

Sleep physiology, circadian rhythms, and the risk for developing psychosis



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Abstract

Sleep and circadian rhythm disruption (SCRD) is frequently found to co-occur in psychotic disorders. This can include a range of phenotypes such as insomnia, circadian delays, deficits in sleep spindles, and sleep-dependent cognitive impairments. However, increasing evidence suggests that psychosis occurs across a continuum of severity within the general population, yet few studies have investigated sleep and circadian rhythms prior to clinical diagnosis. Furthermore, SCRD and psychosis are posited to share underlying neuropathologies and although increasing evidence implicates shared genetic influence, little is known of the shared environmental risk. This thesis investigates sleep and circadian rhythms at multiple levels, from their occurrence in the general population, their disruption in high risk individuals, and a focus on sleep spindle oscillations in the brain. Firstly, the relationship between subjective measures of sleep and circadian rhythms, risk factors for psychosis, and psychotic-like experiences (PLEs) were studied in a large population sample. All three were highly related, with a subset of risk factors showing a strong association to both PLEs and SCRD. Secondly, sleep and circadian rhythms were assessed in individuals at high risk for developing psychosis based upon having a high load of risk factors and sub-clinical psychotic symptoms. High risk individuals showed subjective SCRD but this was not reflected in objective measures assessed by actigraphy and polysomnography. A subset of high risk individuals further showed substantially later melatonin rhythms compared to a low risk group. Thirdly, high and low risk individuals were assessed for measures of declarative and procedural sleep dependent memory consolidation. High risk individuals showed no evidence for sleep dependent cognitive impairment but did show a potential sensitivity in performance to the amount of sleep, not seen in low risk individuals. Finally, recordings in the somatosensory cortex (SCx) of mice were used as a model to explore the spatio-temporal dynamics and functional significance of sleep spindles. Distinct highly localised spindle events were discovered in the anterior SCx, with their complete absence just a fraction of a millimetre away, and their occurrence of which coincided with unique responses to global vigilance states. Together, this work pioneers research into sleep and circadian phenotypes associated with both sub-clinical psychotic symptoms and risk factors for disease and furthers our knowledge of particular sleep processes which could collectively help us to understand why SCRD and psychosis develop.

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Attributions

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Chapter contents

0 Glossary	4
1 Chapter I: An introduction to psychosis, sleep, and circadian rhythms	7
1.1 An introduction to psychosis.....	8
1.2 An introduction to sleep	16
1.3 An introduction to circadian rhythms	32
1.4 Sleep and circadian rhythms in psychosis.....	37
1.5 Thesis aims	43
2 Chapter II: The interplay between sleep disturbance and risk factors for psychosis: A population survey	47
2.1 Introduction	48
2.2 Methodology	50
2.3 Results	58
2.4 Discussion	75
3 Chapter III: Phenotyping sleep and circadian rhythms in individuals at high risk for developing psychosis	85
3.1 Introduction	86
3.2 Methodology	88
3.3 Results	99
3.4 Discussion	112
4 Chapter IV: Sleep dependent memory consolidation in individuals at high risk for developing psychosis	123
4.1 Introduction	124
4.2 Methodology	125
4.3 Results	129
4.4 Discussion	135
5 Chapter V: Hidden cortical spindles during sleep in mice	143
5.1 Introduction	144
5.2 Methodology	146
5.3 Results	156
5.4 Discussion	167
6 Chapter VI: Impact of findings and future directions.....	173
6.1 Summary of findings.....	173
6.2 The shared pathology between SCRD and psychosis.....	174
6.3 Improving the identification, prediction, and prevention of psychosis	176
6.4 Future directions	179
6.5 Conclusions	181
7 Bibliography.....	183
8 Supplementary Material.....	206

Glossary and abbreviations

Explanations of the most important abbreviations and terminology used in this thesis are explained below.

Acrophase: Time of peak in (circadian) rhythm

Actiwatch: A device used to measure wrist movement - a proxy for sleep-wake cycles

aMT6: 6-hydroxymelatonin sulphate; a maker of melatonin output

CAs: Childhood adversities

CAARMS: Comprehensive Assessment of At Risk Mental States

CAR: Cortisol awakening response

Chronotype: A behavioural manifestation of an individual's underlying circadian rhythm

Circadian clock: A biochemical oscillator which acts as an internal time keeping system

Circadian rhythm: A biological rhythm of around 24 hours

DASS-21: Depression, anxiety, and stress scale (21 item version)

Declarative memory: Based upon conscious recollection (e.g. the capital of France)

EEG: Electroencephalography

EMG: Electromyography

Entrainment: Synchronisation of the circadian clock with the external environment

ERFs: Environmental risk factors

FFT: Fast Fourier Transform

Firing rate: The rate at which a neuron spikes (action potentials)

HPA axis: Hypothalamic-Pituitary-Adrenal Axis

LFP: Local field potential; an electrophysiological signal from multiple neurons within a small volume of neuronal tissue

MCTQ: Munich Chronotype Questionnaire

Mid-sleep point: Mid-point in time between sleep onset and sleep offset

MINI: Mini Neuropsychiatric Interview

MSFsc: Mid-sleep point on free days corrected for oversleep

MST: Motor sequence task

MUA: Multi-unit activity; neuronal activity from a small number of cells

Non-declarative memory: Not based upon conscious recollection (e.g. riding a bike)

NREM sleep: Non-rapid eye movement sleep

PLEs: Psychotic-like experiences

PQ-16: Prodromal Questionnaire-16

PSG: Polysomnography

PSQI: Pittsburgh Sleep Quality Index

REM sleep: Rapid eye movement sleep

SCRD: Sleep and circadian rhythm disruption

SCN: Suprachiasmatic nucleus

SCx: Somatosensory cortex

SFA: Spindle frequency activity

SJL: Social jetlag

Sleep homeostasis: A biochemical system controlling sleep pressure

Spike: A neuronal action potential

Spindle density: The number of sleep spindles per time interval (e.g. per minute)

Spindle frequency: The speed of a sleep spindle oscillation (in Hz)

SUA: Single-unit activity; neuronal activity (spikes) from one (putative) cell

SWA: Slow-wave activity

SWS: Slow-wave sleep

UHR: Ultra-high risk (for developing psychosis)

Vigilance state: The cortical state of the brain, in wake, NREM or REM sleep

WPL: Word-pair learning task

Chapter I: An introduction to psychosis, sleep, and circadian rhythms

Contents

1.1 An introduction to psychosis	8
1.1.1 Psychotic disorders	8
1.1.2 Predicting psychosis	10
1.1.3 The psychosis continuum	11
1.1.4 Risk factors and the development of psychosis	12
1.1.5 The impact of risk factors	13
1.1.6 The specificity of risk factors	15
1.2 An introduction to sleep	16
1.2.1 What is sleep?	16
1.2.2 Measuring sleep	18
1.2.3 The electroencephalogram	19
1.2.4 Oscillations of sleep	21
1.2.5 The function of sleep and its role in memory	23
1.2.6 The neural circuits of sleep and wakefulness	27
1.2.7 Local and global sleep	30
1.2.8 The regulation of sleep	31
1.3 An introduction to circadian rhythms	32
1.3.1 What are circadian rhythms?	32
1.3.2 Melatonin and entrainment of the SCN	34
1.3.3 Chronotype	36
1.4 Sleep and circadian rhythms in psychosis	37
1.4.1 Sleep and circadian rhythm disruption in psychotic disorders	37
1.4.2 SCRD in the development of psychosis	39
1.4.3 Underlying mechanisms of SCRD and psychosis	40
1.4.4 The role of sleep spindles	42
1.5 Thesis aims	43

1.1 An introduction to psychosis

1.1.1 Psychotic disorders

Psychosis is a broad mental health problem defined by a loss of contact with reality. It is largely characterised by positive symptoms including hallucinations, delusions, and disorganised thinking, although negative symptoms and cognitive impairments also commonly co-occur¹ (Figure 1.1). Psychosis is a characteristic feature of schizophrenia but can be found across multiple mental health disorders including affective disorders such as bipolar type I disorder and some forms of unipolar depression. It therefore occurs across a 'schizoaffective spectrum', identified by particular shared symptoms and traits across disorders². The lifetime prevalence rate for all psychotic disorders is estimated to be around 3.48%³. Psychosis onset can range anywhere between 12 to 60 years of age but most commonly occurs between the ages of 19 to 25 with a median age around 22⁴. Psychosis severely affects cognitive and social functioning often causing social withdrawal, depression, and impaired physical health, ultimately leading to a poorer quality of life⁵. Psychotic disorders are also associated with premature mortality which may be the result of greater risk for suicide, heart disease and cancer⁶. These factors generate substantial social and financial burden worldwide with an estimated cost across countries ranging from \$94 million to \$102 billion US dollars per year⁷. Psychosis is primarily treated with antipsychotic medication such as clozapine, risperidone and haloperidol. However these are impermanent treatments that largely focus on the positive symptoms, and although effective at reducing them, are also associated with several, sometimes serious side effects⁸. There is currently no cure or effective prevention of psychosis or psychotic disorders.

Positive symptoms	
Hallucinations	Perceptual experiences that occur in any sensory modality without external stimulus e.g. hearing things such as whispering, seeing things such as a face in the mirror, or tasting metallic substances when none of which actually exist
Delusions	Having fixed irrational beliefs that are not open to change despite conflicting evidence. This can include 'non-bizarre' ideas such as persecutory ideas, feelings of grandeur and jealousy and 'bizarre' ideas such as mind reading
Disorganized thinking	Inferred from disorganised speech which can be unrelated to the conversation, frequently involve switching from one topic to another, and is sometimes incomprehensible
Catatonia	A marked decrease in reactivity to the environment ranging from bizarre postures to a complete lack of verbal and motor response
Negative symptoms	
	Including alogia (inability to speak), avolition (severe lack of motivation) and anhedonia (inability to feel pleasure)
Cognitive symptoms	
	Including deficits in attention, working memory, executive functions

Figure 1.1: Descriptions of the most common symptoms of schizophrenia¹. Psychosis most commonly includes the positive symptoms.

Psychosis does not exist as a binary, irreversible state. Individuals with a psychotic disorder such as schizophrenia can experience fluctuations in psychotic symptoms throughout their lives from changes within a day to changes across seasons or even years^{9,10}. Psychotic episodes often manifest as brief periods of severe positive symptoms which may be broken by periods of manageable, low symptom severity, or relatively good health (remission). The first episode of psychosis is often reported as the most severe and hardest to manage, since the individual is often naïve to treatment and unaware of its development¹¹. Subsequent episodes may thus be under tighter control from medication and are managed at an earlier stage. The development of a psychotic episode is generally thought to arise from a gradual process that is initially seen from behavioural changes in the individual before the acute psychotic symptoms manifest. This period, known as the prodromal phase, is therefore a useful marker for predicting the onset of or relapse in psychotic disorders¹¹.

1.1.2 Predicting psychosis

Predicting the occurrence of a psychotic episode is invaluable for improving treatment and intervention strategies^{12,13}. Because of this, increasing research is now focusing on identifying individuals at high risk for developing psychosis. Currently, the two most common methods for identifying individuals at high risk rely upon either genetic risk or symptomatic risk¹⁴. Genetic risk is based upon the high heritability of psychotic disorders and identifies a 'vulnerability group' of first-degree relatives of individuals with psychotic disorders. Symptomatic risk is largely based upon identifying the initial prodrome before the onset of a first psychotic episode. Approximately 80-90% of patients with schizophrenia retrospectively report subclinical symptoms in the months or years preceding psychosis including changes in attention, perception and mood¹⁵. When prospectively assessed, the initial prodrome can be referred as the 'At Risk Mental State', or 'Ultra High Risk' (UHR), defined by the presence of premorbid or sub-clinical symptoms of psychosis which may affect the stability of an individual's mental state and level of functioning, but not necessarily lead to a diagnosed disorder¹⁶. This state is most commonly assessed through the use of questionnaires and interviews, such as the Comprehensive Assessment of At Risk Mental States (CAARMS¹⁶). Both the genetic and symptomatic approaches provide an important methodology for identifying individuals who are likely to transition to a first episode and those who may require immediate treatment or intervention. However, the effective and accurate identification of symptomatic UHR individuals is a difficult task and genetic risk is not always sensitive enough to identify those who will develop psychosis. Further, although intervention could alleviate or delay future psychotic symptoms, it is unlikely to prevent them at this stage of disease progression. Finding additional markers of mental health or identifying individuals at even earlier stages is therefore essential for improving treatment and prevention strategies.

1.1.3 The psychosis continuum

There is now an increasing body of literature which suggests that rather than a dichotomous clinical diagnosis, psychosis exists along a continuum of symptomatology, present across the whole general population¹⁷. In this sense, the presence of a disorder or even poor health is not inevitably associated with experiencing symptoms of psychosis, but rather depends on factors such as the amount of distress, the frequency of occurrence, or coping mechanisms associated with these experiences. These sub-clinical symptoms are known as psychotic-like experiences (PLEs) and can be defined as symptoms that resemble psychotic symptoms but are often far milder in nature, occur less frequently, and are not necessarily associated with distress or impairment to daily functioning¹⁷. Psychosis may therefore exist across a continuum that includes psychotic-like experiences (PLEs), psychotic symptoms (when experienced at a clinically relevant level) and clinical psychotic disorders (when all criteria are met for a clinical diagnosis). PLEs are estimated to occur in around 5-8% of the general population and although they are not necessarily clinically relevant in terms of treatment need, they are associated with increased risk for developing a psychotic disorder^{14,18}. Using brief self-report questionnaires to assess PLEs therefore has an advantage over screening for prodromal/UHR states since they can be easily administered to the general population or vulnerable groups, allowing a quick, large scale screening of individuals for risk levels. Furthermore, PLEs can occur at any age, and most commonly manifest during adolescence allowing individuals to be assessed at an earlier stage, before psychotic symptoms develop¹⁴. Why some individuals develop PLEs and why some go on to develop psychosis is unclear, but is thought to involve particular traits or experiences that could cause, trigger, or exacerbate psychotic symptoms¹⁹. These are collectively known as risk factors and are critically implicated in the development of psychosis, and likely psychotic-like experiences^{20,21}.

1.1.4 Risk factors and the development of psychosis

Risk factors are defined as traits or exposures that increase the risk for developing a disorder. Since the causative association between some risk factors and psychosis is unclear, many 'symptom-like' traits such as PLEs, personality, or cognitive impairments can be thought of as both risk factors and comorbidities, or potential developmental stages of psychosis. In general, risk factors are most broadly split into those that are of either genetic or environmental origin. Genetic risk factors commonly arise from hereditary mutations or polymorphisms. Both twin and family studies have shown that individuals with a family member who have a psychotic disorder, are at increased risk for developing a psychotic disorder themselves. This heritability in schizophrenia has been estimated to be up to 80% and genetics are known to play a major role in the development of psychotic disorders^{22,23}. For example, one of the strongest genetic abnormalities associated with the development of schizophrenia is a microdeletion on chromosome 22. 22q11.2 Deletion Syndrome occurs in between 1 in 2500 to 4000 live births and includes multiple clinical features with as much as 30% of individuals going on to develop schizophrenia²⁴. In general, major gene effects are uncommon in complex disorders such as schizophrenia and are instead thought to arise from multiple genes of small effect which may act additively or multiplicatively to increase risk²⁵. Single nucleotide polymorphisms (SNPs) denote variations in single nucleotides within genes and commonly occur within the general population where they are widely investigated for their potential influence on disease. With the introduction of genome-wide association studies and subsequent meta-analyses it is now possible to achieve a powerful detection of SNPs in genes that are associated with psychotic disorders. A range of risk genes have thus been identified such as *COMT*, *DISC1*, and *NRG1* which have a range of functions from involvement in the inactivation of neurotransmitters, neural development and synaptic plasticity²⁵.

An abundance of evidence has shown that environmental factors also play a key role in the development of psychosis. These environmental risk factors (ERFs) can be defined as any attribute, characteristic, exposure, or personal experience that an individual may have which increases their likelihood of developing a psychotic disorder²⁰. ERFs therefore encompass a broad range of factors involving personality traits, social interactions, growth and development or the physical environment. ERFs also have a broad temporal influence and can impact the individual prenatally, during infancy, childhood, adolescence and adulthood²⁰. Examples of ERFs commonly associated with psychotic disorders include cannabis use²⁶, childhood abuse²⁷, migration²⁸, and obstetric complications²⁹. Since there is often a large degree of heterogeneity between studies in terms of assessment methods, cohorts, and study design (with many relying upon retrospective reports), the relative risk for each ERF is unclear. Nevertheless they are still a topic of ongoing research and can provide invaluable insight into the aetiology of psychosis and for identifying individuals who are at risk for developing disorders.

1.1.5 The impact of risk factors

How do risk factors interact and lead to the development of psychosis? The underlying mechanisms behind the impact of genetic and environmental factors remain largely undetermined. The central theory is that psychosis arises from alterations in neural signalling. The finding that many antipsychotic drugs have antagonistic effects on dopamine D₂ (and D₂-like) receptors has led to a large body of research supporting the 'dopamine hypothesis'. This model proposes that positive symptoms of psychosis arise from the hyperactivity of dopaminergic subcortical mesolimbic transmission³⁰. However this is not well understood and many other pathways and neurotransmitters are thought to contribute including the hypofunction of glutamatergic signalling via NMDA receptors (the 'glutamate hypothesis')³¹. The influence of genetic and environmental factors on these neuronal signalling pathways is a complex, multifaceted process. Genetic factors may

moderate the sensitivity of neural cells or circuits whereas environmental factors such as those during childhood are largely thought to enhance stress signalling pathways which can then lead to impaired neuronal responsiveness^{32,33}. Specifically it is hypothesised that repeated exposure of stressful ERFs causes progressively greater responses over time (e.g. from catecholamine stress hormones), which ultimately impact dopamine reactivity, as a final common pathway³⁴. This process of 'sensitisation' is inconclusive but several lines of research are in support. For example, studies in animals have shown that exposure of many relevant environmental factors lead to altered dopamine neurotransmission^{35,36}.

Although genetic mutations are thought to account for a large proportion of risk in developing psychosis, classical twin studies may underestimate the influence of environmental factors³⁷. For example, studies have shown that the level of familial risk is higher when assessing individuals in risk environments such as within urban areas or minority groups^{38,39}. The 'stress-vulnerability' model is one of the most popular models for the aetiology of psychosis and proposes that genetic risk factors operate by making individuals more vulnerable to the effects of environmental risks⁴⁰. However, several other explanations could account for the development of psychosis and it could also be that genetic factors increase the probability that the individual becomes exposed to the environmental factors⁴¹. Further to this, both genetic and environmental risk factors are most commonly studied for their impact upon psychotic disorders in isolation. However given that genetic risk is thought to arise from several genes of small effect, and only few cases have shown a direct cause from environmental factors (e.g. cannabis-induced), these risk factors rarely act alone in psychosis development^{25,42}. Despite this, few studies have directly assessed gene and environment interactions, holistically assessed interactions across multiple risk factors, or quantified their use for identifying high risk populations.

1.1.6 The specificity of risk factors

Although a wide range of risk factors have been associated with psychotic disorders, the specificity of this risk is unclear. Increasing evidence has shown that risk factors are commonly associated with several psychiatric disorders. For example a family history of schizophrenia is associated with a high genetic risk for schizophrenia. However, almost all psychiatric disorders in first degree relatives are associated with increased risk for schizophrenia and many other psychiatric diagnoses²³. Indeed, data from over 60,000 patients and controls, as part of the psychiatric genomics consortium, identified four genetic loci to be associated with several disorders including depression, autism and schizophrenia⁴³. Similarly, ERFs have also been found to be shared across multiple disorders. Laursen *et al.* undertook a cohort study of more than 2 million individuals identifying a range of risk factors including loss of a parent that was associated with depression, bipolar disorder, schizoaffective disorder and schizophrenia⁴⁴. With the vast overlap in symptoms, high-degree of comorbidity between disorders, and common misdiagnoses, it is unsurprising that risk factors are commonly shared across psychiatric disorders. Stepping away from clinical diagnoses may therefore be necessary for understanding how particular mental health problems form.

The transdiagnostic theory of mental health provides an alternative approach for understanding psychiatric disorders. Specifically, the 2010 Research Domain Criteria Project (RDoC) from the National Institute of Mental Health describes this idea for classifying mental health problems based upon observable phenotypic dimensions including biological and psychological constructs, that may span several clinical disorders⁴⁵. This breaking down of clinical diagnoses and focus on specific phenotypes, particularly prior to diagnosis, could therefore provide a clearer and more relevant understanding of the neuropathology of mental health problems such as psychosis. One such transdiagnostic phenotypic construct, frequently found to co-occur with psychosis,

and which could provide greater insight into its aetiology, is sleep and circadian rhythm disruption (SCRD).

1.2 An introduction to sleep

In order to understand the importance of SCRD in the development of psychosis it is essential to first acknowledge what is already known about sleep and circadian rhythms. Furthermore, expanding this view to other species, including rodents, provides an invaluable framework to model human physiology since it allows a greater depth of methodology and highlights fundamental aspects of these complex processes.

1.2.1 What is sleep?

Sleep is primarily a process of the brain. Behaviourally it can be defined as a state of immobility with reduced responsiveness to external stimuli, but one that is rapidly reversible (unlike anaesthesia or hibernation). Sleep is a universal process found in all animals studied, from complex organisms such as dolphins and geese to more primitive flatworms and fruit flies which at least show 'sleep-like' states^{46,47}. The complexity, duration, and timing of sleep may vastly differ between species. For example bats may sleep up to 18-20 hours a day whereas an elephant may sleep as little as 3-4⁴⁸. Mice are nocturnal species that sleep during daylight hours; humans are diurnal and sleep during the night.

Sleep can be broadly separated into different stages based upon the changes in global neuronal activity that can be detected from an electroencephalogram (EEG). In mammals, sleep can be most broadly separated into non-rapid eye movement (NREM) sleep and rapid-eye movement (REM) sleep. In rodents, such as mice, both waking and REM are characterised by low voltage, mixed or high frequency EEG oscillations and

a predominance of activity in the theta frequency range (6-9Hz). During NREM sleep, the EEG is commonly differentiated by high voltage low frequency slow waves (0.5-4Hz) and periodic waxing and waning waveforms known as sleep spindles (8-16Hz)⁴⁹. In humans sleep can be separated into 5 stages. As sleep initiates, stage 1 NREM sleep occurs, characterised by mid frequency waveforms and slow-rolling eye movements detected from an electrooculogram (EOG). Stage 2 NREM sleep or 'light sleep' is characterised by the occurrence of periodic sleep spindles, which are often coupled with high amplitude low frequency waveforms known as K-complexes. As sleep deepens the occurrence of high amplitude low frequency delta waves (0.5-4Hz) characterise stage 3 NREM sleep (>20 but <50% of the EEG), which dominates the EEG during stage 4 sleep (>50% of the EEG). In contrast, REM sleep shows EEG similar to waking, with low amplitude high frequency waves combined with a drop in muscle tone recorded from the electromyogram (EMG). REM sleep is also known as paradoxical sleep due to its wake-like activity concurrent with muscle atonia. The traditional classification of these sleep stages in humans was formalised by Rechtschaffen and Kales with a separation of sleep into 5 stages⁵⁰. This was later amended by the American Society for Sleep Research with the major change of collapsing NREM stage 3 and 4 into one stage (collectively known as slow-wave sleep; SWS), although both classifications are still widely used today⁵¹. The 5 stages of sleep cycle with an ultradian rhythm (rhythms less than a day) of approximately 90 minutes generally transitioning from stage 1 to 4, to REM and back again. These 90 minute cycles may repeat between 4-6 times in a night resulting in around 8 hours of sleep⁵². Further, the relative amount of each sleep stage changes across the night with an initial high amount of SWS which gradually decreases with increasing amounts of stage 2 and REM sleep towards the later cycles of the sleep period. In contrast to this, mice have polyphasic sleep with around 100 successive cycles of NREM to REM sleep lasting an average of 8 minutes and interrupted by periods of waking throughout the 24 hour day⁴⁹. Figure 1.2 shows a summary of these sleep patterns between humans and rodents.

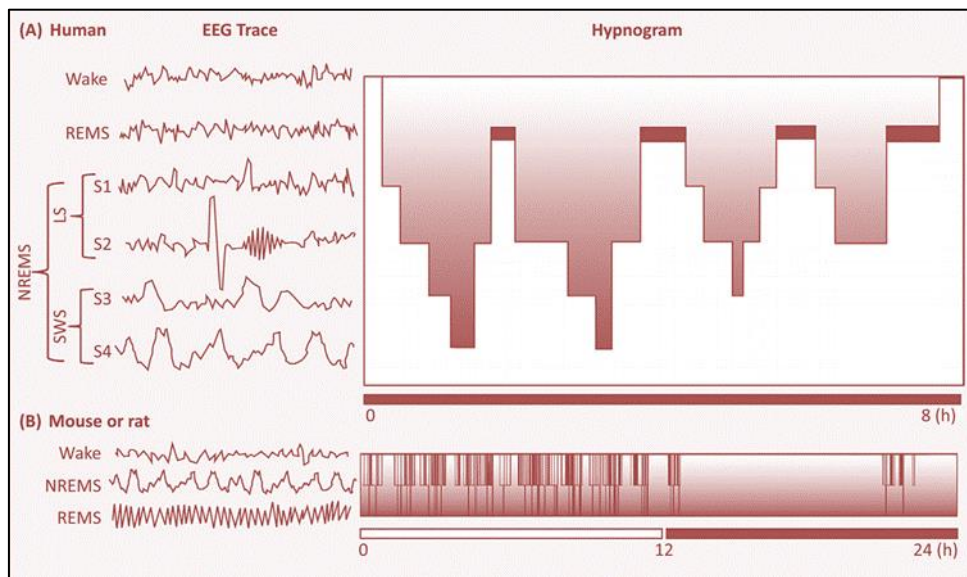


Figure 1.2: The varying stages of sleep between humans and rodents. Left shows electroencephalogram traces of characteristic brain activity during each stage of sleep. Right shows hypnogram traces of the ultradian cycle between sleep stages throughout the sleep period. Humans generally sleep for around 8 hours during the night. Rodents sleep in short bouts across the 24 hour day, but mostly confined to daylight hours. REMS=rapid eye movement sleep, NREMS=non-rapid eye movement sleep, S1/2/3/4= Stage 1/2/3/4, LS=light sleep, SWS=sleep wave sleep (figure adapted from Genzel et al⁵³).

1.2.2 Measuring sleep

Sleep can be measured through an array of techniques encompassing both subjective and objective outputs. Subjective measures of sleep involve the individual's perception of their sleep, largely based upon how long they think it took them to fall asleep (sleep onset latency) and the number and duration of awakenings during the night. Subjective measures are commonly assessed using interviews, self-report questionnaires, and sleep diaries. One of the most widely used sleep questionnaires is the Pittsburgh Sleep Quality Index (PSQI) which assesses subjective sleep quality over a one month time interval⁵⁴. However, since sleep involves reduced consciousness, subjective measures such as these are often inaccurate and individuals may overestimate or underestimate sleep latency and awakenings during the night⁵⁵. Nevertheless, subjective measures still provide important insight to the individual's psychology. For example, a subset of individuals who suffer from insomnia have problems with their sleep solely based upon their perception, with no apparent deficits when objectively measured. This classification of 'paradoxical

insomnia' or 'sleep state misperception' is estimated to occur in 5-9% of individuals with insomnia and not only affects sleep perception, but can also severely impact upon daily functioning and overall quality of life⁵⁶.

Actigraphy is an objective measure that provides a good representation of sleep and circadian timing based upon rest-activity rhythms which are used as a proxy measurement. Actigraphs are small watch-like devices which are typically worn on the wrist and contain an accelerometer sensor to detect movement across the day. They are often used in combination with subjective sleep diaries allowing an accurate estimation of when an individual falls asleep, based upon minimal activity levels. Actigraphy is advantageous since it provides a minimally invasive objective tool for assessing sleep and circadian quality over long periods of time from days to months⁵⁷. However, in order to assess the changes in brain activity, the defining feature of sleep, alternative measures are required. One of the most commonly used cortical measure of sleep is the electroencephalogram (EEG).

1.2.3 The electroencephalogram

First developed for humans in 1924 by Hans Berger, the EEG allows the recording of electrical activity from the brain generated from the summed activity of its billions of neurons. An individual neuron is electrically charged from its constant exchange of ions with the extracellular milieu during the maintenance of resting potential or action potentials. Since ions of a similar charge repel each other, the collective pumping of ions out of many neurons can push neighbouring ions causing a wave. This wave eventually reaches scalp electrodes through the movement of electrons in a process known as volume conductance. These electrodes conduct the push or pull of the ionic waves, and the difference between electrodes are measured by a voltmeter which is amplified giving rise to the EEG signal⁵⁸. Since electrodes are commonly recorded from the scalp, cortical

pyramidal cells are a major contributor to the synaptic potentials recorded from the EEG. Furthermore, EEG signals are largely a reflection of postsynaptic potentials which are longer in duration than fast spiking action potentials⁵⁸.

Although the EEG provides good temporal resolution and allows the recording of global cortical activity across time, it has poor spatial resolution and cannot localise well to specific areas of the brain including deeper layers of the cortex or subcortical areas. Intracranial EEG, placing electrodes into specific areas of the brain rather than the scalp, is one technique that improves the spatial resolution. However it is highly invasive, requiring extensive brain surgery and is thus often reserved for specific medical purposes and limited to patients with brain disorders such as epilepsy. This technique is therefore much more accessible using animals where electrodes can be placed in any region of the brain to record extracellular local field potentials (LFPs). LFPs are similar to EEG signals but allow the recording of summed electrical current from localised neuronal populations. The size of the neuronal population recorded is then dependent upon the size of the electrode used. The use of microwire electrodes (e.g. around 30µm in diameter) allows the recording of not only LFPs but also multi-unit activity (MUA) and single unit activity (SUA). By applying high-pass filters, which process the signal and remove lower frequency components (which make up the LFP), multi-unit activity can measure fast spiking activity (action potentials) of groups of neurons rather than the more sustained slower currents comprised largely of postsynaptic potentials in the LFP. Using a process known as spike sorting, waveforms taken from MUA can then be sorted and clustered according to particular features such as amplitude and shape allowing their assignment to specific neurons generating the single-unit activity. Thus the spiking activity of an individual neuron can easily be compared to the summated activity of the whole neuronal population or whole brain activity, providing substantial insight into sleep processes^{59,60}.

1.2.4 Oscillations of sleep

NREM sleep is comprised of several oscillations primarily differentiated by their frequency distributions and localisation within the brain. The $\sim 0.75\text{Hz}$ slow oscillation (SO) is arguably the most fundamental. The SO arises from global fluctuations in cellular excitability where large populations of cells alternate between active depolarised 'up-states' and inactive hyperpolarised 'down-states'⁶¹. It is thought to be cortically generated and travels across the cortex and to subcortical regions including the thalamus where it is critically involved in the coordination of both delta waves and sleep spindles⁶² (Figure 1.3).

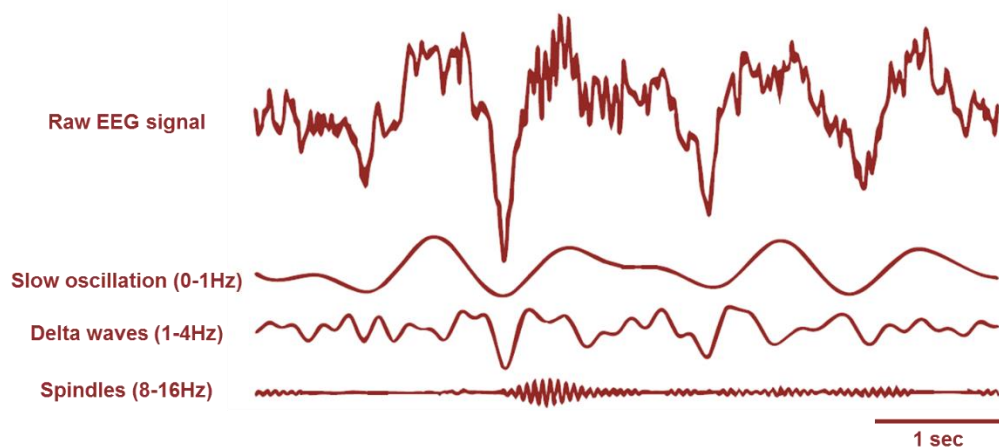


Figure 1.3: The cardinal oscillations of non-rapid eye movement sleep. The top trace shows a raw signal from an EEG with successive filtering of the signal in the below traces revealing the three primary oscillations, slow oscillations, delta waves and sleep spindles (adapted from Hobson & Pace-Schott⁶³).

Sleep spindles manifest as periodic, waxing and waning bursts of activity between 8-16Hz in rodents and 11-16Hz in humans. They occur during NREM sleep and primarily during stage 2 in humans⁶⁴. Sleep spindles are generated from the reticular nucleus (RE) of the thalamus although are likely under the synchronising control of the cortex. RE neurons are GABAergic and receive excitatory input from corticothalamic cells during cortical up-states. These neurons then release GABA at synapses with thalamocortical neurons leading to inhibitory postsynaptic potentials (IPSPs) which cause a hyperpolarisation of the postsynaptic membrane. This hyperpolarisation activates a unique ion channel leading to a high threshold calcium current (I_H) followed by a low threshold calcium current (I_T)

causing the thalamocortical neurons to switch their firing pattern from tonic single spiking to a burst of action potentials. This burst firing mode impinges upon the cortex at spindle frequency where it is then propagated throughout. Spindles are thought to be terminated from either the gradual depolarisation induced by the I_H current or the depolarising action of the corticothalamic neurons which may disrupt thalamic synchrony therefore governing spindle duration⁶⁵. Corticothalamic, thalamic reticular and thalamocortical neurons synapse with one another allowing a tightly regulated feedback loop for both spindle generation and termination (Figure 1.4).

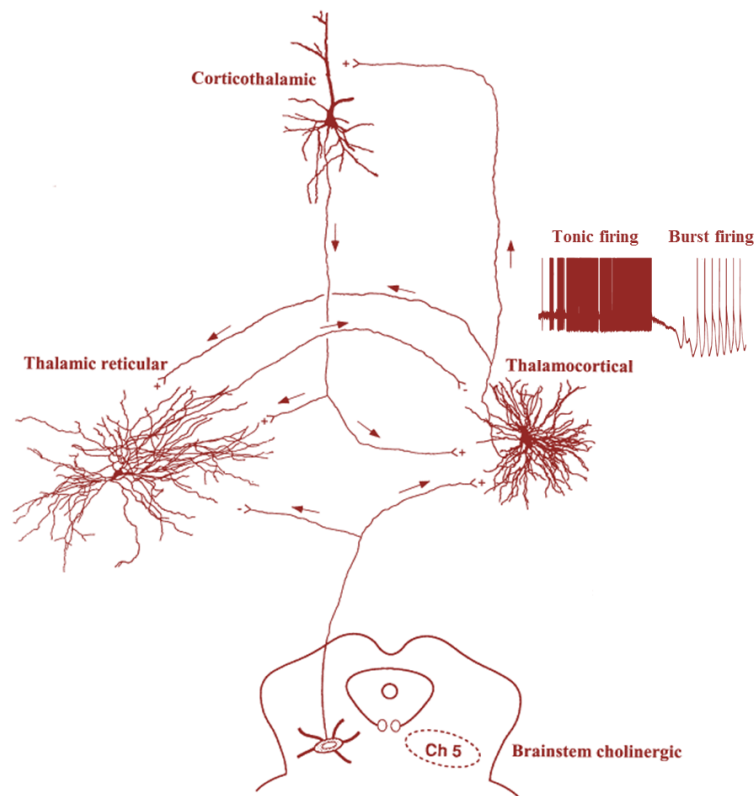


Figure 1.4: Generation of sleep spindles. Sleep spindles arise from a tightly regulated feedback loop between thalamic reticular, thalamocortical and corticothalamic neurons. Thalamic reticular neurons send inhibitory inputs to thalamocortical neurons. This leads to a hyperpolarisation and activation of calcium currents causing a switch from tonic to burst firing. This burst firing impinges upon the cortex at spindle frequency. Cortical neurons and cholinergic neurons of the brainstem additionally send projections back to the thalamus controlling spindle generation and termination. The direction of axons are indicated from the arrows (figure adapted from Steriade⁶⁶).

Delta waves are 1-4Hz oscillations and along with slow oscillations (where they are grouped collectively as slow-waves) are a second hallmark of NREM sleep, occurring primarily during stage 3 and 4 in humans. Delta waves are thought to have two sites of

origin with different underlying cellular mechanisms. Thalamically generated delta waves, similar to spindles, rely upon hyperpolarisation of thalamocortical neurons. However unlike spindles, they are intrinsically generated and are not thought to rely on the reticular nucleus. The shared reliance on thalamocortical neurons means that spindles and delta waves are incompatible at least at the level of a single neuron (and not necessarily the EEG). Spindles arise from a hyperpolarisation of around -60mV whereas delta waves occur at more negative membrane potentials⁶⁵. The second type of delta wave is thought to be of cortical origin, since they have been shown to persist after thalamectomy, although less is understood on its underlying mechanisms and could be an alteration of the slow oscillation⁶⁷.

Overall the neuronal oscillations during sleep are highly regulated and occur under tight temporal coordination. For example, it has been shown that sharp-wave ripples (80-200Hz), another oscillation found in the hippocampus, are coupled by thalamic spindles which are in turn grouped by cortical slow waves⁶⁸. This nesting between fast and slower oscillations is thought to be critical for the fundamental processes and function of sleep.

1.2.5 The function of sleep and its role in memory

The function of sleep is one of the key undetermined questions of modern neuroscience. Although several theories and evidence exist, there is no consensus over its ultimate role. Sleep has been related to several processes including cellular restoration, energy conservation, immune function, memory consolidation, emotional regulation, and the clearance of waste via the glymphatic system⁶⁹. Because sleep has been related to these wide-ranging processes, a central question arises of what its true primary function is over potential secondary add-ons or epiphenomena that may have emerged later. Its most basic fundamental function, that has arisen and sustained through evolution, and occurs across all animals studied, must therefore outweigh the negative consequences of

minimally conscious states. The synaptic homeostasis (SHY) hypothesis is one such theory for the fundamental function of sleep.

According to the SHY hypothesis, during wakefulness animals receive sensory input for learning and encoding of memories which relies upon the strength and number of connections between neurons. This process of synaptic plasticity leads to an overall increase in net synaptic strength. The synaptic strength is predicted to be related to the homeostatic regulation of sleep whereby the longer the animal stays awake, the greater the increase in synaptic strength and the more subsequent slow wave activity that occurs. Slow wave activity during sleep may therefore be required for renormalizing overused synapses and eliminating weak connections improving the signal to noise ratio and preventing metabolic and cellular costs (Figure 1.5). This process of synaptic downscaling consequently aids information processing providing beneficial effects on waking performance⁷⁰. Although further work is required to discern the mechanisms behind synaptic homeostasis, the SHY hypothesis provides a worthy explanation for the function of sleep and sleep homeostasis, and there is increasing evidence to support its claims⁷¹. Furthermore, it provides a viable explanation for the observed importance of sleep in memory consolidation.

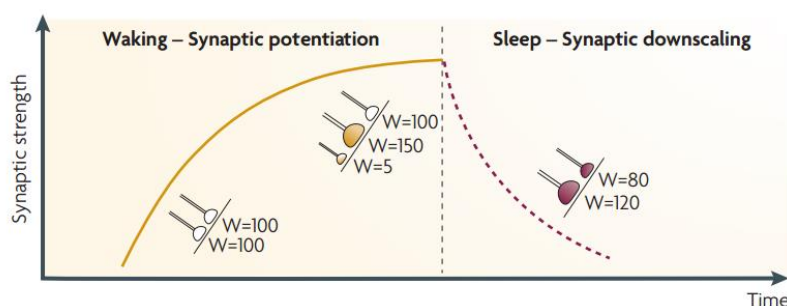


Figure 1.5: Synaptic homeostasis hypothesis. During waking, the strength of connections between local populations of neurons increases due to encoding of information through synaptic potentiation. Some synapses may increase in strength whilst others remain inactive. Sleep functions by downscaling these connections so that the relative strength is maintained, but the signal to noise ratio is enhanced. By reducing synaptic strength, sleep also promotes healthy cellular function and metabolism (figure taken from Diekelmann & Born⁷²).

The physiological basis of memory, how they are formed (encoded), processed (consolidated) and recollected (retrieved) is a topic of ongoing investigation. Memories can be broadly separated into declarative memories, which require conscious recollection, and non-declarative memories, which do not commonly require conscious recollection. Both of these categories can be further subdivided. For example, declarative memories can be divided into semantic memories (knowledge/facts, e.g. capital of France) and episodic memories (specific events or experiences e.g. what did you eat for lunch). Non-declarative memories most notably include procedural skills (e.g. how to ride a bike)⁷³. The development of these memories is thought to rely upon different regions of the brain and different levels of processing, from alterations between synapses (synaptic plasticity) to alterations in the interaction between different neuronal populations (systems-level processing)⁷². Declarative memories are characterised by their crucial dependence on the hippocampus and interactions with the cortex, whereas procedural memories are thought to rely upon cortico-striatal and cortico-cerebellar loops⁷⁴. Both the encoding and retrieval of memories occur most effectively during waking whereas memory consolidation is thought to be most effective during sleep.

The process of memory consolidation involves the transformation of newly encoded labile short-term memories into more stable long-term representations that can become integrated into surrounding networks⁷². Ample evidence has shown that sleep plays a role in enhancing this process for both procedural learning^{75,76} and declarative memories^{73,77–79}. Specifically, performance on several tasks has been shown to be significantly enhanced after a period of sleep but not equivalent periods of wake, suggesting a process of sleep-dependent memory consolidation (SDMC). However, not all studies and not all types of memories have shown strong or consistent results. It is an ongoing debate as to whether sleep plays a passive role in protecting memories from interference (that occurs during wake), a permissive role where conditions during sleep favour consolidation, or an active direct role in the consolidation process⁷⁹. The active system consolidation hypothesis is

one of the most widely researched theories for how sleep is involved in memory consolidation, and although not necessarily mutually exclusive, is often contrasted with the SHY hypothesis. The two-step model largely focuses on memory transfer between the hippocampus and cortex, thought to be required for declarative memories. New memories are first encoded in both the hippocampus, which acts as a temporary but fast processing store, and the neocortex, which acts as a long-term but slower processor. During subsequent NREM sleep the newly encoded memories are repeatedly reactivated in the hippocampus. Further, the coordination of neuronal oscillations (slow waves, spindles, ripples), are thought to drive a redistribution and re-activation of respective representations in the cortex, strengthening the memory, and promoting the long-term storage. These memory traces are further strengthened in the cortex during REM sleep as it becomes ‘disentangled’ from the short term store (Figure 1.6). How these representations are formed and become strengthened is largely thought to arise from the activities of local neuronal populations where sleep may favour synaptic plasticity or even synaptic homeostasis to strengthen or weaken connections between neurons⁷².

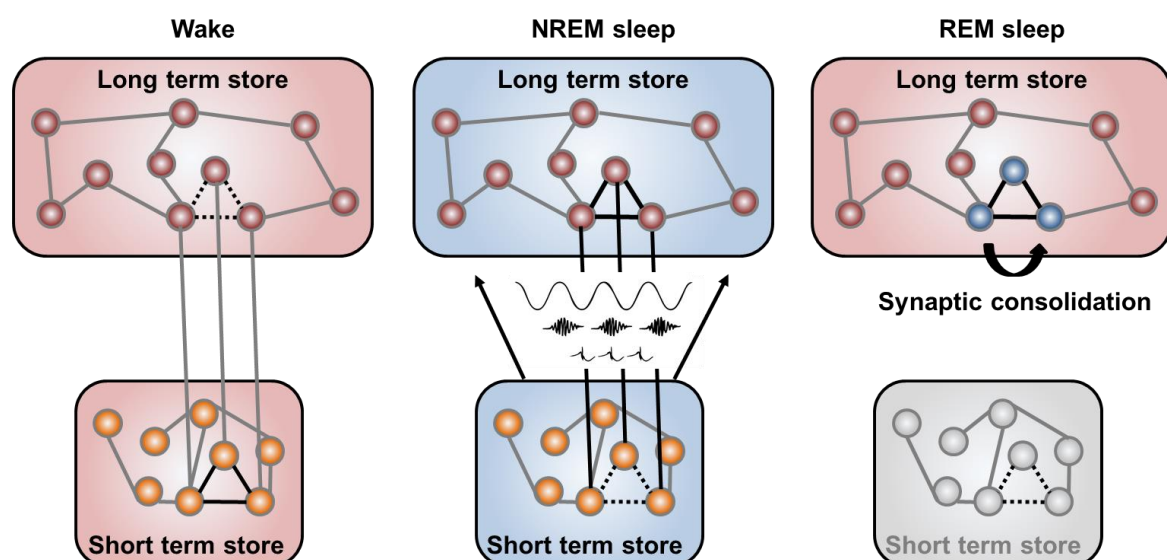


Figure 1.6: Active systems hypothesis for memory consolidation during sleep. During waking, memories are encoded in both a fast acting short term store (hippocampus) and slow acting long term store (cortex) – shown by the black and dotted lines representing the memory trace. During NREM sleep, the memory trace becomes reinforced and re-organised into the long term store from the re-activation of the memory trace and coordination of sleep oscillations including slow waves, spindles and ripples. During REM sleep the long term store is hypothesised to be disconnected from the short term store allowing local encapsulated processes of synaptic consolidation and further strengthening of the memory^{68,72}.

In summary, a brief explanation of just two of the many theories for the function of sleep has been provided, one based upon a global downscaling of synaptic connections, the other, a local strengthening of networks. Although there is still a lot to learn on the function of sleep, there is a growing understanding of how it is regulated both at the level of neural circuits and overall global states, which may provide clues on why it occurs.

1.2.6 The neural circuits of sleep and wakefulness

The balance between sleep and wakefulness is tightly controlled, involving multiple nuclei neuronal pathways, and neurotransmitters. The brainstem, hypothalamus and thalamus are the major regulators of this process⁸⁰. Wakefulness is maintained from activities of the ascending arousal system (AAS) which has two major pathways. The first involves the pedunculo pontine and laterodorsal tegmental nuclei (PPT/LDT). Both nuclei contain acetylcholine producing cells and send projections to the thalamic relay nuclei and reticular nucleus of the thalamus blocking the generation of thalamocortical rhythms and promoting a state of excitability⁸¹. The second pathway activates neurons in the lateral hypothalamus, basal forebrain and throughout the cerebral cortex. This pathway involves several nuclei of the upper brainstem and caudal hypothalamus which contain monoaminergic neurons. Specifically, the noradrenergic locus coeruleus (LC), serotonergic dorsal and median raphe nuclei, histaminergic tuberomammillary nucleus (TMN) and dopaminergic ventral periaqueductal grey matter (vPAG). These pathways are further regulated by orexin/melanin-concentrating hormone containing neurons of the lateral hypothalamus and GABA/acetylcholine containing neurons of the basal forebrain which input to the cerebral cortex^{80,82}.

In opposition to the AAS, the ventrolateral preoptic nucleus (VLPO) is thought to be the primary promoter of sleep. The VLPO is a major nucleus situated in the anterior

hypothalamus and sends inhibitory inputs via GABA and galanin neurotransmitters to all major cell groups that make up the AAS (Figure 1.7).

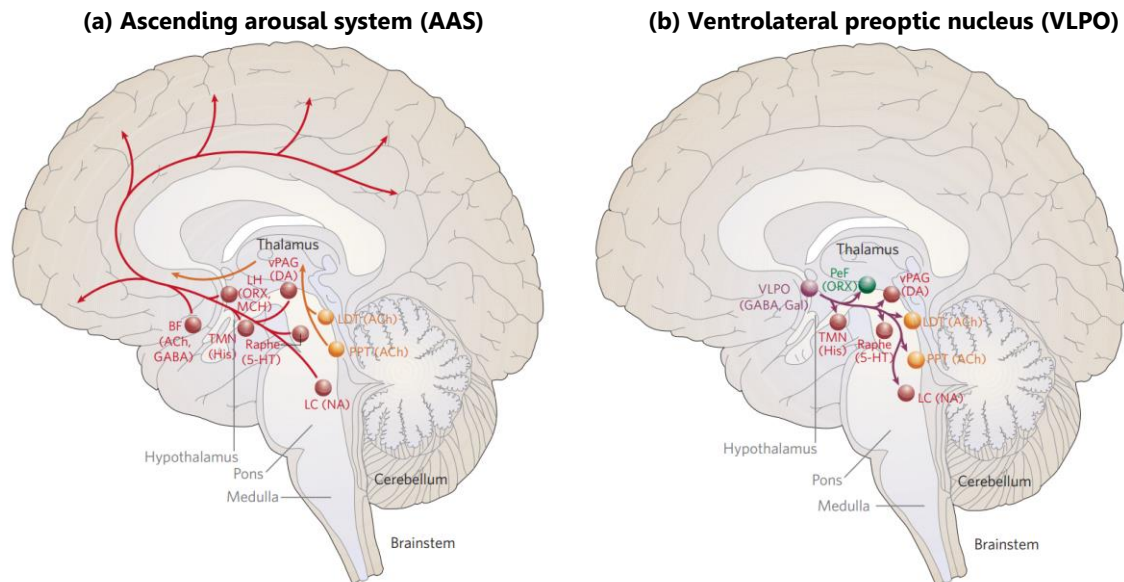


Figure 1.7: Neural circuits of sleep and wakefulness. (a) The wake promoting ascending arousal system (AAS) is comprised of several nuclei including the acetylcholine (ACh) containing pedunculopontine and laterodorsal tegmental nuclei (PPT/LDT) which project to the thalamus. In addition, the noradrenergic (NA) locus coeruleus (LC), serotonergic (5-HT) dorsal and median raphe nuclei, histaminergic (His) tuberomammillary nucleus (TMN) and dopaminergic (DA) ventral periaqueductal grey matter (vPAG) are regulated by orexin/melanin-concentrating hormone (ORX/MCH) containing neurons of the lateral hypothalamus (LH) and GABA/ACh containing neurons of the basal forebrain (BF) which altogether input to the cerebral cortex. (b) The ventrolateral preoptic nucleus (VLPO) is the primary sleep promoting nucleus and sends inhibitory projections to the AAS (figure adapted from Saper et al⁸⁰).

The transition between sleep and wake has been extensively studied and is thought to be controlled by a ‘flip-flop’ switch akin to an on/off light switch between sleep and wakefulness, rather than a gradual transition. During sleep the VLPO sends inhibitory projections to monoaminergic nuclei of the AAS which further relieves the inhibition of VLPO cells from these monoaminergic nuclei. When waking, the balance between this inhibition of monoaminergic nuclei and activation of VLPO switches. Monoaminergic neurons now inhibit VLPO, relieving further inhibition of these cells in addition to orexinergic neurons of the lateral hypothalamus which can then reinforce the monoaminergic tone. Since orexinergic neurons promote and reinforce the

precoeruleus region (PC). During NREM sleep REM-off nuclei are active and inhibit the REM-on nuclei. Both the LC and DR and ORX neurons mediate and reinforce this inhibition. During REM sleep REM-on nuclei become active and inhibit the REM-off nuclei. Neurons of the pedunculo pontine and laterodorsal tegmental nuclei (PPT/LDT) and VLPO mediate and reinforce this inhibition. A separate population of SLD neurons activate medullary and spinal interneurons (MI/SI) which inhibit α -motor neurons leading to muscle atonia⁸³.

Due to the varying activation and inhibition of nuclei, overall activity of monoaminergic tone varies between waking, NREM and REM sleep. Compared to wake, activity of the cholinergic, noradrenergic and serotonergic neurons become decreased during NREM sleep. During REM sleep many cholinergic neurons activate again, whereas the serotonergic and noradrenergic neurons become inactive. In the lateral hypothalamus, orexinergic neurons are most active during wakefulness whereas melanin-concentrating hormone containing neurons are most active during REM sleep⁸⁰.

1.2.7 Local and global sleep

Sleep is often considered a global state with synchronised neuronal activity across the cortex. However, recent work has shown this is not always the case and synchronised neuronal activity is in fact often local, occurring within specific regions of the brain at specific times⁸⁴. In an extreme example, certain species such as dolphins are known to undergo unihemispheric sleep, with only one hemisphere of the brain in a sleep state at any one time⁸⁵. However all mammals are likely to show some level of locality. For example, slow wave activity (SWA) is highest in frontal regions of the brain which is particularly evident during early sleep or after sleep deprivation^{86,87}. In humans, sleep spindles have also been shown to have distinct spatial characteristics across the cortex with slow frontal (~11-13Hz) and fast centro-parietal (~13-15Hz) classifications which may reflect alternative topographical sources of generation^{88,89}. Localised differences also occur at the level of individual oscillations and even single neurons⁹⁰. Intra-cranial EEG in both humans and rodents have shown that slow waves and spindles can occur within specific local regions of the brain which are not detected in other regions or in global scalp/cranial recordings^{84,91,92}. This regional occurrence of sleep oscillations has been shown to have local homeostatic dynamics whereby areas that have higher activity during

waking, also show greater SWA during sleep⁹³. Finally from studies in rats, sleep-like states have also been shown to occur locally during wake, particularly during sleep deprivation. Vyazovskiy *et al.* showed that when rats were sleep deprived, cortical neurons showed brief periods of inactivity which were accompanied by slow waves in the EEG. However, these periods were often local in nature with other cortical regions showing normal wake-like states of activity and the global EEG state of the animal remaining in wakefulness⁹⁴. The growing literature on local activity during sleep (and wake) provides substantial evidence that sleep may arise from the activity of local circuitry which is required for the regulation and processing of sleep at global cortical states.

1.2.8 The regulation of sleep

The regulation of sleep including its timing, duration and structure throughout the day is thought to involve two intertwined processes. As proposed by Borbély and others, sleep regulation relies upon both a homeostatic and circadian process^{95,96}. Homeostatic regulation also known as process S, produces a drive for sleep which increases with prolonged wakefulness and decreases with sleep. Thus the longer the time spent awake, the greater the sleep pressure and the more probable that sleep occurs. The longer time spent asleep, the lower the sleep pressure and the more probable that waking occurs. One of the clearest markers of the sleep homeostat is slow wave activity (SWA) which is at its highest upon sleep onset and gradually dissipates with prolonged sleep. Furthermore, increasing wakefulness through sleep deprivation produces a proportional increase in sleep pressure and SWA⁹⁷. Although much remains for understanding the molecular mechanisms behind sleep homeostasis, which likely involves different processes between NREM and REM sleep, it is thought to rely upon the accumulation of substrates during waking, which subsequently dissipate during sleep. Currently the most favourable candidate for this substrate is adenosine^{98,99}. Adenosine is a key output of energy expenditure and accumulates during prolonged wakefulness from the continued

breakdown of adenosine triphosphate (ATP) and the exhaustion of the brains glycogen reserves¹⁰⁰. This accumulation may enhance the activity of sleep promoting nuclei and reduce the activity of wake promoting nuclei. With prolonged sleep, extracellular adenosine dissipates promoting wakefulness^{101,102}.

A second regulation of sleep and wakefulness arises from a circadian component, also known as process C. Using forced desynchrony protocols where the 24 hour day is experimentally altered by removing external time cues, it has been shown in humans that they continue to show an endogenous approximate 24 hour rhythm in sleep drive (see section 1.3)¹⁰³. Both process S and process C therefore work in tandem regulating the drive for sleep and wakefulness across the day (Figure 1.9).

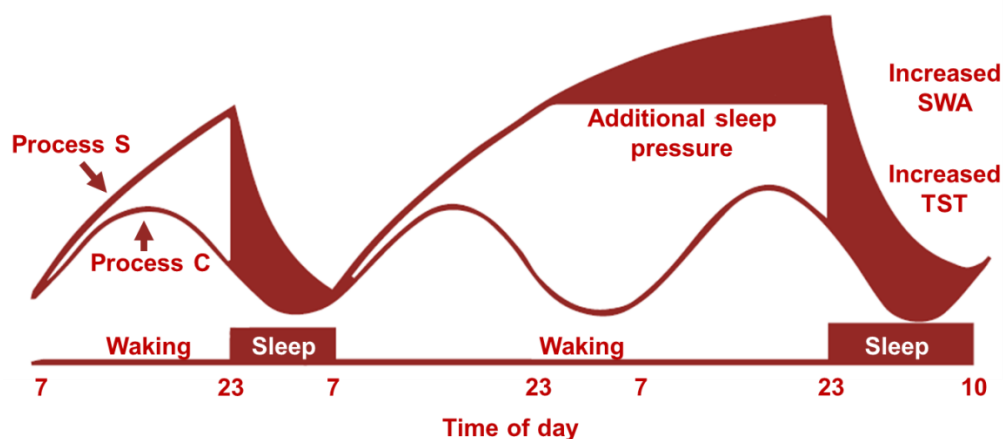


Figure 1.9: The regulation of sleep and wakefulness by process S and C. Sleep is homeostatically regulated from an increase in certain substances during wakefulness leading to a gradual drive to sleep. Upon entering sleep this increase in sleep pressure gradually dissipates until wake occurs again. Sleep is under circadian regulation from an internal molecular clock system which entrains the cycle to external light so that the drive for sleep is lowest during the middle of the night and gradually increases throughout the day. Homeostatic sleep pressure can be increased from sleep deprivation leading to a conflict between the homeostatic and circadian regulation. This can cause greater slow wave activity (SWA) during sleep and a longer total sleep time (TST) against the circadian system. This renormalizing of sleep is known as recovery sleep⁹⁵ (figure adapted from Moens¹⁰⁴).

1.3 An introduction to circadian rhythms

1.3.1 What are circadian rhythms?

Circadian rhythms describe any biological process which follows an endogenous internal rhythm of about 24 hours (*circa diēm* – Latin for ‘around a day’). The underlying

mechanisms of circadian rhythms are driven by molecular clocks: biochemical oscillators that receive input to allow entrainment, and produce outputs to regulate changes in biochemistry, physiology and behaviour. Although peripheral clocks exist in virtually all mammalian cells, they are governed (either directly or indirectly) by a central master clock in the brain known as the suprachiasmatic nucleus (SCN)¹⁰⁵. The SCN is situated in the anterior hypothalamus where neurons fire in an approximate 24 hour cycle. This cycle is regulated by a conserved molecular system involving autoregulatory transcriptional-translational loops of 'clock genes'¹⁰⁶. In mammals, two proteins, CLOCK and BMAL1 are expressed at the beginning of the cycle and act as transcriptional enhancers for several genes including *Per*, and *Cry*. These genes are translated into proteins which serve several metabolic and physiological functions, entraining peripheral clocks and leading to coordinated rhythmic outputs such as core body temperature, sleep timing, and hormone secretion including cortisol and melatonin¹⁰⁷. In addition, these proteins also function in the autoregulation of their own transcription through binding and gradual inhibition of CLOCK/BMAL1 proteins^{106,108} (Figure 1.10). This subsequently resets the system and the cycle repeats at an approximate 24 hour rhythm. Since the circadian system must respond to a changing external environment, it receives many inputs that entrain this system. These can include anything from external temperature to daily meal times and are collectively known as zeitgebers¹⁰⁶. Most notably, the resetting of the circadian rhythm to conform to the external day-night cycles is under the control of light inputs during the day and subsequent rise in melatonin secretion during the night.

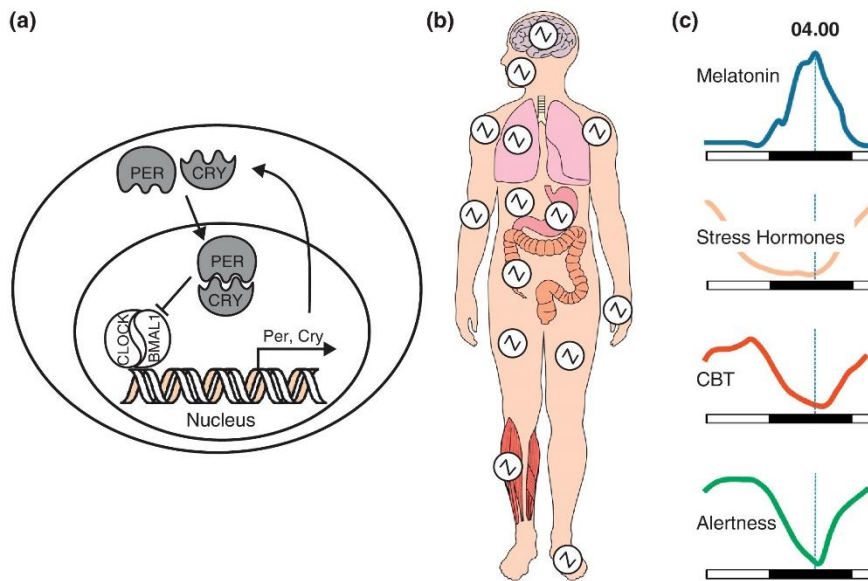


Figure 1.10: The generation of circadian rhythms. (a) Circadian regulation is controlled by an autoregulatory transcription-translation feedback loop in cells of the suprachiasmatic nucleus which acts as a master clock. Transcription factors CLOCK and BMAL1 drive the expression of other genes including *Per* and *Cry* which are involved in the regulation of physiology and metabolism and the inhibition of CLOCK/BMAL1. This consequently inhibits their own expression in approximate 24 hour rhythm. (b) The physiological outputs of the master clock are coordinated by peripheral clocks throughout the body. (c) Some examples of circadian regulated physiological outputs include melatonin and stress hormones secretion, core body temperature (CBT) and alertness levels (figure taken from Jagannath et al.¹⁰⁹).

1.3.2 Melatonin and entrainment of the SCN

Light is arguably the most important zeitgeber for circadian entrainment in mammals and is received by photosensitive cells in the retina of the eye. Although the classical rods and cones, photoreceptive cells required for vision, are thought to be involved in circadian entrainment, an additional cell type, the photosensitive retinal ganglion cells, are also important. Indeed, animals lacking both rods and cones can still be entrained to the daily light/dark cycle through functioning retinal ganglion cells¹¹⁰. These cells contain the photopigment melanopsin which is maximally sensitive to blue light and able to detect light inputs which are converted into neuronal excitations that propagate to the SCN via the retinohypothalamic tract (RHT)¹¹¹. The modulation of SCN activity by light leads to transcription of the CLOCK/BMAL1 genes and initiates the molecular clock cycle. This has subsequent effects on SCN downstream outputs, one of the primary examples being melatonin.

Melatonin is a derivative of serotonin and L-tryptophan and is synthesised in pinealocytes of the pineal gland where it is secreted into the blood and cerebrospinal fluid¹¹². In humans it exhibits a robust circadian rhythm rising during the evening, reaching a peak around 4am, and remaining low during daylight hours¹¹³. Light during the day inputs to the SCN which inhibits secretion of melatonin from the pineal gland through an indirect pathway involving the paraventricular nucleus of the hypothalamus (PVN), preganglionic neurons of the intermediolateral cell column (IML) and the superior cervical ganglion (SCG; Figure 1.11). A reduction of light as daylight ends prevents this inhibition allowing increased melatonin secretion. Melatonin reciprocally acts on the SCN via MT1 and MT2 G-protein coupled receptors (GPCRs) which inhibits SCN firing and resets the rhythm¹¹². Since melatonin levels can be measured from blood, or less invasive proxy measures from saliva and urine using melatonin derivatives (e.g. 6-sulphatoxymelatonin), it is regarded as the best peripheral index of internal circadian timing¹¹². Furthermore, due to its potential for advancing the circadian rhythm and promoting sleep, exogenous melatonin is increasingly used as a medication for sleep problems, although its usefulness is largely undetermined¹¹⁴.

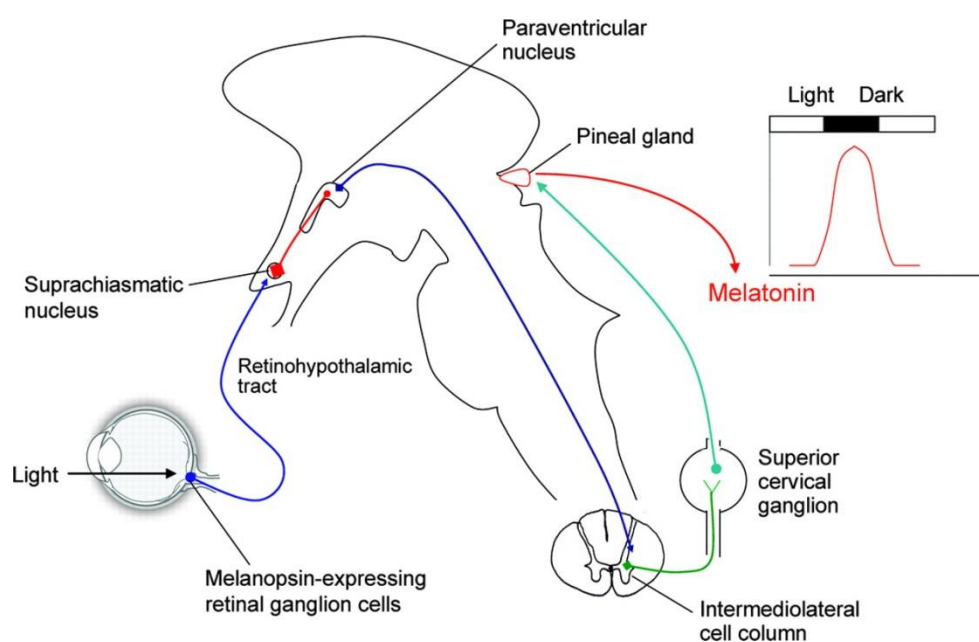


Figure 1.11: Circadian regulation of melatonin. Light information detected by retinal ganglion cells of the eye pass to the suprachiasmatic nucleus (SCN) via the retinohypothalamic tract. The SCN indirectly inhibits the secretion of melatonin via the paraventricular nucleus, intermediolateral cell column and superior cervical ganglion. In the absence of light, melatonin is secreted from the pineal gland and can act on the SCN to promote sleep (figure taken from Benarroch¹¹²).

1.3.3 Chronotype

In humans, the approximate 24 hour rhythm of the molecular clock is influenced by genetic background, sex, and age, producing slight changes in the overall period length between individuals¹¹⁵. These differences in period length define the chronotype, and results in variations in circadian outputs and processes, including the timing of the sleep/wake cycle. Since chronotype and sleep timings are heavily interrelated, sleep timings and more specifically the mid-sleep point (the mid-point between sleep onset and sleep offset) is often used as an indicator of the entrained internal circadian rhythm. Chronotype based upon an individuals preferred timing for sleep can be broadly separated into early, late and intermediate types¹¹⁵. Early types tend to go to sleep earlier and wake up earlier whereas later types tend to go to sleep later and wake up later. However, the majority of the population can be categorised as intermediate types which are generally more flexible in their sleep/wake timing. Various external factors influence sleep timing including social factors and artificial lighting which are particularly prominent in today's industrialised societies. Work and social commitments commonly lead to later bed times or earlier wake times producing a 'sleep debt' as individuals are forced to desynchronise from their endogenous circadian rhythm. When work and social commitments cause this desynchrony, a deficit in sleep gradually builds up until it is allowed to recover with extended sleep periods when these commitments cease (typically at weekends). Social jetlag is a term commonly used to describe this difference between sleep timing on work days, where social commitments impact the circadian rhythm, and free days where these social commitments are removed. A higher social jetlag thus indicates a greater desynchrony between our endogenous circadian rhythm and external social rhythm which may lead to a greater sleep debt¹¹⁶. With an increasing '24 hour' society and a drive for activity and productivity at the expense of sleep need, understanding normal sleep physiology and the impact of its disruption on health and disease is a pertinent topic for further investigation.

1.4 Sleep and circadian rhythms in psychosis

1.4.1 Sleep and circadian rhythm disruption in psychotic disorders

Sleep physiology and circadian rhythms are fundamental processes in normal healthy functioning and there is now an extensive range of literature showing a tight association between sleep and circadian rhythm disruption (SCRD) in mental health disorders^{117,118}. Sleep disturbances are a common complaint in psychotic disorders and include a range of phenotypes such as insomnia with impaired sleep continuity, insomnia with delayed sleep onset, and hypersomnia (excessive sleep)^{119–121} (Figure 1.12). EEG studies have shown that most patients with schizophrenia show increased sleep latency, decreased total sleep time and lower sleep efficiency¹²¹. Further, schizophrenia has also been associated with reduced slow-wave activity (SWA) during NREM sleep, decreased REM sleep onset latency and a deficit in the density of NREM sleep spindles^{122–126}. From the use of actigraphy and melatonin profiles it has also been shown that patients with schizophrenia have a wide range of circadian disruptions including delayed sleep phases, advanced sleep phases, or irregular sleep-wake timings (Figure 1.12)^{119,127,128}. Furthermore, a subgroup of individuals show substantially later melatonin peaks indicative of more delayed circadian rhythms¹¹⁹. SCRD in schizophrenia is unlikely a result of medication, since they have been found to exist in unmedicated individuals¹²¹, are exacerbated after medication withdrawal¹²⁹, and the sedatory effects of the antipsychotics often improve sleep problems¹³⁰.

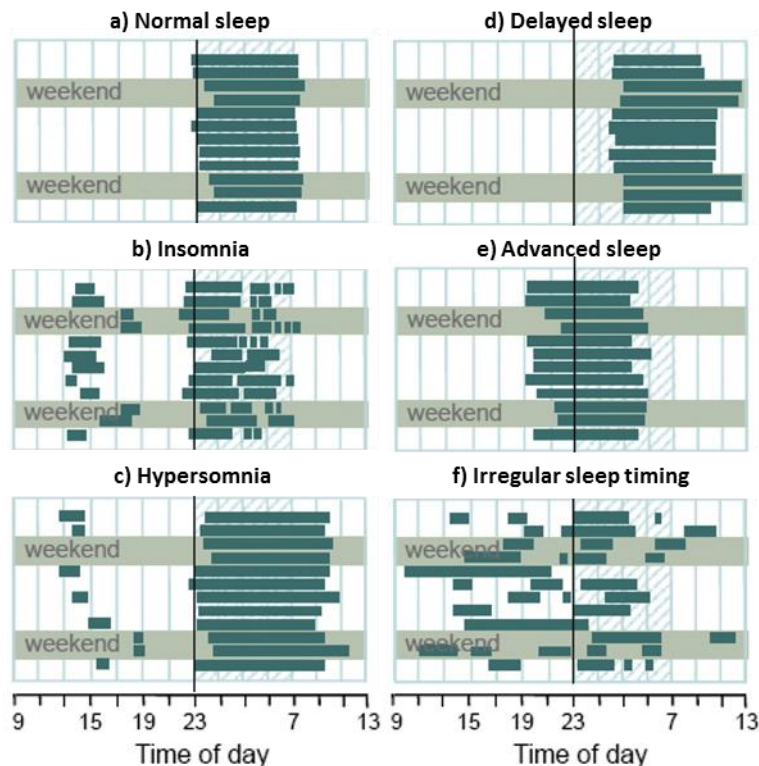


Figure 1.12: Sleep and circadian phenotypes in schizophrenia. Individuals with schizophrenia have been shown to have a range of sleep and circadian patterns including (a) normal sleep-wake patterns, (b) shorter sleep periods or fragmented sleep, (c) longer sleep periods, (d) later sleep onsets and offsets, (e) earlier sleep onsets and offsets, (f) a complete lack of any pattern in sleep-wake timing (figure adapted from Wulff et al.¹²⁰).

The established overlap between SCRD and psychosis also posits an intriguing possibility of alternative treatment strategies for psychotic disorders. There is, so far, a paucity of research assessing whether treating sleep problems in psychotic disorders may also have beneficial effects on other symptoms of the disorder. The use of cognitive behavioural therapy for insomnia (CBT-I) has proved effective at reducing sleep problems and symptoms of insomnia across a range of psychiatric conditions including depression, schizophrenia and bipolar disorder¹³¹. The impact of this treatment on the psychiatric symptoms has shown mixed results. Of the two trials that have assessed this in psychotic disorders, both found a significant reduction of insomnia symptoms. However in one this was also associated with substantial reductions in the level of persecutory delusions, while the other showed either reduced or increased levels of delusions and hallucinations^{132,133}. Further work is required to fully delineate these effects, and the use of objective EEG measures of sleep may provide improved insight into this relationship¹³⁴.

1.4.2 SCRD in the development of psychosis

Although SCRD in psychotic disorders is well established, at what stage these disturbances occur is less understood. To examine this, there is now increasing research on SCRD during early and prodromal stages of psychosis. Similarly to chronic schizophrenia, studies on first episode psychosis have shown both subjective^{135,136} and objective^{137,138} sleep disturbance, although often to a lesser extent and with inconsistencies between studies¹³⁹. Of the few studies that have assessed sleep disturbances in individuals at ultra-high risk for developing psychosis, all have identified some form of sleep or circadian disturbance compared to healthy controls. This included poorer subjective sleep quality, less synchronised rest-activity rhythms, and decreased sleep efficiency^{140–143}. In addition to this work, SCRD has also been found to be associated with psychotic-like experiences within the general population^{144–146}. For example, in a recent large population based cross-sectional study encompassing data from over 260,000 individuals, Koyanagi and Stickley identified that general sleep problems including problems falling asleep and night awakenings were associated with a 2.41 times higher odds of having a PLE¹⁴⁴. Overall these studies provide support that SCRD occurs across the whole spectrum of psychotic symptoms. However further work is necessary to characterise this occurrence prior to disease onset.

Can SCRD predict future mental health problems? Studies on individuals with schizophrenia have shown that those who relapse after stopping medication have larger decreases in total sleep time and sleep efficiency compared to individuals who do not relapse^{129,147}. Further, sleep disturbance has been shown to predict next day functioning and psychotic symptom severity¹⁴⁸. Evidence from longitudinal studies have also shown that sleep problems are predictive of future psychotic experiences. For example, findings from a UK birth cohort of 4720 individuals identified an association between the presence of nightmares in children at age 12 and psychotic experiences at age 18¹⁴⁹. Ritter *et al.*,

similarly identified poor sleep quality as a significant risk for the subsequent development of bipolar disorder, which may include psychotic symptoms¹⁵⁰. Characterising sleep and circadian rhythms in individuals at high risk could therefore provide additional information and potential for predicting transition to first episode. Indeed, two longitudinal studies have assessed this although results are currently inconclusive^{151,152}.

1.4.3 Underlying mechanisms of SCRD and psychosis

The underlying mechanisms linking SCRD and psychosis is a topic of ongoing investigation. From the use of animal models and genome wide association studies, knockouts or polymorphisms of several genes have now been found to cause symptoms of both SCRD and psychosis or these genes are associated with a psychotic disorder¹⁵³. Furthermore several of these genes are known to be involved in normal sleep/circadian regulation or the development of schizophrenia. For example associations have been identified with a range of polymorphisms in circadian genes including CLOCK, TIMELESS, and PERIOD3 across psychotic disorders^{154–156}. CLOCK mutant mice show both decreased sleep, hyperactivity and increase in reward value of cocaine, analogous to manic-like behaviours in bipolar disorder¹⁵⁷. The *Snap25* gene, encodes a protein necessary for normal synaptic vesicle release, and polymorphisms in this gene have been linked to schizophrenia¹⁵⁸. Mutant mouse models of the *Snap25* gene show distinct sleep and circadian phenotypes including phase shifts and fragmented activity¹⁵⁹. Intriguingly these mice also show psychiatric-like symptoms including hyperactivity and abnormalities in social interaction making them viable models for both SCRD and psychosis¹⁶⁰.

This work is further supported by the finding of regional abnormalities in brain structures in patients with schizophrenia. For example, bilateral reductions in grey matter have been observed in multiple areas including the thalamus, cortex, hypothalamus, brainstem and basal forebrain – all of which play a role in sleep and circadian regulation (see section

1.2.6, pg27)^{161–165}. On a molecular level, the interaction between SCRD and psychosis is hypothesised to results from abnormal synaptic neurotransmission¹⁰⁹. Mutations in the CLOCK gene for example, have also been associated with dopamine hyperactivity¹⁶⁶ and dopamine receptor D₂ signalling has been shown to increase clock gene expression¹⁶⁷. Further, the use of dopamine receptor D₂ agonists such as apomorphine have been shown to enhance wakefulness whereas YM-09151-2, a D₂ blocking agent, has been shown to increase sleep^{168,169}. Dopamine transmission is also known to be under the modulation of melatonin and abnormalities in melatonin synthesis or release could account for the joint effects of both SCRD and positive symptoms for psychosis^{169,170}. These findings provide interesting support for the dysregulation of dopamine in schizophrenia which may also lead to sleep and circadian phenotypes. However, they are still largely speculative and further work is required to directly assess pathways linking SCRD with psychosis, which likely relies upon more than just dopaminergic transmission.

Similarly to psychosis, sleep disturbances and sleep disorders have also been associated with several environmental risk factors. For example a prospective study on risk factors for insomnia identified an array of risk factors including gender, ethnic minorities and social introversion, which are also known to be associated with schizophrenia¹⁷¹. Studies in rodents have shown exposure to prenatal stress, a known risk factor for schizophrenia, was associated with reduced social interaction, increased anxiety-related behaviour, and reduced dopamine turnover, consistent with schizophrenia. These animals further showed sleep and circadian abnormalities including reduced slow wave sleep and phase shifts in hormone secretion¹⁷². Only one study has assessed shared genetic and environmental components between both sleep disturbances and psychotic-like experiences¹⁷³. In this study approximately 5000 monozygotic and dizygotic twin pairs were compared revealing a significant overlap in genetic and environmental influence across PLEs and sleep disturbances. Overall these studies support the proposed overlap in genetic and environmental risk factors for SCRD and psychosis. However, further work is necessary

to directly test these relationships and assess how specific risk factors are involved in and interact with the development of both SCRD and psychosis.

1.4.4 The role of sleep spindles

A common symptom of psychotic disorders is cognitive impairment which can include deficits in attention, working memory and executive function¹⁷⁴. Given the importance of sleep for learning and memory, and the severe disruption to sleep and circadian cycles in psychotic disorders, it is somewhat unsurprising that schizophrenia has also been associated with deficits in sleep-dependent memory consolidation (SDMC). Specifically, it has consistently been shown that patients trained on a non-declarative procedural motor sequence task, show learning of the task which is equivalent to that of healthy controls, but without the significant improvement after sleep^{124,125,137,175}. The cause of deficits in SDMC in patients with schizophrenia is largely unknown but has been most commonly associated with the amount of stage 2 NREM sleep, and specifically correlated to sleep spindle density^{124,125}.

Sleep spindles in schizophrenia have consistently been shown to be reduced, both in density and total number throughout the sleep period^{125,137,176–180}. These findings have led to an increased interest of spindle dysfunction in schizophrenia where they could provide an important new endophenotype¹⁸⁰. However little is known about the development of spindle dysfunction and studies in individuals during earlier stages of disease have shown variable results. In first episode patients, spindle density has either been shown to be reduced¹³⁷ or show no difference to healthy controls^{138,181}. Further investigation is therefore needed to confirm this endophenotype and whether it exists in individuals at high risk for developing psychosis.

What is the importance of reduced spindles in schizophrenia? Current evidence has implicated a role of spindles in various functions including the gating of sensory information and sleep continuity^{182–184}, cortical development¹⁸⁵, synaptic plasticity¹⁸⁶, and memory consolidation^{187,188}. It has been hypothesised that spindle abnormalities in schizophrenia arises from thalamic dysfunction and their proposed role in memory consolidation could account for the deficits associated with sleep dependent memory consolidation¹⁸⁰. However, the precise function of sleep spindles remains elusive and evidence for a role in memory consolidation is largely based upon correlative data. Further, recordings in humans are largely based on gross EEG which lacks the specificity between brain regions or cortical networks. Arguably, understanding the spatio-temporal pattern of spindle occurrence during sleep is essential for elucidating their function. Some evidence has shown that spindles have topographical differences across the cortex, and show local dynamics within specific regions. However, existing knowledge of how and when spindles emerge is surprisingly fragmentary, particularly within deeper layers of the cortex or subcortical areas. To fully understand the importance of reduced spindles in schizophrenia we must understand how, when, and ultimately why they occur.

1.5 Thesis aims

This overview of sleep, circadian rhythms and their relationship to psychosis highlights several key unanswered questions that this thesis aims to cover. Firstly, risk factors are likely to be directly involved in the aetiology of psychosis and psychotic-like experiences. Sleep and circadian rhythm disruption is frequently found to co-occur in psychotic disorders with evidence, largely based on genetic data, suggesting shared neuropathological pathways. However, little is known on the extent of environmental influence on this overlap, and how risk factors and SCRD may interact to increase the risk for developing psychosis. Therefore, the first key research question of this thesis and the topic of investigation for chapter II was:

1. What is the relationship between risk factors and sleep and circadian rhythm disruption in the development of psychotic-like experiences?

Secondly, psychotic disorders are associated with an array of sleep and circadian dysregulation including deficits in total sleep time, slow wave sleep, irregular sleep timing, delayed melatonin secretion, and deficits in sleep spindles. Little is known on whether these phenotypes occur in individuals at high risk for developing psychosis, and it has never been investigated in relation to both sub-clinical symptoms and risk factors for psychosis. The second key research question and topic of investigation for chapter III was:

2. How does sleep and circadian rhythm disruption manifest in individuals who are at high risk for developing psychosis?

Thirdly, sleep has been shown to play an important role in memory consolidation and deficits in sleep-dependent memory consolidation have repeatedly been shown in patients with schizophrenia. However it is unknown at what stage these deficits may occur in relation to disease onset. The third key research question and topic of investigation for chapter IV was:

3. Do individuals at high risk for developing psychosis show deficits in sleep dependent memory consolidation?

Finally, deficits in sleep spindles associated with schizophrenia are thought to arise from thalamic dysfunctions yet their functional role and importance for sleep regulation and memory consolidation remains unclear. Using animal models is a valuable method for gaining a greater detail of the local nature of these sleep oscillations which may provide

insight into their functional role. The final key research question and topic of investigation for chapter V was:

4. How are sleep spindles distributed within local regions of the brain, and what can this tell us about their functional role in sleep processing and normal functioning?

Through these questions this thesis aims to further our knowledge on the function and expression of sleep and circadian rhythms and how this can better our understanding on the development of psychosis.

Chapter II: The interplay between sleep disturbance and risk factors for psychosis: A population survey

Contents

2.1 Introduction	48
2.1.1 The psychosis continuum	48
2.1.2 The importance of risk factors	48
2.1.3 Sleep and circadian rhythms in psychosis	49
2.1.4 Study aims	50
2.2 Methodology	50
2.2.1 Identifying risk factors for psychosis	50
2.2.2 Developing the survey	51
2.2.3 Follow-up survey	55
2.2.4 Quantifying risk factors	55
2.2.5 Data processing	56
2.2.6 Statistical analyses	57
2.3 Results	58
2.3.1 Assessment of mental state	58
2.3.2 Risk factors within the population	59
2.3.3 The association between risk factors and PLEs	62
2.3.4 The effects of risk factor severity or frequency on PLEs	64
2.3.5 The relationship to sleep disturbance	67
2.3.6 Longitudinal relationship between risk factors, sleep quality and PLEs	69
2.3.7 Relationship between all mental state measures and risk factors	73
2.4 Discussion	75
2.4.1 Risk factors associated with PLEs	75
2.4.2 Interactions between risk factors	77
2.4.3 Risk factors are associated with sleep disturbance	78
2.4.4 Longitudinal associations between risk factors, sleep disturbance and PLEs	79
2.4.5 Overlapping symptoms and shared pathways	81
2.4.6 Risk factors unassociated with PLEs	81
2.4.7 Limitations	83
2.4.8 Summary of findings	84

Foreword: The risk factor literature search, compilation of questionnaires and initial advertising of this survey was a joint project between myself and Jan Cosgrave, another DPhil student.

2.1 Introduction

2.1.1 The psychosis continuum

As discussed in Chapter I (section 1.1.3, pg11), psychosis is thought to exist along a continuum of symptomatology, with sub-clinical symptoms known as psychotic-like experiences (PLEs) occurring in around 5-8% of the general population¹⁸. Several large scale population surveys support this continuum of psychosis from PLEs to psychotic disorders. Data collected from 7076 men and women aged 18-64 in the Netherlands Mental Health Survey and Incidence Study (NEMESIS) revealed a prevalence of 2.1% for a diagnosis of a non-affective psychotic disorder and 4.2% for experiencing clinically relevant psychotic symptoms (but not necessarily a diagnosis). Expanding this to include psychotic-like experiences unassociated with distress revealed a sample prevalence of 12.9% and the total prevalence for any positive psychosis rating (across the whole continuum) was 17.5%¹⁷. Similarly the US National Comorbidity Survey of 5877 individuals revealed lifetime prevalence rates of clinically diagnosed non-affective psychosis at 0.7%, yet up to 28% of the population reported one or more PLEs¹⁸⁹. These findings highlight the existence of non-clinically relevant symptoms in healthy individuals and suggest clinical diagnoses explain only a small proportion of psychotic experiences within the general population. Furthermore, several studies have shown that PLEs are associated with an increased risk for developing psychosis and this 'symptomatic' approach is often used to identify individuals at risk^{14,190-192}. Given PLEs and psychosis may also share a similar developmental trajectory (see below), PLEs are thought to be a viable measure for investigating the aetiology of psychosis

2.1.2 The importance of risk factors

Why do PLEs develop and what causes the transition from PLEs to clinically relevant symptoms? As discussed in Chapter I (section 1.1.4, pg12), the aetiology of psychosis is thought to depend on the occurrence of genetic and environmental risk factors. Increasing

evidence has shown that risk factors for psychosis are also associated with PLEs¹⁴. For example, Johns *et al.*²¹ assessed a general population sample of 8580 individuals identifying cannabis dependence, ethnicity, and traumatic life events amongst several risk factors associated with PLEs. Similarly another large scale population survey on 5427 Chinese adolescents revealed a selection of risk factors associated with increased PLEs including traumatic events, urbanicity and family history of a psychiatric illness¹⁹³. Familial and twin studies have shown that PLEs are also heritable, although there is currently little evidence identifying shared risk genes to psychotic disorders^{194–196}. Despite the identified associations between risk factors and PLEs, there is a paucity of research assessing how risk factors may interact with one another and how they may influence the transition from PLEs to psychotic symptoms and diagnosed disorders. The use of longitudinal studies that comprehensively assess the interaction between mental state and risk factors is fundamental to furthering our understanding and improving prediction in the development of psychosis.

2.1.3 Sleep and circadian rhythms in psychosis

As previously discussed (Chapter I section 1.4, pg37), sleep and circadian rhythm disruption (SCRD) is frequently found to co-occur with psychotic disorders^{117,119,127,139}. Although studies are limited, evidence also suggests that SCRD exists prior to the onset of a psychotic disorder, during prodromal stages^{135,136}, ultra-high risk predictive of disease onset^{141,142,197}, and in first degree relatives of people with a psychotic disorder^{137,198}. Increased sleep disturbance has also been reported to be associated with PLEs amongst the general population^{144,145}. SCRD could therefore be a fundamental feature across the psychosis continuum. Furthermore, increasing evidence, largely based upon shared genetic risks, suggests that SCRD and psychosis share a common underlying neuropathology^{120,153}. Given the proposed role of risk factors in the development of psychosis and PLEs, it is plausible that risk factors for psychosis are also involved in the development of SCRD. Interestingly one study on twins identified both a genetic and

environmental influence on the degree of PLEs and subjective sleep disturbances reported¹⁷³. However, no other studies have assessed this or identified a relationship with specific risk factors. Investigating the temporal relationship between SCRD and psychosis and how they both may be influenced by risk factors could therefore provide critical insight into their aetiology and aid the identification of vulnerable individuals.

2.1.4 Study aims

This was an explorative study aimed to provide further knowledge on the interaction between sleep and circadian rhythm disruption, psychotic-like experiences and risk factors for psychosis. This study had three aims. Firstly this study aimed to identify the prevalence of risk factors for psychosis across the general population, how they may interact with one another, and how they relate to the occurrence of psychotic-like experiences. Secondly, this study aimed to investigate the relationship to sleep disturbance and whether particular sleep or circadian phenotypes are associated with both PLEs and risk factors. Finally, this study aimed to identify, longitudinally, whether the occurrence of risk factors were also predictive of future changes in PLEs and sleep disturbance one year later.

2.2 Methodology

To understand the relationship between psychotic-like experiences (PLEs), sleep disturbance and risk factors, a population based online survey was created.

2.2.1 Identifying risk factors for psychosis

A systematic literature search was undertaken to identify all risk factors associated with psychosis (Figure 2.1). Factors of interest were considered 'risk factors' if they met a predefined definition of being any attribute, characteristic, or exposure associated with an increased likelihood for developing a psychotic disorder. Searches were limited to systematic reviews and/or meta-analyses showing a clear, established positive

association (regardless of strength) between the risk factor and a psychotic disorder. From screening a total of 17,187 articles, 49 articles were identified and used to select risk factors for assessment in the online survey (Figure 2.1).

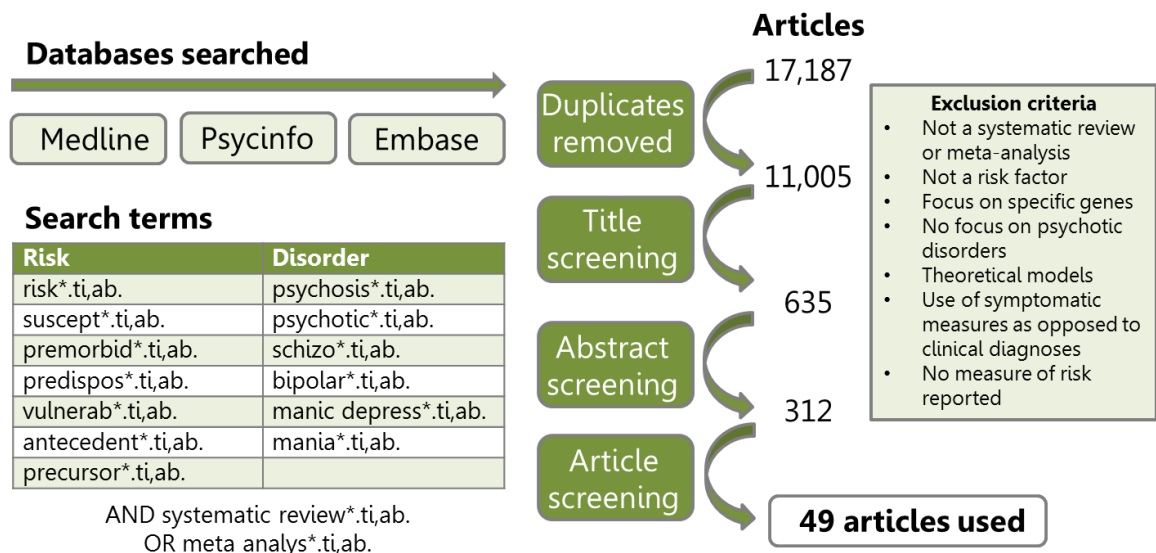


Figure 2.1: Systematic literature search for risk factors associated with the development of psychotic disorders. A total of three databases (MEDLINE, PsycINFO, Embase) were searched from inception to February 2014. Boolean mapping was used to search for articles based upon the title/abstract and database-specific subject headings. The risk factor search terms were grouped to disorder-specific search terms and limited to systematic reviews or meta-analyses. This produced 17,187 articles which were narrowed down with increasing detail based on specific exclusion criteria. A total of 49 articles were used to select risk factors for inclusion in the survey.

2.2.2 Developing the survey

The Oxford Wellbeing, Life Experiences and Sleep Survey (OWLS Survey) was created using LimeSurvey¹⁹⁹. From the 49 articles identified in the literature search, a total of 18 risk factors were selected for inclusion in the survey. Table 2.1 outlines all 18 risk factors and how they were assessed in the survey. Only risk factors that could be adequately and efficiently assessed through an online questionnaire were selected. Thus associations such as personality traits or specific gene mutations were excluded due to lengthy measures required or inability for assessment (see Supplementary Table S1 for the list of excluded risk factors).

Table 2.1: Risk factors associated with psychosis, identified from the literature search, and chosen to be included in the online survey. The justification for the use of the risk factor in the survey and how the risk factor was assessed is shown. OR=Odds ratio, RR=Relative risk.

Risk factor	Justification	Ref.	Assessment method
Brain infection	Infections that affect the brain are associated with increased risk (RR=1.7).	[²⁰⁰]	'Have you ever suffered from encephalitis, meningitis or an infection of the brain before the age of 16 which required hospitalisation for more than one day?
Brain injury	A traumatic brain injury of any kind is associated with increased risk (OR=1.65).	[²⁰¹]	'Have you ever suffered from a brain injury that required hospitalisation for more than one day?'
Cannabis use	Increased usage causes increased risk, and is particularly pertinent during development (OR=2.09)	[²⁰²]	A series of questions relating to current frequency of cannabis use, highest frequency of use, duration of most frequent use, and age of most frequent use
Childhood abuse	Physical, sexual, emotional and psychological abuse are associated with increased risk (OR=2.78)	[²⁷]	Questions on frequency of physical, sexual, psychological and emotional abuse before the age of 16 as used by Cuijpers <i>et al</i> ²⁰³
Childhood bullying	Frequent childhood bullying is associated with increased risk (OR=2.3)	[²⁰⁴]	'When you were at school were you the victim of frequent bullying?'
Childhood social withdrawal	Social withdrawal is associated with increased risk (OR=6.54)	[²⁰⁵]	Frequency (never, sometimes, often) of four measures of social withdrawal as a child with a score ranging between 0-8. Taken from items 42, 65, 88 and 111 of the Child Behavioural Checklist (CBCL) ²⁰⁶ and edited to make appropriate for retrospective report
Diagnosis of a non-psychotic psychiatric disorder	Comorbidity is associated with increased risk	[²⁰⁷⁻²¹⁰]	Diagnoses and treatment used if applicable
Ethnicity	Black ethnicities are associated with increased risk (OR range from 4.9-5.9)	[²¹¹]	Ethnicity question taken from the national census
Family history of psychiatric disorders	A family history of psychiatric disorders are associated with increased risk (OR=4.07 for schizophrenia and OR=4.4 for bipolar disorder)	[²¹²]	Number of blood relatives diagnosed with a mental health disorder, their diagnosis, and treatment if applicable
1 st of 2 nd generation migrant	Being a migrant is associated with increased risk (1 st gen OR=2.33, 2 nd gen OR=2.13). Moving from a less developed country and/or a country of primarily ethnically black origin are particularly pertinent	[^{28,213}]	Participants country of birth, participants country of permanent residence, parents country of birth
Gender	Males are associated with increased risk (RR=1.42)	[²¹⁴]	'What is your sex/gender?'

Latitude	Growing up in higher latitudes is associated with an increased risk ^[215,216]	Participants clicked which region on a world map where they have lived the longest between the ages of 0-18 yrs
Obstetric complications	Obstetric complications are associated with increased risk (OR range from 1.36-7.76) ^[29]	List of obstetric complications given. Participants were asked to ring their mother if willing
Paternal age	A paternal age of more than 50 upon conception is associated with an increased risk (RR=1.66) ^[217]	'How old was your father when you were conceived?'
Season of birth	Being born in winter or spring months are associated with an increased risk (OR=1.07) ^[218]	'What is your date of birth?'
Traumatic experiences	Having traumatic life experiences are associated with an increased risk (OR=3.19) ^[219]	Life Threatening Experiences (LTE) scale with distress levels added ²²⁰
Urbanicity	Being raised in a densely populated urban area is associated with an increased risk. RR=2.24 for being born in an urban area, and RR=2.75 for being raised in an urban area ^[221,222]	'Is where you have lived the longest between the ages of 0-18 years a densely populated crowded city?' Examples given
22q11.2 Deletion Syndrome	Having a diagnosis of 22q11.2 deletion syndrome is associated with a 30% increased risk ^[24]	Diagnosis present: Y/N

Psychotic-like experiences, were assessed using the Prodromal Questionnaire-16 (PQ-16)²²³. This scale is comprised of 16 questions including 9 items to assess perceptual abnormalities and hallucinations, 5 items to assess unusual thought content and delusions and 2 items to assess negative symptoms. A score of more than 5 indicates clinical risk for developing psychosis. A Likert scale of distress levels for each item were also included to better distinguish clinical relevance. Help seeking behaviour for PLEs was additionally assessed by asking participants if they have ever sought help from a counsellor or GP for these experiences. Symptoms of depression, anxiety and stress were assessed using the Depression Anxiety Stress Scale-21 (DASS-21)²²⁴. This questionnaire is comprised of 21 Likert scale questions with 7 items each to assess the frequency of depression, anxiety and stress symptoms. A score of >9 indicates at least moderate distress for anxiety and depression. A score of >18 indicates at least moderate distress from symptoms of stress.

Subjective sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI)⁵⁴. This questionnaire assesses sleep quality with a score of more than 5 being indicative of

poor sleep quality. The PSQI can be further broken down into 7 components assessing subjective sleep quality, sleep onset latency, sleep duration, sleep efficiency, daytime dysfunction, medication for sleep problems, and sleep disturbances. Due to the vast heterogeneity in sleep disturbances and medication use, the latter two components were excluded from analysis. A short version of the Sleep Condition Indicator (SCI-2)²²⁵ was used to more specifically look at symptoms of insomnia. The SCI-2 is comprised of two items which assess insomnia severity with a score of less than 4 indicative of diagnostic criteria met for insomnia disorder. Finally chronotype and social jetlag were assessed using the Munich Chronotype Questionnaire (MCTQ)²²⁶. This scale is comprised of 6 questions to assess habitual sleep timings. The primary outcome, mid-sleep point on free days, corrected for oversleep (MSFsc) is the midpoint between sleep start and sleep end corrected for oversleep which may result from sleep deprivation on work days (days with scheduled commitments). It is calculated from the following formula:

$$MSFsc = MSF - (0.5 * (SDf - (((nWD \times SDw) + ((7 - nWD) \times SDf) / 7)))$$

Where MSF is the mid-sleep point on free days, SDf is the sleep duration on free days, SDw is the sleep duration on work days, and nWD is the number of work days. The MSFsc provides a correlate of chronotype – the circadian phase relative to time of day. Social jetlag (SJL) refers to the amount of discrepancy between the timing of the sleep phase that is influenced by social and work commitments and that of the endogenous circadian rhythm¹¹⁶. It is calculated from the following formula:

$$SJL = MSF - MSW$$

Where MSW is the mid sleep point on work days.

In total, the OWLS Survey was comprised of around 100 questions taking roughly 15 minutes to complete. The survey was advertised using posters around Oxford on noticeboards in hospitals, community centres, medical practices and Oxford university colleges. In addition, online advertisement was carried out through university mailing lists

and various social media websites, including Facebook. This study was approved by the Central University Research Ethics Committee (Reference number MSD-IDREC-C1-2014-054).

2.2.3 Follow-up survey

To identify how mental state, and sleep habits changed over time, a follow-up survey was additionally created for participants to complete 12 months later. This survey was a duplicate of the original survey with the removal of questions relating to risk factors that occurred during childhood. Thus all experiences were specific to the past 12 months. Participants who consented to being contacted 12 months later in the original survey were contacted through email with a link to the follow-up survey.

2.2.4 Quantifying risk factors

To quantify risk factors endorsed by individuals, binary scores were created for each risk factor. To account for varying risk levels, some risk factors were separated into more than one variable (e.g. childhood abuse was split into psychological, physical, sexual and emotional abuse). From the 18 risk factors assessed, a total of 23 items were therefore selected for use as a binary score. From this, a total risk score was created from the sum of each individual binary score. Table 2.2 provides information on how the binary score was given for each risk factor variable. Criteria were based upon the literature and agreed by the whole research team.

Table 2.2: Criteria for creating binary scores for each of the 23 risk factor variables. In certain cases more than one item was selected to adequately assess the risk factor.

Risk Factor	Binary score criteria (for a score of 1)
Brain infection	Has suffered from encephalitis, meningitis or an infection of the brain before the age of 16 which required hospitalisation for more than 1 day
Brain injury	Has suffered from a brain injury that required hospitalisation for more than 1 day
Current cannabis usage	Currently use cannabis weekly or more
Childhood cannabis usage	Past usage of cannabis weekly or more and before the age of 18 (if more frequent than current usage)
Childhood physical abuse	Experience of childhood physical abuse regularly or more
Childhood emotional abuse	Experience of childhood emotional abuse regularly or more
Childhood sexual abuse	Experience of childhood sexual abuse regularly or more
Childhood psychological abuse	Experience of childhood psychological abuse regularly or more
Childhood bullying	Has experienced frequent bullying at school
Childhood social withdrawal	Score of 5 or above on social withdrawal questions
Diagnosis of non-psychotic psychiatric disorder	Has been diagnosed with a non-psychotic psychiatric disorder
Ethnicity	Is of black ethnicity
Family history of psychiatric disorders	Has a family member with a diagnosis of a psychiatric disorder
1 st or 2 nd generation migrant	Individual or parents have emigrated and changed permanent residence to a country other than individual's country of birth
1 st or 2 nd generation migrant from risk area	Individual or parents have emigrated and changed permanent residence from a country of primarily black ethnic origin to a developed country
Gender	Is male
Latitude	Grew up in a region of 60° latitude or more
Obstetric complications	Is aware of at least one serious obstetric complication during pregnancy/birth
Paternal age	Paternal age of more than 50 upon conception
Season of birth	Was born in winter or spring months
Traumatic experiences	Has experienced a trauma that caused at least moderate distress
Urbanicity	Grew up in a [subjectively measured] densely populated urban area
22q11.2 deletion syndrome	Has a diagnosis of 22q11.2 deletion syndrome

2.2.5 Data processing

All data was stored using a university online server. This survey is an ongoing project expected to complete within 5 years from inception. For the data presented in this thesis, survey data from May 2014 to June 2016 were included.

133 (7%) individuals who completed the survey were removed due to email duplications.

26 (1%) individuals were removed for reporting a previous diagnosis of a psychotic

disorder. 145 (7%) individuals were removed from all analyses involving the PSQI and MCTQ due to unreliable responses including a mismatch in lights off and sleep timing, negative sleep durations, or sleep durations greater than 16 hours (data averaged between work and free days).

2.2.6 Statistical analyses

The statistical program 'R' was used for all data analysis²²⁷. To assess the relationship between risk factors, a maximum-likelihood exploratory factor analysis was undertaken. This technique was used since it allows the assessment across a large set of variables enabling them to be grouped based upon potential underlying shared factors. Using the in-built R-function *factanal*, risk factors were grouped into factors based upon their loadings (analogous to a Pearson's r correlation) onto the constructed factors. The number of factors chosen were determined by a step-wise increase in number until an acceptable fit of the model was reached (where $p > 0.05$).

Spearman's rho correlation coefficients were used to assess relationships between the PSQI, PQ-16 and the total number of risk factors endorsed as a non-parametric assessment of non-normal data. To assess the relationship between risk factor binary scores, PSQI, PQ-16 and DASS-21, linear models were fitted, with stepwise backward elimination for non-significant variables. A folded transformation was applied to the dependent variable to provide log-normal distributions. To assess longitudinal effects of risk factor binary scores on PQ-16 and PSQI scores, a repeated measures ANOVA (a binomial linear mixed effect model with multivariate normal random effects using Penalized Quasi-Likelihood) was fitted. Finally group differences were calculated using ANOVA and post-hoc t-tests and non-parametric Wilcoxon rank sum tests for non-normal data.

2.3 Results

2.3.1 Assessment of mental state

A total of 1898 individuals were included in this analysis. The median age was 23±9 (interquartile range), 71% (1355) were female, and 69% (1305) were students. 26% (488) of individuals reported at least one lifetime psychiatric diagnosis (including comorbidities: 51% depressive disorder, 24% anxiety related disorder, 7% obsessive-compulsive or related disorder, and 18% other/unknown cases were reported). 37% (704), 35% (663) and 27% (505) of individuals experienced moderate or greater symptoms of depression, anxiety and stress, respectively (DASS-21 scores >9, >9 and >18). In addition, 26% (496) reported a 1st degree relative having a psychiatric disorder with 3% (66) reporting this to be a psychotic disorder.

79% (1501) of individuals endorsed at least one psychotic-like experience (PQ-16 score >0) and 21% (395) of individuals scored positively for a clinical risk of psychosis (PQ-16 score >5). All items of the PQ-16 were endorsed by 5% to 36% of the population with social anxiety, avolition, thought insertion, and delusional mood amongst the most commonly reported PLEs (Figure 2.2). Finally, 8% (157) of individuals reported seeking help for these experiences including counselling or talking with a general practitioner.

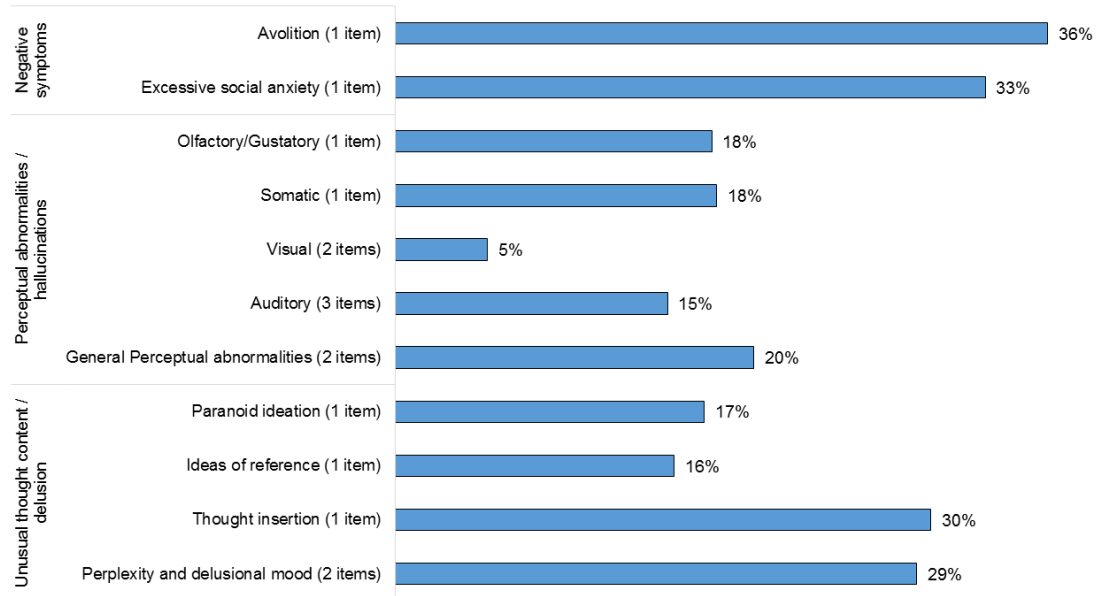


Figure 2.2: Percent endorsement of the Prodromal Questionnaire 16 components assessing psychotic-like experiences (n=1898).

2.3.2 Risk factors within the population

Individuals reported an average of 4 ± 2 risk factors (median 4, mode 4) with only 1% (11) of individuals reporting no risk factors. Experiencing a traumatic event causing at least moderate distress was the most prevalent risk factor, endorsed by 84% of the population (Figure 2.3). These traumatic experiences included a range of events such as a serious personal injury, ending a steady relationship or death of a relative, and occurred at any time throughout the individual's life (for further details see Table 2.5). A winter/spring season of birth was endorsed by 51% of the population closely followed by a family history of a psychiatric illness (42%) and having migrant status (37%). The remaining risk factors were endorsed between 1-30% of the population with the exception of 22q11.2 Deletion Syndrome which was not endorsed by any individual (Figure 2.3).

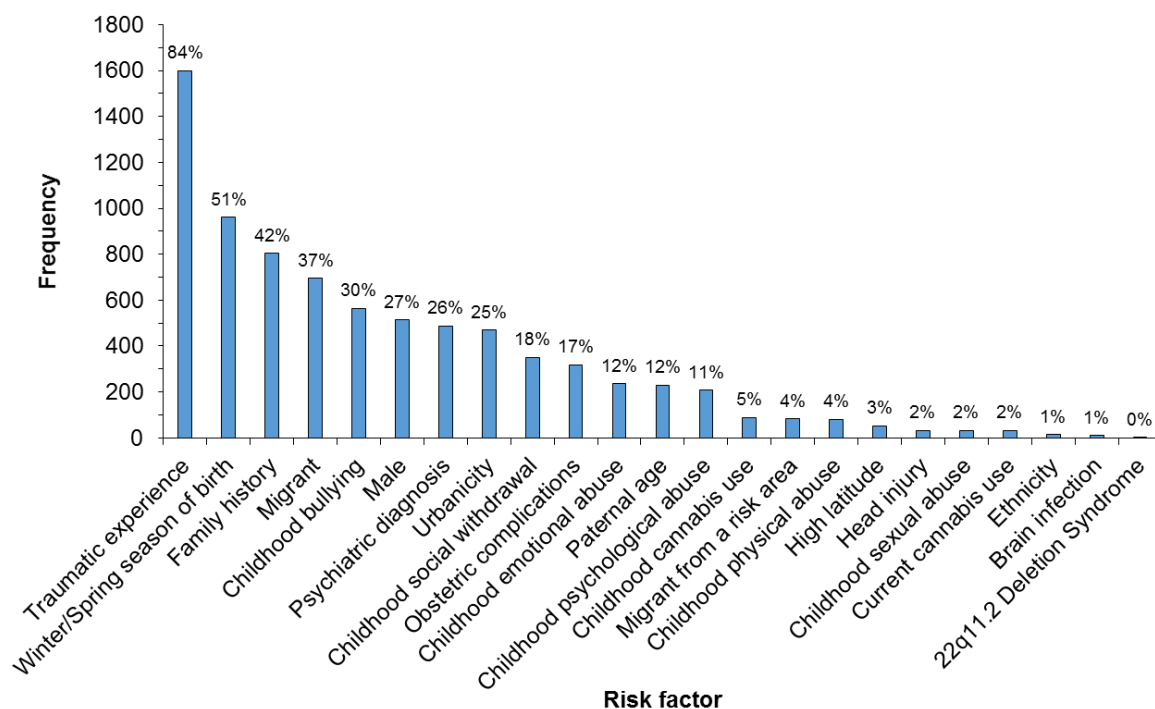


Figure 2.3: Risk factor prevalence. Childhood abuse was assessed using 4 items separating physical, sexual, psychological and emotional abuse. Cannabis use was assessed using 2 items separating current use and childhood use. Migration was assessed using 2 items separating migration from any country to any country and migration specifically from areas of greatest risk. The remaining risk factors were assessed with 1 item only (see methods). N=1898.

A factor analysis was used to identify the relationship between risk factors. This analysis grouped risk factors into eight clusters ordered from 1 to 8 based on the strength of association between each risk factor. Cluster 1 contained risk factors with the highest loadings, and cluster 8 with the lowest loadings. The higher the loading, the more likely the risk factors were associated with one another. Cluster 1 indicated that a psychiatric diagnosis, childhood abuse, childhood social withdrawal, and childhood bullying were most strongly associated with one another (Figure 2.4). These risk factors were also present across multiple clusters (cluster 1, 4, 5, 6, 8). Both migrant status and ethnicity were clustered together (cluster 2, 3) but were not associated with any of the other risk factors. Male gender and urbanicity were negatively associated with trauma, childhood abuse, psychiatric diagnoses and family history (cluster 4). Cannabis use was associated with family history and psychiatric diagnoses (albeit with a low factor loading, cluster 8).

Season of birth, latitude, paternal age and obstetric complications were unrelated to all other risk factors.

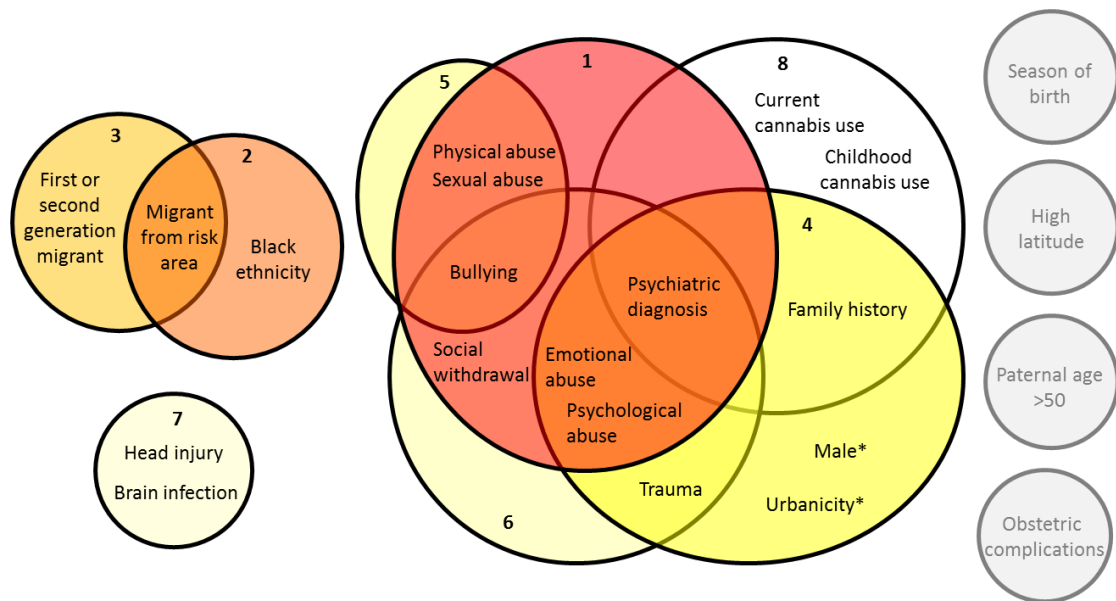


Figure 2.4: Factor analysis overview. Each circle represents a separate cluster of relatedness (correlational co-occurrence), with some risk factors appearing in multiple clusters. Numbers denote clusters in order of greatest loadings (1 to 8). *Risk factors were negatively associated with the other risk factors in that cluster. Note that all risk factors in cluster 1 except a psychiatric diagnosis refer to childhood experiences.

Details of the factor loadings can be found in Table 2.3.

Table 2.3: Factor analysis between risk factors. A minimum of 8 clusters was required to adequately model the data ($p=0.393$). Data show loadings for each risk factor within each of 8 clusters (C1-8). Only values more than 0.1 or less than -0.1 are shown. $N=1898$.

Risk factor	C1	C2	C3	C4	C5	C6	C7	C8
Psychiatric diagnosis	0.118			0.538		0.214		0.125
Childhood bullying	0.118				0.113	0.451		
Childhood psychological abuse	0.897			0.123		0.185		
Childhood emotional abuse	0.559			0.138		0.248		
Childhood physical abuse	0.455				0.170			
Childhood sexual abuse	0.170				0.774			
Childhood social withdrawal	0.155					0.520		
Family history of psychiatric illness				0.441				0.111
Trauma				0.178		0.152		
Migrant from a risk area		0.299	0.265					
Black ethnicity		0.989						
First or second generation migrant			0.990					
Male				-0.298				
Urbanicity				-0.159				
Head injury							0.469	
Brain infection							0.429	
Current cannabis use								0.430
Childhood cannabis use								0.431

2.3.3 The association between risk factors and PLEs

A moderate but significant positive correlation was identified for the total number of risk factors with both the number of psychotic-like experiences (rho=0.32, p<0.001; Figure 2.5a) and distress levels from these experiences (rho=0.34, p<0.001; Figure 2.5b).

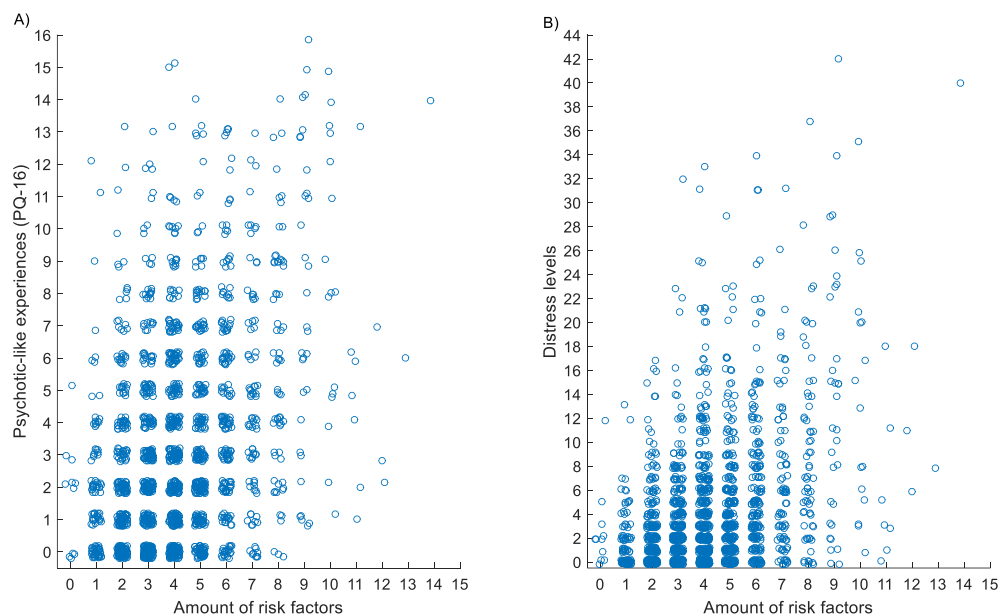


Figure 2.5: Total amount of risk factors in relation to (A) psychotic-like experiences (PQ-16) and (B) distress levels from these experiences (n=1898).

To identify the effects of individual risk factors on the number of PLEs, a linear regression model was fitted using individual risk factor binary scores. This analysis revealed that current cannabis use (OR[CI]=2.35[1.58-3.49]), a lifetime psychiatric diagnosis (OR[CI]=1.83[1.62-2.06]) and childhood emotional abuse (OR[CI]=1.80[1.53-2.13]) were the most predictive risk factors for the number of PLEs reported (Table 2.4). In addition, childhood social withdrawal, trauma, childhood physical abuse, migration from a risk area, childhood bullying and 1st/2nd generation migrant were also significantly associated with the number of PLEs. These risk factors will henceforth be referred as Group I risk factors. The remaining risk factors, which did not survive step-wise backward elimination from the model, are referred to as Group II risk factors (see Table 2.4).

Comparing these results with the previous factor analysis (Figure 2.4) revealed that 5 out of the 7 Cluster 1 risk factors were also Group I risk factors. These risk factors were therefore both strongly associated with one another and associated with an increasing number of PLEs. Childhood psychological and sexual abuse were two exceptions in Cluster 1 unassociated with PLEs. Furthermore cannabis use showed the strongest association with the number of PLEs but was weakly related to other risk factors, appearing in Cluster 8.

Next, possible interactions between risk factors associated with the number of PLEs were assessed. For this, interactions between any two risk factors were sequentially added to the linear model and removed if not significant. This analysis revealed a significant interaction between childhood social withdrawal and emotional abuse (Linear model, $t=-2.32$, $p=0.021$) and childhood social withdrawal and childhood bullying (Linear model, $t=-2.07$, $p=0.039$). A greater than additive effect is therefore implied for these risk factor combinations. However no other interactions were identified between any of the remaining risk factor combinations suggesting a general additive rather than multiplicative effect on the number of PLEs.

Finally, since the severity of PLEs is an important contributor for assessing psychosis and at risk states, a second model was fitted to identify the effects of risk factor binary scores on distress levels from PLEs. All Group I risk factors were significantly associated with an increasing amount of distress from PLEs with the addition of childhood psychological abuse ($OR[CI]=1.23[1.03-1.59]$), obstetric complications ($1.17[1.02-1.34]$) and brain injury ($1.50[1.01-2.24]$; Table 2.4).

Table 2.4: The association between risk factors and psychotic-like experiences. Risk factors were given a binary score based upon their frequency (regularly or more) and degree of distress (moderate or more). A linear model was used to calculate t, p and OR (Odds ratios) for each risk factor (n=1898). *denote significant differences

	Risk factor	Number of PLEs			Amount of distress from PLEs		
		t	p	OR (CI)	t	p	OR (CI)
Group I risk factors	Current cannabis use	4.24	<0.001***	2.35 (1.58 – 3.49)	2.91	0.004**	1.82 (1.22 – 2.73)
	Lifetime psychiatric diagnosis	9.72	<0.001***	1.83 (1.62 – 2.06)	12.07	<0.001***	2.15 (1.90 – 2.44)
	Childhood emotional abuse	6.91	<0.001***	1.80 (1.53 – 2.13)	5.54	<0.001***	1.73 (1.42 – 2.10)
	Childhood social withdrawal	6.60	<0.001***	1.59 (1.39 – 1.83)	6.32	<0.001***	1.58 (1.37 – 1.82)
	Trauma	5.75	<0.001***	1.52 (1.32 – 1.75)	4.74	<0.001***	1.42 (1.23 – 1.65)
	Childhood physical abuse	2.85	0.004**	1.47 (1.13 – 1.91)	2.39	0.017*	1.42 (1.06 – 1.89)
	Migrant from a risk area	2.31	0.021*	1.35 (1.05 – 1.75)	2.68	0.007**	1.43 (1.10 – 1.86)
	Childhood bullying	3.35	0.001***	1.22 (1.09 – 1.37)	2.92	0.004**	1.20 (1.06 – 1.35)
	First or second generation migrant	-2.27	0.023*	0.88 (0.79 – 0.98)	-2.31	0.021*	0.88 (0.78 – 0.98)
Group II risk factors	Childhood psychological abuse	-	-	-	2.20	0.028*	1.23 (1.03 – 1.59)
	Obstetric complications	-	-	-	2.18	0.029*	1.17 (1.02 – 1.34)
	Brain injury	-	-	-	2.01	0.045*	1.50 (1.01 – 2.24)
	Season of birth	-	-	-	-	-	-
	Family history	-	-	-	-	-	-
	Male	-	-	-	-	-	-
	Urbanicity	-	-	-	-	-	-
	Paternal age	-	-	-	-	-	-
	Childhood cannabis use	-	-	-	-	-	-
	High latitude	-	-	-	-	-	-
	Childhood sexual abuse	-	-	-	-	-	-
	Black ethnicity	-	-	-	-	-	-
	Brain infection	-	-	-	-	-	-
	22q11.2 Deletion Syndrome	-	-	-	-	-	-

Since the distress scale used in the PQ-16 has not yet been validated amongst the general population, the remainder of these analyses focused on the *number* of psychotic-like experiences endorsed.

2.3.4 The effects of risk factor severity or frequency on PLEs

For risk factors that included a scale of severity or frequency (such as childhood abuse), a further analysis was conducted to determine if the level of severity or frequency influenced the association with PLEs. For this analysis, linear models were created which included all Group I risk factors in addition to the risk factor of interest split based on its severity or frequency level.

Results revealed that some of these risk factors have a dose-response relationship to PLEs. Specifically, a dose response relationship was identified for the severity of childhood social withdrawal, with low levels (score of 1-2), medium levels (score 3-4), high levels (score 5-6) and very high levels (score 7-8) being increasingly predictive of PLEs (OR[CI]=1.22[1.06-1.42], 1.82[1.55-2.13], 2.17[1.79-2.62], 2.47[1.89-3.23] respectively). A dose response relationship was identified for the frequency of current cannabis use with weekly use (OR[CI]=2.14[1.30-3.50]) and daily use (OR[CI]=2.37[1.21-4.65]) having the strongest effect on PLEs over having ever tried cannabis. No effect of irregular or monthly use was found. A dose response relationship was also identified for the amount of distress caused by any traumatic experience on PLEs, with moderate distress (OR[CI]=1.48[1.20-1.83]) and severe distress (OR[CI]=1.57[1.27-1.96]) having the greatest effect (no effect of no distress or mild distress was found). A further assessment of individual traumatic events revealed a financial crisis, death of a family friend and a personal illness, injury or assault were most predictive of PLEs. An inverse relationship was identified for marital separation (Table 2.5).

Table 2.5: Prevalence of traumatic experiences causing at least moderate distress and their effect on psychotic-like experiences (n=1898). *denote significant differences.

Traumatic experience	Prevalence	t	p
A serious personal illness, injury or an assault	393 (21%)	2.288	0.022*
Ending a steady relationship	326 (17%)	-	-
Death of parent, child or spouse	238 (13%)	-1.786	0.074
A serious problem with a close friend, neighbour or relative	189 (10%)	-	-
A serious illness, injury or assault to a close relative	170 (9%)	-	-
Lost or stolen valuable	146 (8%)	-	-
Marital separation	100 (5%)	-3.288	0.001**
Death of a close family friend or another relative	79 (4%)	2.568	0.010*
Unemployment	67 (4%)	-	-
Dismissed from employment	45 (2%)	-	-
Problems with the police and court appearance	30 (2%)	-	-
Major financial crisis	27 (1%)	3.554	<0.001***

A second set of risk factors showed a significant association to PLEs but this was independent of how frequent or severe they were and thus no dose-response relationship was identified. Childhood emotional abuse had a significant effect on PLEs for all

frequencies, whether it occurred “one time” (OR[CI]=1.89[1.35-2.65]), “sometimes” (1.48[1.28-1.71]), “regularly” (1.72[1.34-2.20]), “often” (2.42[1.84-3.18]) or “very often” (2.17[1.65-2.85]). Childhood physical abuse had a significant effect if it occurred “often” (OR[CI]=1.64[1.03-2.61]), but no effect for any other frequencies. Childhood psychological abuse had a significant effect if it occurred “one time” (OR[CI]=1.72[1.18-2.50]), but no effect for any other frequencies. The inverse relationship with migration and PLEs was identified to be specific for first generation migrants (OR[CI]=0.85[0.75-0.96]). No effect was found for second generation migrants.

A final set of risk factors presented no association to PLEs regardless of their frequency or severity. Specifically, childhood sexual abuse presented no effect on PLEs for any frequency of occurrence. Further, no effect of family history on amount of PLEs was found for any type of relative (n=803), first-degree relatives (n=495) or first-degree relatives with a psychotic disorder (n=65). Details of all models used in these analyses can be found in supplementary material (Table S2).

2.3.5 The relationship to sleep disturbance

42% (806) of individuals scored more than 5 on the PSQI indicative of poor sleep quality. Higher PSQI scores were moderately correlated with an increasing number of risk factors ($\rho=0.27$ $p<0.01$, Figure 2.6a) and greater psychotic-like experiences (PQ-16, $\rho=0.44$, $p<0.001$, Figure 2.6b).

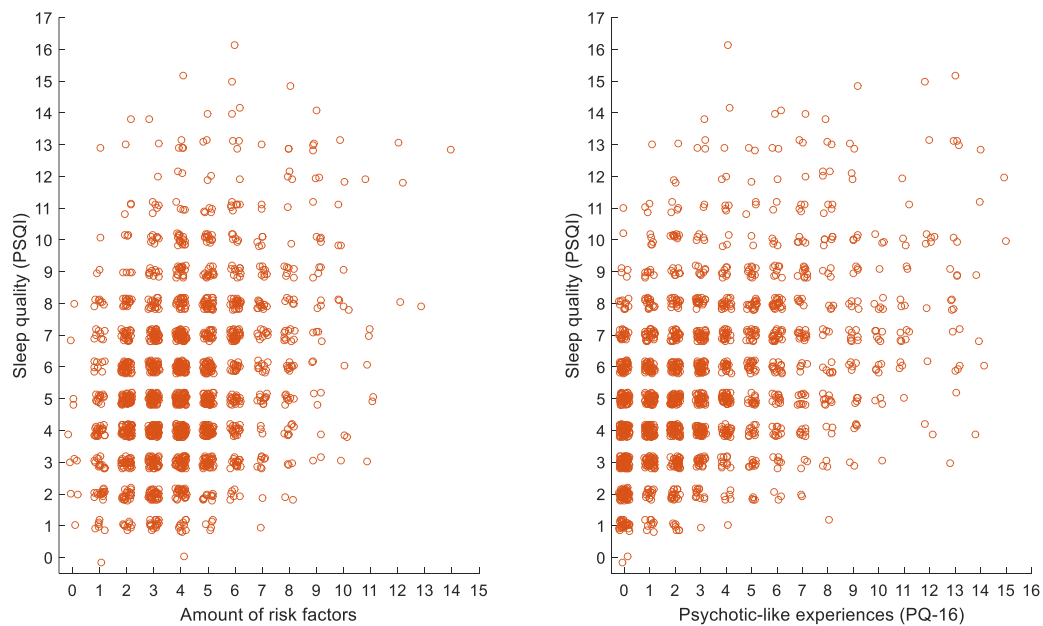


Figure 2.6: Sleep quality (PSQI) in relation to (A) the total number of risk factors and (B) the number of psychotic-like experiences (PQ-16; $n=1753$).

To assess whether specific sleep or circadian measures were associated with PLEs and risk factors, a linear model was fitted using individual components of the PSQI, and outputs from the SCI-2 and the MCTQ. This model revealed increased day-time dysfunction from poor sleep (PSQI), higher sleep-onset latencies (PSQI), greater insomnia severity (SCI-2) and later mid-sleep points (MCTQ) had the greatest association to both psychotic-like experiences and the total number of risk factors endorsed (Table 2.6). The SCI-2 was moderately correlated with PSQI daytime dysfunction ($r=-0.45$, $p<0.001$), and PSQI sleep onset latency ($r=-0.44$, $p<0.001$), with no correlation to MCTQ mid-sleep points ($r=-0.02$, $p=0.437$).

Table 2.6: Summary of sleep and circadian parameters and their influence on psychotic-like experiences and total number of risk factors. n=1753. *denote significant differences.

Sleep/circadian component	Mean	Median	IQR	Number of PLEs		Number of risk factors	
				t	p	t	p
Global sleep quality (PSQI)	5.6	5.0	3.0	-	-	-	-
Self-rated sleep quality	1.23	1.00	1.00	2.04	0.042*	-	-
Day-time dysfunction	1.35	1.00	1.00	14.00	<0.001***	6.68	<0.001***
Sleep efficiency (%)	96.3	97.5	5.6	-	-	-	-
Sleep latency (hrs)	00:29	00:20	00:25	5.40	<0.001***	2.70	0.007**
Sleep duration (hrs)	08:25	08:23	01:15	-	-	-	-
Insomnia severity (SCI-2)	4.9	5.0	4.0	-2.79	0.005**	-6.52	<0.001***
Mid-sleep point on free days (hrs; MCTQ)	04:26	04:16	01:50	4.84	<0.001***	2.16	0.031*
Social jetlag (hrs; MCTQ)	01:15	01:08	01:08	-	-	-	-

To identify whether risk factors were related to sleep disturbance independent of PLEs, a second analysis assessed sleep quality in individuals with high and low amounts of risk factors and PLEs. A high amount of PLEs was defined as a PQ-16 score more than 5, a low amount of PLEs was defined as a PQ-16 score less than 6. A high and low amount of risk factors was based upon a median split of risk factor prevalence from the total sample with those reporting more than 4 (high amount) and less than 5 (low amount) respectively. Four groups were therefore identified: those with a low amount of risk factors and PLEs (n=1027), a high amount of risk factors and low amount of PLEs (n=476), a low amount of risk factors and high amount of PLEs (n=173) and a high amount of risk factors and PLEs (n=222). A non-parametric analysis of variance revealed a significant group difference in sleep quality (PSQI scores; Kruskal-wallis rank sum test, $X^2=245$, $p<0.001$; Figure 2.7). Post-hoc non-parametric t-tests revealed a significant difference in sleep quality between each group where an increased amount of both risk factors and PLEs was associated with significantly poorer subjective sleep quality. Of note, individuals who had a high amount of risk factors but low amount of PLEs still reported significantly poorer sleep quality compared to those with both a low amount of risk factors and PLEs (Wilcoxon rank sum test; $W=174160$, $p<0.001$; Figure 2.7 and see Supplementary Table S3 for all post-hoc comparisons).

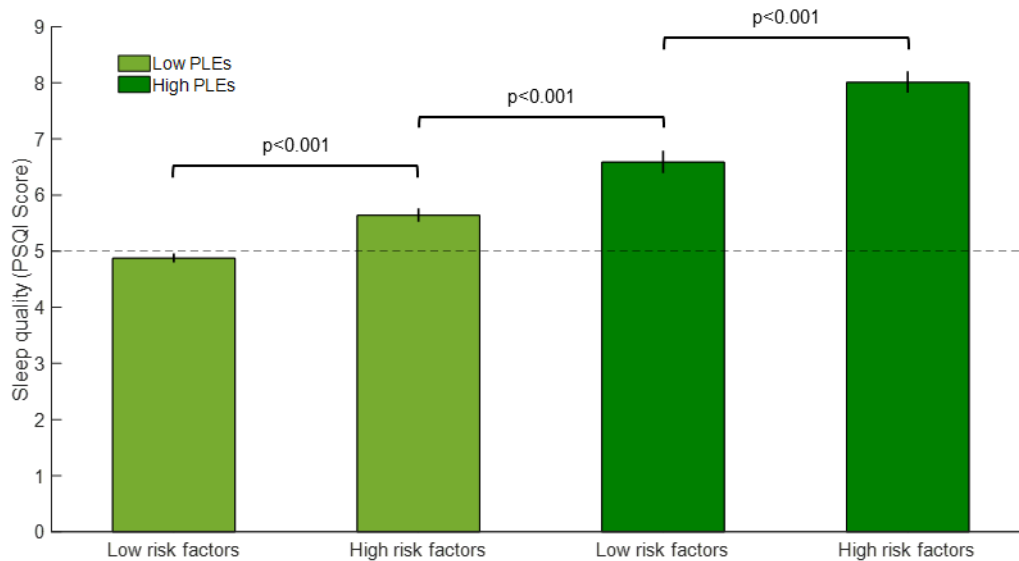


Figure 2.7: Effect of the number of risk factors and psychotic-like experiences (PLEs; PQ-16 score) on sleep quality (PSQI score). An increasing number of risk factors and psychotic-like experiences were associated with significantly poorer sleep quality (Kruskal-wallis rank sum test, $X^2=245$, $p<0.001$). Note the cut-off score for significant poor sleep is >5 . A high amount of PLEs corresponds to a PQ-16 score of more than 5, a high amount of risk factors corresponds to those reporting more than 4.

2.3.6 Longitudinal relationship between risk factors, sleep quality and PLEs

501 individuals completed the 12 month follow-up survey (after an average of 12.66 ± 0.63 months). Using a repeated measures analysis of variance, risk factors endorsed in the original survey were analysed in relation to changes in PLEs and sleep quality from the original survey (time 1) to the 12 month follow-up (time 2). No risk factors were predictive of future changes in both PLEs and sleep quality. Childhood bullying and childhood sexual abuse were the only risk factors endorsed at time 1 that were predictive of future increases in PLEs (Table 2.7). Childhood social withdrawal was the only risk factor endorsed at time 1 that was predictive of poorer sleep quality with time.

Results from the main effects of the model (associations between risk factors at time 1 to sleep quality and PLEs including both time points) revealed a significant main effect for childhood emotional abuse, childhood bullying and a lifetime psychiatric diagnosis on both PLEs and sleep quality (Table 2.7). Poorer sleep quality was additionally associated with

being female, obstetric complications, and childhood physical abuse. A greater number of PLEs was additionally associated with cannabis use, childhood social withdrawal, migration and traumatic experiences. These results comply with the previous analysis from the original survey at time 1 (section 2.3.3 , pg62) but in contrast, no main effect was identified for migration from a risk area or childhood physical abuse on PLEs. Further, a significant negative association was identified to brain injury.

Table 2.7: Effect of risk factors on the change in amount of psychotic-like experiences (change in PQ-16 score) and change in sleep quality (change in PSQI score) 12 months later. ^ Refer to group I risk factors, those that were significantly associated to PLEs in the original analysis at time point 1. SE=standard error. *denote significant differences, bold indicates significant time effects.

Risk factor	Psychotic-like experiences			Sleep quality		
	β -coeff.	SE	p-value	β -coeff.	SE	p-value
<i>Time effects</i>						
Time	-0.126	0.135	0.351	-0.115	0.045	0.011*
Brain injury: Time	1.333	0.544	0.015*	-	-	-
Childhood bullying: Time	-0.026	0.096	0.784	-0.022	0.062	0.723
Childhood social withdrawal: Time	-0.097	0.108	0.370	0.152	0.075	0.043*
Current cannabis use: Time	<0.001	0.271	0.999	-0.330	0.205	0.109
Childhood emotional abuse: Time	0.029	0.125	0.818	0.036	0.091	0.691
Lifetime psychiatric diagnosis: Time	0.034	0.100	0.732	-0.008	0.067	0.908
Male: Time	-	-	-	0.064	0.067	0.334
1 st /2 nd generation migrant: Time	-0.026	0.095	0.785	-	-	-
Obstetric complications: Time	-	-	-	-0.106	0.074	0.155
Childhood physical abuse: Time	-	-	-	-0.219	0.142	0.125
Childhood sexual abuse: Time	0.815	0.331	0.014*	-	-	-
Traumatic experience: Time	-0.062	0.138	0.653	-	-	-
<i>Main effects</i>						
Brain injury	-1.386	0.684	0.043*	-	-	-
Childhood bullying^	0.206	0.123	0.096*	0.154	0.08	0.023*
Childhood social withdrawal^	0.414	0.144	0.004*	-0.047	0.081	0.562
Current cannabis use^	0.772	0.376	0.041*	0.441	0.229	0.054
Childhood emotional abuse^	0.501	0.173	0.004*	0.234	0.100	0.020*
Lifetime psychiatric diagnosis^	0.548	0.131	<0.001**	0.283	0.073	<0.001**
Male	-	-	-	-0.173	0.071	0.016*
1 st /2 nd generation migrant^	-0.198	0.117	0.092*	-	-	-
Obstetric complications	-	-	-	0.262	0.081	0.001*
Childhood physical abuse^	-	-	-	0.395	0.156	0.012*
Childhood sexual abuse	0.599	0.462	0.196	-	-	-
Traumatic experience^	0.321	0.163	0.049*	-	-	-

To further understand changes in mental health status after 12 months, a descriptive analysis was carried out to identify individuals who had transitioned to a positive screen on the PQ-16 or received a psychiatric diagnosis. From the follow-up survey, 5% (26) of individuals endorsed a positive screen on the PQ-16 after previously scoring below this threshold (PQ-16<6) at time 1. 4% (20) of individuals reported a new psychiatric diagnosis within the past 12 months, regardless of whether they had been previously diagnosed with another disorder. 2% (10) reported this to be their first diagnosis and 2 individuals reported a diagnosis of a psychotic disorder. Further, 50% (10) of these individuals reporting a diagnosis within the past 12 months had a positive screen on the PQ-16 (>5) at time 2. An assessment of new risk factors endorsed revealed 15% (74) of individuals reporting a new psychiatric diagnosis in a family member and 49% (246) reporting a traumatic experience causing at least moderate distress in the past 12 months (Table 2.8).

Table 2.8: Changes in risk factor occurrence and symptom scores across time. --, not present at either time points, +- occurred only at time point 1, -+, occurred only at time point 2, ++, occurred at both time points. Total prevalence = occurred in the last 12 months regardless of whether it had occurred before (sum of --+ and ++). Psychiatric diagnoses at time point 1 indicates a lifetime diagnosis, and at time point 2 a diagnosis within the last 12 months. N=501.

Measure	Total prevalence	--	+-	-+	++
Symptoms					
<i>Psychotic-like experiences (PQ-16>5)</i>	76 (15%)	378 (75%)	47 (9%)	26 (5%)	50 (10%)
<i>Symptoms of depression (DASS-21>9)</i>	160 (32%)	267 (53%)	74 (15%)	45 (9%)	115 (23%)
<i>Symptoms of anxiety (DASS-21>9)</i>	154 (31%)	287 (57%)	60 (12%)	47 (9%)	107 (21%)
<i>Symptoms of stress (DASS-21>18)</i>	124 (25%)	321 (64%)	56 (11%)	53 (11%)	71 (14%)
<i>Sleep quality (PSQI>5)</i>	163 (33%)	202 (40%)	79 (16%)	41 (8%)	122 (24%)
Risk factors					
<i>Psychiatric diagnosis</i>	20 (4%)	360 (72%)	121 (24%)	10 (2%)	10 (2%)
<i>Family history of psychiatric illness</i>	74 (15%)	246 (49%)	181 (36%)	23 (5%)	51 (10%)
<i>Traumatic experience</i>	246 (49%)	52 (10%)	203 (41%)	25 (5%)	221 (44%)
<i>Current cannabis use</i>	11 (2%)	485 (97%)	5 (1%)	6 (1%)	5 (1%)
<i>Head injury</i>	1 (0%)	496 (99%)	4 (1%)	0 (0%)	1 (0%)
<i>First or second generation migrant</i>	64 (13%)	290 (58%)	147 (29%)	27 (5%)	37 (7%)
<i>22q11.2 Deletion Syndrome</i>	1 (0%)	500 (100%)	0 (0%)	1 (0%)	0 (0%)

Individuals were next assessed based upon risk factors and sleep reported at time 1 and their subsequent transition status for clinical risk of psychosis at time 2 (PQ-16 score>5). Individuals who had low levels of PLEs at time 1 but exceeded the threshold for clinical risk at time 2 were classed as ‘converters’ (n=26), i.e. individuals who have developed a clinical risk for psychosis within the study period. Individuals who consistently presented low levels of PLEs at both time points were classed as ‘non-converters’ (n=378), i.e.

individuals who remained at low risk for developing psychosis throughout the study period. The total number of risk factors was significantly greater in converters (mean number in converters=5.1, mean number in non-converters=3.8; Wilcoxon rank sum test: $W=3799$, $p=0.048$). Figure 2.8 highlights differences in the prevalence of risk factors reported at time 1 between converters and non-converters. 18 out of the 23 risk factor variables presented a higher prevalence in converters compared to non-converters. Ethnicity and 22q11.2 deletion syndrome presented no difference. In contrast, gender, childhood cannabis use, and migrant status, presented a higher prevalence in non-converters.

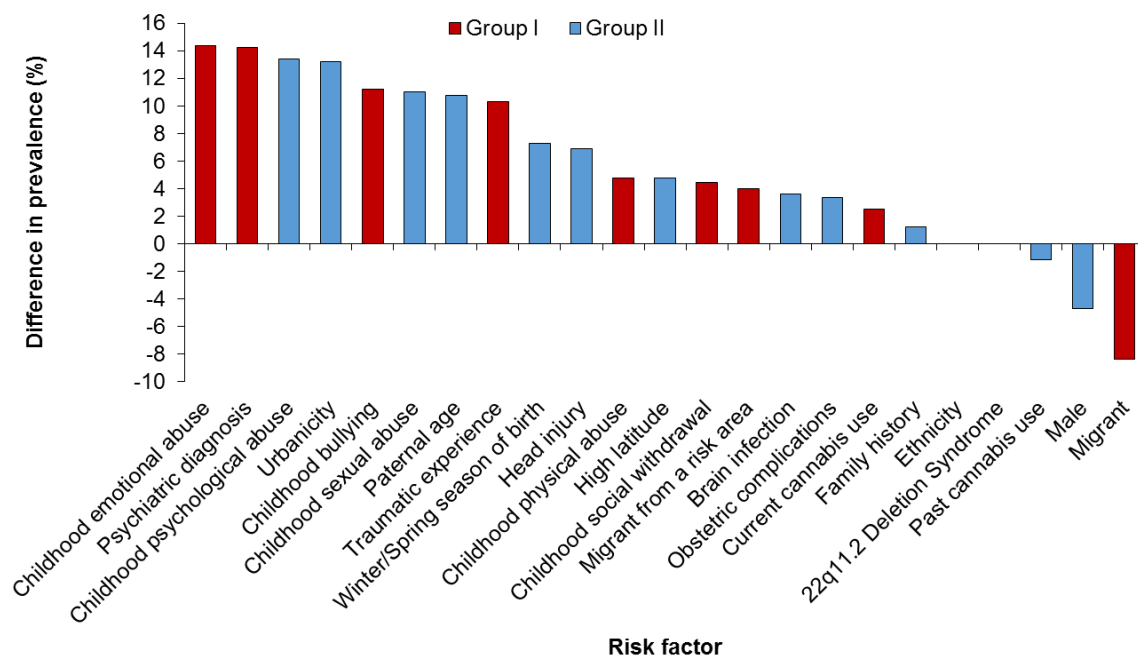


Figure 2.8: Difference in prevalence rate of risk factors at time point 1 between individuals with (converters) and without (non-converters) a transition to positive screen for clinical risk of psychosis ($PQ-16 > 5$). A positive difference indicates a higher prevalence in converters; a negative difference indicates a higher prevalence in non-converters. Bar colours indicate risk factor groupings based on previous analysis (see section 2.3.3). $N=404$.

Figure 2.9 shows measures of sleep quality, insomnia severity and mid-sleep points reported at time 1 between converters and non-converters. Converters reported significantly poorer sleep quality ($W=2722$, $p=0.002$), greater insomnia severity ($W=6501$, $p=0.005$), but no differences in mid-sleep points ($W=3852$, $p=0.148$) compared to non-converters.

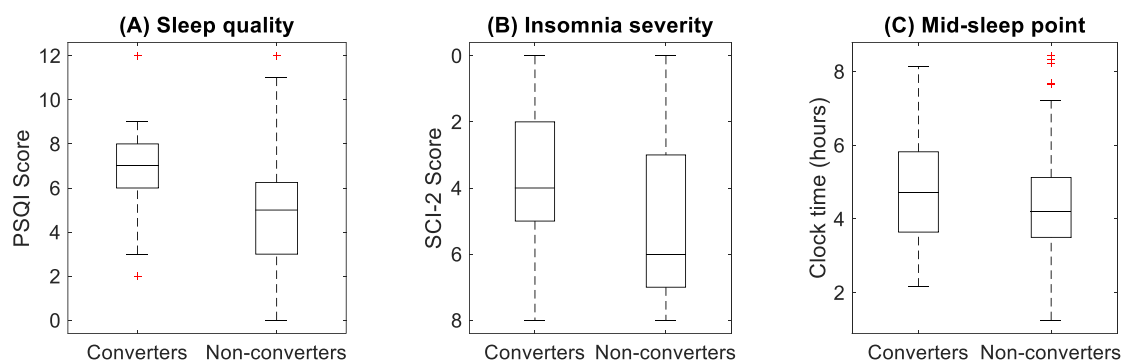


Figure 2.9: Sleep measures at time 1 in individuals who transitioned to clinical risk for levels of psychotic-like experiences (PLEs; PQ-16>5) at time 2 (converters, n=26) and those who maintained low levels of PLEs (PQ-16<6) across time (non-converters, n=378).

2.3.7 Relationship between all mental state measures and risk factors

Since mental health problems often involve many overlapping symptoms and aetiologies, a final analysis was carried out on the effect of risk factors across all measures of mental state including PLEs, depression, anxiety, stress, and sleep quality (using the original sample, n=1898). Psychotic-like experiences were strongly correlated to symptom-based measures of depression (DASS-21, $\rho=0.57$, $p<0.001$), anxiety (DASS-21, $\rho=0.57$, $p<0.001$) and stress (DASS-21, $\rho=0.56$, $p<0.001$). Table 2.9 outlines results from linear model regression analyses of shared risk factors across all mental state measures. Results revealed a lifetime psychiatric diagnosis, experiencing childhood emotional abuse and a traumatic experience were shared across all measures. With the exception of psychological and sexual abuse, all risk factors related to childhood affect (emotional/physical abuse, withdrawal, bullying), a psychiatric diagnosis, trauma, and gender were shared across at least 4 of the mental state measures. Interestingly, this closely followed the previous factor analysis showing these risk factors to be highly associated with one another (Figure 2.4). Finally, risk factors most predictive of psychotic-like experiences were all shared with symptoms of anxiety.

Table 2.9: Linear model regression analysis of shared risk factors across symptom-based measures of psychotic-like experiences (PLEs, PQ-16), depression, anxiety and stress (DASS-21) and poor sleep (PSQI). Numbers denote odds ratio, with brackets indicating confidence intervals.

Risk factor	PLEs	Depression	Anxiety	Stress	Poor sleep
Psychiatric diagnosis	1.83 (1.62 – 2.06)	2.24 (1.97 – 2.54)	1.61 (1.46 – 1.79)	1.77 (1.60 – 1.95)	1.30 (1.24 – 1.37)
Childhood emotional abuse	1.80 (1.53 – 2.13)	1.89 (1.57 – 2.20)	1.23 (1.10 – 1.51)	1.55 (1.35 – 1.78)	1.15 (1.06 – 1.25)
Traumatic experience	1.52 (1.32 – 1.75)	1.31 (1.14 – 1.52)	1.78 (1.05 – 1.33)	1.37 (1.22 – 1.54)	1.08 (1.02 – 1.15)
Childhood social withdrawal	1.59 (1.39 – 1.83)	-	1.26 (1.13 – 1.42)	1.28 (1.14 – 1.43)	1.12 (1.05 – 1.19)
Childhood physical abuse	1.47 (1.13 – 1.91)	1.37 (1.05 – 1.79)	1.36 (1.08 – 1.72)	1.45 (1.20 – 1.84)	-
Childhood bullying	1.22 (1.09 – 1.37)	-	1.12 (1.01 – 1.23)	1.25 (1.14 – 1.38)	1.06 (1.01 – 1.11)
Male	-	0.88 (0.78 – 0.98)	0.78 (0.71 – 0.86)	0.73 (0.66 – 0.80)	0.91 (0.87 – 0.96)
Obstetric complications	-	-	1.21 (1.08 – 1.36)	1.14 (1.02 – 1.27)	1.12 (1.06 – 1.19)
Current cannabis use	2.35 (1.58 – 3.49)	-	1.73 (1.23 – 2.42)	-	1.21 (1.02 – 1.43)
Childhood psychological abuse	-	-	1.36 (1.14 – 1.62)	-	1.13 (1.04 – 1.23)
Migrant from a risk area	1.35 (1.05 – 1.75)	-	1.25 (1.01 – 1.55)	-	-
1 st /2 nd generation migrant	0.88 (0.79 – 0.98)	-	0.91 (0.83 – 0.99)	-	-
Childhood cannabis use	-	-	-	1.25 (1.02 – 1.52)	-
Season of birth	-	-	-	1.09 (1.01 – 1.19)	-
Black ethnicity	-	-	-	-	1.55 (1.15 – 2.10)
Brain infection	-	-	-	-	-
Childhood sexual abuse	-	-	-	-	-
Head injury	-	-	-	-	-
High latitude	-	-	-	-	-
Paternal age >50	-	-	-	-	-
Urbanicity	-	-	-	-	-
Family history	-	-	-	-	-
22q11.2 Deletion Syndrome	-	-	-	-	-

2.4 Discussion

This study is the first to directly assess the interaction between risk factors for developing psychosis, psychotic-like experiences and subjective sleep disturbance. Subjective sleep disturbance was additively associated with both increasing PLEs and risk factors. Risk factors also showed a distinct pattern of co-occurrence with a subset predictive of both a greater number of PLEs, distress levels from PLEs and greater sleep disturbance. Finally, individual risk factors did not predict future changes in PLEs and sleep but both risk factors and sleep disturbance were significantly more common in individuals who showed an increase in PLEs after 12 months.

2.4.1 Risk factors associated with PLEs

Overall, risk factors were highly prevalent in this population being endorsed by up to 84% of individuals. A greater amount of PLEs were associated with cannabis use, a comorbid psychiatric diagnosis, traumatic life events, emotional abuse, physical abuse, bullying, and social withdrawal, the latter four of which were specific to childhood experiences. Further to this, these primarily psychological experiences were commonly associated with one another and reflected a more additive rather than multiplicative effect on PLEs. This data are in line with previous literature associating these risk factors with the development of psychotic disorders²⁰ and PLEs¹⁴ and provides one of the few studies assessing their complex interactions. These findings highlight the importance of assessing a multitude of risk factors which may be collectively implicated in the development of psychosis and are often overlooked when assessing at risk states.

One of the key findings from the current data was the association of PLEs with various risk factors related to childhood adversity including abuse, bullying and social withdrawal. Further, not only were these risk factors related to PLEs, but they also commonly co-occurred with one another. Previously it has been shown that a broad range of childhood

adversities (CAs) including physical abuse, sexual abuse, parental death, and bullying increase the risk for developing psychosis, regardless of the nature of the adversity²⁷, and these have also been related to PLEs²²⁸. A study on over 9000 adults identified a high co-occurrence of a range of childhood adversities with an average of 3.2 CAs in individuals reporting more than 1 CA²²⁹. Interestingly, a factor analysis used in this study also identified a 'maladaptive family functioning' (MFF) as the strongest cluster of CAs which included parental mental illness, criminality, family violence and childhood abuse. These childhood adversities were unspecific and associated with a wide range of psychiatric disorders. The current findings could similarly suggest that CAs collectively arise from MFF which may also make individuals more vulnerable to other risk factors such as bullying at school, social withdrawal and psychiatric diagnoses. Further to this, the current study showed no dose-response relationship between PLEs and the frequency of childhood abuse suggesting any degree of exposure could have substantial implications on the development of PLEs. Overall these findings provide important insight into the potential developmental trajectory of psychosis and highlight areas for targeted intervention. Specifically, prevention of such childhood exposures and implementing family intervention through targeting at risk families (e.g. parents who have a serious mental health problem) could have crucial effects on childhood development and mental health.

Regular cannabis use was identified as having the strongest association to PLEs with an adjusted odds ratio of 2.35. This finding was also dosage-dependent and in line with previous studies showing a significant effect for having ever tried cannabis (OR=2.23²³⁰) and for cannabis dependence (OR=2.94²¹). Furthermore, in the latter study, cannabis dependence had one of the strongest effects on PLEs in comparison to other risk factors which included dependence on other drugs. Interestingly in the present study, despite cannabis use showing the greatest association to PLEs, it was relatively independent of the other risk factors showing only a weak co-occurrence with a psychiatric diagnoses and family history. This association could be explained since cannabis dependence has been

shown to be a heritable trait²³¹ and cannabis use has also been associated with non-psychotic disorders^{232,233}. Although the causative relationship between cannabis and the development of psychosis remains undetermined, cannabis has been shown to reduce dopamine synthesis and increase sensitivity of the D₂ dopamine receptor which could impact psychotic symptoms^{234,235}. Furthermore, cannabis can directly induce transient psychotic symptoms⁴². The cannabis users in the current study may therefore represent a sub-group of individuals at greater risk for developing psychosis. Since there was little evidence for cannabis use on future changes in PLEs, further investigation using longer time windows is necessary to identify whether there is a causal relationship and how this association may affect the outcome of a clinical psychotic diagnosis.

2.4.2 Interactions between risk factors

Previous studies that have looked into interactions between risk factors have provided evidence that the presence of two risk factors can increase the likelihood of psychotic symptoms to a greater degree than if the risk factors were acting independently. Harley *et al* identified a greater than additive effect for the presence of both childhood trauma and cannabis use on clinical psychotic symptoms²³⁶. As Harley *et al* show, the presence of both risk factors produced a far greater effect (OR=20.9) than each risk factor alone (cannabis use only, OR=1.9; trauma only, OR=2.6) and that would be expected if they were both present but acted independently. Zammit *et al*²³⁷, examined outcomes between five risk factors on non-affective psychosis diagnosis identifying support for multiplicative interactions in 8 out of the 10 models used, including cannabis use and psychiatric comorbidity. Results from the current study provided little evidence that endorsing multiple risk factors had greater than additive effects on the frequency of PLEs which suggests these risk factors are not as influential on one another as expected. However our findings did reveal that many of these risk factors commonly co-occur and the additive effect could still be detrimental to developing PLEs and psychosis. Since both of the previous studies

looked into the effects on clinical outcomes, it is possible the present findings were unable to distinguish these effects on non-clinical PLEs in the general population. Indeed, the number of PLEs may not be the best representation of psychosis risk which could for example be more dependent on distress levels. Alternatively, since these previous studies only assessed two risk factors at any one time, it is possible the assessment of a large number of risk factors could explain any greater than additive effects. For example, endorsing both cannabis use and childhood trauma may be associated with also having another risk factor such as social withdrawal. A more directed hypothesis based approach, selecting individuals with and without specific risk factor combinations would be required to fully delineate these findings and the interaction between risk factors specific to PLEs.

2.4.3 Risk factors are associated with sleep disturbance

Sleep disturbance showed a significant positive correlation to both the number of PLEs and risk factors for developing psychosis. Specifically, greater daytime dysfunction from poor sleep, longer sleep onset latencies, greater insomnia severity, and later mid-sleep points indicative of a later circadian phase, had the strongest association. Furthermore, poorer sleep quality was still associated with a higher amount of risk factors in individuals with a low amount of PLEs, and having both a high amount of risk factors and PLEs resulted in the greatest sleep disturbance. This data provides the first evidence that sleep disturbance is not only associated with psychotic-like experiences, but is also associated with specific risk factors implicated in the development of psychosis.

In line with these findings, a recent large population based cross-sectional study encompassing data from over 260,000 individuals identified that general sleep problems including problems falling asleep and night awakenings were associated with a 2.41 times higher odds of having a PLE¹⁴⁴. Importantly this was observed across almost all of the 56 countries assessed covering multiple continents and middle to low income countries

providing immense support of a global relationship between sleep problems and PLEs. In another survey based study within a student population, Sheaves *et al.*, similarly identified increased rates of insomnia, nightmares and circadian phase delay in individuals experiencing a broad range of psychiatric symptoms that included hallucinations and paranoia²³⁸. Since sleep disturbance and psychosis commonly co-occur and are hypothesised to share underlying neuropathologies, it could be expected that risk factors for psychosis also interact with sleep and circadian physiology¹⁰⁹. Limited studies however have directly assessed these predictions. Taylor *et al* collected data from a sample of approximately 5000 twin pairs and similarly identified that both sleep disturbance and PLEs were influenced by genetic (correlation of 0.54) and environmental (correlation of 0.24) factors¹⁷³. However, specific factors contributing to this environmental risk were not assessed. No other studies have directly assessed shared risk factors between sleep disturbance and psychosis. Nevertheless, the current findings further support the involvement of risk factors in the development of sleep disturbance and add to the growing literature on shared pathways between sleep and psychosis. Assessing this relationship using objective measures in individuals with a high load of risk factors is an essential next step for establishing whether they are implicated in the development of sleep disturbance (see Chapter III).

2.4.4 Longitudinal associations between risk factors, sleep disturbance and PLEs

In the current study, there was little evidence to support a prediction of future increases in PLEs or sleep disturbance from the presence of specific risk factors. The small sample size upon follow-up (N=501), the assessment of changes in PLEs rather than psychotic symptoms, and the small amount of changes in PLEs and sleep that can be expected after only 1 year, could have prevented the finding of a significant association. Nevertheless, a greater total number of risk factors was identified in people who screened positive for

clinical risk of psychosis after 12 months compared to consistently negative screens suggesting a potential collective predictive power. Furthermore, the main effects of risk factors on both PLEs and sleep disturbance still remained for this follow-up data.

Several prospective studies have identified associations between risk factors and future psychosis including childhood adversities²⁷, ethnic minorities²⁴⁰ and cannabis use²⁴¹. Although a paucity of research exists, there is also some evidence to suggest shared risk factors for future sleep problems. Singareddy *et al.*, conducted a general population prospective study into risk factors predictive of future incident chronic insomnia¹⁷¹. In this study, insomnia was associated with an array of risk factors including gender, ethnic minorities and social introversion, also known to be associated with schizophrenia. Retrospective studies have also identified a significant association between physical, emotional and sexual childhood abuse as well as substance abuse and victimisation as risk factors for significant sleep problems^{242,243}. The current finding that individuals with risk factors already show changes in sleep and mental state, given that risk factors are commonly already present by childhood, provides valuable insight for predicting psychosis risk and effects on physiology. This may allow detection and intervention at earlier stages of disease progression; potentially before even PLEs arise.

Although the sample size was small, an additional finding of this study was that sleep disturbances were significantly greater in individuals who later developed a clinically relevant level of PLEs compared to individuals who maintained low levels of PLEs across time. This finding provides some evidence that sleep disturbances could occur before the development of symptoms. Previous studies have shown that sleep disturbance predicts relapse in psychotic disorders, and increasing evidence supports the presence of sleep disturbance in individuals at ultra-high risk^{142,147,197}. If this can be extended to individuals developing PLEs, there is substantial implication for preventative strategies. For example, since the presence of PLEs and sleep disturbance is common within the general

population, disseminating a wider awareness of the importance of sleep and tips for better sleep hygiene (habits for improving sleep) from general practitioners or even news articles could help alleviate such problems at a population level.

2.4.5 Overlapping symptoms and shared pathways

The causality and underlying mechanisms between risk factors, psychosis, and sleep disturbance requires substantial further investigation. A significant point of consideration for this is the vast overlap and comorbidities for other mental health problems. All of the risk factors identified to be associated with PLEs in this study were also associated with other symptomatic measures including depression, anxiety and stress. Both psychotic disorders and sleep disorders have known associations with depression and anxiety disorders, and clinical definitions commonly involve overlapping symptomatology. Moreover, studies have also identified symptoms of anxiety and depression as risk factors for the development of psychosis and sleep disturbance including insomnia^{171,244,245}. Collectively this data highlights the tight interplay between risk factors, sleep disturbance and broader psychiatric symptoms. These findings emphasise the necessity to encapsulate these broad constructs or rather hone direction towards particular behavioural and physiological phenotypes over multifaceted clinical definitions.

2.4.6 Risk factors unassociated with PLEs

Although all risk factors assessed in this study have been associated with psychotic disorders, several risk factors including family history, obstetric complications and urbanicity were not associated with PLEs in the current sample. Similarly, previous population studies on socio-demographic factors associated with PLEs have also identified no such relationship to family history²⁴⁶, urbanicity²¹, black ethnicity^{21,193,195}, advanced paternal age^{195,246} or winter and spring births¹⁹⁵. A possible explanation for

these findings is that weaker associations between certain risk factors and psychosis may limit their capacity as markers at the level of psychotic-like experiences. As an example of this, season of birth has been shown to have a significant but weak association to schizophrenia with an odds ratio of 1.1²¹⁸. In comparison, traumatic experiences may have an effect of up to 3.2²¹⁹. Since the population of individuals that PLEs capture will be much larger and unspecific compared to those who actually develop a disorder, a significant relationship to these weakly associated risk factors may remain undetected. Alternatively these risk factors may simply have no association to the number or distress levels from PLEs and only to the presence of psychotic symptoms where some neuroanatomical change may have occurred²⁴⁷. Despite these possibilities, several studies have found significant associations between these risk factors and PLEs, including family history^{195,248}, obstetric complications²⁴⁹, black ethnicity^{250,251}, urbanicity^{193,252} and childhood sexual abuse²⁵³. Further explanations for these inconsistencies could be down to the use of assessing an extensive range of risk factors in the current study, where their close co-occurrence to one another may have masked the effect of certain risk factors on PLEs. For example 18 out of the 23 risk factor variables were grouped in the factor analysis, including several childhood adversities which could have a greater impact on PLEs and therefore masked the association between PLEs and sexual abuse. Alternatively, for certain risk factors, including black ethnicity, brain infection, and sexual abuse, the low prevalence, below 3%, may have prevented a statistically significant association. However perhaps a more likely explanation is that the lack of association was due to the different assessment methods used compared to previous literature. Two examples of this include data on obstetric complications which are often taken from medical birth records²⁹, and data on urbanicity, which is often based on specific objective criteria²⁵⁴. In the present study, subjective and retrospective methods were instead used, to allow feasibility in assessing these risk factors through an online questionnaire.

Also of note, migration from any background has been associated with psychosis, although evidence tends to support a greater effect more specifically to ethnic minorities and particularly migrants of black Caribbean descent²¹³. The negative finding identified in the current study between migration and PLEs may therefore not be as unexpected since this risk factor encompassed all migrant backgrounds, and a significant positive effect was found when specifically selecting migrants from areas of black ethnicity. The negative association, which was driven by first rather than second generation migrants, may instead reflect some indirect protective factor associated with a drive to migrate, such as improved employment opportunities.

A final point to highlight is that all risk factors that were related to the number of psychotic-like experiences in this study were also related to higher distress levels from these experiences. Further, childhood psychological abuse, obstetric complications and a brain injury were also associated with increased distress from PLEs, but not with an increased number of PLEs. Since PLEs exist along a continuum within the general population and do not necessarily reflect clinical risk, identifying distress levels from these experiences may be a more informative measure¹⁸. For example, in a recent study Kline *et al* used a distress scale into the Prodromal-Questionnaire which improved prediction of individuals at clinical high-risk for psychosis²⁵⁵. Future work should therefore aim to incorporate distress levels in PLE assessments which may further help to establish the association between risk factors and the risk for developing psychosis.

2.4.7 Limitations

Several limitations to this study should be addressed. Firstly, this study aimed to assess the general population. However a large proportion of the sample were students and female and the level of sleep disturbance and psychiatric symptoms may not be representative. For example, Doi *et al* identified a PSQI score of more than 5 in 29% of a

general population sample of 1871 individuals compared to 42% in the current population²⁵⁶. Expanding the catchment area away from a university central town could have improved the sample variance. However even if symptoms were higher in this population, this research still provides valuable insight into how risk factors are associated with sleep disturbance and PLEs which could be easier to detect in less healthy individuals. Two further limitations as mentioned previously include the short longitudinal assessment (although the study is expected to run for 5 years) and the potential inadequacy to assess certain risk factors or symptoms in an online questionnaire. The use of objective assessments could solve this issue (as used in Chapter III) however the methods of this study were necessary for gathering data from a large group of individuals.

2.4.8 Summary of findings

The primary finding that subjective sleep disturbance was associated with both psychotic-like experiences and risk factors provides the first direct evidence that risk factors could be implicated in the development of sleep disturbance. Secondly, specific risk factors commonly co-occurred. Their relationship to PLEs and sleep disturbance highlights both the collective importance of risk factors but also the specificity of particular domains such as childhood adversity which should be addressed further for intervention strategies and tackling disease progression at an earlier stage. Whether sleep and circadian disturbance manifests at an objective physiological level in individuals with a high load of risk factors and how this may relate to clinical risk and future mental health outcomes poses a compelling question that remains to be determined.

Chapter III: Phenotyping sleep and circadian rhythms in individuals at high risk for developing psychosis

Contents

3.1 Introduction	86
3.1.1 SCRD in at risk states	86
3.1.2 A common link between SCRD and psychosis?	86
3.1.3 Study aims	88
3.2 Methodology	88
3.2.1 High and low risk selection criteria	88
3.2.2 Study participants	89
3.2.3 Experimental design	90
3.2.4 Assessments	90
3.2.5 Data analysis	94
3.3 Results	99
3.3.1 Demographics and recruitment characteristics	99
3.3.2 Mental state	100
3.3.3 Sleep profiles	101
3.3.4 Circadian profiles	106
3.3.5 HPA Function	111
3.4 Discussion	112
3.4.1 High risk individuals show distinct circadian phenotypes	112
3.4.2 High risk individuals show subjective but not objective sleep disruption	115
3.4.3 High risk individuals show subtle changes in spindle activity	118
3.4.4 High risk individuals and HPA function	119
3.4.5 High risk individuals as a transdiagnostic group	120
3.4.6 Limitations	121
3.4.7 Summary of findings	122

3.1 Introduction

3.1.1 SCRD in at risk states

Little is known about the temporal relationship between sleep and circadian rhythm disruption (SCRD) and psychosis and few studies have assessed SCRD in individuals at high risk for developing psychosis. Familial high risk studies are limited but have indicated that relatives of individuals with schizophrenia show reductions in slow-wave sleep (SWS) and decreased sleep spindle density^{137,198,257}. Studies on individuals at Ultra-High Risk (UHR) have also identified subjective and objective sleep and circadian disturbances^{140–142,197,258}. However, further investigation is essential to replicate and expand on this work. For example, currently only one study has assessed electroencephalographic (EEG) measures in UHR individuals¹⁴⁰ and no studies have assessed sleep spindle dynamics in these individuals. Further, although abnormalities in chronotype, objectively assessed through melatonin profiles, have been identified in patients with schizophrenia¹¹⁹, it is unknown whether such differences may also occur in individuals at risk.

3.1.2 A common link between SCRD and psychosis?

Increasing evidence has shown that SCRD and psychosis may have common underlying mechanisms with shared risk genes that lead to changes in neuronal transmission and consequent dysfunction^{109,117}. Given that environmental risk factors (ERFs) are strongly implicated in the development of psychosis, it is plausible that they also influence sleep and circadian disturbance. As shown in Chapter II, several genetic and environmental risk factors were associated with both sleep disturbance and increased psychotic-like experiences (PLEs, sub-clinical symptoms of psychosis) in a general population of individuals. Combining methods from familial risk, based upon genetic risk factors, ultra-high risk, based upon symptomatic factors, and adding ERFs could thus provide a powerful tool for identifying at risk groups. Furthermore assessing sleep and circadian

rhythms in these individuals could provide additional insight into not only the extent of SCRD in at risk populations but also potential shared pathways.

Through what mechanisms could risk factors impact upon SCRD and psychosis? Although unclear, genetic factors may moderate the sensitivity of neural cells whereas environmental factors are largely thought to enhance stress signalling pathways which can then lead to impaired neuronal responsiveness (see Chapter I, section 1.1.4, pg12)^{32,33}. The hypothalamus-pituitary-adrenal (HPA) axis is a major endocrine system involved in many physiological and homeostatic processes including the response to stress. The glucocorticoid cortisol is the primary output of the HPA axis and plays a crucial role in mediating physiological responses to stressors²⁵⁹. Cortisol secretion is also under circadian regulation with a distinct diurnal rhythm that peaks in the morning and is lowest during the first half of the night. Within the first 30 minutes of awakening, cortisol levels also show a second peak known as the cortisol awakening response (CAR) which is thought to be a reliable measure for HPA axis function²⁵⁹. Conversely the CAR has been shown to be associated with sleep disturbance, and is blunted in patients with insomnia, schizophrenia and UHR individuals^{260–262}. This could overall reflect a reduced responsiveness to stress due to a hyperactive HPA axis. Whether abnormalities are associated with a high load of risk factors has yet to be determined but could provide further insight into the pathways leading to sleep disturbance and psychosis.

Further to this, it has recently been shown that the interaction between risk factors, sleep disturbance, and PLEs are influenced by other symptomatic domains including negative affect such as depression and anxiety¹⁷³. Personality traits such as schizotypy or neuroticism, and dissociative experiences have also been associated with risk factors, sleep disturbance, and the increased risk for psychosis^{263–267}. Investigating broader constructs of mental state and physiology is therefore necessary for understanding the specificity of this interaction. Collectively, ‘phenotyping’ individuals in at risk states using

a range of physiological, behavioural and psychological measures could provide invaluable insight into how risk factors, SCRD and changes in mental state mediate the onset of psychosis.

3.1.3 Study aims

Using a broad range of subjective and objective measures, the primary aim of this study was to phenotype sleep and circadian rhythms in individuals at high risk for developing psychosis based upon having both a high load of risk factors and sub-clinical psychotic symptoms. A secondary aim was to investigate additional correlates including negative affect, personality and HPA function to further delineate the relationship between risk factors, sleep, and psychosis.

3.2 Methodology

3.2.1 High and low risk selection criteria

Two groups of participants were selected and invited to partake in this study from a database of 1898 individuals of the general population who had previously participated in an online survey (see Chapter II). The high and low risk groups, individuals predicted to be at highest/lowest risk for developing psychosis in this population, were selected based upon both the total amount of risk factors for psychosis, and the number of psychotic-like experiences endorsed. Since psychotic disorders are associated with high comorbidity and overlapping symptoms to many other psychiatric disorders, individuals were additionally assessed for levels of depression, anxiety and stress covering general Axis I disorder symptomatology²⁰⁷. Finally help-seeking behaviour was also assessed to identify heightened distress levels.

To select individuals, symptoms of depression, anxiety and stress were assessed by the DASS-21 questionnaire and individuals were given a binary score based on severity, with moderate or more (DASS-21 score of >9 , >9 or >18 respectively) having a score of 1. Help-seeking behaviour was given a binary score with a score of 1 if individuals were seeking help for a psychotic-like experience from either counselling, therapy or a general practitioner. The binary scores for depression, anxiety, stress and help seeking were then summed with the total number of risk factors reported to produce a composite score. High/low risk participants were selected from the top/bottom 15% of this composite score from the collective database sample (N=1898). In addition high/low risk participants had to also score at least/less than 5 on the prodromal questionnaire 16 to assess the level of sub-clinical psychotic symptoms. High risk participants were thus selected for a high load of risk factors, sub-clinical psychotic experiences and general negative affect. Full details of the data obtained from participants from this survey, additionally including the Pittsburgh Sleep Quality Index (PSQI), Sleep Condition Indicator-2 (SCI-2) and Munich Chronotype Questionnaire (MCTQ) can be found in Chapter II Section 2.2.2, pg51.

3.2.2 Study participants

Individuals aged between 18-30 years were recruited to either the high risk group or low risk group. Participants were excluded if they had travelled across more than one time zone in the past two weeks, were pregnant, had epilepsy, or had taken any medication for a mental health problem (including sleep problems) within the last 3 months. Low risk participants were excluded if they had any history of mental health disorders. High risk participants were excluded if they had any history of a diagnosis of a psychotic disorder or psychotic episode, including bipolar disorder and schizophrenia. None of the participants were taking any medication (with the exception of the contraceptive pill). All participants provided written informed consent and received an honorarium for their time.

The study was conducted in accordance with the Declaration of Helsinki and approved by the North West Liverpool Central NHS Research Ethics Committee (REC: 14/NW/1142).

3.2.3 Experimental design

The 21 day study included four sessions spaced across the first 3 days with interviews, questionnaires and cognitive testing. The first two nights involved domiciliary polysomnography (PSG), day 2 and day 3 involved a 45 minute morning saliva collection to assess cortisol awakening responses and a 48 hour urine collection to assess melatonin profiles. Actigraphy and diary keeping was recorded for the duration of the 21 days. An outline of the study protocol and timings of assessment can be found in Figure 3.1.

Time of session	Day 1	Day 2	Day 3	Days 4-21	
Morning		45min saliva collection	45min saliva collection	Actigraphy & diary keeping	
		Session 2 MST/WPL1 training	Session 3/4 MST/WPL2 testing		
			MST/WPL2 training		
Evening	Session 1 Information & consent General health questionnaire MINI interview CAARMS interview Questionnaires PSG set-up	Session 2/3 PSG set-up Resting EEG RVP task MST/WPL1 training WPL2 training	Session 4 MST/WPL2 testing		
		MST/WPL1 training			
	Night	Overnight PSG	Overnight PSG		
	All	Actigraphy start	48 hour urine collection		

Figure 3.1: Study protocol and timings. For the sleep-dependent memory consolidation (SDMC) tasks (see Chapter IV) blue indicates the AM-PM-AM group, who were first tested in the morning; green indicates the PM-AM-PM group, who were first tested in the evening. CAARMS= Comprehensive Assessment of At Risk Mental States, MINI= Mini Neuropsychiatric interview, MST= Motor Sequence Task, WPL= Word Pair Learning Task.

3.2.4 Assessments

Mental state assessments

At first admission into the study, participants were assessed on an array of mental and physical health, personality and sleep measures. A general health questionnaire was administered to record demographics, to screen for medical conditions, medication use,

caffeine and alcohol intake, stage of menstruation cycle (for females) and other factors that can affect circadian and sleep behaviour including shift work and jetlag. General mental health problems were assessed using the Mini International Neuropsychological Interview (MINI), a brief structured “yes/no” interview to screen for major Axis I psychiatric disorders in DSM-IV and ICD-10²⁶⁸. The Comprehensive Assessment of At Risk Mental States (CAARMS) was used to assess clinical risk (ultra-high risk status) for psychosis¹⁶. The CAARMS is a semi-structured interview involving a range of subscales targeting various areas of psychopathology and functioning. For the purpose of this study, only positive symptoms of psychosis were assessed. Participants were categorised as ultra-high risk with a subthreshold psychotic syndrome (with or without a drop in social functioning) if they presented symptoms but did not reach threshold levels for psychosis due to subthreshold severity or frequency. Participants were assessed on a case-by-case basis and borderline cases were discussed with the research team and a clinical psychologist. After interviews participants completed a battery of questionnaires assessing mood, personality, daily functioning and sleep.

Mood and personality: This included the Mood Disorder Questionnaire (MDQ) as a baseline assessment of manic symptomology with a positive screen scored if at least 7 symptoms have been experienced, occurring at the same time, and causing at least moderate distress²⁶⁹; 23 items of the 90 item “yes/no” Eysenck Personality Questionnaire (EPQ) to specifically assess neuroticism²⁷⁰; and the Schizotypal Personality Questionnaire Brief Revised (SPQ-BR), a 16 item scale assessing schizotypal personality traits on a Likert scale (from strongly agree to strongly disagree)²⁷¹. For both the EPQ and SPQ-BR a total score is created from the sum of all item scores.

Daily experiences and functioning: This included the Dissociative Experiences Scale (DES), to assess trait dissociative symptoms (based upon a 28 item, 0 to 100 assessment of how often they occur, with a final score given as the mean of all item scores²⁷²), the Cognitive Failures Questionnaire (CFQ) to assess absent mindedness (based upon 25 items of everyday experiences with a Likert scale of frequency from very often to never²⁷³), the Mind Wandering Questionnaire (MWQ) to assess trait mind wandering/day-dreaming behaviour (based upon 5 items with a Likert scale of frequency, from almost never to almost always²⁷⁴). For both the CFQ and MWQ a total score is created from the sum of all item scores. The Edinburgh Handedness Inventory Revised (EHI-R), an 8 item Likert scale (from always left to always right) was used to assess handedness, with a score from -100 to +100 for left to right dominance respectively²⁷⁵.

Subjective sleep: In addition to the PSQI, SCI-2 and MCTQ (see Chapter II section 2.2.2, pg51), further assessments of subjective sleep and sleep experiences were measured. This included the SLEEP-50, a 50 item Likert scale of symptom frequency to screen for various sleep disorders²⁷⁶, the IOWA Sleep Experiences Survey (ISES) an 18 item Likert scale of frequency (from never to multiple times per week) to assess unusual sleep experiences including hypnagogic hallucinations and sleep paralysis²⁷⁷. A final total score is given as the mean of all item scores, with 15 items used for general sleep experiences, and 3 items specifically for lucid dreaming. The Nightmare Frequency Questionnaire (NFQ) was used based on two questions assessing the frequency of nightmares²⁷⁸. Finally the Nightmare Distress Questionnaire (NDQ) a 13 item Likert scale of frequency was used to assess the impact of nightmares on everyday functioning²⁷⁹. For the NFQ a total score is created from the average of the item scores. For the NDS a total score is created from the sum of the item scores.

Polysomnography

Two consecutive nights of domiciliary polysomnography (PSG) was carried out on all participants (SomnoScreen+PSQ, SOMNOmedics GmbH). This recorded the brain's electrical activity (electroencephalography, EEG), muscle activity (electromyography, EMG), and eye movements (electrooculography, EOG). A standard bilateral montage of 11 channels (Fp1, Fp2, F3, F4, C3, Cz, C4, P3, P4, O1, O2) referenced to the mastoids was used. Signals were sampled at 128 Hz and digitally filtered using finite impulse response (FIR) band pass filtering between 0.2 Hz and 35Hz with a Hamming window function applied. All PSG was set up in the laboratory and data collected in the participant's home environment where they were encouraged to follow their usual daily routines. The first night acted as an adaptation night, and the data were extracted and analysed from the second night's recording²⁸⁰.

Actigraphy

The sleep-wake cycle and light exposure of each participant was simultaneously monitored for a period of 3 weeks using an actiwatch with an integrated light sensor (AWL; CamNtech Ltd, Cambridge, UK) worn on their non-dominant wrist. For the duration of actigraphic monitoring, participants kept a standardized diary of daily bed times, get-up times and daytime activities including meal times, caffeine intake and periods of high activity (e.g. exercise) and low activity (e.g. reading). The consistency and accuracy of diary entries was monitored approximately half-way through the study with engagement and inspection of actigraphy data with the participant.

Cognitive tests

Two measures of sleep-dependent memory consolidation and one measure of sustained attention were taken. The results will be reported and discussed in Chapter IV.

Cortisol awakening response

The cortisol awakening response (CAR), was recorded from salivary cortisol levels²⁵⁹. Sampling was carried out on the first two mornings of the study, after overnight PSG in participant's home environment under normal routine. For each morning, saliva was collected at four time points: immediately after waking then 15, 30 and 45 minutes after waking. Throughout this period, participants were asked to maintain low activity levels, not to eat, drink, brush their teeth, smoke or consume alcohol the night before.

Melatonin rhythms

A two day profile of 6-sulphatoxymelatonin (aMT6s, a metabolite of melatonin) was taken to estimate the circadian period and phase of the participant's circadian pacemaker. Each participant was asked to pass a full volume of urine into a bottle as often as needed (approximately every 4 hours, or 8 hours when asleep) over a 48 hour period and noted the date and time of collection. The volume of each bottle was recorded and two 5ml aliquots of each sample were stored at -20°C. Participants were given full instruction on how to collect the urine, measure the volume and aliquot the two samples.

3.2.5 Data analysis

Actigraphy analysis

One participant was removed from actigraphy analysis due to insufficient wearing of the actiwatch. Actigraphy data were sampled in 1 minute epochs and analysed with version 1.1.15 of the CamNtech MotionWare software (CamNtech Ltd.). Rest-activity patterns were annotated using diary data and a "bed time" and "get-up time" were manually entered with "sleep start" and "sleep end" calculated automatically using medium sensitivity. Recordings were adjusted for daylight saving time by adding or removing an hour (based on whole day average) of data during the sleep period of the relevant night where

applicable. If the watch had been removed for more than half an hour but less than three hours, the data was edited and replaced with average activity count for that day. Full days were excluded if the watch had been taken off for more than three consecutive hours. Average sleep parameters were calculated by averaging across days whereas variability in sleep parameters was calculated by taking the standard deviation across days. A non-parametric circadian rhythm analysis was also performed using the MotionWare software to calculate an average 24 hour profile from an overlay of each day from midnight to midnight. A minimum of two consecutive days were required for this analysis and a weighted average was performed across each 'block' of days if not continuous. Details of the actigraphic sleep and circadian parameters are shown in Table 3.1.

Table 3.1: Actigraphic sleep and circadian parameters. Analysis provided by CamNtech MotionWare software.

Sleep parameter	Calculation
Total sleep time (TST)	Total time spent asleep (actual sleep time)
Sleep efficiency (SE)	Actual sleep time expressed as a percentage of time in bed
Sleep onset latency (SOL)	The time from bed time to sleep start
Fragmentation index	The degree of fragmentation of the sleep period providing an indication of sleep quality and based upon the sum of activity time and immobile bouts less than/equal to one minute during the sleep period.
Interdaily stability (IS)	The degree of regularity in the activity-rest pattern scored between 0 to 1. A value of 0 indicates a complete lack of rhythm, a value of 1 indicates a perfectly stable rhythm
Intradaily variability (IV)	The degree of fragmentation of activity-rest periods scored between 0 to 2. The higher score indicates higher fragmentation
L5 counts	The average activity level for the least active 5 hours of the day
M10 counts	The average activity level for the most active 10 hours of the day
Mid sleep point corrected for oversleep (MSFsc)	Provides an indication of chronotype. Please see Chapter II section 2.2.2 for further details. For this calculation, work days included Monday, Tuesday, Wednesday and Thursday nights. Free days included Friday and Saturday nights.
Social Jetlag (SJL)	Provides an indication of the discrepancy between the sleep-wake cycle that is influenced by social and work commitments and the internal circadian clock. Please see Chapter II Section 2.2.2 for further details.

Sleep scoring and spectral analysis

PSG data were analysed using the DOMINO version 2.6.0 software (SOMNOmedics GmbH) and scored at 30 second epochs according to the American Academy of Sleep Medicine criteria with the separation of both stage 3 and stage 4 sleep to gain greater macrostructural detail⁵¹. Spectral and sleep spindle analysis was performed using

MATLAB (The MathWorks Inc, Natick, Massachusetts, USA). Power density was calculated using a Fast Fourier Transform (FFT) with a 5 second window length, 0% overlap and a frequency resolution of 0.2Hz. Artefacts were automatically removed as whole epochs using a low (0.6-4.4Hz) and high frequency (20-40Hz) moving median filter with a 10 second window length. For NREM sleep, an average of $84.42 \pm 8.63\%$ of epochs were kept after artefact removal. To compare power densities, the FFT data was binned into delta (0.6-4Hz), theta (4.2-8Hz), alpha (8.2-12Hz), sigma (12.2-16Hz), and beta (16.2-20Hz) frequency bands.

Spindle analysis

To detect sleep spindles, an automatic detection algorithm was used, a modified version as used in Chapter V, and based upon previous methodologies¹⁷⁶. Analysis was limited to artefact free NREM sleep. The raw signal was filtered using an Infinite Impulse Response (IIR) bandpass Butterworth filter between 11-16Hz. Next, the instantaneous amplitude was computed using a Hilbert transform and an upper threshold was applied to detect spindle events, with a lower threshold used to determine the start and end of the spindle event. Visual scoring of sleep spindles, which is the gold standard criteria for assessing spindle events, was carried out on 10% of the recordings (n=4) and compared with the automatic detection algorithm to determine the optimum thresholds. The upper threshold was set at the mean amplitude + 2 * standard deviation. The lower threshold was set at the mean amplitude + 1.5 * standard deviation. A maximal interruption interval of 300ms was allowed if the amplitude fell below the lower threshold and above again, ensuring a single spindle would not be detected as two. A duration threshold was also set limiting spindles to be detected between 0.5-2 seconds. Compared to visual detection, this algorithm produced a sensitivity of 78%, comparable to performance of many previously used algorithms²⁸¹. Further, a previously published and validated algorithm²⁸¹ was also applied to the visually scored sample producing a poorer sensitivity of 66%. Spindle characteristics were

determined as follows: Amplitude, maximum amplitude within the spindle event; Duration, the difference between spindle start and end; Density, number of spindles per minute of NREM sleep; Frequency, number of amplitude peaks within the spindle event divided by the duration. Both spindle detection and spectral analysis was carried out on electrodes Fp2, F4, C4, P4 and O2.

Cortisol analysis

A radioimmunoassay of cortisol using ^{125}I cortisol was performed by Stockgrand Ltd (University of Surrey). To calculate the cortisol awakening response the area under the curve with respect to increase (AUC_i) was used since it emphasises changes over time and sensitivity of the response²⁸². The AUC_i was computed using the following formula:

$$\text{AUC}_i = \left(\sum_{i=1}^{n-1} \frac{(m_{(i+1)} + m_i)}{2} \right) - (n - 1) \times m_1$$

Where m_i denotes a single measurement and n denotes the total amount of measurements²⁸³. Data from one participant was excluded due to unusable results.

Melatonin analysis

A radioimmunoassay of melatonin sulphate aMT6s using AN^{123} as a labelled tracer was performed by Stockgrand Ltd (University of Surrey)²⁸⁴. As used in previous studies, a cosinor analysis was carried out to determine melatonin sulphate rhythms^{285,286}. Using a Microsoft Excel macro, a cosine function was applied based on the least square approximation of the time series with a period of 24 hours. This provided the following parameters: acrophase time, the time of aMT6s concentration peak taken as the fitted cosinor function maximum; mesor, the mean aMT6s value for all included samples; amplitude, the difference between the acrophase time and the mesor (Figure 3.2). To determine the validity of the cosinor output two measures of fit were assessed. Firstly, the

percentage of data variability accounted for by the cosine curve with 100% indicating all data points fall on the curve and 0% no data points fall on the curve. The cosine output was considered acceptable if at least 50% of the data fit on the curve. In the current sample an average of $91 \pm 6\%$ of data variability was accounted for by the cosine curve. The second measure assessed the likelihood of the data fitting a straight line rather than a cosine curve. This was expressed as a p-value with data only used if the cosinor fit was significant at the 95% level ($p < 0.05$)²⁸⁵. Data from 6 individuals were excluded for not meeting these criteria.

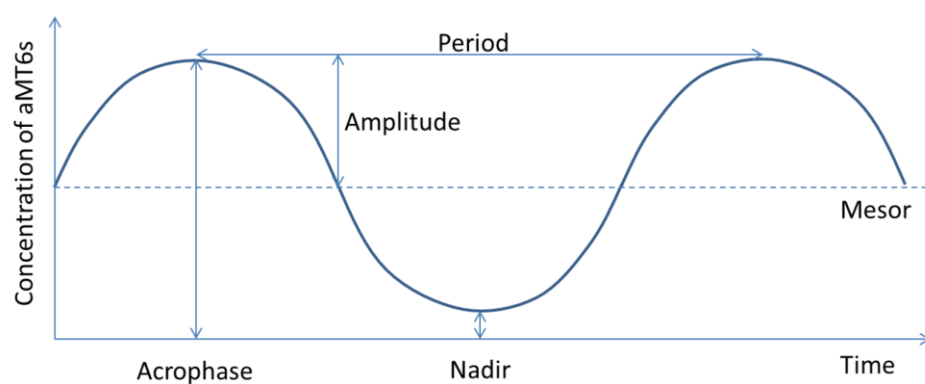


Figure 3.2: Parameters used for calculating melatonin sulphate circadian rhythm (Figure taken with permission from Dr Kate Porcheret).

Statistical analysis

All statistical analysis was computed using 'R' software²²⁷. Group differences were calculated using ANOVA, post-hoc t-tests for normal data, non-parametric Wilcoxon rank sum tests for non-normal data and Chi-squared tests for count data. For cortisol awakening response, a repeated measures analysis of variance was used to compare cortisol levels across the four time points. A linear model was fit to identify correlates of melatonin acrophase. A Bonferroni correction was applied to α -values to control for multiple testing, where appropriate.

3.3 Results

3.3.1 Demographics and recruitment characteristics

A total of 44 individuals (high risk, $n=22$; low risk, $n=22$) completed the study. Participants had a mean age of 22 ± 3 years (high risk, 22 ± 3 ; low risk 22 ± 3), 70% (31) of participants were female (high risk, 16; low risk, 15) and 68% (30) of participants were students (high risk, 13; low risk, 17). The average amount of risk factors reported in the high risk group was 6.8 ± 1.5 compared to 2.1 ± 0.9 in the low risk group. A traumatic experience, winter/spring season of birth and a family history of a psychiatric disorder were amongst the most prevalent risk factors identified in the sample (Figure 3.3a). These prevalence rates closely matched results from the previous online population based survey suggesting an accurate representation of the more general population (Figure 3.3b, see Chapter II, section 2.3.2, pg59).

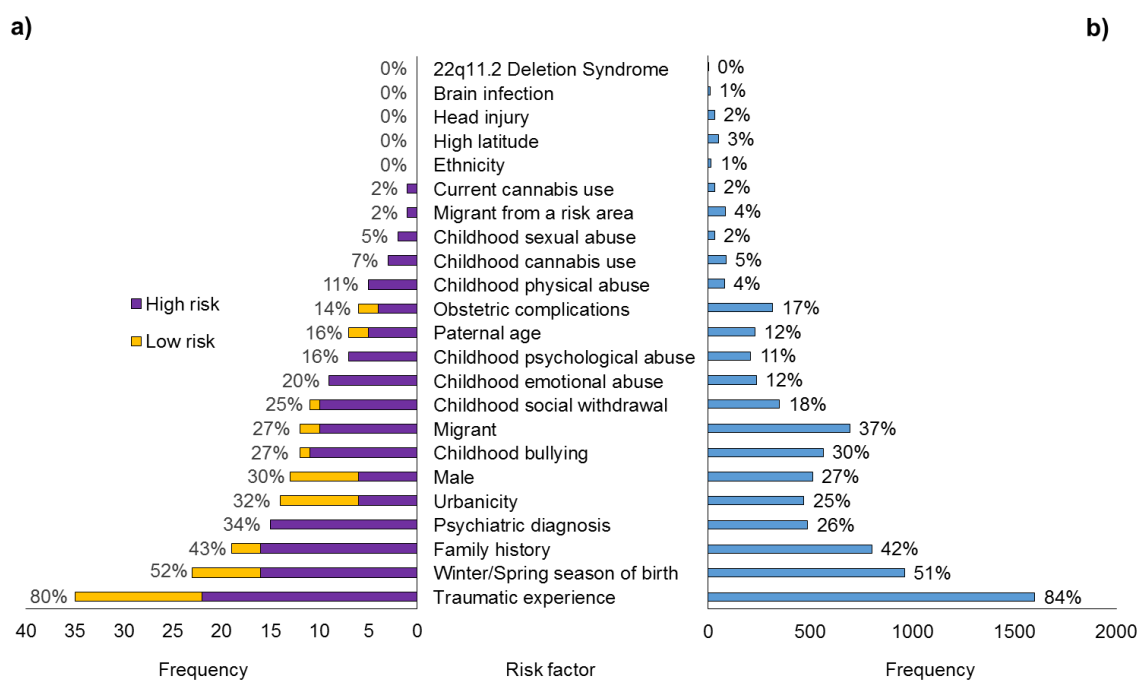


Figure 3.3: Prevalence of risk factors for developing psychosis between (a) high and low risk groups recruited into the study ($n=22$, $n=22$) and (b) all individuals who completed the online survey ($n=1898$). Note the similarities with a high prevalence of traumatic experience, winter/spring season of birth, family history and low prevalence of cannabis use, childhood physical and sexual abuse between samples.

In addition to having a significant amount of sub-clinical symptoms for psychosis, high risk individuals also reported high levels of depression anxiety, stress and help seeking behaviour (Table 3.2).

Table 3.2: Positive screens for measures of psychotic-like experiences, depression, anxiety, stress, help-seeking behaviour and number of risk factors, used to select participants. High risk, n=22, low risk, n=22.

Positive screens	High risk	Low risk
Psychotic-like experiences (PQ-16>5)	22 (100%)	0 (0%)
Depression (DASS-21>9)	18 (82%)	1 (5%)
Anxiety (DASS-21>9)	19 (86%)	2 (9%)
Stress (DASS-21>18)	20 (91%)	0 (0%)
Help seeking (yes)	14 (64%)	0 (0%)
Risk factors (mean number)	6.8±1.5	2.1±0.9

3.3.2 Mental state

The Comprehensive Assessment of At Risk Mental States (CAARMS) was used to assess the level of clinical risk for developing psychosis and the MINI neuropsychiatric interview, to assess general mental health problems. In the high risk group, 9 (41%) individuals screened positive for ultra-high risk for psychosis with a deficit in social functioning, 8 (36%) individuals screened positive for ultra-high risk for psychosis without a deficit in social functioning, and 5 (23%) individuals screened negative based on the CAARMS interview. No individuals scored positive for ultra-high risk for psychosis in the low risk group (Table 3.3). As assessed through the MINI, high risk individuals reported a significantly greater amount of total mental health problems (chi-squared, $X^2=20.46$, $p<0.001$) with anxiety being the most prevalent mental health problem (endorsed by 73% of high risk individuals). Furthermore high risk individuals scored significantly greater for measures of schizotypy, neuroticism, dissociation, cognitive failures and mind wandering (Table 3.3).

Table 3.3: Mental state measures. Data shows positive screens for the Comprehensive Assessment of At Risk Mental States (CAARMS) interview for assessing clinical risk for developing psychosis and the MINI Neuropsychiatric interview for screening of general mental health problems. Positive screens on the CAARMS in the high risk group includes both with (n=9) and without (n=8) a deficit in social functioning. A battery of self-completed questionnaires were also undertaken to assess personality, and waking experiences involved with daily functioning. N=44.

Mental health	High risk	Low risk	X ²	p
Clinical Psychosis Risk (CAARMS)	77% [17]	0% [0]	24.54	<0.001*
Total mental health problems (MINI)	86% [19]	14% [3]	20.46	<0.001*
<i>Depressive disorder</i>	32% [7]	0% [0]	-	-
<i>Anxiety disorder</i>	73% [16]	5% [1]	-	-
<i>PTSD</i>	9% [2]	0% [0]	-	-
<i>OCD</i>	14% [3]	0% [0]	-	-
<i>Eating disorder</i>	14% [3]	0% [0]	-	-
<i>Alcohol/substance dependence</i>	32% [7]	9% [2]	-	-
<i>Suicidality</i>	41% [9]	0% [0]	-	-
<i>(Hypo) Manic episode</i>	64% [14]	0% [0]	-	-
Mania (MDQ)	23% [5]	0% [0]	3.610	0.057
Personality	High risk	Low risk	W	p
Schizotypy (SPQ-BR)	67.59±12.79	24.36±13.59	480	<0.001*
Neuroticism (EPQ)	16.41±3.54	4.96±4.73	462.5	<0.001*
Daily functioning	High risk	Low risk	W	p
Dissociation (DES)	13.21±6.61	4.01±3.40	437	<0.001*
Cognitive failures (CFQ)	53.68±12.76	33.45±12.82	421	<0.001*
Mind wandering (MWQ)	21.09±4.07	16.36±4.58	372.5	0.002*
Other	High risk	Low risk	W	p
Handedness (EHI-R)	82.67±16.36	87.50±16.25	193	0.241

3.3.3 Sleep profiles

Sleep was assessed using subjective questionnaires, actigraphy and electroencephalography (EEG; Table 3.4). High risk individuals reported significantly poorer subjective sleep quality (greater global PSQI scores) and greater symptoms of insomnia (lower SCI-2 scores) with an average score of 7.00 and 3.09 respectively, indicative of both poor sleep (PSQI cut off >5) and possible insomnia disorder (SCI cut-off <4). A breakdown of the PSQI into component scores revealed high risk individuals reported significantly poorer sleep quality, longer sleep onset latencies, and greater daytime dysfunction due to sleep. High risk individuals also experienced significantly more nightmares (higher NFQ scores) but no other differences in terms of nightmare distress, unusual sleep experiences or screens for possible sleep disorders (Table 3.4). An analysis of objective sleep parameters revealed significantly greater sleep onset latency in high risk

individuals based upon actigraphy, but this did not withstand controls for multiple testing. Comparing the variability between days for the actigraphic measures (taken as the standard deviation between days) again revealed significantly greater variability in sleep onset latency in high risk individuals. No other actigraphic differences were identified between groups. Further, no differences in polysomnography were identified between groups including total sleep times, sleep efficiency, wake after sleep onset, or time spent in each sleep stage (Table 3.4).

Table 3.4: Subjective and objective sleep parameters between high/low risk groups. Data show means $\pm$ st.dev. or % [count] where relevant. *denote significant differences, with a Bonferroni corrected α for multiple testing. Underlining denotes significance at the 0.05 level but not after controlling for multiple testing.

Subjective sleep quality	High risk	Low risk	W-value	p-value
Global sleep quality (PSQI)	7.00 $\pm$ 2.16	3.40 $\pm$ 1.97	428.5	<0.001*
<i>Subjective sleep quality</i>	1.73 $\pm$ 0.77	0.59 $\pm$ 0.67	411	<0.001*
<i>Total sleep time (TST; hr)</i>	8.64 $\pm$ 0.91	8.69 $\pm$ 1.09	228.5	0.76
<i>Sleep onset latency (SL; min)</i>	44.77 $\pm$ 34.96	23.00 $\pm$ 26.42	372	0.002*
<i>Sleep efficiency (SE; %)</i>	95.58 $\pm$ 5.01	97.62 $\pm$ 3.08	171	0.09
<i>Daytime dysfunction</i>	1.73 $\pm$ 0.88	0.64 $\pm$ 0.66	398	<0.001*
Sleep condition indicator-2 (SCI-2)	3.09 $\pm$ 2.20	6.91 $\pm$ 1.82	51	<0.001*
Subjective sleep experiences	High risk	Low risk	W/X ² -value	p-value
Nightmare frequency (NFQ, per month)	2.99 $\pm$ 4.17	0.59 $\pm$ 0.75	362	0.005*
Nightmare distress (NDS)	7.86 $\pm$ 9.59	2.55 $\pm$ 4.93	309	0.092
Unusual sleep experiences (IOWA)	1.75 $\pm$ 1.78	1.14 $\pm$ 1.35	290	0.233
Total sleep 'disorders' (SLEEP-50)	59% [13]	23% [5]	4.61	<u>0.032</u>
<i>Sleep apnoea</i>	50% [11]	5% [1]	-	-
<i>Insomnia</i>	50% [11]	5% [1]	-	-
<i>Narcolepsy</i>	64% [14]	27% [6]	-	-
<i>RLS</i>	41% [9]	9% [2]	-	-
<i>CRSD</i>	23% [5]	0% [0]	-	-
<i>Affective disorders</i>	23% [5]	0% [0]	-	-
<i>Hypersomnia</i>	5% [1]	5% [1]	-	-
Actigraphy sleep parameter	High risk	Low risk	t-value	p-value
Total sleep time (TST; hr)	6.86 $\pm$ 0.77	6.79 $\pm$ 0.63	0.34	0.74
Sleep onset latency (SOL; min)	9.44 $\pm$ 4.21	6.94 $\pm$ 2.58	2.36	<u>0.02</u>
Sleep efficiency (SE; %)	81.76 $\pm$ 4.01	81.94 $\pm$ 4.84	-0.13	0.90
Wake after sleep onset (WASO; min)	81.04 $\pm$ 22.65	82.01 $\pm$ 26.03	-0.13	0.90
Fragmentation index (FI)	28.01 $\pm$ 5.82	27.86 $\pm$ 6.98	0.08	0.94
<i>Sleep variability (St.Dev between days)</i>				
<i>Total sleep time (TST; hr)</i>	1.27 $\pm$ 0.44	1.06 $\pm$ 0.53	1.38	0.18
<i>Sleep onset latency (SL; min)</i>	11.31 $\pm$ 4.38	6.77 $\pm$ 3.48	3.77	<0.001*
<i>Sleep efficiency (SE; %)</i>	4.41 $\pm$ 1.41	3.88 $\pm$ 1.23	1.32	0.19
<i>Wake after sleep onset (WASO; min)</i>	28.09 $\pm$ 10.35	25.77 $\pm$ 10.94	0.71	0.48
<i>Fragmentation index (FI)</i>	8.84 $\pm$ 1.85	8.18 $\pm$ 2.42	1.01	0.32
EEG sleep parameter	High risk	Low risk	t-value	p-value
Total sleep time (TST; hr)	6.80 $\pm$ 1.21	6.56 $\pm$ 1.03	0.71	0.48
Sleep onset latency (SOL; min)	18.90 $\pm$ 15.73	16.26 $\pm$ 18.17	0.52	0.61
REM onset latency (ROL; min)	81.41 $\pm$ 38.90	84.39 $\pm$ 28.10	-0.29	0.77
Sleep efficiency (SE; %)	90.97 $\pm$ 6.53	91.66 $\pm$ 4.51	-0.41	0.69
Time in stage 1 (min)	21.20 $\pm$ 13.79	26.33 $\pm$ 11.57	-1.34	0.19
Time in stage 2 (min)	189.36 $\pm$ 44.03	181.66 $\pm$ 44.66	0.58	0.57
Time in stage 3 (min)	43.00 $\pm$ 16.38	41.23 $\pm$ 19.21	0.33	0.74
Time in stage 4 (min)	59.91 $\pm$ 29.21	55.61 $\pm$ 30.06	0.48	0.63
Time in REM (min)	94.31 $\pm$ 28.64	88.50 $\pm$ 21.50	0.76	0.45
Wake after sleep onset (WASO; min)	20.43 $\pm$ 15.29	19.15 $\pm$ 10.34	0.33	0.75
Number of sleep stage changes (/hr)	15.67 $\pm$ 4.33	18.81 $\pm$ 4.37	-2.40	<u>0.02</u>

To assess microstructural EEG differences, a further analysis of power density and sleep spindles was conducted. Using a Fast Fourier Transform (FFT), power densities for delta, theta, alpha, and sigma frequency bands were calculated during NREM sleep revealing no differences between high and low risk groups (Figure 3.4a, Table 3.5). Despite this, a slight non-significant shift in power was identified within the sigma frequency range (11-16 Hz) where high risk individuals presented higher power at higher sigma frequencies. Since sigma frequency power does not separate sleep spindle events from general background sigma frequency activity, an automatic detection algorithm was next used to specifically detect spindle events. This analysis revealed a small yet significant increase in spindle frequency in high risk individuals ($t=2.32$, $p=0.03$) which closely matched the identified shift in sigma power. No differences in average spindle density, amplitude, duration or total number was identified between groups (Figure 3.4b, Table 3.5). All results were calculated from a central C4 channel, with similar findings observed for P4 and no differences identified for Fp2, F4 and O2 (see Supplementary Table S4).

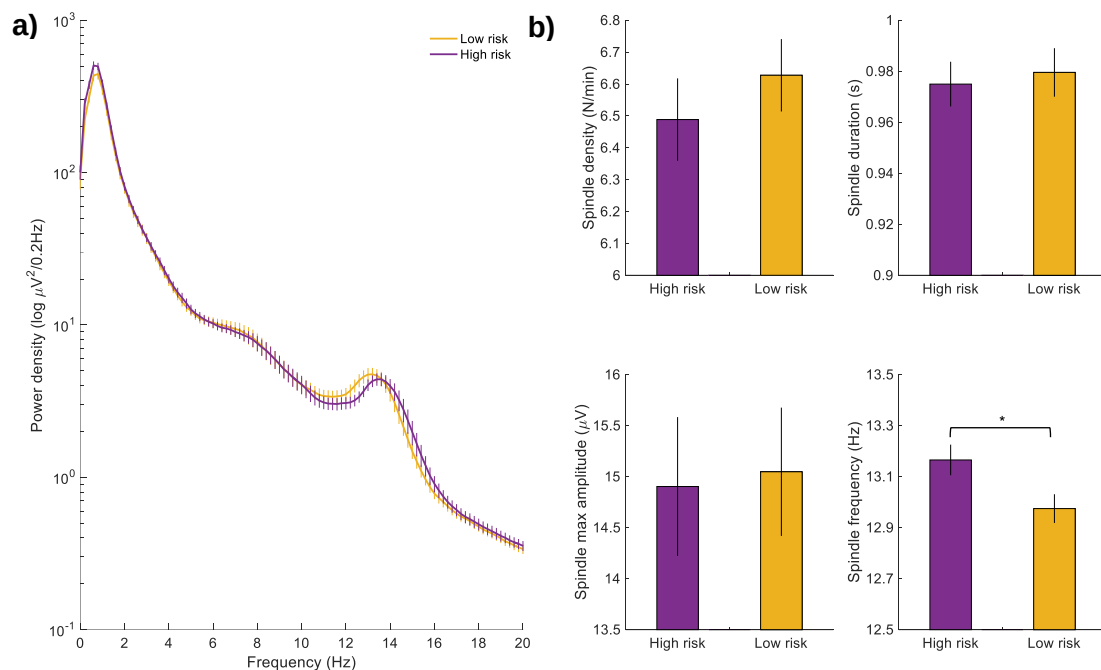


Figure 3.4: a) Power density during artefact-free NREM sleep (0.2Hz resolution). b) Average density, duration, amplitude and frequency of spindles during NREM sleep. Data is from central channel C4. Bars denote S.E.M. High risk, $n=22$; low risk, $n=22$.

Table 3.5: Group differences in average spectral power density and spindle characteristics from central channel C4 during NREM sleep. High risk, n=22; low risk, n=22.

Parameter	High risk	Low risk	t	p
Power density				
<i>Delta power (0.6-4Hz)</i>	142.20±31.06	130.30±43.98	1.04	0.31
<i>Theta power (4.2-8Hz)</i>	10.96±3.34	10.92±4.12	0.04	0.97
<i>Alpha power (8.2-12Hz)</i>	4.34±2.38	4.50±2.36	-0.21	0.83
<i>Sigma power (12.2-16Hz)</i>	2.94±1.35	2.94±1.19	-0.00	1.00
<i>Beta power (16.2-20Hz)</i>	0.51±0.21	0.49±0.17	0.45	0.65
Spindle characteristics				
<i>Total number</i>	1548±304	1551±295	-0.03	0.98
<i>Density (N/min)</i>	6.49±0.61	6.63±0.53	-0.81	0.42
<i>Duration (s)</i>	0.97±0.04	0.98±0.04	-0.35	0.73
<i>Amplitude (μV)</i>	14.90±3.19	15.05±2.95	-0.16	0.88
<i>Frequency (Hz)</i>	13.17±0.28	12.97±0.26	2.32	0.03*

Previous literature has most specifically identified schizophrenia to be associated with reduced spindle density^{125,137,176}. Since there is little known of whether this deficit is present prior to disorder onset, high risk individuals were additionally assessed according to their level of clinical risk in order to identify individuals most likely to develop a disorder. This descriptive analysis revealed a trend towards lower spindle density with increased clinical risk (Figure 3.5). However sample sizes were likely not high enough to confirm this finding (ANOVA, $F=2.31$, $p=0.14$). Further, spindle density was not correlated with the number of, or distress levels from psychotic-like experiences ($p>0.05$).

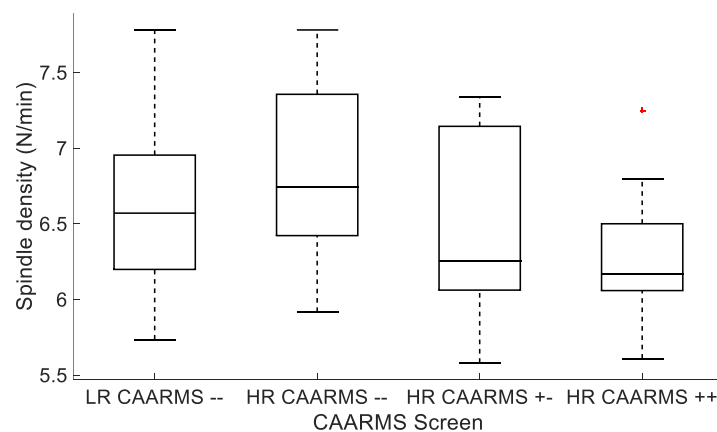


Figure 3.5: Spindle density as a function of clinical risk for developing psychosis. LR=low risk group, HR= high risk group, CAARMS= Comprehensive Assessment of At Risk Mental States, -- = negative screen, += positive screen for symptoms of psychosis, but no deficit in social functioning, ++= positive screen for symptoms of psychosis and a deficit in social functioning. From left to right: n=22, n=5, n=8, n=9.

3.3.4 Circadian profiles

Circadian parameters based upon subjective questionnaires revealed significantly later mid-sleep points on free days (MSFsc, corrected for work days) in high risk individuals by an average of 51 minutes, indicative of later chronotypes. No differences were identified between groups for subjectively reported social jetlag (SJL; Table 3.6). Objective measures from actigraphy showed no differences in terms of MSFsc, SJL, sleep onset or sleep offset. Further, parameters assessing the level and rhythmicity of activity throughout the day revealed no differences between groups (level of activity, level of inactivity, interdaily stability and intradaily variability; Table 3.6).

Table 3.6: Group differences in subjective and objective circadian parameters. MCTQ= Munich Chronotype Questionnaire. N=43.

MCTQ	High risk	Low risk	W-value	p-value
Social jetlag (SJL, hrs)	1.46±0.90	1.14±0.83	287.5	0.29
Mid-sleep point (MSFsc, time)	5.47±1.14	4.62±1.16	339.5	0.02*
Actigraphy	High risk	Low risk	t-value	p-value
Social jetlag (SJL, hrs)	0.51±0.73	0.57±0.75	-0.30	0.76
Mid-sleep point (MSFsc, time)	5.32±1.28	4.73±1.24	1.54	0.13
Sleep onset (hr:min)	00:52±01:05	00:23±01:10	1.39	0.17
Sleep offset (hr:min)	9:05±1:14	8:32±1:19	1.37	0.18
Level of activity (M10 counts)	16096±3103	16934±4507	-0.71	0.48
Level of inactivity (L5 counts)	1083±473	1027±463	0.39	0.70
Interdaily stability (IS)	0.39±0.10	0.36±0.07	1.01	0.32
Intradaily variability (IV)	0.94±0.22	1.00±0.19	-1.03	0.31

Individuals at high risk showed a significantly later melatonin acrophase by an average of 1.11 hours (mean acrophase clock time in low risk=4.11, high risk=5.22; $t=2.83$, $p=0.008$; Figure 3.6) similar to the subjective mid-sleep points. No differences were identified in the average melatonin level (mean mesor, low risk=14.96, high risk=16.25; $t=0.55$, $p=0.58$) or amplitude (mean amplitude, low risk=19.24, high risk=20.86; $t=0.52$, $p=0.61$). Melatonin acrophase was strongly correlated to both actigraphic sleep onset ($r=0.64$, $p<0.001$) and sleep offset ($r=0.45$, $p=0.005$).

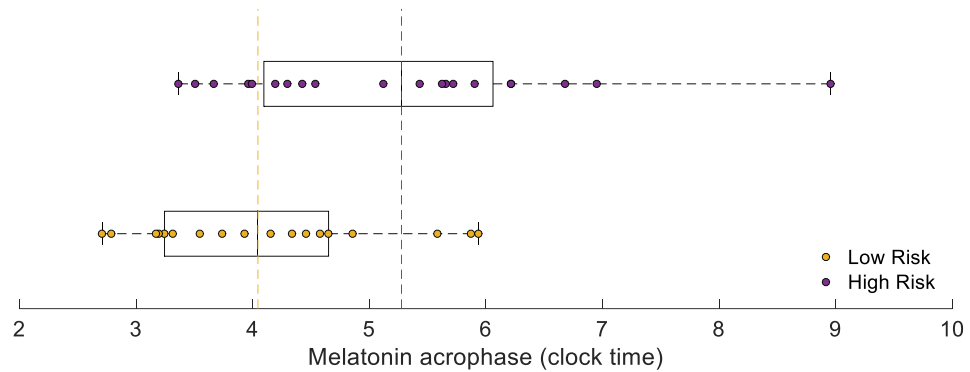


Figure 3.6: Melatonin acrophase between groups. Purple and orange dots represent individual data points. High risk individuals presented a significantly later melatonin acrophase compared to low risk (mean acrophase in low risk=4.11, high risk=5.22; $t=2.83$, $p=0.008$). $n=38$.

An interesting observation from these results was a clustering of high risk individuals, with those of early melatonin peaks (ranging from 3.37 to 4.54, $n=9$) and late melatonin peaks (ranging from 5.12-8.96, $n=11$), suggestive of two distinct profiles. High risk individuals were therefore separated based on these profiles (referred as 'high risk early', HRE and 'high risk late', HRL) and a further analysis of habitual sleep-wake timings and circadian phase was conducted (Figure 3.7a). Results revealed and confirmed a significantly later melatonin acrophase in the HRL group by 2.2 hours compared to both the HRE and low risk (LR) individuals (ANOVA: $F=23.46$, $p<0.001$; post-hoc t-test: HRL.HRE, $t=-6.45$, $p<0.001$; HRL.LR, $t=-5.57$, $p<0.001$). Further, HRL individuals also showed significantly later actigraphic sleep onset to HRE and a trend to LR individuals after controlling for multiple testing (ANOVA: $F=4.81$, $p=0.015$; post-hoc t-test: HRL.HRE, $t=-3.35$, $p=0.004$; HRL.LR, $t=-2.42$, $p=0.024$). No differences were identified between HRE and LR individuals for either melatonin acrophase ($t=0.12$, $p=0.903$) or sleep onset ($t=0.73$, $p=0.476$). Finally, no group differences were identified for sleep offset (ANOVA: $F=2.07$, $p=0.14$). This analysis was repeated with removal of a potential outlier in the HRL group (with a melatonin acrophase of 8.96), producing no differences in the aforementioned findings.

To further distinguish the relationship between melatonin acrophase, subjective, and actigraphic mid-sleep points, a correlational analysis was conducted (Figure 3.7b). Melatonin acrophase was strongly correlated to subjective mid-sleep points ($r=0.70$, $p<0.001$) and moderately correlated to actigraphic mid-sleep points ($r=0.49$, $p=0.002$). Group averages revealed that actigraphic mid-sleep points in low risk and HRE groups were later compared to both subjective mid-sleep points and melatonin acrophase. However in the HRL group, actigraphic mid-sleep points were earlier compared to subjective mid-sleep points and melatonin acrophase (Figure 3.7c).

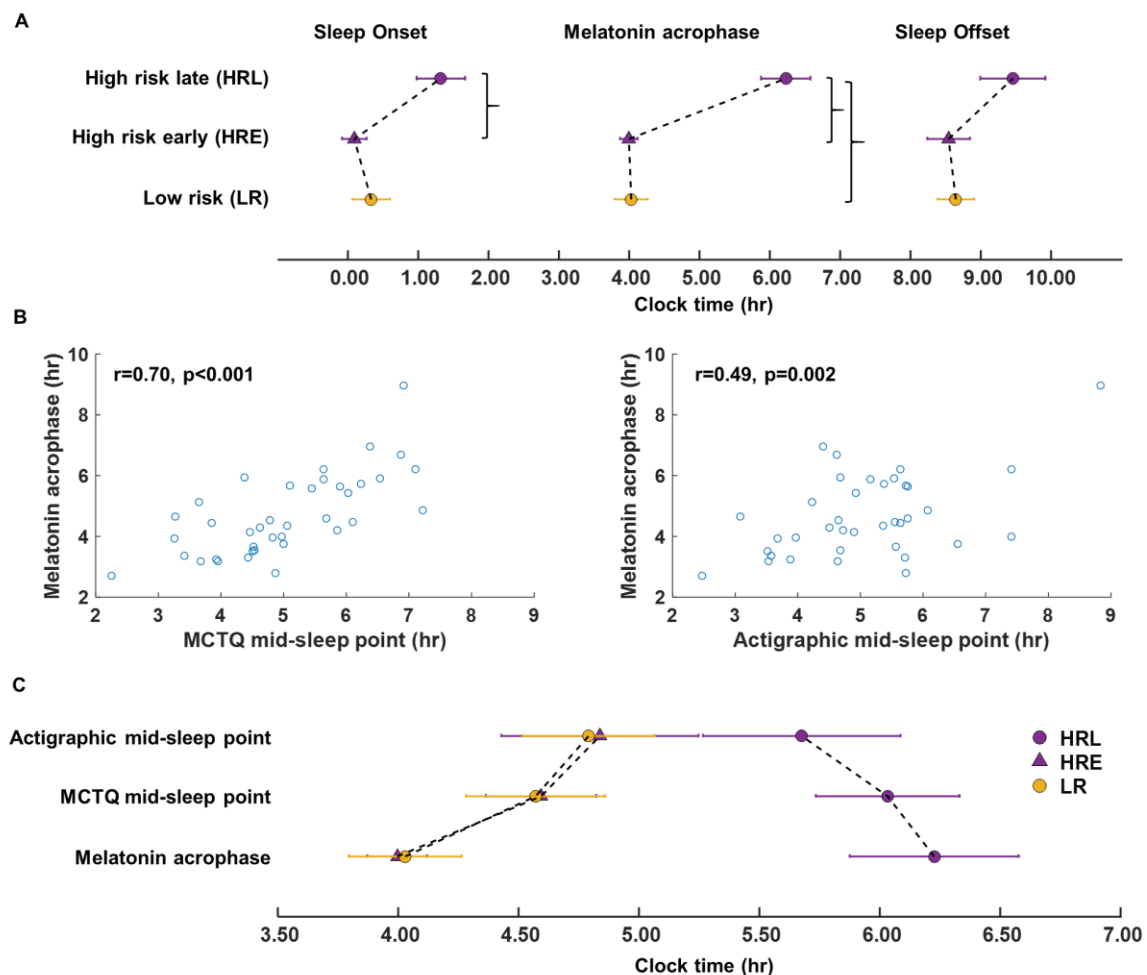


Figure 3.7: (A) Relationship between habitual sleep-wake and internal circadian timings derived from actigraphic sleep onset/offset and melatonin sulphate peaks. Error bars denote SEM, brackets denote significant group differences from post-hoc t-tests. (B) Melatonin sulphate peaks in relation to subjective (Munich Chronotype Questionnaire, MCTQ) and objective (actigraphic) mid-sleep points ($N=37$). (C) Group averages in melatonin sulphate peaks and subjective and objective mid-sleep points. HRL=High risk late, HRE=High risk early, LR=Low risk. Error bars denote SEM. $N=37$ (HRL=11, HRE=9, LR=17).

To determine whether the HRL late group presented a specific phenotype associated with the later chronotype, the three groups were next compared according to measures of sleep physiology and mental state. No differences were identified between HRL and HRE groups for any actigraphic measures including total sleep time, sleep onset latency, sleep efficiency or level of activity/inactivity during the day (see Supplementary Table S5). For measures of mental state, again no group differences were identified between the HRL and HRE groups in terms of clinical risk status, psychotic experiences, depression, anxiety, stress, mania or neuroticism (see Supplementary Table S6). However HRL individuals did show a trend toward a higher number of risk factors (mean±sd: HRL=7.18±1.25, HRE=6±1.4; $t=1.96$, $p=0.068$). To identify on a broader level what might predict chronotype, a linear model was created using melatonin acrophase as a continuous measure. In addition to measures of mental state and risk factors; age, gender and the level of cortisol upon awakening were also included since these have been previously linked to chronotype^{287–289}. A later melatonin acrophase, was moderately associated with a higher number of risk factors (OR[CI]= 1.30[1.06-1.61]) and younger age (OR[CI]=0.84[0.74-0.96]). The cortisol awakening response and the level of depressive symptoms were also related to melatonin acrophase, but the degree of effect was negligible. No other associations were identified from this model (Table 3.7).

Table 3.7: Predictors of melatonin acrophase. Predictors where no t/p value is shown did not survive stepwise backward elimination. Linear model, N=37.

Predictor	t	p	OR (CI)
Number of risk factors	2.49	0.018	1.30 (1.06-1.61)
Cortisol awakening response	-2.10	0.044	1.00 (1.00-1.00)
Depression	-2.07	0.047	0.95 (0.91-1.00)
Age	-2.55	0.016	0.84 (0.74-0.96)
Stress	1.67	0.104	-
Number of psychotic-like experiences	-	-	-
Distress from psychotic-like experiences	-	-	-
Mania	-	-	-
Neuroticism	-	-	-
Anxiety	-	-	-
Gender	-	-	-

Since a trend towards a higher number of risk factors was identified in the HRL group, and a later melatonin acrophase was associated with a higher number of risk factors, the prevalence of risk factors was compared between HRL and HRE individuals (Figure 3.8). Results revealed no single risk factor that completely separated HRL and HRE individuals. Growing up in a highly urbanised area showed the greatest difference in prevalence with 46% endorsement in HRL individuals compared to 0% in the HRE group. A family history of a psychiatric disorder and childhood psychological abuse also showed a large difference in prevalence endorsed by 91% and 45% in the HRL group compared to 56% and 11% in the HRE group respectively. Similarly reporting a psychiatric diagnosis also showed a large difference between groups but instead, was most highly endorsed by the HRE group (HRL: 55%, HRE: 89%).

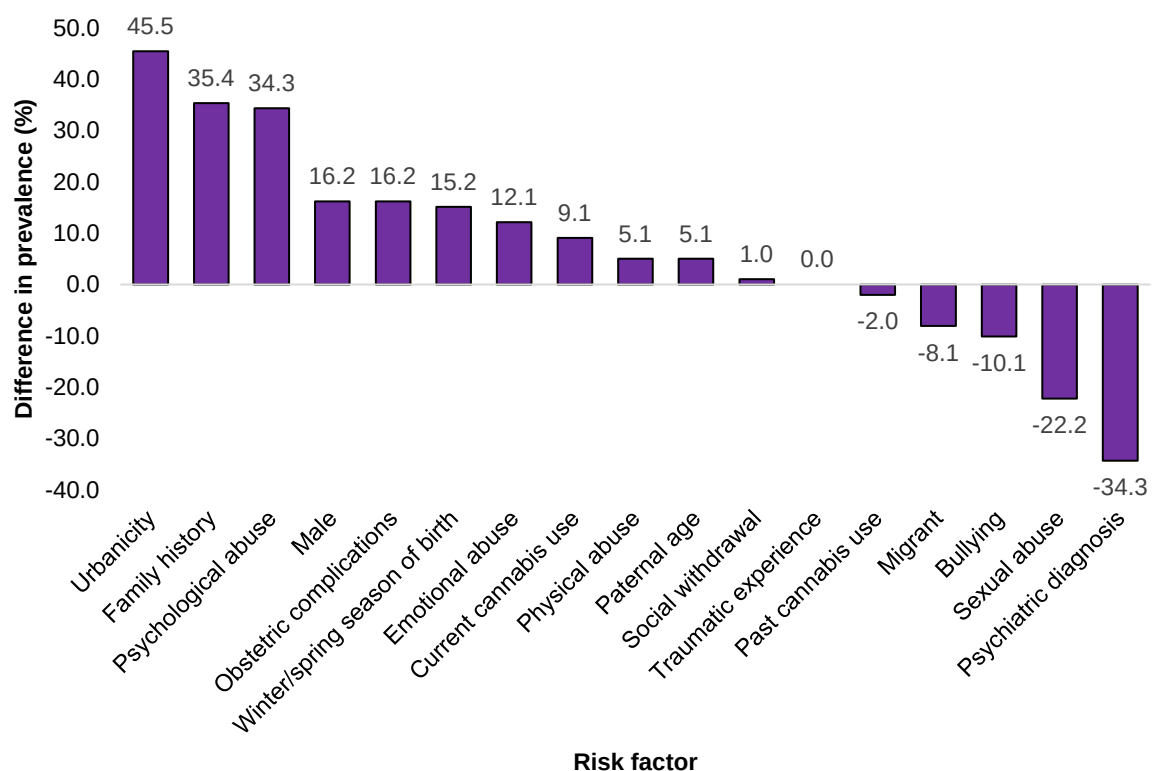


Figure 3.8: Difference in risk factor prevalence between high risk late (HRL, n=11) and high risk early (HRE, n=9) groups separated based upon whether they showed a late or early melatonin peak. A positive difference indicates a greater prevalence in HRL individuals; a negative difference indicates greater prevalence in HRE individuals.

3.3.5 HPA Function

Finally the cortisol awakening response (CAR), an increase in cortisol in the first half hour after waking, was recorded from salivary cortisol levels. No differences were identified in the average CAR between groups in terms of either delta changes across the recording period (average AUC_i across days; $W=215$, $p=0.706$), or the concentration of cortisol (nmol/L) at each time point (repeated-measures ANOVA, $p>0.05$ in all cases, Figure 3.9a). However, a comparison of intra-individual variability in the CAR revealed significantly less variability in high risk individuals between the two days of data collection (difference in AUC_i between days; $W=128$, $p=0.013$; Figure 3.9b/c).

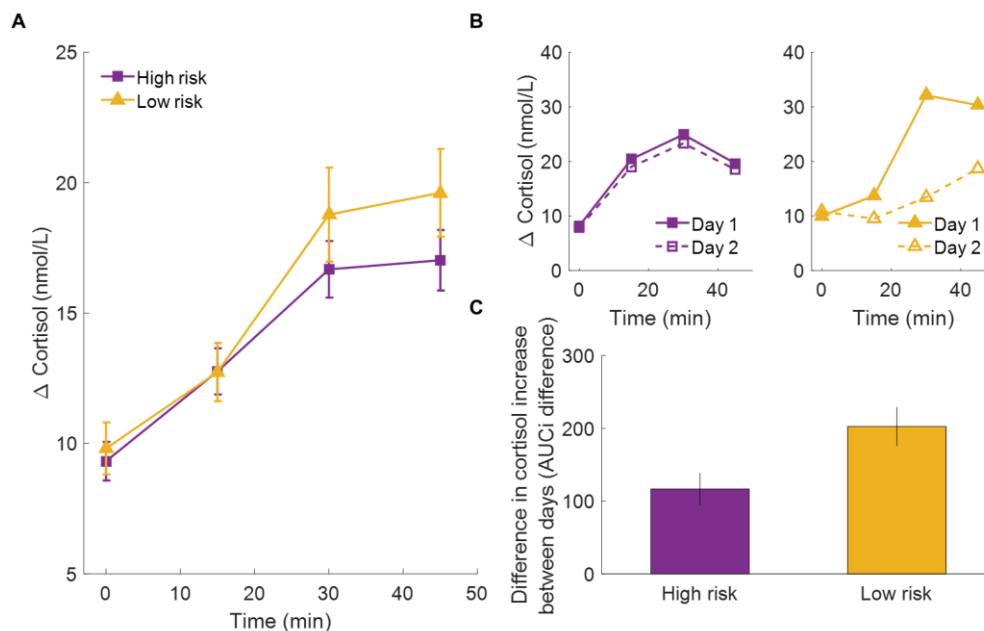


Figure 3.9: Cortisol awakening response. (A) Group differences in cortisol levels, presented as delta changes from 0, 15, 30 and 45 minutes after awakening (N=43). Bars denote S.E.M. (B) Representative examples from a high risk individual (left) and low risk individual (right) showing cortisol levels upon awakening between days. (C) Average difference in cortisol between days as measured by differences in area under the curve with respect to increase (AUC_i ; $W=128$, $p=0.013$).

3.4 Discussion

This study is the first to assess sleep and circadian phenotypes in individuals at high risk for developing psychosis based upon not only the presence of sub-clinical psychotic symptoms, but also a high load of risk factors for psychosis. High risk individuals showed clear subjective sleep disruption, with reduced overall sleep quality, longer sleep latency, increased nightmares and were overall more likely to meet criteria for insomnia disorder. Surprisingly this was not seen in objective polysomnographic and actigraphic measures. However, circadian parameters revealed substantial differences between groups with later subjective chronotype in high risk individuals and a subset showing considerably later melatonin peaks.

3.4.1 High risk individuals show distinct circadian phenotypes

In a recent study, Lunsford-Avery *et al* assessed circadian rhythms in ultra-high risk (UHR) individuals identifying more fragmented circadian rhythms, lower daily activity and later onset of nocturnal rest²⁵⁸. The circadian disruptions also predicted the severity of psychotic symptoms and psychosocial impairment at 1 year follow-ups. Castro *et al* similarly identified circadian rhythm disturbances including more fragmented and less regular rest-activity rhythms and lower activity levels in UHR individuals¹⁴¹. In the current study, although no differences in actigraphic circadian measures were identified between groups, significantly later chronotypes were identified from both subjective questionnaires and later melatonin peaks in high risk individuals. Wulff *et al* identified significant sleep and circadian rhythm disruption (SCRD) in patients with schizophrenia with a sub-group of individuals having later melatonin peaks. Further, this melatonin shift was associated with free running and delayed sleep-wake cycles¹¹⁹. Surprisingly, the current study similarly identified a sub-group of high risk individuals (HRL group) with substantially later melatonin peaks which were in line with a significantly delayed sleep onset but not sleep offset. Considering the sample were all either students or employed, the lack of difference in sleep offset was

likely due to work or social commitments contrasting to the unemployed population in the previous study. The importance of these findings is unclear but could suggest that the later melatonin peaks represent a primary abnormality with the circadian clock. HRL individuals may thus have a longer endogenous circadian rhythm or a reduced responsiveness to cues from the external environment preventing the normal resetting of the clock²⁹⁰. The normal objective sleep physiology in this high risk group could therefore represent an intact homeostatic sleep regulation, but one that is desynchronised (e.g. due to social commitments) with the complementary circadian rhythm leading to subjective sleep complaints and potentially premonitory of future objective sleep disruption with chronic desynchrony. In contrast, perhaps the HRE group, with normal circadian rhythms and objective sleep, represent a different subgroup where subjective sleep and circadian disturbances arise from a more negative psychology (see section 3.4.2, pg115). An alternative explanation for the delayed circadian rhythms in the HRL group could be due to abnormal light exposure. For example, perhaps these individuals are more prone to higher light levels in the evening or to falling asleep with the lights on, which could delay the melatonin rhythm and perhaps even their perception of sleep in contrast to their objective sleep timings. Since circadian disorders such as delayed sleep phase disorder (DSPS) has previously been linked to genetic mutations including a shorter repeat region of the *Per3* gene²⁹¹, future work using genetic analysis or the use of constant routine protocols would help determine these explanations in high risk individuals.

Given the strong associations between SCRD and psychosis which may both involve underlying neurotransmitter dysfunction¹⁰⁹, this circadian phenotype could be an important new marker for psychosis pathology in at least a subset of individuals. Although no relationship between melatonin acrophase and mental state was identified, the number of risk factors was the most predictive variable and showed a trend increase in the HRL compared to the HRE group. Melatonin rhythms, or more generally chronotype, is thought to have a large genetic influence¹¹⁵ and abnormal circadian gene expression including an

absence of rhythmic expression in PER2 and CRY1 genes have been identified in patients with schizophrenia²⁹². It is therefore likely that underlying genetic differences explain the delayed rhythm in HRL individuals and could account for the increased family history of psychiatric disorders compared to HRE individuals (given that circadian rhythms and psychiatric disorders share risk genes¹⁵³). However, chronotype is also influenced by the environment and may be related to developmental exposures²²⁶. For example, obstetric complications were marginally increased in HRL individuals and it has previously been shown that these can affect 6-sulphatoxymelatonin excretion in adult life²⁹³. The most common risk factor associated with HRL individuals was urbanicity during childhood. With increased light pollution and more 24 hour lifestyles, urban environments have been associated with later chronotypes, and this could also have a role in delaying melatonin rhythmicity^{294,295}. It is speculative whether urban exposure during development would have such an effect on adulthood physiology, however environmental light exposure during development has been shown to have long lasting effects. For example although not a prominent difference in the current finding, season of birth has been associated with morning-evening preference in humans^{296,297} and the length of the perinatal photoperiod has been experimentally shown to have lasting effects on adult circadian rhythms in mice²⁹⁸. In addition, living in urban environments during development could also promote 'urban-like' behaviours in later life (e.g. staying up later with more social activities in the evening). Overall, although there is little evidence that delayed melatonin adds additional impact on mental state at this stage, it could be an indication of a subgroup with particular shared genetic or environmental factors. These factors may exacerbate the vulnerability to sleep disturbance, circadian rhythm disruption, and potentially lead to specific symptoms upon development of psychosis. Future studies assessing particular circadian risk genes, a higher sample size to identify trends in environmental risk factors, and further investigation into melatonin rhythms in psychotic disorders would help determine this.

3.4.2 High risk individuals show subjective but not objective sleep disruption

High risk individuals showed significantly poorer subjective sleep quality, longer sleep onset latencies, greater daytime dysfunction due to poor sleep and greater symptoms of insomnia. These findings are consistent with previously conducted studies on UHR individuals, with all studies having identified some form of sleep disturbances^{140–142,197}. Castro *et al* and Zanini *et al* identified a similar global PSQI score of 7.7 in UHR individuals compared to 7.0 in the current sample^{140,141}. Lunsford-Avery *et al* also identified significantly longer sleep onset latencies and greater sleep disturbances in UHR individuals compared to healthy controls¹⁹⁷. This consistent evidence suggests that subjective sleep disturbance is a clear phenotype of individuals at high risk for developing psychosis including those presenting a high load of environmental risk factors, and could be a valuable additional marker for improving identification of risk groups.

Interestingly, the current study also identified a greater frequency of nightmares in high risk individuals. Nightmares have been shown to be more frequent in schizophrenia, are predictive of developing future psychotic experiences, and have recently been shown to be elevated in UHR individuals^{149,299,300}. It has been suggested that rather than mirroring the positive symptoms of schizophrenia elevated nightmares may instead reflect greater daytime distress³⁰⁰. Further, nightmares are also associated with schizotypal personality and dissociative experiences which have collectively been linked to traumatic childhood experiences which may disrupt an underlying shared pathway^{263,266}. Since experiencing a traumatic event including childhood abuse was highly prevalent in the high risk group, the current findings are consistent with this argument. Elevated nightmare frequency may thus be an important additional feature of high risk groups and should be investigated further. For example, routine assessments of nightmares may help identify risk groups which could be targeted for specific treatments such as image rehearsal therapy to alleviate symptoms and improve sleep³⁰¹.

In contrast to the subjective sleep disruption, polysomnographic measures revealed no clear differences between groups in terms of total sleep times, sleep onset latency or amount of time in each sleep stage. Although previous studies using PSG have identified differences in at risk groups, they have not been consistent. Only one other study has assessed polysomnographic measures in a UHR group. In this study, Zanini *et al.* measured sleep in 20 UHR individuals identifying significantly longer sleep latencies and REM onset latencies compared to controls¹⁴⁰. One of the main differences in the current study to all previous studies on UHR individuals is the cohort and recruitment methods used. Previous studies have recruited at risk individuals who are already seeking some form of formal help or who self-refer for having psychotic-like experiences. In the current study, individuals were not informed as to why they were selected to take part and they were unlikely to have sought help for PLEs. Furthermore only 41% of the high risk individuals would be classed as UHR in line with previous studies and it is unknown whether the remaining participants would also transition to UHR status. These individuals may thus represent an earlier stage of disease progression, a different subgroup of at risk individuals, or the recruitment sensitivity is not high enough to identify individuals who will transition to psychosis. All of these explanations could explain the lack of PSG disturbances in the current high risk group. Interestingly, Manoach *et al* assessed PSG variables in 19 familial high risk individuals identifying increased Wake After Sleep Onset, reduced sleep efficiency, and increased stage 1 and 2 sleep¹³⁷. It is therefore possible that changes in sleep architecture are more related to a high genetic risk rather than the greater emphasis on environmental risk as in the current study. Considering the limited studies on PSG in high risk groups, and the inconsistent evidence for sleep architectural changes in schizophrenia^{121,139}, these results provide important further insight into objective sleep in at risk states and how this may relate to risk factors.

High risk individuals similarly showed a lack of objective sleep disturbances based on actigraphic measures, but did show greater variability in sleep onset latency. Only one

other study has directly assessed sleep disturbance in UHR individuals from actigraphy. In this study, Lunsford-Avery *et al.* identified significantly reduced sleep efficiency, greater wake after sleep onset and more total movements during sleep in UHR individuals¹⁴². These differences between studies could again be due to the differences in cohorts but further replication, such as with longitudinal assessments would help confirm this. Further, Lunsford-Avery *et al.* did not assess sleep onset latency in their study so it cannot be determined if it was also a feature of the UHR cohort. Regardless, caution should be taken when assessing sleep onset latency from actigraphy since it uses activity levels as a proxy for sleep-wake cycles, and PSG is often more appropriate. Overall, the current findings provide little evidence for objective sleep disturbances in individuals with a high load of risk factors.

Why would individuals at high risk show strong subjective but not objective sleep disturbances? It is thought that subjective and objective assessments likely measure different aspects of sleep physiology and it has previously been shown that the PSQI does not correlate to either EEG or actigraphic measures^{302,303}. Instead, the PSQI is thought to be a greater measure of psychology which could reflect more negative cognitive viewpoints or general dissatisfaction³⁰³. In this sense, the higher PSQI scores in high risk individuals may instead be a reflection of the observed increase in anxiety and depression. Sleep-state misperception is a subtype or particular extreme of insomnia disorder where no objective differences are observed, but individuals still suffer from substantial complaints of sleep disturbance³⁰⁴. A previous study showed that individuals with sleep-state misperception have characteristically higher depression and anxiety levels compared to insomnia sufferers with objective disruption³⁰⁵. It is possible then that high risk individuals in the current study have an altered or more negative perception of sleep which likely still impacts daily functioning³⁰⁶. Alternatively, it is also possible that subjective disturbances are a marker of a transitional or prodromal state between normal sleep and future sleep disturbances which could develop with increasing psychotic symptoms³⁰⁴.

Investigating the longitudinal relationship between objective sleep disturbance and psychotic symptoms is an important task for disentangling this further.

3.4.3 High risk individuals show subtle changes in spindle activity

Although sleep spindle deficits are widely reported in schizophrenia^{125,176,177}, few studies have looked into the early stages of disease and none during high symptomatic risk. This study is the first to characterise spindles in individuals at high risk, where no deficits in spindle density or number were identified. Previously, two studies have shown a reduction in spindle density, one in antipsychotic naïve first episode patients¹³⁷, and another in healthy first degree relatives²⁵⁷. However two other studies have shown no differences in spindle density in antipsychotic naïve first episode patients^{138,181}, providing inconclusive evidence of this reduction in early and at risk stages. It is possible therefore that the spindle deficits seen in schizophrenia manifest at a later development of the disease, or are not severe enough to be detected in high risk individuals. Indeed, when high risk individuals were separated into ultra-high risk status (based upon the CAARMS interview), those with the greatest symptom severity did show a trend towards lower spindle densities, supporting this explanation. Future studies using longitudinal assessments would help to discern this. Furthermore improving assessment strategies such as through the use of high density EEG to identify spatial or temporal differences could provide further insight as to whether the deficit is a global phenomenon.

Although no changes in density were identified, high risk individuals showed a significantly higher frequency (Hz) in central and parietal spindles. Spindle frequency has not been previously reported to differ in schizophrenia and although significant, the difference between high and low risk groups was small. Nevertheless this phenotype could be important. Previous studies have similarly identified small but significant increases in spindle frequency with age with one showing similar frequencies in 30-39 year olds as the

high risk group, while 20-29 year olds were more comparable to the low risk group^{307,308}. Spindle frequency is thought to be dependent upon the duration of hyperpolarisation of thalamocortical cells, indicates lighter sleep, and may reflect changes in sleep pressure^{309–311}. Higher spindle frequencies in high risk individuals could therefore reflect a subtle sleep disturbance involving disrupted sleep homeostasis that may only be detected with a manipulation to this system (such as through sleep deprivation). Interestingly, homeostatic abnormalities in spindles have been previously associated with sleep maintenance insomnia³¹². Further replication would be required to identify the importance of these microstructural differences in high risk individuals.

3.4.4 High risk individuals and HPA function

High risk individuals showed significantly less intra-individual variability in the cortisol awakening response (CAR) but no overall differences with respect to cortisol increase upon awakening. With previous work in schizophrenia showing a blunted CAR²⁶⁰, the lack of change in the high risk group suggests that these individuals maintain a normal functioning HPA axis, despite the high load of risk factors which likely act as chronic stressors throughout development. HPA axis dysfunction may not therefore be an adequate predictor of future transition, and inconsistencies in the CAR response have been identified in UHR populations^{261,313}. Perhaps HPA dysfunction manifests with severity of psychotic symptoms or arises from recent stressful life events that may trigger the development of psychosis. Nevertheless, a non-significant decrease in cortisol levels was identified during the last 30-45 minutes which could reflect an attenuation of the blunted response seen in UHR and first episode individuals^{260,261}. Furthermore the novel finding of less intra-individual variability in high risk, has not been reported or assessed previously in relation to psychosis, but could have a physiological significance. The CAR response is largely a trait measure, but has also been shown to vary with state factors including time of awakening, sleep duration, sleep quality, intensity of morning light and stress load of

the prior day³¹⁴. It has also been suggested that the CAR presents a minor stress response to the anticipated demands of the upcoming day²⁵⁹. It is unclear how this may relate to high risk individuals, but could suggest the lack of variability across days reflects a blunted response to changing stressors across the week. A potential limitation of this is that the variability could reflect or at least partially present a measurement error, although there is no reason to believe that compliance differed between groups³¹⁵. Future work should therefore take intra-individual variability into account when assessing the CAR response in psychiatric disorders.

3.4.5 High risk individuals as a transdiagnostic group

With the high comorbidity and overlapping symptoms across psychiatric disorders high risk individuals were not only selected for sub-clinical psychotic symptoms but also increased levels of depression, anxiety and stress. High risk individuals further showed significantly greater comorbid psychiatric diagnoses, dissociative experiences, mind-wandering, cognitive failures, and neurotic and schizotypal personality traits. Therefore this population of individuals largely presents an elevated psychopathology. These findings are not unexpected, but could suggest that a high load of environmental and genetic risk factors are not specific for the development of psychosis or SCRD (also see Chapter II section 2.4.5, pg81). Interestingly a recent twin study identified that the genetic and environmental influences on PLEs and sleep disturbance were substantially mediated by negative affect¹⁷³. However, this did not account for all variance and another study of over 400 UHR individuals did not find such an association between depression, anxiety or dissociation in mediating the interaction between childhood abuse and transition to psychosis³¹⁶. The current study aimed to target individuals at risk for psychosis, and the methods provide focus for this. However it is also clearly beneficial for identifying vulnerabilities to a range of mental health problems which, given the shared pathologies and comorbidities, is not a disadvantage. Future studies that require higher specificity will

likely benefit from a focus on more specific psychotic symptoms such as persecutory delusions.

Following this, sleep and circadian rhythm disruption is found not only in psychotic disorders, but across several psychiatric disorders including depression, anxiety and alcoholism¹¹⁷. The usefulness of identifying sleep and circadian phenotypes therefore provides great potential for disentangling symptomatic domains where common pathways can be identified. For example, the later melatonin subgroup, although not evident yet, could develop specific psychiatric symptoms that are associated with particular overlapping genetic mutations and neural circuit dysfunction. Furthermore the benefit of treating SCRD such as through the use of cognitive behavioural therapy for insomnia (CBT-I) has been shown to improve both sleep and symptoms in some disorders¹³¹. Implementing sleep and circadian intervention in these high risk populations and increasing routine practice for assessing sleep and circadian disturbance, even with quickly administered questionnaires, could prove an effective strategy in ameliorating a wide range of mental health problems.

3.4.6 Limitations

Certain limitations should be addressed for the interpretation of these findings. Firstly, as identified in Chapter II, not all risk factors assessed were equally contributory for the prediction of sub-clinical symptoms. It is therefore possible that the level of risk factors was not specific or high enough to adequately identify high risk groups. However, since 77% of the high risk sample screened positively for subthreshold symptoms of psychosis and 41% screened as UHR, this sample is still a relevant at risk group given the combination of genetic, environmental and symptomatic factors which could represent an even earlier stage of disease progression. As with all high risk studies, without longitudinal data, it cannot be determined what proportion of individuals will go on to develop a disorder and therefore what proportion represent relevant phenotypes (if any) specific for psychosis.

On the reverse of this, it is unknown how representative low risk individuals were. The low amount of risk factors could represent an extreme group of individuals however all were assessed as reasonably healthy, and there is no evidence to support a difference to the general population. Overall, the study could have benefited from a larger sample size and more non-student individuals.

3.4.7 Summary of findings

This is the first study to assess sleep and circadian rhythms in individuals at high risk for developing psychosis based upon having a high load of risk factors and sub-clinical symptoms. This population exhibited substantial subjective sleep disturbance and differentiated circadian phenotypes with later circadian rhythms in a subset of individuals. This work further exemplifies the tight relationship between SCRD across the psychosis continuum, which may be related to the occurrence of risk factors, and could benefit intervention strategies of individuals at high risk. Longitudinal studies are a crucial next step for identifying whether these findings highlight a premorbid sleep and circadian phenotype associated with the development of psychotic symptoms and objective sleep disturbances.

Chapter IV: Sleep dependent memory consolidation in individuals at high risk for developing psychosis

Contents

4.1 Introduction	124
4.1.1 Sleep dependent memory consolidation	124
4.1.2 SDMC in schizophrenia and high risk	124
4.1.3 Study aims	125
4.2 Methodology	125
4.2.1 Non-declarative procedural learning	125
4.2.2 Declarative learning	126
4.2.3 Study design	127
4.2.4 Attention task	128
4.2.5 Statistical analysis	128
4.3 Results	129
4.3.1 No differences in MST learning	129
4.3.2 MST performance and sleep architecture	132
4.3.3 No differences in word-pair learning	133
4.3.4 Sustained attention	134
4.4 Discussion	135
4.4.1 Sleep-dependent procedural learning	135
4.4.2 Sleep-dependent declarative learning	137
4.4.3 Is there a dependence on sleep?	138
4.4.4 No deficits in sustained attention	140
4.4.5 Limitations	140
4.4.6 Impact of findings and future directions	141

4.1 Introduction

4.1.1 Sleep dependent memory consolidation

Given the importance of sleep for a broad array of functions including restoration, immune function, and neuronal plasticity, changes in sleep physiology associated with psychosis is likely to have an extensive impact⁶⁹. One area of increasing evidence suggests that sleep plays an important role in the consolidation and processing of memory^{72,73}. Several studies have shown that performance on both declarative and non-declarative learning tasks is significantly improved after a period of sleep but not after an equivalent period of wake (see Chapter 1 Section 1.2.5, pg23)^{73,75,317}. Learning tasks such as those involving procedural memory may therefore benefit from specific processes during sleep, such as slow waves or sleep spindles, that lead to faster more automated performance, and a decrease in dependence on voluntary attention³¹⁸.

4.1.2 SDMC in schizophrenia and high risk

Interestingly in schizophrenia, with at least some types of memory tasks, this sleep dependent improvement is largely absent^{124,177,319}. Although the underlying mechanisms for the role of sleep in memory consolidation are unclear, these impairments have been related to both the duration of stage 2 sleep and deficits in sleep spindle density^{124,125}. Further to this, sleep disruption has also been implicated in the exacerbation of attentional deficits which is in itself, a key cognitive symptom of schizophrenia^{181,320–322}. It is therefore possible that sleep and circadian rhythm disruption has a key influence on the widely known cognitive impairments associated with schizophrenia, either directly through its importance for memory consolidation, or indirectly through its impact on sustained attention³¹⁸. However, further work is needed to delineate the importance of sleep for memory processing in psychosis and when deficits in SDMC occur in relation to psychotic symptoms and other cognitive impairments. Recently, two studies have assessed learning in relation to measures of sleep in Ultra-High Risk individuals and first degree relatives,

both finding correlations between sleep parameters and memory performance^{257,323}. This emerging literature rouses a link between sleep and memory performance prior to disorder onset, however, neither study tested changes in performance across sleep and thus cannot determine the impact on memory consolidation.

4.1.3 Study aims

This study aimed to assess sleep dependent memory consolidation in individuals at high risk for developing psychosis. A secondary aim was to identify levels of attention to further identify what impact sleep may have on cognition. Overall this work adds to chapter III providing further phenotyping of high risk individuals selected for a high load of risk factors and sub-clinical psychotic symptoms.

4.2 Methodology

Please refer to Chapter III Section 3.2 (pg88) for an outline of participant recruitment and the full study protocol. To test for differences between high and low risk groups, two measures of sleep-dependent memory consolidation (SDMC) were administered using a within-subjects design to allow for both a wake and sleep condition in each participant.

4.2.1 Non-declarative procedural learning

The finger tapping motor sequence task (MST) is a classical measure of non-declarative procedural memory⁷⁵. In this task participants are asked to type a five element sequence (4-1-3-2-4) “as quickly and accurately as possible” for a period of 30 seconds for a total of 12 trials with a 30 second break period between each trial. During the task, the numeric sequence was displayed on a computer screen in white against a green background to minimise working memory requirements. A white asterisk was used to underline which key should be pressed next (and moved as the sequence progressed). During the 30 second

interlude between trials, a red screen was displayed with the word “Break”. Approximately 12 hours and 24 hours later the same task was repeated for a further 2 trials each. Each 30 second trial was scored for the number of correct sequences and errors. One participant was excluded from this analysis for inadequate completion of the task.

4.2.2 Declarative learning

A Word-Pair Learning (WPL) task was used as a measure of declarative memory as used previously^{317,324}. 64 high-frequency monosyllable concrete nouns were selected and paired to create 32 semantically unrelated word pairs (e.g. Ice-Dog). The task involved 4 stages. During encoding, participants were presented with each of the 32 word pairs successively, each lasting 4 seconds (inter-stimulus interval of 250ms), with one word centred on each half of the computer screen. Participants were instructed to pay close attention to each word pair since they were to be tested on them later. Specifically participants were instructed “To remember the pairs, it is helpful to think of associations between the pairs. For example, if the words were frame-shoe, you might imagine a framed painting of a shoe”. Immediately after, participants began an ‘immediate recall phase’. During this phase participants were randomly presented with the first word of each word pair only and asked to call out the corresponding word. The researcher then typed this on the screen. Feedback was provided as “correct” or “wrong” followed by the correct pairing, displayed for 750ms if wrong. Participants were encouraged to guess if they did not know. Participants repeated this for 30 of the word-pairs (excluding the first and last for primacy and recency effects³²⁵) until at least 62% accuracy had been reached or until the word list had cycled 5 times. The order of word presentation was randomized across each cycle. A delayed recall phase then followed with the words presented a final time, without any feedback. Approximately 12 hours later, the delayed recall was repeated once more. Participants were then told a new list (again with 32 word pairs) would be given, and the protocol was repeated (to allow both a sleep and wake condition). Since performance

in the immediate recall phase was very high for the first ten participants (average 84% recall accuracy compared to 74% in a previous study³¹⁷), for the remaining participants the word lists were increased to 40 to prevent potential ceiling effects. Since the delayed recall output expresses recall accuracy as a percentage of original immediate recall accuracy, all participants were included in statistical analysis. In the results of this study, no differences were identified when taking the initial ten participants out of the statistical analysis.

4.2.3 Study design

For both SDMC tasks, participants were randomly split into an AM-PM-AM group and a PM-AM-PM group, counterbalanced for high risk and low risk groupings. The AM-PM-AM group completed the tasks on day 2 of the study at ~9am, 9pm and day 3 at 9am. The PM-AM-PM group completed the tasks on day 2 of the study at ~9pm, and on day 3 at ~9am and ~9pm. Times were adjusted to fit participant's normal routines, but were always maintained with a 12 ± 1 hour difference between sessions. The MST and WPL task were given in random order between participants, but kept in the same order across trials for each participant. Please see Figure 4.1 for an outline of the full SDMC protocol.

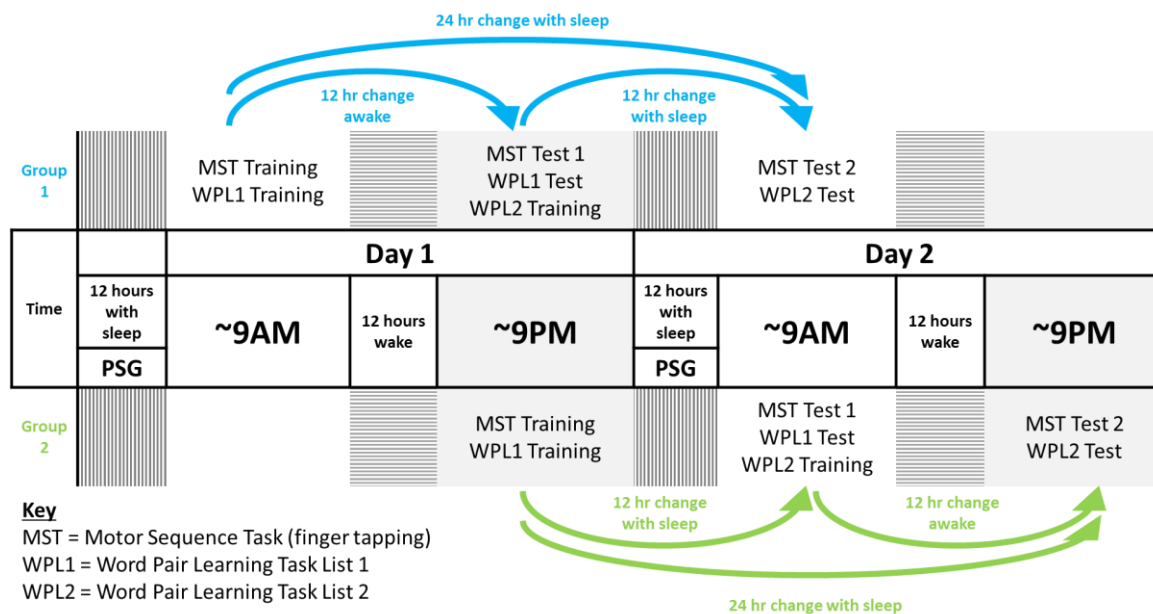


Figure 4.1: Sleep dependent memory consolidation (SDMC) protocol. Both Group 1 (AM-PM-AM group) and Group 2 (PM-AM-PM group) underwent training and testing for two measures of SDMC. In the motor-sequence task (MST) participants repeat a 5-digit numerical sequence on a keyboard for 30 seconds per trial for a total of 10 trials. This is then immediately tested for a further two trials and again 12 hours and 24 hours later. In the word-pair verbal memory task, participants learned one of two lists of word pairs (WPL1 = Word list 1, WPL2 = Word list 2) and were then immediately tested through recall of the second word from the pair when presented with the first word. This was repeated after 12 hours. After this, participants then learnt the second word pair list, were immediately tested and then retested after a further 12 hours. The order of the motor-sequence and word-pair tasks was randomized across participants. Overnight polysomnography (PSG) was recorded during the participant's sleep period. Arrows indicate the test of interest from those timings.

4.2.4 Attention task

To measure sustained attention, the CANTAB (Cambridge Cognition Ltd, Cambridge, UK) Rapid Visual Information Processing (RVP) test was used. This 10 minute test requires participants to watch a sequence of pseudo-random digits from 2 to 9 appearing one at a time within a white box in the centre of the screen. Participants are asked to detect a target sequence of digits (e.g. 3-5-7) and respond as quickly as possible, by clicking a button, upon seeing the final digit of the target sequence. The RVP produces nine outcome measures covering latency, probabilities and sensitivity. All participants completed the RVP on the second evening of beginning the study after set-up of the polysomnography.

4.2.5 Statistical analysis

All statistical analysis was computed using 'R' software²²⁷. Group differences were calculated using ANOVA, post-hoc t-tests for normal data, and non-parametric Wilcoxon

rank sum tests for non-normal data. For the motor-sequence task, a repeated measures analysis of variance was used to compare performance across the three time points. Pearson's product moment correlation coefficients were used to assess the relationship between the memory tasks and sleep parameters.

4.3 Results

4.3.1 No differences in MST learning

Performance on the MST was assessed in terms of improvement across the 12 hours and 24 hours. A sleep effect was identified if participants significantly improved after a period of sleep but not with the equivalent period of wake. For high risk participants who trained in the evening (PM-AM-PM), a clear sleep effect was identified where the number of sequences typed was significantly improved after 12 hours with sleep, but not from the additional 12 hours of wake (Figure 4.2c). Similarly, for low risk participants who trained in the morning (AM-PM-AM), a clear sleep effect was identified, where the number of sequences typed was significantly improved after 12 hours with sleep, but not from the initial 12 hours of wake (Figure 4.2b). Low risk individuals trained in the evening (PM-AM-PM, Figure 4.2d) and high risk individuals trained in the morning (AM-PM-AM, Figure 4.2a) showed an improvement after both 12 hours with sleep and 12 hours of wake, and thus it could not be clearly determined if sleep had an additional effect on motor sequence improvement over simple practice of the task.

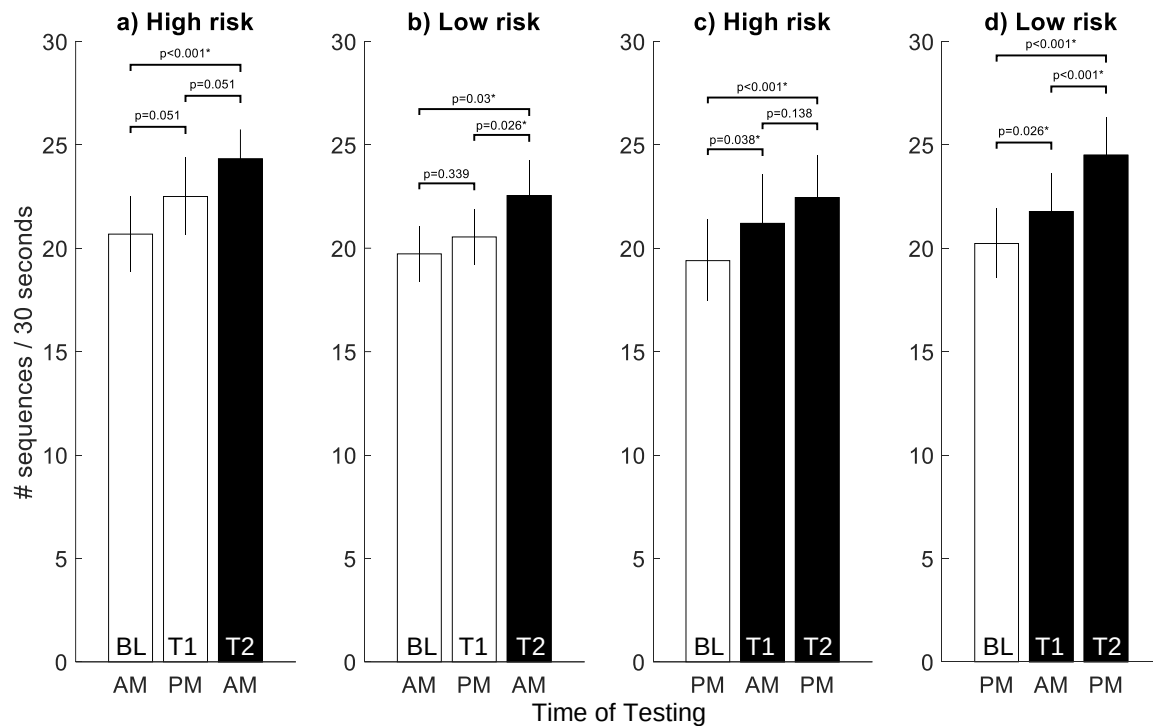


Figure 4.2: Effects of sleep and wake on motor skill learning. Half of participants began training in the morning (a, high risk, $n=11$; b, low risk, $n=11$) and were tested 12 hours later across wake and retested a further 12 hours later across sleep. Half of participants began training in the evening (c, high risk, $n=10$; d, low risk, $n=11$) and were tested 12 hours later across sleep and retested a further 12 hours later across wake. BL= baseline learning, T1= Test 1, T2=Test 2. P-values refer to a repeated measures analysis of variance. Bars denote SEM.

All four of the AM-PM groups presented a significant increase in the number of correct sequences typed across the total 24 hours (from baseline learning to test 2), suggesting that there were no deficits in learning of the task between high and low risk (Figure 4.2; Figure 4.3a). Collapsing AM-PM-AM and PM-AM-PM groups together revealed no overall group differences between high and low risk in the percent improvement of the task after 12 hours with sleep (Figure 4.3b) or 12 hours of wake (Figure 4.3c) suggesting that overall, there were no deficits in SDMC for the motor-sequence task.

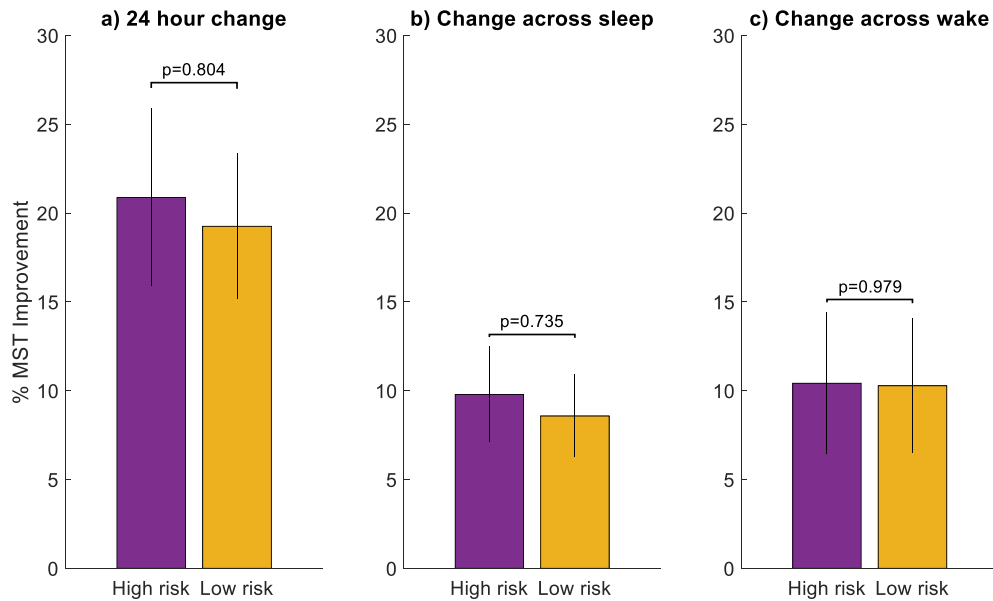


Figure 4.3: Group differences in motor skill learning. No differences were identified in the percentage of improvement on the motor sequence task (MST) between high and low risk individuals after 24 hours, 12 hours with sleep or 12 hours with wake (two-sample t-test, $p > 0.05$; high risk $n = 21$, low risk, $n = 22$). Bars denote SEM.

Since high risk individuals presented a clear sleep effect when trained in the evening and low risk when trained in the morning, MST improvement was next correlated to subjective mid-sleep points (MSFsc) to identify a potential circadian influence. No significant associations were identified between MSFsc and MST improvement based upon the time of testing (AM-PM-AM MST improvement with sleep, $r = -0.96$, $p = 0.669$; PM-AM-PM improvement with sleep, $r = 0.25$, $p = 0.283$). To test for subjective alertness between trials the Stanford Sleepiness Scale (SSS) was employed. For individuals trained in the morning (AM-PM-AM), high risk individuals reported significantly less alertness levels during the evening test 1 (mean values: high risk, 3.91 ± 1.04 , low risk, 2.55 ± 1.04 ; $t = 3.07$, $p = 0.006$) but no difference in the morning training (mean values: high risk, 2.64 ± 0.81 , low risk, 2.27 ± 0.79 ; $t = 1.07$, $p = 0.298$) or morning test 2 (mean values: high risk, 2.45 ± 0.93 , low risk, 2.09 ± 0.70 ; $t = 1.03$, $p = 0.315$). In contrast, for individuals trained in the evening (PM-AM-PM), high risk individuals reported significantly less alertness levels during the morning test 1 (mean values: high risk, 2.55 ± 0.52 , low risk, 1.73 ± 0.79 ; $t = 2.87$, $p = 0.010$) but no difference in the evening training (mean values: high risk, 2.91 ± 1.14 , low risk, 3.00 ± 1.10 ;

$t=-0.19$, $p=0.850$) or evening test 2 (mean values: high risk, 2.55 ± 1.13 , low risk, 2.09 ± 0.83 ; $t=1.08$, $p=0.296$). Since high risk individuals performed overall better on this task, these differences in alertness are unlikely to have affected learning outcomes.

4.3.2 MST performance and sleep architecture

Next, to identify whether the amount of improvement in motor sequence learning after a 12 hour period with sleep was related to particular polysomnographic sleep parameters (see Chapter III section 3.3.3, pg101), a correlational analysis was conducted. High risk individuals showed a significant positive correlation between total sleep time and overnight improvement in the motor sequence task. An analysis of individual sleep stages revealed this to be specifically driven by the total amount of REM sleep ($r=0.56$, $p=0.008$), with no significant correlations identified for the amount of stage 1, stage 2 or stage 3 (combined stage 3 and 4). This correlation was maintained when comparing the amount of REM sleep as a percentage of the total sleep time ($r=0.54$, $p=0.012$). Low risk individuals showed no significant correlations in sleep time and MST improvement (Figure 4.4). MST improvement was not correlated with either the number of spindles or spindle density for either group (spindle number: High risk, $r=0.36$, $p=0.105$, Low risk, $r=0.10$, $p=0.656$; spindle density: High risk, $r=0.08$, $p=0.741$, Low risk, $r=0.01$, $p=0.972$).

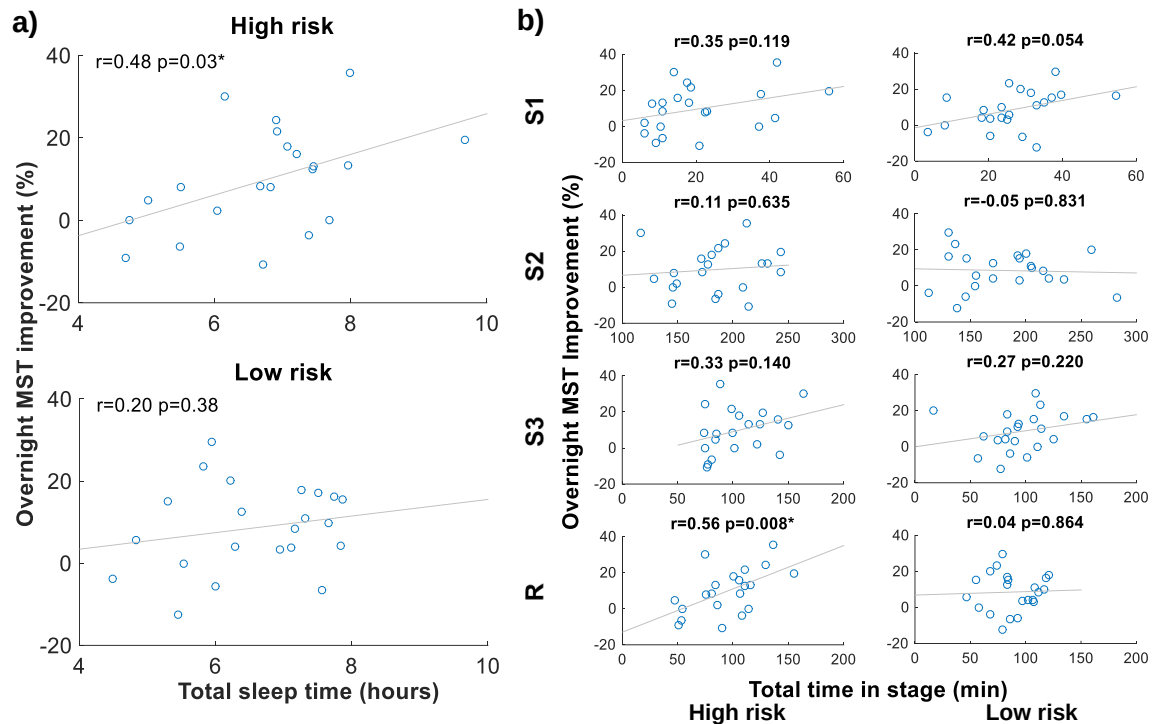


Figure 4.4: Correlation between total sleep time (a) and total time in each sleep stage (b) with the percent of overnight improvement in the motor sequence task (MST). Significant correlations were limited to high risk individuals which showed a positive association in MST improvement and total sleep time, related to the total amount of REM sleep. S1=stage 1, S2=stage 2, S3=stage 3/4, R=REM sleep. High risk, $n=21$; low risk, $n=22$.

4.3.3 No differences in word-pair learning

In the second task a declarative word-pair learning (WPL) task was employed. The average immediate recall accuracy (immediately after training) was $81 \pm 13\%$ in the high risk group and $88 \pm 8\%$ in the low risk group across both word lists. There were no differences in immediate recall accuracy between word lists (Wilcoxon test: $W=835$, $p=0.267$) but a trend towards lower accuracy in high risk groups (average of both lists, Wilcoxon test: $W=157$, $p=0.05$). No sleep effect was identified in either high or low risk groups when re-tested 12 hours later across sleep compared to 12 hours across wake (no difference in recall accuracy; Figure 4.5). In addition, the percentage of words recalled across sleep was not correlated to EEG total sleep time, the amount of time in each stage of sleep or sleep spindle characteristics for either high or low risk groups (Supplementary Table S7).

The task order between the MST and WPL tasks had no effect on the learning of these tasks (MST change with sleep, $t=0.08$, $p=0.93$; MST change with wake, $t=-1.67$, $p=0.10$; WPL change with sleep, $t=-1.66$, $p=0.10$; WPL change with wake, $t=1.37$, $p=0.18$).

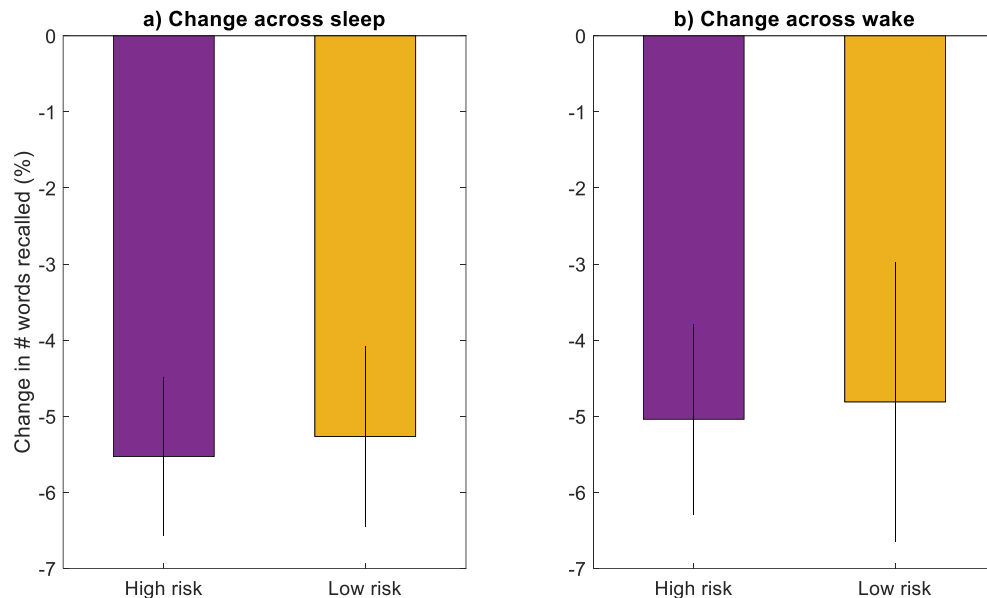


Figure 4.5: Word-pair learning across sleep and wake. No differences were identified in the percentage of words recalled between high and low risk individuals across sleep (two-sample t-test, $t=0.17$, $p=0.867$) or wake (two-sample t-test, $t=0.10$, $p=0.919$). Further, no differences were identified within groups between sleep and wake (High risk, $t=-0.30$, $p=0.767$; low risk, $t=-0.21$, $p=0.836$). High risk, $n=22$, low risk, $n=22$. Bars denote SEM.

4.3.4 Sustained attention

In addition to measures of sleep-dependent memory consolidation, a rapid visual information processing (RVP) task was employed to measure baseline levels of sustained attention. No differences were identified between groups on any measure of this task including the total number of correctly detected target sequences (two sample t-test, $t=-1.54$, $p=0.13$) or the mean latency in detecting these sequences (two sample t-test, $t=0.72$, $p=0.47$) suggesting no attentional differences between groups.

4.4 Discussion

This is the first study to assess measures of sleep dependent memory consolidation in individuals at high risk for developing psychosis. Interestingly, no differences were found in performance of either procedural or declarative learning tasks between high and low risk groups. Further, there was weak evidence for a sleep-dependent nature. However, performance of the motor sequence task was strongly correlated to the amount of REM sleep specifically in high risk individuals.

4.4.1 Sleep-dependent procedural learning

Multiple studies have identified that patients with schizophrenia show significantly worse performance on procedural tasks after a period of sleep compared to both healthy controls and other psychiatric conditions^{124,125,175,178,319,326}. The current study provides no evidence that performance on the motor sequence task (MST) is reduced in individuals at high risk. Several explanations could account for the current findings. Firstly, deficits in SDMC may only become apparent with a clinically relevant pathology, or with substantial chronicity of disease. Alternatively, the lack of objective sleep deficits between high and low risk could also imply that sleep disruption in high risk is not severe enough to cause impairments to memory consolidation. However, the previous studies on SDMC in schizophrenia similarly reported no objective changes in sleep architecture compared to healthy controls^{124,125}. Two factors that contrast to the previous studies on SDMC in schizophrenia are medication use and age related changes. All studies were conducted on chronically medicated individuals and it cannot be discerned what effects medication may have on procedural learning and SDMC. Further, the average age amongst patients ranged between 34 to 45 across studies compared to the average age of 22 in the current study^{124,125,175,319}. Architectural changes in sleep are known to occur with age, including decreased slow wave activity, which may hinder memory consolidation processes. Indeed, previous studies have shown that healthy older adults fail to show sleep dependent improvements

on this task³²⁷. Therefore it is possible patients with schizophrenia are more susceptible to the age related changes in SDMC compared to age matched controls and younger individuals.

Interestingly, despite no overall group differences in performance of procedural learning, the percentage of improvement on the motor sequence task in high risk individuals was positively correlated to total sleep time and specifically the amount of REM sleep. Previously, Wamsley *et al* and Manoach *et al* found that the motor sequence task correlated to NREM stage 2 sleep, and to both sleep spindle number and density in patients with schizophrenia^{75,125}. However several other studies in healthy individuals have shown improvements in procedural memory, including the finger tapping task, correlated with REM sleep^{325,328,329}. The current findings build upon the hypothesis that procedural memory, with discrete cortical cerebellar and striatal processing, benefits more from REM sleep since it allows locally encapsulated synaptic consolidation to strengthen memories. This is in contrast to hippocampal-dependent declarative memories which may be more reliant on NREM sleep for hippocampal-cortical cross-talk^{72,325}. However both forms of memory likely benefit from both NREM and REM sleep⁷². Perhaps the disparity between the current association with REM sleep and previous association to NREM stage 2 and spindles is a result of a developed pathology such as a dysfunction to thalamocortical transmission in patients, which has not developed in high risk. This possibility is further supported by the lack of changes in spindle density identified in the high risk group (see Chapter III section 3.3.3, pg101). Given the hypothesised two-step process in memory consolidation during sleep, abnormalities associated with disease pathology may put greater emphasis on NREM sleep processing, which occurs first, and mask the influence of REM sleep³³⁰.

Further to this, and perhaps most interestingly, the correlation with REM sleep was only identified in the high risk group. Remarkably, several previous studies have identified a

significant association between various sleep parameters and task performance that was only found in the schizophrenia group, and not controls. Specifically, decreased spindle density¹²⁵ and a weaker phase relationship between spindles and slow waves³³¹ has been associated with poorer performance for the MST task, and decreased slow-wave sleep has been associated with poorer performance of visuospatial memory³²⁶, specific to patient groups. Wamsley *et al* suggest the lack of correlation in the control group could be due to the restricted range of overnight improvement and spindle parameters in the controls compared to the patient group¹²⁵. In the current study, both high and low risk groups exhibited a similar range of MST improvement and REM sleep duration rendering this explanation unlikely. Alternatively, the difference between high and low risk groups could be that high risk individuals have less resilience and are more vulnerable to the effects of changes in sleep. Therefore, having less REM sleep could have a greater impact on SDMC in high risk individuals. An obvious limitation of these findings is that they are based upon correlative evidence which cannot determine the causative nature. Further work such as using sleep deprivation specific to NREM and REM sleep, or pharmacological agents to manipulate particular sleep oscillations could help disentangle these processes.

4.4.2 Sleep-dependent declarative learning

No difference in declarative word-pair learning was identified between high and low risk groups and there was no evidence for a sleep effect. Göder *et al* have shown from several studies, that patients with schizophrenia have deficits in performance for declarative tasks across sleep which have also been associated with lower amounts of slow-wave sleep and spindle density^{177,326,332}. Since performance in the current study was not correlated to any sleep parameters in either low or high risk groups, it is difficult to discern the potential importance of particular sleep parameters in this process. For example, memory consolidation of declarative memories during sleep is most strongly thought to rely upon

hippocampal to cortical transfer, facilitated by NREM oscillations including sleep spindles^{68,72}, but there was no association in this study. It is therefore possible that this association only becomes apparent with pathology, and high risk individuals have not developed this dysfunction.

A confounding factor in the present study could be the difficulty of the task. Seeck-Hirschner *et al* assessed declarative memory between wake conditions and NREM sleep naps across three groups: individuals with schizophrenia, major depression and healthy controls¹⁷⁸. Interestingly performance on the Rey–Osterrieth Complex Figure Test, revealed a significant sleep effect in both diagnosis groups but not the healthy controls. As the authors suggest, since there was a clear difference in recall on the task between groups, with the schizophrenia group showing lowest performance, the lack of sleep effect in controls could be caused by a ceiling effect, with the task being too easy. Indeed, in a similar study using a different declarative task Göder *et al* instead identified a sleep effect specific to healthy controls with no significant differences between sleep and wake conditions in patients with schizophrenia¹⁷⁷. In the current study the average baseline learning was as high as 88% in the low risk group compared to 74% in a previous study employing a similar finger tapping/word-pair learning protocol in healthy individuals³¹⁷. This issue could also explain the small degree of change in performance after either sleep or wake and further work would be required, either using different declarative tasks, or increasing the difficulty, to clarify these findings.

4.4.3 Is there a dependence on sleep?

This study provided inconclusive evidence of the importance of sleep for either declarative or non-declarative learning, and only two out of the four AM-PM groups showed a definite sleep effect. Only one out of the four published studies of sleep-dependent MST learning in schizophrenia included a wake control condition^{124,125,175,319}. In this study Manoach *et al*.

identified no improvement across either the sleep or wake interval in patients with schizophrenia. However, in contrast, healthy controls showed a significant improvement on performance of the task in both the sleep and wake condition¹²⁴. In another study using a different procedural task, Ramakrishnan *et al.* identified no sleep effect in schizophrenia patients, however without a control group it is difficult to draw conclusions on whether this was related to pathology or instead a lack of sleep dependence³³³. Interestingly, one study on a student population identified enhanced performance across nocturnal sleep compared to a diurnal wake condition, but no differences to nocturnal wakefulness, suggesting that circadian phase is also contributory³³⁴. Overall these findings challenge previous literature of the importance of sleep in memory consolidation, or at least highlight that very specific experimental conditions must be present to reveal the effect of sleep.

It is therefore useful to consider other alternatives for the lack of deficits in SDMC in high risk individuals or the presence of deficits in schizophrenia. For example deficits in SDMC in schizophrenia may be more related to the general cognitive impairments associated with the disorder, producing deficits across both sleep and wake³³⁵. Seeck-Hirschner *et al* and Goder *et al* both employed sleep and wake conditions identifying a sleep effect for both procedural and declarative learning in healthy controls but not patients with schizophrenia^{177,178}. However, although the schizophrenia group did not show this improvement, their performance across both sleep and wake was lower than controls. In general, procedural learning is known to be impaired in schizophrenia³³⁵ and although baseline learning is usually comparable between patients and controls, the ability for patients to improve may not be comparable. There is inconsistent evidence on procedural learning impairment during early stages of psychosis and high risk and perhaps these milder symptoms, or lack of pathology, can account for normal performance^{336–338}. Nevertheless, sleep-dependent memory consolidation is a well-established finding⁷³. Deficits in SDMC in schizophrenia are therefore most likely a combination of both

psychiatric symptoms and sleep and circadian rhythm disruption which essentially results from an underlying shared neuropathology¹¹⁷.

4.4.4 No deficits in sustained attention

Finally, high risk individuals showed no deficits in sustained attention. Although attentional impairments are a strong characteristic of schizophrenia³²¹, previous work has revealed inconsistent findings in UHR groups^{339,340}. It has been shown that attentional deficits in schizophrenia are associated with various sleep parameters including less slow wave sleep and decreased spindle density^{181,322}. The lack of objective sleep disturbances in high risk could therefore explain the normal levels of attention and it seems likely that attentional impairment manifests with more severe symptoms. Further work is needed to discern the interplay between sleep processing and attention and how this may become pathological in psychotic disorders.

4.4.5 Limitations

Certain limitations should be addressed for the interpretation of these findings. Firstly, the cohort selected in the current study may not be specific enough for those who develop psychosis (see Chapter III section 3.4.2, pg115) and, being largely University of Oxford students, may not be representative of the general population. Furthermore, given the small sample size (especially using a within-participant design) and potential ceiling effects from the declarative learning task, the interpretation of these results requires some caution. It is also not possible to determine the effects of interference on memory consolidation as participants were not controlled for activities during the day. A larger sample size, the use of different learning tasks and a wider cohort could strengthen these findings.

4.4.6 Impact of findings and future directions

This study identified no deficits in measures of sleep-dependent memory consolidation or sustained attention in individuals at high risk for developing psychosis. Having a high load of risk factors in combination with sub-clinical symptoms does not imply a premorbid state for these cognitive impairments. Instead they likely develop with a greater level of symptom severity, sleep disturbance and ultimately pathology. However, a correlation between procedural learning and REM sleep was found specific to high risk individuals which could suggest a vulnerability to sleep disruption at this stage. Overall, further work is needed to understand the links between sleep processes and memory consolidation which largely requires a movement away from solely correlative evidence. For example, the use of eszopiclone to increase spindles³⁴¹ or transcranial direct current stimulation to manipulate slow-wave activity³⁴² have been used in two pioneering studies that have attempted to directly identify whether enhancing particular sleep processes can improve memory consolidation in schizophrenia. Further, investigating particular oscillations during natural sleep, such as theta oscillations during REM or sleep spindles during NREM, and gaining a greater basic understanding of their spatial and temporal organisation could help us better understand their functional role (if any) in memory consolidation, and ultimately why it is impaired in schizophrenia.

Chapter V: Hidden cortical spindles during sleep in mice

Contents

5.1 Introduction	144
5.1.1 The function of sleep spindles	144
5.1.2 The spatio-temporal dynamics of sleep spindles	145
5.1.3 Study aims	146
5.2 Methodology	146
5.2.1 Animals and surgical procedures	146
5.2.2 Signal processing and analysis of vigilance states	149
5.2.3 Sleep spindle detection	150
5.2.4 Data analysis	154
5.3 Results	156
5.3.1 Spindles are local events within the somatosensory cortex	156
5.3.2 Sleep spindles are absent in parts of the SCx	157
5.3.3 The temporal dynamics of distinct spindle events	159
5.3.4 DS LFPs show NREM-like activity during REM sleep	163
5.3.5 Spiking activity from both DS and IS channels are related to spindle events	165
5.4 Discussion	167
5.4.1 The spatial occurrence of spindles	167
5.4.2 The role of spindles in memory consolidation	168
5.4.3 Spindle bursts during development	169
5.4.4 The role of spindles in sleep regulation	169
5.4.5 Sleep as a local unitary state	171
5.4.6 Impact of findings	171
5.4.7 Limitations	172
5.4.8 Summary	172

Foreword: For this project, all surgeries and collection of the EEG/LFP data were conducted by Laura McKillop, Simon Fisher, Sibah Hasan, and Ashna Ramkisoensing. Vladyslav Vyazovskiy performed spike sorting analysis of MUA data. I carried out the histological analysis, sleep scoring, created the spindle detection algorithm, and performed all data and statistical analysis.

5.1 Introduction

5.1.1 The function of sleep spindles

The sleep spindle is a hallmark neuronal oscillation of non-rapid eye movement (NREM) sleep and is believed to be one of its critically important features. Sleep spindles manifest as periodic, waxing and waning bursts of activity between 8-16Hz which are generated from the thalamus and are propagated to the cortex where they are observed on an electroencephalogram (EEG; see Chapter I section 1.2.4, pg21)^{64,66,343}. Sleep spindles are known to decline with age³⁴⁴, and abnormalities in spindle activity are associated with several pathological conditions including epilepsy³⁴⁵, Alzheimer's disease³⁴⁴ and schizophrenia¹⁷⁶. As discussed in Chapter III, they may also be present prior to the onset of disorders and thus represent a phenotypic trait associated with an underlying neuropathology¹⁸⁰. However, the precise function of the sleep spindle remains elusive, with mostly correlative and sometimes contradictory evidence suggesting a role in the gating of sensory information and sleep continuity^{182–184,346}, cortical development¹⁸⁵, synaptic plasticity^{186,188} and memory consolidation¹⁸⁷.

The role of sleep spindles in memory consolidation has garnered extensive interest and numerous studies have shown an association of spindle activity with learning in both declarative^{347,348} and procedural tasks^{349,350} as well as predicting their overnight improvement^{347,351,352}. This evidence has been extended to rodents using associative learning paradigms^{353,354} and deficits in spindles, such as those identified in patients suffering from schizophrenia, have also been associated with impairments in sleep dependent learning (see Chapter IV)^{124,125}. For spindles to be actively involved in memory consolidation, the spatial-temporal pattern of spindle occurrence during sleep should not be random, but instead reflect preceding learning experience and involve specific functionally related cortical circuits. The prefrontal cortex is the area which is typically investigated in this context, and conclusions are often based on spindles recorded with

gross EEG, which precludes reliable localisation of the occurrence of individual spindle events across the rest of the neocortex.

5.1.2 The spatio-temporal dynamics of sleep spindles

Arguably, understanding the spatio-temporal pattern of spindle occurrence during sleep is essential for elucidating their function, both for healthy functioning and with pathology. However, existing knowledge of how and when spindles emerge on the EEG or the local field potential (LFP) is surprisingly fragmentary. Temporally, spindles have been shown to have a highly coordinated interaction to other major network oscillations such as slow oscillations (SO) and sharp-wave ripples with a particular association to SO up-states, where large neuronal populations become depolarised^{68,355,356}. Within a NREM episode, spindles change in frequency between light and deep sleep³¹¹, increase in density during NREM to REM transitions^{357,358} and most recently have been shown to occur periodically following an infraslow oscillation of 0.02Hz in both mice and humans³⁵⁵. On a longer time-scale, existing evidence in humans suggests that sleep spindles undergo both circadian and homeostatic regulation. For example, spindle density is known to show an initial decrease after increased sleep pressure from sleep deprivation^{357,359}.

The temporal pattern of occurrence of spindles is also closely related to their spatial distribution. In humans, sleep spindles have been shown to have distinct spatial characteristics across the cortex with slow frontal (~11-13Hz) and fast centro-parietal (~13-15Hz) classifications which may reflect alternative topographical sources of generation^{88,89}. It is hypothesised that projections from the thalamus that lead to the propagation of cortical spindles are widespread and can be divided into core and matrix pathways. The core pathway is spatially selective and impinges upon middle layers of the cortex. In contrast, matrix projections are often diffuse and synapse to superficial layers of the cortex³⁶⁰. Although the functional significance is largely undetermined, these pathways

may be important for influencing cortical states at both local and widespread spatial scales. Indeed from more recent work on intracranial EEG studies in epileptic patients, it is now known that spindles are local events, rarely occurring across large distributed networks^{84,91}. In rodents, spindles have been recorded in several cortical areas including primary visual, somatosensory, motor and auditory cortices^{92,346,355}. EEG recordings have also identified mostly anterior occurrence of spindles in both rats^{361,362} and mice³⁶³, although the functional significance of this pattern remains unclear and detailed mapping studies of LFP spindles recorded from deep cortical layers are lacking.

5.1.3 Study aims

This study was based upon an initial finding from multi-electrode arrays placed into deep layers of the somatosensory cortex. Highly local spindles were identified that occurred within small circumscribed territories and were often undetectable in neighbouring recording channels just a fraction of a millimetre away and further, were absent in the EEG. The aims of this study were therefore to characterise these distinct territories where spindles occur in terms of their spatio-temporal dynamics and relationship across vigilance states, with sleep homeostasis, and to the global EEG.

5.2 Methodology

5.2.1 Animals and surgical procedures

Animals

Experiments were performed in adult male C57BL/6J mice (Harlan UK Ltd, Bicester, United Kingdom, N=11) aged between 3-5 months. Mice were individually housed in custom-made clear plexiglass cages (20.3 x 32 x 35 cm) with free access to a running wheel and food and water available *ad libitum*. Cages were housed in ventilated, sound attenuated Faraday chambers (Campden Instruments, Loughborough, UK, two cages per chamber) under a 12:12 hour light-dark cycle (lights on 0900, ZT0, light levels ~120-180

lux). Room temperature and relative humidity were maintained at $22\pm 1^{\circ}\text{C}$ and $50\pm 20\%$ respectively. Mice were habituated to both the cage and the recording cables for a minimum of four days prior to recording. All procedures conformed to the Animal (Scientific Procedures) Act 1986 and were performed under a UK Home Office Project Licence in accordance with institutional guidelines.

Surgical procedures

Surgical procedures were carried out using aseptic techniques under isoflurane anaesthesia (3-5% induction, 1-2% maintenance). During surgery animals were head fixed using a stereotaxic frame (David Kopf Instruments, CA, USA) and liquid gel (Viscotears, Alcon Laboratories Ltd, UK) was applied to protect the eyes. One day prior to surgery animals received dexamethasone (0.2 mg/kg, intraperitoneal) to suppress the local immunological response. Metacam (1-2 mg/kg, subcutaneous (s.c.), Boehringer Ingelheim Ltd, UK) and dexamethasone (0.2 mg/kg, s.c.) were administered preoperatively (and for at least three days after surgery). Post operatively animals were administered saline (0.1ml/20g body weight, s.c.) and provided thermal support throughout and following surgery. A minimum two week recovery period was permitted prior to cabling the animals for recording EEG and LFP.

Mice were implanted with a single polyimide-insulated tungsten microwire array (Tucker-Davis Technologies Inc (TDT), Alachua, FL, USA) into deep layers of the somatosensory cortex. The array consisted of 16-channels (2 rows each of 8 wires), the wire diameter was $33\mu\text{m}$, electrode spacing $250\mu\text{m}$, row separation L-R: $375\mu\text{m}$ and tip angle 45 degrees. Customised arrays were used so that the lateral row of wires was longer by $250\mu\text{m}$. A $1\times 2\text{mm}$ craniotomy was made using a high-speed drill (carbon burr drill bits, 0.7mm, InterFocus Ltd, Cambridge, UK) with the midpoint of the craniotomy -1mm anteroposterior (AP) and 3.25mm mediolateral (ML) relative to bregma. The dura was dissected using a 25 gauge needle and saline was applied to the cranial opening to keep

the exposed brain moist. Gelfoam soaked in sterile saline was used in rare cases when removal of the dura caused bleeding. The microwire array was advanced into the brain until the longer row of microwires was at the level of cortical layer 5 (~0.5-0.6mm below the pial surface). A two-component silicon gel (KwikSil; World Precision Instruments, FL, USA) was used to seal the craniotomy and protect the surface of the brain from dental acrylic. After 5-10 minutes, required for the gel to polymerise, dental acrylic was applied to fix the array to the skull. In addition, stainless steel screw electrodes were implanted epidurally over frontal (motor area, M1, AP +2 mm, ML 2 mm) and occipital (visual area, V1, AP -3.5-4mm, ML, 2.5mm) cortical areas contralateral to the array, using procedures previously described³⁶⁴. An additional screw was implanted over the cerebellum to act as a reference and an anchor screw was placed behind or opposite to the array to ensure implant stability. EEG screws were soldered (prior to implantation) to custom-made headmounts (Pinnacle Technology Inc. Lawrence, USA) and all screws and wires were secured to the skull using dental acrylic. Finally, an electromyogram (EMG) was recorded from two single stranded, stainless steel wires inserted either side of the nuchal muscle.

Verification of recording sites

A histological analysis was used to confirm the location of the microwire arrays placed into layers 4/5 of the somatosensory cortex. All arrays were coated with a thin layer of Dil fluorescent dye (DilC18(3), Invitrogen) to facilitate subsequent localisation. Upon completion of the experiments mice were deeply anesthetized and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde solution. Brains were photographed, and the location of the site of implantation was calculated in correspondence with a mouse brain atlas. The brains were then sectioned using a freezing Microtome (Leica, Germany) to produce 50µm coronal slice throughout the region of interest and mounted on slides. Because of well-known anatomical variability between individual animals and since it was not possible to perform functional mapping of cortical regions in this study, the exact

location of the arrays with respect to precise borders or functionally differentiated cortical areas cannot be ascertained.

5.2.2 Signal processing and analysis of vigilance states

Data acquisition was performed using the Multichannel Neurophysiology Recording System (TDT, Alachua FL, USA). Extracellular neuronal spike data were collected continuously (25 kHz, 300 Hz - 5kHz) concomitantly with local field potentials (LFPs) from the same electrodes. Online spike sorting was performed primarily to eliminate artefactual waveforms caused by electrical or mechanical noise. This was performed with OpenEx software (TDT), by manually applying an amplitude threshold for online spike detection. Whenever the recorded voltage exceeded this predefined threshold ($2 \times$ noise level, at least $-25 \mu\text{V}$), a segment of 46 samples (0.48 ms before, 1.36 ms after the threshold crossing) was extracted and stored for later use, together with the corresponding time stamps. Cortical EEG was recorded from frontal and occipital derivations. EEG/EMG data were filtered between 0.1-100 Hz, amplified (PZ5 NeuroDigitizer pre-amplifier, TDT Alachua FL, USA) and stored on a local computer at a sampling rate of 256.9 Hz. LFP/EEG/EMG data were resampled offline at a sampling rate of 256 Hz. Signal conversion was performed using custom-written Matlab (The MathWorks Inc, Natick, Massachusetts, USA) scripts and was then transformed into European Data Format (EDF) using open source Neurotraces software (www.neurotraces.com). For each recording, EEG and LFP power spectra were computed by a Fast Fourier Transform (FFT) routine for 4-s epochs (fast Fourier transform routine, Hanning window), with a 0.25 Hz resolution (SleepSign Kissei Comtec Co, Nagano, Japan).

Vigilance states were scored offline through manual visual inspection of consecutive 4-s epochs (SleepSign, Kissei Comtec Co, Nagano, Japan). Two EEG channels (frontal and occipital), EMG and running wheel (RW) activity were displayed simultaneously to aid

vigilance state scoring. Vigilance states were classified as waking (low voltage, high frequency EEG with a high level of phasic EMG activity), NREM sleep (presence of EEG slow waves, a signal of a high amplitude and low frequency) or REM sleep (low voltage, high frequency EEG with a low level of EMG activity). Great care was taken to eliminate epochs contaminated by eating, drinking or gross movements resulting in artefacts in at least one of the two EEG derivations or in any of the MUA channels. Detailed analyses of neuronal activity (see below) were based on selected time intervals to ensure stability of neuronal waveforms within the time window specified.

5.2.3 Sleep spindle detection

Spindle detection

For seven mice, after visual inspection of LFP data from all 16 channels, prominent clearly defined spindle events could be identified in only a subset of LFPs. From these mice, LFPs were categorised into ‘distinct spindle’ (DS) channels, where spindles were defined as visually clear, unambiguous events (as confirmed by two independent scorers), and ‘indistinct spindle’ (IS) channels, where spindles could not be visually detected. To quantify this observation, spindle events were detected automatically using basic principles from previously reported methods¹⁷⁶ with analysis restricted to NREM and REM sleep. This analysis was further restricted to only include recordings where visually detected spindles (as a gold standard measure) were observed. Since clearly defined spindles could not be visually identified in LFPs for 4 of the 11 mice, this analysis focused on the remaining 7 mice where frontal EEG and (a subset of) LFPs showed visually distinct spindle events.

Preliminary spectral analysis (n=4) of 4-second epochs with visually detected spindles revealed a pronounced peak between 10-15 Hz, which was absent in the epochs without spindles or corresponding epochs recorded from traces in the IS channels (Figure 5.1). Signals were therefore filtered using an IIR band-pass Butterworth filter in a sigma

frequency range of 10-15Hz. Since large amplitude differences were identified between EEG/LFP signals, signals were normalised using a z-score transformation as follows:

$$z = \frac{x - \mu}{\sigma} \quad \begin{array}{l} \text{where } x = \text{signal value} \\ \mu = \text{signal mean} \\ \sigma = \text{standard deviation} \end{array}$$

The instantaneous amplitude was then computed using a Hilbert transform. Spindle events were detected if the instantaneous amplitude surpassed an upper threshold. The start and end of the spindle event was defined as the point at which the amplitude fell below a lower threshold (see below for threshold definitions). Putative spindles were excluded if their duration fell below 0.4 seconds. A maximal interruption interval of <300ms was allowed to account for waxing and waning in the signal (see Figure 5.2a).

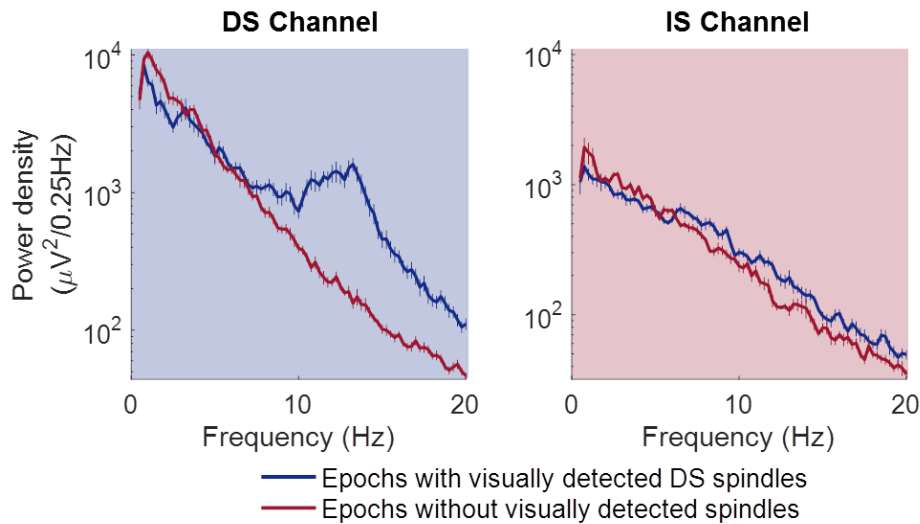


Figure 5.1: Mean LFP power density in 'distinct spindle' DS (left) and 'indistinct spindle' IS channels (right) during 4s epochs with and without visually detected spindles in the DS channel. Data is taken from a subset of mice (n=4) using 75 epochs with spindles and 75 epochs without spindles (5 minutes each) during NREM sleep. Bars denote SEM.

Defining the spindle detection threshold

Several tests were used to identify suitable thresholds for spindle detection based on subject specific differences in putative spindle characteristics. After visual inspection of LFP data from all 16 channels, prominent clearly defined spindle events could be identified in only a subset of LFPs. For each mouse, a visually 'distinct spindle' (DS) LFP channel and visually 'indistinct spindle' (IS) LFP channel were selected. For each selected channel,

peaks in sigma frequency (10-15Hz) signal amplitude (sigma peaks) were identified across the 12-hour signal during NREM sleep. These sigma peaks were then binned according to the frequency of their occurrence and the two channels were compared.

As shown in Figure 5.2b, a characteristic pattern in the number of sigma peaks between DS and IS channels was identified for all 7 mice. DS channels presented markedly increased occurrences of low amplitude sigma peaks (z-score between 0~2) and high amplitude sigma peaks (z-score above ~3) compared to IS channels. In contrast IS channels presented markedly increased occurrences of mid amplitude sigma peaks (z-score between 2~3) compared to DS channels. Furthermore, this pattern was also observed when comparing frontal EEG (similar to a 'DS' channel) to occipital EEG (similar to an 'IS' channel). These results suggest that the occurrence of sleep spindles is associated with both high and low sigma amplitudes with mid-range sigma amplitudes representative of more general background sigma activity.

Comparing the difference between DS and IS channels (Figure 5.2c) provided a clear-cut definition for threshold optimisation. In this study, the lower threshold was defined as the point of greatest difference in mid amplitude sigma peaks between DS and IS channels (i.e. the point at which sigma peaks with larger amplitudes in IS channels begin to decrease compared to DS channels). The upper threshold was defined as the intersection where the occurrence of high amplitude sigma peaks was greater in the DS channel.

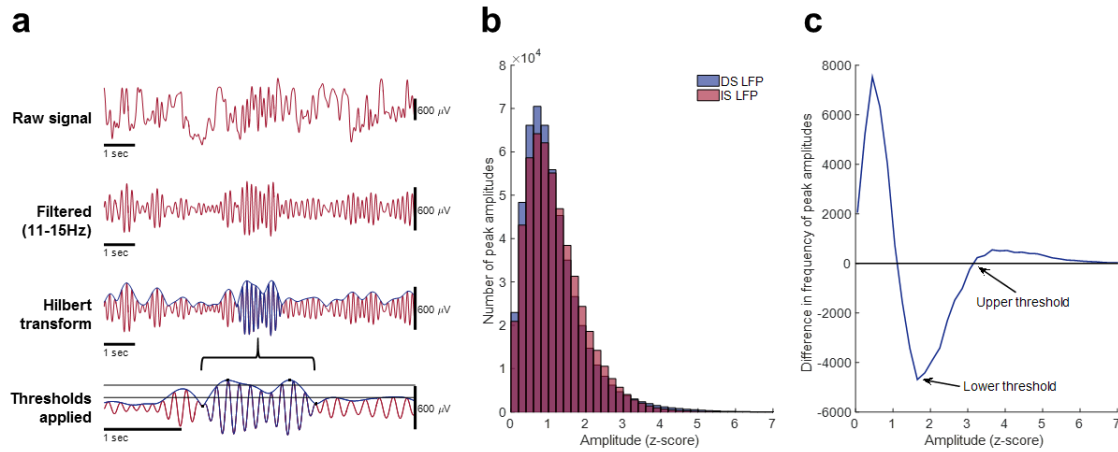


Figure 5.2: Spindle detection and threshold identification. (a) Spindle detection: Raw signals were band-pass filtered between 10-15Hz and the instantaneous amplitude was extracted using the Hilbert transform. Peaks exceeding an upper threshold were used to detect spindle events. The start and end of the spindle event was defined when the amplitude fell below a lower threshold. (b) A representative example comparing the occurrence of sigma range (10-15Hz) amplitude peaks between a 'distinct spindle' (DS) and 'indistinct spindle' (IS) LFP channel. (c) The difference between DS and IS LFPs in the number of sigma peaks at varying amplitudes allowed identification of thresholds for spindle detection.

For 6 out of the 7 mice, using the frontal and occipital EEG as a DS and IS channel respectively, successfully replicated the peak sigma frequency differences and thresholds were subsequently selected. For the remaining mouse, the occipital EEG channel was omitted due to poor signal and the upper and lower thresholds were calculated based upon averages from the other mice (3.36 and 1.86 SD's above mean respectively).

To validate the performance of the algorithm, automatic spindle detection was compared with visual detection from the first or second hour after light onset for all 7 mice where a distinct spindle channel could be identified. The automatic algorithm produced a mean sensitivity of $80 \pm 11\%$ and false discovery rate of $54 \pm 12\%$ in line with several other algorithms used in previous research²⁸¹.

5.2.4 Data analysis

Spindle analysis

Spindle characteristics were calculated as follows: spindle density, the number of spindles detected per minute of NREM sleep; spindle duration, the difference between spindle start and end; spindle amplitude, the maximum signal amplitude within a detected spindle event; spindle frequency, the number of sigma filtered (10-15Hz) amplitude peaks within a detected spindle divided by the duration of the spindle. Spectrograms of individual spindle events were computed using 2 second intervals around the spindle midpoint. A short-time Fourier transform was applied using a 500ms window length with a 95% overlap at a frequency resolution of 0.25Hz. The average power spectral density was calculated from all detected spindles within each mouse for IS and DS channels separately.

Analysis of extracellular neuronal activity

To investigate the relationship between LFP activity and individual putative single units' firing, an offline spike sorting procedure was used as previously described⁶⁰. Briefly, an artefact removal procedure was implemented in order to eliminate remaining artefactual waveforms and to facilitate subsequent clustering. After removal of the artefactual spikes, principal components were computed using principal component analysis (PCA) on a segment of the spike waveform between the 5th and the 35th time sample, because this is the segment that contains the initial negative peak after threshold crossing and subsequent after hyperpolarization, and is, therefore, more informative about the overall spike waveform. After performing PCA, each spike between samples 5 and 35 was thus described by 31 variables, each being a linear combination of the original sampling values. Clustering was then performed based on the k-means algorithm³⁶⁵. This is a partitioning method that aims to divide n observations into k clusters in which each observation belongs to the cluster with the nearest mean, serving as a prototype of the cluster. All selected clusters were screened and visually classified according to their signal to noise

ratio, waveshape of the action potential, stability of the amplitude across time and ISI distribution histogram, and spurious or unstable clusters were excluded from the analysis. Since putative single units varied greatly in absolute firing rates from <1 Hz to more than 60 Hz, in most cases average absolute values were avoided. In this case, mean values would be biased by high-frequency spiking putative single units, even if slow-spiking neurons show relatively more pronounced changes. One way to account for this is to express firing rates of putative single units as a percentage of their mean firing rates across all artefact free epochs as has been done for all analyses.

LFP waves and single unit firing

To compare putative single-unit activity between DS and IS channels during sleep oscillations, LFP signals were filtered (IIR bandpass Butterworth filter) between 0.5-4Hz for detection of slow waves during NREM sleep. Waves were detected as positive deflections of the filtered LFP signal between two consecutive negative deflections below the zero-crossing. Only LFP waves with the peak amplitude larger than the median amplitude across all detected waves within the same vigilance state were included. Sigma waves (10-15Hz) were based upon detected spindle midpoints.

Statistical analysis

Unless specified otherwise in the results, analysis was based upon one representative DS and IS channel from each mouse. Data were analysed using Matlab (The Math Works, Inc., Natick, MA, USA) and R (R Core Team, Vienna, Austria). ANOVAs and two-sample t-tests were used to identify differences between channels. Wilcoxon signed ranked tests were used for non-normal data where appropriate. Two-way repeated measures ANOVAs were used to identify differences in spindle frequency activity and slow wave activity across time. All values reported are mean $\pm$ s.e.m.

5.3 Results

5.3.1 Spindles are local events within the somatosensory cortex

Traditionally, waking, NREM sleep and REM sleep are distinguished by characteristic cortical activity. In rodents, both waking and REM are typically assumed to involve low voltage, mixed or high frequency EEG and LFP oscillations and a predominance of activity in the theta frequency range (6-9Hz). In contrast, during NREM sleep, the EEG and LFP are commonly differentiated by high voltage slow waves (0.5-4Hz) and sleep spindles (8-16Hz). In this study an array of 16 microwire electrodes were implanted for chronic multichannel recordings of LFPs and multi-unit activity (MUA) from deep layers of the somatosensory cortex (SCx; Figure 5.3a). All mice (N=11) showed typical EEG signatures of waking, NREM and REM sleep (representative animal: Figure 5.3b/c). However, in a subset of animals (n=7), visual inspection of LFPs during NREM sleep revealed a striking occurrence of clearly distinct local spindle events that were present in some recording channels, with no visible sign of spindles just a few hundred micrometres away. This finding was unexpected and suggested that there may be other factors beyond merely sleep state that determine spindle occurrence even within a small cortical area. To investigate this aspect further, LFPs in these animals were categorised into 'distinct spindle' (DS) channels, where spindles were defined as visually clear, unambiguous events (as confirmed by two independent scorers), and 'indistinct spindle' (IS) channels, where spindles could not be visually detected (individual examples: Figure 5.3d/e). Mapping the position of DS and IS channels revealed a tendency towards more anterior clustering of the former within the primary somatosensory cortex (Figure 5.3f). The DS channels were encountered less frequently as compared to IS channels (DS: 5.0 ± 0.49 ; IS: 10.7 ± 0.7 channels per animal, $p < 0.05$, Wilcoxon signed-rank test).

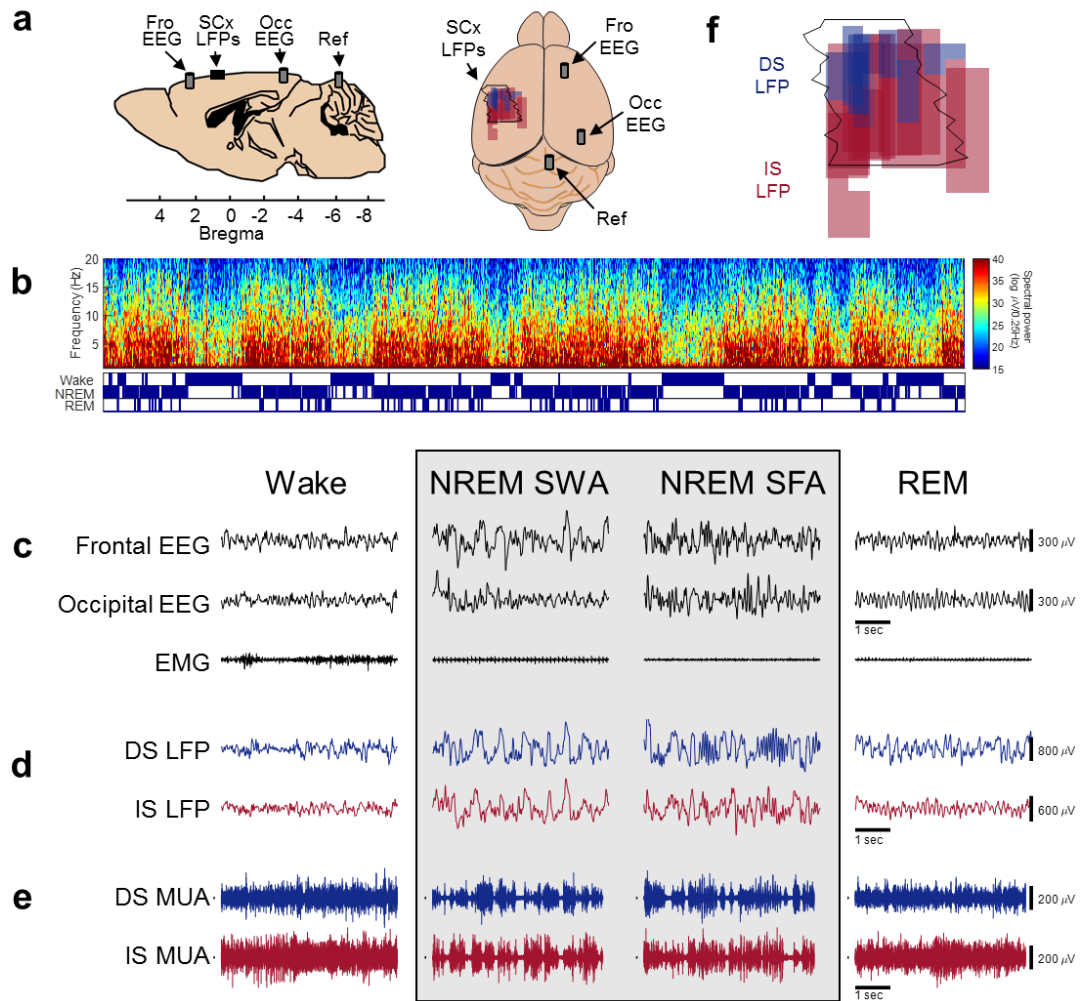


Figure 5.3: Electrode positions and recordings of cortical activity during wake and sleep. (a) A 16-ch microwire array was implanted into infragranular layers of the somatosensory cortex (SCx) to record local field potentials (LFP) and multi-unit activity (MUA) in addition to three stainless steel screws implanted epidurally over frontal and occipital cortical areas, referenced to the cerebellum. (b) A representative spectrogram and corresponding hypnogram from one mouse during a 12 hour undisturbed light period. (c) A 5 second representative example from electroencephalogram (EEG) and electromyogram (EMG) recordings in one mouse during wake (high frequency activity and high muscle tone), NREM sleep with high slow-wave activity (SWA; 0.5-4Hz), NREM sleep with high spindle-frequency activity (SFA; 11-15Hz) and REM sleep (with high theta activity, 6-9Hz). (d) LFP recordings from 2 of the 16 microwires and (e) corresponding MUA. Note the visually distinct spindle events during NREM SFA in the 'distinct spindle' (DS) LFP and absence in the 'indistinct spindle' (IS) LFP just 750 μm posterior. (f) Spatial distribution of DS and IS LFPs between all 11 mice measured from the approximate position of each microwire array centred on the SCx (outlined in black, and shown on the brain in (a)).

5.3.2 Sleep spindles are absent in parts of the SCx

To further explore the occurrence of visually distinct spindles using objective criteria, an automated spindle detection algorithm was applied to the LFP signals filtered between 10-15 Hz. Since spindles could not be visually detected in IS channels, automatic detection was restricted to DS channels. An average of 7.65 ± 2.52 spindles per minute of NREM sleep were detected in DS LFPs with corresponding IS LFPs showing a complete lack of

spindle activity (individual examples: Figure 5.4a). As expected, average LFP power spectra calculated for epochs with automatically detected spindle events in the DS LFPs presented markedly higher spectral power specifically in the frequency range between 11-15Hz (Figure 5.4b, top). Concurrently, IS LFPs showed an absence of SFA increase during the same epochs (Figure 5.4b, bottom).

Next, based on the well-known temporal coupling between spindles and slow-wave oscillations, which often precede or succeed spindle events³⁵⁶, spectrograms for individual putative spindle events detected in DS channels were calculated. A prominent increase in LFP power within sigma frequency range (10-15 Hz) was associated with a suppression of low power in the slow wave frequency range (0.5-4Hz). In contrast, the same time period in IS channels showed no such increase in sigma power and instead showed a continuous level of LFP activity in the slow frequency range (Figure 5.4c). Overall these analyses suggest that true spindle events are indeed restricted to a small circumscribed territory of the SCx.

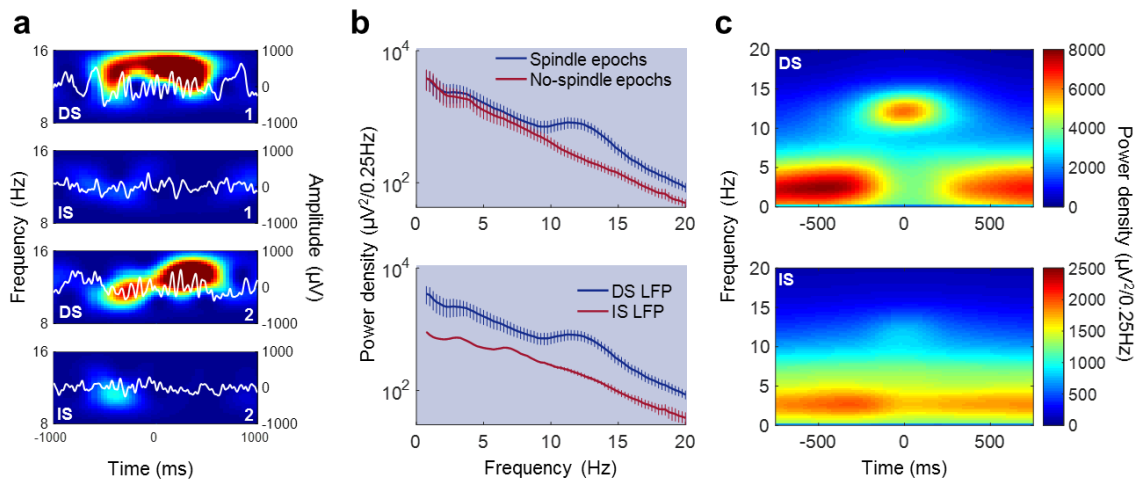


Figure 5.4: Spindle frequency activity in 'distinct spindle' (DS) and 'indistinct spindle' (IS) channels. (a) Representative examples of automatically detected spindle events in DS channels and corresponding data during the same time period in IS channels. Example 1 shows a typical, well defined high frequency spindle event (~13-15Hz) in a DS channel which is preceded and succeeded by a slow (0.5-4Hz) delta wave. Example 2 shows a spindle which develops with increasing frequency. Note the complete lack of sigma frequency activity in terms of both spectral power and amplitude in IS channels. (b) Mean LFP power density in DS channels during 4s epochs with or without detected spindles (top) and mean LFP power density in DS and IS channels during 4s epochs with spindles detected in DS channels (bottom). Bars denote SEM. N=7. (c) Average spectrogram aligned to detected spindle midpoints in DS channels (top) with respective spectrogram during the same time window in IS channels (bottom). Note the difference in spectral power scales between DS and IS channels. All data is during NREM sleep over a 12 hour light period. N=7.

5.3.3 The temporal dynamics of distinct spindle events

As sleep progresses, the concurrent dissipation of sleep pressure is associated with characteristic changes in underlying cortical activity^{366,367}. This is manifested in a progressive decrease in slow wave activity (SWA, 0.5-4 Hz) but is also accompanied by an increase in spindle frequency activity (SFA) as sleep endures^{357,359}. The functional significance of the reciprocal relationship between SWA and SFA across sleep is still largely unknown, but highlights a potentially important physiological relationship between these two types of network oscillations for sleep regulation. To compare the time course of SWA and SFA between LFP and EEG channels, average SWA/SFA was calculated based upon 2 hour intervals across the 12 hour recording period. Consistent with this, a declining trend of SWA across the 12-h light period was identified in LFP channels, including both those with or without spindles (two-way RM-ANOVA; time: $F(5,66)=32.34$, $p<0.001$, DS vs IS: $F(1,66)=0.00$, $p=1.00$, time.DSvsIS: $F(5,66)=1.31$, $p=0.27$; Figure 5.5a, Supplementary Figure S1). This declining trend in SWA was also identified in the EEG (time: $F(5,60)=24.79$, $p<0.001$, Fro vs Occ: $F(1,60)=0.00$, $p=1.00$, time.FrovsOcc: $F(5,60)=0.81$, $p=0.551$). The magnitude of change in SWA was in the range of 40-50% as previously reported^{357,359}. Comparing the rate of decline in SWA between DS and IS LFP channels revealed a significantly faster decline in IS channels (paired t-test, $t=-5.51$, $p=0.002$; Figure 5.5b).

Also consistent with earlier reports³⁵⁷, a significant increase in spindle density across time was observed in both the DS LFP and frontal EEG channels, but there were no differences between channels (two-way RM-ANOVA; time: $F(5,66)=9.95$, $p<0.001$, DS vs Fro: $F(1,66)=0.001$, $p=0.981$, time.DSvsFro: $F(5,66)=1.27$, $p=0.290$; Figure 5.5c). It has been shown previously in humans that SWA and SFA correlate positively when SWA is low, but negatively when SWA is high^{91,368}. To examine the relationship between SWA and SFA, all 4-sec epochs in artifact-free NREM sleep occurring during the 12-h light period were

subdivided into twenty 5% bins as a function of SWA sorted from lowest to highest values, and corresponding SFA values were averaged for the same epochs prior to calculating means between animals (Figure 5.5d). Pearson's product moment correlations between SFA and SWA were calculated for $SWA < 100\%$ (= below the mean value of SWA) and $> 100\%$ (= above the mean value of SWA). Interestingly, and consistent with human EEG data³⁶⁸, DS LFPs presented a sharp increase in SFA ($SWA < 100\%$, $r=0.94$, $p<0.001$) followed by a decrease ($SWA > 100\%$, $r=-0.94$, $p<0.001$) in relation to increasing SWA. Notably, this relationship was abolished in IS LFPs ($SWA < 100\%$, $r=0.95$, $p<0.001$; $SWA > 100\%$, $r=0.63$, $p=0.093$). The relationship between SFA and SWA in the frontal EEG displayed a similar pattern to DS LFPs ($SWA < 100\%$, $r=0.97$, $p<0.001$; $SWA > 100\%$, $r=-0.70$, $p=0.056$). In contrast, the relationship between SFA and SWA in the occipital EEG, where spindles are typically absent, was similar to the pattern observed in IS LFP channels ($SWA < 100\%$, $r=0.91$, $p<0.001$; $SWA > 100\%$, $r=0.50$, $p=0.210$). Finally, calculating the correlation between SWA and SFA across all 16 LFP channels in a representative animal revealed that the inverted U-shaped curve of SWA vs SFA relationship maps precisely on the channels with visually and automatically detected DS spindle events (Figure 5.5e). This analysis does not only suggest that local cortical areas within the SCx differ with respect to the co-existence and functional relationship between spindles and slow waves but also suggest the latter as a potentially useful objective criterion for identifying 'true' spindle activity.

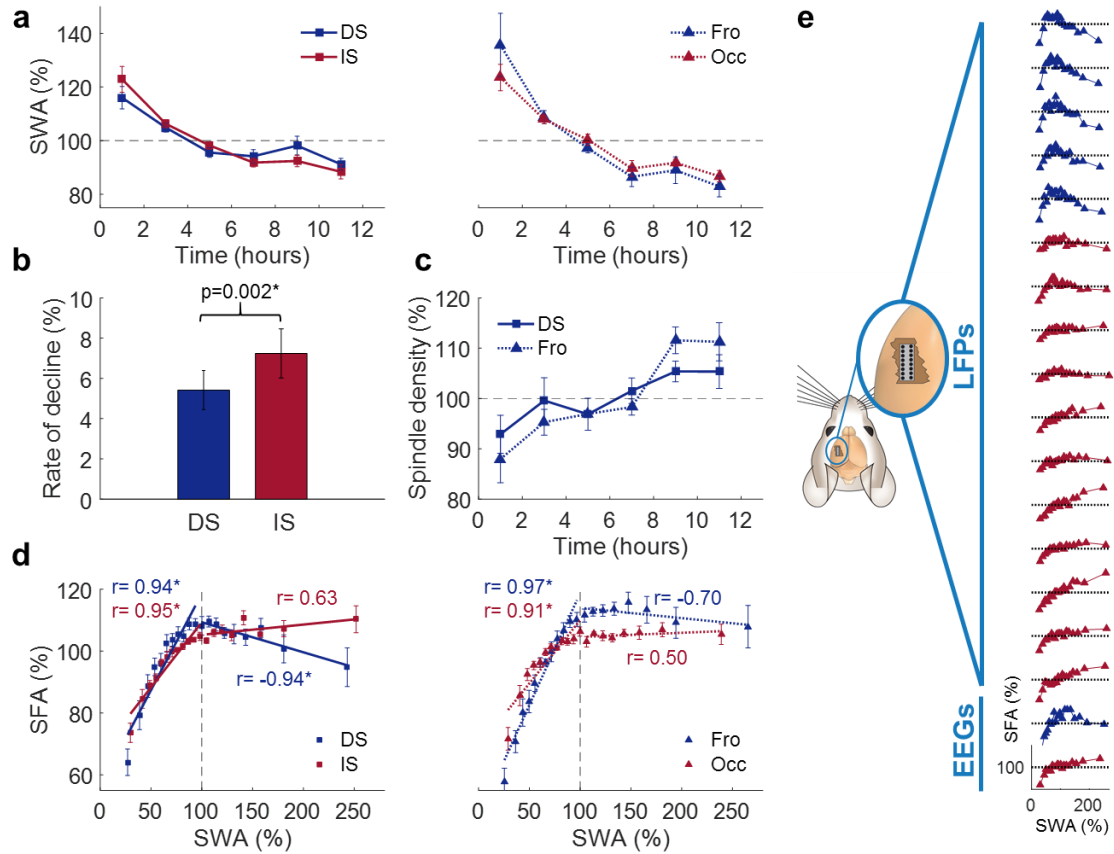


Figure 5.5: The relationship between slow wave activity (SWA, LFP/EEG power between 0.5-4Hz) and spindle frequency activity (SFA, 10-15Hz). (a) Time course of average SWA during NREM sleep plotted for 2 hour intervals across a 12 hour light period from all 16 'distinct spindle' (DS) or 'indistinct spindle' (IS) LFPs (left), and frontal (Fro) and occipital (Occ) EEGs (right). SWA values are expressed as % of mean value during NREM sleep. Bars denote SEM. N=7. (b) Average rate of decline of SWA per 2 hour interval of the 12 hour light period in DS and IS LFPs. SWA decreased significantly faster in IS LFPs (paired t-test, $t=-5.51$, $p=0.002$). Bars denote SEM. N=7. (c) Time course of average spindle density automatically detected in DS LFPs and frontal EEGs plotted for 2 hour intervals across a 12 hour light period. Spindle density is expressed as % of mean spindle density per minute of NREM sleep. Bars denote SEM. N=7. (d) Average SFA as a function of SWA (0.5-4Hz) based on power density during NREM sleep for DS/IS LFPs (left) and frontal/occipital EEGs (right). Straight lines depict linear regressions separately for epochs with SWA < 100% (= below the mean value of SWA) and SWA > 100% (= above the mean value of SWA). R values correspond to Pearson's product moment correlation, asterisks denote significance ($p < 0.05$). Bars denote SEM. N=7. (e) A representative example from one animal of SFA as a function of SWA across all 16 LFPs (top 16 plots, blue=DS channels, red=IS channels) and EEG (bottom 2 plots, blue=frontal EEG, red=occipital EEG). Note the characteristic increase in SFA followed by a decrease as SWA increases in primarily DS and frontal EEG channels compared to a linear increase in primarily IS and occipital EEG channels.

Next, spindles occurring within local cortical areas in the LFP were examined in relation to the global cortical network activity in the EEG. Local spindle events were significantly more common in DS LFPs compared to frontal EEG (average $\pm$ SEM: [DS LFP: 7.65 ± 2.32 /min, frontal EEG: 5.13 ± 0.60 /min]; paired t-test: $t=2.79$, $p=0.031$; Figure 5.6a). From this, spindle events were then separated according to their temporal overlap between the LFP and EEG. Similar to previously used terminology⁸⁴, spindles that overlapped in both the

EEG and DS LFP were classed as 'concordant' spindle events, while those that occurred with no overlap between channels were classed as 'isolated' spindle events (Figure 5.6b). This analysis revealed that isolated, localised spindles detected in DS LFPs were significantly more common than those concordant with EEG (average $\pm$ SEM: [concordant: 2.81 $\pm$ 0.75/min, isolated: 4.85 $\pm$ 1.80/min]; $t=-3.60$, $p=0.011$; Figure 5.6c). Further, isolated spindles remained more common across the light period, but there were no differences in spindle density between isolated and concordant events across time (repeated measures ANOVA, $p>0.05$; Figure 5.6d). Overall these results could suggest that in studies with epidural EEG recordings only, most spindle activity remains undetected.

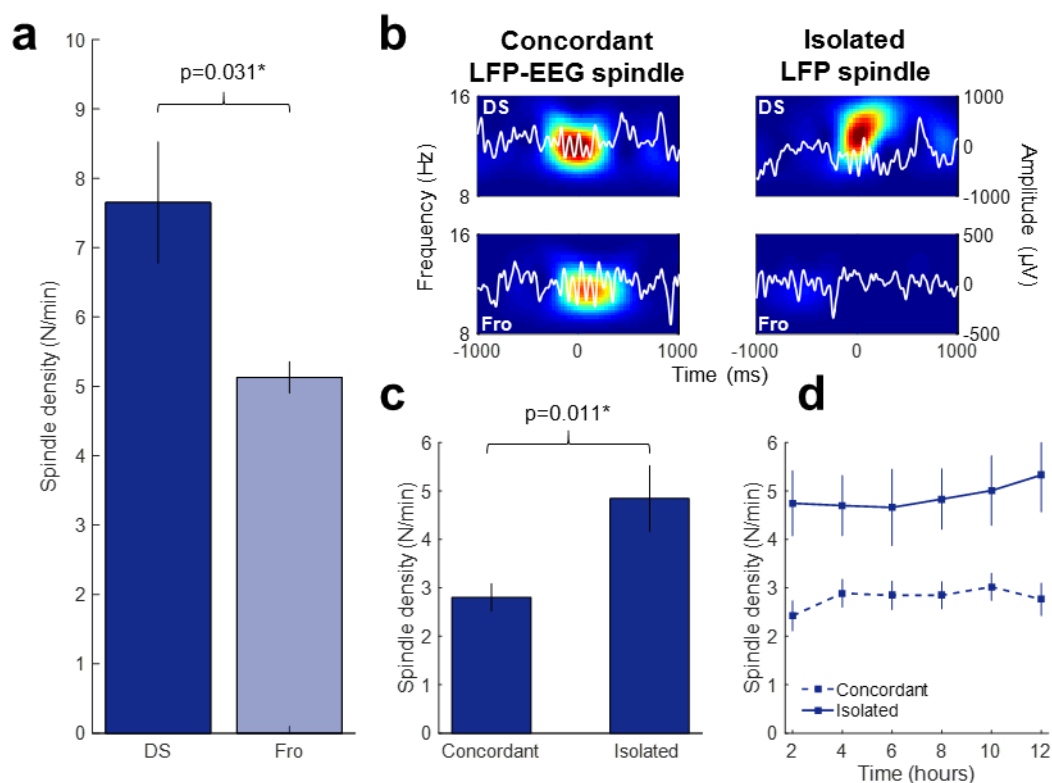


Figure 5.6: The relationship between EEG and LFP spindles. (a) Average spindle density across NREM sleep between 'distinct spindle' (DS) LFPs and frontal (Fro) EEG. DS LFP spindles were significantly more common than frontal EEG spindles (paired t-test; $t=2.79$, $p=0.031$). Error bars denote SEM, $N=7$. (b) Representative examples of a concordant spindle (left) occurring with temporal overlap in both DS LFP channels (top) and frontal EEG (bottom), and an isolated spindle (right) occurring in only DS LFPs with no temporal overlap. (c) Mean spindle density for concordant and isolated LFP-EEG events. The average spindle density was significantly higher for LFP isolated spindles compared to LFP-EEG concordant spindles (paired t-test; $t=-3.60$, $p=0.011$). Error bars denote SEM, $N=7$. (e) Time course of spindle density across the 12 hour light period for concordant and isolated LFP-EEG events. No differences were identified across time for either concordant or isolated spindles (repeated measure ANOVA, $p>0.05$). Error bars denote SEM, $N=7$.

5.3.4 DS LFPs show NREM-like activity during REM sleep

As well known, spindles in rodents are most prominent immediately prior to the NREM-REM transition^{357,358,369}. To assess the dynamics of cortical state at this point, spectral changes specifically during the last 2 min of NREM sleep episodes and the following 1 min or REM sleep was investigated (Figure 5.7). In both DS LFP and frontal EEG channels, a surge of spectral power in the spindle frequency range (10-15Hz) was apparent during the last 30 seconds before transitioning into REM sleep. However, this increase in SFA was not observed in the IS LFPs, as well as in the occipital EEG channels. Interestingly, it was also observed that upon entering REM sleep, IS LFPs and frontal and occipital EEGs showed a characteristic dominance in theta power (6-9Hz) which was completely absent in DS LFPs.

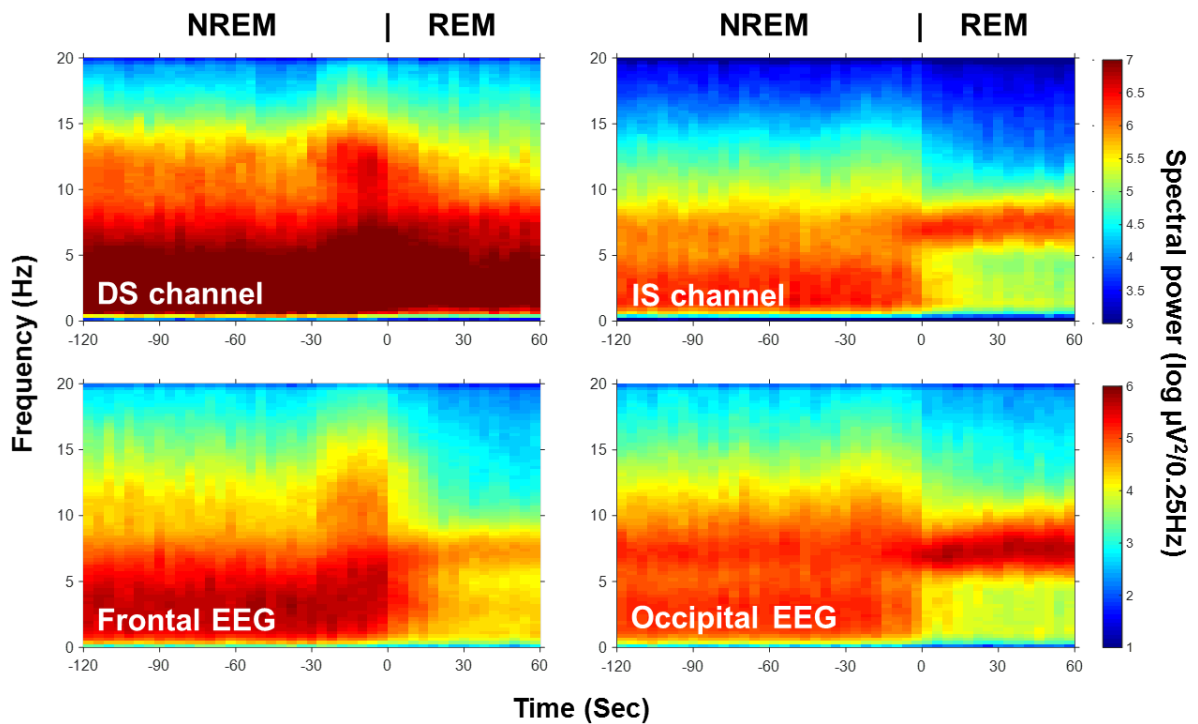


Figure 5.7: Spectral power dynamics during the transition from NREM to REM sleep. Spectrograms show average data from ‘distinct spindle’ (DS) LFP channels (top left), ‘indistinct spindle’ (IS) LFP channels (top right), frontal EEG channels (bottom left) and occipital EEG channels (bottom right). Only REM episodes >1 min were included in this analysis (28.7 ± 4.7 episodes per animal). Two minutes prior to the transition (=0) and one minute after the transition is illustrated based on 4-s epochs (x-axis: in seconds). Power density is color-coded according to the log scale ($\mu V^2/0.25$ Hz) on the right. Note a surge of LFP/EEG power in spindle-frequency range immediately prior to transition in DS LFP and frontal EEG channels which is absent in IS LFP and occipital EEG channels. N=7.

The lack of dominance in theta power upon transition to REM sleep in DS LFPs provoked a further inspection of local network activity during REM sleep. Unexpectedly, visual inspection of DS LFPs showed a persistence of slow waves resembling NREM sleep and a clear lack of distinct theta activity which was visible in EEG and IS LFPs (Figure 5.8a). Calculating the average power density in the three vigilance states (wake, NREM sleep, REM sleep) supported this notion. In all animals in which DS channels were identified, the LFP power spectra were virtually indistinguishable between NREM and REM sleep. In contrast, typical sleep state specific spectra, characterised by pronounced theta-frequency peak, were readily apparent in the IS channels (Figure 5.8b/c, Supplementary Figure 2).

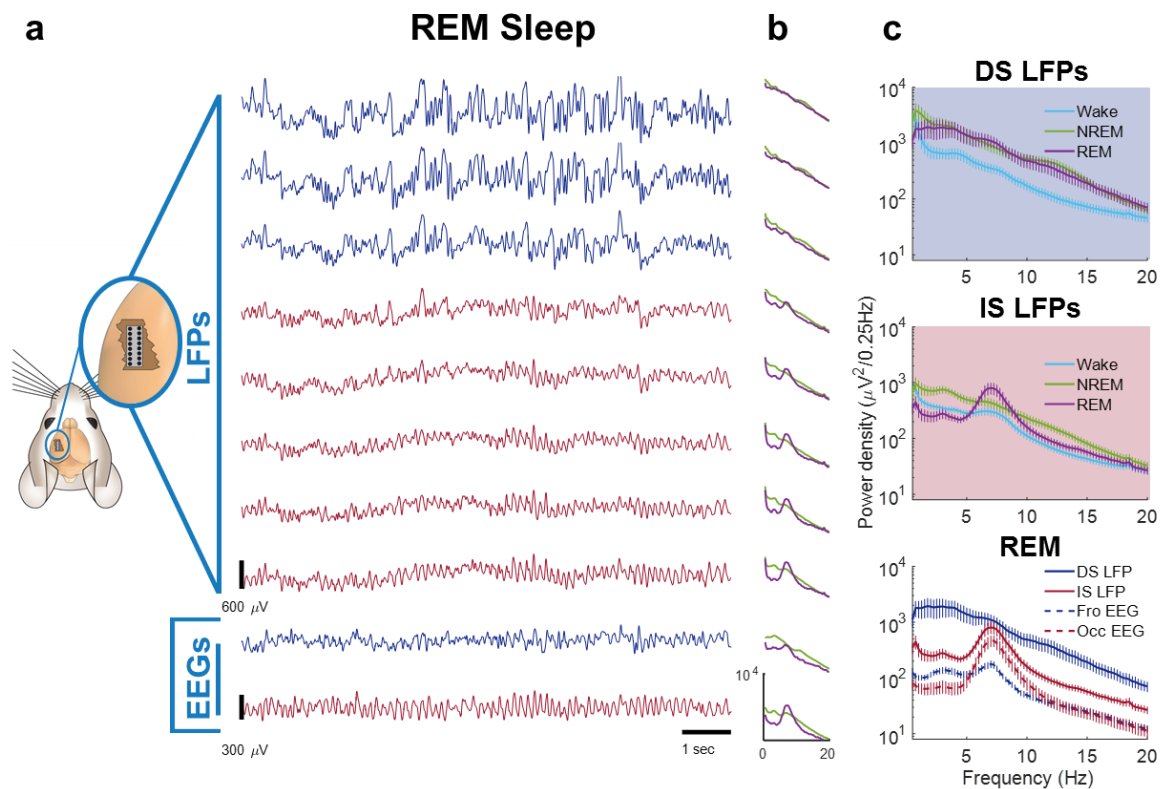


Figure 5.8: Local cortical activity during REM sleep. (a) A representative 10 second example from one mouse showing DS (top 3 LFP channels), IS (bottom 5 LFP channels) LFPs and frontal (top EEG), occipital (bottom EEG) EEGs during REM sleep. (b) Corresponding power density ($\mu\text{V}^2/0.25\text{Hz}$) during NREM (green) and REM (purple) sleep. Note the presence of slow-wave activity in DS channels and lack of increased spectral power within theta frequency range (6-9Hz) during REM sleep. (c) Mean power density across vigilance states in DS LFPs (top), IS LFPs (middle) and in comparison to frontal and occipital EEGs specifically during REM sleep (bottom). Data corresponds to a 12 hour undisturbed light period. Bars denote SEM. N=7.

5.3.5 Spiking activity from both DS and IS channels are related to spindle events

There is evidence that spindles are involved in the modulation of cortical neuronal firing but this is not well established in contrast to the clear activity and inactivity in response to the up and down states of slow waves^{90,91}. The spiking activity of putative cortical neurons was therefore examined during NREM and REM sleep oscillations to identify whether this differed between DS and IS channels. Since the number of DS channels was less than the number of IS channels in each animal, the number of identified putative single units was higher in the latter (4.6 ± 1.6 and 12.7 ± 3.6 putative units per animal in DS and IS channels respectively; $p < 0.05$, Wilcoxon signed-rank test). However, the number of putative neurons recorded per channel was similar between the DS and IS channels (DS: 1.8 ± 0.2 , IS: 1.69 ± 0.2), suggesting that the lack of spindles and overall lower LFP power in the IS channels cannot be accounted for by technical confounds and overall low signal quality. For this analysis a total of 23 DS and 24 IS single-units were selected from the 7 mice with at least 3 units per channel in each mouse. Overall average firing rates were lower in putative neurons from IS channels, but there were no differences between DS and IS channels within wake, REM and NREM sleep (ANOVA; vigilance state: $F(2,36)=18.47$, $p < 0.001$; DSvsIS: $F(1,36)=12.23$, $p=0.001$; vigilance state.DSvsIS: $F(2,36)=0.64$, $p=0.53$; Figure 5.9a). An assessment of MUA before, during, and after individual spindle events detected in the DS channels revealed similar average firing rates between DS and IS putative neurons during spindle events, which was on average 153% higher than mean firing rates across NREM sleep (ANOVA; time period: $F(2,36)=172.19$, $p < 0.001$; DSvsIS: $F(1,36)=0.13$, $p=0.720$; time period.DSvsIS: $F(2,36)=8.37$, $p=0.001$). Firing rates following 500ms after spindle termination were significantly lower in DS compared to IS putative neurons, possibly suggesting a consistent occurrence of a slow wave (post-hoc t-test; $t=-9.85$, $p < 0.001$; Figure 5.9b). From visual inspection, within spindle events multi-unit activity showed clear modulation with successive rhythmic increases and decreases in firing (Figure 5.9c). Calculating average firing rates during all

spindle events confirmed that firing rates of DS putative neurons were clearly modulated by LFP spindle oscillation, showing increased spiking during spindle troughs and a reduction at spindle peaks. In contrast, IS putative neurons showed a similar but attenuated pattern of firing during these DS detected spindle events (Figure 5.9d). Finally, spiking activity was assessed during high amplitude LFP slow waves (0.5-4Hz) during NREM sleep. As expected, in both DS and IS channels neuronal firing was substantially reduced during positive LFP waves, but this was attenuated in IS channels (Figure 5.9e).

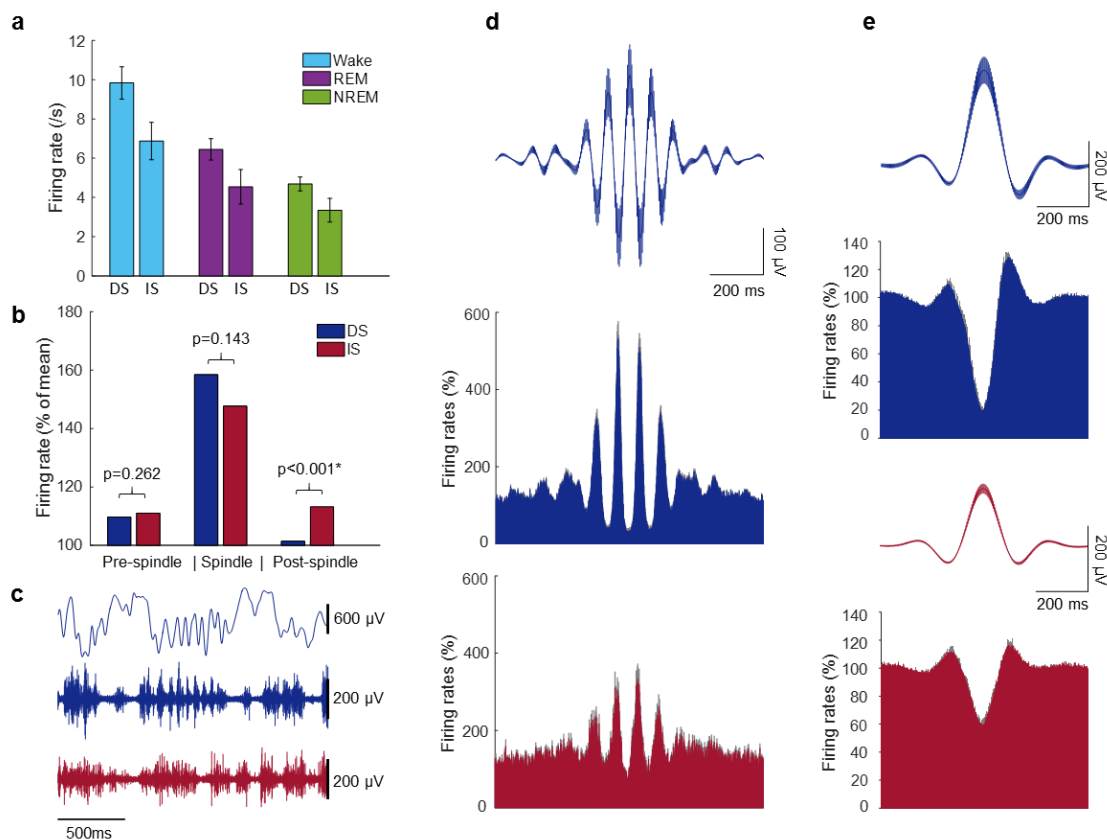


Figure 5.9: Putative neuronal spiking activity in 'distinct spindle' (DS) and 'indistinct spindle' (IS) channels. (a) Average firing rates during wake, REM and NREM sleep. Firing rates were overall lower in IS channels but there were no significant differences within each vigilance state (ANOVA; vigilance state: $F(2,36)=18.47$, $p<0.001$; DSvsIS: $F(1,36)=12.23$, $p=0.001$; vigilance state.DSvsIS: $F(2,36)=0.64$, $p=0.53$). (b) Average firing rates between DS and IS putative neurons during spindle events, 500ms preceding spindle onset (pre-spindle) and 500ms succeeding spindle termination (post-spindle). P-values correspond to post-hoc t-tests (pre-spindle: $t=-1.18$, $p=0.262$; spindle: $t=1.64$, $p=0.143$; post-spindle: $t=-9.85$, $p<0.001$). (c) An example of a visually detected spindle event in a DS channel (top) and corresponding multi-unit activity in the DS (middle) and an IS (bottom) channel. (d) Average local field potential (LFP) during automatically detected spindle events in a DS channel (top) and corresponding average MUA (shown as % mean MUA during NREM sleep) separately for DS (middle) and IS (bottom) channels. (e) Average local field potential (LFP) slow wave recorded during NREM sleep (0.5-4 Hz), and corresponding average MUA (shown as % mean MUA over a 300 ms window before the slow wave) separately for DS (top; blue) and IS (bottom; red) channels. Mean values, $N=7$ mice.

5.4 Discussion

This study is the first to describe the occurrence of spindle events that are highly localised to a fraction of a millimetre within the somatosensory cortex (SCx). These ‘hyper’ local spindle events were common, often remained undetected at the EEG level, showed specific temporal and homeostatic dynamics, and exhibited substantial modulation of neuronal firing rates. Further to this, the occurrence of LFP spindles coincided with unique responses to global vigilance states and state transitions where LFPs showed an absence of response to global theta activity during REM sleep.

5.4.1 The spatial occurrence of spindles

From intracranial EEG studies in humans, it has previously been shown that spindle events are mostly local and occur in region-specific areas throughout the neocortex⁹¹. The current findings extend this and reveal that even within a region, spindle events can occur in a highly localised manner. Although the precise locations of spindle active regions within the somatosensory cortex could not be identified, a dispersed band of occurrence was observed that was clearly anteriorly oriented. Thus, there is an intriguing possibility of localised spindles within somatosensory-motor cortical boundaries which could arise from the localisation of thalamocortical synaptic connections (e.g. core cells) or the activities of specific thalamic reticular nuclei³⁶⁰. Overall, these results indicate that spindles commonly arise from activities of local circuitry and support the importance of local processing in sleep function^{70,93,370,371}.

Further to this, cortical neurons fired in close phase relation to LFP spindle events. Surprisingly previous literature has provided inconsistent results, either showing similar findings^{372,373}, or only weak modulation of cortical neurons to spindle activity^{91,374}. Since LFP data primarily represents input postsynaptic potentials, the clear phase relation to output spiking neuronal activity provides further support for a functional role of spindles in

modulating local circuitry. Determining the nature of this modulation, such as if it is for specific neuronal cell types or networks of neurons, could provide further insight into sleep spindle function.

5.4.2 The role of spindles in memory consolidation

Recordings from gross EEG of global sleep spindle events have repeatedly suggested spindles have an active role in the process of memory consolidation^{43–45}. Specifically, spindles, slow waves and ripples are thought to act in a highly regulated coordination to promote hippocampal-cortical cross talk allowing long term memory storage (see Chapter I section 1.2.5, pg23)^{46,47}. The finding of highly distinct and frequent hyper-localised spindle events provides an intriguing possibility that spindles reflect specific anatomically defined routes of communication between brain areas, clustered within localised cortical areas which are implicated in the local processing of information³⁷⁵. However, since there was no explicit novel experience or prior learning, these results suggest that the anatomical or functional organisation of cortical areas, rather than preceding learning or experience, is the key determinant of spindle expression. It is an ongoing debate as to whether spindles are actively involved in memory processing, or merely serve a permissive role providing its favourable conditions⁵³. For example studies in birds, have thus far provided no evidence for the existence of spindles during sleep. Although there is also no evidence for hippocampal to extra-hippocampal transfer of memory traces in birds, systems-level processing during sleep has still been shown for certain behaviours including filial imprinting and song learning³⁷⁶. Therefore in at least some species, the sleep dependent processing of hippocampal memories, if present at all, does not rely upon spindles. Nevertheless, the current study provides no negation that spindles are involved in memory processing and they could have multiple functions with a more active role in other regions of the brain such as the prefrontal cortex where memory processing may be more relevant.

5.4.3 Spindle bursts during development

Why else could we see such distinct spindle events within the somatosensory cortex? During development, spindle-like events known as spindle bursts have been shown to be selectively triggered in a somatotopic manner through spontaneous muscle twitches¹⁸⁵. These bursts present the first organised pattern within the SCx and likely play a role in the development of sensorimotor coordination. In a previous study, the placement of an array of eight electrodes into the SCx of 15 rat pups revealed a high spatial localisation of spindle bursts, similar to spindle events in the current study¹⁸⁵. Perhaps the finding of distinct spatially localised spindle events in adult rodents is a reflection of this developmental role and continued maintenance of sensorimotor processing, which is specific to the SCx. One caveat of this is that spindle bursts during development are most commonly associated with REM sleep³⁷⁷. Thus, the substantial increase in spontaneous muscle twitching during REM sleep, compared to both NREM and wake³⁷⁸, suggests a stronger involvement of spindle bursts in this function compared to adult NREM sleep spindles. However, there is an intriguing possibility, that there is a shift in the function of spindles from their role in cortical maturation early in development, which is facilitated by REM sleep³⁷⁹, to a more advanced involvement in information processing during adulthood, which is fulfilled during NREM sleep.

5.4.4 The role of spindles in sleep regulation

An alternative explanation for presence of localised spindle events, is for their role in global sleep regulation. These results replicate previous findings of an increase in overall spindle activity with time, which was in reciprocal coordination with SWA and likely reflects a decrease in the homeostatic drive for sleep^{357,368}. It has been proposed that the homeostatic decrease of SWA is a result of a decrease in synaptic strength of cortico-cortical connections^{70,366}. Considering research into the function of sleep spindles, it seems intuitive to predict that the role of spindles in maintaining³⁸⁰, or even prolonging

sleep³⁸¹ could not only prevent sensory intrusion, but also moderate this net decrease in synaptic connectivity as sleep pressure is reduced. With this, sleep spindles could instead provide an indirect function for supporting the prolongation of other oscillations such as slow waves in synaptic downscaling or active memory processing.

Two further findings support a role of sleep spindles in the regulatory processes of sleep. Firstly, DS LFPs showed large increases in spindle frequency activity (SFA) during NREM-REM transitions. This 'intermediate stage' of sleep has been shown previously in mice, rats, cats and to some extent humans where the large thalamic bursting activity producing spindles is evidenced to disconnect the forebrain from the brainstem likely aiding the transition process^{358,369}. Secondly, distinct spindle LFPs showed an absence of oscillatory theta dominance during REM sleep, and instead, continued to show a persistence of local NREM-like SWA, despite the previous response to state-transitions. Since theta activity is generated from the hippocampus and related areas³⁸², the lack of theta dominance in DS LFPs could reflect the decay of theta amplitude due to distance from the hippocampus. However the disappearance of theta frequency peak was observed within just a few hundred millimetres (from IS channels) rendering this unlikely. It is instead possible that the intense local activities in the DS channels, as reflected in overall higher spectral power, may arise from stronger connectivity with thalamic areas which predominates over the weak volume conducted signal arriving from the hippocampus. Funk *et al.*, for the first time, showed the presence of local slow waves during REM sleep within superficial layers of the primary sensory and motor cortex of mice⁹². As suggested by Funk and colleagues the occurrence of local slow waves may be required during REM sleep to sustain sensory disconnection whilst still allowing paradoxical wake-like cortical activation.

5.4.5 Sleep as a local unitary state

This finding of local NREM-like activity during REM sleep resulted in a clear temporal dissociation between local and global cortical states. In recent years, there has been an increasing amount of evidence showing that wake, NREM sleep and REM sleep are not mutually exclusive events but can instead emerge in a mixed or local manner irrespective of the global cortical state^{60,92,94,383,384}. In relation to the current findings, it is hypothesised that it is not the identification of distinct spindle events *per se*, but rather a recording of a broader spatially specific local cortical circuitry within the SCx that may assist the regulatory process of global sleep across NREM and REM sleep. These local networks, capable of generating spindles and emerging as ‘unitary states’, may be both anatomically and functionally unique from other cortical regions which do not show spindles. As has been previously theorised, NREM and REM sleep likely function in fundamentally different aspects of information processing⁷². It is therefore possible that these processes are not only under temporal but also spatial separation.

5.4.6 Impact of findings

These findings may have substantial implications not only for our understanding of normal sleep functioning but also in pathology. As discussed in Chapter III, sleep disruption and deficits in sleep spindles are a known characteristic of schizophrenia and also feature to some level in individuals at high risk^{117,176}. These abnormalities could reflect a disruption to the developmental cortical organisation or thalamocortical communication that specifically target local cortical areas where sleep is expressed as a unitary state. This disruption of local cortical circuits could then affect global sleep continuity leading to the associated sleep disruption seen in schizophrenia. Currently, the local aspect of sleep is often overlooked or difficult to measure in humans. However, further work on the local aspects of sleep in relevant animal models could provide essential further insight into the sleep abnormalities that may arise in psychiatric disorders. Supporting this with the use of

high density EEG or combining EEG with other methods such as fMRI or magnetoencephalography (MEG) in humans could further translate this work.

5.4.7 Limitations

Without the use of any memory tasks or manipulation of sleep including sleep deprivation, the interpretation of the function of local sleep spindles for either memory consolidation or sleep regulation, is limited. Furthermore the accuracy of mapping the location of array placement could not be determined and it is unknown whether differences in surgeries between mice accounted for the differences in presence of spindle channels across mice. Without the aim of recording from other layers of the cortex, it also cannot be determined how specific the area of spindle occurrence was. Further work using memory tasks or sleep deprivation, increasing the sample size, and widening the field of cortical recording may help strengthen these findings.

5.4.8 Summary

To conclude, this study is the first to show that spindles can occur in a hyper-local manner and the presence of which may predict the emergence of unitary states. These findings suggest that spindles are not merely implicated in the processing of information acquired during immediately preceding waking experience, but may have a more fundamental role in regulation of global and local cortical states. Further investigation into the connectivity of cortico-subcortical networks underpinning spindle dynamics and the involvement of this in sleep regulatory processes is a necessity.

Chapter VI: Impact of findings and future directions

Contents

6.1 Summary of findings	173
6.2 The shared pathology between SCRD and psychosis	174
6.3 Improving the identification, prediction, and prevention of psychosis	176
6.4 Future directions	179
6.5 Conclusions	181

6.1 Summary of findings

The aim of this thesis was to investigate the contribution of sleep physiology and circadian rhythms in the development of psychosis. The first study identified a clear positive association between sleep disturbance, psychotic-like experiences (PLEs) and risk factors associated with the development of psychosis in a large population of individuals. Furthermore, these risk factors were not equally related, with a subset including cannabis use, traumatic experiences and childhood abuse being significantly more predictive of both PLEs and sleep disturbance.

In the second study, a sample of these individuals, selected for being at high risk for developing psychosis based upon having a high load of risk factors and sub-clinical psychotic symptoms, were extensively phenotyped for sleep and circadian rhythms. High risk individuals showed substantial subjective sleep disturbance and differentiated circadian phenotypes including a delayed melatonin rhythm in a subset of individuals. High risk individuals also showed a general lack of objective differences as assessed by actigraphy and polysomnography including comparable sleep efficiency, sleep architecture, and sleep spindle density to the low risk group.

In light of the sleep and cognitive deficits associated with schizophrenia, in the third study, high risk individuals were assessed for measures of sleep dependent memory consolidation. No differences were identified for either procedural or declarative memory between high and low risk groups. Further, only weak evidence supported sleep having a greater enhancement of memory consolidation over wake. However a positive correlation was found for procedural learning and REM sleep duration, specific to the high risk group, with no such associations with sleep spindle density as previously identified.

Finally, since deficits in sleep spindles are a known characteristic of schizophrenia, the spatio-temporal dynamics of sleep spindles were investigated in the mouse as a model organism. Distinct spindle events were found within small circumscribed areas of the somatosensory cortex, with their complete absence just a fraction of a millimetre away. Further, the presence of spindles predicted responses to global cortical states with the persistence of NREM-like activity during REM sleep which could reflect a role in sleep maintenance.

6.2 The shared pathology between SCRD and psychosis

As discussed in Chapter I, the strong association between SCRD and psychosis is posited to arise from underlying shared pathologies which are primarily evidenced by the expression of particular shared genetic mutations and brain abnormalities^{120,239}. The research in this thesis is one of the first to provide evidence that environmental risk factors, and particularly those associated with childhood, are also associated with the development of both psychotic-like experiences and sleep and circadian rhythm disruption. Further, these associations are present across varying degrees of symptom severity, both in the general population and in individuals at high risk for developing psychosis (Figure 6.1A). From this work, a hypothetical model of how genetic and environmental factors may lead to SCRD and psychosis can be conceptualised. Given the early occurrence of risk factors

and gradual onset of a disorder, the aetiology of psychosis and SCRD likely follows a developmental pathway²³⁹. Genetic and environmental factors generate vulnerabilities and biological or psychological stress across the prenatal, infant, childhood and adolescent period leading to altered neuromaturational factors and neurotransmission. Abnormalities during this period, critical for the establishment and maturation of normal physiology including sleep, circadian rhythms, cognitive functioning and psychosocial development could then conceivably lead to the manifestation of psychotic-like experiences and subjective sleep and circadian disturbance. SCRD and sub-clinical symptoms are reciprocally deleterious; an altered mental state can cause poorer sleep and poorer sleep can cause an altered mental state. Furthermore the repeated exposure of environmental insults causes further sensitisation of stress and neural responses. This progressive exacerbation of sub-clinical symptoms and SCRD eventually leads the onset of a psychotic episode (Figure 6.1B). Although this thesis supports and adds to the hypothesised framework, further work is needed to establish the causative nature of risk factors and how SCRD and PLEs progress to clinical symptoms upon transition to disorder (see section 6.4 **Future directions**, pg179).

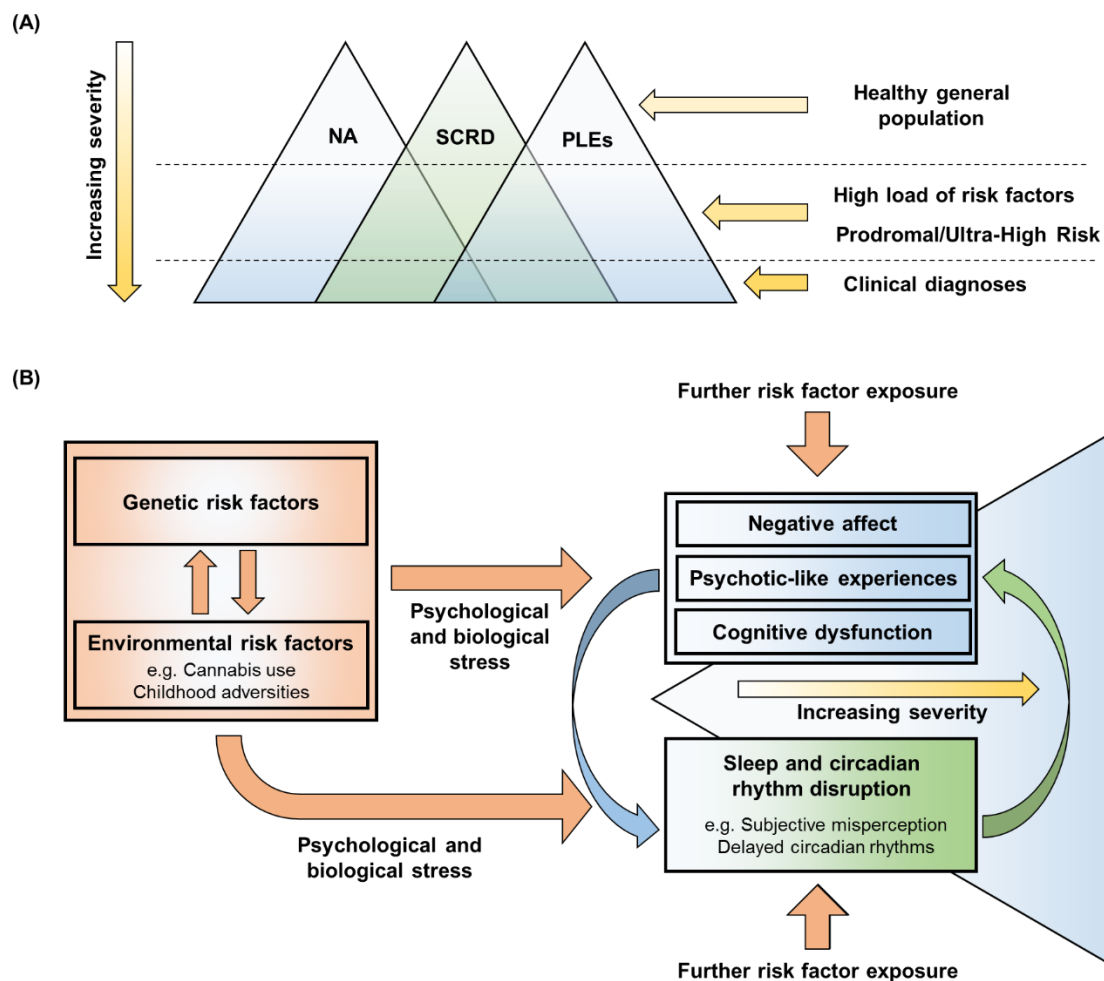


Figure 6.1: (A) Spectrum of negative affect (NA, e.g. depression and anxiety), sleep and circadian rhythm disruption (SCRD) and psychotic-like experiences (PLEs) within the general population. Both NA, SCRD and PLEs are interrelated and exist with varying degrees from healthy individuals to those at risk, to those with clinical diagnoses of psychiatric disorders. (B) Proposed model of interaction between risk factors, PLEs and SCRD in the development of a psychotic disorder. Both environmental and genetic risk factors lead to increased biological and psychological stress. This leads to physiological changes such as hyperactivity of the hypothalamic-pituitary-adrenal axis and changes in neurotransmission predisposing an individual to negative mental states including the development of PLEs. This thesis additionally identified an effect of risk factors on sleep and circadian rhythms which may similarly lead to abnormalities such as subjective sleep disturbance. Poor sleep and circadian misalignment may consequently exacerbate psychiatric symptoms and vice versa in addition to further environmental risk factors causing a progressive build-up and eventual development of disease.

6.3 Improving the identification, prediction, and prevention of psychosis

Surprisingly, given the importance of risk factors in the aetiology of psychosis, the use of screening for risk factors when assessing mental health or at risk individuals is minimal¹. The current findings highlight the impact of risk factors in both general and at risk populations and indicate that specific risk factors such as childhood adversities, are of particular importance in relation to PLEs. Although the research of this thesis cannot

determine the predictiveness of transitioning to psychosis it does provide the first study to directly select for and assess individuals based upon more than just symptom based measures. Given that risk factors commonly occur during development, this alternative method could help in the identification of at risk individuals at even earlier stages of disease progression which may have substantial implications for intervention and management strategies. Disseminating this work to the scientific community, clinicians, and more general healthcare community could therefore improve the identification of at risk groups and prevention of mental health problems. For example, screening of particular risk factors (e.g. childhood abuse, cannabis use) in at risk groups could provide knowledge of where early intervention should be targeted. Perhaps some individuals would benefit from family management whereas others may require rehabilitation of drug addiction.

Following this, this thesis also revealed that subjective sleep disturbances were a clear feature of high risk individuals and associated with PLEs in a general population sample. This data supports a need for assessment of sleep problems in relation to mental health where it could even become routine practice given the relatively quick assessments available and its importance for a wide range of mental health problems¹¹⁷. The hypothesised shared neuropathology between SCRD and psychosis could mean that treating SCRD has a beneficial impact for attenuating the progression of psychotic symptoms and overall mental health outcomes. At a population level, implementing sleep education and increasing awareness of the importance of sleep could be used to alleviate mild sleep problems which may benefit the prevention of mental health problems. For example, methods to improve sleep hygiene such as ensuring no light-emitting technology before bed, or maintaining regular sleep-wake routines are simple, self-administered strategies. Furthermore, although studies are currently limited, the use of cognitive behavioural therapy for insomnia (CBT-I) has been shown to alleviate sleep problems in schizophrenia and could also be targeted for vulnerable at-risk individuals and individuals with more severe sleep problems^{132,133}.

The prospect that SCRD may be a crucial factor in at least some aspects of cognitive dysfunction associated with psychosis is largely undetermined. This thesis has also provided the first assessment of sleep relevant cognitive measures including declarative memory, procedural memory and sustained attention in individuals in at risk states. Given that general cognitive deficits are well established in high risk groups³⁸⁵, the lack of differences in sleep dependent measures could importantly suggest that impairments as found in schizophrenia are only associated with the progressive worsening of sleep or psychiatric symptoms. For example, the lack of deficits in sleep spindles identified in the high risk group may reflect a lack of change in brain morphology such as alterations in the hippocampus or thalamus associated with schizophrenia and implicated in sleep and cognitive processes^{161,165}. This improved understanding of cognitive impairment in disorder progression could help direct targets for treatment where it is conceivable that treating developing sleep and circadian rhythm disruption may also attenuate cognitive impairments.

However, this thesis also highlights that sleep spindles, thought to be critically implicated in memory consolidation, are far less understood than what is currently concluded. Rather than an occurrence of global processes during sleep, sleep may rely on specific local networks that are essential for the regulation and development of sleep at a global level. Pathology in schizophrenia such as thalamic dysfunction could therefore specifically affect these local networks leading to a disrupted continuity of sleep and global state changes. These findings emphasize the necessity to understand these local processes and how they may be implicated in disease. Further, there is a crucial importance of cortical location in studies, which are usually based on a limited number of electrodes or gross EEG recordings only, as marked differences in network state may be observed even within a small cortical area.

6.4 Future directions

Several lines of future investigation would help complement the work of this thesis. Firstly, further work is needed to identify the influence of risk factors on both SCRD and psychosis. Perhaps the most essential future direction from this thesis is the use of longitudinal studies. Assessing the progression of both SCRD and psychotic-like experiences in individuals with a high load of risk factors could help determine whether particular risk factors or the occurrence of new risk factors exacerbate this development and are associated with the transition to clinical diagnoses. For example, do individuals who have experienced childhood adversities go on to develop different sleep, circadian and psychosis phenotypes compared to those who regularly take cannabis? Concurrent with this, further work is also necessary to identify the causative nature of risk factors, particularly in relation to SCRD. Animal models provide a useful method to examine this. For example Oliver *et al* have shown a mutation in the synaptosomal-associated protein of 25 kDa (Snap-25) gene and environmental prenatal stress in the Blind-Drunk mouse model of schizophrenia leads to further psychosis-like phenotypes including social interaction abnormalities, not seen with the genetic mutation alone¹⁶⁰. Moreover, this mouse model has also been shown to have distinct sleep and circadian phenotypes including a fragmented rest-activity rhythm reminiscent of SCRD in schizophrenia¹⁵⁹. Investigating whether risk factors also modify or worsen the sleep and circadian phenotypes could provide a more substantial link between the shared pathways between SCRD and psychosis. Extending this to other genetic and environmental risk factors, inducing them at different stages of development, and measuring underlying physiology and neurotransmitter systems provides an unexplored but immensely insightful avenue for further investigation.

Secondly, further work is needed to understand the temporal relationship between SCRD and psychosis. Again, longitudinal studies would help determine for example whether

objective sleep disturbances develop in individuals with a high load of risk factors and if these co-occur with the development of clinically relevant psychotic symptoms. Further investigation is also needed to identify whether improving SCRD also improves psychotic and cognitive symptoms (and vice versa). Cognitive behavioural therapy for insomnia is a viable method to explore this, but research should also focus on the circadian abnormalities associated with psychosis including the use of light therapy or exogenous melatonin to advance or synchronise the circadian clock. The use of experimental work in non-clinical samples may also be useful for understanding the causative interaction between SCRD and psychosis. Only three studies have tested the effects of manipulating sleep on psychotic-like experiences in non-clinical samples. All have identified increases in at least some forms of PLEs but all were based on limited sample sizes and limited measures of psychotic experiences³⁸⁶.

Following this, further work should aim to discern the underlying shared pathology of SCRD and psychosis and particularly how the local nature of sleep is associated with psychosis. Since they are costly, time-intensive or highly invasive, studies using high-density EEG, intra-cranial EEG or magnetoencephalography (MEG) are limited in schizophrenia and no research has used these techniques in individuals at high risk. However, it would be useful to identify whether sleep abnormalities occur at a local level within specific regions of the cortex or subcortical layers and if this is a driving factor for global sleep disruption. Animal models provide a viable method for studying this further through targeting specific regions of the brain or local circuitry. For example, the *Glua1* mutant mouse model of schizophrenia associated with glutamatergic dysfunction is the first to show a lack of global sleep spindles (Ang *et al*, unpublished data). Using models such as these to examine local dynamics, and identifying whether deficits in spindles are also found in local hotspots such as the anterior somatosensory cortex could provide clues on specific thalamic dysfunction associated with both SCRD and psychosis-like phenotypes.

Finally, further work is also needed to address the functional impact of SCRD in psychotic disorders and at risk populations. Limited work has tested the sleep homeostat and response to changes in sleep pressure including sleep deprivation in these populations. However, this work could reveal crucial additional phenotypes such as blunted responses to increased sleep pressure which may account for the inconsistency of objective disturbances identified in these populations and could explain an underlying vulnerability to sleep and circadian disruption (rather than a distinct sleep or circadian phenotype in itself). The importance of sleep and sleep disruption in memory consolidation should also be further examined such as through the use of trans-cranial direct current stimulation, auditory closed-loop stimulation or relevant pharmacological substances to manipulate particular sleep processes. This work should be further complemented using experimental approaches in animals such as pharmacogenetics or optogenetics to directly excite or inhibit specific neuronal populations or events such as spindles to test their functional importance in sleep regulation or memory consolidation.

6.5 Conclusions

This thesis has shown for the first time that risk factors associated with the development of psychosis, are also associated with sleep and circadian rhythm disruption. Sleep and circadian rhythm disruption occurs in individuals at high risk for developing psychosis covering several phenotypes including subjective disturbance and delayed circadian rhythms. There is no evidence for a deficit in sleep dependent memory consolidation in individuals at high risk, although further work is necessary for this to be concluded. Finally, sleep spindles occur in a highly local manner during sleep which may reflect an importance of local processes for global sleep regulation and may have implications for abnormalities of local circuitry in psychosis. Overall, this work provides a broad direction for future

research initiatives and furthers our knowledge of the importance of sleep and circadian rhythms in health and disease.

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Supplementary Material

Table S1: Risk factors for psychosis identified from the literature search that were not included in the online survey. Justification for their exclusion is given. Time restraints refer to ensuring the survey was short enough to allow adequate completion from participants.

Risk factor	Ref.	Justification for exclusion
Personality	387	Requires time-consuming assessment
Socioeconomic status	205	Unable to adequately measure in an online survey
Biological markers including olfactory deficits, pituitary gland volume	388	Unable to adequately measure in an online survey
Medication or recreational substances including antidepressants and tobacco use	389,390	Partly assessed in the survey but not conclusively due to time constraints
Early developmental abnormalities	391	Requires time-consuming assessment
Parental communication	392	Unable to adequately measure in an online survey
Non-psychiatric comorbidities including toxoplasmosis, obesity, cardiovascular disease, cancer	221,389,393,394	Requires time-consuming assessment and direction of association is often unclear
Maternal influenza	395	Unable to adequately measure in an online survey
Vitamin D deficiency	396	Unable to adequately measure in an online survey
Specific genetic mutations	25	Unable to adequately measure in an online survey

Table S2: Linear models testing individual risk factor components in relation to psychotic-like experiences. Each model controlled for all other risk factors.

Model	Factor	B	SE	t	p	Sig.	OR	CI
1	1st degree Sz/Bp	-0.035	0.146	-0.239	0.811	-	-	-
	1st degree all disorders	0.036	0.067	0.532	0.595	-	-	-
	Other degree all disorders	0.046	0.073	0.633	0.527	-	-	-
2	Physical abuse (once)	-	-	-	-	-	-	-
	Physical abuse (sometimes)	-	-	-	-	-	-	-
	Physical abuse (regularly)	-	-	-	-	-	-	-
	Physical abuse (often)	0.494	0.238	2.075	0.038	*	1.64	1.03-2.61
	Physical abuse (very often)	0.502	0.293	1.717	0.086	.	-	-
	Psychological abuse (once)	0.541	0.192	2.826	0.005	**	1.72	1.18-2.50
	Psychological abuse (sometimes)	-	-	-	-	-	-	-
	Psychological abuse (regularly)	-	-	-	-	-	-	-
	Psychological abuse (often)	-	-	-	-	-	-	-
	Psychological abuse (very often)	-	-	-	-	-	-	-
	Emotional abuse (once)	0.638	0.172	3.703	<0.001	***	1.89	1.35-2.65
	Emotional abuse (sometimes)	0.389	0.074	5.267	<0.001	***	1.48	1.28-1.71
	Emotional abuse (regularly)	0.539	0.127	4.235	<0.001	***	1.72	1.34-2.20
	Emotional abuse (often)	0.884	0.139	6.368	<0.001	***	2.42	1.84-3.18
	Emotional abuse (very often)	0.774	0.140	5.527	<0.001	***	2.17	1.65-2.85
	Sexual abuse (once)	-	-	-	-	-	-	-
	Sexual abuse (sometimes)	-	-	-	-	-	-	-

	Sexual abuse (regularly)	-	-	-	-	-	-	-
	Sexual abuse (often)	-	-	-	-	-	-	-
	Sexual abuse (very often)	-	-	-	-	-	-	-
3	Ever tried cannabis	0.131	0.081	1.616	0.106	-	-	-
	Cannabis use (sometimes)	0.194	0.106	1.828	0.068	.	-	-
	Cannabis use (monthly)	0.177	0.197	0.899	0.369	-	-	-
	Cannabis use (weekly)	0.759	0.252	3.015	0.003	**	2.14	1.30-3.50
	Cannabis use (daily)	0.863	0.344	2.510	0.012	*	2.37	1.21-4.65
	Cannabis use (aged under 15)	0.163	0.102	1.601	0.110	-	-	-
	Cannabis use (aged 15-18)	0.194	0.116	1.663	0.097	.	-	-
	Cannabis use (aged 18+)	-	-	-	-	-	-	-
4	Trauma (aged 0-12)	0.151	0.062	2.435	0.015	*	1.16	1.03-1.31
	Trauma (aged 13-18)	0.336	0.058	5.832	<0.001	***	1.40	1.25-1.57
	Trauma (aged 18+)	-0.179	0.062	-2.903	0.004	**	0.84	0.74-0.94
	Trauma (within past year)	0.147	0.061	2.410	0.016	*	1.16	1.03-1.31
	Trauma (within past 6 months)	0.151	0.060	2.529	0.012	*	1.16	1.03-1.31
5	Trauma (total number)	0.032	0.017	1.863	0.063	.	-	-
	Trauma (slightly stressful)	0.251	0.131	1.912	0.056	.	-	-
	Trauma (moderately stressful)	0.393	0.109	3.608	<0.001	***	1.48	1.20-1.83
	Trauma (very stressful)	0.455	0.110	4.143	<0.001	****	1.58	1.27-1.96
6	1st generation migrant	0.168	0.064	-2.639	0.008	**	0.85	0.75-0.96
	2nd generation migrant	0.056	0.067	-0.833	0.405	-	-	-
7	Social withdrawal (low)	0.202	0.076	2.677	0.007	**	1.22	1.06-1.42
	Social withdrawal (medium)	0.598	0.081	7.395	<0.001	***	1.82	1.55-2.13
	Social withdrawal (high)	0.772	0.098	7.885	<0.001	***	2.17	1.79-2.62
	Social withdrawal (very high)	0.904	0.137	6.593	<0.001	***	2.47	1.89-3.23

Table S3: Effect of the number of risk factors and psychotic-like experiences (PLEs; PQ-16 score) on sleep quality (PSQI score). Table shows group comparisons in PSQI score (average±standard deviation) between individuals with a low or high amount of PLEs and a low or high amount of risk factors. A high amount of PLEs corresponds to a PQ-16 score of more than 5, a high amount of risk factors corresponds to those reporting more than 4. A Kruskal-Wallis analysis of variance was used to identify overall group differences with post-hoc Wilcoxon rank sum tests (W-value, p-value) to compare between groups.

Group comparison: PLEs Risk factors vs PLEs Risk factors	PSQI score Group1 vs Group2	W-value	p-value
KW-Anova: $X^2=245$, $p<0.001$			
Low Low vs Low High	4.87±2.23 vs 5.64±2.42	174160	<0.001
Low Low vs High Low	4.87±2.23 vs 6.58±2.51	45130	<0.001
Low Low vs High High	4.87±2.23 vs 8.00±2.69	34630	<0.001
Low High vs High Low	5.64±2.42 vs 6.58±2.51	26585	<0.001
Low High vs High High	5.64±2.42 vs 8.00±2.69	21946	<0.001
High Low vs High High	6.58±2.51 vs 8.00±2.69	10618	<0.001

Table S4: Group differences in average spectral power density and spindle characteristics from frontal channel Fp2, F4, parietal channel P4 and occipital channel O2 during NREM sleep. High risk, n=22; low risk, n=22.

Parameter	Fp2		F4		P4		O2	
	t	p	t	p	t	p	t	p
Power density								
<i>Delta power (0.6-4Hz)</i>	1.75	0.088	1.45	0.156	0.66	0.512	-0.16	0.874
<i>Theta power (4.2-8Hz)</i>	-0.37	0.712	0.04	0.969	-0.24	0.814	-0.61	0.548
<i>Alpha power (8.2-12Hz)</i>	-0.06	0.955	-0.34	0.739	-0.34	0.737	-0.27	0.788
<i>Sigma power (12.2-16Hz)</i>	0.60	0.550	-0.21	0.833	-0.14	0.888	-0.68	0.503
<i>Beta power (16.2-20Hz)</i>	-0.12	0.902	-0.51	0.613	0.27	0.789	-0.29	0.776
Spindle characteristics								
<i>Density (N/min)</i>	-0.12	0.902	-0.05	0.958	-0.76	0.453	-0.15	0.885
<i>Duration (s)</i>	1.04	0.305	0.26	0.797	-0.26	0.793	-0.22	0.826
<i>Amplitude (μV)</i>	0.56	0.576	-0.05	0.958	-0.25	0.801	-0.68	0.498
<i>Frequency (Hz)</i>	-0.18	0.857	0.57	0.574	2.08	0.043*	1.09	0.283

Table S5: Group differences in actigraphic measures between high risk late (HRL, n=11), high risk early (HRE, n=9) and low risk (LR, n=17) groups separated based upon whether they showed an early or late melatonin peak. Data show ANOVA tests (F, p).

Parameter	HRL	HRE	LR	F	p
Total sleep time (TST; hr)	6.81	7.06	6.85	0.35	0.706
Sleep onset latency (SL; min)	0.17	0.14	0.12	2.73	0.079
Sleep efficiency (SE; %)	81.71	81.99	80.94	0.20	0.819
Level of activity (M10 counts)	15206	16602	17315	0.95	0.397
Level of inactivity (L5 counts)	1031	1146	978	0.37	0.691
Wake after sleep onset (WASO; min)	79.57	83.48	87.96	0.41	0.664

Table S6: Group differences in measures of mental state between high risk late (HRL, n=11), high risk early (HRE, n=9) and low risk (LR, n=17) groups separated based upon whether they showed an early or late melatonin peak. Data show ANOVA (F, p) and post-hoc t-tests (t, p).

Measure	HRL	HRE	LR	F	p	Comparison	t	p
Number of risk factors	7.18	6.00	2.06	76.5	<0.001	HRL:LR	11.76	<0.001
						HRE:LR	7.59	<0.001
						HRL:HRE	1.96	0.067
Number of psychotic-like experiences (PQ-16)	9.09	8.22	1.00	66.39	<0.001	HRL:LR	9.47	<0.001
						HRE:LR	8.58	<0.001
						HRL:HRE	0.78	0.444
Distress from psychotic-like experiences (PQ-16 distress)	14.09	13.22	0.82	26.41	<0.001	HRL:LR	5.93	<0.001
						HRE:LR	5.11	<0.001
						HRL:HRE	17.35	0.793
At Risk Mental Status (CAARMS score)	2.27	2.00	0.00	59.79	<0.001	HRL:LR	9.59	<0.001
						HRE:LR	6.93	<0.001
						HRL:HRE	16.45	0.476
Depression (DASS-21 Dep)	21.45	23.78	3.76	23.96	<0.001	HRL:LR	4.52	<0.001
						HRE:LR	7.56	<0.001
						HRL:HRE	-0.52	0.608
Anxiety (DASS-21 Anx)	17.82	16.67	2.59	19.34	<0.001	HRL:LR	5.25	<0.001
						HRE:LR	4.24	0.002
						HRL:HRE	0.27	0.793

Stress (DASS-21 Str)	28.00	23.33	4.47	49.84	<0.001	HRL:LR	7.77	<0.001
						HRE:LR	8.51	<0.001
						HRL:HRE	1.37	0.189
Mania (MDQ)	7.64	6.00	1.71	12.98	<0.001	HRL:LR	4.86	<0.001
						HRE:LR	2.89	0.017
						HRL:HRE	0.90	0.379
Neuroticism (EPQ Neu)	17.18	15.44	4.71	42.77	<0.001	HRL:LR	8.03	<0.001
						HRE:LR	7.33	<0.001
						HRL:HRE	1.06	0.302

Table S7: Correlation between total sleep time, total time in each sleep stage and sleep spindle characteristics with the percent of words recalled after a night of sleep following a word-pair learning task. No significant correlations were identified for either high or low risk groups. High risk, n=22; low risk, n=22.

Parameter	High risk		Low risk	
	r	p	r	p
Total sleep time	-0.06	0.798	0.28	0.202
Time in stage 1	-0.19	0.393	0.18	0.426
Time in stage 2	-0.12	0.592	0.02	0.927
Time in stage 3/4	0.18	0.415	0.31	0.157
Time in REM (min)	-0.05	0.815	0.21	0.354
Spindle number	0.10	0.672	0.05	0.842
Spindle density (N/min)	-0.13	0.551	-0.08	0.725

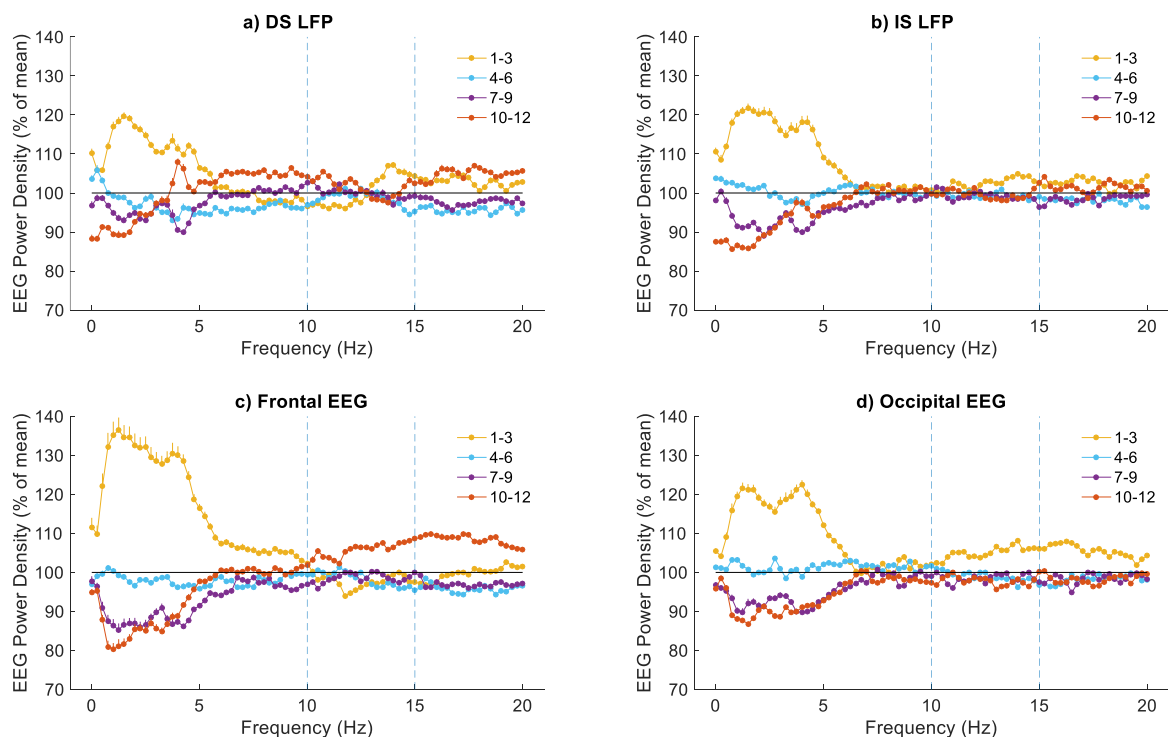


Figure S1: Mean power density during 3 hour intervals across a 12 hour light period. All data is during NREM sleep from a visually distinct spindle (DS) LFP (a), indistinct spindle (IS) LFP (b), frontal EEG channel (c) and occipital EEG channel (d). Bars denote SEM. N=7.

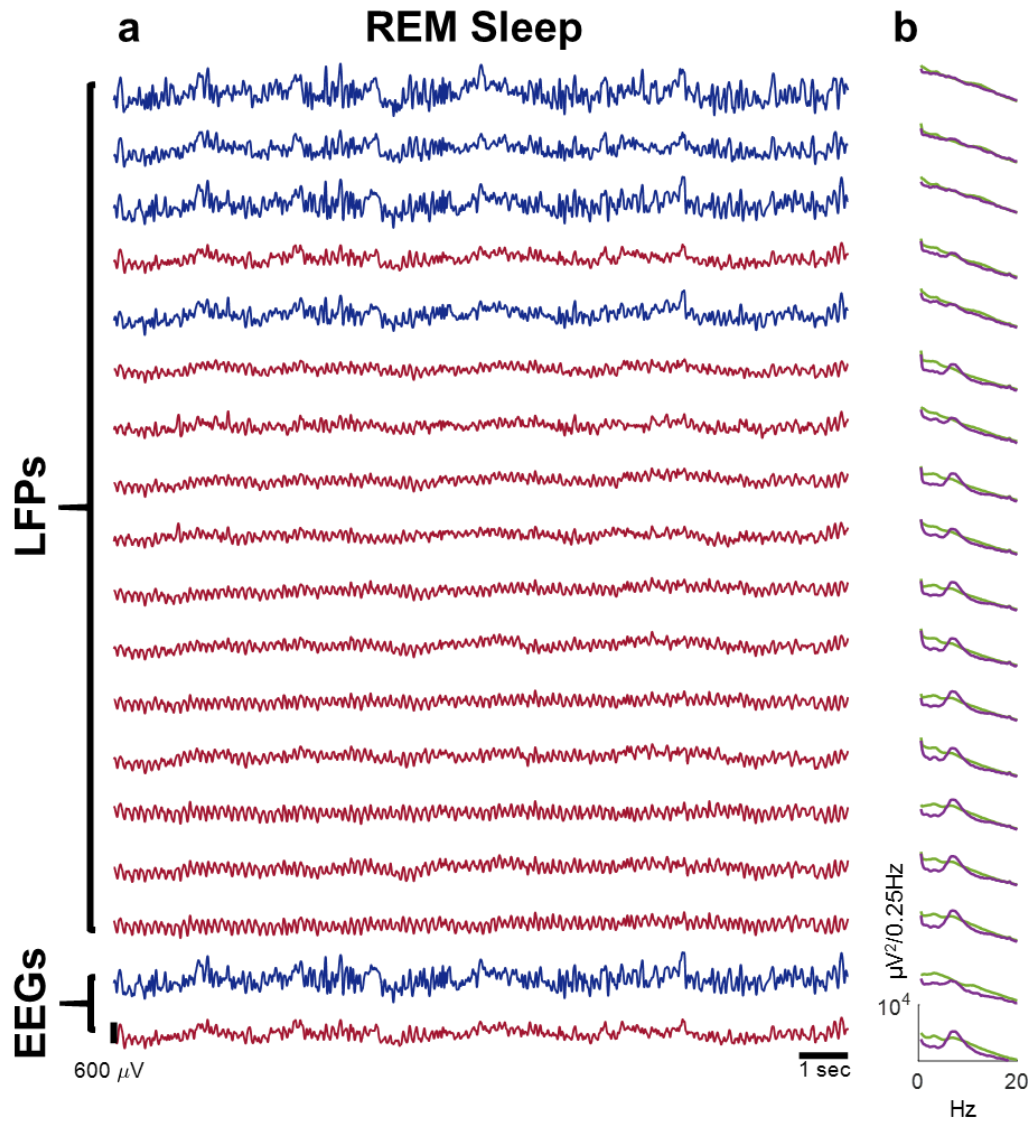


Figure S2: (a) A representative 15 second example from all 16 LFP channels showing DS (blue LFP channels), IS (red LFP channels) LFPs and frontal (top EEG), occipital (bottom EEG) EEGs in one mouse during REM sleep. (b) Corresponding power density during NREM (green) and REM (purple) sleep. Note the presence of slow-wave activity in DS channels and lack of increased spectral power within theta frequency range (6-9Hz) during REM sleep.