.12*	.29	*.25*	-.06	*.95*	b
	Change	.07	*.06*	.08	*.12*	-.13	*.91*	b
Gamma in REM in late night								
interval 6	Retest	.12	*.07*	.13	*.10*	-.93	*.35*	b
	Change	.01	*.04*	.04	*.13*	-3.83	***.00***	b
interval 7	Retest	.11	*.08*	.16	*.35*	-.48	*.63*	b
	Change	.00	*.06*	.03	*.33*	-.43	*.66*	b
interval 8	Retest	.12	*.07*	.14	*.14*	-.18	*.56*	b
	Change	.03	*.07*	-.01	*.19*	-.11	*.91*	b
Occipitial sigma in NREM	Retest	2.77	*1.32*	2.67	*1.52*	-.743	*.458*	a
	Change	.71	*1.36*	.70	*1.15*	-.039	*.969*	a
Occipitial theta in NREM	Retest	16.36	*7.35*	14.59	*9.33*	-2.227	*.026*	a
	Change	4.81	*9.88*	4.63	*6.94*	-.225	*.822*	a
Occipitial alpha in NREM	Retest	6.41	*3.72*	6.02	*6.22*	-1.85	*.07*	a
	Change	1.79	*3.61*	1.81	*2.67*	-1.79	*.07*	b
Occipitial beta1 in NREM	Retest	.58	*.33*	.52	*.45*	-2.07	*.04*	a
	Change	.14	*.29*	.13	*.35*	-.33	*.74*	b
Parietal beta1 in NREM	Retest	.25	*.13*	.23	*.13*	-1.54	*.13*	a
	Change	.03	*.12*	.02	*.11*	-.464	*.643*	a
Prefrontal beta 2 in REM	Retest	.25	*.18*	.27	*.18*	-.45	*.65*	b
	Change	.01	*.22*	.02	*.37*	.38	*.72*	a
Frontal beta 2 in REM	Retest	.00	*.00*	.02	*.13*	-1.49	*.14*	b
	Change	.00	*.00*	-.07	*.31*	-2.49	*.01*	b
Parietal beta2 in REM	Retest	.20	*.11*	.18	*.14*	-3.38	***.00***	b
	Change	.04	*.12*	.03	*.20*	-.81	*.42*	b

Table 5.11. Indirect associations with intrusive memories. The average levels of each sleep characteristic that changes for the sleep deprived or non-sleep deprived participants for the retest night and difference from the baseline to retest night. The statistical comparison between the groups is also given ([a]t-test and [b]Mann Whitney). Significance level following Bonferroni correction: [a] 0.05/29=0.0017, [b] 0.05/9=0.0056.

No change in REM sleep was found for REM latency, duration or density in this thesis study. Although there have been several studies that have reported alterations to REM sleep following emotional experiences (Vandekerckhove et al. 2011, Baekeland et al. 1968, Germain et al. 2003, Koulack et al. 1985) this finding is not consistent and no alterations to REM sleep have also been reported (Lauer et al. 1987, Goodenough et al. 1975, Baekeland et al. 1968). Therefore, the effect of an emotional event on sleep physiology remains to be fully determined. These inconsistencies are likely to be due to different methodologies used by these studies. Firstly, various different methods were used to create an emotionally stressful situation including film clips as in this study (Baekeland et al. 1968, Goodenough et al. 1975, Lauer et al. 1987), cognitive failure tasks (Koulack et al. 1985, Vandekerckhove et al. 2011), being instructed to give an evaluated speech the following morning (Germain et al. 2003) and also the monitoring of real-life stress levels of the participants (Akerstedt et al. 2007). Sleep recordings were also conducted in different environments: at home (Akerstedt et al. 2007) or in a laboratory (Vandekerckhove et al. 2011, Germain et al. 2003, Goodenough et al. 1975, Lauer et al. 1987, Baekeland et al. 1968, Koulack et al. 1985). In this study, sleep was recorded at home, to ensure as natural sleep as possible. However, it is possible that by removing the participants from the environment in which the emotional experience was created (the laboratory), the emotional impact of the event was diminished. Although Akerstedt and colleagues (Akerstedt et al. 2007) also recorded sleep at home, in their study the participants' real-life emotional stress was assessed, which would therefore be more connected to the home environment where the recordings took place. Finally Germain and colleagues (Germain et al. 2003) have demonstrated that state (i.e. reactions to emotional event) and trait (i.e. personality and coping styles) factors can influence the effect of an emotional event on sleep, indicating that individual variation in state and trait parameters could contribute to the inconsistences seen.

5.4.3. Effect of trauma film and sleep deprivation on sleep physiology

The rebound effect of sleep deprivation on sleep observed in this study is consistent with previous studies: an increase in the total sleep time, percentage of stage 4 and sleep efficiency, and a decrease in the percentage of stage1 and SWS latency (Carskadon and Dement 1979, Marzano et al. 2011, Marzano et al. 2010, Borbely et al. 1981). No change in REM sleep percentage was found, which has previously been reported by some studies (Borbely et al. 1981, Carskadon and Dement 1979), while an decrease in the percentage of REM sleep has been reported by others (Marzano et al. 2010, Marzano et al. 2011). In terms of absolute sleep time, an increase was seen in the total sleep time, and the amount of NREM and REM sleep. This finding is actually inconsistent with previous findings, where no change in REM sleep time was found (Carskadon and Dement 1979, Marzano et al. 2010, Marzano et al. 2011). These discrepancies, especially in the amount of REM sleep, are probably due to the sleep periods for the recovery and baseline nights being restricted in these previous studies (Carskadon and Dement 1979, Marzano et al. 2010, Marzano et al. 2011, Borbely et al. 1981). By wakening the participants early, especially on the recovery night the full sleep rebound effect is lost, especially for REM sleep, which is concentrated in the second half of the night. In this study the participants sleep period was not restricted allowing the full sleep rebound to be recorded. Since it appears common practice to restrict the sleep period following sleep deprivation, further characterisation needs to be conducted to assess the true rebound effect of sleep deprivation.

REM density was not found to differ from the baseline to retest night. This is in contrast to previous studies that have consistently shown that REM density is lower on the recovery night following total sleep deprivation (Marzano et al. 2011, Feinberg et al. 1987, De Gennaro et al. 2000). Moreover, previous studies have also shown that REM density remains at a constant level throughout the recovery night, unlike on the baseline night when it progressively increases with each REM period (Marzano et al. 2011, Feinberg et al. 1987). The results in this study conflict with this finding, as REM density progressively increased on the recovery night, as it did on the

baseline night. Although not consistently, an increase in REM density has been reported following a stressful event by a number of studies in healthy individuals (Germain et al. 2003, Baekeland et al. 1968) and also increased REM density is often seen in PTSD patients (Pillar et al. 2000). This suggests that REM density is increased following a traumatic or highly emotional event. Therefore, the higher than expected levels of REM density following sleep deprivation, could be in response to the analogue traumatic event in this study.

REM density is thought to have an inverse relationship with slow wave sleep (SWS) amount (Marzano et al. 2011, De Gennaro et al. 2000). In this study, although SWS was found to increase, it was not to the level previously reported as only the percentage of stage 4 sleep increased: a result that did not remain following Bonferroni correction. Therefore it is possible that the high level of REM density seen may be preventing the level of SWS rebound that has previously been reported. If this is the case it raises the question: what is the role of REM density? There is no clear understanding of the function of REM density, however there is evidence for a role in emotional processing. Increased REM density has been reported following emotional events including a distressing film (Baekeland et al. 1968) and exams (Smith and Lapp 1991), and has also been associated with more intense and vivid dreams (Baekeland et al. 1968). REM density signifies the amount of rapid eye movements that occur during REM sleep. Rapid eye movements during REM sleep have been hypothesised to be following the visual component of dreams i.e. looking at the dream world (known as the scanning hypothesis; (Leclair-Visonneau et al. 2010, Hong et al. 2009). Although not consistently supported (Arnulf 2011, Zhou and King 1997), evidence for this hypothesis is: that dreaming involves the same brain systems and mechanisms as waking visual perception (Farah 1984) and that REM density correlates to the amount of visual imagery in dreams (Hong et al. 1997). The direction of eye movements (up and down, left and right) have also been found to have an 82% concordance with the direction of movement of dream scenes as assessed in patients with REM sleep behaviour disorder: a disorder where individuals act out their dreams (Leclair-Visonneau et al. 2010). This hypothesis implicates REM

density in dreaming. Since dreaming has been associated with mood regulation (Cartwright et al. 1998, Lara-Carrasco et al. 2009), it could be that REM density is acting through dreaming to process emotional events. Hence in this study the higher than expected levels of REM density in the sleep deprived participants following sleep deprivation could be an indication of more dreaming and hence more emotional processing.

Spectral frequency activity was found to change from baseline to recovery night. In REM sleep lower beta2 activity was found on the retest night as a whole and lower gamma activity was found for the second half of the retest night. Lower gamma activity in REM sleep has previously been correlated with decreased emotional reactivity (van der Helm et al. 2011). This would therefore support the hypothesis, along with increased REM density, that increased emotional processing is occurring after the trauma film for the sleep deprived participants. In terms of beta activity, to date no study has reported any interaction between beta activity and emotional processing. Moreover, during wake inconsistent results have been reported regarding beta activity and emotional processing (Balconi and Pozzoli 2009), therefore the significance of lower beta2 activity on the retest night in REM sleep is unclear.

Slow frequency EEG activity (i.e. delta and theta), like slow wave sleep, have previously been reported to increase in REM and NREM sleep following sleep deprivation (Borbely et al. 1981, Tinguely et al. 2006, Marzano et al. 2010). In this thesis study a decrease in theta activity was observed in NREM sleep in the occipital region but no change in delta activity was observed, which is consistent with the diminished SWS rebound that was observed in this study. This suggests that delta activity as well as SWS may have been affected by the higher than expected levels of REM density. Sigma activity was also found to be lower in the occipital region during NREM sleep, while beta 1 and alpha activity were higher. Decreased sigma activity has been previously described during NREM sleep following sleep deprivation (Marzano et al. 2010), however this was reported to be in the fronto-central region rather than the occipital. However

an increase in beta1 and alpha activity has not previously been reported following sleep deprivation. These increases in activity were in the occipital region in NREM sleep. The occipital lobe contains the visual processing centre of the mammalian brain (Crossman and Neary 2005) and is also involved, particularly the primary visual cortex (areas 17 or 18), in mental imagery (Kosslyn and Thompson 2003, Ishai et al. 2000, Ishai et al. 2002, O'Craven and Kanwisher 2000), which is the mental visualisation of objects and scenes. The increased alpha and beta activity in the occipital region during NREM sleep in this study could therefore be indicative of increased activation of this region and possibly increased processing of visual information. In light of the importance of visual informational processing in the frequency of intrusive memories, this increase in occipital brain activity may be a consequence of the trauma film. Mental imagery is also the most common aspect of intrusive memories (Kosslyn et al. 2001). The association between these changes in sleep physiology and intrusive memories will be discussed in detail below.

In summary the changes in sleep physiology seen in the sleep deprived participants cannot be solely explained by the period of extended wakefulness. Moreover the changes seen, including higher than expected levels of REM density and increased brain activity in the occipital region, suggest that these changes may be the result of increased emotional processing in response to the analogue traumatic event. What is interesting however is why these changes, which are possibly related to the trauma film, were observed in the sleep deprived participants and not the non-sleep deprived participants. Firstly sleep deprivation affects mood and emotion itself (Banks and Dinges 2007) and could therefore add to the emotional load the participants experienced. It is also possible that the sleep deprivation impacted on how the traumatic event was processed. The data presented in Chapter 4 demonstrated that the sleep deprived participants experienced fewer intrusive memories than those non-sleep deprived. The changes in sleep physiology observed in the sleep deprived participants may reflect this difference in emotional processing. In contrast it is also possible that the trauma film had more of an impact on the sleep deprived

participants as they remained in the sleep lab following the analogue traumatic event while they were being sleep deprived. It has previously been discussed that the relatively unchanged sleep physiology of the non-sleep deprived participants could have been a consequence of leaving the sleep lab following the trauma film. Hence since the sleep deprived participants remained in the same environment for longer, the analogue traumatic event could have had more of an effect. However this seems unlikely considering that the sleep deprived participants reported fewer intrusive memories related to the trauma film.

5.4.4. Sleep parameters and intrusive memories

To date no study has investigated associations between sleep physiology and intrusive memories. Based on research investigating sleep and intrusive memories in isolation and the results presented in Chapter 4 (p.91), there are three possible mechanisms that may underlie an association between sleep physiology and intrusive memory frequency: 1) memory consolidation, 2) hyperarousal and 3) information processing.

5.4.4.1. Memory consolidation

Sleep enhances memory consolidation, especially emotional memory consolidation (see Chapter 1 for details, (Walker and Stickgold 2004, Born and Wilhelm 2012, Hu et al. 2006, Walker and van der Helm 2009, Cartwright et al. 1975, Greenberg et al. 1983, Wagner et al. 2001, Nishida et al. 2009). In Chapter 4 it was postulated that the lower level of intrusions experienced by the sleep deprived participants, could be due to the lack of memory consolidation, following the analogue traumatic event, as a result of extended wakefulness. Therefore, it is possible that participants who have a greater opportunity for memory consolidation (more SWS and REM sleep) following the trauma film could experience more intrusive memories. In this study the sleep deprived participants who had more stage 4 sleep on the retest night had a higher frequency of intrusive memories. This suggests that individuals who had more opportunity for memory consolidation had more intrusions. Dreams have also been hypothesised to be involved in memory

consolidation (Payne and Nadel 2004) and it is possible that REM density reflects the amount of dreaming that is taking place (Leclair-Visonneau et al. 2010, Hong et al. 2009). In this study increased REM density was associated with more intrusions in the sleep deprived participants, which may also support the hypothesis that more memory consolidation results in more intrusive memories. Finally support for this theory comes from the initial finding that the sleep deprived participants, who had no sleep enhancement of memory as a result of extended wakefulness (i.e. sleep deprivation), reported fewer intrusive memories.

5.4.4.2. Hyperarousal

Increased arousal or hyper-arousal is one of the key symptomologies of post-traumatic stress disorder (PTSD) (American Psychological Association 2000). Hyperarousal is also thought to be involved in the development of intrusive memories following trauma. In patients with PTSD, increased physiological arousal is observed during intrusive memories compared to ordinary memories (Hellawell and Brewin 2002) and the level of arousal experienced during the trauma predicts the development of intrusive memories (Resnick 1997). Experimentally induced hyperarousal, using the hyperventilation provocation test, increases the frequency of intrusive memories in individuals with acute stress disorder, an acute trauma response that is predictive for PTSD (Nixon and Bryant 2005, Hopwood and Bryant 2006). The same response has also been reported in healthy individuals; however in this study the effect was short lived and only affected the frequency of intrusions immediately following the induced hyperarousal (Nixon et al. 2007). Moreover, since increased arousal impacts on the sleep-wake cycle, it is possible that hyperarousal might affect sleep physiology. In this study evidence for increased levels of arousal during sleep, were associated with increased levels of intrusions. Individuals, in the sleep deprived group, who had an increase in sleep latency, stage 1 sleep and wake and a decrease in sleep efficiency on the retest night compared with the baseline night experienced more intrusions. Indirect associations also support this theory. On the retest night the sleep deprived participants, who reported fewer intrusions, had higher sleep efficiency and a trend for less time

spent awake and in stage 1 sleep than the non-sleep deprived participants. A lower SWS latency on the retest night and a bigger decrease in SWS latency was also seen for the sleep deprived participants. Since SWS is the deepest sleep stage, a quicker entry into this sleep stage indicates a lower level of arousal. However, a shorter sleep latency was also associated with more intrusions being experienced in the sleep deprived individuals. A shorter sleep latency is indicative of a quicker decent into sleep and therefore a lower level of arousal. Sleep latency is calculated from lights off to entry into the first period of persistent sleep. As the participants were monitored at home, lights off was based on self-reported times. Therefore it is possible that the timings given were not as accurate as those collected from a lab based study. However overall, there is more convincing evidence in support of increased hyperarousal causing an increase in the frequency of intrusive memories. What remains unclear is whether it is increased daytime hyperarousal, which is reflected in reduced sleep quality, that is impacting on the number of intrusions experienced, or whether poorer sleep itself is having an impact.

5.4.4.3. Information processing

As previously described in Chapters 1 and 4, interference in visual-spatial and verbal processing affects intrusion frequency, causing fewer and more intrusions to be experienced respectively (Nixon et al. 2007, Holmes et al. 2004, Stuart et al. 2006). This suggests that the type of information processes that occurs during and after trauma influences the frequency of intrusive memories. Since, there is increasing evidence that waking experiences are replayed during sleep (O'Neill et al. 2010), information processing during sleep may also influence intrusive memory frequency. Visual spatial information is processed by the primary visual cortex, which is located in the occipital lobe (Crossman and Neary 2005). The primary visual cortex is also involved in mental imagery, which involves the processing of visual information without external visual stimuli. Lower brain activity was observed in the occipital region for the sleep deprived participants compared with the non-sleep deprived participants on the retest night. This suggests that higher activation of the occipital region and hence increased visual spatial processing may result in more

intrusive memories. This supports the theory of visual-spatial processing influencing the frequency of intrusive memories, and also suggests that this processing continues during sleep.

In summary there is evidence supporting all three hypothesis for the mechanism underlying the frequency of intrusive memories. It is possible that all these potential mechanisms interact and therefore further investigations need to be carried out to establish which mechanisms are involved.

5.4.5. Study limitations and improvements

There are two potential limitations for this study. Firstly as the non-sleep deprived participants did not remain in the sleep lab following the trauma film this may have diminished the impact of the film. As discussed above this is a possible reason for little change to sleep physiology being observed following the trauma film in the non-sleep deprived participants, compared to previous studies. Assessment of non-sleep deprived participants who remained in the sleep lab to sleep following the trauma film, would address this situation. Secondly no direct assessment of the effect of the trauma film and the effect of sleep deprivation on sleep physiology was possible. A crossover design whereby the same individual experiences the trauma film, sleep deprivation and the trauma film and sleep deprivation together, would enable this comparison. Moreover this study design would remove any inter-individual differences between the comparisons.

5.5. Key findings

- The non-sleep deprived participants spent more time awake during the sleep period on the night following the trauma film than at baseline
- The sleep deprived participants showed the expected rebound of sleep, although with less rebound of slow wave sleep than expected
- The sleep deprived participants did not show a decrease in REM density following sleep deprivation as has previously been reported

- The sleep deprived participants showed higher brain activity in the occipital region following sleep deprivation and the trauma film
- The data support three theories for the frequency of intrusive memories: i) memory consolidation ii) hyperarousal and iii) information processing

5.6. Conclusions

The aim of this study was to assess the effect of the trauma film on sleep physiology. The trauma film was found to have little effect on sleep physiology for the non-sleep deprived participants. The sleep physiology of the sleep deprived participants after the trauma film and sleep deprivation, was not completely consistent with the expected effects of sleep deprivation on recovery sleep. Both higher levels of REM density and brain activity in the occipital region were observed that may be associated with increased emotional processing. Aspects of sleep physiology and intrusive memory frequency were also correlated. Although evidence for all three theories for the mechanism underlying intrusive memory frequency were reported, increased hyperarousal received the most support.

6. High risk for mood disorders, sleep and intrusive memories

6.1. Introduction

This chapter, reports on the study which investigated the relationship between sleep and emotional processing, in healthy students with a family history of a mood disorder as an extension to the study reported in Chapter 4 (p.91). This allows the final question of this thesis to be addressed: **is the relationship between sleep and emotional processing different in people at-risk of a mood disorder?** The primary hypothesis for this study was that the at-risk participants would experience more intrusive memoires than the participants without a family history of mood disorders. It was also hypothesised that sleep and circadian disruptions, and a negative emotional bias would be observed in the at-risk participants.

This study is on-going and the preliminary data are presented on the three participants recruited so far as case studies. Although the inclusion criterion for the study was for family history of any mood disorder, two of the participants' relatives only had a family history of depression and the one participant that had a distant relative with bipolar disorder also had an extensive family history of depression. Hence the remainder of this chapter will focus on the impact of depression and an elevated risk for depression has on sleep and emotional processing.

As described in Chapter 1 (section 1.4. p.24) similar sleep disruptions and emotional biases are seen in people with depression (Rubinow and Post 1992, Surguladze et al. 2004) and those with an increased risk of developing depression (Chan et al. 2007, Brotman et al. 2008). Although intrusive memories are often experienced by people with depression, experimental investigations simulating trauma (analogue trauma) to explore the intrusive memories are not possible for ethical reasons. To date only one study has looked at intrusive memories in high risk individuals: individuals with an elevated risk for a mood disorder were found to experience more intrusive memories to an analogue traumatic event than controls (Malik et al. Manuscript submitted).

The circadian clock is involved in the regulation of the sleep-wake cycle (Borbely 1982, Borbély 2009, Borbely and Achermann 1999) hence, the sleep disruptions observed in depression and in at-risk individuals suggests the circadian clock may also be disrupted. Evidence for the disruption of the circadian clock however is tentative. In depression, abnormalities in robust circadian rhythms, including core body temperature (Souetre et al. 1989) and melatonin concentration (Claustrat et al. 1984), have only been reported by these two studies. Depression has been associated with more 'eveningness' as assessed by the Horne-Ostberg morningness-eveningness questionnaire (Drennan et al. 1991), which suggests a delayed phase of the clock. However, the difference in scores between depressed and control groups was small and in fact both groups remained with the intermediate type classification for diurnal preference (chronotype). Indicating that, although the depressed individuals were later than the controls using this questionnaire, this did not equate to a real behavioural difference between the groups. Finally, chronotherapeutic strategies (treatments that act to stabilise the circadian clock such as bright light therapy, sleep-phase advancement and sleep deprivation) have been used to treat depression, though with varying levels of success (e.g. (Benedetti et al. 2007, Echizenya et al. 2012)).

In high risk individuals, very few studies into circadian disruptions have been investigated and with inconsistent results. Moreover these studies have focused on those at risk of bipolar disorder. However, an increased risk of bipolar disorder whether familial (DelBello and Geller 2001) or behavioural (Lewinsohn et al 03), is associated with an increased risk of depression. In individuals with a behavioural risk of bipolar disorder (and hence depression) an attenuated relative amplitude of the rest/activity rhythm has been reported from actigraphic recordings (Ankers and Jones 2009, Rock et al. Manuscript). However, in individuals with a familial risk no difference in any actigraphic measures of the rest/activity cycle were found (Jones et al. 2006).

The stress response is also thought to be disrupted in depression. The hypothalamic-pituitary-adrenal (HPA) axis is the primary neuroendocrine pathway involved in the coordination of the stress response (for review see (de Kloet et al. 2005). Briefly, activation of the HPA axis results in the synthesis and release of glucocorticoids, the most prominent of which is cortisol, which allows a coordinated response from the brain and body to react and recover from the stressor (de Kloet et al. 2005). An increase in the release of cortisol occurs in the first half an hour after waking, known as the cortisol awakening response (CAR) (Fries et al. 2009). The role of the CAR is still unclear, however this response is thought to be distinct from the circadian modulation of the HPA axis and directly linked to the sleep-wake transition (Wilhelm et al. 2007). It is thought that it may provide an initial boost to an individual to begin the day (Adam et al. 2006). In depression enhanced cortisol release after waking has been reported in many studies (Bhagwagar et al. 2005, Deuschle et al. 1997, Vreeburg et al. 2009, Burke et al. 2005) though not all (Strickland et al. 2002, Posener et al. 2000). Cortisol levels have also been found in the majority of studies to be higher in the children of individuals with a mood disorder (Deshauer et al. 2006, Ellenbogen et al. 2004, Ellenbogen et al. 2006, Lupien et al. 2000, Mannie et al. 2007). There is growing evidence that the functioning of the HPA axis is influenced by early prenatal and postnatal environmental conditions (Levine 2005, Halligan et al. 2004, Lundy et al. 1999). Hence the combination of elevated cortisol levels during pregnancy in women with depression along with the additional stressful environment often created by a parent with a mood disorder, could explain the increased cortisol levels seen in these children. Moreover, in individuals with only a behavioural risk for depression and therefore without the confounding effects of pre- and post-partum depression, waking cortisol levels are not elevated (Chan et al. 2007).

In summary, depression is associated with disruptions in sleep, the stress response and possibly the circadian clock. The occurrence of these disruptions within individuals before the onset of the condition i.e. those with an elevated risk, indicates that these disruptions may be trait characteristics of those predisposed to the condition rather than consequences of either the

condition or treatment. Further investigation of these systems may not only help predict the development of depression but also provide targets for treatment and potentially even prevention, with the stabilisation of one or a combination of these systems. Moreover research with at-risk but otherwise healthy individuals removes any potential cofounding effects of medication.

6.2. Methods

The methods and techniques used to produce the results of this chapter are presented in detail in Chapter 2 (section 2.5.2. p.54). The study protocol used is the same as outlined in Chapter 4 (p.91), the only difference being the inclusion and exclusion criteria for the participants.

6.2.1. Inclusion and exclusion criteria

The key inclusion criterion for this study was that the participants must have a positive family history of a mood disorder. The family member must be a blood relative with a diagnosed mood disorder. Initially a first degree relative was required. However, due to difficulties in the recruitment of participants, second degree relatives were also allowed. A first degree relative is a relative that shares 50% of the genetic material, while a second degree relative still shares 25%. To determine the participant's family history, the participants were required to complete the family history recording form before beginning the study. In addition several of the exclusion criteria were adapted for this population: participants were included with any score on the PSQI and MEQ. This was to take in to account the fact that individuals at-risk may have disrupted sleep and circadian rhythms, as previously reported.

6.2.2. Sleep physiology

The polysomnographic (PSG) recordings were analysed in the same way as for the non-family history (nFH) participants (for details see Chapter 2 section 2.5.2.2. p.58 and Chapter 5 section 5.2.1. p.132). However unlike the nFH participants the spectral frequency data is not reported

here. The differences in the spectral frequency data reported in Chapter 4 are not visually discernible and required statistical analysis to be determined. In the data presented here statistical comparisons were not possible with only three participants therefore the spectral frequency data is not presented as determining differences was not possible.

6.3. Results

Of 34 individuals who were interested in taking part in the study, only three that agreed to take part were found to be eligible: two non-sleep deprived participants (FH1 and FH2) and one sleep deprived participant (FH3). Each participant was required to have at least one blood relative with a current or past diagnosis of a mood disorder, and have no personal history of any psychological or psychiatric disorders, which was confirmed with the mini international neuropsychological interview (MINI), mood disorder questionnaire (MDQ) and general health questionnaire (GHQ) (results in **table 6.1**). All the participants were female, non-smokers and had no history of drug or alcohol abuse. The remaining data is presented as three case studies. For comparison all the data are represented in tables and figures alongside the mean data from the previous experiments described in Chapters 4 (p.91) and 5 (p.129): sleep deprived and non-sleep deprived healthy individuals without a family history of depression (no family history: nFH).

6.3.1. FH1

Participant FH1 had an extensive family history of depression: both parents had a current diagnosis of depression, as well as an extensive maternal (an aunt, uncle, grandmother, great-aunt and great-uncle) and paternal (a paternal aunt) family history of depression. FH1 also had a maternal great-uncle with bipolar disorder that had resulted in periods of hospitalisation.

		Non-sleep deprived				Sleep deprived		
		FH1	FH2	nFH mean [count]	*s.d.*	FH3	nFH mean [count]	*s.d.*
	Age	**19**	**23**	21.59	*2.17*	**19**	21.53	*1.71*
	Sex (female)	**yes**	**yes**	[15]		**yes**	[13]	
	Alcohol (units per week)	**4**	**5**	2.82	*4.09*	**20**	3.79	*3.58*
	Caffeine (mg per day)	**19**	**3.2**	122.86	*96.16*	**0**	111.25	*98.32*
	Menstral cycle (regular)	**no**	**yes**	[14]		**yes**	[9]	
MDQ	Symptoms	**3**	**4**	2.00	*1.80*	**0**	1.32	*2.19*
	Experienced at same time (yes)	**yes**	**no**					
	Problem	**no**	**no**					
	MEQ	**49**	**53**	54.39	*9.44*	**40**	51.58	*9.82*
	GHQtotal	**7**	**7**	9.00	*2.83*	**8**	7.47	*2.25*
	PSQIglobal	**6**	**4**	2.86	*1.25*	**3**	2.95	*1.22*
EPQ	Neuroticism	**12**	**3**	6.00	*3.13*	**9**	5.42	*3.31*
	Psychoticism	**2**	**8**	3.55	*1.90*	**7**	2.05	*2.01*
	Lie	**10**	**11**	8.73	*4.04*	**5**	9.42	*4.03*
	Extraversion	**8**	**14**	14.18	*3.67*	**9**	14.58	*4.32*
POMS	Tension-Anxiety	**11**	**2**	5.81	*4.29*	**5**	5.68	*3.25*
	Depression-Dejection	**13**	**3**	6.29	*5.20*	**8**	4.74	*3.86*
	Anger-Hostility	**8**	**3**	4.48	*4.00*	**3**	2.84	*2.89*
	Vigour-Activity	**12**	**20**	16.57	*4.57*	**17**	16.95	*4.53*
	Fatigue	**7**	**2**	3.95	*3.22*	**7**	6.32	*3.62*
	Confusion	**6**	**8**	4.24	*2.43*	**8**	4.05	*2.48*

Table 6.1 Demographic data. The demographic data along with psychological assesments are presented for each at-risk participant (FH1, FH2 and FH3), along with the mean data (and standard deviaiton) for the sleep deprived and non-sleep deprived participants without a family history (nFH). Count data for alcohol and menstral cycle are presented in sqaure brackets.

6.3.1.1. Baseline assessment

FH1 was 19 years old, and had low alcohol and caffeine consumption. Baseline assessment revealed a poor sleep quality (PSQI), later diurnal preference (MEQ), higher levels of neuroticism and lie (EPQ) as well as higher levels of depression and tension and anxiety (POMS) as compared with the nFH participants (**table 6.1**). Indicating that along with a familial risk of depression, FH1 also had a heightened risk due to increased neuroticism, depression and poor sleep quality.

6.3.1.2. Circadian measures

Despite a poor sleep quality reported on the PSQI, actigraphic assessment of the rest/activity cycle revealed no difference in sleep timing, duration or quality from the nFH participants (**table 6.2**).

	Non-sleep deprived				Sleep deprived		
	FH1	FH2	nFH		FH3	nFH	
			mean	*s.d.*		mean	*s.d.*
Sleep start	**01:11**	**23:42**	00:26	*00:53*	**00:37**	00:21	*01:00*
Sleep end	**08:32**	**08:31**	08:16	*00:51*	**08:37**	08:18	*00:58*
Total sleep time	**07:21**	**08:49**	07:49	*00:37*	**08:00**	07:55	*00:40*
Actual wake time	**00:38**	**01:30**	00:58	*00:26*	**00:44**	00:59	*00:13*
Actual wake (%)	**9.04**	**16.24**	12.42	*5.04*	**9.35**	13.03	*3.46*
Sleep efficiency	**87.93**	**82.60**	86.34	*5.18*	**88.39**	85.55	*4.12*
Sleep latency	**00:12:00**	**00:05:39**	00:06:01	*00:02:42*	**00:11:13**	00:06:52	*00:05:13*
Wake bouts	**23**	**36**	28.60	*8.74*	**21**	30.99	*7.13*
Mean sleep bout time	**00:18:00**	**00:12:24**	00:16:24	*00:05:10*	**00:21:02**	00:14:45	*00:03:51*
Mean wake bout time	**00:01:42**	**00:02:31**	00:01:59	*00:00:20*	**00:02:04**	00:01:57	*00:00:17*
Fragmentation index	**27.0**	**33.5**	25.10	*8.08*	**25.7**	27.48	*6.44*
Interdaily stability	**0.50**	**0.38**	0.49	*0.09*	**0.30**	0.47	*0.11*
Intradaily variability	**0.72**	**0.93**	1.02	*0.21*	**0.96**	0.93	*0.21*
Rest phase (L5) onset	**02:16**	**02:27**	01:39	*00:12*	**02:00**	01:40	*00:15*
Rest phase (L5) activity level	**1544**	**2312**	1298	709	**1997**	1284	1068
Active phase (M10) onset	**12:05**	**10:09**	10:49	*01:06*	**11:09**	10:55	*01:50*
Active phase (M10) activity level	**22909**	**20331**	21172	3645	**19147**	22915	5172
Relative amplitude	**0.88**	**0.78**	0.88	*0.06*	**0.82**	0.89	*0.08*

Table 6.2 Actigraphic data. The actigraphic assessment of the rest/activity cycle is presented for each at-risk participant (FH1, FH2 and FH3), along with the mean data (and standard deviation) for the sleep deprived and non-sleep deprived participants without a family history.

FH1 also showed a stable circadian rhythm of the rest/activity cycle that was comparable with the nFH participants. However, the rhythm was slightly delayed as the onset of activity (M10) and rest (L5) was later than the nFH participants by 1.27 hours and 0.62 hours respectively. The acrophase of the melatonin metabolite, 6-sulfatoxymelatonin (aMT6) was also found to be delayed at 07:21 (**figure 6.1**).

6.3.1.3. Diurnal mood

Participant FH1 showed greater changes in mood over the day compared with the nFH participants (**figure 6.2**). FH1 reported below the mean nFH levels of happiness and calmness throughout the day, with the lowest levels being reached in the middle of the day. Increases above the mean nFH levels in hopelessness, tension, awkwardness, anxiety and restlessness were also reported. These changes in mood again tended to be reported in the middle of the day.

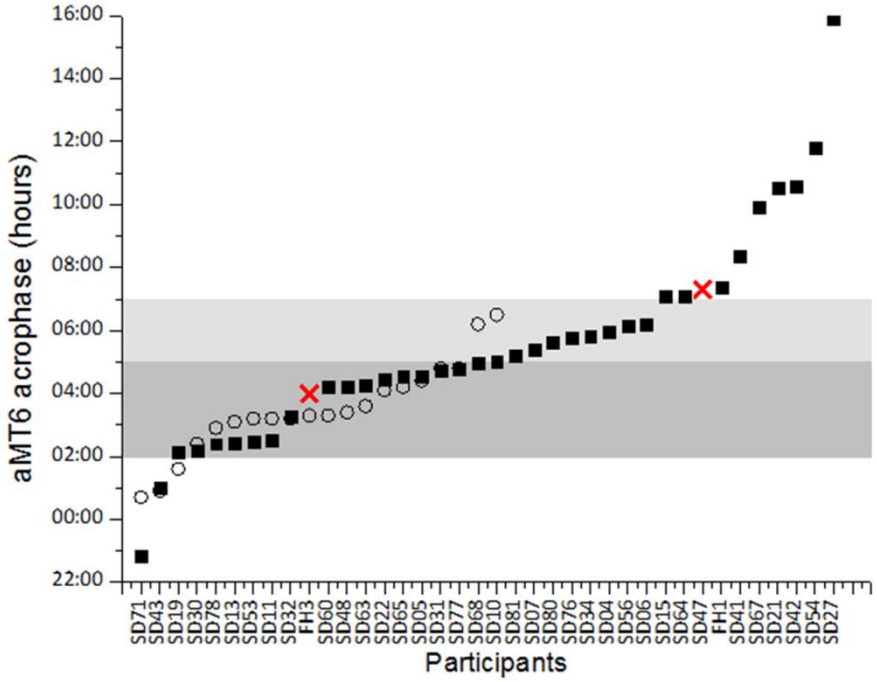


Figure 6.1 aMT6s acrophase. The aMT6s acrophase for each at-risk participant (red cross) is presented along side the nFH participants (black squares) and control data (open circles) provided by Benita Middleton. The dark grey area represents the normal range for aMT6s acrophase while the light grey area represents the slightly dealyed range.

Changes in levels of reported sleepiness and alertness were consistent with those seen for the nFH participants. As a result of these changes in mood, FH1 ranked highly in the distribution of mood fluctuations (**figure 6.3**). However FH1 was not the highest ranking participant, indicating that although FH1 show high levels of mood fluctuation, it was still inside the range seen for the nFH participants.

6.3.1.4. Cortisol awaking response

Waking salivary cortisol levels were analysed at four time points on two days (**figure 6.4**). FH1 failed to provide enough saliva for analysis at the first time point on day one. The same profile of cortisol levels was seen on both sampling days: initial increase post waking, followed by a decrease 45 minutes after waking.

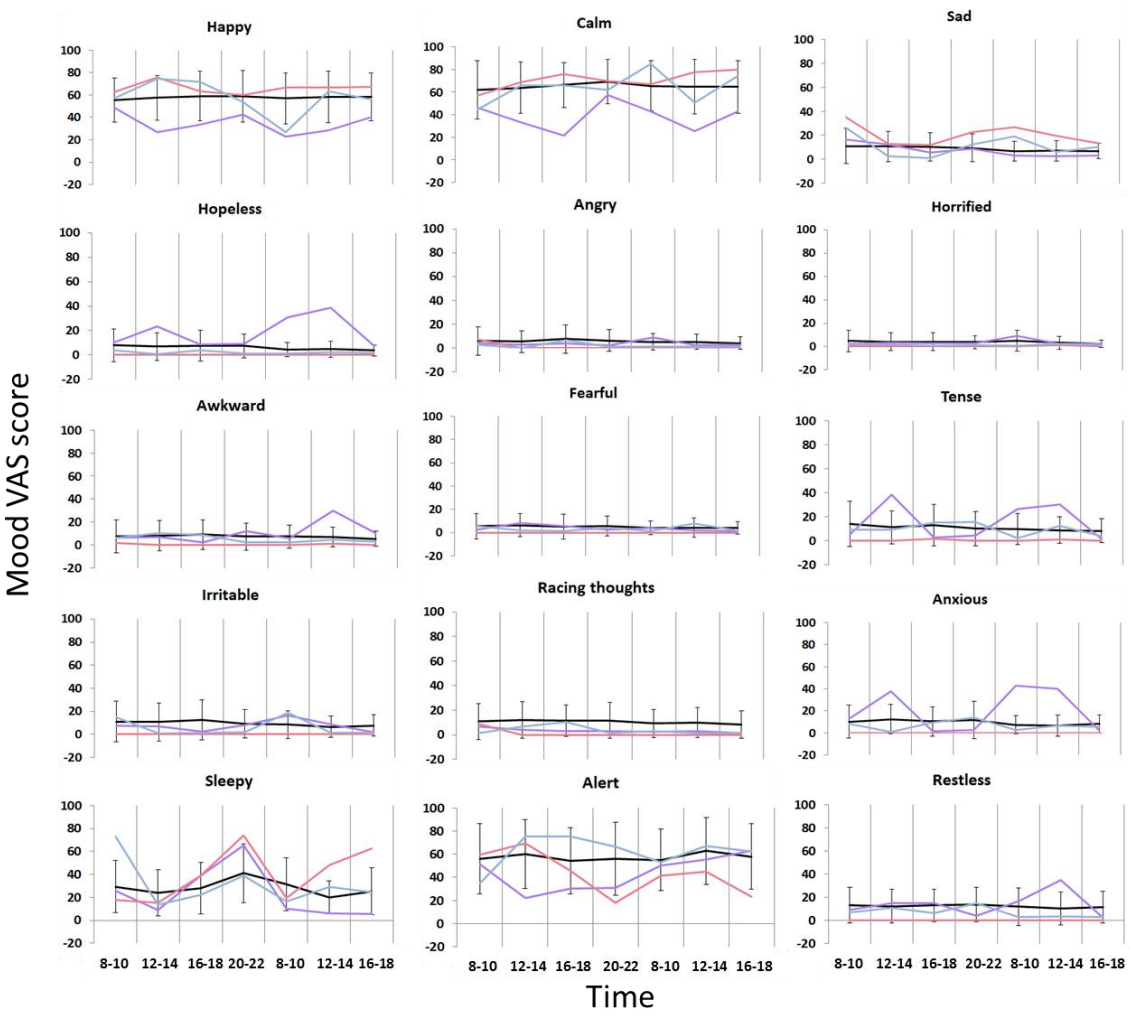


Figure 6.2 Diurnal mood. The mean score for each mood is given in four hour bins over two days for each at-risk participant (FH1: purple, FH2: pink, FH3: blue) and the nFH participants (black). Error bars represent the standard deviation of the mean for the nFH participants.

This differed from the average profile seen with the FH participants which showed a gradual increase with little or no decrease 45 minutes post waking. The concentration of cortisol seen for FH1 was also higher at its peak (30 minutes post waking) than the mean (filled squares) and standard deviation (box plot whiskers) concentration of the nFH participants.

6.3.1.5. Response to trauma film

Immediately following the trauma film, FH1 reported a decrease in positive affect to a similar extent as the mean nFH level (**figure 6.5a**).

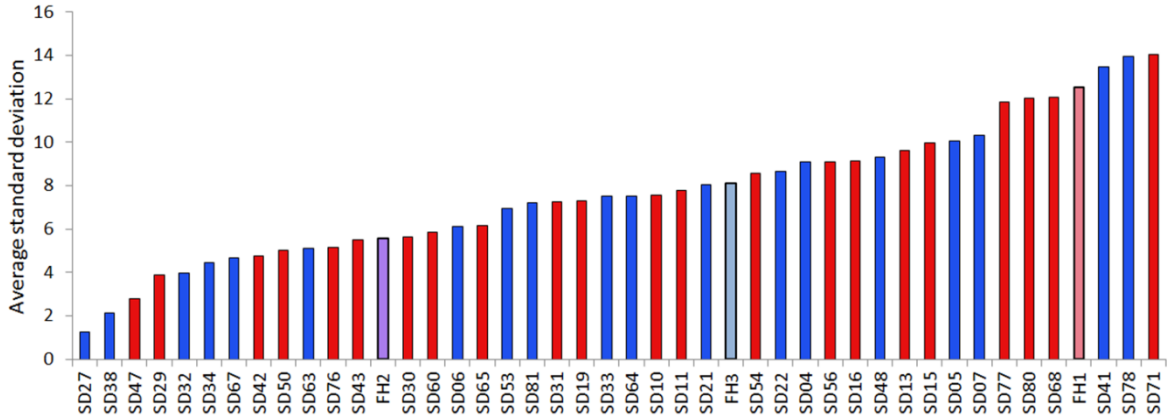


Figure 6.3 Mood fluctuation. The mean stardard deviaiton of the combined moods is presented for the each at-risk participants (FH1: purple, FH2: pink, FH3: blue) and the nFH participants (sleep deprived (blue) and non-sleep deprived (red) participants).

However, although an increase in negative affect was also reported this was to a much lesser extent than in the nFH participants. Participant FH1 also showed an increase in dissociation as a result of watching the trauma film (**figure 6.5b**), which is consistent with the nFH participants and reported the same level of relevancy and familiarity to the trauma film as the nFH participants (**figure 6.5c**).

6.3.1.6. Response to stressor

Participant FH1 scored above average on the stressful task (remote associates task) (**figure6.6a**). Despite this she reported the difficulty of the task and her performance at a similar level as the nFH participants (**figure6.6b**). In terms of mood, FH1 showed a slight increase in negative affect after the stressor and a large decrease in positive affect (**figure6.6c**). In relation to the nFH participants the change in mood reported by FH1, although smaller for negative affect, was much larger for positive affect. This suggests that despite the small change in negative affect, FH1 was more affected by the stressor than the nFH participants.

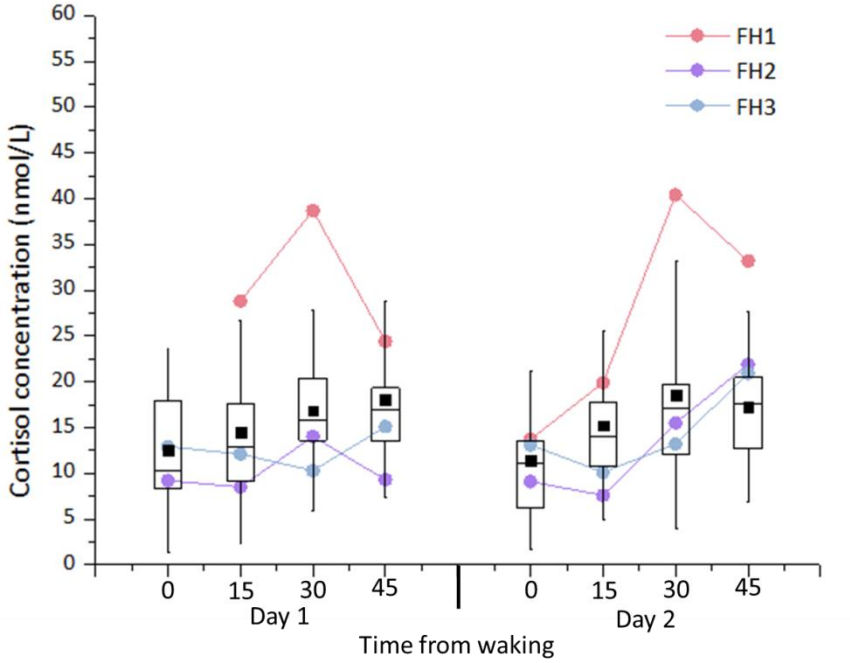


Figure 6.4 Cortisol awakening response. The concentration of cortisol at 0, 15, 30 and 45 minutes after waking is shown for both collection days for each at-risk participant (FH1: purple, FH2: pink, FH3: blue) along with the mean values of the nFH participants (black). For the nFH participants the mean cortisol concentration (open squares) is presented along with the 25, 50 and 75 percentiles (box) and standard deviation (whiskers).

6.3.1.7. Change in mood over experiment

Over the course of the experiment FH1 experienced similar changes in alertness (**figure 6.7a**), sleepiness (**figure 6.7b**), anxiety (**figure 6.7c**) and positive and negative affect (**figure 6.7d**) compared with the non-sleep deprived nFH participants, in whom all moods were improved following a night of sleep. The only exception being negative affect, which like the non-sleep deprived nFH participants showed very little change of the course of the experiment.

6.3.1.8. Activities

Participant FH1 spent more time doing verbal activities than visual spatial activities (**figure 6.8**). Moreover the time spent doing visual spatial activities was higher than the non-sleep deprived nFH participants.

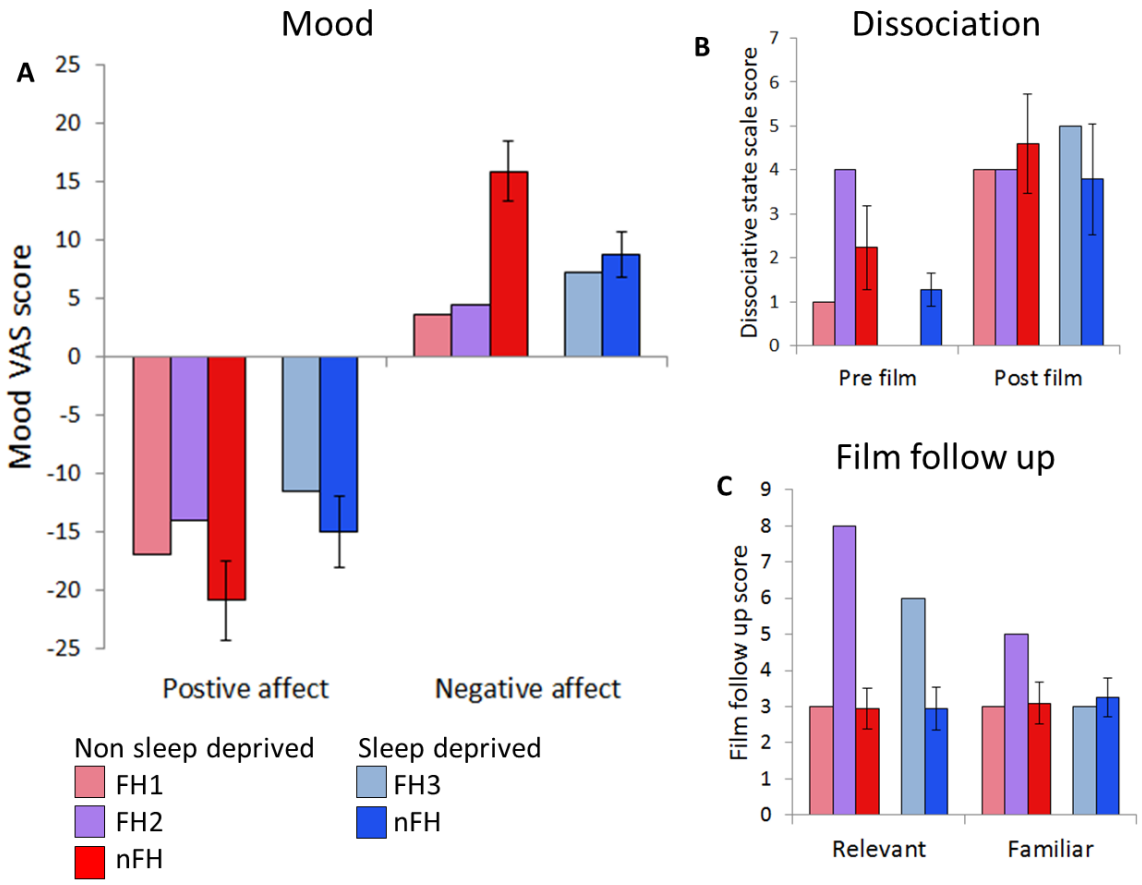


Figure 6.5 Response to trauma film. The change in positive and negative affect (A), and dissociation (B) after watching the trauma film, along with the relevance and familiarity with the content of the trauma film (C) is shown for each at-risk participants (FH1: purple, FH2: pink, FH3: blue) and the nFH participants: sleep deprived (blue) and non-sleep deprived (red). Error bars represent the standard error of the mean.

6.3.1.9. Intrusive memories

In the week following the trauma film, FH1 recorded three intrusions in the intrusion diary (**figure 6.9a**), which was below the mean number of intrusions reported by the non-sleep deprived nFH participants (mean = 4.14, SD = 2.03), but higher than the sleep deprived nFH participants (mean = 2.35, SD = 1.06). Day by day analysis of the intrusions revealed, FH1 experienced one intrusion on day 1 and the remaining two intrusions both on day 4 (**figure 6.9b**). This is in contrast to the nFH participants who tended to experience more intrusions in the first few days after exposure to the trauma film.

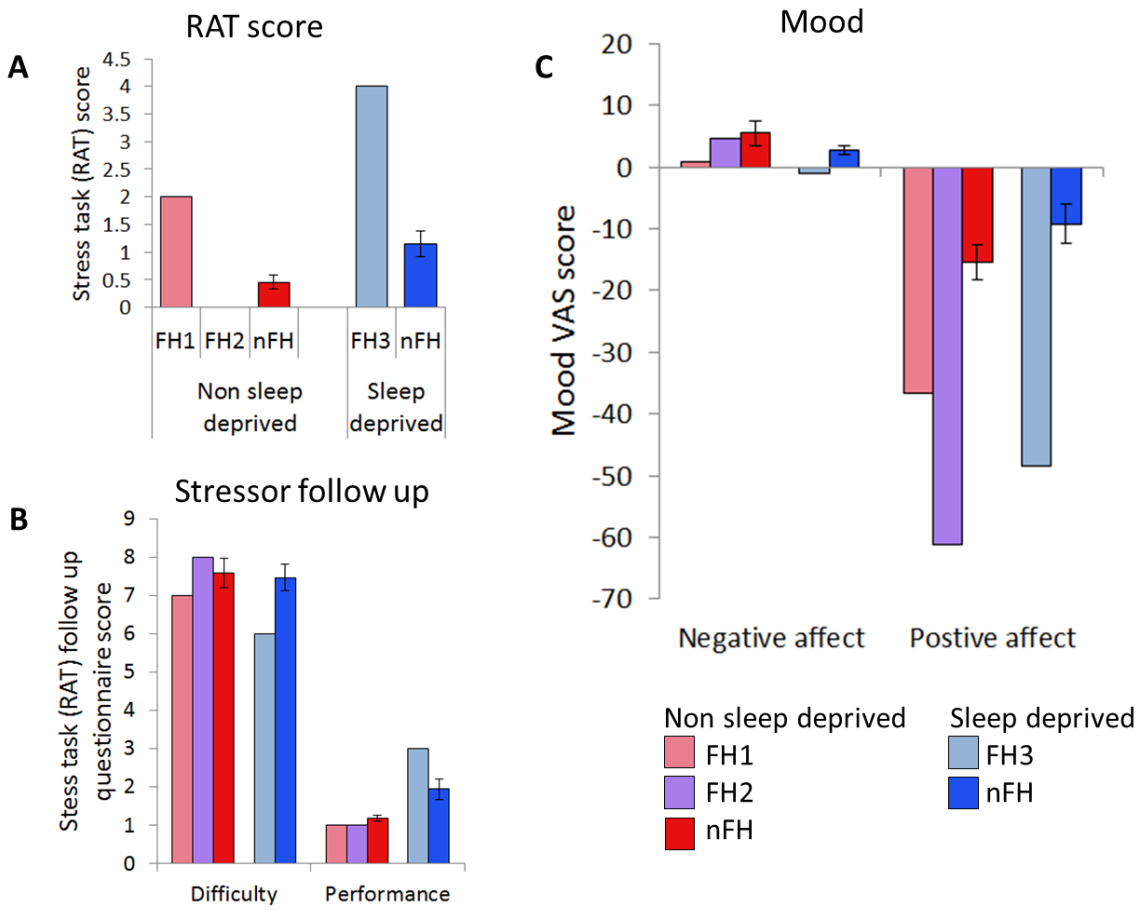


Figure 6.6 Response to stressor. The score on the RAT (A), self-rated difficulty and performance of the RAT (B) and change in positive and negative affect after the RAT (C) are illustrated for the at-risk participants (FH1: purple, FH2: pink, FH3: blue), alongside the mean values for the nFH participants: sleep deprived (blue) and non-sleep deprived (red). Error bars represent the standard error of the mean.

On the impact of event scale, however FH1 reported experiencing no intrusions, though she did report experiencing a similar level of avoidance to the nFH participants, and no hyperarousal.

6.3.1.10. Sleep physiology

The sleep hypnogram for the baseline and retest night are shown in **figure 6.10**. FH1 showed a consistent profile on both nights. Overall participant FH1 showed similar sleep physiology to the nFH participants.

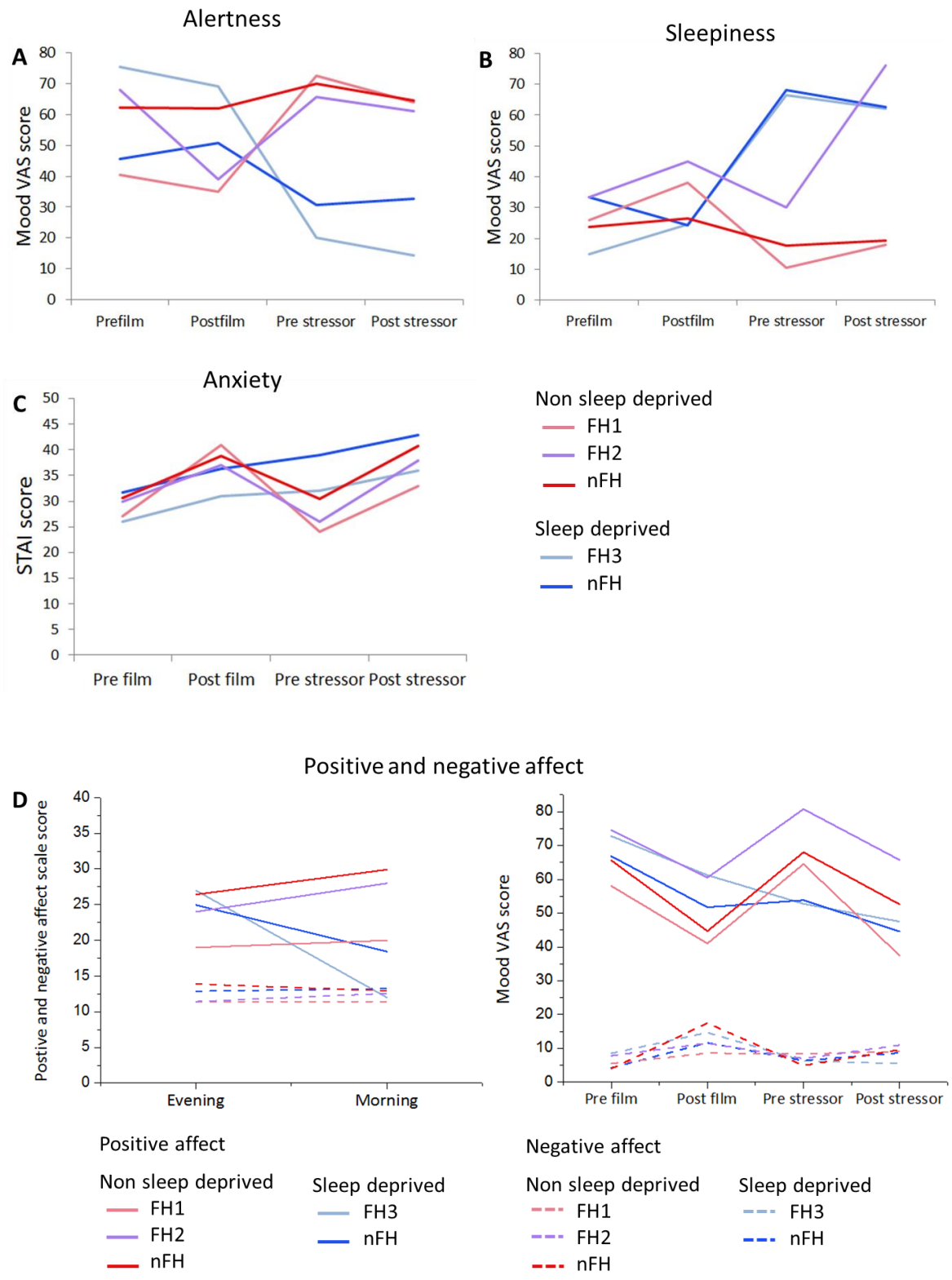


Figure 6.7 Effect of sleep deprivation on mood. The change in alertness (A), sleepiness (B), anxiety (C) and positive (solid line) and negative affect (broken line) as assessed by the mood VAS and PANAS (D) are shown for the each at-risk participant (FH1: purple, FH2: pink, FH3: blue), along with the mean values for the nFH participants: sleep deprived (blue) and non-sleep deprived (red).

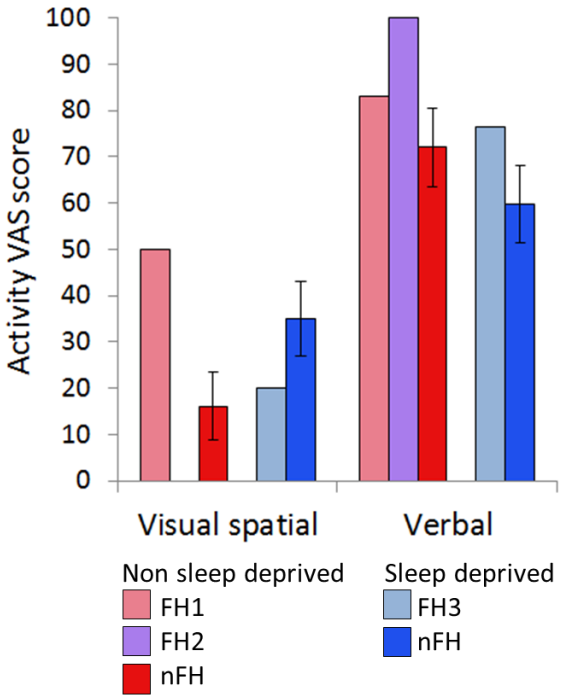


Figure 6.8 Activities. The percentage of time spent doing visual-spatial and verbal activities are shown for each at-risk participant (FH1: purple, FH2: pink, FH3: blue), as well as the nFH participants: sleep deprived (blue) and non-sleep deprived (red). Error bars represent the standard error of the mean.

In terms of sleep structure FH1 had a slightly lower total sleep time, a lower sleep latency on the retest night and no wake was observed during the sleep period for the baseline and retest night (**figure 6.11**). Slightly less stage 1, 2 and REM sleep was recorded for both nights and very little stage 4 sleep was seen for either night (**figure 6.12** and **table 6.3**). Finally in terms of REM density, FH1 showed approximately half the amount of REM density on the baseline night compared with the mean for the nFH participants, and an increase on the retest night (**figure 6.13a**). REM density increased over the course of the night (**figure 6.13b**).

6.3.2. FH2

Participant FH2 had one first degree relative with depression: a younger sister who received cognitive behavioural therapy for depression from 14 to 20 years of age.

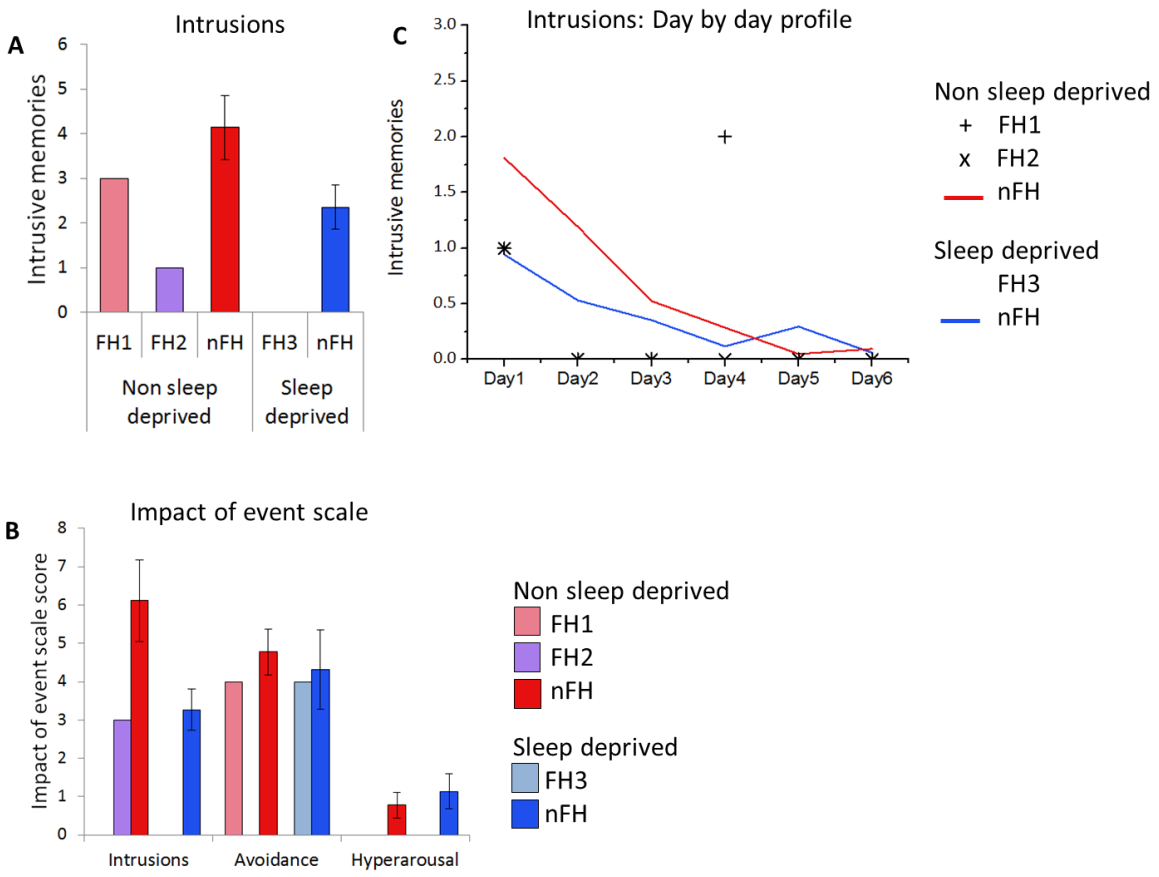


Figure 6.9 Intrusive memories. The intrusive memories reported over the week following the trauma film (A), the day by day profile of intrusion frequency (B) and the impact of event scale scores (C) for each at-risk participant (FH1: purple, FH2: pink, FH3: blue), and the mean values for the nFH participants: sleep deprived (blue) and non-sleep deprived (red). Error bars represent the standard error of the mean.There was no data avalible for FH3.

6.3.2.1. Baseline assessment

FH2 was 23 years old, and had low alcohol and caffeine consumption. Baseline assessment revealed very little difference from the average nFH participants. FH2 reported a slightly poorer sleep quality (PSQI), though at a level that was still considered good quality; no difference in diurnal mood (MEQ); a higher level of lie and psychoticism, but actually a lower level of neuroticism (EPQ); and better mood as assessed by the POMS with lower negative affect subscales (e.g. tension and depression) and higher positive affect (vigour) (**table 6.1**).

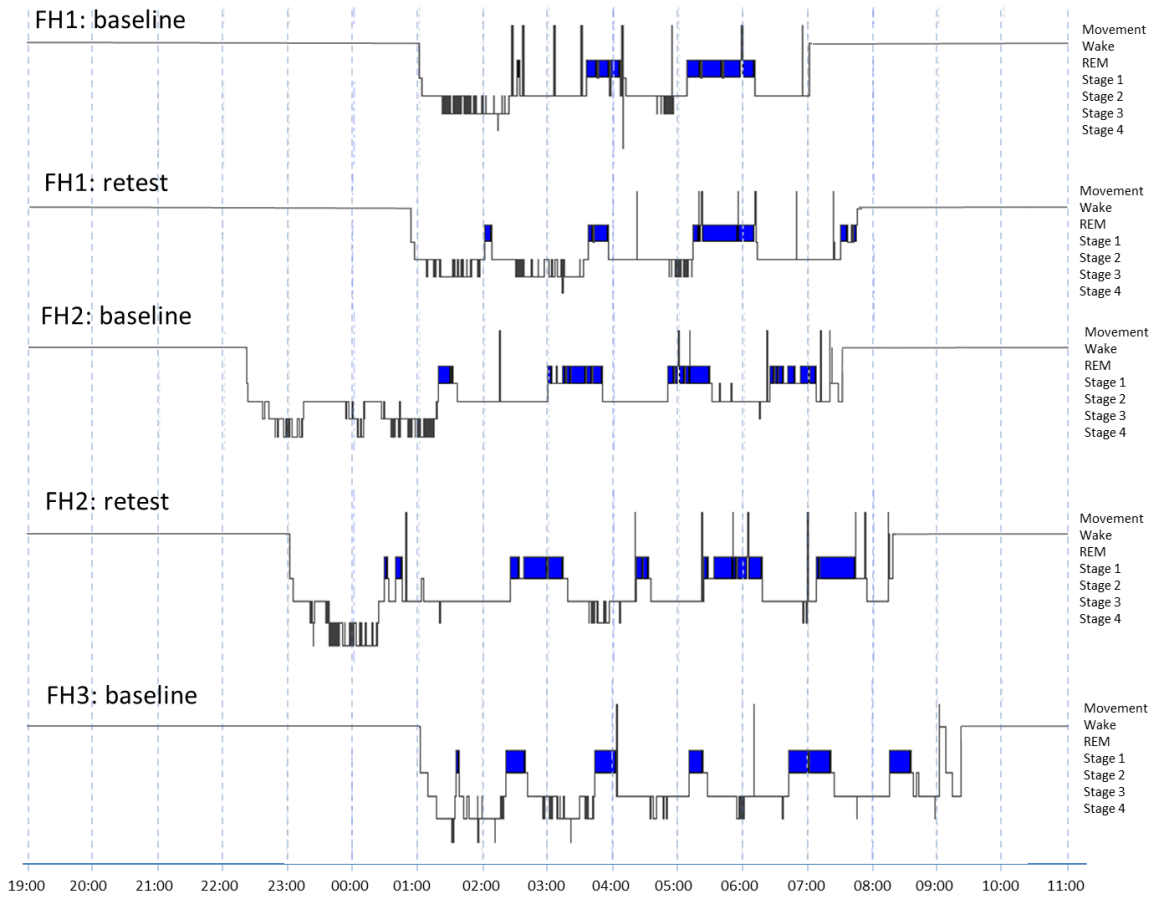


Figure 6.10 Sleep hypnograms. The hypnograms for each participant for both the baseline and retest night is presented. No hypnogram for FH3 is presented for the retest night as the PSG recording failed.

6.3.2.2. Circadian measures

Actigraphic analysis revealed that FH2 had a slightly worse sleep quality than the nFH participants and the other FH participants (**table 6.2**). Despite actually spending around an hour longer in bed, FH2 had lower sleep efficiency than the other participants. This was reflected in a higher amount of time spent awake during the sleep period, more wake bouts, a shorter mean sleep bout time, and a higher fragmentation index. Although FH2 did show a lower sleep quality compared with the other participants, with a sleep efficiency of 83% this is still considered good sleep quality. FH2 also had a slightly less stable circadian rest/activity rhythm, with lower relative amplitude, and also a delayed onset of the rest phase (L5) by 0.8 hours.

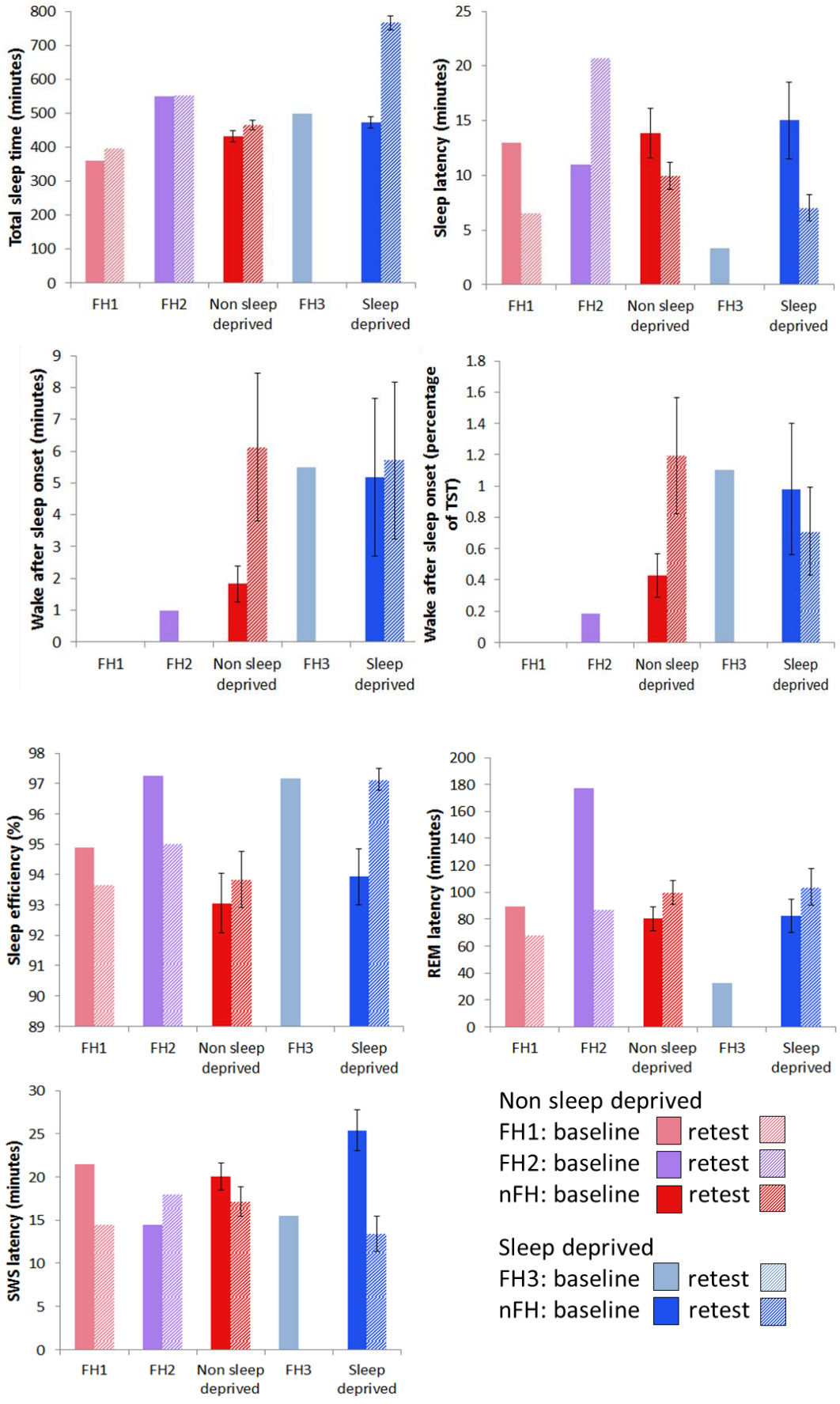

Total sleep time (minutes)
800
700
600
500
400
300
200
100
0
FH1
FH2
Non sleep deprived
FH3
Sleep deprived
Sleep latency (minutes)
25
20
15
10
5
0
Wake after sleep onset (minutes)
9
8
7
6
5
4
3
2
1
0
Wake after sleep onset (percentage of TST)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Sleep efficiency (%)
98
97
96
95
94
93
92
91
90
89
REM latency (minutes)
200
180
160
140
120
100
80
60
40
20
0
SWS latency (minutes)
30
25
20
15
10
5
0
Non sleep deprived
FH1: baseline
retest
FH2: baseline
retest
nFH: baseline
retest
Sleep deprived
FH3: baseline
retest
nFH: baseline
retest

Figure 6.11 Sleep parameters. The data for each at-risk participant (FH1: purple, FH2: pink, FH3: blue), is plotted for all the sleep parameters alongside the mean values for the nFH participants: sleep deprived (blue) and non-sleep deprived (red), for the baseline (solid colour) and retest (striped) nights. Error bars represent standard error of mean.

The amount of activity recorded during the five hours of least activity i.e. the rest phase, was also higher in participant FH2 than the mean levels for nFH participants. Since the level of activity was not higher during the activity phase (i.e. 10 hours of most activity), the attenuation in the relative amplitude was due to the higher level of activity during the rest phase. Unfortunately the delay in the activity/rest cycle could not be investigated in a more robust marker of the circadian clock: the aMT6 circadian rhythm. This was due to insufficient time points to allow the non-linear regression model to be fitted.

6.3.2.3. Diurnal mood

Although participant FH2 showed changes in mood over the two collection days these were largley within the variation seen for the nFH participants (**figure 6.2**). Only levels of self-reported sadness were found to be outside one standard deviation from the nFH mean: FH2 reported slightly higher levels throughout the day. The two moods which showed the most diurnal change were sleepiness and alertness, which was consistent with the nFH participants. Hence FH2 ranked fairly low in the distribution of mood fluctuation (**figure 6.3**).

6.3.2.4. Cortisol awaking response

The cortisol awaking response in FH2 showed a different profile between the two collection days (**figure 6.4**). On the first day, the cortisol concentration peaked at 30 minutes post waking before decreasing, while on day 2 it continued to increase at 45 minutes post waking. The concentrations of cortisol however remained well within the range of the nFH participants.

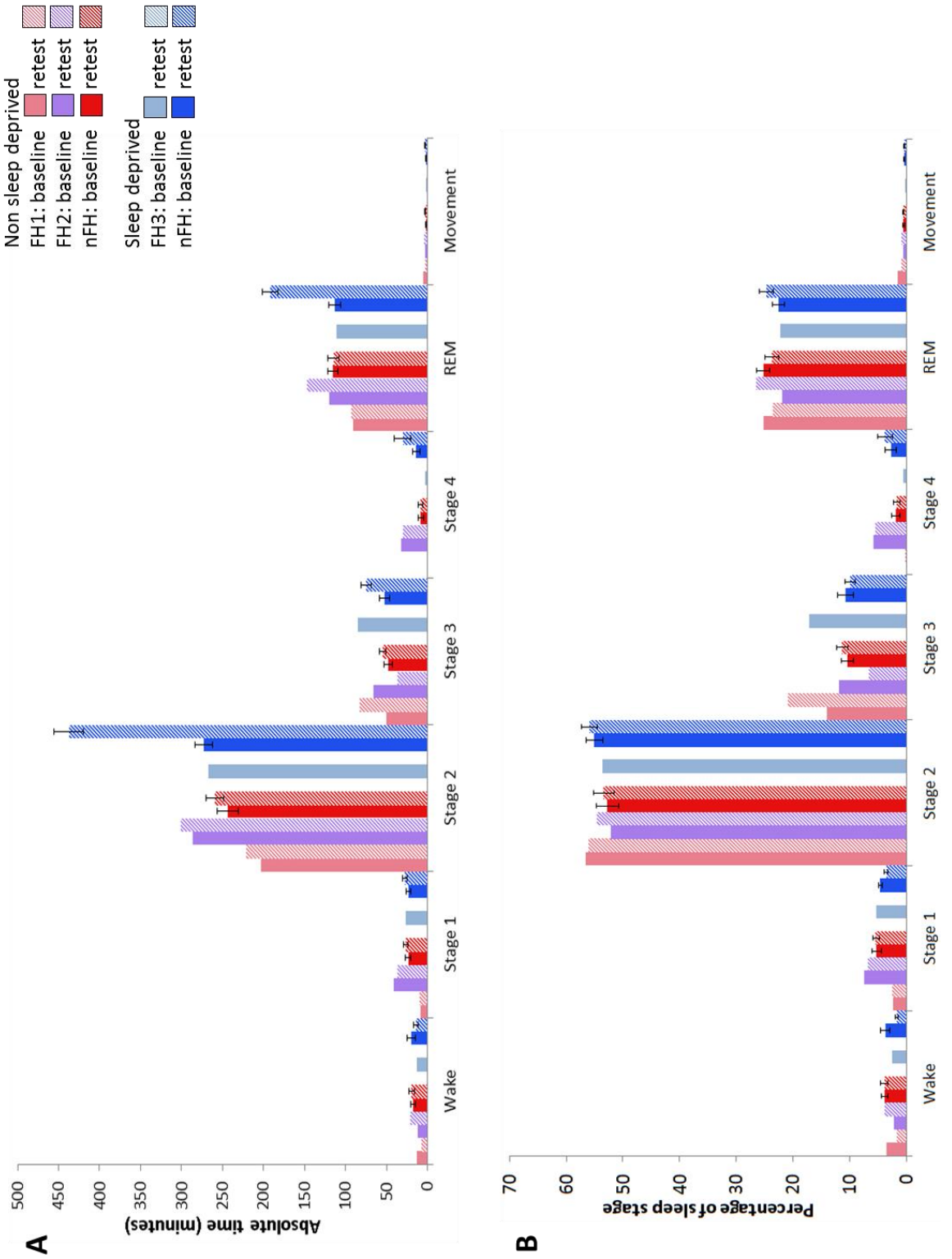


Figure 6.12. Sleep stages. The absolute (A) and percentage of the total sleep time (B) amount for each sleep stage for each at-risk participant (FH1: purple, FH2: pink, FH3: blue), is plotted alongside the mean values for the nFH participants: sleep deprived (blue) and non-sleep deprived (red), for the baseline (solid colour) and retest (striped) nights. Error bars represent standard error of mean.

		Wake		Stage 1		Stage 2		Stage 3		Stage 4		REM		Movement	
		minutes	%	minutes	%	minutes	%	minutes	%	minutes	%	minutes	%	minutes	%
FH1	Baseline	13.0	3.6	8.5	2.36	203.5	56.61	50.5	14.05	0.5	0.14	90.5	25.17	5.5	1.53
	Retest	7.0	1.8	10.0	2.53	221.7	56.02	83.0	20.98	0.1	0.02	93.0	23.50	3.5	0.88
FH2	Baseline	12.0	2.2	41.0	7.45	286.7	52.06	66.0	11.99	32.0	5.81	120.5	21.88	3.5	0.64
	Retest	21.2	3.8	37.2	6.72	301.2	54.49	37.0	6.69	30.0	5.43	146.5	26.51	4.5	0.81
nFH non-sleep deprived	Baseline	17.7	3.9	23.6	5.32	243.7	52.75	47.6	10.41	8.2	1.89	115.8	25.27	2.3	0.50
	Retest	19.4	3.9	26.6	5.42	259.1	53.35	54.9	11.36	8.6	1.72	114.7	23.69	2.9	0.57
FH3	Baseline	12.8	2.6	27.0	5.41	267.7	53.62	85.5	17.13	2.5	0.50	111.0	22.24	1.5	0.30
	Retest														
nFH sleep deprived	Baseline	20.1	3.7	23.3	4.62	273.0	55.04	52.4	10.80	13.7	2.78	113.0	22.62	1.9	0.39
	Retest	14.2	1.7	28.0	3.58	437.5	55.93	75.4	9.87	30.4	3.81	192.3	24.70	2.6	0.35

Table 6.3 Sleep stages. The absolute (minutes) and percentage (%) of each sleep stage are given for each at-risk participant alongside the mean for the nFH participants. FH 3 did not have retest PSG data due to the recording failing.

6.3.2.5. Response to trauma film

The change in mood reported by FH2 in response to the trauma film was very similar to FH1: a decrease in positive affect of a similar magnitude to the nFH participants, but a smaller increase in negative affect (**figure 6.5a**). FH2 however, showed no change in dissociation after watching the trauma film, though this may be because FH2 reported a high level of dissociation before watching the film (**figure 6.5b**). This suggests that FH2 may have a high trait level of dissociation. Furthermore FH2 reported a much higher level of relevance and familiarity with the trauma film than the mean levels for the nFH participants (**figure 6.5c**). However, despite this these scores were not outside of the range reported by the nFH participants (range = 0 to 8).

6.3.2.6. Response to stressor

Participant FH2 failed to score on the stressful task (remote associated task) (**figure6.6a**), which was consistent with nFH participants. FH2's rating of the task's difficulty and her performance, were also consistent with the nFH participants (**figure 6.6b**). In terms of mood, FH2 showed the same profile as FH1: small increase in negative affect, larger decrease in positive affect (**figure 6.6c**). Moreover these changes in mood are larger than FH1, suggesting that FH2 was more affected than FH1 to the stressor.

6.3.2.7. Change in mood over experiment

Over the course of the experiment FH2 showed a very similar change in mood to FH1: an improvement in alertness, anxiety, and positive affect following a night of sleep, and little change in negative affect (**figure 6.7a, c and d**). However, in terms of sleepiness despite reporting a decrease in sleepiness following a night of sleep and before the stressor, an increase in sleepiness was reported following the stressor (**figure 6.7b**). It is unclear what the reason for this, but it may have been due to the emotional response to the stressor.

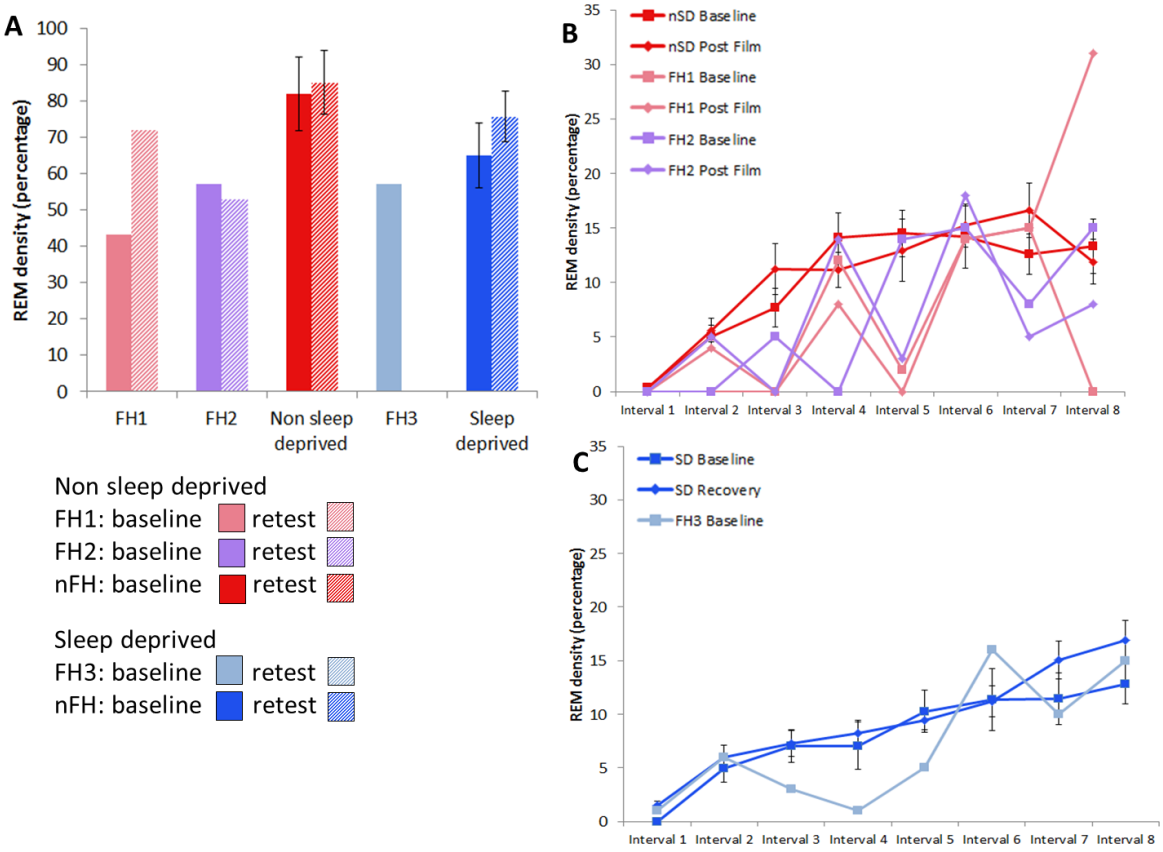


Figure 6.13. REM density. The total REM density for the night (A) and over time (B) is plotted for each at-risk participant (FH1: purple, FH2: pink, FH3: blue), is plotted alongside the mean values for the nFH participants: sleep deprived (blue) and non-sleep deprived (red), for the baseline (solid colour/ squares) and retest (striped/diamonds) nights. Error bars represent standard error of mean.

6.3.2.8. Activities

Participant FH1 reported spending all of her time after watching the trauma film doing verbal activities (**figure 6.8**).

6.3.2.9. Intrusive memories

In total FH2 recorded only one intrusive memory, which was on day one following the trauma film (**figure 6.9a and b**). This was below the mean number of intrusions reported by both the non-sleep deprived nFH participants (mean = 4.14, SD = 2.03), and the sleep deprived nFH participants

(mean = 2.35, SD = 1.06). On the impact of event scale, FH2 also reported experiencing intrusions, but no avoidance or hyperarousal (**figure 6.9c**).

6.3.2.10. Sleep physiology

The sleep hypnograms for FH2 (**figure 6.10**) show a similar profile for the baseline and retest nights. In terms of sleep structure participant FH2 had a slightly higher total sleep time, higher sleep latency on the retest night, very little wake during the sleep period on either night and a higher sleep efficiency and REM latency on the baseline night (**figure 6.11**). During the experiment an increase in sleep latency and a decrease in REM latency were seen between baseline and retest. Very little difference was seen in terms of sleep stages, other than FH2 had over 20 minutes more stage 4 sleep than the average nFH participants (**figure 6.12** and **table 6.3**). FH2 also showed lower levels of REM density on both nights compared with the mean of the nFH participants (**figure 6.13a**), though REM density was still seen to increase over the night (**figure 6.13b**).

6.3.3. FH3

Participant FH3 had one second degree relative with depression: an uncle with current depression for the past two years.

6.3.3.1. Baseline assessment

FH3 was 19 years old, and had low caffeine consumption. FH3 did have a high weekly intake of alcohol but screened negatively for alcohol abuse on the MINI. FH2 reported a good sleep quality (PSQI); a later diurnal preference (MEQ); a higher level of neuroticism and psychoticism (EPQ); and a higher level of depression (POMS) (**table 6.1**). Therefore although FH3 had a weaker familial risk for depression, behavioural risk factors were also present.

6.3.3.2. Circadian measures

From the actigraphic analysis FH3 was found to have good sleep quality, with very little deviation from the mean values for nFH participants for all the sleep and circadian parameters assessed apart from a slightly lower relative amplitude of the rest/activity cycle. This again could be attributed to a slightly higher activity level during the rest phase (five hours of least activity) (**table 6.2**). FH3 showed a slight delay in the rest onset by 0.3 hours, however this was only 5 minutes outside one standard deviation of the nFH mean for the nFH participants. Moreover the aMT6 acrophase was found to be 03:59, which is well within the average time window (**figure 6.1**). Taken together with the stable circadian parameters, this suggests that FH3 had a stable, and well entrained circadian clock albeit with a slightly lower amplitude.

6.3.3.3. Diurnal mood

The change in mood over the two day collection period reported by FH3 was very similar to FH2: fluctuation was reported for happy, calm, sad, sleepy and alert (**figure 6.2**). However the levels rarely went outside one standard deviation from the mean for the nFH participants. Despite this FH3 ranked fairly centrally within the mood fluctuation distribution (**figure 6.3**).

6.3.3.4. Cortisol awaking response

FH3 showed the same cortisol awaking response on the two collection days: slight decrease from waking to 15-30 minutes post waking before increasing at 45 minutes post waking (**figure 6.4**). The concentrations of cortisol were again very consistent with those of the nFH participants.

6.3.3.5. Response to trauma film

Although very similar to the other family history (FH) participants, FH3 showed a slightly smaller decrease in positive affect and larger increase in negative affect as a result of the trauma film (**figure 6.5a**). FH3 also showed an increase in dissociation after watching the film, like FH1 (**figure 6.5b**). In terms of recognition of the film, FH3 found it slightly more relevant than the nFH mean,

but reported the same level of familiarity (**figure 6.5c**). Nevertheless as reported previously these scores are not outside of the range reported by the nFH participants.

6.3.3.6. Response to stressor

FH3 scored the highest score ever recorded in this study on the stressful task, though two nFH participants also achieved the same score (**figure6.6a**). As a result of this, FH3 rated the task's difficulty as lower than the mean for the nFH participants and her performance better (**figure 6.6b**). This result may also explain the decrease in negative affect seen as a result of the task (**figure 6.6c**). However, the large decrease in positive affect also reported by FH1 and FH2 was seen. Therefore despite a good score on the stressful task, FH3 still responded more in terms of positive affect than the average nFH participant.

6.3.3.7. Change in mood over experiment

As a result of being sleep deprived, FH3 showed a different mood profile over the course of the experiment than FH1 and FH2, however it was consistent with the sleep deprived nFH participants. A decline was reported in alertness (**figure 6.7a**) and positive affect (**figure 6.7d**), while an increase in sleepiness (**figure 6.7b**) and anxiety (**figure 6.7c**) was seen. However yet again very little change in negative affect was seen (**figure 6.7d**).

6.3.3.8. Activities

Participant FH3 reported spending more time doing verbal activities than visual spatial, to a level that was consistent with the nFH participants (**figure 6.8**).

6.3.3.9. Intrusive memories

Participant FH3 failed to return the intrusion dairy, so there is no data to present. However, on the impact of event scale FH3 reported experiencing no intrusions, as well as similar levels of avoidance as the nFH participants and no hyperarousal (**figure 6.9c**).

6.3.3.10. Sleep physiology

Due to technical difficulties the PSG recording on the retest night for FH3, following sleep deprivation failed to record. The sleep hypnogram for the baseline night is shown in **figure 6.10**. On the baseline night FH3 showed fairly similar sleep to the nFH participants, however FH3 had a much lower sleep and REM latency, and higher sleep efficiency (**figure 6.11**). In terms of sleep stages FH3 had less stage 4 sleep (**figure 6.12** and **table 6.3**). REM density was also slightly lower for the whole night (**figure 6.13a**), but again it was found to increase over the night (**figure 6.13c**).

6.4. Discussion

The primary aim of this investigation was to extend the investigation described in Chapter 4 (p.91) to individuals with a familial risk of mood disorders. Although the three participants reported on in this chapter had a family history for depression, there was variability between the participants. Firstly the extent of the family history varied widely, with FH1 having the most extensive and FH3 having the weakest. In addition other risk factors for depression were also observed. Both FH1 and FH3 had higher levels of neuroticism than the mean levels of the nFH participants. High levels of neuroticism have been associated with an increased risk of developing a mood disorder (Clayton et al. 1994, Kendler et al. 1993). In this study the levels of neuroticism were within the range (8-12 on the EPQ) that is considered high and has been used as a criterion for elevated risk of depression in previous studies (Chan et al. 2007, Chan et al. 2009). FH1 and FH3 also reported higher levels of depression. Subclinical depressive symptoms have also been associated with an increased risk of depression (Ernst et al. 1992). FH2 by contrast, reported low neuroticism and depression scores. Mood liability, the ups and downs of mood, is a strong risk factor of depression (Angst et al. 2003). Participant FH1 showed greater changes in diurnal mood than the average for nFH participants and was one of the highest ranking participants out of all the nFH and FH participants, in terms of mood fluctuation. Hence overall participant FH1 appeared to have the most risk factors associated with mood disorders: extensive family history,

high neuroticism, depression and mood liability. Conversely participant FH2 had the fewest risk factors: family history but low neuroticism, depression and mood liability.

6.4.1. Baseline assessment of participants

6.4.1.1. Sleep disruptions

Sleep disruptions are a common feature of depression and in individuals at-risk of depression (Armitage 2007, Harvey 2008, Hudson et al. 1988, Hudson et al. 1992, Rao et al. 2009, Friess et al. 2008). Poor sleep quality was reported by FH1, however it was only participant FH2 who showed any sleep disturbances using actigraphic recordings. Moreover although the sleep patterns of FH2 were worse than the other FH and nFH participants, they were not very disrupted and a sleep efficiency of 82% is still considered reasonable sleep (Akerstedt et al. 1997). In terms of sleep physiology the at-risk participants showed little evidence for more disturbed sleep than the nFH participants. FH1 and FH3 did show slightly less stage 4 sleep which may be an indication of higher arousal and less deep sleep, but FH2 showed approximately 20 minutes more stage 4 sleep. Moreover both FH1 and 2 showed little time spent awake during the sleep period, and they all showed good sleep efficiency with FH2 and FH3 showing much higher sleep efficiency than the nFH participants. Therefore based on one night of PSG recordings the at-risk participants showed if anything, slightly better than average sleep quality.

Individuals at-risk for depression have previously been found to have a shorter REM latency and higher REM density (Rao et al. 2009, Friess et al. 2008). At baseline FH3 did show a shorter REM latency, while FH2 showed a REM latency similar to the nFH participants and FH2 had a longer REM latency. However, in terms of REM density all the at-risk participants showed a lower level of REM density at baseline than the mean level for the nFH participants. Although this is inconsistent with previous research this is only from data on three individuals and highlights the need for this investigation to be continued with more participants.

6.4.1.2. Circadian abnormalities

Some abnormalities were seen in the circadian system. Both FH1 and FH3 had a later diurnal preference score than the mean for nFH participants, however only FH3 was classified as a moderately evening type (FH1 and FH2 were intermediate types) and none of the scores were outside of the range for the nFH participants (MEQ: nFH participants range = 37-72). All the nFH participants showed a delay, of varying extents, in the timing of the rest/activity cycle, especially the timing of rest. The acrophase of the urinary melatonin metabolite (aMT6) was also delayed for FH1. Finally the relative amplitude of the rest-activity cycle was attenuated in FH2 and FH3 to an extent that is consistent with previous research in people with a behavioural risk for a mood disorder (Ankers and Jones 2009, Rock et al. Manuscript). The attenuation of the rhythm was due to higher levels of activity during rest which is again consistent with Rock and colleagues (Rock et al. Manuscript). Despite this, from the PSG recordings no differences were found in the amount of movement during sleep between the at-risk participants and those without an elevated risk.

6.4.1.3. Cortisol awakening response

The cortisol awakening response (CAR) showed an elevated concentration of cortisol for FH1, while FH2 and FH3 were well within the average range of the nFH participants. The result for FH1 is consistent with previous studies in individuals with a familial history of depression and was perhaps only seen in FH1 as this participant had the most risk factors for depression. Although the majority of research has focused on the magnitude of the CAR i.e. the concentrations of cortisol released, it is possible that the flexibility of the CAR is also important. Mikolajczak and colleagues (Mikolajczak et al. 2010) measured the CAR in men on three days, two work days compared with one weekend day: work days are assumed to provide a more stressful experience than weekends. They found participants who were happier, less stressed and less neurotic had a more flexible CAR, with higher levels of cortisol on work days than free days. Unhappy, stressed and neurotic participants however had a less flexible CAR with similar levels of cortisol on work and free days. In the investigation reported in this thesis the participants showed similar concentrations of

cortisol for both collection periods, suggesting an inflexible CAR response, which is consistent with less psychologically healthy individuals. However, the collection periods were not defined and the participants were free to choose on which days they did the collections. Furthermore no information was gathered regarding the participants schedules for those days. Since the participants in the study reported here are university students it is not possible to assume work days and weekends have different workloads and stress levels. Therefore it is not possible to conclude that the participants have an inflexible CAR response and hence more work is needed to investigate this further.

In summary the three at-risk participants in this study were found to be variable in the baseline measures of sleep and the circadian system. However, the disruptions that were observed are consistent with previous research. Overall participant FH1 presented with the most risk factors for depression, while FH2 was found to have the most disrupted sleep and circadian system. Participant FH3 lies between the two, with some additional risk factors (high neuroticism and depression) as well as slightly attenuated rest/activity cycle. Hence the participants within this study showed similar characteristics to at-risk individuals previously described.

6.4.2. Experimental manipulation

The at-risk participants underwent exactly the same experimental protocol as the nFH participants. The participants responded to the trauma film largely to the same extent as the nFH participants. However, in terms of the stressor, the cognitive task, all the at-risk participants responded with a much greater decrease in positive affect compared with the nFH participants. This suggests that the at-risk participants were more affected by the stressor, an affect that was not influenced by sleep deprivation as both the sleep deprived and non-sleep deprived participants showed the same response. This is perhaps not surprising considering the fact that the HPA axis is disrupted in depressed and at-risk individuals and the response to stressful life events is implicated in the development and maintenance of depression (Mazure 1998, Stroud et

al. 2008). Although in this study the cortisol awakening response was only elevated in one participant, this is not the only measure of the HPA axis and does not give an indication of the sensitivity to stressful situations. Analysis of cortisol levels following the stressor is needed to properly assess the situation.

In never depressed individuals, higher cortisol levels following a stressor are associated with an increased risk of developing depressive symptoms within six months (Morris et al. 2012). However, no difference in the cortisol response to a social stress test (Trier social stress test) is seen in individuals already at-risk with a behavioural risk for depression due to high levels of rumination, and passive and repetitive focus on personal distress (Young and Nolen-Hoeksema 2001). Furthermore no difference in the cortisol response was found in a group of children with a family history of bipolar disorder (and hence depression) using the same social stress test (Ellenbogen et al. 2006). This suggests that both a behavioural and familial risk for depression result in the same outcome in terms of the response to the stressor. Therefore these studies are inconsistent with the findings of this study. However it must be remembered that in this study the participants were assessed in terms of their subjective self-reported mood to the stressor, rather than an objective biological response as in the concentration of cortisol. Moreover different tasks were used as a stressor. The stressor used in this study is a cognitive task in which the participants are designed to fail, whereas the Trier social stress test involves the preparation and delivery of a public speech as well as mental arithmetic.

The at-risk participants reported fewer intrusive memories than the nFH participants on both the intrusion diary and impact of event scale. This finding is the opposite of the original hypothesis: at-risk individuals were expected to report more intrusive memories than the nFH participants, since people with depression (Kuyken and Brewin 1994) and individuals with a behavioural risk for depression (Malik et al. Manuscript) experience more intrusions. However, very little work has been conducted in this field and to date only one other study has looked at intrusive memories in

at-risk individuals. Furthermore the frequency of intrusive memories are very variable and the results in this study are not outside of the range previously reported for the nFH participants in Chapter 4 (p.91) of this thesis. To draw meaningful conclusions this study need to be repeated with a larger sample size.

Finally the effect of the experiment on sleep physiology can only be assessed for the non-sleep deprived FH participants. As reported in Chapter 5, the trauma film had very little effect on sleep physiology in the non-sleep deprived nFH participants. There was limited evidence for increased arousal as participants tended to spend more time awake after the trauma film than at baseline, but no changes to REM sleep were observed, which has previously been reported to be affected by emotionally stressful daytime events in some studies (Vandekerckhove et al. 2011, Baekeland et al. 1968, Germain et al. 2003, Koulack et al. 1985). In the at-risk participants, FH2 did show an increase in sleep latency after the trauma film, which suggests increased arousal, though FH1 showed a decrease. FH2 also showed a decreased in REM latency after the trauma film and FH1 showed an increase in REM density. Both of these changes could be indicative of increased emotional processing after the trauma film as discussed in Chapter 5 (section 5.4.3. p.165). However these findings are obviously inconclusive due to the limited study numbers.

6.5. Study limitations

Since the data presented here are only for three participants, the main limitation is the small sample size, which prevents statistical analysis and drawing any general conclusions. The same study limitations that apply to the data presented in Chapter 4 also apply to this data.

6.6. Key findings

- The individuals in this study had different risk factors for depression despite all having a family history of depression
- Subjective sleep disruptions were reported, but not seen objectively in PSG recordings

- An attenuated rest-activity rhythm was observed in two of the participants, due to higher activity levels during the rest period (a proxy of sleep). However higher activity levels were not observed in PSG recordings of sleep
- An increased cortisol awakening response was observed in the participant deemed to have the most risk factors for depression
- Higher emotional reactively to the stressor was observed for all the at-risk participants compared with the nFH participants
- Fewer intrusive memories were reported by at-risk participants compared with nFH participants
- Inconsistent effects of the trauma film on sleep physiology were observed

6.7. Conclusions

This study has demonstrated that in the three individuals studied there was considerable variability between individuals at risk for depression. Considering the variation in the risk factors experienced by the participants in this study, this highlights the need to study as homologous population as possible. Despite this all the at-risk participants showed a stronger emotional response to the stressor than the participants without a family history. However due to the low study numbers, any conclusions drawn from this data are highly tentative and therefore the study should be continued in additional at-risk individuals.

7. Concluding remarks

The aim of this thesis was to investigate sleep and sleep timing in relation to light and emotional processing. Study 1 was designed to answer the question: do environmental light levels influence sleep timing and chronotype? Study 2 addressed three aspects of the relationship between sleep and emotional processing: i) what is the relationship between sleep and intrusive memory frequency, ii) what is the effect of an analogue traumatic event on sleep physiology, and iii) is the relationship between sleep and emotional processing different in people at-risk of a mood disorder? The aim of this chapter is to determine whether these questions have been answered, to discuss the implications of these findings for society and to detail suggestions for future research.

7.1. Study 1: influence of light on sleep timing

7.1.1. Do environmental light levels influence sleep timing and chronotype?

The principal finding of study 1 was that the sleep midpoint, a marker for sleep timing and chronotype, was earlier in southern hemisphere cities (Melbourne, Perth and Auckland) when compared with northern hemisphere cities (Oxford, Munich and Groningen). The daily light irradiance also differed between the hemispheres: higher in the southern than in the northern cities. However, light intensity did not have an effect on the timing of the sleep midpoint. The variance in sleep timing was found to be influenced by the length of time spent outside, age and sex, which is consistent with previous research (Roenneberg et al. 2007a). Study 1 has also demonstrated that the sleep midpoint is more related to dawn than dusk. Although the sleep midpoint has previously been shown to track to the timing of dawn (Kantermann et al. 2007), this is the first study to assess the impact of dusk. Therefore study 1 has demonstrated that although the intensity of light did not influence sleep timing and chronotype, environmental light does affect sleep timing by not only the duration of exposure to daylight, but also that the timing of sunrise has a stronger impact on sleep timing in humans than the timing of sunset. Furthermore

this study has added to the body of knowledge that many different factors can influence sleep timing. These factors as demonstrated by the findings presented in this thesis and previous research include: light exposure, timing of dawn, age, sex, and social and work commitments (Duffy and Czeisler 2002, Dijk et al. 2000, Roenneberg et al. 2003, Randler and Diaz-Morales 2007, Roenneberg et al. 2004, Adan and Natale 2002, Roenneberg et al. 2007a, Archer et al. 2003, Toh et al. 2001, Archer et al. 2010). The implication of this is that the sleep wake cycle can become out of synchrony with the natural rhythm regulated by the body. The degree of this misalignment of the sleep-wake cycle and the circadian clock, known as social jet lag, was also investigated by this study.

7.1.2. Social jet lag

In this study social jet lag was strongly associated with shorter sleep duration on work days: more social jet lag the shorter the work day sleep duration. Although social jet lag is presumed to occur most often due to waking up earlier than preferred on work days to get to work, this is the first time this has actually been demonstrated. An average of 1.3-1.7 hours of social jet lag was reported in this study. Therefore the sleep midpoint of free days was on average 1.3-1.7 hours later than on work days. Although this is not a big discrepancy between the sleep-wake cycle (mid sleep point on work days) and circadian clock (mid sleep point on free days), social jet lag of one to two hours has been correlated with an increase in percentage of smokers (up to approximately 30%) compared with those with no social jet lag (10%) (Wittman et al 06). In terms of the global distribution of social jet lag, in this study the levels of social jet lag were found to be fairly consistent between the southern (1.4-1.7 hours) and northern (1.3-1.7 hours) hemisphere cities. Therefore this study has demonstrated that social jetlag occurs in a student population to a level that has previously been reported to be associated with poorer health.

In summary research findings from study 1 add to the knowledge that sleep timing is influenced by many factors including light exposure, age, sex, social and work commitments, but not light

intensity. These factors can cause the sleep-wake cycle to become out of synchrony with the internal body clock and that this social jet lag results in people sleeping less on work days. Our society is progressively becoming one that functions around the clock, thus the implications of living out of synchrony with our own body clocks and the resulting sleep loss on long term health must be considered.

7.1.3. Implications on society

We now live in a world that functions around the clock: productivity never stops as factories enforce shifts to cover the entire 24 hour period and consumerism is also 24/7 as a result of online shopping and supermarkets being open 24 hours a day. In England and Wales even socialising can now continue all night since the introduction of 24 hour licences for the selling of alcohol in 2005 (Antoniades and Thompson 2010). Along with this change in society, in the last 50 years the average sleep duration in adults has decreased by approximately 0.2-2 hours in developed nations (Finland: (Kronholm et al. 2008); America: (National Health Interview Survey 2005). There is some debate as to whether these changes in sleep duration are the sole consequence of our 24/7 world. Shorter sleep durations have been associated with longer working hours (Williams et al. 2012). However Bixler (Bixler 2009) argues that the change in sleep duration is more likely a combined effect of multiple factors including socioeconomic status, lifestyle (smoking, alcohol and exercise), sleep disorders, stress and age. However these factors could also be influenced by our changing society: for example a low socioeconomic status has been linked to short sleep duration (Bixler 2009), which could the due to longer working hours and more shift work being undertaken by this demographic. Therefore an integration of many factors is likely to impact on sleep duration, which are all exacerbated by the pressures of a 24/7 world.

Regardless of the reason behind the changes in sleeping patterns, the fact remains that they are changing and that the long term effects on health are becoming increasingly apparent. Shift work,

especially night shift work results in people working and sleeping at the wrong biological time as even people on permanent night shift have been found not to fully shift to their work schedule (Drake et al. 2004). Shift work has been associated with breast cancer and cardiovascular disease, and also to a lesser degree with other conditions such as diabetes and other forms of cancer (Wang et al. 2011). Social jet lag has also been associated with increased stimulant consumption, smoking and obesity (Wittmann et al. 2006, Roenneberg et al. 2012). The findings in this thesis demonstrated that more social jet lag was associated with shorter sleep durations on work days, and previously night shift workers have been reported to have a higher prevalence of insomnia or excessive sleepiness compared to day works: 32% compared with 18% (Drake et al. 2004). Therefore sleeping at the wrong time of day results in sleep loss, which has also been associated with obesity, diabetes, hypertension and cardiovascular disease (Knutson and Van Cauter 2008, King et al. 2008, Cappuccio et al. 2007, Knutson et al. 2009). Thus, sleeping at the wrong biological time whether due to shift work or social jet lag can have deleterious effects on long term health. What remains unclear is whether these effects arise from the misalignment of the sleep-wake cycle and the circadian clock or the loss of sleep.

Despite the increasing evidence for sleeping at the wrong time of day and for restricted sleep having deleterious effects on health, there is still a general consensus in society to prioritise work and social commitments over sleep. In a recent survey of over 14,000 individuals from 28 countries, 40% of respondents rated work hours as the major factor dictating their sleep schedule and 16% rated family and children as the major influence (ACNielsen 2004). Therefore not only do we need to better understand the effect of sleep loss and misalignment of the sleep-wake cycle and the circadian clock on long term health, but also and more importantly we need to raise awareness of the importance of sleep and the consequences of disregarding it. In light of this there are two areas that warrant particular attention in future research: i) the contribution of the different factors that influence sleep timing and ii) the impact of sleep at the wrong day of time compared with sleep loss on health.

7.1.3.1. Future work: do different factors have different impacts on the regulation on sleep timing?

Although many factors are known to influence sleep timing including light exposure, age and sex, the interactions between these factors is less clear. Is one factor a stronger predictor of sleep timing than others? Do some factors have an additive effect? Knowing which factor(s) are most influential on the regulation of sleep timing, would enable better targets for the stabilisation of sleep patterns to be identified. Given the importance of maintaining the synchrony of the sleep-wake cycle and the circadian clock, this could be beneficial for long term good health.

7.1.3.2. Future work: does sleeping at the wrong biological time have a different effect to just sleep loss?

Both shift work and social jet lag result in people sleeping at the wrong time of the day, and as a consequence sleeping less. Moreover, shift work, social jet lag and sleep loss have all been associated with poorer health. However, what is unclear is whether it is sleeping at the wrong time of day, sleep loss, or both that affects health. As it is not possible to address long term health in laboratory based experiments, real life situations need to be investigated. In shift workers, variability has been reported in the amount of sleep disturbance experienced (Axelsson et al. 2004). Therefore it might be possible to follow shift workers who show high or low levels of sleep disturbance and assess their long term health. Answering this question could help determine targets for the prevention of the deleterious effects of shift work and social jet lag.

7.2. Sleep and emotional processing

7.2.1. <u>What is the relationship between sleep and intrusive memory frequency?</u>

Sleep and intrusive memories were investigated in terms of the effect of a period of sleep or no sleep following an analogue traumatic event, a trauma film. A period of sleep deprivation following the trauma film resulted in healthy students experiencing fewer intrusive memories than those who had slept. The amount of time the participants spent doing visual-spatial activities was also found to influence the frequency of intrusive memories, but not over the effect

of sleep or sleep deprivation. Participants who had experienced at least one symptom of mania reported more intrusive memories than those who have not experienced any. Furthermore, sleep deprived participants who show an increase in dissociation following the trauma film reported fewer intrusions than non-sleep deprived participants who did not show a change in dissociation. In this study the participants were also subjected to a stressful situation, a cognitive task. The sleep deprived participants were found to respond less to the stressor than the non-sleep deprived. In summary the frequency of intrusive memories was found to be influenced by sleep, visual-spatial activities, manic symptomology and dissociation. However the principal finding was that a period of sleep deprivation appeared to be beneficial not only on the frequency of intrusive memories, but also on the response to a stressor.

7.2.2. What is the effect of an analogue traumatic event on sleep physiology?

The effect of the analogue traumatic event on sleep physiology was found to be most pronounced in recovery sleep following sleep deprivation. Whilst the expected rebound effects of sleep deprivation were observed: increased total sleep time, slow wave sleep and decreased stage 1 sleep, additional changes in sleep physiology were also recorded: higher than expected levels of REM density and increased brain activity in the occipital region. These findings could possibly reflect an increase in emotional processing following the analogue traumatic event. Aspects of sleep physiology were associated with intrusive memories, suggesting that a higher frequency of intrusive memories could be as a result of increased emotional processing, hyperarousal or increased visual-spatial processing during sleep. Therefore these findings suggest that sleep physiology is altered following trauma, although more so following sleep deprivation, and that these changes may be associated with increased emotional processing.

7.2.3. Is the relationship between sleep and emotional processing different in people at-risk for mood disorder?

Subjective sleep disruptions and attenuated rest-activity rhythms were observed in some but not all of the three at-risk participants. All of the at-risk participants were found to have higher emotional reactivity in response to the stressor (cognitive task) compared with the participants without a family history: larger decrease in positive affect following the task. Therefore emotional processing does appear to be different in at-risk individuals. However the low sample size for this investigation meant that no definitive conclusions can be drawn from this data.

7.2.4. Implications of sleep following trauma

Despite the deleterious effects of sleep loss and sleeping at the wrong time of day, there are some situations where not sleeping may actually be beneficial. As discussed in Chapter 4 (p.91) a period of sleep deprivation appears to be beneficial in reducing the number of intrusive memories to an analogue traumatic event (study 2) and extinguishing fear responses (Kuriyama et al. 2010). Moreover, sedation following trauma has been associated with an increased risk for the development of post-traumatic stress disorder (PTSD) (Gelpin et al. 1996, Matar et al. 2009, Mellman et al. 2002). The mechanisms for these actions are not yet fully understood. The effect of sleep deprivation on intrusive memory frequency could be due to the disruption of memory consolidation or visual-spatial and verbal information processing (for details see Chapter 4 section 4.4.2. p.116). Increased hyperarousal has also been implicated as a possible mechanism (for details see Chapter 5 section 5.4.4.2. p.170). Kuriyama and colleagues (Kuriyama et al. 2010) implicated a lack of memory consolidation as the mechanism underling the extinction of fear responses by sleep deprivation. However in their study Kuriyama and colleagues (Kuriyama et al. 2010) demonstrated that sleep deprivation affected the implicit memory (focused on emotional aspects) of the event but not the explicit memory (focused on facts and details). In studies not involving sleep deprivation explicit memory has also previously not been found to influence intrusive memory frequency (Holmes et al. 2004, Nixon et al. 2007). Thus it is possible that it is

the impact on implicit memory that underlies the effect of sleep deprivation on intrusive memories. However this mechanism required further investigation to be fully elucidated.

7.2.4.1. Future work: what is the mechanism underlying the beneficial effect of sleep deprivation?

To fully determine the mechanism underlying the effect of sleep deprivation following trauma, the three theories postulated need to be investigated: i) memory consolidation, ii) information processing iii) hyperarousal.

Memory consolidation: To investigate memory consolidation the effect of the implicit and explicit memory of an analogue traumatic event following sleep deprivation should be determined. Furthermore it must be established whether either of these memory types influence the frequency of intrusive memory following sleep deprivation.

Information processing: The effect of information processing on intrusion frequency in conjunction with sleep deprivation could be determined by preventing either visual-spatial or verbal activities during the sleep deprivation period following the trauma film. Moreover to determine whether sleep deprivation preferentially affects either type of information processing (visual-spatial or verbal) the performance of sleep deprived participants could be assessed on tasks testing each type of information processing separately.

Hyperarousal: To assess whether hyperarousal affects the frequency of intrusive memories, the level of arousal of the participants during and after the trauma film could be assessed and correlated to the number of intrusive memories experienced. This could also be extended to assess arousal levels during sleep deprivation and before and after sleep for the non-sleep deprived participants to assess the impact of sleep and sleep deprivation on arousal.

In addition it needs to be definitely established that the fewer intrusive memories reported by the sleep deprived participants was due to the lack of sleep rather than the length of time spent awake following the trauma film and subsequent sleep. To investigate this, the same experiment

could be conducted with the trauma film being shown in the morning and the evening to assess not only time of day effects, but also if the amount of time between watching the film and going to sleep has an effect.

7.2.5. Implications of sedation following trauma

Despite clinical evidence for increased incidence of PTSD following sedation after trauma, there are currently no clinical guidelines for the treatment of the non-physical aspects of trauma. Evidence based guidelines for the management of PTSD however, advise against the use of benzodiazepines (sedatives) in acutely traumatised people for the prevention of acute stress disorder (ASD) and posttraumatic stress disorder (PTSD) (National Collaborating Centre for Mental Health 2005, The Management of Post-Traumatic Stress Working Group 2010). The prescription of benzodiazepines has decreased in U.S. war veterans since 1999, however they were still the second most common drug prescribed for PTSD in 2009 (Lund et al. 2012). This reluctance to stop prescribing sedatives following trauma, against the advice of clinical guidelines suggests that more work is need to support the recommendation that sedation is not beneficial following trauma.

7.2.5.1. Future work: what is the effect of sedation on the frequency if intrusive memories?

To date two studies in humans (Gelpin et al. 1996, Mellman et al. 2002) and one in animals (Matar et al. 2009) have suggested that sedation following trauma increases the incidence of PTSD. However the studies in humans include only a small sample of individuals (26 and 22) therefore larger studies need to be conducted to support these findings. In addition experimental investigations could be conducted to investigate the effect of sedation on PTSD symptomology including intrusive memories. The sedation of health individuals following an analogue traumatic event would enable the investigation into the frequency of intrusive memories and other symptoms of PTSD such as hyperarousal. The experimental investigation of sedation following trauma would also allow the underlying mechanism to be explored.

7.2.6. Implications for real life trauma

As discussed in Chapter 4, from an evolutionary perspective it could be postulated that following an emotional or traumatic event sleep deprivation is beneficial. What remains unknown is whether or not sleeping following a trauma is a natural mechanism to reduce the negative effects of trauma. Sleep disturbances are integral to a diagnosis of PTSD (American Psychological Association 2000) and acute stress disorder (Bryant and Harvey 1997). Acute stress disorder (ACD) is an acute form of PTSD that occurs between 2 days and 4 weeks following a trauma, whereas PSTD is only diagnosed once symptoms have been experienced for at least one month (American Psychological Association 2000). People with ACD experience many of the symptoms of those with PTSD: re-experiencing of the trauma (intrusive memories); avoidance and hyperarousal. However, there is also an emphasis on dissociative symptoms: feelings of detachment or amnesia of the trauma (Bryant and Harvey 1997). Insomnia has been reported in 44-68% of individuals with ACD after a variety of different traumatic events including plane crash, ambush and earthquake (Bryant and Harvey 1997, Cardeña and Spiegel 1993), suggesting that sleep is prevented following trauma. Greater sleep disturbances, have however been reported to be a predictor for the development of PTSD in motor vehicle accident survivors (Koren et al. 2002), suggesting that the lack of sleep following trauma is a causal factor for PTSD rather than preventative. However all these studies report chronic sleep disruption symptoms post-trauma rather than immediate acute sleep deprivation following the trauma. Therefore studies into the sleeping patterns following trauma need to be conducted.

7.2.6.1. Future work: what happens in real life following trauma?

Subjecting people to real life trauma for research is unethical, however, observations could be made in cases of real life trauma. For example victims of trauma could be observed in terms of their sleep habits immediately after the trauma, to determine if some people naturally sleep deprive themselves following trauma and if so whether these individuals go on to experience fewer consequences of the trauma than those that sleep.

7.3. Conclusion

Overall this thesis has added to the knowledge that sleep timing is influenced by many factors including light exposure, age, sex, social and work commitments, and that the effect of these factors can result in social jet lag, sleep loss and deleterious effects on health. Furthermore, it has added to the increasing body of evidence that sleep deprivation in certain situations, such as following trauma, may be beneficial for the emotional processing of that event. These findings suggest that in different situations different functioning of the sleep-wake cycle may be beneficial, such as alignment of the sleep wake cycle with the circadian clock for everyday functioning, and a period of extended wakefulness following trauma.

8. References

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9. Appendices

9.1. Appendix I: data used to produce graphical representations of the results as presented in chapters 3, 4, 5 and 6

9.1.1. Chapter 3

Figure 3.1 Geographical coordinates of participating cities

	Latitude	Longitude
Oxford	51.75° N	1.26° W
Groningen	53.22° N	6.57° E
Munich	48.13° N	11.57° E
Perth	31.96° S	115.86° E
Melbourne	37.78° S	144.97° E
Auckland	36.85° S	174.78° E

Figure 3.2 Mean and standard deviation (SD) of MSFsc relative to local clock time for each city and season

	MSFsc			
	Spring		Autumn	
	Mean	*SD*	Mean	*SD*
Oxford	5.30	*1.30*	5.22	*1.23*
Groningen	5.23	*1.25*	5.30	*1.27*
Munich	5.30	*1.23*	5.22	*1.28*
Perth	4.67	*1.42*	4.93	*1.72*
Melbourne	4.75	*1.50*	4.89	*1.40*
Auckland	4.53	*1.50*	4.54	*1.29*

Figure 3.3 Mean and standard error (SE) of MSFsc for each age for total population, northern and southern hemispheres.

		MSFsc					
		Worldwide		Northern		Southern	
		Mean	*SE*	Mean	*SE*	Mean	*SE*
Age (years)	17	4.74	*.22*	5.18	*.31*	4.16	*.23*
	18	4.94	*.06*	5.30	*.08*	4.68	*.08*
	19	5.18	*.05*	5.46	*.06*	4.66	*.09*
	20	5.18	*.04*	5.26	*.05*	4.83	*.11*
	21	5.27	*.04*	5.34	*.04*	4.87	*.12*
	22	5.07	*.04*	5.10	*.04*	4.80	*.15*
	23	5.03	*.04*	5.08	*.04*	4.39	*.14*
	24	5.04	*.05*	5.07	*.05*	4.46	*.21*
	25	5.05	*.06*	5.11	*.06*	4.13	*.18*
	26	4.96	*.08*	4.97	*.08*	4.55	*.48*

Figure 3.4 Mean and standard error (SE) of MSFsc for men and women for each city

	MSFsc			
	Women		Men	
	Mean	*SE*	Mean	*SE*
Oxford	5.19	*.11*	5.35	*.10*
Groningen	5.02	*.03*	5.72	*.04*
Munich	4.88	*.03*	5.60	*.06*
Perth	4.43	*.09*	5.10	*.14*
Melbourne	4.76	*.08*	5.20	*.17*
Auckland	4.39	*.07*	4.87	*.11*

Figure 3.5 Mean and standard error (SE) light exposure and light dose for each city and season

	Light exposure				Light Dose			
	Spring		Autumn		Spring		Autumn	
	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
Oxford	2.01	*.10*	1.90	*.08*	27.29	*1.41*	12.70	*.54*
Groningen	2.25	*.03*	2.11	*.04*	32.62	*.47*	14.00	*.24*
Munich	2.30	*.04*	2.15	*.04*	25.13	*.43*	16.24	*.32*
Perth	2.80	*.10*	2.48	*.25*	66.44	*2.32*	38.48	*3.86*
Melbourne	1.95	*.20*	1.88	*.09*	36.15	*3.66*	24.95	*1.13*
Auckland	2.10	*.13*	2.20	*.08*	36.26	*2.25*	19.86	*.71*

Figure 3.6 Timings of sunrise and sunset for each city and season

	Spring		Autumn	
	Sunrise	Sunset	Sunrise	Sunset
Oxford	5.21	-3.15	7.47	-5.78
Groningen	5.34	-2.29	7.79	-5.09
Munich	5.67	-3.32	7.26	-5.19
Perth	5.72	-5.58	7.00	-6.56
Melbourne	5.78	-4.60	7.29	-6.74
Auckland	5.92	-4.57	7.17	-6.59

Figure 3.7 Mean MSFsc for each 30 minute bin of light exposure for northern and southern hemisphere

Light exposure	MSFsc	
(30 minute bins)	Northern	Southern
0.5	5.19	4.39
1	5.19	4.81
1.5	5.13	4.75
2	5.17	4.57
2.5	5.15	4.65
3	5.25	4.52
3.5	5.16	4.74
4	5.42	4.77
4.5	5.20	4.86
5	5.14	5.35
5.5	5.19	5.02
6	5.35	5.57
6.5	5.84	4.26
7	5.29	4.57
7.5	5.80	4.00
8	5.11	4.73
8.5	5.56	4.01
9	5.47	4.95
9.5	5.76	3.14
10	4.29	4.00
10.5	4.10	6.30
11	2.96	3.55
11.5	5.64	
12	3.61	

Figure 3.8 Mean and standard deviation (SD) MSFsc relative to sunrise and sunset for each city and season

	MSFsc_SR				MSFsc_SS			
	Spring		Autumn		Spring		Autumn	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
Oxford	.10	*1.28*	-2.24	*1.26*	8.45	*1.32*	11.01	*1.22*
Groningen	-.11	*1.25*	-2.49	*1.27*	7.52	*1.25*	10.39	*1.27*
Munich	-.54	*1.23*	-2.25	*1.28*	8.45	*1.23*	10.19	*1.29*
Perth	-1.05	*1.42*	-2.07	*1.72*	10.25	*1.42*	11.49	*1.72*
Melbourne	-1.03	*1.49*	-2.40	*1.40*	9.35	*1.60*	11.63	*1.40*
Auckland	-1.39	*1.51*	-2.63	*1.29*	9.10	*1.50*	11.13	*1.30*

Figure 3.8 Percentage change of MSFsc_SR and MSFsc_SS from spring to autumn for each city

	MSFsc_SR	MSFsc_SS
Oxford	104.25	23.24
Groningen	95.45	27.63
Munich	75.97	17.07
Perth	49.32	10.78
Melbourne	56.86	19.59
Auckland	47.08	18.23

Figure 3.9 Mean and standard error (SE) social jet lag for each city and season

	Social jet lag			
	Spring		Autumn	
	Mean	*SE*	Mean	*SE*
Oxford	1.42	*.08*	1.46	*.06*
Groningen	1.34	*.02*	1.45	*.02*
Munich	1.66	*.03*	1.67	*.03*
Perth	1.41	*.05*	1.73	*.16*
Melbourne	1.63	*.17*	1.65	*.05*
Auckland	1.64	*.09*	1.62	*.05*

Figure 3.10a The mean sleep duration on work days for 30 minutes bins of social jet lag for northern and southern hemisphere

Social jet lag	Sleep duration on work days	
	Northern	Southern
0.5	7.693	7.576
1	7.670	7.694
1.5	7.604	7.434
2	7.422	7.245
2.5	7.158	7.017
3	7.005	6.485
3.5	6.989	6.194
4	6.975	6.425
4.5	6.770	
5	6.732	

Figure 3.10b The mean and standard error (SE) of social jet lag for each age for the whole population

	Social jet lag	
Age	Mean	*SE*
17	1.60	*.14*
18	1.63	*.04*
19	1.62	*.03*
20	1.52	*.03*
21	1.54	*.03*
22	1.53	*.03*
23	1.39	*.03*
24	1.42	*.03*
25	1.45	*.04*
26	1.46	*.06*

Figure 3.10c The mean and standard error (SE) of social jet lag for men and women for the whole population

	Social jet lag	
	Mean	*SE*
Women	1.45	*.01*
Men	1.64	*.02*

9.1.2. Chapter 4

Figure 4.1 aMT6s acrophase for each participant and control data provided by Benita Middleton

Study data		Control data	
Participant ID	Acrophase	Participant ID	Acrophase
SD71	22:50	1	3.2
SD43	00:59	2	4.8
SD19	02:07	3	4.8
SD30	02:09	4	6.5
SD78	02:23	5	4.4
SD13	02:24	6	0.9
SD53	02:28	7	2.9
SD11	02:30	8	3.2
SD32	03:17	9	0.7
SD60	04:12	10	2.4
SD48	04:13	11	3.2
SD63	04:16	12	1.6
SD22	04:25	13	4.1
SD65	04:31	14	3.1
SD05	04:31	15	3.3
SD31	04:44	16	4.2
SD77	04:46	17	3.3
SD68	04:58	18	3.4
SD10	04:59	19	6.2
SD81	05:11	20	3.6
SD07	05:24		
SD80	05:36		
SD76	05:46		
SD34	05:47		
SD04	05:58		
SD56	06:09		
SD06	06:10		
SD15	07:04		
SD64	07:05		
SD47	07:18		
SD41	08:20		
SD67	09:56		
SD21	10:32		
SD42	10:34		
SD54	11:48		
SD27	15:52		

Figure 4.2 Diurnal mood: mean and standard deviation (SD) of each mood at each time point.

	Day 1								Day 2							
	8am to 10am		12pm to 2pm		4pm to 6pm		8pm to 10pm		8am to 10am		12pm tp 2pm		4pm to 6pm			
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*		
Sad	11.24	*14.82*	10.78	*12.74*	10.53	*11.79*	9.69	*11.55*	6.90	*8.11*	7.32	*8.46*	7.13	*6.62*		
Calm	61.92	*25.68*	63.68	*22.82*	66.07	*19.89*	69.15	*19.63*	65.30	*22.11*	64.83	*24.04*	64.55	*23.22*		
Horrified	4.76	*9.09*	4.13	*7.78*	4.00	*7.65*	3.77	*5.53*	4.82	*8.86*	3.37	*5.54*	2.26	*3.00*		
Happy	55.63	*19.72*	57.65	*19.99*	59.07	*22.12*	58.62	*23.02*	57.01	*22.71*	58.16	*22.93*	58.11	*21.51*		
Fearful	5.46	*11.17*	6.37	*9.91*	5.13	*10.53*	5.54	*8.53*	3.85	*5.93*	4.22	*8.24*	4.02	*5.38*		
Hopeless	7.85	*13.30*	6.84	*11.53*	7.74	*12.68*	7.38	*9.84*	4.42	*5.79*	4.74	*6.41*	3.74	*4.53*		
Angry	6.01	*11.73*	5.42	*9.13*	7.71	*11.81*	6.49	*9.05*	5.30	*6.86*	5.23	*6.35*	4.23	*5.12*		
Sleepy	29.24	*22.87*	23.80	*20.28*	27.92	*22.64*	40.91	*25.67*	31.25	*22.91*	20.03	*14.51*	25.34	*20.46*		
Awkward	7.60	*14.41*	8.16	*13.26*	8.84	*13.12*	7.19	*11.69*	7.15	*9.84*	6.65	*8.62*	5.32	*6.60*		
Tense	13.96	*18.83*	11.10	*13.78*	13.03	*17.29*	9.97	*14.15*	9.54	*12.81*	8.76	*11.12*	8.14	*9.96*		
Restless	13.18	*15.37*	12.00	*14.64*	12.80	*14.21*	13.52	*14.97*	11.72	*16.42*	10.19	*14.28*	11.42	*13.91*		
Irritable	11.15	*17.64*	10.64	*16.42*	12.58	*17.09*	9.22	*12.54*	8.42	*12.00*	6.58	*9.09*	7.80	*8.98*		
Racing thoughts	10.76	*14.70*	11.98	*14.87*	11.40	*12.73*	11.72	*14.46*	9.22	*11.41*	10.12	*12.24*	8.45	*11.11*		
Anxious	10.23	*15.11*	12.38	*13.32*	10.39	*13.40*	11.69	*16.88*	7.40	*8.42*	6.41	*9.65*	8.11	*8.15*		
Alert	55.93	*30.21*	59.88	*29.88*	54.12	*28.76*	55.93	*31.59*	54.85	*26.64*	62.77	*28.85*	57.99	*28.23*		

Figure 4.3 Mood fluctuation: the mean standard deviation of each mood for each participant

Participant ID	Average standard deviation of diurnal mood
SD27	1.25
SD38	2.11
SD47	2.79
SD29	3.88
SD32	3.99
SD34	4.45
SD67	4.67
SD42	4.77
SD50	5.01
SD63	5.12
SD76	5.18
SD43	5.53
SD30	5.65
SD60	5.86
SD06	6.11
SD65	6.16
SD53	6.97
SD81	7.23
SD31	7.25
SD19	7.31
SD33	7.50
SD64	7.54
SD10	7.56
SD11	7.80
SD21	8.03
SD54	8.57
SD22	8.68
SD04	9.10
SD56	9.12
SD16	9.12
SD48	9.33
SD13	9.62
SD15	9.99
SD05	10.05
SD07	10.32
SD77	11.87
SD80	12.05
SD68	12.08
SD41	13.48
SD78	13.98
SD71	14.07

Figure 4.4 Cortisol awakening response: the average statistical parameters of the concentration of cortisol for each time point for the sleep deprived and non-sleep deprived groups

		Day 1				Day 2			
		0	15	30	45	0	15	30	45
Sleep deprived	Mean	1.03	1.13	15.27	16.20	3.22	13.11	16.04	16.58
	25th percentile	.93	1.02	13.60	13.20	2.51	8.40	11.80	13.30
	50th percentile	.99	1.11	15.10	15.60	3.15	13.50	14.60	17.60
	75th percentile	1.16	1.14	16.40	17.70	3.54	17.60	17.70	19.90
	Standard Error of Mean	.06	.04	1.80	1.27	.24	1.69	1.66	1.28
	Standard Deviation	.24	.17	7.43	5.24	.98	6.96	6.86	5.28
Non sleep deprived	Mean	1.00	1.04	17.32	19.43	3.30	16.17	19.67	17.81
	25th percentile	.83	.86	12.80	14.50	2.76	11.70	12.10	12.70
	50th percentile	1.04	1.09	16.25	17.85	3.41	13.95	17.30	17.80
	75th percentile	1.30	1.25	20.40	21.10	3.69	17.80	21.60	21.10
	of Mean	.07	.08	1.68	1.74	.18	1.55	2.53	1.71
	Deviation	.33	.36	7.87	8.16	.83	7.25	11.87	8.04

Figure 4.5 Response to trauma film: the mean and standard error (SE) of the scales used to assess the response to the trauma film for the sleep deprived and non-sleep deprived groups

		Sleep deprived		Non- sleep deprived	
		Mean	*SE*	Mean	*SE*
Mood VAS	Postive affect	-15.07	*3.09*	-20.92	*3.37*
	Negative affect	8.70	*1.92*	15.87	*2.61*
Dissociaitive	Pre Film	1.26	*.37*	2.23	*.95*
state scale	Post Film	3.79	*1.26*	4.59	*1.12*
Film follow up	Relevance	2.95	*.60*	2.95	*.56*
	Familiarity	3.26	*.53*	3.09	*.57*

Figure 4.6 Response to stressor: the mean and standard error (SE) of the scales used to assess the response to the stressor for the sleep deprived and non-sleep deprived groups

		Sleep deprived		Non- sleep deprived	
		Mean	*SE*	Mean	*SE*
Score		1.16	*.23*	.45	*.13*
Mood VAS	Negative affect	2.70	*.75*	5.46	*1.92*
	Postive affect	-9.21	*3.11*	-15.35	*2.82*
RAT follow up	Difficulty	7.47	*.35*	7.59	*.38*
	Performance	1.95	*.27*	1.18	*.08*

Figure 4.7 Effect of sleep deprivation on mood: the mean and standard error (SE) of the scales used to assess the effect of the experiment on mood for the sleep deprived and non-sleep deprived groups

			Sleep deprived		Non- sleep deprived	
			Mean	*SE*	Mean	*SE*
Mood VAS	Alertness	Pre film	45.47	*5.79*	62.32	*5.28*
		Post film	50.82	*5.83*	61.95	*5.51*
		Pre stressor	30.71	*5.12*	70.05	*5.78*
		Post stressor	32.76	*4.23*	64.39	*5.51*
	Sleepiness	Pre film	33.31	*5.78*	23.89	*4.03*
		Post film	24.34	*4.27*	26.48	*4.19*
		Pre stressor	68.24	*4.72*	17.77	*3.55*
		Post stressor	62.50	*5.07*	19.30	*4.37*
	Positive affect	Pre film	66.82	*4.08*	65.60	*3.49*
		Post film	51.75	*4.19*	44.68	*3.52*
		Pre stressor	53.83	*4.43*	67.99	*4.08*
		Post stressor	44.62	*4.55*	52.64	*4.69*
	Negative affect	Pre film	5.03	*1.78*	4.72	*.94*
		Post film	13.73	*2.50*	20.59	*2.58*
		Pre stressor	7.50	*2.44*	5.82	*1.41*
		Post stressor	10.20	*2.39*	11.28	*2.50*
State trait anxiety index	Anxiety	Pre film	31.71	*1.63*	30.57	*1.14*
		Post film	36.32	*1.45*	38.73	*1.77*
		Pre stressor	39.05	*1.77*	30.50	*1.25*
		Post stressor	42.89	*1.60*	40.77	*2.01*
Positive and negative affect scale	Positive affect	PM	24.95	*1.94*	26.43	*1.31*
		AM	18.42	*1.30*	29.91	*1.30*
	Negative affect	PM	11.26	*.35*	12.14	*.54*
		AM	11.58	*.44*	11.32	*.44*

Figure 4.8 Activities: the mean and standard error (SE) for the amount of time spent doing visual-spatial and verbal activities by the sleep deprived and non-sleep deprived groups

		Sleep deprived		Non- sleep deprived	
		Mean	*SE*	Mean	*SE*
Activities VAS	Visual-spatial	35.000	*8.16*	16.03	*7.35*
	Verbal	59.750	*8.40*	72.00	*8.37*

Figure 4.9 Intrusive memories: the mean and standard error (SE) for both measures of intrusive memoires for the sleep deprived and non-sleep deprived groups

		Sleep deprived		Non- sleep deprived	
		Mean	*SE*	Mean	*SE*
Intrusions	Total	2.35	*.49*	4.14	*.72*
	Day1	.941	*.375*	1.810	*.338*
	Day2	.529	*.254*	1.190	*.229*
	Day3	.353	*.167*	.524	*.150*
	Day4	.118	*.115*	.286	*.103*
	Day5	.294	*.131*	.048	*.118*
	Day6	.059	*.067*	.095	*.060*
Impact of event	Intrusions	3.200	*1.076*	6.472	*.982*
scale	Avoidance	3.800	*.841*	4.444	*.768*
	Hyperarousal	1.133	*.412*	.778	*.376*

Figure 4.10 Predictors of intrusion frequency: the mean and standard error (SE) of intrusion frequency for the additional grouping factors and sleep deprived and non-sleep deprived groups were applicable

		Truama		Emotional response to film		MDQ	DSS and sleep deprivation			
		No trauma	Trauma	Low responders	High responde	no MDQ	MDQ	Sleep deprived		Non sleep deprived	
								Low DSS	High DSS	Low DSS	High DSS
Intrusions	Mean	3.30	2.88	2.80	3.67	2.18	4.05	3.57	1.40	2.33	5.17
	SE	*.61*	*1.16*	*.63*	*.89*	*.54*	*.82*	*1.46*	*.50*	*.85*	*1.05*

9.1.3. Chapter 5

Figure 5.2 Sleep parameters: the mean and standard error (SE) for each sleep parameter on the baseline and retest nights for the sleep deprived and non-sleep deprived groups

		Non sleep deprived		Sleep deprived	
		Mean	*SE*	Mean	*SE*
Total sleep time	Baseline	431.62	*16.07*	473.42	*17.22*
	Retest	465.06	*14.95*	765.66	*19.81*
Sleep latency	Baseline	13.87	*2.29*	15.04	*3.51*
	Retest	9.97	*1.22*	7.03	*1.21*
Wake after sleep onset	Baseline	1.83	*.57*	5.18	*2.47*
(absolute time)	Retest	6.12	*2.32*	5.71	*2.45*
Wake after sleep onset	Baseline	.43	*.14*	.98	*.42*
(percentage of TST)	Retest	1.19	*.37*	.71	*.28*
Sleep efficiency	Baseline	93.05	*.98*	93.93	*.92*
	Retest	93.84	*.92*	97.13	*.36*
SWS latency	Baseline	20.05	*1.58*	25.38	*2.37*
	Retest	17.17	*1.71*	13.41	*2.06*
REM latency	Baseline	80.33	*8.99*	82.32	*12.36*
	Retest	99.94	*8.95*	103.88	*13.66*

Figure 5.3 Sleep stages: the mean and standard error (SE) of the absolute and percentage of each sleep stage on the baseline or retest night for the sleep deprived and non-sleep deprived groups

			Wake		Stage 1		Stage 2		Stage 3		Stage 4		REM		Movement	
			Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
Non-sleep deprived	Baseline	Absolute	17.75	*2.49*	23.60	*3.49*	243.72	*13.36*	47.64	*4.99*	8.19	*3.44*	115.79	*6.38*	2.31	*.53*
		Precentage	3.86	*.56*	5.32	*.83*	52.75	*2.00*	10.41	*1.05*	1.89	*.76*	25.27	*1.10*	.50	*.11*
	Retest	Absolute	19.40	*3.48*	26.60	*3.00*	259.05	*10.74*	54.87	*4.24*	8.64	*3.22*	114.69	*6.61*	2.90	*.80*
		Precentage	3.89	*.65*	5.42	*.57*	53.35	*1.74*	11.36	*.94*	1.72	*.63*	23.69	*1.25*	.57	*.13*
Sleep deprived	Baseline	Absolute	20.09	*4.84*	23.32	*2.35*	272.95	*11.19*	52.44	*6.21*	13.74	*4.57*	113.00	*6.99*	1.85	*.42*
		Precentage	3.74	*.79*	4.62	*.38*	55.04	*1.50*	10.80	*1.40*	2.78	*.93*	22.62	*1.05*	.39	*.09*
	Retest	Absolute	14.17	*2.82*	28.03	*2.84*	437.45	*17.63*	75.35	*6.17*	30.44	*10.37*	192.26	*9.51*	2.62	*.64*
		Precentage	1.75	*.31*	3.58	*.34*	55.93	*1.45*	9.87	*.91*	3.81	*1.26*	24.70	*1.17*	.35	*.09*

Figure 5.4 Absolute amounts of sleep stages per interval: the mean and standard error (SE) of the absolute amount of time of each sleep stage for each interval on the baseline and retest night for the sleep deprived and non-sleep deprived groups

			Wake		Stage 1		Stage 2		Stage 3		Stage 4		REM		Movement	
			Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
Sleep deprived	Baseline	Interval 1	17.45	*4.31*	7.74	*1.31*	19.86	*1.87*	13.09	*1.95*	3.93	*1.51*	.00	*.00*	.15	*.07*
		Interval 2	.14	*.14*	.38	*.30*	32.20	*2.47*	15.45	*1.81*	4.86	*1.86*	9.00	*2.22*	.15	*.07*
		Interval 3	.36	*.31*	1.47	*.82*	31.46	*2.71*	12.27	*2.31*	3.76	*1.70*	12.61	*2.81*	.24	*.10*
		Interval 4	.00	*.00*	.84	*.38*	40.97	*3.85*	5.17	*1.53*	.59	*.40*	14.40	*2.75*	.18	*.06*
		Interval 5	.00	*.00*	1.16	*.40*	39.95	*3.21*	2.78	*1.41*	.53	*.53*	17.57	*3.23*	.24	*.08*
		Interval 6	.00	*.00*	1.50	*.63*	40.28	*3.68*	1.74	*.80*	.00	*.00*	18.36	*3.25*	.26	*.12*
		Interval 7	.79	*.49*	4.23	*.81*	37.49	*2.83*	.56	*.47*	.00	*.00*	18.75	*2.48*	.38	*.12*
		Interval 8	1.34	*.61*	6.01	*1.61*	30.74	*2.67*	1.38	*1.29*	.06	*.06*	22.30	*2.75*	.26	*.09*
	Retest	Interval 1	7.32	*1.16*	6.27	*.81*	29.58	*4.72*	30.23	*3.85*	18.63	*5.96*	5.54	*1.91*	.06	*.04*
		Interval 2	.09	*.06*	1.05	*.45*	47.59	*4.21*	24.54	*2.67*	9.52	*3.61*	14.52	*2.49*	.21	*.09*
		Interval 3	.00	*.00*	.06	*.04*	62.26	*4.33*	12.56	*2.31*	2.24	*1.59*	20.17	*2.45*	.26	*.17*
		Interval 4	.09	*.05*	.95	*.41*	67.80	*3.62*	4.85	*1.85*	.06	*.06*	23.34	*2.78*	.41	*.15*
		Interval 5	.15	*.12*	1.75	*.53*	57.90	*3.48*	1.79	*.99*	.00	*.00*	35.54	*2.98*	.44	*.16*
		Interval 6	.71	*.47*	3.59	*.97*	65.62	*2.99*	.59	*.22*	.00	*.00*	26.66	*2.97*	.35	*.09*
		Interval 7	.18	*.15*	4.10	*.79*	54.88	*3.62*	.56	*.47*	.00	*.00*	37.30	*2.71*	.53	*.18*
		Interval 8	5.64	*2.84*	10.25	*2.07*	51.81	*2.89*	.24	*.14*	.00	*.00*	29.20	*2.98*	.35	*.10*
Non-sleep deprived	Baseline	Interval 1	13.47	*2.25*	5.38	*.71*	21.92	*2.32*	14.49	*2.48*	1.48	*.66*	.56	*.39*	.14	*.07*
		Interval 2	.21	*.15*	1.26	*.62*	31.96	*2.87*	11.70	*1.84*	2.75	*1.32*	9.33	*2.02*	.14	*.07*
		Interval 3	.00	*.00*	.52	*.31*	32.76	*2.92*	11.29	*1.52*	2.72	*1.23*	9.87	*2.09*	.24	*.11*
		Interval 4	.17	*.12*	1.62	*.68*	31.63	*3.11*	5.13	*1.37*	1.16	*.78*	17.47	*2.56*	.14	*.05*
		Interval 5	.05	*.05*	1.13	*.53*	38.93	*2.65*	2.20	*.77*	.02	*.02*	14.75	*2.55*	.33	*.11*
		Interval 6	.00	*.00*	1.21	*.58*	32.79	*3.25*	.94	*.41*	.00	*.00*	22.06	*2.95*	.36	*.12*
		Interval 7	.36	*.18*	4.10	*.96*	27.59	*3.41*	1.01	*.62*	.02	*.02*	23.95	*2.98*	.36	*.12*
		Interval 8	3.49	*.98*	8.37	*1.56*	26.13	*3.05*	.88	*.50*	.02	*.02*	17.81	*2.50*	.60	*.14*
	Retest	Interval 1	9.28	*1.24*	5.60	*.73*	20.84	*2.10*	19.38	*1.99*	4.24	*1.67*	1.49	*1.05*	.02	*.02*
		Interval 2	.14	*.07*	.69	*.29*	35.42	*2.63*	14.01	*1.75*	1.08	*.32*	9.08	*1.90*	.36	*.18*
		Interval 3	.45	*.28*	1.79	*.75*	30.40	*3.08*	11.40	*1.95*	3.13	*1.53*	13.21	*2.23*	.40	*.24*
		Interval 4	.17	*.12*	1.18	*.49*	37.29	*2.63*	4.20	*1.36*	.07	*.07*	17.48	*2.37*	.34	*.13*
		Interval 5	.12	*.08*	1.08	*.35*	39.18	*3.20*	3.71	*1.25*	.12	*.08*	16.25	*3.25*	.34	*.12*
		Interval 6	.53	*.28*	4.12	*1.05*	33.26	*3.09*	.76	*.43*	.00	*.00*	21.71	*2.31*	.39	*.17*
		Interval 7	1.71	*1.53*	3.47	*.88*	34.77	*2.20*	1.00	*.39*	.00	*.00*	19.25	*2.61*	.60	*.18*
		Interval 8	7.00	*2.41*	8.67	*1.47*	27.88	*3.00*	.40	*.24*	.00	*.00*	16.23	*2.65*	.45	*.17*

Figure 5.5 Percentage of sleep stages per interval: the mean and standard error (SE) of the percentage of each sleep stage for each interval on the baseline and retest night for the sleep deprived and non-sleep deprived groups

			Wake		Stage 1		Stage 2		Stage 3		Stage 4		REM		Movement	
			Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
Sleep deprived	Baseline	Interval 1	25.80	*5.49*	12.59	*2.16*	32.80	*3.36*	21.99	*3.53*	6.56	*2.56*	.00	*.00*	.26	*.13*
		Interval 2	.20	*.20*	.49	*.35*	52.31	*4.22*	24.73	*2.81*	7.50	*2.69*	14.55	*3.50*	.23	*.11*
		Interval 3	.54	*.44*	2.16	*1.18*	51.06	*4.62*	20.37	*4.11*	6.23	*2.89*	19.23	*3.95*	.41	*.17*
		Interval 4	.00	*.00*	1.35	*.61*	65.51	*5.53*	8.40	*2.63*	.99	*.68*	23.43	*4.48*	.31	*.11*
		Interval 5	.00	*.00*	1.84	*.63*	64.08	*4.44*	4.43	*2.29*	.89	*.89*	28.36	*5.26*	.40	*.13*
		Interval 6	.00	*.00*	2.39	*1.02*	64.55	*5.73*	3.22	*1.54*	.00	*.00*	29.40	*5.56*	.43	*.20*
		Interval 7	1.23	*.73*	6.80	*1.34*	60.66	*4.70*	.89	*.74*	.00	*.00*	29.77	*3.93*	.65	*.21*
		Interval 8	2.13	*.97*	9.38	*2.22*	49.37	*4.22*	2.33	*2.18*	.10	*.10*	36.25	*4.78*	.45	*.15*
	Retest	Interval 1	15.34	*2.07*	9.19	*1.19*	35.06	*3.79*	31.59	*3.14*	6.70	*2.61*	2.09	*1.44*	.03	*.03*
		Interval 2	.24	*.12*	1.09	*.48*	58.30	*4.10*	22.86	*2.80*	1.78	*.54*	15.18	*3.28*	.54	*.25*
		Interval 3	.66	*.36*	3.03	*1.29*	50.50	*5.38*	18.97	*3.27*	4.93	*2.36*	21.34	*3.60*	.57	*.29*
		Interval 4	.25	*.18*	1.92	*.80*	60.78	*3.58*	7.47	*2.60*	.14	*.14*	28.91	*4.12*	.52	*.18*
		Interval 5	.21	*.16*	1.70	*.54*	64.70	*5.07*	6.44	*2.14*	.23	*.15*	26.16	*5.18*	.56	*.19*
		Interval 6	.82	*.39*	6.68	*1.65*	54.05	*4.84*	1.29	*.76*	.00	*.00*	36.60	*4.40*	.57	*.21*
		Interval 7	2.15	*1.84*	5.51	*1.40*	57.69	*3.88*	1.58	*.61*	.00	*.00*	32.07	*4.41*	1.00	*.30*
		Interval 8	11.43	*3.98*	14.25	*2.49*	45.75	*4.93*	.63	*.37*	.00	*.00*	27.16	*4.78*	.78	*.32*
Non-sleep deprived	Baseline	Interval 1	23.54	*4.06*	9.97	*1.52*	38.90	*4.27*	23.65	*3.79*	2.45	*1.10*	1.23	*.89*	.26	*.13*
		Interval 2	.33	*.23*	2.62	*1.32*	54.78	*4.12*	20.27	*3.17*	4.84	*2.21*	16.91	*3.72*	.25	*.13*
		Interval 3	.00	*.00*	.91	*.55*	55.90	*4.84*	21.23	*3.51*	5.65	*2.66*	15.93	*3.27*	.38	*.17*
		Interval 4	.26	*.19*	2.61	*1.09*	54.65	*4.98*	9.58	*2.63*	2.01	*1.36*	30.60	*4.82*	.28	*.10*
		Interval 5	.08	*.08*	2.05	*1.02*	68.90	*4.79*	3.45	*1.17*	.07	*.07*	24.84	*4.26*	.62	*.22*
		Interval 6	.00	*.00*	2.00	*.88*	56.37	*5.08*	1.78	*.76*	.00	*.00*	39.23	*5.24*	.61	*.21*
		Interval 7	.61	*.32*	7.41	*1.78*	47.11	*5.97*	1.63	*1.00*	.04	*.04*	42.59	*5.66*	.60	*.19*
		Interval 8	6.05	*1.70*	14.97	*3.06*	45.39	*5.13*	1.62	*.93*	.04	*.04*	30.91	*4.33*	1.02	*.23*
	Retest	Interval 1	7.54	*1.18*	6.52	*.91*	29.74	*4.24*	31.69	*4.12*	18.80	*5.86*	5.64	*1.87*	.07	*.05*
		Interval 2	.09	*.06*	1.12	*.49*	48.83	*4.03*	25.31	*2.75*	9.42	*3.55*	15.02	*2.55*	.22	*.09*
		Interval 3	.00	*.00*	.06	*.04*	63.42	*3.72*	13.09	*2.40*	2.19	*1.56*	20.96	*2.89*	.28	*.18*
		Interval 4	.10	*.05*	.98	*.43*	69.36	*3.29*	5.41	*2.32*	.08	*.08*	23.63	*2.63*	.44	*.16*
		Interval 5	.14	*.11*	1.82	*.55*	58.99	*2.81*	1.95	*1.12*	.00	*.00*	36.63	*3.11*	.47	*.17*
		Interval 6	.74	*.49*	3.70	*1.05*	67.49	*2.77*	.64	*.24*	.00	*.00*	27.06	*2.82*	.36	*.10*
		Interval 7	.17	*.14*	4.24	*.83*	55.77	*2.76*	.62	*.53*	.00	*.00*	38.64	*2.95*	.57	*.19*
		Interval 8	5.21	*2.52*	10.21	*1.91*	53.89	*3.29*	.24	*.14*	.00	*.00*	30.06	*2.90*	.38	*.12*

Figure 5.6 REM density for whole night: the mean and standard error (SE) for the total REM density of the baseline and retest nights for the sleep deprived and non-sleep deprived groups

	Non sleep deprived		Sleep deprived	
	Mean	*SE*	Mean	*SE*
Baseline	81.95	*10.17*	64.93	*9.04*
Retest	85.00	*8.74*	75.59	*6.98*

Figure 5.7 REM density over the course of the night: the mean and standard error (SE) for the REM density for each interval of the baseline and retest nights for the sleep deprived and non-sleep deprived groups

	Non-sleep deprived				Sleep deprived			
	Baseline		Retest		Baseline		Retest	
	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
Interval 1	.40	*.28*	.29	*.29*	.00	*.00*	1.47	*.46*
Interval 2	5.05	*1.00*	5.62	*1.09*	4.93	*1.27*	6.00	*1.13*
Interval 3	7.70	*1.77*	11.24	*2.31*	7.00	*1.53*	7.29	*1.18*
Interval 4	14.15	*2.20*	11.14	*1.60*	7.07	*2.20*	8.24	*1.24*
Interval 5	14.50	*2.15*	12.95	*2.87*	10.27	*1.98*	9.41	*.89*
Interval 6	14.20	*2.85*	15.24	*1.98*	11.40	*2.89*	11.24	*1.45*
Interval 7	12.60	*1.82*	16.62	*2.47*	11.47	*2.40*	15.06	*1.75*
Interval 8	13.35	*2.48*	11.90	*2.05*	12.80	*1.84*	16.88	*1.84*

Figure 5.8 Spectral frequency bands for NREM and REM sleep: the mean and standard error (SE) of each frequency band for REM and NREM sleep for the baseline and retest night for the sleep deprived and non-sleep deprived groups

		Sleep deprived				Non-sleep deprived			
		Baseline		Retest		Baseline		Retest	
		Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
REM	Delta	17.93	*.53*	17.92	*.46*	20.37	*.58*	19.75	*.59*
	Theta	4.08	*.16*	4.13	*.15*	4.68	*.14*	4.60	*.14*
	Alpha	2.03	*.10*	1.95	*.10*	2.13	*.08*	2.11	*.08*
	Sigma	.66	*.03*	.64	*.03*	.70	*.02*	.68	*.02*
	Beta1	.37	*.01*	.33	*.02*	.38	*.01*	.37	*.01*
	Beta2	.29	*.02*	.22	*.01*	.23	*.01*	.24	*.01*
	Gamma	.15	*.01*	.13	*.01*	.12	*.00*	.12	*.00*
NREM	Delta	139.65	*4.25*	137.53	*4.52*	136.78	*3.65*	132.03	*3.54*
	Theta	7.73	*.18*	8.08	*.21*	8.32	*.16*	8.51	*.18*
	Alpha	3.15	*.10*	3.41	*.13*	3.25	*.07*	3.32	*.07*
	Sigma	1.89	*.04*	1.99	*.05*	1.81	*.03*	1.82	*.04*
	Beta1	.34	*.01*	.33	*.01*	.32	*.01*	.32	*.01*
	Beta2	.21	*.01*	.24	*.02*	.17	*.00*	.17	*.00*
	Gamma	.11	*.00*	.10	*.00*	.10	*.00*	.10	*.00*

Figure 5.9 Time course of each spectral frequency band NREM sleep: the mean and standard error (SE) of each frequency band for NREM sleep for each interval for the baseline and retest night for the sleep deprived and non-sleep deprived groups

			Interval 1		Interval 2		Interval 3		Interval 4		Interval 5		Interval 6		Interval 7		Interval 8	
			Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
Non-sleep deprived	Delta	Baseline	171.71	*9.64*	179.64	*10.65*	175.33	*9.84*	132.47	*9.26*	103.99	*8.65*	81.33	*5.58*	68.37	*5.63*	93.09	*11.66*
		Retest	181.15	*9.77*	176.92	*10.37*	159.77	*10.11*	101.13	*6.77*	123.06	*9.42*	84.75	*8.57*	83.78	*5.72*	54.50	*4.07*
	Theta	Baseline	11.47	*.51*	9.63	*.42*	8.25	*.38*	7.50	*.37*	6.83	*.41*	6.53	*.37*	6.36	*.46*	7.02	*.45*
		Retest	11.82	*.54*	9.48	*.43*	8.12	*.38*	7.61	*.45*	7.31	*.43*	7.33	*.57*	7.07	*.45*	6.61	*.49*
	Alpha	Baseline	3.67	*.20*	3.61	*.18*	3.44	*.19*	3.06	*.17*	2.94	*.20*	3.07	*.22*	2.65	*.18*	2.88	*.20*
		Retest	3.91	*.20*	3.48	*.18*	3.23	*.18*	3.45	*.23*	3.13	*.20*	3.13	*.25*	2.92	*.19*	2.74	*.20*
	Sigma	Baseline	2.03	*.10*	1.85	*.08*	1.72	*.08*	1.68	*.08*	1.72	*.09*	1.79	*.10*	1.78	*.13*	1.85	*.12*
		Retest	2.10	*.10*	1.78	*.08*	1.59	*.08*	1.90	*.10*	1.78	*.09*	1.95	*.13*	1.78	*.11*	1.65	*.12*
	Beta1	Baseline	.33	*.01*	.34	*.02*	.29	*.01*	.33	*.02*	.31	*.02*	.35	*.02*	.30	*.02*	.36	*.02*
		Retest	.33	*.01*	.32	*.02*	.29	*.02*	.31	*.01*	.31	*.02*	.38	*.03*	.33	*.02*	.35	*.02*
	Beta2	Baseline	.17	*.01*	.18	*.01*	.16	*.01*	.17	*.01*	.16	*.01*	.18	*.01*	.16	*.01*	.18	*.01*
		Retest	.17	*.01*	.17	*.01*	.16	*.01*	.16	*.01*	.16	*.01*	.19	*.01*	.18	*.01*	.18	*.01*
	Gamma	Baseline	.10	*.00*	.10	*.00*	.09	*.00*	.10	*.00*	.09	*.01*	.10	*.00*	.09	*.00*	.11	*.00*
		Retest	.10	*.00*	.09	*.00*	.09	*.00*	.10	*.00*	.09	*.00*	.11	*.01*	.10	*.01*	.10	*.01*
Sleep deprived	Delta	Baseline	181.51	*11.67*	189.08	*12.57*	183.22	*12.81*	121.51	*9.06*	103.92	*9.24*	90.28	*6.03*	64.11	*6.66*	74.75	*8.72*
		Retest	237.62	*14.36*	195.22	*12.30*	134.81	*8.95*	104.65	*7.91*	84.66	*8.53*	72.80	*7.03*	61.02	*8.58*	53.97	*4.98*
	Theta	Baseline	10.57	*.55*	8.97	*.50*	7.85	*.41*	6.79	*.41*	6.79	*.50*	5.93	*.37*	5.81	*.50*	5.88	*.45*
		Retest	13.17	*.69*	8.88	*.48*	7.12	*.43*	6.65	*.43*	6.12	*.50*	5.57	*.40*	6.18	*.60*	6.06	*.57*
	Alpha	Baseline	4.12	*.38*	3.46	*.27*	3.07	*.21*	3.07	*.25*	3.03	*.28*	2.64	*.18*	2.39	*.21*	2.32	*.20*
		Retest	5.06	*.43*	3.63	*.28*	2.98	*.26*	3.17	*.32*	3.12	*.37*	2.31	*.20*	2.75	*.33*	2.65	*.33*
	Sigma	Baseline	2.24	*.12*	1.78	*.09*	1.64	*.08*	1.85	*.11*	1.90	*.13*	1.97	*.12*	1.95	*.15*	1.70	*.13*
		Retest	2.23	*.12*	1.69	*.08*	1.66	*.09*	2.09	*.12*	2.02	*.14*	2.20	*.15*	2.25	*.18*	2.00	*.18*
	Beta1	Baseline	.40	*.02*	.33	*.01*	.30	*.01*	.31	*.01*	.32	*.02*	.32	*.02*	.38	*.03*	.38	*.03*
		Retest	.40	*.02*	.30	*.01*	.28	*.01*	.33	*.02*	.32	*.02*	.31	*.02*	.36	*.03*	.41	*.06*
	Beta2	Baseline	.19	*.01*	.17	*.01*	.18	*.01*	.16	*.01*	.16	*.01*	.17	*.01*	.28	*.04*	.55	*.14*
		Retest	.60	*.12*	.15	*.01*	.14	*.01*	.17	*.01*	.16	*.01*	.16	*.01*	.19	*.03*	.21	*.04*
	Gamma	Baseline	.11	*.00*	.10	*.00*	.09	*.00*	.09	*.00*	.11	*.01*	.11	*.01*	.14	*.02*	.16	*.02*
		Retest	.11	*.00*	.09	*.00*	.09	*.00*	.10	*.00*	.09	*.00*	.10	*.00*	.13	*.04*	.14	*.04*

Figure 5.10 Time course of each spectral frequency band REM sleep: the mean and standard error (SE) of each frequency band for REM sleep for each interval for the baseline and retest night for the sleep deprived and non-sleep deprived groups

			Interval 1		Interval 2		Interval 3		Interval 4		Interval 5		Interval 6		Interval 7		Interval 8	
			Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
Non-sleep deprived	Delta	Baseline	23.78	*4.09*	23.18	*1.96*	20.20	*1.46*	20.14	*1.29*	19.29	*1.26*	20.05	*1.43*	19.95	*1.48*	19.80	*1.92*
		Retest	23.83	*6.59*	21.42	*1.61*	19.92	*1.44*	18.80	*1.36*	19.16	*1.41*	21.12	*2.18*	19.01	*1.30*	18.40	*1.22*
	Theta	Baseline	5.83	*1.33*	5.53	*.48*	4.76	*.42*	4.58	*.34*	4.42	*.36*	4.54	*.36*	4.44	*.35*	4.53	*.39*
		Retest	7.49	*2.18*	5.31	*.44*	4.54	*.37*	4.30	*.36*	4.36	*.36*	4.57	*.37*	4.57	*.41*	4.33	*.35*
	Alpha	Baseline	2.59	*.82*	2.30	*.25*	2.43	*.30*	2.30	*.23*	2.08	*.21*	1.97	*.18*	1.88	*.16*	2.03	*.20*
		Retest	3.63	*1.00*	2.25	*.23*	2.45	*.25*	2.14	*.22*	2.04	*.21*	2.07	*.18*	1.86	*.18*	1.84	*.18*
	Sigma	Baseline	.73	*.22*	.91	*.09*	.69	*.06*	.74	*.08*	.60	*.04*	.67	*.06*	.64	*.05*	.65	*.06*
		Retest	1.21	*.33*	.75	*.06*	.73	*.07*	.65	*.05*	.67	*.07*	.66	*.07*	.60	*.06*	.61	*.05*
	Beta1	Baseline	.29	*.05*	.48	*.05*	.36	*.03*	.41	*.04*	.34	*.02*	.38	*.03*	.36	*.03*	.34	*.03*
		Retest	.70	*.13*	.40	*.03*	.42	*.04*	.34	*.02*	.38	*.04*	.36	*.03*	.33	*.03*	.31	*.02*
	Beta2	Baseline	.20	*.03*	.27	*.02*	.22	*.01*	.24	*.02*	.22	*.01*	.24	*.02*	.23	*.02*	.21	*.02*
		Retest	.37	*.04*	.24	*.02*	.27	*.02*	.22	*.01*	.24	*.02*	.23	*.02*	.22	*.02*	.22	*.02*
	Gamma	Baseline	.12	*.02*	.13	*.01*	.12	*.01*	.12	*.01*	.11	*.01*	.13	*.01*	.12	*.01*	.11	*.01*
		Retest	.19	*.02*	.12	*.01*	.13	*.01*	.12	*.01*	.12	*.01*	.12	*.01*	.12	*.01*	.12	*.01*
Sleep deprived	Delta	Baseline		.	20.60	*2.06*	17.68	*1.41*	17.78	*1.41*	17.99	*1.41*	18.45	*1.44*	17.07	*1.21*	16.70	*1.09*
		Retest	19.10	*1.95*	20.47	*1.47*	19.53	*1.39*	17.77	*1.23*	16.65	*1.14*	16.68	*1.11*	16.93	*1.16*	17.09	*1.23*
	Theta	Baseline		.	4.43	*.46*	3.88	*.41*	4.17	*.42*	4.19	*.50*	4.14	*.42*	3.91	*.36*	3.90	*.34*
		Retest	4.47	*.56*	4.61	*.40*	4.56	*.44*	4.10	*.39*	3.84	*.36*	3.77	*.35*	3.90	*.41*	4.00	*.45*
	Alpha	Baseline		.	2.15	*.29*	2.13	*.30*	2.05	*.28*	2.13	*.30*	1.98	*.25*	1.87	*.22*	1.96	*.22*
		Retest	1.88	*.32*	2.17	*.28*	2.29	*.34*	2.03	*.31*	1.78	*.23*	1.74	*.23*	1.86	*.35*	1.82	*.25*
	Sigma	Baseline		.	.77	*.09*	.74	*.08*	.69	*.07*	.65	*.06*	.63	*.06*	.60	*.05*	.61	*.05*
		Retest	.77	*.11*	.76	*.07*	.71	*.07*	.61	*.06*	.57	*.06*	.55	*.05*	.61	*.11*	.61	*.08*
	Beta1	Baseline		.	.42	*.04*	.42	*.05*	.38	*.04*	.38	*.03*	.33	*.03*	.35	*.03*	.34	*.03*
		Retest	.29	*.03*	.33	*.03*	.36	*.03*	.33	*.03*	.31	*.03*	.31	*.03*	.34	*.08*	.32	*.04*
	Beta2	Baseline		.	.25	*.03*	.28	*.03*	.25	*.03*	.26	*.03*	.21	*.02*	.29	*.03*	.44	*.09*
		Retest	.17	*.02*	.20	*.02*	.23	*.02*	.22	*.02*	.22	*.02*	.23	*.02*	.25	*.06*	.24	*.03*
	Gamma	Baseline		.	.13	*.01*	.13	*.01*	.14	*.01*	.15	*.01*	.14	*.02*	.16	*.02*	.19	*.03*
		Retest	.09	*.01*	.11	*.01*	.12	*.01*	.12	*.01*	.13	*.01*	.13	*.01*	.16	*.04*	.14	*.02*

Figure 5.11 Topographical analysis of each spectral frequency band for NREM sleep: the mean and standard error (SE) of each frequency band for NREM sleep for each EEG channel for the baseline and retest night for the sleep deprived and non-sleep deprived groups

			Pre frontal		Frontal		Central		Parietal		Occipital	
			Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
Sleep deprived	Delta	Baseline	232.47	*12.64*	123.26	*7.01*	53.57	*2.76*	87.74	*4.74*	212.94	*10.72*
		Retest	232.60	*13.14*	117.87	*7.39*	54.21	*2.83*	77.52	*4.45*	208.17	*11.89*
	Theta	Baseline	10.80	*.35*	4.95	*.18*	3.45	*.12*	5.23	*.19*	15.63	*.51*
		Retest	11.64	*.45*	5.04	*.22*	3.69	*.15*	5.18	*.24*	15.62	*.62*
	Alpha	Baseline	4.28	*.25*	1.97	*.10*	1.36	*.06*	2.46	*.15*	6.25	*.36*
		Retest	4.79	*.31*	2.08	*.13*	1.49	*.08*	2.58	*.20*	6.45	*.44*
	Sigma	Baseline	3.27	*.10*	1.30	*.04*	.96	*.03*	1.14	*.04*	2.90	*.08*
		Retest	3.61	*.11*	1.33	*.04*	1.03	*.03*	1.08	*.04*	2.86	*.10*
	Beta1	Baseline	.45	*.01*	.24	*.01*	.22	*.01*	.27	*.01*	.55	*.02*
		Retest	.46	*.01*	.22	*.01*	.21	*.01*	.23	*.01*	.56	*.03*
	Beta2	Baseline	.26	*.03*	.16	*.02*	.16	*.03*	.16	*.01*	.34	*.05*
		Retest	.30	*.05*	.12	*.01*	.19	*.05*	.19	*.04*	.41	*.08*
	Gamma	Baseline	.14	*.01*	.08	*.00*	.08	*.00*	.09	*.01*	.16	*.01*
		Retest	.13	*.00*	.07	*.00*	.07	*.00*	.08	*.00*	.17	*.02*
Non-sleep deprived	Delta	Baseline	224.37	*10.82*	124.79	*6.86*	56.25	*2.55*	87.23	*4.14*	195.76	*8.16*
		Retest	216.67	*10.72*	120.87	*6.06*	55.75	*2.62*	86.78	*4.09*	195.16	*8.81*
	Theta	Baseline	11.31	*.28*	5.84	*.17*	3.71	*.10*	5.98	*.17*	15.49	*.46*
		Retest	11.60	*.32*	5.94	*.17*	3.66	*.10*	6.05	*.17*	17.12	*.51*
	Alpha	Baseline	4.31	*.14*	2.18	*.08*	1.39	*.04*	2.63	*.09*	6.04	*.21*
		Retest	4.42	*.15*	2.31	*.08*	1.41	*.04*	2.73	*.10*	6.41	*.25*
	Sigma	Baseline	2.99	*.08*	1.31	*.04*	.90	*.02*	1.22	*.04*	2.67	*.08*
		Retest	3.08	*.08*	1.36	*.04*	.92	*.02*	1.18	*.03*	2.77	*.09*
	Beta1	Baseline	.42	*.01*	.22	*.01*	.20	*.01*	.25	*.01*	.55	*.02*
		Retest	.41	*.01*	.22	*.01*	.20	*.01*	.25	*.01*	.58	*.02*
	Beta2	Baseline	.22	*.01*	.12	*.00*	.11	*.00*	.13	*.00*	.27	*.01*
		Retest	.21	*.01*	.12	*.00*	.11	*.00*	.13	*.00*	.29	*.01*
	Gamma	Baseline	.13	*.00*	.07	*.00*	.07	*.00*	.08	*.00*	.15	*.00*
		Retest	.12	*.00*	.07	*.00*	.07	*.00*	.07	*.00*	.16	*.00*

Figure 5.12 Topographical analysis of each spectral frequency band for REM sleep: the mean and standard error (SE) of each frequency band for REM sleep for each EEG channel for the baseline and retest night for the sleep deprived and non-sleep deprived groups

			Pre frontal		Frontal		Central		Parietal		Occipital	
			Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
Sleep deprived	Delta	Baseline	24.48	*.71*	10.48	*.45*	10.03	*.62*	14.55	*.70*	33.45	*1.37*
		Retest	26.18	*.64*	10.35	*.28*	9.98	*.38*	13.05	*.47*	31.46	*1.18*
	Theta	Baseline	4.87	*.20*	2.07	*.11*	2.08	*.24*	3.58	*.19*	8.87	*.46*
		Retest	5.25	*.19*	2.11	*.10*	1.84	*.08*	3.32	*.17*	8.74	*.49*
	Alpha	Baseline	1.90	*.11*	.88	*.05*	.95	*.06*	2.06	*.16*	5.09	*.38*
		Retest	1.86	*.09*	.81	*.04*	.86	*.05*	1.70	*.13*	4.96	*.44*
	Sigma	Baseline	.71	*.04*	.35	*.02*	.38	*.02*	.64	*.04*	1.42	*.08*
		Retest	.71	*.04*	.30	*.01*	.35	*.02*	.52	*.03*	1.41	*.11*
	Beta1	Baseline	.43	*.03*	.24	*.02*	.24	*.01*	.35	*.03*	.67	*.04*
		Retest	.38	*.02*	.19	*.01*	.20	*.01*	.26	*.02*	.65	*.07*
	Beta2	Baseline	.35	*.04*	.21	*.03*	.22	*.04*	.25	*.03*	.46	*.06*
		Retest	.27	*.02*	.14	*.01*	.15	*.01*	.18	*.01*	.40	*.05*
	Gamma	Baseline	.19	*.02*	.11	*.01*	.11	*.01*	.13	*.02*	.21	*.02*
		Retest	.15	*.01*	.09	*.00*	.09	*.00*	.10	*.01*	.22	*.03*
Non-sleep deprived	Delta	Baseline	29.09	*.93*	12.10	*.42*	10.19	*.34*	15.44	*.58*	37.11	*1.79*
		Retest	28.63	*.96*	11.96	*.40*	10.12	*.40*	14.79	*.52*	37.52	*2.01*
	Theta	Baseline	5.80	*.23*	2.63	*.12*	2.09	*.10*	4.16	*.18*	9.44	*.42*
		Retest	5.81	*.25*	2.74	*.13*	2.01	*.10*	4.00	*.16*	9.72	*.41*
	Alpha	Baseline	2.04	*.11*	1.05	*.05*	.97	*.05*	2.06	*.10*	5.02	*.27*
		Retest	2.06	*.11*	1.09	*.06*	.96	*.05*	2.03	*.09*	5.17	*.25*
	Sigma	Baseline	.76	*.03*	.38	*.02*	.38	*.02*	.64	*.03*	1.45	*.09*
		Retest	.74	*.03*	.38	*.02*	.37	*.02*	.61	*.03*	1.46	*.09*
	Beta1	Baseline	.43	*.02*	.24	*.01*	.23	*.01*	.34	*.02*	.71	*.05*
		Retest	.43	*.02*	.24	*.01*	.23	*.01*	.32	*.02*	.70	*.05*
	Beta2	Baseline	.29	*.01*	.16	*.01*	.15	*.01*	.20	*.01*	.38	*.02*
		Retest	.30	*.01*	.17	*.01*	.16	*.01*	.20	*.01*	.40	*.02*
	Gamma	Baseline	.15	*.01*	.09	*.00*	.08	*.00*	.10	*.00*	.18	*.01*
		Retest	.16	*.01*	.09	*.00*	.09	*.00*	.10	*.00*	.19	*.01*

Figure 5.13 Sleep parameters and intrusive memories: the mean and standard error (SE) of intrusive memories for high and low levels of sleep parameters on baseline and retest nights and changes in sleep parameters between the baseline and retest nights

			Sleep deprived		Non sleep deprived	
			Mean	*SE*	Mean	*SE*
Sleep latency	Baseline	Low	3.89	*1.10*	3.70	*.73*
		High	.75	*.31*	3.64	*1.35*
	Retest	Low	3.70	*1.00*	2.25	*.62*
		High	.67	*.33*	4.54	*1.14*
	Change	Decrease	1.44	*.47*	3.38	*1.15*
		Increase	4.00	*1.43*	4.13	*.83*
REM latency	Baseline	Low	3.71	*1.52*	3.08	*.77*
		High	1.50	*.43*	4.44	*1.49*
	Retest	Low	4.14	*1.45*	2.75	*.77*
		High	1.33	*.33*	4.89	*1.43*
	Change	Decrease	1.89	*.61*	4.57	*1.63*
		Increase	3.43	*1.48*	3.00	*1.05*
Wake percentage	Baseline	Low	3.33	*1.24*	3.23	*.69*
		High	1.38	*.38*	4.38	*1.73*
Sleep efficiency	Change	Decrease	4.75	*1.25*	3.91	*1.25*
		Increase	1.83	*.80*	3.40	*.92*

Figure 5.14 sleep stages and intrusive memories: the mean and standard error (SE) of intrusive memories for high and low levels of sleep stages on baseline and retest nights and changes in sleep stages between the baseline and retest nights

			Sleep deprived		Non sleep deprived	
			Mean	*SE*	Mean	*SE*
Movement	Change	Decrease	3.67	*1.17*	3.29	*.81*
		Increase	1.00	*.33*	4.15	*1.14*
Stage 1	Change	Decrease	1.50	*.43*	3.63	*1.60*
		Increase	6.67	*2.40*	4.00	*.83*
Stage 2	Baseline	Low	3.29	*1.52*	3.17	*.85*
		High	1.80	*.55*	4.88	*1.49*
Stage 4	Retest	Low	1.11	*.45*	3.10	*.89*
		High	3.88	*1.25*	4.60	*1.30*
Wake	Baseline	Low	4.00	*1.24*	3.50	*.78*
		High	1.00	*.37*	4.20	*1.40*
	Change	Decrease	1.00	*.33*	4.56	*1.45*
		Increase	5.00	*1.41*	3.27	*.82*

Figure 5.15 REM density and intrusive memories: the mean and standard error (SE) of intrusive memories for high and low levels of REM density on baseline and retest nights and changes in REM density between the baseline and retest nights

			Sleep deprived		Non sleep deprived	
			Mean	*SE*	Mean	*SE*
REM density	Baseline	Low	3.44	*1.21*	3.56	*1.08*
		High	1.33	*.49*	3.75	*1.12*
	Change	Decrease	1.00	*.63*	3.00	*.91*
		Increase	3.78	*1.12*	4.08	*1.12*

Figure 5.16 Spectral frequency and intrusive memories: the mean and standard error (SE) of intrusive memories for high and low levels of spectral frequencies on baseline and retest nights and changes in spectral frequencies between the baseline and retest nights

			Sleep deprived		Non sleep deprived	
			Mean	*SE*	Mean	*SE*
Alpha	Retest	Low	1.67	*.60*	3.90	*.95*
		High	3.71	*1.43*	3.80	*1.31*
Sigma	Baseline	Low	2.71	*1.32*	2.82	*.67*
		High	2.20	*.81*	5.11	*1.49*
Beta1	Change	Decrease	3.17	*1.17*	3.09	*.89*
		Increase	2.20	*.96*	4.78	*1.36*
Gamma	Baseline	Low	1.75	*.83*	4.25	*1.19*
		High	4.00	*1.14*	3.25	*.86*

9.1.4. Chapter 6

The data for the FH participants is provided which were presented alongside the mean data for the nFH participants which are provided in the table above.

Figure 6.1 aMT6s acrophase for each FH participant

ID	Acrophase
FH1	07:21
FH2	
FH3	03:59

Figure 6.2 Diurnal mood: mood VAS data for each FH participant at each timepoint

	Day 1												Day 2											
	8am to 10am			12pm to 2pm			4pm to 6pm			8pm to 10pm			8am to 10am			12pm tp 2pm			4pm to 6pm					
	FH1	FH2	FH3	FH1	FH2	FH3	FH1	FH2	FH3	FH1	FH2	FH3	FH1	FH2	FH3	FH1	FH2	FH3	FH1	FH2	FH3			
Sad	16.5	35.25	26.5	12.75	13	2.75	5.75	12	1	9	22.75	12.5	3.5	27	19	3	19.5	6.25	3.5	13.75	10.5			
Calm	46.25	56.75	44.5	33	68.5	65.75	21.25	75.75	66	57.5	69.75	62	42.75	66.75	85	25.5	77.75	50.75	43	79.5	74			
Horrified	2.25	0	2	3.5	0	2	3	0	0.75	2.75	0	1.5	9	0	0.5	2.5	1.25	2.5	2.25	0	1.5			
Happy	48.75	62.75	57	26.75	75.75	74.5	33.5	63.25	71.5	42.75	60	53.5	22.75	66.5	26.5	28.5	66.75	63.5	40.5	67.25	55.75			
Fearful	2.5	0	5.5	8.5	0	1.75	5.5	0	1.5	2.25	0	4.25	4.25	0	1.5	1.75	0	8	1.5	0	1.75			
Hopeless	10	0	4	23.25	0	0.5	8.5	0	4	9	0	1	30.75	0	1	38.75	0	2.5	7.5	0	2			
Angry	4	7.5	3	3	0	0	4	0	6.75	2.5	0	1	9	0	1	2.5	0	1.25	2.75	0	1.5			
Sleepy	25.5	17.75	73	9	15.25	13.75	39	39	22	65.25	74.25	38.75	10	19.5	16.5	6.25	48.25	29.25	5.75	62.75	24.5			
Awkward	6.5	1.5	6	6.75	0	10.25	2.5	0	8.5	11.75	0	2.25	5.5	0	2.5	30	1.25	4.75	10.5	0	2.75			
Tense	5.5	0	9	38	0	9	2.5	1.75	15	4	0	15.5	26.5	0	2	30	1	12.5	1.25	0	3.5			
Restless	9	0	7	14.5	0	11	15	0	6.25	4	0	14.5	16.25	0	3	35	0	3.5	2	0	2.5			
Irritable	7.75	0	15	7	0	0.75	2.5	0	1.25	8.25	0	1.75	16.5	0	19	8.5	0	1.25	2	0	1.5			
Racing thoughts	6.5	9	1.5	4.25	0	6.5	3	0	10.25	2.75	0	1.5	2.5	0	3	3.25	0	1.5	1.25	0	1.25			
Anxious	12.75	0	8.5	37.75	0	1	1.75	0	9.5	2.5	0	14	43	0	2.5	40	0	6.5	2	0	5.5			
Alert	51.25	59.25	35	22	69.25	75.25	30	45.5	75.5	31	18.25	66.5	50	41.5	53	55.5	45	67.25	63	23	62.25			

Figure 6.3 Mood fluctuation: the average standard deviation of diurnal mood for each FH participant

Participant ID	Average standard deviation of dirunal mood
FH2	5.59
FH3	8.09
FH1	12.53

Figure 6.4 Cortisol awakening response: the concentration of salivary cortisol for each FH participant for each time point

	Day 1				Day 2			
	0	15	30	45	0	15	30	45
FH1		28.8	38.7	24.4	13.7	19.9	40.4	33.2
FH2	9.2	8.5	14.0	9.3	9.1	7.6	15.5	21.9
FH3	12.9	12.1	10.3	15.1	13.1	10.1	13.2	20.9

Figure 6.5 Response to trauma film: the data relating to the response to the trauma film for each FH participant

		FH1	FH2	FH3
Mood VAS	Postive affect	-17	-14	-11.5
	Negative affect	3.6	4.4	7.3
Dissociaitive state scale	Pre Film	1	4	0
	Post Film	4	4	5
Film follow up	Relevance	3	8	6
	Familiarity	3	5	3

Figure 6.6 Response to stressor: the data relating to the response to the stressor for each FH participant

		FH1	FH2	FH3
Score		2	0	4
Mood VAS	Negative affect	0.86	4.64	-0.93
	Postive affect	-36.64	-61.11	-48.43
RAT follow up	Difficulty	7	8	6
	Performance	1	1	3

Figure 6.7 Effect of sleep deprivation on mood: the data relating to the change in mood in response to the experiment for each FH participant

			FH1	FH2	FH3
Mood VAS	Alertness	Pre film	40.5	68.0	75.5
		Post film	35.0	39.0	69.0
		Pre stressor	72.5	65.5	20.0
		Post stressor	64.0	61.0	14.5
	Sleepiness	Pre film	26.0	33.5	15.0
		Post film	38.0	45.0	24.5
		Pre stressor	10.5	30.0	66.5
		Post stressor	18.0	76.0	62.0
	Postive affect	Pre film	58.0	74.5	72.8
		Post film	41.0	60.5	61.3
		Pre stressor	64.5	80.8	52.8
		Post stressor	37.5	65.8	47.5
	Negative affect	Pre film	6.5	9.2	10.0
		Post film	10.1	13.6	17.3
		Pre stressor	9.9	8.3	7.4
		Post stressor	10.7	12.9	6.5
State trait anxiety index	Anxitey	Pre film	27.0	30.0	26.0
		Post film	41.0	37.0	31.0
		Pre stressor	24.0	26.0	32.0
		Post stressor	33.0	38.0	36.0
Postive and negative affect scale	Positive affect	PM	19.0	24.0	27.0
		AM	20.0	28.0	12.0
	Negative affect	PM	10.0	10.0	10.0
		AM	10.0	11.0	10.0

Figure 6.8 Activities: the amount of time spent doing visual-spatial and verbal activities for each FH participant

		FH1	FH2	FH3
Activities VAS	Visual-spatial	50	0	20
	Verbal	83	100	76.5

Figure 6.9 Intrusive memories: the data for each FH participant for both measures of intrusive memories

		FH1	FH2	FH3
Intrusions	Total	3	1	
	Day1	1	1	
	Day2	0	0	
	Day3	0	0	
	Day4	2	0	
	Day5	0	0	
	Day6	0	0	
Impact of event scale	Intrusions	0	3	0
	Avoidance	4	0	4
	Hyperarousal	0	0	0

Figure 6.10 sleep parameters: the data for each FH participants for both the baseline and retest night

		FH1	FH2	FH3
Total sleep time	Baseline	359.5	550.667	499.167
	Retest	395.667	552.667	
Sleep latency	Baseline	13	11	3.3333
	Retest	6.5	20.6667	
Wake after sleep onset (absolute time)	Baseline	0	1	5.5
	Retest	0	0	
Wake after sleep onset (percentage of TST)	Baseline	0	0.181598	1.101836
	Retest	0	0	
Sleep efficiency	Baseline	94.8993	97.2404	97.1701
	Retest	93.6704	95.0072	
SWS latency	Baseline	21.5	14.5	15.5
	Retest	14.5	18	
REM latency	Baseline	89.5	177.5	32.5
	Retest	68.5	87.5	

Figure 6.11 sleep stages. The data used to produce this figure were also presented in the data chapter: table 6.3.

Figure 6.12a REM density for whole night: data for each FH participants for both the baseline and retest night

	Baseline	Retest
FH1	43	72
FH2	57	53
FH3	57	

Figure 6.12b and c REM density of each interval: data for each FH participants for both the baseline and retest night for each interval

		Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8
FH1	Baseline	0	0	0	12	2	14	15	0
	Post Film	0	4	0	8	0	14	15	31
FH2	Baseline	0	0	5	0	14	15	8	15
	Post Film	.00	5.00	.00	14.00	3.00	18.00	5.00	8.00
FH3	Baseline	1	6	3	1	5	16	10	15
	Post Film								

9.2. Appendix II: results of non-linear model fitted to aMT6 data

9.2.1. Non Family history participants

	Model			Error			Corrected				
Subject	Degrees of freedom	sum of squares	Mean squares	Degrees of freedom	sum of squares	Mean squares	Degrees of freedom	sum of squares	Mean squares	F value	p value
SD04	2	878860.45	439430.23	5	34646.85	6929.37	7	913507.31	130501.04	63.42	0.00
SD05	2	16031368.05	8015684.02	8	11746286.43	1468285.80	10	27777654.47	2777765.45	5.46	0.03
SD06	2	10917609.92	5458804.96	11	8233329.35	748484.49	13	19150939.28	1473149.18	7.29	0.01
SD07	2	1456921.45	728460.72	12	197640.90	16470.08	14	1654562.35	118183.02	44.23	0.00
SD10	2	4002459.21	2001229.60	2	41904.20	20952.10	4	4044363.40	1011090.85	95.51	0.01
SD11	2	791925.55	395962.77	5	167929.72	33585.94	7	959855.26	137122.18	11.79	0.01
SD13	2	5588491.42	2794245.71	9	1501908.87	166878.76	11	7090400.29	644581.84	16.74	0.00
SD15	2	25452970.83	12726485.42	5	6109155.12	1221831.02	7	31562125.96	4508875.14	10.42	0.02
SD16	2	1487099.22	743549.61	10	2039566.13	203956.61	12	3526665.35	293888.78	3.65	0.06
SD19	2	1632517.13	816258.56	8	716552.18	89569.02	10	2349069.31	234906.93	9.11	0.01
SD21	2	1873965.42	936982.71	4	194388.06	48597.01	6	2068353.47	344725.58	19.28	0.01
SD22	2	3649362.93	1824681.46	9	935076.62	103897.40	11	4584439.55	416767.23	17.56	0.00
SD26	2	1736504.29	868252.15	4	278440.47	69610.12	6	2014944.77	335824.13	12.47	0.02
SD27	3	785571.50	261857.17	4	166349.42	41587.36	7	951920.93	135988.70	6.30	0.05
SD29	2	621954.82	310977.41	3	2778623.98	926207.99	5	3400578.81	680115.76	0.34	0.74
SD30	2	11629965.73	5814982.86	6	4749105.82	791517.64	8	16379071.55	2047383.94	7.35	0.02
SD31	2	7036006.87	3518003.43	7	7313682.78	1044811.83	9	14349689.65	1594409.96	3.37	0.09
SD32	2	4625565.06	2312782.53	8	4410460.59	551307.57	10	9036025.64	903602.56	4.20	0.06
SD33	2	3977724.70	1988862.35	14	19961036.01	1425788.29	16	23938760.71	1496172.54	1.39	0.28
SD34	2	6114428.83	3057214.42	8	3249937.71	406242.21	10	9364366.54	936436.65	7.53	0.01
SD38	2	6292.36	3146.18	7	1088254.01	155464.86	9	1094546.37	121616.26	0.02	0.98
SD41	2	375777.08	187888.54	3	54721.15	18240.38	5	430498.23	86099.65	10.30	0.05
SD42	2	297174.90	148587.45	4	81240.88	20310.22	6	378415.78	63069.30	7.32	0.05
SD43	2	12941896.45	6470948.23	9	12805771.44	1422863.49	11	25747667.90	2340697.08	4.55	0.04
SD47	2	4265961.44	2132980.72	7	1838799.65	262685.66	9	6104761.09	678306.79	8.12	0.01
SD48	2	7207264.76	3603632.38	10	6912071.50	691207.15	12	14119336.27	1176611.36	5.21	0.03
SD53	2	3795663.67	1897831.83	6	393095.27	65515.88	8	4188758.94	523594.87	28.97	0.00
SD54	2	166797769.45	83398884.72	7	49880977.53	7125853.93	9	216678746.98	24075416.33	11.70	0.01
SD56	2	46332362.71	23166181.36	11	5170513.44	470046.68	13	51502876.15	3961759.70	49.28	0.00
SD60	2	6082335.02	3041167.51	7	452537.79	64648.26	9	6534872.81	726096.98	47.04	0.00
SD63	2	15134513.99	7567256.99	4	2060502.80	515125.70	6	17195016.79	2865836.13	14.69	0.01
SD64	2	7691692.11	3845846.05	2	95736.74	47868.37	4	7787428.85	1946857.21	80.34	0.01
SD65	2	12468960.47	6234480.23	13	2548075.01	196005.77	15	15017035.48	1001135.70	31.81	0.00
SD67	2	43465555.48	21732777.74	4	4387370.20	1096842.55	6	47852925.69	7975487.61	19.81	0.01
SD68	2	2885373.43	1442686.71	4	107988.83	26997.21	6	2993362.26	498893.71	53.44	0.00
SD71	2	316824809.37	158412404.68	7	195629432.26	27947061.75	9	512454241.63	56939360.18	5.67	0.03
SD76	2	21573145.19	10786572.60	3	3433825.87	1144608.62	5	25006971.07	5001394.21	9.42	0.05
SD77	2	10422855.15	5211427.58	8	1778779.79	222347.47	10	12201634.94	1220163.49	23.44	0.00
SD78	2	53406705.21	26703352.60	9	2977058.15	330784.24	11	56383763.36	5125796.67	80.73	0.00
SD80	2	15770141.52	7885070.76	4	2009314.99	502328.75	6	17779456.51	2963242.75	15.70	0.01
SD81	2	24423306.16	12211653.08	7	2079888.00	297126.86	9	26503194.17	2944799.35	41.10	0.00

9.2.2. Family history participants

	Model			Error			Corrected				
Subject	Degrees of freedom	sum of squares	Mean squares	Degrees of freedom	sum of squares	Mean squares	Degrees of freedom	sum of squares	Mean squares	F value	p value
SD51	2	807905.19	403952.59	8	301824.02	37728.00	10	1109729.20	110972.92	10.71	0.01
SD55	2	9540310.19	4770155.09	8	4385397.86	548174.73	10	13925708.04	1392570.80	8.70	0.01
SD70	2	24704785.98	12352392.99	4	47041751.10	11760437.77	6	71746537.08	11957756.18	1.05	0.43

9.3. Appendix III: participants included in analysis for study 2 part 1: participants without a family history of a mood disorder

		Sleep deprived	Non sleep deprived
Total number of participants		19	22
Health Questionnaire		19	22
Morningness-eveningness questionnaire		19	22
Mood disorder questionnaire		19	22
General health questionnaire		19	22
Pittsburgh sleep quality index		19	22
Eysenck personality questionnaire		19	22
Profile of mood state		19	21
Actigraphy		17	20
aMT6s acrophase		17	20
Mood VAS: diurnal mood	Day 1: 8am to 10am	18	21
	Day 1: 12pm to 2pm	17	22
	Day 1: 4pm to 6pm	18	22
	Day 1: 8pm to 10pm	18	22
	Day 2: 8am to 10am	19	22
	Day 2: 12pm to 2pm	19	22
	Day 2: 4pm to 6pm	18	22
Mood fluctuation		19	22
Cortisol awakening response		17	22
Mood VAS	pre film	19	22
	post film	19	22
	pre stressor	19	22
	post stressor	19	22
Dissociative state scale		19	22
Film follow up questionnaire		19	22
RAT		19	22
RAT follow up questionnaire		19	22
State trait anxiety index	pre film	19	22
	post film	19	22
	pre stressor	19	22
	post stressor	19	22
Postive and negative affect scale	PM	19	21
	AM	19	22
Activity VAS		12	16
Intrusion diary		18	21
Impact of event scale		19	22

9.4. Appendix IV: Post hoc analysis for each frequency band that showed a time/night interaction for REM and NREM sleep for each interval and channel

Table 9.4.1 Post hoc analysis for each frequency band that showed a time/night interaction for REM and NREM sleep for each interval for the sleep deprived group

Sleep deprived							
NREM sleep				REM sleep			
		F score	p value			F score	p value
Delta	Interval 1	.349	.555	Delta	Interval 2	.679	.412
	Interval 2	.033	.857		Interval 3	.129	.721
	Interval 3	2.880	.091		Interval 4	.048	.827
	Interval 4	1.934	.166		Interval 5	1.774	.186
	Interval 5	5.106	.025		Interval 6	1.499	.224
	Interval 6	1.028	.312		Interval 7	.057	.811
	Interval 7	.797	.373		Interval 8	.028	.868
	Interval 8	4.090	.045	Alpha	Interval 2	.922	.339
Theta	Interval 1	3.597	.059		Interval 3	1.230	.270
	Interval 2	1.618	.205		Interval 4	.613	.436
	Interval 3	.114	.736		Interval 5	.043	.836
	Interval 4	.081	.776		Interval 6	.006	.937
	Interval 5	.009	.924		Interval 7	.246	.621
	Interval 6	.369	.544		Interval 8	.366	.547
	Interval 7	.356	.552	Sigma	Interval 2	.195	.660
	Interval 8	.060	.806		Interval 3	.547	.462
Alpha	Interval 1	5.287	.023		Interval 4	.139	.711
	Interval 2	3.039	.083		Interval 5	.221	.639
	Interval 3	1.802	.181		Interval 6	.016	.899
	Interval 4	.574	.450		Interval 7	.040	.843
	Interval 5	1.672	.198		Interval 8	.237	.627
	Interval 6	.002	.964	Beta1	Interval 2	.042	.838
	Interval 7	1.104	.295		Interval 3	.399	.529
	Interval 8	.764	.383		Interval 4	.025	.876
Beta1	Interval 1	.837	.361		Interval 5	.064	.800
	Interval 2	1.751	.187		Interval 6	.271	.604
	Interval 3	.657	.419		Interval 7	.267	.606
	Interval 4	.213	.645		Interval 8	1.678	.198
	Interval 5	.045	.833	Gamma	Interval 2	.005	.942
	Interval 6	.320	.572		Interval 3	.694	.407
	Interval 7	.006	.936		Interval 4	.002	.965
	Interval 8	.193	.661		Interval 5	.283	.596
Beta2	Interval 1	3.702	.056		Interval 6	8.232	.005
	Interval 2	2.643	.106		Interval 7	11.280	.001
	Interval 3	.751	.387		Interval 8	17.932	.000
	Interval 4	.830	.364				
	Interval 5	.012	.914				
	Interval 6	.142	.707				
	Interval 7	2.974	.086				
	Interval 8	4.929	.028				
Gamma	Interval 1	.837	.361				
	Interval 2	1.715	.192				
	Interval 3	.053	.818				
	Interval 4	.581	.447				
	Interval 5	.698	.405				
	Interval 6	.119	.730				
	Interval 7	.037	.848				
	Interval 8	.302	.583				

Table 9.4.2 Post hoc analysis for each frequency band that showed a time/night interaction for REM and NREM sleep for each interval for the non- sleep deprived group

		Non-sleep deprived					
		NREM sleep				REM sleep	
		F value	p value			F value	p value
Beta 2	Interval 1	.010	.919	Theta	Interval 2	.267	.607
	Interval 2	.178	.674		Interval 3	.073	.788
	Interval 3	.099	.754		Interval 4	.053	.818
	Interval 4	1.500	.222		Interval 5	.004	.950
	Interval 5	.751	.387		Interval 6	.883	.351
	Interval 6	.249	.619		Interval 7	.724	.398
	Interval 7	.576	.449		Interval 8	.034	.853
	Interval 8	.572	.450	Alpha	Interval 2	.684	.411
Gamma	Interval 1	.064	.800		Interval 3	.131	.719
	Interval 2	.034	.855		Interval 4	.238	.627
	Interval 3	.204	.652		Interval 5	.326	.570
	Interval 4	.457	.500		Interval 6	.032	.858
	Interval 5	1.346	.248		Interval 7	.139	.711
	Interval 6	.001	.974		Interval 8	.682	.412
	Interval 7	.619	.433	Beta 1	Interval 2	.483	.490
	Interval 8	.191	.663		Interval 3	1.866	.177
					Interval 4	.708	.403
					Interval 5	.148	.701
					Interval 6	.734	.395
					Interval 7	.058	.811
					Interval 8	.585	.447
				Beta2	Interval 2	2.066	.156
					Interval 3	4.790	.032
					Interval 4	2.374	.128
					Interval 5	1.096	.299
					Interval 6	3.046	.086
					Interval 7	2.946	.091
					Interval 8	1.747	.191
				Gamma	Interval 2	2.600	.112
					Interval 3	3.681	.059
					Interval 4	3.544	.064
					Interval 5	.892	.349
					Interval 6	3.203	.078
					Interval 7	3.818	.055
					Interval 8	3.523	.065

Table 9.4.3 Post hoc analysis for each frequency band that showed a time/night interaction for REM and NREM sleep for each channel for the sleep deprived group

		Sleep deprived					
		NREM sleep				REM sleep	
		F score	p value			F score	p value
Delta	Central	.211	.646	Beta 2	Central	3.519	.062
	PreFronta	.216	.642		PreFronta	4.888	.028
	Frontal	.261	.610		Frontal	6.094	.015
	Occipitial	4.581	.033		Occipitial	.759	.385
	Paritial	2.435	.119		Paritial	4.071	.045
Theta	Central	.632	.427				
	PreFronta	1.697	.193				
	Frontal	.401	.527				
	Occipitial	14.343	.000				
	Paritial	.030	.863				
Alpha	Central	.835	.361				
	PreFronta	1.288	.257				
	Frontal	.647	.422				
	Occipitial	6.657	.010				
	Paritial	.234	.629				
Sigma	Central	.052	.820				
	PreFronta	1.942	.164				
	Frontal	.292	.589				
	Occipitial	13.670	.000				
	Paritial	1.103	.294				
Beta 1	Central	2.610	.107				
	PreFronta	.026	.871				
	Frontal	1.912	.167				
	Occipitial	7.430	.007				
	Paritial	5.364	.021				

Table 9.4.4 Post hoc analysis for each frequency band that showed a time/night interaction for REM and NREM sleep for each channel for the non- sleep deprived group

	Non-sleep deprived		
	NREM sleep		
	F score	p value	
Sigma	Central	.912	.340
	PreFronta	.776	.379
	Frontal	.662	.416
	Occipitial	.289	.591
	Paritial	1.696	.194

9.5. Appendix V: study protocol

Study interview:

- Go over study, show watch, describe details of urine and saliva collection
- Describe mood and sleep experiment
- Detail risks: SD, Film, EEG paste sensitivity test
- Get consent x2
- Get contact details
- Complete check list
- MINI - score
- PSQI - score
- MDQ

Email contact:

- Check doing urine and saliva collections
- Remind when must stop caffeine/alcohol
- Arrange time to set up EEG: remind must come to JR on bus (not number 10), wear comfy t-shirt to sleep in, wear jacket to cover equipment
- Food choices for SD participants
- Buy water

Day two (after first EEG set up):

- Get participants to complete VAS every two hours and as soon as wake up and just before going to bed.

VAS

DIRECTIONS: A number of words describing several moods are given below. Read each mood and then mark on the scale to indicate how you FEEL RIGHT NOW, that is AT THIS MOMNET, ranging from not at all to very. There are no right or wrong answers. Do not spend too much time on each mood but give the answer which seems to describe your present feelings best. Ty to use the full range of the scale and don't look at the scales you

Day three:

- Completion of VAS and KSS

KSS

DIRECTIONS: Please indicate how sleepy you feel AT THE MOMENT on the scale below. Please circle the number.

- Participant wakes up at normal time, EEG removed.

- Ask not to nap during day
- Tell participant to eat dinner before arrive
- Ask participant to arrive at JR between 7-9pm
- Collect food – 5pm
- Buy water

Emotional experience questionnaire order for SD:

- Set up room: close blinds, turn on all lights (260 lux), set up computer (set comfy chair, check volume level (Speakers only: full volume), put sign on door
- Ring floor coordinator: ring switchboard (01865 741166) and ask to bleep 6556 OR dial 89 6556 and extension of phone calling from.
- Collect participant: get them settled in, show bathroom (light switch on outside).
- Ask participant to provide urine sample for drug screening
- Sit down participant in front of computer
- Women only: check day of menstrual cycle

1. Before film:
    a. **State anxiety**

DIRECTONS: A number of statements which people have used to describe themselves are given below. Read each statement and then mark the appropriate number to the right of the statement to indicate how you FEEL RIGHT NOW, that is AT THIS MOMENT. There are no right or wrong answers. Do not spend too much time on any one statement but give the

    b. **VAS**
    c. **DSS**

DIRECTONS: The following questions concern dissociation and how you FEEL RIGHT NOW, that is during THE LAST FEW MINUTES IN THIS ROOM. Read each question and then circle

2. Explanation of film:

You will now see a short film of about 15 minutes. Please sit still and pay close attention. Imagine you are there at the scene watching events unfold in front of your eyes. Please pay attention to the film and try not to look away or shut your eyes. I'm going to be just outside if there are any problems, and I will come in when the film ends. So you don't need to get up once the film has ended. Also you are free to stop watching the film at anytime, just shut down the computer. Any questions?

    a. Turn off the lights
    b. Start film and press start on stop watch
    c. Experimenter exits room and waits outside. Film duration is exactly 15:01 min. Make sure you stay outside of the room in case there are any problems.

3. After film
    a. Experimenter enters room
    b. Turn on the lights and turn off film

c. **DSS –** reiterate that answer questionnaires to how feeling right now, at this moment.
d. **VAS**
e. State anxiety
f. Film follow up
g. Intrusion diary

It may be that over the next few days images or thoughts about the film you have just seen will pop into your mind. This is called an intrusion. What goes through our minds can either take the form of words and phrases ("verbal thoughts"), or it can be like mental images. Although mental images often take the form of pictures in your minds eye they can actually include any of the five senses, so you can imagine sounds too.

Mental images can be fuzzy or fragmented as well as clear; they can also be brief and very fleeting – like a quick flashback of the film. We are interested in knowing about **all** these types of images

[Check participants understands by asking one or several of following & giving feedback.]
Do you understand what I mean by mental images?
Have you had any at all since the film?
Please give me an example of a mental image you have had [if not of the film then from another time].
Please give example of a verbal thought.

So, if you have any intrusions of the film you have just watched over the next week, please record each one as soon as possible in this diary. Keep the diary in your bag or next to your bed and make sure you fill it in **at least** once a day, starting from now. It's really important that you keep it as accurately as possible.
On page 3 and page 4, fill in one of the little boxes every single time you have an intrusion *[demonstrate what to do on part of day that will not be used];* **I** for image, **T** for thought or **IT** if it was both. So if you have 3 intrusions in the afternoon, then 3 of the little boxes should be filled. If you have had none during that time period then write zero or cross that section of the day out.

Next, fill in the details of each intrusion you have had on page 5, putting down the day and time, whether it was an image, thought or both. We then need to know the content of the intrusion. This can be brief but we need to be able to match any intrusions you have to the film so please be as clear as possible. For every box you fill in after having an intrusion there should be a matching line in the back of the diary.
If you are unsure of any of the details, please just make a note in the diary anyway and we will go over it with you later when you return.

Day 1 is today, which is XXX *[Fill in all boxes with the day and date for the participant]*. My details are also on the diary – if you have any questions please get in contact with me. This diary is vital for the study as we won't be able to get any results without it. Remember, if you don't have any intrusions; please still return it as we are interested in those results too. Do you understand how to use it?
Don't forget:
Mental images can be pictures in your mind but also sounds and sensations
Mental images maybe fuzzy, very brief or almost like a flash in your minds eye.
Even if you have several images that are the same please record each individual one in the diary

4. EEG set up

5. EEG bio-calibration:

Sit quietly: look forward (no movement in EOG)
Blink 5 times (see in EOG)

Look left
Look right
Grind teeth (EMG more tense)
Close eyes (Alpha activity in Oz can change)

6. PANAS

This scale consists of a number of words that describe different feelings and emotions. Read each item and then mark the appropriate answer in the space next to the word. Indicate to what extent you feel this way **right now**, that is, **at the present moment**.

7. Activity Log

8. SD:

a. VAS/KSS every two hours (10pm, 12 (midnight), 2am, 4, 6, 8, 10, 12 (midday), 2pm, 4, 6)

b. remind participant to fill in intrusion diary

c. food: 10pm (sandwich), 12 (fruit), 2 (sandwich), 4 (fruit), 6 (sandwich), 8 (sandwich), 10 (fruit), 12 (sandwich), 2 (fruit), 4 (sandwich)

non SD: give **VAS/KSS** to start in morning

Day four:

- Completion of VAS and KSS every two hours and as soon as wake up and just before go to bed.
- Remove EEG
- 10am:
  a. Activity VAS
  b. **PANAS**
  c. **Impact of event scale**

DIRECTONS: Below is a list of comments made by people after stressful life events. Please read each item, indicating how distressing each difficulty has been SINCE YESTERDAY EVENING ***in relation to the topics in the film you saw last night***. If they did not occur during that time, please circle the "not at all" answer.

1. Before RAT
   a. **State anxiety**
   b. **VAS**
   c. **Response sheet**
2. RAT - you are now going to complete a test of creative intelligence

In the following experiment, you will be given a test designed to measure aspects of creativity. Although brief, the test is highly correlated with measures of creative intelligence. Creative intelligence is important in many areas of life and is associated with innovative thinking, improved problem-solving ability, wit and verbal humour, and the ability to communicate in an interesting and persuasive manner through writing or speech. (= as in Moberly & Watkins, 2006)

In each trial, you will be shown three words that are all semantically related to a fourth, unreported word. Please define this word and record it on the response sheet as quickly as possible. Each item will be presented for 30s.

Please press the space bar for two examples.

1. WIDOW – BITE – MONKEY (spider)
2. HEAD- STREET – DARK (light)

Now you will be shown 10 experimental trials. You have 30s available for each item. If there are any more questions please let the experimenter know now. If you are ready to continue please press the space bar.

1. BASS - COMPLEX – SLEEP (deep)
2. CHAMBER - STAFF – BOX (music)
3. DESERT – ICE – SPELL (dry)
4. BASE – SHOW – DANCE (ball)
5. INCH – DEAL – PEG (square)
6. SOAP – SHOE – TISSUE (box)
7. BLOOD – MUSIC – CHEESE (blue)
8. SKUNK – KINGS – BOILED (cabbage)
9. JUMP – KILL – BLISS (joy)
10. SHOPPING – WASHER – PICTURE (window)

Thank you very much!

Previous studies indicate that amongst university students, scores of 7 or more indicate above-average levels of intelligence. (= as in Moberly & Watkins, 2006)
Please wait for the experimenter to analyse and report your test result.

[give feedback, then RAT Follow-up Questionnaire]

3. After RAT
    a. **State anxiety**
    b. **VAS**
    c. **Follow up**
4. Debrief
5. Allow shower
6. Continue with VAS/KSS every two hours
7. Book taxi
8. 6pm EEG set up
9. Get to sign expense forms/ collect bus tickets/ bank details/ NI number

Day five:

1. Remove EEG
2. Go over intrusion diary
3. Give new intrusion diary

Day seven:

1. Collect intrusion diary
2. Go over intrusion diary to check understand all entries.

END OF STUDY

9.6. Appendix VI: questionnaires and scales

9.6.1. Munich chronotype questionnaire

How and why does the biological clock tick? We all know that individuals show distinct preferences for various activities over the course of a day. A simple example is the time at which an individual prefers to go to bed and to get up. With the help of this questionnaire, we aim to understand the underlying complexity of the biological clock and individual differences in the biological clock, as shown in everyday behaviour. Once your questionnaire has been submitted, an automatic evaluation of your CHRONOTYPE (your personal profile) will be sent to you via email. You will see how your results compare to the ones of more than 50,000 other individuals that have so far filled out the questionnaire. After submission of your questionnaire, you will also be provided with a link to relevant literature. If you are interested in this feedback, make sure to provide a correct email address.

We kindly ask you to provide your name and address to enable us to contact you again in case your CHRONOTYPE is of particular interest to our study. Please also give your exact postal code, so that we can compare chronotypes living in different geographical locations (longitude and latitude).

We ask you to provide information as to your age, height and weight, in order to see whether these influence your sleep habits. ALL personal information is strictly protected by the rules of the University of Munich Ethics Commission and will not be visible during data analysis. Your address can only be accessed by Prof. Roenneberg and will only be retrieved in case we are interested in more specific information from your part. Please read the separate document about confidentiality prior to filling out the questionnaire.

Important!!! Do not fill out this questionnaire if you have been working on a shift work schedule within the last 3 months. Please go to the following link if you are a shift worker.

This questionnaire is also available in German, Dutch and French. As of yet, we do only have personal chronotype evaluations in German and English. Evaluations for the French and Dutch questionnaire will therefore be sent in English.

Please try to answer ALL questions, even when an answer seems difficult! Spontaneous answers are often the best.

Please help us in the evaluation of your data by providing unambiguous time references (e.g. 23:00 rather than 11:00 PM).

Confidentiality policy

All of the information you provide in the Chronotype questionnaire is covered by the provisions of the Ethics Commission of the University of Munich and will be

treated with the utmost confidentiality.

Your personal information (name and address, etc.) will be coded and stored separately from your sleep and activity data, allowing an anonymous analysis of your data. Only the head of the Centre for Chronobiology, Prof. Till Roenneberg, has access to your personal information which will solely be retrieved in case we wish to contact you for further inquiries. This will only be the case if your information indicates that you are of outstanding interest to our study (typically less than 5% of all participants). By filling out the questionnaire, you are under no obligation to any further participation. Your personal information will under no circumstances be released to a third party, and will be erased at completion of the study.

I do not want to continue.

Continue to the questionnaire.

Page two of MCTQ website – only accessed in participant contents to continue.

Instructions: The following questionnaire will ask you questions in regards to your sleep and wake behaviour. Please respond to the questions according to your perception of a standard week, based on your most current living conditions. All fields are required unless otherwise specified.

Personal Information

Date	2009.8.18 - 12:35
Name	
Email	(We cannot send you a personal assessment of your chronotype if you do do not provide an email address.)
Age	
Gender	Female Male
Height	cm
Weight	kg
Country	
City	

Postal Code

Referral Information

If you were referred to this site by a particular doctor or if you are part of a specific project, then please enter the keyword or key phrase that you were given below:

Work Schedule

I have a regular work schedule (this includes being a housewife or househusband):

Yes No

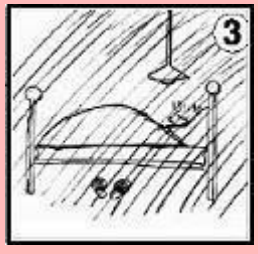
If 'YES', how many days per week?

Instructions: Please complete all of the following sections, regardless of whether you are working on a regular basis or not. Use the 24 hour scale, for example 23:00 instead of 11:00PM!!!!

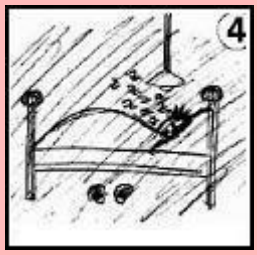
Work Days

I go to bed at : o'clock.

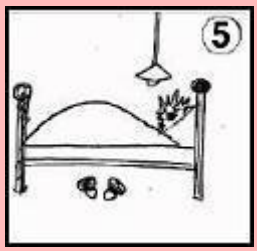
Note that some people stay awake for some time when in bed!



I actually get ready to fall asleep at : o'clock.



I need ____ minutes to fall asleep.



I wake up at ____ : ____ o'clock.,

- with an alarm clock
- without an alarm clock

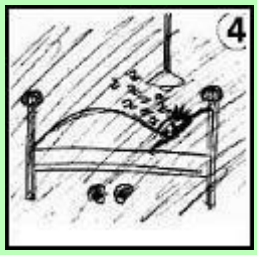
After ____ minutes, I get up.

Free Days

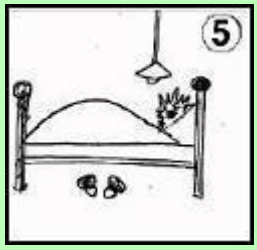
I go to bed at ____ : ____ o'clock.

Note that some people stay awake for some time when in bed!

I actually get ready to fall asleep at ____ : ____ o'clock.



I need ____ minutes to fall asleep.



I wake up at ____ : ____ o'clock.

- with an alarm clock
- without an alarm clock

After ____ minutes, I get up.

Comment Field: Please leave a comment if you currently have NO possibility of freely choosing your sleep times (e.g. because of pet(s), child(ren) etc.). Use this field also to provide additional information, if the system asks for it:

Daylight Exposure

On average, I spend the following amount of time outdoors in daylight (without a roof above my head):

on workdays	____ hours	____ minutes
on free days	____ hours	____ minutes

Submit Reset

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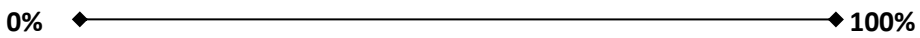
9.6.2. Activity visual analogue scale

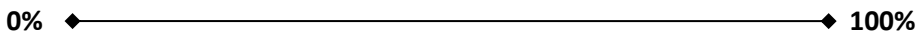
Activities rating

Study code_____Date_____

Please rate how much of your **time**, from 0% to 100%, has been taken up by the following activities since you watched the film last night.



Using your imagination e.g. jigsaw, cards, board games



Talking to other people, reading, writing

9.6.3. Dissociative state scale

DSS RATING SCALE

Subject ID_____Date_____

		Not at all	Slightly	Moderately	Considerably	Extremely
1.	Do things seem to be moving in slow motion? ..…….	0	1	2	3	4
2.	Do things seem unreal to you, as if you are in a dream? ..……..	0	1	2	3	4
3.	Do you have some experience that separates you from what is happening; for instance, do you feel as if you are in a movie or a play, or as if you are a robot? ……..	0	1	2	3	4
4.	Do you feel as if you are looking at things from outside of your body? ……..	0	1	2	3	4
5.	Do you feel as if you are watching the situation as an observer or spectator? …..……..	0	1	2	3	4
6.	Do you feel disconnected from your own body? …….	0	1	2	3	4
7.	Does your sense of your own body feel changed; for instance, does your own body feel unusually large or unusually small?	0	1	2	3	4
8.	Do people seem motionless, dead, or mechanical? ….……	0	1	2	3	4
9.	Do objects look different than you would expect? ….…….	0	1	2	3	4
10.	Do colours seem diminished in intensity? ……...	0	1	2	3	4
11.	Do you see things as if you were in a tunnel or looking through a wide angle photographic lense? ..……..	0	1	2	3	4
12.	Does this experience seem to take much longer than you would have expected? ……	0	1	2	3	4
13.	Do things seem to be happening very quickly, as if there is a lifetime in a moment? …….	0	1	2	3	4
14.	Do things happen that you later cannot account for? …….	0	1	2	3	4
15.	Do you space out, or in some other way, lose track of what is going on? ……..	0	1	2	3	4
16.	Do sounds almost disappear or become much stronger that you would have expected? …….	0	1	2	3	4
17.	Do things seem to be very real, as if there is a special sense of clarity? …….	0	1	2	3	4
18.	Does it seem as if you are looking at the world through a fog, so that people or objects seem far away or unclear? ……	0	1	2	3	4
19.	Do colours seem much brighter than you would have expected?	0	1	2	3	4

9.6.4. Eysenck personality questionnaire

Study code_____Date_____

INSTRUCTIONS Please answer each question by putting a circle around the "YES" or the "NO" following the question. There are no right or wrong answers, and no trick questions. Work quickly and do not think too long about the exact meaning of the questions.

PLEASE REMEMBER TO ANSWER EACH QUESTION

1. Do you have many different hobbies?	YES	NO
2. Do you stop to think things over before doing anything?	YES	NO
3. Does your mood often go up and down?	YES	NO
4. Have you ever taken the praise for something you knew someone else had really done?	YES	NO
5. Are you a talkative person?	YES	NO
6. Would you being in debt worry you?	YES	NO
7. Do you ever feel "just miserable" for no reason?	YES	NO
8. Were you ever greedy by helping yourself to more than your share of anything?	YES	NO
9. Do you lock up your house carefully at night?	YES	NO
10. Are you rather lively?	YES	NO
11. Would it upset you a lot to see a child or an animal suffer?	YES	NO
12. Do you often worry about things you should not have done or said?	YES	NO
13. If you say you will do something, do you always keep your promise no matter how inconvenient it might be?	YES	NO
14. Can you usually let yourself go and enjoy yourself at a lively party?	YES	NO
15. Are you an irritable person?	YES	NO
16. Have you ever blamed someone for doing something you knew was really your fault?	YES	NO
17. Do you enjoy meeting new people?	YES	NO
18. Do you believe insurance schemes are a good idea?	YES	NO
19. Are your feelings easily hurt?	YES	NO
20. Are *all* your habits good and desirable ones?	YES	NO
21. Do you tend to keep in the background on social occasions?	YES	NO
22. Would you take drugs which may have strange or dangerous effects?	YES	NO
23. Do you often feel "fed-up"?	YES	NO

PLEASE TURN OVER Page 1

24. Have you ever taken anything (even a pin or button) that belonged to someone else?	YES	NO
25. Do you like going out a lot?	YES	NO
26. Do you enjoy hurting people you love?	YES	NO
27. Are you often troubled about feelings of guilt?	YES	NO

28. Do you sometimes talk about things you know nothing about? YES NO
29. Do you prefer reading to meeting people? YES NO
30. Do you have enemies who want to harm you? YES NO
31. Would you call yourself a nervous person? YES NO
32. Do you have many friends? YES NO
33. Do you enjoy practical jokes that can sometimes really hurt people? YES NO
34. Are you a worrier? YES NO
35. As a child did you do as you were told immediately and without grumbling? YES NO
36. Would you call yourself happy-go-lucky? YES NO
37. Do good manners and cleanliness matter much to you? YES NO
38. Do you worry about awful things that might happen? YES NO
39. Have you ever broken or lost something belonging to someone else? YES NO
40. Do you usually take the initiative in making new friends? YES NO
41. Would you call yourself tense or “highly-strung”? YES NO
42. Are you mostly quiet when you are with other people? YES NO
43. Do you think marriage is old-fashioned and should be done away with? YES NO
44. Do you sometimes boast a little? YES NO
45. Can you easily get some life into a rather dull party? YES NO
46. Do people who drive carefully annoy you? YES NO
47. Do you worry about your health? YES NO
48. Have you ever said anything bad or nasty about anyone? YES NO
49. Do you like telling jokes and funny stories to your friends? YES NO
50. Do most things taste the same to you? YES NO
51. As a child were you ever cheeky to your parents? YES NO
52. Do you like mixing with people? YES NO
53. Does it worry you if you know there are mistakes in your work? YES NO
54. Do you suffer from sleeplessness? YES NO
55. Do you always wash before a meal? YES NO
56. Do you nearly always have a “ready answer” when people talk to you? YES NO
57. Do you like to arrive at appointments in plenty of time? YES NO
58. Have you often felt listless and tired for no reason? YES NO
59. Have you ever cheated at a game? YES NO
60. Do you like doing things in which you have to act quickly? YES NO
61. Is (or was) your mother a good woman? YES NO

62. Do you often feel life is very dull? YES NO
63. Have you ever taken advantage of someone? YES NO
64. Do you often take on more activities than you have time for? YES NO
65. Are there several people who keep trying to avoid you? YES NO

66. Do you worry a lot about your looks?	YES	NO
67. Do you think people spend too much time safeguarding their future with savings and insurances?	YES	NO
68. Have you ever wished that you were dead?	YES	NO
69. Would you dodge paying taxes if you were sure you could never be found out?	YES	NO
70. Can you get a party going?	YES	NO
71. Do you try not to be rude to people?	YES	NO
72. Do you worry too long after an embarrassing experience?	YES	NO
73. Have you ever insisted on having your own way?	YES	NO
74. When you catch a train do you often arrive at the last minute?	YES	NO
75. Do you suffer from “nerves”?	YES	NO
76. Do your friendships break up easily without it being your fault?	YES	NO
77. Do you often feel lonely?	YES	NO
78. Do you always practice what you preach?	YES	NO
79. Do you sometimes like teasing animals?	YES	NO
80. Are you easily hurt when people find fault with you or the work you do?	YES	NO
81. Have you ever been late for an appointment or work?	YES	NO
82. Do you like plenty of bustle and excitement around you?	YES	NO
83. Would you like other people to be afraid of you?	YES	NO
84. Are you sometimes bubbling over with energy and sometimes very sluggish?	YES	NO
85. Do you sometimes put off until tomorrow what you ought to do today?	YES	NO
86. Do other people think of you as being very lively?	YES	NO
87. Do people tell you a lot of lies?	YES	NO
88. Are you touchy about some things?	YES	NO
89. Are you always willing to admit it when you have made a mistake?	YES	NO
90. Would you feel very sorry for an animal caught in a trap?	YES	NO

PLEASE CHECK TO SEE THAT YOU HAVE ANSWERED ALL THE QUESTIONS

9.6.5. General health questionnaire

Participant number:…….

Date completed:……..

Have you recently (last two weeks):

1. been able to concentrate on what you're doing?
    - better than usual
    - same as usual
    - less than usual
    - much less than usual
2. lost much sleep over worry?
    - not at all
    - no more than usual
    - rather more than usual
    - much more than usual
3. felt that you are playing a useful part in things?
    - more so than usual
    - same as usual
    - less so than usual
    - much less than usual
4. felt capable of making decisions about things?
    - more so than usual
    - same as usual
    - less so than usual
    - much less than usual
5. felt constantly under strain?
    - not at all
    - no more than usual
    - rather more than usual
    - much more than usual
6. felt you couldn't overcome your difficulties?
    - not at all
    - no more than usual
    - rather more than usual
    - much more than usual
7. been able to enjoy your normal day to day activities?
    - more so than usual

- same as usual
- less so than usual
- much less than usual

8. been able to face up to your problems?
    - more so than usual
    - same as usual
    - less so than usual
    - much less than usual
9. been feeling unhappy or depressed?
    - not at all
    - no more than usual
    - rather more than usual
    - much more than usual
10. been losing confidence in yourself?
    - not at all
    - no more than usual
    - rather more than usual
    - much more than usual
11. been thinking of yourself as a worthless person?
    - not at all
    - no more than usual
    - rather more than usual
    - much more than usual
12. been feeling reasonable happy, all things considered?
    - more so than usual
    - same as usual
    - less so than usual
    - much less than usual

9.6.6. Health questionnaire

Health Questionnaire

Participant number:…….
Date completed:……..

General Part

1. Date of birth…….
2. Age……...
3. Weight……..
4. Height:……..
5. Sex: Male/Female
6. Ethic group: Please tick one appropriate group

A White
☐ British
☐ Any Other White background

B Mixed
☐ White and Black Caribbean
☐ White and Black African
☐ White and Asian
☐ Any Other Mixed background

C Asian or Asian British
☐ Indian
☐ Pakistani
☐ Bangladeshi
☐ Any Other Asian background

D Black or Black British
☐ Caribbean
☐ African
☐ Any Other Black background

E Chinese or other ethnic group
☐ Chinese
☐ Any Other

7. Collar size: Male: Less than 17 inches ( )
17 inches or greater ( )
Female: Less than 16 inches ( )
16 inches or greater ( )

8. Current status: Please circle which describes you best:

Single (never married) Married Partnership
In a relationship Divorced Widowed
Separated

Children: Yes/No

9. Current living situation: Please circle which best describes your living situation:

Alone With Partner With Friends
With Family With Parents

10. What type of accommodation do you live in:

House Nursing Home Supported Housing
Flat Shared Accommodation Hospital

11. What is the highest level of education you have ever completed: Please circle one.

Secondary school Polytechnic
Apprenticeship Bachelor degree (BSc/BA)
Grammar school Masters degree
Doctoral degree

Other, please describe:…….

Medical Part

12. Have you ever been told that you have high blood pressure?
Yes No

If Yes, please give details
……
……..

13. Have you had any history of heart trouble?
Yes No

If Yes, please give details

…….....
.....

14. Have you a family history of heart disease/stroke?

Yes No

If Yes, please give details

……...……..……
…….....

15. Have you ever been told by a doctor that you have asthma?

Yes No

16. Do you suffer from a wheezy chest?

Yes No

17. Do you ever have pains in your heart and chest?

Yes No

18. Do you ever feel faint or have spells of dizziness?

Yes No

19. Has a doctor ever told you that you have a bone or joint problem which could be made worse by exercise?

Yes No

20. Have you ever been told by a doctor that you have restless leg syndrome?

Yes No

If yes, please give details

……..……
…….....

21. Have you, or a member of your family, ever been told by a doctor that you have any mental health disorders such as depression?

Yes No

If yes, please give details

……..……
…….....

22. Have you ever received any treatment for mental health problems including medication, psychological therapy or counselling?

Yes No

If yes, please give details

……..……
……..

23. Have you been in hospital at all in the last two years?
Yes No

If Yes, please give reason and outcome
……
……..

24. Have you had any operations or major illness in the last 6 months?
Yes No

If Yes, please give details
……
…….....**.....**

25. Are you undergoing treatment or having any regular medication at the moment?
Yes No

If Yes, please give details
……
……
……..

26. Are you taking any pills or any other medicines, including inhalers, for any of the following:

Heart trouble/angina?……..Yes/No
Chest pains or blood pressure?…….Yes/No
Asthma or other chest diseases?**.....**Yes/No
Contraceptives or enhancing pills?**.....**Yes/No
Or for anything else?**.....**Yes/No

If Yes, please give full details:
……
……
……..

27. Do you have any physical disabilities of any kind?
Yes No

If Yes, please give details
……
……..

28. Have you ever suffered any complications from previous trials or treatments?
Yes No

If Yes, please give details

……
……..

29. Do you suffer from any allergies?

Yes No

If Yes, please give details

……
……..

30. Have you ever had an adverse reaction to a medicine or food?

Yes No

If Yes, please give details

……
……..

31. What is your average weekly intake of alcohol?

……..units per week

(One unit is half a pint of beer/one glass of wine/one measure of spirits)

32. Has there been a time when you regularly consumed more than 14 units of alcohol per week?

Yes No

If Yes, please give details

……
……..

33. How many cigarettes, cigars and/or tobacco do you smoke:

None 1-5 a day 5-10 a day

10-20 a day more than 20 per day

Less than 10 a month

Other, please specify:

……
……..

34. Do you consume caffeine?

If Yes please give details of the type and amount of caffeine consumed

……
……..

35. Have you, or a member of your family, ever been told by a doctor that you had epilepsy problems?

Yes No

If Yes, please give details

……
……..

36. Have you recently been checked for glaucoma by an optician?

Yes No

If Yes, have you been diagnosed with glaucoma?

Yes No

37. Have you ever been told by a medical professional that you have any other problems with your eyes or vision?

Yes No

If Yes, please give details

……
……..

38. Have you done any shift work in the last year?

Yes No

39. Have you travelled across more than one time zone in the past 3 months?

Yes No

40. *(Women only)* Do you have regular menstrual cycles? (ranging in length from 26-35 days with a maximum of three days variation month to month).

Yes No

9.6.7. Impact of event scale

DIRECTONS: Below is a list of comments made by people after stressful life events. Please read each item, indicating how distressing each difficulty has been SINCE YESTERDAY EVENING ***in relation to the topics in the film you saw last night***. If they did not occur during that time, please circle the "not at all" answer.

		Not at all	A little bit	Moderately	Quite a bit	Extremely
1	Any reminder brought back feelings about the film	0	1	2	3	4
2	I had trouble remaining in a restful state	0	1	2	3	4
3	Other things kept making me think about the film	0	1	2	3	4
4	I felt irritable and angry.	0	1	2	3	4
5	I avoided letting myself get upset when I thought about the film or was reminded of the film.	0	1	2	3	4
6	I thought about the film when I didn't mean to.	0	1	2	3	4
7	I felt as if it hadn't happened or wasn't real.	0	1	2	3	4
8	I stayed away from reminders about the film	0	1	2	3	4
9	Pictures about it popped into my mind.	0	1	2	3	4
10	I was jumpy and easily startled.	0	1	2	3	4
11	I tried not to think about the film	0	1	2	3	4
12	I was aware that I still had a lot of feelings about the film, but I didn't deal with them.	0	1	2	3	4
13	My feelings about the film were kind of numb.	0	1	2	3	4
14	I found myself acting or feeling like I was back at that time.	0	1	2	3	4
15	I had trouble falling into a rested state.	0	1	2	3	4
16	I had waves of strong feelings about the film	0	1	2	3	4
17	I tried to remove the film from my memory.	0	1	2	3	4
18	I had trouble concentrating.	0	1	2	3	4
19	Reminders of the film caused me to have physical reactions, such as sweating, trouble breathing, nausea, or a pounding heart.	0	1	2	3	4
20	I had dream-like experiences about the film.	0	1	2	3	4
21	I felt watchful and on guard.	0	1	2	3	4
22	I tried not to talk about the film	0	1	2	3	4

9.6.8. Intrusion diary

16.10.09
Version 2
REC number:09/H0603/2

Thank you for completing the diary, even if you have zero intrusions, we are still interested. Your participation is very much appreciated.

Thank you!!!

Follow up Session Appointment Card

Date:
Time:
Duration:

If you have any questions or problems, please do not hesitate to contact me at kate.porcheret@eye.ox.ac.uk

If you would like to discuss anything related to the experiment or your reactions to it, please do not hesitate to contact the responsible Investigator, Dr. Katharina Wulff, at any point in the week after the film or beyond. Dr. Katharina Wulff can be contacted at the Nuffield Laboratory of Ophtalmology, Level 6 West Wing, The John Radcliffe Hospital, Oxford, OX3 9DU. Tel 01865 234780. email katharina.wulff@eye.ox.ac.uk

p8

PARTICIPANT DIARY

Participant No.:

Date started:

Date completed:

p1

Introduction

❖If over the next few days you experience any **spontaneously occurring intrusions/memories** about the film you have just watched, I would be very grateful if you could note them down in the diary.

❖Note that this is only for intrusions/memories that pop into your mind **spontaneously**, not times when you deliberately think about it.

❖What goes through our minds can either take the form of words and phrases ("verbal thoughts"), or it can be like mental pictures ("images"). Although mental images often take the form of pictures they can actually include any of the five senses, so you can imagine sounds too.

❖For each intrusion, mark 'I', 'T' or 'IT' on pages 3 and 4 for the corresponding time, or **write down that you have had Zero** in that time frame, and then fill in the details of the content of intrusion pages 5, 6 and 7.

❖If you are on occasion unable to record details, please make sure you record that an intrusion has occurred and the date.

**PLEASE KEEP THIS DIARY – IT IS VITAL FOR THE EXPERIMENT.
THANK YOU!!!!**

p2

Date / Day of Intrusion	Was it an Image (I) Thought (T) or both (IT)?	What was the content of the intrusion? (e.g. subject matter)	What, if anything, triggered the intrusion?	How distressed were you at the moment? 0 (not at all) - 10 (extremely)

p7

Date / Day of Intrusion	Was it an Image (I) Thought (T) or both (IT)?	What was the **content** of the intrusion? (e.g. subject matter)	What, if anything, triggered the intrusion?	How **distressed** (own scale) were you at the intrusion? 0 (not at all) - 10 (extremely)?

Mark each intrusion as 'I' for image, 'T' for thought or IT if it is a combination of both. Otherwise write '0' for that timeframe.

Example Day 1	
Morning	0
Afternoon	I
Evening	I

Day 1	Date:
Morning	
Afternoon	
Evening	
Night	

Day 2	Date:
Morning	
Afternoon	
Evening	

Day 3	Date:
Morning	
Afternoon	
Evening	

Please remember to fill in the content page for each intrusion indicated. Thank you

Day 4	Date:
Morning	
Afternoon	
Evening	

Day 5	Date:
Morning	
Afternoon	
Evening	

Day 6	Date:
Morning	
Afternoon	
Evening	

Day 7	Date:
Morning	
Afternoon	
Evening	

Please remember to fill in the content page for each intrusion indicated. Thank you

Content Page

Intrusions of the film can include **mental images**, (that is 'see' or 'hear' in your minds eye) and/or **verbal thoughts**, (thoughts about using verbal language when we talk) or combination of both image plus verbal thoughts.

Date / Day of Intrusion	Was it an Image (I) Thought (T) or both (IT)?	What was the **content** of the intrusion? (e.g. subject matter)	What, if anything, triggered the intrusion?	How distressed (own scale) were you at the intrusion? 0 (not at all) - 10 (extremely)?
Day 1	I	Saw underwater driver	Running the tap	8

M.I.N.I.

MINI INTERNATIONAL NEUROPSYCHIATRIC INTERVIEW

English Version 5.0.0

DSM-IV

USA: **D. Sheehan, J. Janavs, R. Baker, K. Harnett-Sheehan, E. Knapp, M. Sheehan**
University of South Florida - Tampa

FRANCE: **Y. Lecrubier, E. Weiller, T. Hergueta, P. Amorim, L. I. Bonora, J. P. Lépine**
Hôpital de la Salpétrière - Paris

M.I.N.I. 5.0.0 (July 1, 2005)

Patient Name:	*Patient Number:*
Date of Birth:	*Time Interview Began:*
Interviewer's Name:	*Time Interview Ended:*
Date of Interview:	*Total Time:*

	MODULES	TIME FRAME	MEETS CRITERIA	DSM-IV	ICD-10
A	MAJOR DEPRESSIVE EPISODE	Current (2 weeks)	☐	296.20-296.26 Single	F32.x
		Recurrent	☐	296.30-296.36 Recurrent	F33.x
	MDE WITH MELANCHOLIC FEATURES Optional	Current (2 weeks)	☐	296.20-296.26 Single 296.30-296.36 Recurrent	F32.x F33.x
B	DYSTHYMIA	Current (Past 2 years)	☐	300.4	F34.1
C	SUICIDALITY	Current (Past Month) Risk: ☐ Low ☐ Medium ☐ High	☐		
D	MANIC EPISODE	Current	☐	296.00-296.06	F30.x-F31.9
		Past	☐		
	HYPOMANIC EPISODE	Current	☐	296.80-296.89	F31.8-F31.9/F34.0
		Past	☐		
E	PANIC DISORDER	Current (Past Month)	☐	300.01/300.21	F40.01-F41.0
		Lifetime	☐		
F	AGORAPHOBIA	Current	☐	300.22	F40.00
G	SOCIAL PHOBIA (Social Anxiety Disorder)	Current (Past Month)	☐	300.23	F40.1
H	OBSESSIVE-COMPULSIVE DISORDER	Current (Past Month)	☐	300.3	F42.8
I	POSTTRAUMATIC STRESS DISORDER Optional	Current (Past Month)	☐	309.81	F43.1
J	ALCOHOL DEPENDENCE	Past 12 Months	☐	303.9	F10.2x
	ALCOHOL ABUSE	Past 12 Months	☐	305.00	F10.1
K	SUBSTANCE DEPENDENCE (Non-alcohol)	Past 12 Months	☐	304.00-.90/305.20-.90	F11.1-F19.1
	SUBSTANCE ABUSE (Non-alcohol)	Past 12 Months	☐	304.00-.90/305.20-.90	F11.1-F19.1
L	PSYCHOTIC DISORDERS	Lifetime	☐	295.10-295.90/297.1/	F20.xx-F29
		Current	☐	297.3/293.81/293.82/ 293.89/298.8/298.9	
	MOOD DISORDER WITH PSYCHOTIC FEATURES	Lifetime	☐	296.24/296.34/296.44	F32.3/F33.3/
		Current	☐	296.24/296.34/296.44	F30.2/F31.2/F31.5/ F31.8/F31.9/F39
M	ANOREXIA NERVOSA	Current (Past 3 Months)	☐	307.1	F50.0
N	BULIMIA NERVOSA	Current (Past 3 Months)	☐	307.51	F50.2
	ANOREXIA NERVOSA, BINGE EATING/PURGING TYPE	Current	☐	307.1	F50.0
O	GENERALIZED ANXIETY DISORDER	Current (Past 6 Months)	☐	300.02	F41.1
P	ANTISOCIAL PERSONALITY DISORDER Optional	Lifetime	☐	301.7	F60.2

DISCLAIMER

Our aim is to assist in the assessment and tracking of patients with greater efficiency and accuracy. Before action is taken on any data collected and processed by this program, it should be reviewed and interpreted by a licensed clinician. This program is not designed or intended to be used in the place of a full medical and psychiatric evaluation by a qualified licensed physician – psychiatrist. It is intended only as a tool to facilitate accurate data collection and processing of symptoms elicited by trained personnel.

GENERAL INSTRUCTIONS

The M.I.N.I. was designed as a brief structured interview for the major Axis I psychiatric disorders in DSM-IV and ICD-10. Validation and reliability studies have been done comparing the M.I.N.I. to the SCID-P for DSM-III-R and the CIDI (a structured interview developed by the World Health Organization for lay interviewers for ICD-10). The results of these studies show that the M.I.N.I. has acceptably high validation and reliability scores, but can be administered in a much shorter period of time (mean 18.7 ± 11.6 minutes, median 15 minutes) than the above referenced instruments. It can be used by clinicians, after a brief training session. Lay interviewers require more extensive training.

INTERVIEW:

In order to keep the interview as brief as possible, inform the patient that you will conduct a clinical interview that is more structured than usual, with very precise questions about psychological problems which require a yes or no answer.

GENERAL FORMAT:

The M.I.N.I. is divided into **modules** identified by letters, each corresponding to a diagnostic category.

- At the beginning of each diagnostic module (except for psychotic disorders module), screening question(s) corresponding to the main criteria of the disorder are presented in a **gray box**.
- At the end of each module, diagnostic box(es) permit the clinician to indicate whether diagnostic criteria are met.

CONVENTIONS:

Sentences written in « normal font » should be read exactly as written to the patient in order to standardize the assessment of diagnostic criteria.

Sentences written in « CAPITALS » should not be read to the patient. They are instructions for the interviewer to assist in the scoring of the diagnostic algorithms.

Sentences written in « **bold** » indicate the time frame being investigated. The interviewer should read them as often as necessary. Only symptoms occurring during the time frame indicated should be considered in scoring the responses.

Answers with an arrow above them (➨) indicate that one of the criteria necessary for the diagnosis(es) is not met. In this case, the interviewer should go to the end of the module, circle « **NO** » in all the diagnostic boxes and move to the next module.

When terms are separated by a *slash (/)* the interviewer should read only those symptoms known to be present in the patient (for example, question H6).

Phrases in (parentheses) are clinical examples of the symptom. These may be read to the patient to clarify the question.

RATING INSTRUCTIONS:

All questions must be rated. The rating is done at the right of each question by circling either Yes or No. Clinical judgment by the rater should be used in coding the responses. The rater should ask for examples when necessary, to ensure accurate coding. The patient should be encouraged to ask for clarification on any question that is not absolutely clear.

The clinician should be sure that each dimension of the question is taken into account by the patient (for example, time frame, frequency, severity, and/or alternatives).
Symptoms better accounted for by an organic cause or by the use of alcohol or drugs should not be coded positive in the M.I.N.I. The M.I.N.I. Plus has questions that investigate these issues.

For any questions, suggestions, need for a training session, or information about updates of the M.I.N.I., please contact :

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A. MAJOR DEPRESSIVE EPISODE

(➨ MEANS : GO TO THE DIAGNOSTIC BOXES, CIRCLE NO IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

A1	Have you been consistently depressed or down, most of the day, nearly every day, for the past two weeks?	NO	YES
A2	In the past two weeks, have you been much less interested in most things or much less able to enjoy the things you used to enjoy most of the time?	NO	YES
	➨ IS **A1** OR **A2** CODED **YES?**	NO	YES

A3 **Over the past two weeks, when you felt depressed or uninterested:**

a	Was your appetite decreased or increased nearly every day? Did your weight decrease or increase without trying intentionally (i.e., by ±5% of body weight or ±8 lbs. or ±3.5 kgs., for a 160 lb/70 kg. person in a month)? IF YES TO EITHER, CODE YES.	NO	YES *
b	Did you have trouble sleeping nearly every night (difficulty falling asleep, waking up in the middle of the night, early morning wakening or sleeping excessively)?	NO	YES
c	Did you talk or move more slowly than normal or were you fidgety, restless or having trouble sitting still almost every day?	NO	YES *
d	Did you feel tired or without energy almost every day?	NO	YES
e	Did you feel worthless or guilty almost every day?	NO	YES
f	Did you have difficulty concentrating or making decisions almost every day?	NO	YES
g	Did you repeatedly consider hurting yourself, feel suicidal, or wish that you were dead?	NO	YES

ARE **5** OR MORE ANSWERS (**A1-A3**) CODED **YES**?

NO **YES** *

MAJOR DEPRESSIVE EPISODE, CURRENT

IF PATIENT HAS CURRENT MAJOR DEPRESSIVE EPISODE CONTINUE TO A4,
OTHERWISE MOVE TO MODULE B:

A4 a	During your lifetime, did you have other periods of two weeks or more when you felt depressed or uninterested in most things, and had most of the problems we just talked about?	NO	YES
b	Did you ever have an interval of at least 2 months without any depression and any loss of interest between 2 episodes of depression?		

NO **YES**

MAJOR DEPRESSIVE EPISODE, RECURRENT

* If patient has Major Depressive Episode, Current, code **YES** in corresponding questions on page 5

B. DYSTHYMIA

(➨ MEANS : GO TO THE DIAGNOSTIC BOX, CIRCLE NO, AND MOVE TO THE NEXT MODULE)

IF PATIENT'S SYMPTOMS CURRENTLY MEET CRITERIA FOR MAJOR DEPRESSIVE EPISODE, DO NOT EXPLORE THIS MODULE.

B1	Have you felt sad, low or depressed most of the time for the last two years?	➨ NO	YES
B2	Was this period interrupted by your feeling OK for two months or more?	NO	➨ YES
B3	**During this period of feeling depressed most of the time:**		
a	Did your appetite change significantly?	NO	YES
b	Did you have trouble sleeping or sleep excessively?	NO	YES
c	Did you feel tired or without energy?	NO	YES
d	Did you lose your self-confidence?	NO	YES
e	Did you have trouble concentrating or making decisions?	NO	YES
f	Did you feel hopeless?	NO	YES
	ARE **2** OR MORE **B3** ANSWERS CODED **YES**?	➨ NO	YES

B4	Did the symptoms of depression cause you significant distress or impair your ability to function at work, socially, or in some other important way?

NO **YES**

DYSTHYMIA
CURRENT

MAJOR DEPRESSIVE EPISODE WITH MELANCHOLIC FEATURES (optional)

(➨ MEANS : GO TO THE DIAGNOSTIC BOX, CIRCLE NO, AND MOVE TO THE NEXT MODULE)

IF THE PATIENT CODES POSITIVE FOR A CURRENT MAJOR DEPRESSIVE EPISODE (**A3 = YES**), EXPLORE THE FOLLOWING:

A5	a During the most severe period of the current depressive episode, did you lose almost completely your ability to enjoy nearly everything?	NO	YES
	b During the most severe period of the current depressive episode, did you lose your ability to respond to things that previously gave you pleasure, or cheered you up? **IF NO:** When something good happens does it fail to make you feel better, even temporarily?	NO	YES
	IS EITHER **A5a** OR **A5b** CODED **YES**?	➨ NO	YES
A6	**Over the past two week period, when you felt depressed and uninterested:**		
	a Did you feel depressed in a way that is different from the kind of feeling you experience when someone close to you dies?	NO	YES
	b Did you feel regularly worse in the morning, almost every day?	NO	YES
	c Did you wake up at least 2 hours before the usual time of awakening and have difficulty getting back to sleep, almost every day?	NO	YES
	d IS **A3c** CODED **YES** (PSYCHOMOTOR RETARDATION OR AGITATION)?	NO	YES
	e IS **A3a** CODED **YES** FOR ANOREXIA OR WEIGHT LOSS?	NO	YES
	f Did you feel excessive guilt or guilt out of proportion to the reality of the situation?	NO	YES

ARE **3** OR MORE **A6** ANSWERS CODED **YES**?

NO **YES**

Major Depressive Episode
with
Melancholic Features
Current

C. SUICIDALITY

In the past month did you:

				Points
C1	Suffer any accident?	NO	YES	0
	IF NO TO C1, SKIP TO C2; IF YES, ASK C1a,:			
C1a	Plan or intend to hurt yourself in that accident either passively or actively?	NO	YES	0
	IF NO TO C1a, SKIP TO C2: IF YES, ASK C1b,:			
C1b	Did you intend to die as a result of this accident?	NO	YES	0
C2	Think that you would be better off dead or wish you were dead?	NO	YES	1
C3	Want to harm yourself or to hurt or to injure yourself?	NO	YES	2
C4	Think about suicide?	NO	YES	6

IF YES, ASK ABOUT THE INTENSITY AND FREQUENCY OF THE SUICIDAL IDEATION:

Frequency		Intensity	
Occasionally	❐	Mild	❐
Often	❐	Moderate	❐
Very often	❐	Severe	❐

C5	Have a suicide plan?	NO	YES	8
C6	Take any active steps to prepare to injure yourself or to prepare for a suicide attempt in which you expected or intended to die?	NO	YES	9
C7	Intentionally injure yourself without suicide intent?	NO	YES	4
C8	Attempt suicide?	NO	YES	10

Hoped to be rescued / survive ❐
Expected / intended to die ❐

In your lifetime:

C9	Did you ever make a suicide attempt?	NO	YES	4

IS AT LEAST **1** OF THE ABOVE CODED **YES**?

NO **YES**

IF YES, ADD THE TOTAL NUMBER OF POINTS FOR THE ANSWERS (C1-C9) CHECKED 'YES' AND SPECIFY THE LEVEL OF SUICIDE RISK AS FOLLOWS:

SUICIDE RISK CURRENT

1-8 points	Low	❐
9-16 points	Moderate	❐
≥ 17 points	High	❐

MAKE ANY ADDITIONAL COMMENTS ABOUT YOUR ASSESSMENT OF THIS PATIENT'S CURRENT AND NEAR FUTURE SUICIDE RISK IN THE SPACE BELOW:

D. (HYPO) MANIC EPISODE

(➨ MEANS : GO TO THE DIAGNOSTIC BOXES, CIRCLE **NO** IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

D1	a	Have you **ever** had a period of time when you were feeling 'up' or 'high' or 'hyper' or so full of energy or full of yourself that you got into trouble, or that other people thought you were not your usual self? (Do not consider times when you were intoxicated on drugs or alcohol.)	NO	YES
		IF PATIENT IS PUZZLED OR UNCLEAR ABOUT WHAT YOU MEAN BY 'UP' OR 'HIGH' OR 'HYPER', CLARIFY AS FOLLOWS: By 'up' or 'high' or 'hyper' I mean: having elated mood; increased energy; needing less sleep; having rapid thoughts; being full of ideas; having an increase in productivity, motivation, creativity, or impulsive behavior.		
		IF NO, CODE NO TO **D1b**: IF **YES** ASK:		
	b	Are you currently feeling 'up' or 'high' or 'hyper' or full of energy?		NO YES
D2	a	Have you **ever** been persistently irritable, for several days, so that you had arguments or verbal or physical fights, or shouted at people outside your family? Have you or others noticed that you have been more irritable or over reacted, compared to other people, even in situations that you felt were justified?	NO	YES
		IF NO, CODE NO TO **D2b**: IF **YES** ASK:		
	b	Are you currently feeling persistently irritable?	NO	YES
			➨	
		IS **D1a** OR **D2a** CODED **YES**?	NO	YES

D3 IF **D1b** OR **D2b** = **YES**: EXPLORE ONLY **CURRENT** EPISODE, OTHERWISE
IF **D1b** AND **D2b** = **NO**: EXPLORE THE MOST SYMPTOMATIC **PAST** EPISODE

During the times when you felt high, full of energy, or irritable did you:

a	Feel that you could do things others couldn't do, or that you were an especially important person?	NO	YES
b	Need less sleep (for example, feel rested after only a few hours sleep)?	NO	YES
c	Talk too much without stopping, or so fast that people had difficulty understanding?	NO	YES
d	Have racing thoughts?	NO	YES
e	Become easily distracted so that any little interruption could distract you?	NO	YES
f	Become so active or physically restless that others were worried about you?	NO	YES
g	Want so much to engage in pleasurable activities that you ignored the risks or consequences (for example, spending sprees, reckless driving, or sexual indiscretions)?	NO	YES
		➨	
	ARE **3** OR MORE **D3** ANSWERS CODED **YES** (OR **4** OR MORE IF **D1a** IS **NO** (IN RATING PAST EPISODE) AND IF **D1b** IS **NO** (IN RATING CURRENT EPISODE)) ?	NO	YES

D4 Did these symptoms last at least a week **and** cause significant problems at home, at work, socially, or at school, **or** were you hospitalized for these problems? NO YES

↓ ↓

THE EPISODE EXPLORED WAS A: ❒ ❒

HYPOMANIC EPISODE *MANIC EPISODE*

IS **D4** CODED **NO**?

SPECIFY IF THE EPISODE IS CURRENT OR PAST.

NO	**YES**
HYPOMANIC EPISODE	
CURRENT	❒
PAST	❒

IS **D4** CODED **YES**?

SPECIFY IF THE EPISODE IS CURRENT OR PAST.

NO	**YES**
MANIC EPISODE	
CURRENT	❒
PAST	❒

E. PANIC DISORDER

(➨ MEANS : CIRCLE **NO** IN **E5, E6** AND **E7** AND SKIP TO **F1**)

			➨	
E1	a	Have you, on more than one occasion, had spells or attacks when you **suddenly** felt anxious, frightened, uncomfortable or uneasy, even in situations where most people would not feel that way?	NO	YES
	b	Did the spells surge to a peak within 10 minutes of starting?	NO	YES
E2		At any time in the past, did any of those spells or attacks come on unexpectedly or occur in an unpredictable or unprovoked manner?	NO	YES
E3		Have you ever had one such attack followed by a month or more of persistent concern about having another attack, or worries about the consequences of the attack?	NO	YES
E4		**During the worst spell that you can remember:**		
	a	Did you have skipping, racing or pounding of your heart?	NO	YES
	b	Did you have sweating or clammy hands?	NO	YES
	c	Were you trembling or shaking?	NO	YES
	d	Did you have shortness of breath or difficulty breathing?	NO	YES
	e	Did you have a choking sensation or a lump in your throat?	NO	YES
	f	Did you have chest pain, pressure or discomfort?	NO	YES
	g	Did you have nausea, stomach problems or sudden diarrhea?	NO	YES
	h	Did you feel dizzy, unsteady, lightheaded or faint?	NO	YES
	i	Did things around you feel strange, unreal, detached or unfamiliar, or did you feel outside of or detached from part or all of your body?	NO	YES
	j	Did you fear that you were losing control or going crazy?	NO	YES
	k	Did you fear that you were dying?	NO	YES
	l	Did you have tingling or numbness in parts of your body?	NO	YES
	m	Did you have hot flushes or chills?	NO	YES
E5		ARE BOTH **E3,** AND **4** OR MORE **E4** ANSWERS, CODED **YES**?	NO	YES *PANIC DISORDER LIFETIME*
		IF YES TO E5, SKIP TO E7.		
E6		IF **E5 = NO,** ARE ANY E4 ANSWERS CODED **YES**?	NO	YES *LIMITED SYMPTOM ATTACKS LIFETIME*
		THEN SKIP TO **F1.**		
E7		In the past month, did you have such attacks repeatedly (2 or more) followed by persistent concern about having another attack?	NO	YES *PANIC DISORDER CURRENT*

F. AGORAPHOBIA

F1	Do you feel anxious or uneasy in places or situations where you might have a panic attack or the panic-like symptoms we just spoke about, or where help might not be available or escape might be difficult: like being in a crowd, standing in a line (queue), when you are alone away from home or alone at home, or when crossing a bridge, traveling in a bus, train or car?	NO YES
	IF **F1** = **NO**, CIRCLE **NO** IN **F2**.	
F2	Do you fear these situations so much that you avoid them, or suffer through them, or need a companion to face them?	NO YES *AGORAPHOBIA CURRENT*

IS **F2** (CURRENT AGORAPHOBIA) CODED **NO**

and

IS **E7** (CURRENT PANIC DISORDER) CODED **YES**?

NO **YES**

PANIC DISORDER without Agoraphobia CURRENT

IS **F2** (CURRENT AGORAPHOBIA) CODED **YES**

and

IS **E7** (CURRENT PANIC DISORDER) CODED **YES**?

NO **YES**

PANIC DISORDER with Agoraphobia CURRENT

IS **F2** (CURRENT AGORAPHOBIA) CODED **YES**

and

IS **E5** (PANIC DISORDER LIFETIME) CODED **NO**?

NO **YES**

AGORAPHOBIA, CURRENT without history of Panic Disorder

G. SOCIAL PHOBIA (Social Anxiety Disorder)

(➨ MEANS : GO TO THE DIAGNOSTIC BOX, CIRCLE **NO** AND MOVE TO THE NEXT MODULE)

G1	In the past month, were you fearful or embarrassed being watched, being the focus of attention, or fearful of being humiliated? This includes things like speaking in public, eating in public or with others, writing while someone watches, or being in social situations.	➨ NO YES
G2	Is this social fear excessive or unreasonable?	➨ NO YES
G3	Do you fear these social situations so much that you avoid them or suffer through them?	➨ NO YES
G4	Does this social fear disrupt your normal work or social functioning or cause you significant distress?	**NO** **YES** ***SOCIAL PHOBIA*** *(Social Anxiety Disorder)* ***CURRENT***

H. OBSESSIVE-COMPULSIVE DISORDER

(➨ MEANS: GO TO THE DIAGNOSTIC BOX, CIRCLE NO AND MOVE TO THE NEXT MODULE)

H1	In the past month, have you been bothered by recurrent thoughts, impulses, or images that were unwanted, distasteful, inappropriate, intrusive, or distressing? (For example, the idea that you were dirty, contaminated or had germs, **or** fear of contaminating others, **or** fear of harming someone even though you didn't want to, **or** fearing you would act on some impulse, **or** fear or superstitions that you would be responsible for things going wrong, **or** obsessions with sexual thoughts, images or impulses, **or** hoarding, collecting, **or** religious obsessions.) (DO NOT INCLUDE SIMPLY EXCESSIVE WORRIES ABOUT REAL LIFE PROBLEMS. DO NOT INCLUDE OBSESSIONS DIRECTLY RELATED TO EATING DISORDERS, SEXUAL DEVIATIONS, PATHOLOGICAL GAMBLING, OR ALCOHOL OR DRUG ABUSE BECAUSE THE PATIENT MAY DERIVE PLEASURE FROM THE ACTIVITY AND MAY WANT TO RESIST IT ONLY BECAUSE OF ITS NEGATIVE CONSEQUENCES.)	NO YES ↓ SKIP TO **H4**
H2	Did they keep coming back into your mind even when you tried to ignore or get rid of them?	NO YES ↓ SKIP TO **H4**
H3	Do you think that these obsessions are the product of your own mind and that they are not imposed from the outside?	NO YES obsessions
H4	In the past month, did you do something repeatedly without being able to resist doing it, like washing or cleaning excessively, counting or checking things over and over, or repeating, collecting, arranging things, or other superstitious rituals?	NO YES compulsions
	IS **H3** OR **H4** CODED **YES**?	➨ NO YES
H5	Did you recognize that either these obsessive thoughts or these compulsive behaviors were excessive or unreasonable?	➨ NO YES
H6	Did these obsessive thoughts and/or compulsive behaviors significantly interfere with your normal routine, occupational functioning, usual social activities, or relationships, or did they take more than one hour a day?	**NO** **YES** ***O.C.D. CURRENT***

I. POSTTRAUMATIC STRESS DISORDER (optional)

(➨ MEANS: GO TO THE DIAGNOSTIC BOX, CIRCLE NO, AND MOVE TO THE NEXT MODULE)

I1	Have you ever experienced or witnessed or had to deal with an extremely traumatic event that included actual or threatened death or serious injury to you or someone else? EXAMPLES OF TRAUMATIC EVENTS INCLUDE: SERIOUS ACCIDENTS, SEXUAL OR PHYSICAL ASSAULT, A TERRORIST ATTACK, BEING HELD HOSTAGE, KIDNAPPING, FIRE, DISCOVERING A BODY, SUDDEN DEATH OF SOMEONE CLOSE TO YOU, WAR, OR NATURAL DISASTER.	➨ NO YES
I2	Did you respond with intense fear, helplessness or horror?	➨ NO YES
I3	During the past month, have you re-experienced the event in a distressing way (such as, dreams, intense recollections, flashbacks or physical reactions)?	➨ NO YES
I4	**In the past month:**	
	a Have you avoided thinking about or talking about the event ?	NO YES
	b Have you avoided activities, places or people that remind you of the event?	NO YES
	c Have you had trouble recalling some important part of what happened?	NO YES
	d Have you become much less interested in hobbies or social activities?	NO YES
	e Have you felt detached or estranged from others?	NO YES
	f Have you noticed that your feelings are numbed?	NO YES
	g Have you felt that your life will be shortened or that you will die sooner than other people?	NO YES
	ARE **3** OR MORE **I4** ANSWERS CODED **YES**?	➨ NO YES
I5	**In the past month:**	
	a Have you had difficulty sleeping?	NO YES
	b Were you especially irritable or did you have outbursts of anger?	NO YES
	c Have you had difficulty concentrating?	NO YES
	d Were you nervous or constantly on your guard?	NO YES
	e Were you easily startled?	NO YES
	ARE **2** OR MORE **I5** ANSWERS CODED **YES**?	➨ NO YES
I6	During the past month, have these problems significantly interfered with your work or social activities, or caused significant distress?	**NO** **YES** ***POSTTRAUMATIC STRESS DISORDER CURRENT***

J. ALCOHOL ABUSE AND DEPENDENCE

(➨ MEANS: GO TO DIAGNOSTIC BOXES, CIRCLE NO IN BOTH AND MOVE TO THE NEXT MODULE)

		➨ NO	YES
J1	**In the past 12 months**, have you had 3 or more alcoholic drinks within a 3 hour period on 3 or more occasions?		
J2	**In the past 12 months**:		
a	Did you need to drink more in order to get the same effect that you got when you first started drinking?	NO	YES
b	When you cut down on drinking did your hands shake, did you sweat or feel agitated? Did you drink to avoid these symptoms or to avoid being hungover, for example, "the shakes", sweating or agitation? IF YES TO EITHER, CODE YES.	NO	YES
c	During the times when you drank alcohol, did you end up drinking more than you planned when you started?	NO	YES
d	Have you tried to reduce or stop drinking alcohol but failed?	NO	YES
e	On the days that you drank, did you spend substantial time in obtaining alcohol, drinking, or in recovering from the effects of alcohol?	NO	YES
f	Did you spend less time working, enjoying hobbies, or being with others because of your drinking?	NO	YES
g	Have you continued to drink even though you knew that the drinking caused you health or mental problems?	NO	YES

ARE 3 OR MORE **J2** ANSWERS CODED **YES**?

NO **YES***

* IF YES, SKIP J3 QUESTIONS, CIRCLE N/A IN THE ABUSE BOX AND MOVE TO THE NEXT DISORDER. DEPENDENCE PREEMPTS ABUSE.

ALCOHOL DEPENDENCE
CURRENT

		NO	YES
J3	**In the past 12 months:**		
a	Have you been intoxicated, high, or hungover more than once when you had other responsibilities at school, at work, or at home? Did this cause any problems? (CODE YES ONLY IF THIS CAUSED PROBLEMS.)	NO	YES
b	Were you intoxicated more than once in any situation where you were physically at risk, for example, driving a car, riding a motorbike, using machinery, boating, etc.?	NO	YES
c	Did you have legal problems more than once because of your drinking, for example, an arrest or disorderly conduct?	NO	YES
d	Did you continue to drink even though your drinking caused problems with your family or other people?	NO	YES

ARE 1 OR MORE **J3** ANSWERS CODED **YES**?

NO **N/A** **YES**

ALCOHOL ABUSE
CURRENT

K. NON-ALCOHOL PSYCHOACTIVE SUBSTANCE USE DISORDERS

(➨ MEANS : GO TO THE DIAGNOSTIC BOXES, CIRCLE NO IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

Now I am going to show you / read to you a list of street drugs or medicines.

		➨ NO	YES
K1	a **In the past 12 months**, did you take any of these drugs more than once, to get high, to feel better, or to change your mood?		

CIRCLE EACH DRUG TAKEN:

Stimulants: amphetamines, "speed", crystal meth, "crank", "rush", Dexedrine, Ritalin, diet pills.

Cocaine: snorting, IV, freebase, crack, "speedball".

Narcotics: heroin, morphine, Dilaudid, opium, Demerol, methadone, codeine, Percodan, Darvon, OxyContin.

Hallucinogens: LSD ("acid"), mescaline, peyote, PCP ("angel dust", "peace pill"), psilocybin, STP, "mushrooms", "ecstasy", MDA, MDMA, or ketamine ("special K").

Inhalants: "glue", ethyl chloride, "rush", nitrous oxide ("laughing gas"), amyl or butyl nitrate ("poppers").

Marijuana: hashish ("hash"), THC, "pot", "grass", "weed", "reefer".

Tranquilizers: Quaalude, Seconal ("reds"), Valium, Xanax, Librium, Ativan, Dalmane, Halcion, barbiturates, Miltown, GHB, Roofinol, "Roofies".

Miscellaneous: steroids, nonprescription sleep or diet pills. Any others?

SPECIFY MOST USED DRUG(S): _____

CHECK ONE BOX

ONLY ONE DRUG / DRUG CLASS HAS BEEN USED ☐

ONLY THE MOST USED DRUG CLASS IS INVESTIGATED. ☐

EACH DRUG CLASS USED IS EXAMINED SEPARATELY (PHOTOCOPY K2 AND K3 AS NEEDED) ☐

b SPECIFY WHICH DRUG/DRUG CLASS WILL BE EXPLORED IN THE INTERVIEW BELOW IF THERE IS CONCURRENT OR SEQUENTIAL POLYSUBSTANCE USE: _____

		NO	YES
K2	**Considering your use of (NAME THE DRUG / DRUG CLASS SELECTED), in the past 12 months:**		
a	Have you found that you needed to use more (NAME OF DRUG / DRUG CLASS SELECTED) to get the same effect that you did when you first started taking it?	NO	YES
b	When you reduced or stopped using (NAME OF DRUG / DRUG CLASS SELECTED), did you have withdrawal symptoms (aches, shaking, fever, weakness, diarrhea, nausea, sweating, heart pounding, difficulty sleeping, or feeling agitated, anxious, irritable, or depressed)? Did you use any drug(s) to keep yourself from getting sick (withdrawal symptoms) or so that you would feel better? IF YES TO EITHER, CODE YES.	NO	YES
c	Have you often found that when you used (NAME OF DRUG / DRUG CLASS SELECTED), you ended up taking more than you thought you would?	NO	YES
d	Have you tried to reduce or stop taking (NAME OF DRUG / DRUG CLASS SELECTED) but failed?	NO	YES
e	On the days that you used (NAME OF DRUG / DRUG CLASS SELECTED), did you spend substantial time (>2 HOURS), obtaining, using or in recovering from the drug, or thinking about the drug?	NO	YES

L. PSYCHOTIC DISORDERS AND MOOD DISORDER WITH PSYCHOTIC FEATURES

ASK FOR AN EXAMPLE OF EACH QUESTION ANSWERED POSITIVELY. CODE **YES** ONLY IF THE EXAMPLES CLEARLY SHOW A DISTORTION OF THOUGHT OR OF PERCEPTION OR IF THEY ARE NOT CULTURALLY APPROPRIATE. BEFORE CODING, INVESTIGATE WHETHER DELUSIONS QUALIFY AS "BIZARRE".

DELUSIONS ARE "BIZARRE" IF: CLEARLY IMPLAUSIBLE, ABSURD, NOT UNDERSTANDABLE, AND CANNOT DERIVE FROM ORDINARY LIFE EXPERIENCE.

HALLUCINATIONS ARE SCORED "BIZARRE" IF: A VOICE COMMENTS ON THE PERSON'S THOUGHTS OR BEHAVIOR, OR WHEN TWO OR MORE VOICES ARE CONVERSING WITH EACH OTHER.

Now I am going to ask you about unusual experiences that some people have.

					BIZARRE
L1	a	Have you ever believed that people were spying on you, or that someone was plotting against you, or trying to hurt you? NOTE: ASK FOR EXAMPLES TO RULE OUT ACTUAL STALKING.	NO	YES	YES
	b	**IF YES:** do you currently believe these things?	NO	YES	YES ➡L6
L2	a	Have you ever believed that someone was reading your mind or could hear your thoughts, or that you could actually read someone's mind or hear what another person was thinking?	NO	YES	YES
	b	**IF YES:** do you currently believe these things?	NO	YES	YES ➡L6
L3	a	Have you ever believed that someone or some force outside of yourself put thoughts in your mind that were not your own, or made you act in a way that was not your usual self? Have you ever felt that you were possessed? CLINICIAN: ASK FOR EXAMPLES AND DISCOUNT ANY THAT ARE NOT PSYCHOTIC.	NO	YES	YES
	b	**IF YES:** do you currently believe these things?	NO	YES	YES ➡L6
L4	a	Have you ever believed that you were being sent special messages through the TV, radio, or newspaper, or that a person you did not personally know was particularly interested in you?	NO	YES	YES
	b	**IF YES:** do you currently believe these things?	NO	YES	YES ➡L6
L5	a	Have your relatives or friends ever considered any of your beliefs strange or unusual? INTERVIEWER: ASK FOR EXAMPLES. ONLY CODE **YES** IF THE EXAMPLES ARE **CLEARLY** DELUSIONAL IDEAS NOT EXPLORED IN QUESTIONS L1 TO L4, FOR EXAMPLE, SOMATIC OR RELIGIOUS DELUSIONS OR DELUSIONS OF GRANDIOSITY, JEALOUSY, GUILT, RUIN OR DESTITUTION, ETC.	NO	YES	YES
	b	**IF YES:** do they currently consider your beliefs strange?	NO	YES	YES
L6	a	Have you ever heard things other people couldn't hear, such as voices? HALLUCINATIONS ARE SCORED "BIZARRE" ONLY IF PATIENT ANSWERS **YES** TO THE FOLLOWING:	NO	YES	
		IF YES: Did you hear a voice commenting on your thoughts or behavior or did you hear two or more voices talking to each other?	NO		YES
	b	**IF YES:** have you heard these things in the past month?	NO	YES	YES ➡L8b

	f	Did you spend less time working, enjoying hobbies, or being with family or friends because of your drug use?	NO	YES
	g	Have you continued to use (NAME OF DRUG / DRUG CLASS SELECTED), even though it caused you health or mental problems?	NO	YES

ARE **3** OR MORE **K2** ANSWERS CODED **YES**?

SPECIFY DRUG(S): _____

* IF YES, SKIP K3 QUESTIONS, CIRCLE N/A IN THE ABUSE BOX FOR THIS SUBSTANCE AND MOVE TO THE NEXT DISORDER. DEPENDENCE PREEMPTS ABUSE.

NO **YES**
*

SUBSTANCE DEPENDENCE
CURRENT

Considering your use of (NAME THE DRUG CLASS SELECTED), in the past 12 months:

K3	a	Have you been intoxicated, high, or hungover from (NAME OF DRUG / DRUG CLASS SELECTED) more than once, when you had other responsibilities at school, at work, or at home? Did this cause any problem? (CODE **YES** ONLY IF THIS CAUSED PROBLEMS.)	NO	YES
	b	Have you been high or intoxicated from (NAME OF DRUG / DRUG CLASS SELECTED) more than once in any situation where you were physically at risk (for example, driving a car, riding a motorbike, using machinery, boating, etc.)?	NO	YES
	c	Did you have legal problems more than once because of your drug use, for example, an arrest or disorderly conduct?	NO	YES
	d	Did you continue to use (NAME OF DRUG / DRUG CLASS SELECTED), even though it caused problems with your family or other people?	NO	YES

ARE **1** OR MORE **K3** ANSWERS CODED **YES**?

SPECIFY DRUG(S): _____

NO **N/A** **YES**

SUBSTANCE ABUSE
CURRENT

L7 a Have you ever had visions when you were awake or have you ever seen things other people couldn't see? NO YES
CLINICIAN: CHECK TO SEE IF THESE ARE CULTURALLY INAPPROPRIATE.

b **IF YES:** have you seen these things in the past month? NO YES

CLINICIAN'S JUDGMENT

L8 b IS THE PATIENT CURRENTLY EXHIBITING INCOHERENCE, DISORGANIZED SPEECH, OR MARKED LOOSENING OF ASSOCIATIONS? NO YES

L9 b IS THE PATIENT CURRENTLY EXHIBITING DISORGANIZED OR CATATONIC BEHAVIOR? NO YES

L10 b ARE NEGATIVE SYMPTOMS OF SCHIZOPHRENIA, E.G. SIGNIFICANT AFFECTIVE FLATTENING, POVERTY OF SPEECH (ALOGIA) OR AN INABILITY TO INITIATE OR PERSIST IN GOAL-DIRECTED ACTIVITIES (AVOLITION), PROMINENT DURING THE INTERVIEW? NO YES

L11 a ARE 1 OR MORE « a » QUESTIONS FROM L1a TO L7a CODED **YES OR YES BIZARRE** AND IS EITHER:

MAJOR DEPRESSIVE EPISODE, (CURRENT OR RECURRENT)
OR
MANIC OR HYPOMANIC EPISODE, (CURRENT OR PAST) CODED **YES**? NO ➨L13 YES

IF NO TO L11 a, CIRCLE NO IN BOTH 'MOOD DISORDER WITH PSYCHOTIC FEATURES' DIAGNOSTIC BOXES AND MOVE TO L13.

b You told me earlier that you had period(s) when you felt (depressed/high/persistently irritable).

Were the beliefs and experiences you just described (SYMPTOMS CODED YES FROM L1a TO L7a) restricted exclusively to times when you were feeling depressed/high/irritable?

IF THE PATIENT EVER HAD A PERIOD OF AT LEAST 2 WEEKS OF HAVING THESE BELIEFS OR EXPERIENCES (PSYCHOTIC SYMPTOMS) WHEN THEY WERE NOT DEPRESSED/HIGH/IRRITABLE, CODE NO TO THIS DISORDER.

IF THE ANSWER IS NO TO THIS DISORDER, ALSO CIRCLE NO TO L12 AND MOVE TO L13

NO **YES**

MOOD DISORDER WITH PSYCHOTIC FEATURES

LIFETIME

L12 a ARE 1 OR MORE « b » QUESTIONS FROM L1b TO L7b CODED **YES OR YES BIZARRE** AND IS EITHER:

MAJOR DEPRESSIVE EPISODE, (CURRENT)
OR
MANIC OR HYPOMANIC EPISODE, (CURRENT) CODED **YES**?

IF THE ANSWER IS YES TO THIS DISORDER, CIRCLE NO TO L13 AND L14 AND MOVE TO THE NEXT MODULE

NO **YES**

MOOD DISORDER WITH PSYCHOTIC FEATURES

CURRENT

L13 ARE 1 OR MORE « b » QUESTIONS CODED **YES BIZARRE**?

OR

ARE 2 OR MORE « b » QUESTIONS CODED **YES** (RATHER THAN **YES BIZARRE**)?

AND DID AT LEAST TWO OF THE PSYCHOTIC SYMPTOMS OCCUR DURING THE SAME 1 MONTH PERIOD?

NO **YES**

PSYCHOTIC DISORDER
CURRENT

L14 IS **L13** CODED **YES**

OR

ARE 1 OR MORE « a » QUESTIONS FROM L1a TO L7a, CODED **YES BIZARRE**?

OR

ARE 2 OR MORE « a » QUESTIONS FROM L1a TO L7a, CODED **YES** (RATHER THAN **YES BIZARRE**)

AND DID AT LEAST TWO OF THE PSYCHOTIC SYMPTOMS OCCUR DURING THE SAME 1 MONTH PERIOD?

NO **YES**

PSYCHOTIC DISORDER
LIFETIME

M. ANOREXIA NERVOSA

(➨ MEANS : GO TO THE DIAGNOSTIC BOX, CIRCLE **NO**, AND MOVE TO THE NEXT MODULE)

M1	a	How tall are you?	❑ft ❑❑in.
			❑ ❑❑cm.
	b.	What was your lowest weight in the past 3 months?	❑ ❑❑lbs.
			❑ ❑❑kgs.
	c	IS PATIENT'S WEIGHT EQUAL TO OR BELOW THE THRESHOLD CORRESPONDING TO HIS / HER HEIGHT? (SEE TABLE BELOW)	NO YES

In the past 3 months:

M2		In spite of this low weight, have you tried not to gain weight?	NO YES
M3		Have you intensely feared gaining weight or becoming fat, even though you were underweight?	NO YES
M4	a	Have you considered yourself too big / fat or that part of your body was too big / fat?	NO YES
	b	Has your body weight or shape greatly influenced how you felt about yourself?	NO YES
	c	Have you thought that your current low body weight was normal or excessive?	NO YES
M5		ARE 1 OR MORE ITEMS FROM **M4** CODED YES?	NO YES
M6		FOR WOMEN ONLY: During the last 3 months, did you miss all your menstrual periods when they were expected to occur (when you were not pregnant)?	NO YES

FOR WOMEN: ARE **M5 AND M6** CODED YES?

FOR MEN: IS **M5** CODED **YES**?

NO **YES**

ANOREXIA NERVOSA
CURRENT

HEIGHT / WEIGHT TABLE CORRESPONDING TO A BMI THRESHOLD OF 17.5 KG/M²

Height/Weight														
ft/in	4'9	4'10	4'11	5'0	5'1	5'2	5'3	5'4	5'5	5'6	5'7	5'8	5'9	5'10
lbs.	81	84	87	89	92	96	99	102	105	108	112	115	118	122
cm	145	147	150	152	155	158	160	163	165	168	170	173	175	178
kgs	37	38	39	41	42	43	45	46	48	49	51	52	54	55

Height/Weight					
ft/in	5'11	6'0	6'1	6'2	6'3
lbs.	125	129	132	136	140
cm	180	183	185	188	191
kgs	57	59	60	62	64

The weight thresholds above are calculated using a body mass index (BMI) equal to or below 17.5 kg/m² for the patient's height. This is the threshold guideline below which a person is deemed underweight by the DSM-IV and the ICD-10 Diagnostic Criteria for Research for Anorexia Nervosa.

N. BULIMIA NERVOSA

(➨ MEANS : GO TO THE DIAGNOSTIC BOXES, CIRCLE **NO** IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

N1	In the past three months, did you have eating binges or times when you ate a very large amount of food within a 2-hour period?	➨ NO YES
N2	In the last 3 months, did you have eating binges as often as twice a week?	➨ NO YES
N3	During these binges, did you feel that your eating was out of control?	➨ NO YES
N4	Did you do anything to compensate for, or to prevent a weight gain from these binges, like vomiting, fasting, exercising or taking laxatives, enemas, diuretics (fluid pills), or other medications?	➨ NO YES
N5	Does your body weight or shape greatly influence how you feel about yourself?	➨ NO YES
N6	DO THE PATIENT'S SYMPTOMS MEET CRITERIA FOR ANOREXIA NERVOSA?	➨ NO YES ↓ Skip to N8
N7	Do these binges occur only when you are under (_____lbs./kgs.)? INTERVIEWER: WRITE IN THE ABOVE PARENTHESIS THE THRESHOLD WEIGHT FOR THIS PATIENT'S HEIGHT FROM THE HEIGHT / WEIGHT TABLE IN THE ANOREXIA NERVOSA MODULE.	➨ NO YES

N8 IS **N5** CODED **YES** AND IS EITHER **N6** OR **N7** CODED **NO**?

NO **YES**

BULIMIA NERVOSA
CURRENT

IS **N7** CODED **YES**?

NO **YES**

ANOREXIA NERVOSA
Binge Eating/Purging Type
CURRENT

O. GENERALIZED ANXIETY DISORDER

(➨ MEANS : GO TO THE DIAGNOSTIC BOX, CIRCLE NO, AND MOVE TO THE NEXT MODULE)

O1	a	Have you worried excessively or been anxious about several things over the past 6 months?	NO	YES
	b	Are these worries present most days?	NO	YES
		IS THE PATIENT'S ANXIETY RESTRICTED EXCLUSIVELY TO, OR BETTER EXPLAINED BY, ANY DISORDER PRIOR TO THIS POINT?	NO	YES
O2		Do you find it difficult to control the worries or do they interfere with your ability to focus on what you are doing?	NO	YES
O3		FOR THE FOLLOWING, CODE NO IF THE SYMPTOMS ARE CONFINED TO FEATURES OF ANY DISORDER EXPLORED PRIOR TO THIS POINT.		
		When you were anxious over the past 6 months, did you, most of the time:		
	a	Feel restless, keyed up or on edge?	NO	YES
	b	Feel tense?	NO	YES
	c	Feel tired, weak or exhausted easily?	NO	YES
	d	Have difficulty concentrating or find your mind going blank?	NO	YES
	e	Feel irritable?	NO	YES
	f	Have difficulty sleeping (difficulty falling asleep, waking up in the middle of the night, early morning wakening or sleeping excessively)?	NO	YES

ARE 3 OR MORE O3 ANSWERS CODED YES?

NO **YES**

GENERALIZED ANXIETY DISORDER
CURRENT

P. ANTISOCIAL PERSONALITY DISORDER (optional)

(➨ MEANS : GO TO THE DIAGNOSTIC BOX AND CIRCLE NO.)

P1		**Before you were 15 years old, did you:**		
	a	repeatedly skip school or run away from home overnight?	NO	YES
	b	repeatedly lie, cheat, "con" others, or steal?	NO	YES
	c	start fights or bully, threaten, or intimidate others?	NO	YES
	d	deliberately destroy things or start fires?	NO	YES
	e	deliberately hurt animals or people?	NO	YES
	f	force someone to have sex with you?	NO	YES
		ARE 2 OR MORE P1 ANSWERS CODED YES?	NO	YES
		DO NOT CODE YES TO THE BEHAVIORS BELOW IF THEY ARE EXCLUSIVELY POLITICALLY OR RELIGIOUSLY MOTIVATED.		
P2		**Since you were 15 years old, have you:**		
	a	repeatedly behaved in a way that others would consider irresponsible, like failing to pay for things you owed, deliberately being impulsive or deliberately not working to support yourself?	NO	YES
	b	done things that are illegal even if you didn't get caught (for example, destroying property, shoplifting, stealing, selling drugs, or committing a felony)?	NO	YES
	c	been in physical fights repeatedly (including physical fights with your spouse or children)?	NO	YES
	d	often lied or "conned" other people to get money or pleasure, or lied just for fun?	NO	YES
	e	exposed others to danger without caring?	NO	YES
	f	felt no guilt after hurting, mistreating, lying to, or stealing from others, or after damaging property?	NO	YES

ARE 3 OR MORE P2 QUESTIONS CODED YES?

NO **YES**

ANTISOCIAL PERSONALITY DISORDER
LIFETIME

THIS CONCLUDES THE INTERVIEW

REFERENCES

Sheehan DV, Lecrubier Y, Harnett-Sheehan K, Janavs J, Weiller E, Bonara LI, Keskiner A, Schinka J, Knapp E, Sheehan MF, Dunbar GC. Reliability and Validity of the MINI International Neuropsychiatric Interview (M.I.N.I.): According to the SCID-P. European Psychiatry. 1997; 12:232-241.

Lecrubier Y, Sheehan D, Weiller E, Amorim P, Bonora I, Sheehan K, Janavs J, Dunbar G. The MINI International Neuropsychiatric Interview (M.I.N.I.) A Short Diagnostic Structured Interview: Reliability and Validity According to the CIDI. European Psychiatry. 1997; 12: 224-231.

Sheehan DV, Lecrubier Y, Harnett-Sheehan K, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar G: The Mini International Neuropsychiatric Interview (M.I.N.I.): The Development and Validation of a Structured Diagnostic Psychiatric Interview. J. Clin Psychiatry, 1998;59(suppl 20):22-33.

Amorim P, Lecrubier Y, Weiller E, Hergueta T, Sheehan D: DSM-III-R Psychotic Disorders: procedural validity of the Mini International Neuropsychiatric Interview (M.I.N.I.). Concordance and causes for discordance with the CIDI. European Psychiatry. 1998; 13:26-34.

Translations	M.I.N.I. 4.4 or earlier versions	M.I.N.I. 4.6/5.0, M.I.N.I. Plus 4.6/5.0 and M.I.N.I. Screen 5.0:
Afrikaans		R. Emsley W. Maartens
Arabic		O. Osman, E. Al-Radi
Bengali		H. Banerjee, A. Banerjee
Brazilian Portuguese	P. Amorim	P. Amorim
Bulgarian		L. G.. Hranov
Chinese		L. Carroll, Y-J. Lee, Y-S. Chen, C-C. Chen, C-Y. Liu, C-K. Wu, H-S. Tang, K-D. Juang, Yan-Ping Zheng. In preparation
Croatian		
Czech		P. Zvlosky
Danish	P. Bech	P. Bech, T. Schütze
Dutch/Flemish	E. Griez, K. Shruers, T. Overbeek, K. Demyttenaere	I. Van Vliet, H. Leroy, H. van Megen
English	D. Sheehan, J. Janavs, R. Baker, K. Harnett-Sheehan, E. Knapp, M. Sheehan	D. Sheehan, R. Baker, J. Janavs, K. Harnett-Sheehan, M. Sheehan
Estonian		J. Shlik, A. Aluoja, E. Khil
Farsi/Persian		K. Khooshabi, A. Zomorodi
Finnish	M. Heikkinen, M. Lijeström, O. Tuominen	M. Heikkinen, M. Lijeström, O. Tuominen
French	Y. Lecrubier, E. Weiller, I. Bonora, P. Amorim, J.P. Lepine	Y. Lecrubier, E. Weiller, P. Amorim, T. Hergueta
German	I. v. Denffer, M. Ackenheil, R. Dietz-Bauer	G. Stotz, R. Dietz-Bauer, M. Ackenheil
Greek	S. Beratis	T. Calligas, S. Beratis
Gujarati		M. Patel, B. Patel
Hebrew	J. Zohar, Y. Sasson	R. Bardu, I. Levinson, A. Aviv
Hindi		C. Mittal, K. Batra, S. Gambhir
Hungarian	I. Bitter, J. Balazs	I. Bitter, J. Balazs
Icelandic		J.G. Stefansson
Italian	I. Bonora, L. Conti, M. Piccinelli, M. Tansella, G. Cassano, Y. Lecrubier, P. Donda, E. Weiller	L. Conti, A. Rossi, P. Donda
Japanese		T. Otsubo, H. Watanabe, H. Miyaoka, K. Kamijima, J.Shinoda, K.Tanaka, Y. Okajima
Korean		In preparation, Anxiety Disorder Association of Korea
Latvian	V. Janavs, J. Janavs, I. Nagobads	V. Janavs, J. Janavs
Lithuanian		A. Bacevicius
Norwegian	G. Pedersen, S. Blomhoff	K.A. Leiknes , U. Malt, E. Malt, S. Leganger
Polish	M. Masiak, E. Jasiak	M. Masiak, E. Jasiak
Portuguese	P. Amorim	P. Amorim, T. Guterres
Punjabi		A. Gahunia, S. Gambhir
Romanian		O. Driga
Russian		A. Bystritsky, E. Selivra, M. Bystritsky
Serbian	I. Timotijevic	I. Timotijevic
Setswana		K. Ketlogetswe
Slovenian	M. Kocmur	M. Kocmur
Spanish	L. Ferrando, J. Bobes-Garcia, J. Gilbert-Rahola, Y. Lecrubier	L. Ferrando, L. Franco-Alfonso, M. Soto, J. Bobes-Garcia, O. Soto, L. Franco, G. Heinze, C. Santana
Swedish	M. Waern, S. Andersch, M. Humble	C. Allgulander, M. Waern, A. Brimse, M. Humble, H. Agren
Turkish	T. Örnek, A. Keskiner, I. Vahip	T. Örnek, A. Keskiner, A Engeler
Urdu		A. Taj, S. Gambhir

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9.6.10. Mood disorder questionnaire

1. Has there ever been a period of time when you were not your usual self and...

...you felt so good or so hyper that other people thought you were not your normal self or you
were so hyper that you got into trouble?

Yes No

...you were so irritable that you shouted at people or started fights or arguments?

Yes No

...you felt much more self-confident than usual?

Yes No

...you got much less sleep than usual and found you didn't really miss it?

Yes No

...you were much more talkative or spoke much faster than usual?

Yes No

...thoughts raced through your head or you couldn't slow your mind down?

Yes No

...you were so easily distracted by things around you that you had trouble concentrating or staying on track?

Yes No

...you had much more energy than usual?

Yes No

...you were much more active or did many more things than usual?

Yes No

...you were much more social or outgoing than usual, for example, you telephoned friends in the middle of the night?

Yes No

...you were much more interested in sex than usual?

Yes No

...you did things that were unusual for you or that other people might have thought were excessive, foolish, or risky?

Yes No

...spending money got you or your family into trouble?

Yes No

2. If you checked YES to more than one of the above, have several of these ever happened during the same period of time?

Yes No

3. How much of a problem did any of these cause you—like being unable to work; having family, money, or legal troubles; getting into arguments or fights? Please select one response only.

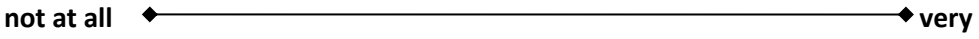
No Problem Minor Problem Moderate Problem Serious Problem

9.6.11. Mood visual analogue scale

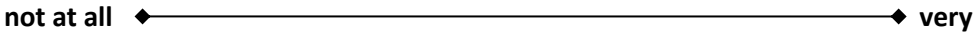
Visual Analogue Scales for Mood ratings

Participant No: _____ Date_____time_____

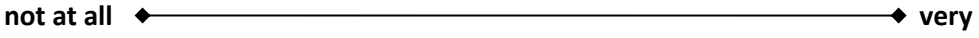
At the moment I am feeling



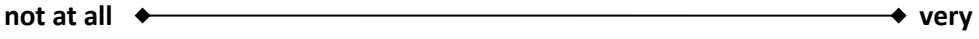
SAD



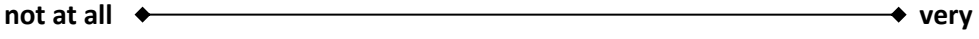
CALM



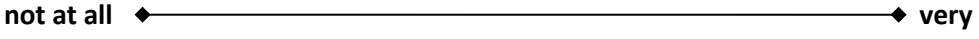
HORRIFIED



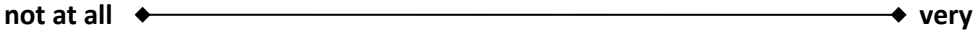
HAPPY



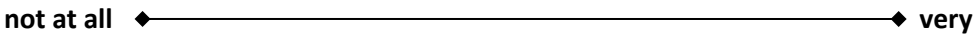
FEARFUL



HOPELESS



ANGRY



ALERT

not at all ◆——————————————————————————◆ very

SLEEPY

not at all ◆——————————————————————————◆ very

AWKWARD

not at all ◆——————————————————————————◆ very

TENSE

not at all ◆——————————————————————————◆ very

RESTLESS

not at all ◆——————————————————————————◆ very

IRRITABLE

not at all ◆——————————————————————————◆ very

RACING THOUGHTS

not at all ◆——————————————————————————◆ very

ANXIOUS

9.6.12. Morningness, eveningness questionnaire

MORNINGNESS-EVENINGNESS (LARK/OWL) QUESTIONNAIRE

SUBJECT CODE:_____ DATE:_____

INSTRUCTIONS

a) Please read each question very carefully before answering.
b) Answer all questions.
c) Answer questions in numerical order.
d) Each question should be answered independently of others. Do **NOT** go back and check your answers.
e) For some questions, you are required to respond by placing a cross alongside your answer. In such cases, select **ONE** answer only.
f) Please answer each question as honestly as possible. Both your answers and results will be kept in strict confidence.

Question 1

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

	or earlier 05:00 – 06:30	…..
	06:31 – 07:45	…..
Time: ……	07:46 – 09:45	…..
	09:46 – 11:00	…..
	11:01 – 12:00 or later	…..

Question 2

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

	20:00 – 21:00	…..
	21:01 – 22:15	…..
	22:16 – 00:30	
Time: ……..	00:31 – 01:45	…..
	01:46 – 03:00 or later	…..

Question 3

If there is a specific time you at which 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 [ ]

Question 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 [ ]

Question 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 [ ]

Question 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 [ ]

Question 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 [ ]

Question 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 [ ]

Question 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 07:00 and 08:00h. 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 [ ]

Question 10

At what time in the evening do you feel tired and as a result in need of sleep?

Time: ……

Question 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, and considering only your own "feeling best" - which ONE of the four testing times would you choose?

a. 08:00 – 10:00 [ ]

b. 11:00 – 13:00 [ ]

c. 15:00 – 17:00 [ ]

d. 19:00 – 21:00 [ ]

Question 12

If you went to bed at 23:00h at what level of tiredness would you be?

a. Not at all tired [ ]

b. A little tired [ ]

c. Fairly tired [ ]

d. Very tired [ ]

Question 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. Which ONE of the following events are you most likely to experience?

a. Will wake up at the usual time and will NOT fall asleep again [ ]

b. Will wake up at the usual time and will doze thereafter [ ]

c. Will wake up at the usual time but will fall asleep again [ ]

d. Will NOT wake up until later than usual [ ]

Question 14

One night you have to remain awake between 04:00 and 06:00h in order to carry out a night watch. You have no commitments the next day. Which ONE of the following alternatives will suit you best?

a. Will NOT go to bed until watch was over [ ]

b. Would take a nap before the watch and sleep after [ ]

c. Would take a good sleep before the watch and nap after [ ]

d. Would take ALL sleep before the night watch [ ]

Question 15

You have to do two hours of hard physical work. You are entirely free to plan your day and considering only your own "feeling best", which ONE of the following times would you choose?

a. 08:00 – 10:00 [ ]

b. 11:00 – 13:00 [ ]

c. 15:00 – 17:00 [ ]

d. 19:00 – 21:00 [ ]

Question 16

You have decided to engage in some physical exercise. A friend suggests that you do this for one hour twice a week and the best time for him is between 22:00 and 23:00h. Bearing in mind nothing else but your own "feeling best" - how well 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 [ ]

Question 17

Suppose that you can choose your own work hours. Assume that you had to work a FIVE-hour day (including breaks) and that your job was interesting and paid by results. Which FIVE CONSECUTIVE HOURS would you choose:

Note hours here: ……

Question 18

At what time of day do you think that you reach your "feeling best" peak?

Note Time here: ……

Question 19

One hears of "morning" and "evening" types. Which ONE of these types do you consider yourself to be?

a.	definitely a “morning type”	[ ]
b.	Rather more a “morning” than an “evening” type	[ ]
c.	Rather more an “evening” than a “morning” type	[ ]
d.	definitely an “evening” type	[ ]

9.6.13. Pittsburgh sleep quality index

Subject code: _____ Study: _____

Date: _____

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

1) During the past month, when have you usually gone to bed at night?

Usual bed time

2) During the past month, how long (in minutes) has it usually take you to fall asleep each 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 from the number of hours spent in bed.)

Hours of sleep per night

For each of the remaining questions, check the one best response. Please answer all questions.

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

a) Cannot get to sleep within 30 minutes

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week____

b) Wake up in the middle of the night or early morning

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week___

c) Have to get up to use the bathroom

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week___

d) Cannot breathe comfortably

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

Not during the	Less than	Once or twice	Three or more

past month_____	once a week_____	a week_____	times a week___

f) Feel too cold

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week___

g) Feel too hot

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week___

h) Had bad dreams

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week___

i) Have pain

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week___

j) Other 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 show enthusiasm to get things done?

No problem at all_____

Only a very slight problem_____

Somewhat of a problem_____

A very big problem_____

10) How often in the past month you have experienced the following:

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___

11) Do you have a bed partner or roommate?

No bed partner or roommate?_____

Partner/roommate in other room_____

Partner in same room, but not same bed_____

Partner in same bed_____

If you have a roommate 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___

9.6.14. Positive and negative effect scale

PANAS(state)

Study no._____Date_____

This scale consists of a number of words that describe different feelings and emotions. Read each item and then mark the appropriate answer in the space next to the word. Indicate to what extent you feel this way **right now**, that is, **at the present moment**.

	1 =	*2 =*	***3 =***	*4 =*	***5 =***	
	Not at all	*A little*	***Moderately***	*Quite a bit*	***extremely***	
Interested						
Distressed						
Excited						
Upset						
Strong						
Guilty						
Scared						
Hostile						
Enthusiastic						
Proud						
Irritable						
Alert						
Ashamed						
Inspired						
Nervous						
Determined						
Attentive						
Jittery						
Active						
Afraid						

9.6.15. Profile of mood state

NCS Trans-Optic® M08-70416-3

NAME _____ DATE _____

SEX: Male (M) Female (F)

IDENTIFICATION

Below is a list of words that describe feelings people have. Please read each one carefully. Then fill in ONE circle under the answer to the right which best describes HOW YOU HAVE BEEN FEELING DURING THE PAST WEEK INCLUDING TODAY.

The numbers refer to these phrases.

0 = Not at all
1 = A little
2 = Moderately
3 = Quite a bit
4 = Extremely

Col (C) O.P. (O)

	NOT AT ALL	A LITTLE	MODERATELY	QUITE A BIT	EXTREMELY
1. Friendly	0	1	2	3	4
2. Tense	0	1	2	3	4
3. Angry	0	1	2	3	4
4. Worn out	0	1	2	3	4
5. Unhappy	0	1	2	3	4
6 Clear-headed	0	1	2	3	4
7. Lively	0	1	2	3	4
8. Confused	0	1	2	3	4
9. Sorry for things done	0	1	2	3	4
10. Shaky	0	1	2	3	4
11. Listless	0	1	2	3	4
12. Peeved	0	1	2	3	4
13. Considerate	0	1	2	3	4
14. Sad	0	1	2	3	4
15. Active	0	1	2	3	4
16. On edge	0	1	2	3	4
17. Grouchy	0	1	2	3	4
18. Blue	0	1	2	3	4
19. Energetic	0	1	2	3	4
20. Panicky	0	1	2	3	4
21. Hopeless	0	1	2	3	4
22 Relaxed	0	1	2	3	4
23. Unworthy	0	1	2	3	4
24. Spiteful	0	1	2	3	4
25. Sympathetic	0	1	2	3	4
26. Uneasy	0	1	2	3	4
27. Restless	0	1	2	3	4
28. Unable to concentrate	0	1	2	3	4
29. Fatigued	0	1	2	3	4
30. Helpful	0	1	2	3	4
31. Annoyed	0	1	2	3	4
32. Discouraged	0	1	2	3	4
33. Resentful	0	1	2	3	4
34. Nervous	0	1	2	3	4
35. Lonely	0	1	2	3	4
36. Miserable	0	1	2	3	4
37. Muddled	0	1	2	3	4
38. Cheerful	0	1	2	3	4
39. Bitter	0	1	2	3	4
40. Exhausted	0	1	2	3	4
41. Anxious	0	1	2	3	4
42. Ready to fight	0	1	2	3	4
43. Good natured	0	1	2	3	4
44. Gloomy	0	1	2	3	4
45. Desperate	0	1	2	3	4
46. Sluggish	0	1	2	3	4
47. Rebellious	0	1	2	3	4
48. Helpless	0	1	2	3	4
49. Weary	0	1	2	3	4
50. Bewildered	0	1	2	3	4
51. Alert	0	1	2	3	4
52. Deceived	0	1	2	3	4
53. Furious	0	1	2	3	4
54. Efficient	0	1	2	3	4
55. Trusting	0	1	2	3	4
56. Full of pep	0	1	2	3	4
57. Bad-tempered	0	1	2	3	4
58. Worthless	0	1	2	3	4
59. Forgetful	0	1	2	3	4
60. Carefree	0	1	2	3	4
61. Terrified	0	1	2	3	4
62. Guilty	0	1	2	3	4
63. Vigorous	0	1	2	3	4
64. Uncertain about things	0	1	2	3	4
65. Bushed	0	1	2	3	4

MAKE SURE YOU HAVE ANSWERED EVERY ITEM.

POM 021

9.6.16. State-trait anxiety inventory

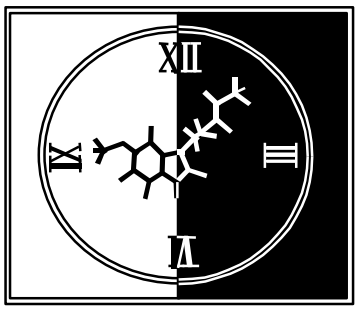
STATE ANXIETY MOOD RATING SCALE

Subject ID_____Date_____

		Not at all	Somewhat	Moderately so	Very much so
1.	I feel calm…….	1	2	3	4
2.	I feel secure……..	1	2	3	4
3.	I am tense…….	1	2	3	4
4.	I feel strained……	1	2	3	4
5.	I feel at ease……..	1	2	3	4
6.	I feel upset…….	1	2	3	4
7.	I am presently worrying over possible misfortunes……..	1	2	3	4
8.	I feel satisfied……	1	2	3	4
9.	I feel frightened…….	1	2	3	4
10.	I feel comfortable……..	1	2	3	4
11.	I feel self-confident……..	1	2	3	4
12.	I feel nervous……	1	2	3	4
13.	I am jittery…….	1	2	3	4
14.	I feel indecisive…….	1	2	3	4
15.	I am relaxed……..	1	2	3	4
16.	I feel content…….	1	2	3	4
17.	I am worried…….	1	2	3	4
18.	I feel confused……..	1	2	3	4
19.	I feel steady……..	1	2	3	4
20.	I feel pleasant……	1	2	3	4

9.7. Appendix VII: hormone assay protocols

9.7.1. Protocol for salivary cortisol assay



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INSTRUCTIONS FOR RADIOIMMUNOASSAY OF CORTISOL IN HUMAN SALIVA

Adapted from: Read GF et al (1977). Ann Clin Biochem 14, 323-349

Seth J et al (1978). Clin Chim Acta 86, 109-120

Riad-Fahmy D et al (1979). Clin Chim 25, 665-668

The technique provides a sensitive, rapid and reproducible procedure. The saliva sample is incubated with a specific antiserum raised in a sheep and trace amounts of ^{125}I cortisol are then added. The free and antibody bound fractions of cortisol are separated using a solid phase linked second antibody and the antibody bound fraction precipitated by centrifugation. The radioactivity in this bound fraction is counted in a gamma counter. A standard curve is constructed at the same time from standards made up in buffer.

REAGENTS

All water used is prepared by reverse osmosis or is double glass distilled.

Buffer: 0.1M citrate/phosphate pH 4.0.

13.0g citric acid)
8.9g Na_2HPO_4anhydrous) to 1L with water
1.0g gelatin)
0.2g thiomersal)

The buffer is stored at 4˚C for up to two weeks

Antiserum: Sheep anti-cortisol-3-O-(carboxymethyl) oxime – bovine serum albumin conjugate obtainable from Scottish Antiserum Production Unit (product no.: S004-201) and used at an initial dilution of 1:8,000.

Each vial of freeze-dried antiserum is reconstituted with 1ml H_2O and allowed to stand at room temperature for 1h, 7ml assay buffer are added and 100µl aliquots kept at -20 ˚C until use. These aliquots are stable for at least 6 months

The working dilution is prepared by diluting one of the above aliquots with 9.9ml assay buffer and is made fresh each day.

Solid phase separation system: cellulose linked donkey anti-sheep IgG (Dapsep, Stockgrand Ltd) is stirred gently for 10min prior to, and during, use. This product is stable for at least 6 months.

Radiolabel: cortisol-3-O-(carboxymethyl)-oximino-^{125}iodohistamine (Amersham International product no: IM 129) is diluted in assau buffer such that 100µl contains approximately 10,000cpm. This product is stable for 2 months.

Standards: a solution of cortisol (Sigma Ltd. Product no: H4001) at 1mg/ml in ethanol is further diluted in assay buffer to give 1500mU/L. 0.5ml aliquots are then stored at -20 ˚C until use and are stable for at least 6 months.

This solution is further diluted to give working standards of 0, 1.4, 2.8, 5.7, 11.5, 23, 47, 984, 188, 375 and 750mU/L. These solutions are prepared freshly for each

Brji/ saline wash: dissolve 9.0g NaCl in 998ml H_2O and add 2.0ml Brji 35.

Saliva samples: saliva samples for analysis are stored at -20 ˚C until assayed. After thawing samples are mixed are then centrifuged at 1500rpm for 5min to remove any particulate matter.

METHOD

Duplicate tubes are set up for all standards, samples, total radioactivity and non-specific binding tubes. The volumes required for the assay are as follows:

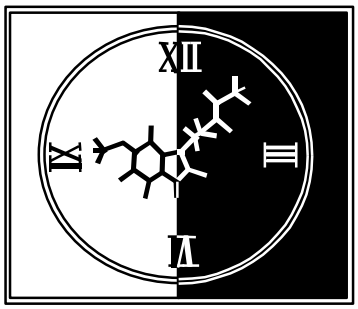
Samples	200µl
Standards	50µl + 150µl assay buffer
Antiserum	100µl
Radiolabel	100µl
Dapsep	900µl

The assay is performed in plastic LP3 tubes.

ASSAY PROTOCOL.

1. Pipette standards to form standard curve.
2. Pipette samples
3. Add 100μl antiserum to all tubes expect NSBs and totals
4. Add 100μl radiolabel to all tubes
5. Vortex and incubate at room temperature for 2h
6. Add 100μl Dabsep to all tubes expect the totals
7. Vortex and incubate at room temperature for 1h, vortexing every 15mins
8. Add 1ml Brij / saline wash to all tubes expect the totals
9. Centrifuge all tubes at 2,000rpm from 15min
10. Decant and discard the supernatant
11. Count the antibody bound fraction in the pellet in a gamma radiation counter
12. Determine the cortisol concentration in the sample from the dose response curve.

9.7.2. Protocol for urinary aMT6s assay



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INSTRUCTIONS FOR RADIOIMMUNOASSAY OF 6-SULPHATOXYMELATONIN (6-HYDROXYMELATONIN SULPHATE, aMT6S) IN HUMAN URINE USING AN ^{125}I LABELLED TRACER.

This method is also suitable for use in rat, hamster, gerbil, guinea-pig and other species. The standard curve should be constructed in charcoal stripped urine from the same species as the samples.

The RIA of aMT6S has been modified from Arendt et al (1985) to use iodinated aMT6S (Aldhous et al (1988)). The technique provides a more sensitive, rapid and economical procedure. Results with the iodinated aMT6S correlate well with the original ^{3}H aMT6S assay. The diluted urine sample is incubated with a specific antiserum to aMT6S raised in a sheep and trace amounts of ^{125}I-aMT6S are then added. The free and antibody-bound fractions of aMT6S are separated using a dextran-coated charcoal suspension. The free aMT6S fraction is precipitated with the charcoal by centrifugation and the radioactivity counted in a gamma counter. A standard curve is constructed at the same time from standards made up in charcoal stripped urine.

REAGENTS

All water used is freshly double-glass distilled and stored in glass.

Buffer: Tricine (Sigma Ltd. product no.T-0377) is made up at 0.1M (pH 5.5) with 0.9% NaCl and 0.1% gelatin. It is necessary to heat to 50^{o}C for 30min to dissolve the gelatin.

17.9g tricine	)	
0.9g NaCl	)	to 1L with DGDW
1.0g gelatin	)	

Buffer is made up fresh weekly.

Antiserum: Sheep anti-aMT6S antiserum, batch number G/S/1118-23884 is supplied freeze-dried in different sized aliquots which should be reconstituted as follows:

AB/S/04 - sufficient for at least 10,000 assay tubes.

Reconstitute the contents of the vial with 1ml DGDW and add 9ml assay buffer to give an intermediate dilution of 1:100. Divide into 100μl aliquots which can be stored at -20^{o}C for up to 6 months: each aliquot is sufficient for 100 assay tubes. The working dilution of 1:20,000 is made up as required by diluting an aliquot to 20ml with assay buffer.

AB/S/05 - sufficient for at least 1000 assay tubes.

Reconstitute the contents of the vial with 1ml DGDW to give a 1:100 dilution. Aliquots can be stored or diluted for use as described above.

AB/S/06 - sufficient for at least 150 assay tubes.

Reconstitute the contents of the vial with 150μl DGDW and add 29.85ml assay buffer to provide the working dilution of 1:20,000.

The working dilution should be prepared on the day of the assay and cannot be stored.

Dextran-coated charcoal: suspend activated charcoal (Sigma Ltd. product number C-5260) at 2% w/v in assay buffer. Stir for 5 minutes. Centrifuge at 4^{o}C for 5min at 1000rpm. Discard the supernatant and any fines around the sides of the vessel. Resuspend the charcoal in the original volume of assay buffer and add 0.02% w/v dextran T-70 (Sigma Ltd. product number D-1390). Stir for at least 1hr at 4^{o}C. Store at 4^{o}C and make up fresh weekly.

Radiolabel: instructions for preparing ^{125}I-aMT6S.

This method is adapted from the method of Vakkuri et al (1984). 10μg aMT6S is supplied in methanol. This should be evaporated to dryness under nitrogen and the residue redissolved in 10μl 0.05M phosphate buffer pH 6.0 (1mg/ml)

Iodogen (Sigma Ltd. product number T-0656) is dissolved at 100μg/ml in chloroform and 10μl (1μg) aliquots dispensed into Eppendorf tubes. The chloroform is allowed to evaporate at room temperature and the tubes stored at 4^{o}C for up to one year prior to use.

5μl of a 1mg/ml solution aMT6S in 0.05M phosphate buffer pH 6.0 (5μg aMT6S) and 2μl Na-^{125}I (0.2mCi) in NaOH are added simultaneously to an Eppendorf tube containing iodogen. After mixing for 2 minutes 100μl methanol is added: the mixture is vortexed for 30sec and left overnight at 4^{o}C.

The MeoH phase is subjected to TLC on cellulose F plastic plates (Merck) using butan-1-ol : glacial acetic acid : DGDW, 4 : 1.5 : 1 and allowed to run for approximately 12 - 15cm. The plate is then removed from the tank, blown dry with O-free nitrogen and re-run to a similar end-point. The plate is then cut into 1cm sections to locate the position of the radiolabelled aMT6S. The plate sections containing a peak of radioactivity occurring at approximate Rf 0.68 are eluted overnight with 5ml MeOH containing 0.1% ascorbic acid.

Each label should be assessed by standard antiserum dilution and displacement curves before use. An average yield for this procedure is 100μCi in the specific ^{125}I-aMT6S fraction and the radiolabel can be stored for up to 4 months at 4^{o}C. The plate sections and cellulose do not have to be removed for storage.

The working solution is prepared by diluting the stock solution with assay buffer to give 8,000 - 10,000 cpm/100μl. This is prepared freshly for each assay.

Charcoal stripped (aMT6S-free) urine; this is supplied freeze-dried (100μl per vial). Reconstitute with 25ml assay buffer to give a dilution of 1:250. Store at -20^{o}C.

Standards: 500pg aMT6S (sufficient for one standard curve) is supplied at 200pg/ml in 1:250 diluted, charcoal stripped urine and in this form is stable for up to 12 weeks if stored at 4^{o}C. Further dilution with charcoal stripped urine (1:250 in assay buffer) provides standards (0, 1, 2, 4, 8, 14, 20, 20, 40 and 100pg) for the standard curve, i.e.,

aMT6S standard 200pg/ml urine	**aMT6S-free urine 1:250 dilution**	**aMT6S pg/tube**	**aMt6S ng/ml**
0	500μl	0	0
5μl	495μl	1	0.5
10μl	490μl	2	1.0
20μl	480μl	4	2.0
40μl	460μl	8	4.0
70μl	430μl	14	7.0
100μl	400μl	20	10.0
200μl	300μl	40	20.0
500μl	0	100	50.0

The standards are treated in exactly the same way as the urine samples in the assay.

Urine samples: urine samples for analysis are stored at -20^{o}C until assayed. aMT6S is stable for at least 2 years in urine stored at -20^{o}C, for at least 5 days at 4^{o}C or at room temperature. Urine is diluted 1:250 with assay buffer prior to assay.

Urine samples can be diluted directly into the assay tubes using an automatic diluter: if this is not available it is suggested that an intermediate dilution (e.g. 1:50) is made for each sample.

General:

i) the radioimmunoassay is performed in plastic tubes.

ii) disposable glass or plastic apparatus is used where possible with essential glassware being sonicated in methanol (AR grade) before use to eliminate contamination. All water used for cleaning and rinsing is DGDW.

iii) the figures given for stability of various reagents are minimum estimates.

METHOD.

Duplicate tubes are set up for all samples, standards, totals and NSBs. The volumes required in the assay are as follows:

Samples/standards	500μl
Antiserum	200μl
Radiolabel	100μl
Dextran-coated charcoal	100μl
Total volume	900μl

The volumes can be added with ordinary microlitre dispensers or preferably with repeating dispensers. It is necessary to restrict assay sizes to 150 tubes or less in order to eliminate assay drift.

ASSAY PROTOCOL.

1. Pipette standards and diluted (1:250) stripped urine to form standard curve.

2. Add 500μl of diluted urine sample to assay tube.

3. Add 200μl of diluted antiserum to all tubes except total count and non-specific binding tubes. Vortex and incubate at room temperature for 30 minutes.

4. Add 100μl ^{125}I-aMT6S to all tubes and vortex. Incubate for 15 - 18 hours at 4^{o}C.

(For a less sensitive , but more rapid assay, this incubation can be reduced to 2 hours).

5. Separate antibody-bound aMT6S from the free fraction by incubation for 15min at 4^{o}C with 100μl dextran-coated charcoal (stirred during and after addition). The charcoal should be stirred for 30min prior to use. Charcoal addition and vortexing should be done quickly to reduce intra-assay variation.

6. Centrifuge at 3500rpm at 4^{o}C for 15min.

7. Decant supernatant over a mesh and discard. This must be done immediately after centrifugation. Blot the tops of the tubes.

8. Count the non-antibody-bound fraction in the charcoal pellet in an appropriate gamma-radiation counter. Determine the aMT6S concentration in the samples from the dose-response curve.

OTHER COMMENTS: those workers wishing to use tritiated aMT6S should follow the procedure described in Arendt et al (1985)

REFERENCES:

Arendt J., Bojkowski C., Franey C., Wright J. and Marks V. (1985). Immunoassay of 6-hydroxymelatonin sulphate in human plasma and urine: abolition of the urinary 24-hour rhythm with atenolol. J. Clin. Endoc. Met. 60, 1166-1173.

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