

Thesis

Anastasia Stuart

2024-07-19

I, Anastasia Stuart confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Contents

Abbreviations

Introduction	1
Problem statement	1
Introduction to sleep disorders	2
Insomnia disorder	2
Non-restorative sleep	3
Mechanisms of sleep	3
Neurophysiological correlates of sleep	3
Sleep homeostasis	4
SWA	4
Daytime impacts	5
SWA in wake	5
Objective Drowsiness	6
Subjective sleepiness	6
Aim	8
Hypotheses	8
Method	9
Study design	9
Participants	9
Protocol	10
Measures	10
KSS	10
KDT	11
HD-EEG	11
Power spectra	11
Statistical analysis	12
Data cleaning	12
FFT	13

Independent components analysis	13
average referencing	14
Process	14
References	15

Abbreviations

AAC	Alpha Activity Coherence
DASS-21	Depression Anxiety Stress Scales - 21 Items
HC	Healthy Controls
HD-EEG	High-Density ElectroEncephaloGraphy
ID-NRS	Insomnia Disorder with Non-Restorative Sleep
ID-SSD	Insomnia Disorder with Short Sleep Duration
ISI	Insomnia Severity Index
KDT	Karolinska Drowsiness Test
KSS	Karolinska Sleepiness Scale
NREM	Non-Rapid Eye Movement
PSG	Polysomnographic
PSQI	Pittsburgh Sleep Quality Index
REM	Rapid Eye Movement
RSQ-D	Restorative Sleep Questionnaire Daily Version
SnPM	Statistical nonParametric Mapping
SR	Slowing Ratio

Introduction

Problem statement

Non-restorative sleep (NRS) is a condition characterised by unrefreshing sleep upon awakening despite normal sleep duration and architecture as measured by polysomnography (PSG), leading to excessive daytime fatigue and significant daytime functional impairment (Roth et al., 2010). Despite the significant impact of this condition, it is poorly diagnosed and not included in the Diagnostic and Statistical Manual-5-TR (Association, 2022), with no established guidelines for clinical management. NRS has previously been clinically managed as a subtype of insomnia disorder (ID) despite evidence suggesting it is phenotypically distinct with a different underlying aetiology (cite?).

Both disorders are associated with increased daytime fatigue and sleepiness, however there may be different causal mechanisms leading to these symptoms.

A major distinction between ID and NRS is sleep architecture, with a marker of ID being shortened overnight sleep duration or frequent overnight arousals, which are not present in NRS. The dysfunctional sleep architecture experienced by ID populations is hypothesised to be a causal factor for daytime sleepiness lead

A potential reason for the lack of restorative sleep experienced by this population may be a dysfunction in slow wave activity (SWA), which is the strongest established marker of reduced sleep pressure (tononi2008?). Low SWA power during sleep leads to ineffective dissipation of accumulated sleep pressure, while increased SWA in wake is associated with increased feelings of sleepiness. Subjective sleepiness is **important because of why**

EEG spectral power during sleep, in contrast to standard polysomnography or questionnaires, may provide a better biomarker for distinguishing insomnia subtypes.

Although this population has normal sleep parameters as measured by traditional PSG methodologies, new technologies and techniques such as HD-EEG and spectral analysis enable exploration of the underlying neural mechanisms in greater resolution, which may reveal differences in sleep processes that result in non-restorative sleep.

In order to explore if NRS is a result of dysfunctions in SWA processes during sleep and wake in comparison to healthy populations and those with ID, this study will high-density electroencephalography (HD-EEG) to examine the power and topographic variance of SWA during resting wake and sleep. Additionally, it will examine if there are group differences in the correlation between subjective sleepiness and SWA

following sleep.

Introduction to sleep disorders

Insomnia disorder

ID is the most common sleep disorder in Australia with an estimated prevalence of 23.2% (Appleton et al., 2022). It is characterised by complaints of shortened overnight sleep, difficulty with sleep initiation, or frequent overnight arousals causing clinically significant distress or dysfunction in daily life (Association, 2022). In contrast to many other sleep disorders that are diagnosed through PSG data, diagnosis of insomnia is recommended based on subjective reports of impairment through self-assessed questionnaires (American Academy of Sleep Medicine, 2005). ID is associated with diminished quality of life (kyle2010?) and increased risk of comorbid psychiatric disorders (hertenstein2019?). Daytime fatigue, being the subjective experience of low energy (raizen2023?), is highly prevalent in this population (kyle2010?), but patients experience decreased sleepiness as measured through sleep latency (Huang et al., 2012; Roehrs et al., 2011).

The contrasting experiences of increased fatigue with reduced sleepiness is hypothesised to be caused by the competing influences of hyperarousal and sleep pressure. ID is proposed to be caused and perpetuated by increased physiological and neurobiological arousal, preventing sleep initiation and leading to increased overnight awakenings (Riemann et al., 2010).

how does this affect daytime sleepiness

ID with short sleep duration is proposed to be caused and perpetuated by increased physiological and neurobiological arousal, preventing sleep initiation and leading to increased overnight awakenings (Riemann et al., 2010). Neurobiological hyperarousal in ID is observed through increased fast frequency EEG activity during sleep and wake (Zhao et al., 2021). During sleep, ID patients have decreased SWA (Kao et al., 2021) and increased fast frequency band activity (Hogan et al., 2020; Merica et al., 1998) during NREM sleep in comparison to healthy controls, leading to diminished overnight dissipation of sleep pressure (Lunsford-Avery et al., 2021). Additionally, high frequency activity is associated with poorer subjective sleep quality (Perlis et al., 2001). Hyperarousal persists during wake, with ID being associated with increased alpha power during resting wake, indicating increased wakefulness (Feige et al., 2017).

Hyperarousal may explain the low levels of excessive daytime sleepiness (EDS) observed in ID despite dysfunctional sleep architecture. Approximately half of the ID population experience EDS (Hein et al., 2017), and often have normal or increased sleep latency during daytime sleep tests (Huang et al., 2012; Roehrs et

al., 2011) These findings support the hypothesis that hyperarousal contributes to ID pathology as a stable construct, rather than just during sleep (Dressle & Riemann, 2023).

Non-restorative sleep

Although both conditions are characterised by complaints of inadequate sleep, NRS is distinct from ID due to having a normal sleep duration and architecture as measured by PSG (Roth et al., 2010). Patients have a primary complaint of sleep being subjectively unrefreshing or unrestorative without a comorbid sleep disorder (Stone et al., 2008). Prevalence in range of 1.4-35% across studies and populations (Zhang et al., 2012) although variation in definitions and a lack of a validated measure poses a challenge for classification. Daytime impairments associated with NRS include significant daytime fatigue, reduced cognitive performance, and reduced psychological well-being, leading to reduced quality of life and impaired daily function (cite?). (Neu et al., 2015)

Despite the significant effects of the condition, the symptom of non-restorative sleep was removed from the diagnostic criteria of ID in the DSM-5, meaning this population is diagnosed as “other specified sleep-wake disorder” (Association, 2022). As NRS may be its own unique disorder with an underlying neurobiological cause, it is essential to develop diagnostic criteria and understand the associated neural mechanisms to improve outcomes for patients.

Although this population has normal sleep duration and architecture, unrefreshing sleep may be a consequence of disruptions in physiological processes occurring during slow-wave sleep, which are critical for neural function (Kao et al., 2021; Tononi & Cirelli, 2006). **this population does not have cortical hyperarousal** Power spectral analysis may present an improved criteria for classifying and understanding the cause of non-restorative sleep in this population. NRS patients exhibit lower SWA during NREM sleep compared to healthy controls, despite having similar objective sleep duration (Kao et al., 2021). This dysfunctional SWA during sleep may be associated with increased SWA during wake (cite?), however further exploration using improved technology is required.

Mechanisms of sleep

Neurophysiological correlates of sleep

Sleep is behaviourally defined as a reversible reduction in responsiveness to external stimuli, accompanied with measurable brain activity patterns (Cirelli & Tononi, 2008). The neurophysiological correlates of sleep and wake in humans can be measured through EEG recordings of brain activity patterns, providing

a spatiotemporally integrated recording of neuronal signals across the cortical surface (Buzsáki et al., 2012). Wakefulness is characterised through low amplitude, high frequency signals in beta and alpha frequencies, accompanied by irregular muscle activity recorded in electromyogram (EMG). Non-rapid eye movement (NREM) sleep is characterised by reduced muscle movement and the appearance of high-amplitude slow oscillations of delta frequency (0.5-4 Hz), deemed slow wave activity (SWA). Sleep progresses through cycles of brain activity throughout the night, with the greatest prevalence of SWA appearing in N3 sleep (Achermann & Borbély, 2003).

Sleep homeostasis

Sleep is regulated by both a homeostatic and circadian system, wherein the homeostatic system increases the level of perceived sleepiness as waking time increases, while the circadian system regulates internal synchrony with the environment (Borbély, 1982). The homeostatic system determines the quantity and intensity of sleep, creating an accumulation of perceived sleepiness deemed “sleep pressure” (Borbély et al., 2016). Sleep pressure increases in proportion to the duration and intensity of the waking episode, evident through increased sleep duration and sleep intensity (Benington, 2000; Borbély, 1982). Sleep pressure can be measured through SWA, being greatest during the first period of N3 sleep and dissipating in response to sleep duration (cite?).

Sleep homeostasis dysfunction may be a causal factor in the impairments observed in ID and NRS patients (Pigeon & Perlis, 2006; cite?). In patients with insomnia with short sleep duration, there is a global reduction in SWA, while insomnia patients with normal sleep duration as measured by PSG can have either reduced delta power or normal delta power (Kao et al., 2021). Overnight SWA has not previously been examined in a NRS population.

SWA

Slow waves are synchronised neuronal oscillations of membrane potential between hyperpolarised and depolarised states originating in thalamocortical loops which propagate through the brain (Achermann & Borbély, 2003; Steriade et al., 2001). Although the precise function of SWA remains unclear, it appears to be critical for cellular maintenance and repair, allowing neurons to reverse minor cellular damage before it becomes irreversible (Vyazovskiy & Harris, 2013). The frequency, amplitude and spatial topography of SWA is additionally influenced by sleep homeostasis, creating measurable variations in underlying neuronal activity (Krueger et al., 2019). Increased sleep pressure leads to longer periods of hyperpolarisation and greater synchrony between brain regions, which are reduced as sleep pressure dissipates (Vyazovskiy et al.,

2011). Increased synchrony can be measured using HD-EEG through cluster analysis, which provides greater spatial resolution than EEG.

SWA has topographic variance across the cortex, varying in a use-dependent manner (Krueger & Obäl Jr., 1993). SWA has an antero-posterior cortical progression, with the greatest activity in the frontal regions at sleep onset (Huber et al., 2000). Increased SWA following sleep deprivation is additionally greatest in the frontal cortex (Cajochen et al., 1999; Werth et al., 1996). Repetitive task performance recruiting functional areas of the brain, such as the motor or sensory cortices, leads to increased regional SWA during subsequent sleep (Huber et al., 2004; Vyazovskiy et al., 2008). These findings suggest that SWA is a localised phenomenon, appearing in response to accumulated sleep pressure and dissipating with sleep.

Daytime impacts

SWA in wake

Although SWA is a characteristic of sleep, intrusions of localised SWA can also be observed during wake in a use and time-dependent manner in response to the accumulation of sleep pressure (Huber et al., 2004; Krueger et al., 2019). Rodent studies have found increased SWA in local cortical networks in response to sleep deprivation despite being physiologically awake, increasing in intensity and synchronicity with the duration of wake (Vyazovskiy & Harris, 2013). Localised increases in SWA have additionally been observed in humans in response to prolonged wakefulness, being greatest in the frontal and lateral centro-parietal regions compared to baseline (Hung et al., 2013; Plante et al., 2016). The increase of slower frequency power during wake is hypothesised to be an adaptive process of cortical downregulation, allowing cells to prevent long-term damage during periods of extended wake by engaging in the restorative processes observed in slow-wave sleep while maintaining consciousness (Vyazovskiy & Harris, 2013). These findings suggest that intrusions of SWA in wake may be representative of accumulated sleep pressure, and therefore a measure of physiological fatigue.

Analysis of SWA during wake can be used to assess arousal and fatigue in ID and NRS. In ID populations, waking EEG

Increased SWA is correlated with subjective and objective markers of fatigue, meaning it is a variable of interest for this study. The appearance of SWA in task-related regions is associated with diminished behavioural performance (Bernardi et al., 2015). HD-EEG recordings observed an increased SWA during wake in the left frontal brain region following a language task and posterior parietal region following a

visuomotor task, which was additionally associated with increased SWA during recovery sleep (Hung et al., 2013). This suggests that the localisation of sleep pressure observed in sleep is also observed during wake.

Objective Drowsiness

Objective drowsiness can be measured through a range of tests, measuring associated but distinct characteristics linked to the accumulation of sleep pressure. The most common measures used in clinical practice and scientific research are the multiple sleep latency test which measures sleep propensity, the maintenance of wakefulness test measuring the consequences of sleepiness, and the psychomotor vigilance task which measures sustained attention and reaction time, known to diminish with increased sleepiness (Basner & Dinges, 2011; Martin et al., 2023). However, these measures do not directly measure the experience of drowsiness, instead measuring its consequence. As the consequences of drowsiness may be create different experiences across populations, it is therefore important that the neural activity of drowsiness itself, rather than its consequences, are measured.

The Karolinska Drowsiness Test (KDT) was developed as a specific and sensitive measure of drowsiness that can provide insight into the neurobiological markers of drowsiness across populations (Åkerstedt et al., 2014; Åkerstedt & Gillberg, 1990). The test uses EEG to measure brain activity during resting wake, which can be transformed into power spectra using a fast Fourier transform and then assessed through power spectral analysis (**cite?**). The test has been validated in healthy populations, being a reliable marker of drowsiness in accordance with sleep pressure and circadian rhythm fluctuations (Kaida et al., 2006).

Subjective sleepiness

Subjective sleepiness is a measure of an individual’s self-assessed level of sleep pressure, objective drowsiness, or sleep propensity, which fluctuates throughout the day in response to the influence of sleep homeostasis and circadian systems (Åkerstedt et al., 2014). There are two dimensions of sleepiness, sleepiness propensity being the likelihood of an individual sleeping in a given situation, and sleepiness perception being the subjective assessment of an individuals feelings of sleepiness (Johns, 2009). However, sleepiness perception is not experienced uniformly across populations, with the differential influences of factors including fatigue and arousal causing individuals to possibly misperceive their internal state (Marques et al., 2019).

In healthy populations, subjective sleepiness scores correlate closely with objective measures of drowsiness, such as sleep latency (**cite?**), reaction time (**cite?**), and EEG spectral power (**cite?**). Subjective sleepiness is predominantly measured through self-reported questionnaires that measure either state or trait sleepiness. The most prevalent measure of trait somnolence is the Epworth Sleepiness Scale (ESS), which

measures an individual’s propensity to sleep in given scenarios robust to variations in sleep pressure and circadian variance (Johns, 1991; Martin et al., 2023). The Karolinska Sleepiness Scale (KSS) measures state sleepiness using a 1-item nine point Likert scale, and is highly correlated with EEG measures of drowsiness in response to sleep deprivation (Åkerstedt et al., 2014; Kaida et al., 2006). This correlation makes the KSS a useful measurement tool for examining the relationship between objective and subjective measures of drowsiness on clinical populations, as it measures sleepiness at a particular point in time which can then be compared to EEG activity.

The feeling of subjective sleepiness is not experienced homogeneously across populations. Excessive daytime sleepiness is one of the most common complaints associated with NRS, with significantly increased daytime fatigue, and self-reported cognitive and psychological impairments (Sarsour et al., 2010; Tinajero et al., 2018). Daytime sleepiness is also present in ID, with excessive daytime sleepiness (EDS) having a prevalence of 45% (Hein et al., 2017). Insomnia symptom severity is correlated to increased EDS scores across the day, particularly in the morning and evening (Balter et al., 2024). However, these symptoms are additionally associated with hyperarousal, leading to a phenomenon of co-activation of the parasympathetic and sympathetic nervous systems. This co-activation leads to high and low arousal symptoms being experienced concurrently, leading to greater variability in symptoms. Examining how the experience of subjective sleepiness varies across disorders will lead to greater understanding of the subjective experience of sleepiness across both disorders.

Although subjective sleepiness scores strongly correlate with objective measures of drowsiness in healthy populations, there is a subjective-objective mismatch observed in individuals with ID, possibly due to increased fast-frequency activity (cite?). ID is associated with a discrepancy between objective sleep as measured by PSG and subjective sleep as reported by a sleep diary. Patients with ID report a reduction in sleep duration of up to 4 hours greater than that measured by PSG, however this discrepancy may be attributable to mismeasurement rather than misperception (Benz et al., 2023; Stephan & Siclari, 2023). Localised spectral power cannot be recorded through traditional PSG methods, which are hypothesised to be a determinant of sleep-related consciousness (Siclari & Tononi, 2017). The relationship between EEG spectral power and subjective state drowsiness has not been explored in clinical populations, and greater understanding of this relationship is needed.

Aim

This study aimed to explore if there are differences in how populations with NRS, ID, and healthy controls experience subjective and objective sleepiness, and if these differences are associated with topographic differences of SWA during resting wake and overnight sleep. Using mixed linear models, we aimed to assess if there was a difference in the correlation between subjective and objective measures based on population group. Finally, to examine if delta power is a potential mechanism for non-refreshing sleep in NRS, we investigated if clusters associated with a higher slowing ratio were associated with reduced delta power during the previous night's sleep.

By examining regional brain activity during resting wake, the study aims to examine if there are differences in how NRS, ID and HC experience and dissipate sleep pressure. Differences in delta power and SWA among groups may reveal differences in how sleep pressure is dissipated and if there are adaptive processes emerging as a result of ongoing sleep deprivation.

Hypotheses

1. KSS scores upon awakening will be highest in the NRS group compared to ID and healthy controls, reporting higher subjective sleepiness following sleep.
2. The correlation between KSS score and global Slowing Ratio will be significantly different between groups.
3. Topographic cluster analysis of SR will reveal cluster differences between groups. We hypothesise that at least one cluster of EEG channels will demonstrate a significantly different slowing ratio power that will differentiate the NRS group from ID and healthy controls.
4. For those with NRS, channel clusters with high values of slowing ratio will also show reduced delta power in NREM3 sleep.

Method

Study design

The study was a cross-sectional, age and sex matched case-control study. The study employed a between-participants mixed linear model design. The independent variables was clinical group and EEG channel, and the dependent variables were KSS score and spectral power. Additionally, topographic analysis of spectral power ...

Participants

12 participants from each clinical population were recruited: individuals with insomnia disorder (ID), individuals with non-restorative sleep (NRS), and healthy controls (HC). Recruitment was conducted through referrals from the Woolcock Institute and the Royal Prince Alfred sleep clinics, in addition to social media advertising. Due to the influence of age and sex on sleep architecture (Mongrain et al., 2005), participants were sex and age matched with a maximum difference of 1 year.

Participants were excluded if they had any comorbid sleep apnoea, as measured by wrist oximetry (oxygen desaturation index above 10 during any night of monitoring) (WristOX has high sensitivity of diagnosing OSAS (Nigro et al., 2009)). Participants were additionally excluded if they had clinically significant depression or anxiety scores as measured through the DASS-21, heavy alcohol use, pregnancy, circadian rhythm disruption through shift work or recent international travel, or a natural sleep time that of less than 6 hours or outside the hours of 21:30 and 8:00. As medications are known to affect sleep architecture, participants taking regular medications affecting sleep were excluded.

The inclusion criteria for the ID group was as set by the DSM-5-TR (Association, 2022) criteria, with difficulty initiating or maintaining sleep persisting for over 1 month causing clinically significant distress or impairment in daily life. They additionally were required to have a Pittsburgh Sleep Quality Index (PSIQ) score of 6 or higher, and an Insomnia Severity Index (ISI) score of 16 or higher.

Individuals in the NRS group could not have a mean Total Sleep Time (TST) below six hours as measured by sleep diary or actigraphy, or a mean refreshed score above 3. Inclusion in this group required a PSQI of 6 or more, with subcomponent scores of at least 2 on the PSQI Component 1 and 10 on PSQI Component 5.

Healthy controls needed to have a PSQI score of 4 or less and an ISI score of 6 or less.

All participants provided written consent and participation could be discontinued at any time. Par-

participants were remunerated \$100 upon successful completion of the study.

Due to the strict inclusion and exclusion criteria, of the N participants that completed the expression of interest form, only N were eligible for inclusion.

Protocol

The study was approved by the Macquarie University Human Research Ethics Committee (FoRA ID 17112).

Participants attended the sleep laboratory at the Woolcock Institute of Medical Research for initial screening by a sleep physician. Participants baseline sleep and activity patterns were measured via an Actigraphy watch (**which one**) for 7 days prior, which was validated against self-reported sleep diaries. Participants additionally completed the Restorative Sleep Questionnaire Daily Version (RSQ-D) for 7 days prior.

Upon arrival at the laboratory at 17:00, participants underwent final medical screening and a series of cognitive assessments. They were then served dinner and fitted with a high-density electroencephalography (HD-EEG) cap **which one**. Further cognitive assessments were conducted before the administration of the Karolinska Drowsiness Test (KDT) approximately 45 minutes prior to their habitual bedtime. Overnight polysomnography using HD-EEG was recorded, in addition to sleep video recording using a AXIS P3225-LV camera.

Lights were turned on at the participant's natural wake time and they were asked if they were already awake or wakened by researchers. The KSS and KDT was administered five minutes post-habitual wake time. Following the morning KDT, participants completed further cognitive testing and an MRI scan.

Measures

KSS

Subjective sleepiness was assessed 15 minutes after natural wake time using the Karolinska Sleepiness Scale (KSS), a 9 point scale with verbal anchors at each step (Åkerstedt & Gillberg, 1990). It is a measure of an individual's perceived sleepiness at a given point and is therefore difficult to assess test-retest reliability, however it has demonstrated reliability over two nights of sleep loss with a one week recovery period (Gillberg et al., 1994). It is sensitive to manipulations affecting sleepiness and is used consistently across individuals (Åkerstedt et al., 2014) The KSS has been validated in healthy populations as being closely related to EEG and behavioural variables of sleepiness (Åkerstedt et al., 1991; Kaida et al., 2006). Correlations between

KSS scores and EEG measures of sleepiness are over $r = .5$ [Åkerstedt & Gillberg (1990); vandenbergh2005] and correlate ($r = .57$) with response times on a vigilance test (Kaida et al., 2006).

KDT

The Karolinska Drowsiness Test (KDT) was administered immediately following the KSS and was used to measure electrophysiological drowsiness as measured through HD-EEG recordings. Participants were instructed “Look at the dot in front of you and be as relaxed as possible while staying awake. Keep your head and body still and minimize blinking. After a few minutes, I’ll ask you to close your eyes and keep them closed for a few minutes. Finally, I’ll ask you to open your eyes again and keep them open for a few minutes.” The test is 7 minutes long with 3 phases (eyes open/eyes closed/eyes open) each lasting 120 seconds. why do we do eyes open and eyes closed

Spectral power is calculated for each 6 second epoch

HD-EEG

High-density EEG data were collected using 256-channel caps (**which one**). The signals were amplified and digitised, impedances, recordings were acquired with electrodes referenced to the vertex processing of original eeg signals was performed

The data was visually inspected for artefacts and arousals using a **semi-automatic process** and was manually verified and cleaned. The record was visually inspected for bad channels and channels identified as poor quality ($2.5\% \pm 1.4\%$ of 164 channels) were replaced with an interpolated EEG signal using a spherical spline interpolation algorithm. After artifact removal and bad channel interpolation, the EEG signals were average-referenced. did we do this?

Power spectra

Get a power spectrum for each 6 second epoch in your data

- PSA based on FFD most common method for processing EEG signal, transforms eeg signal from time domain (amplitude x time) to frequency domain (frequency x time), providing greater insight into brain activity over time
- The most common quantitative method employed in sleep studies is spectral analysis, which decomposes a time series of EEG data into power (squared amplitude) in frequency bins (mV²/bin) , can be

expressed as absolute or relative to the summed power in all bins, spectral analysis may represent an objective method for examining the pathophysiological mechanisms underlying insomnia (Zhao et al., 2021)

- Raw PSD has a straightforward connection to signal amplitude, with channels expressing larger signal amplitudes typically showing larger power, useful when absolute differences in signal amplitude are deemed meaningful (topographical analysis) (Cox & Fell, 2020)

To calculate power spectral density, cleaned EEG signals were analysed using a fast Fourier transform with 50% overlapping between consecutive 4-second windows with a Hanning filter function, resulting in a frequency resolution of 0.25 Hz

EEG spectral power densities were quantified as: delta (1–4.5 Hz), theta (4.5–8 Hz), alpha (8–12 Hz), sigma (12–15 Hz), beta (15–25 Hz), and gamma (25–40 Hz).

Statistical analysis

z-score normalised power spectral data were analysed for eyes open and eyes closed conditions for each participant

To control for Type I error rate in cluster analysis, statistical nonparametric mapping (SnPM) with the suprathreshold cluster test will be used. SnPM uses permutation tests (10 000 random shuffles of the data) to establish a distribution of cluster size findings that occur due to chance. This distribution can then be used to compare cluster size to the a priori set threshold of $p < .05$, determining if it is statistically significant (D’Rozario et al., 2023).

EEG processor

All analyses were performed using MATLAB version r2024a (MathWorks, Natick, MA, USA).

Data cleaning

Gives us discontinuous data (boundary clip)

Bad channels and epochs were manually inspected and replaced with interpolated signals from neighbouring channels Using linear mixing to replace bad channels - from direct neighbours created a new signal that is interpolated from the neigh creates a reconstructed channel that is interpolated from neighbouring channels weighted by non-linear (squared) distance weights

$$\frac{\alpha_{1R}}$$

FFT

done in 6 second epochs

Independent components analysis

concatenated files

ICA estimates how independent the estimated sources are Attempts to make the sources as independent as possible can use components to subtract the artefact from the data take away the time series signal
- remove the component weighted components

algorithm looks at eye, muscle and heart

classifier looks at spatial weights in the time series

Limitations of the approach there is no perfect solution to subtract artefacts and just capture brain activity If you remove artefacts, you also remove brain activity Remove as many artefacts as you can without removing too much brain activity trying to optimise weights - may end up with a global minimum rather than a local minimum

The lack of ICA in PSG means that it is contaminated by ECG artefacts - this is a limitation

ICA assumes that sources are stationary

we are using it to clean the data cocktail party problem - cleans the data so that each electrode is only picking up activity that is specific to that area combines signals in a way so that they combine the activity that they measure and cancel out activity that they do not consistently measure no perfect way of separating the data

Classifier - ICLabel - <https://www.sciencedirect.com/science/article/pii/S1053811919304185>
threshold of 0.8 If a component has a weight of .8 or higher on eye/muscle/heart it is removed, otherwise retained

ICA is NOT done for PSG

average referencing

rereferencing = want to stay close enough to skull but not close enough to pick up brain activity
HD-EEG allows a uniform estimation of all brain activity

(net voltage brain potential should be 0) - meaning that in order to accurately measure total brain activity you need to be able to measure enough activity to know the voltage potential differences between the sites) - using average of brain activity across cortex means that you are measuring the activity of the particular electrode

when to do - it doesn't matter as you are selecting the average reference at each particular point - as long as you don't average across time or apply an FFT first but it does need to be done before ICA!

Process

1. manual inspection and removal of artefacts
2. average referencing
3. ICA
4. concatenate two eyes open channels
5. then apply FFT

References

- Achermann, P., & Borbély, A. A. (2003). Mathematical models of sleep regulation. *Frontiers in Bioscience-Landmark*, 8(6), 683–693. <https://doi.org/10.2741/1064>
- Åkerstedt, T., Anund, A., Axelsson, J., & Kecklund, G. (2014). Subjective sleepiness is a sensitive indicator of insufficient sleep and impaired waking function. *Journal of Sleep Research*, 23(3), 242–254. <https://doi.org/10.1111/jsr.12158>
- Åkerstedt, T., & Gillberg, M. (1990). Subjective and objective sleepiness in the active individual. *International Journal of Neuroscience*, 52(1-2), 29–37. <https://doi.org/10.3109/00207459008994241>
- Åkerstedt, T., Kecklund, G., & Knutsson, A. (1991). Spectral analysis of sleep electroencephalography in rotating three-shift work. *Scandinavian Journal of Work, Environment & Health*, 17(5), 330–336. <https://www.jstor.org/stable/40965913>
- American Academy of Sleep Medicine. (2005). International classification of sleep disorders. *Diagnostic and Coding Manual*, 148–152.
- Appleton, S. L., Reynolds, A. C., Gill, T. K., Melaku, Y. A., & Adams, R. J. (2022). Insomnia Prevalence Varies with Symptom Criteria Used with Implications for Epidemiological Studies: Role of Anthropometrics, Sleep Habit, and Comorbidities. *Nature and Science of Sleep*, 14, 775–790. <https://doi.org/10.2147/NSS.S359437>
- Association, A. P. (2022). *Diagnostic and statistical manual of mental disorders* (5th ed., text revision). American Psychiatric Association.
- Balter, L. J. T., Van Someren, E. J. W., & Axelsson, J. (2024). Insomnia symptom severity and dynamics of arousal-related symptoms across the day. *Journal of Sleep Research*, e14276. <https://doi.org/10.1111/jsr.14276>
- Basner, M., & Dinges, D. F. (2011). Maximizing Sensitivity of the Psychomotor Vigilance Test (PVT) to Sleep Loss. *Sleep*, 34(5), 581–591. <https://doi.org/10.1093/sleep/34.5.581>
- Benington, J. H. (2000). Sleep homeostasis and the function of sleep. *Sleep*, 23(7), 959–966.
- Benz, F., Riemann, D., Domschke, K., Spiegelhalder, K., Johann, A. F., Marshall, N. S., & Feige, B. (2023). How many hours do you sleep? A comparison of subjective and objective sleep duration measures in a sample of insomnia patients and good sleepers. *Journal of Sleep Research*, 32(2), e13802. <https://doi.org/10.1111/jsr.13802>
- Bernardi, G., Siclari, F., Yu, X., Zennig, C., Bellesi, M., Ricciardi, E., Cirelli, C., Ghilardi, M. F., Pietrini, P., & Tononi, G. (2015). Neural and Behavioral Correlates of Extended Training during Sleep Deprivation in Humans: Evidence for Local, Task-Specific Effects. *Journal of Neuroscience*, 35(11), 4487–4500. <https://doi.org/10.1523/JNEUROSCI.4567-14.2015>
- Borbély, A. A. (1982). A two process model of sleep regulation. *Human Neurobiology*, 1(3), 195–204.
- Borbély, A. A., Daan, S., Wirz-Justice, A., & Deboer, T. (2016). The two-process model of sleep regulation: A reappraisal. *Journal of Sleep Research*, 25(2), 131–143. <https://doi.org/10.1111/jsr.12371>
- Buzsáki, G., Anastassiou, C. A., & Koch, C. (2012). The origin of extracellular fields and currents — EEG, ECoG, LFP and spikes. *Nature Reviews Neuroscience*, 13(6), 407–420. <https://doi.org/10.1038/nrn3241>
- Cajochen, C., Foy, R., & Dijk, D.-J. (1999). Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. *Sleep Research Online : SRO*, 2, 65–69.
- Cirelli, C., & Tononi, G. (2008). Is sleep essential? *PLOS Biology*, 6(8), e216. <https://doi.org/10.1371/journal.pbio.0060216>
- Cox, R., & Fell, J. (2020). Analyzing human sleep EEG: A methodological primer with code implementation. *Sleep Medicine Reviews*, 54, 101353. <https://doi.org/10.1016/j.smrv.2020.101353>
- Dressle, R. J., & Riemann, D. (2023). Hyperarousal in insomnia disorder: Current evidence and potential mechanisms. *Journal of Sleep Research*, 32(6), e13928. <https://doi.org/10.1111/jsr.13928>
- Feige, B., Spiegelhalder, K., Kiemen, A., Bosch, O. G., Tebartz van Elst, L., Hennig, J., Seifritz, E., & Riemann, D. (2017). Distinctive time-lagged resting-state networks revealed by simultaneous EEG-fMRI. *NeuroImage*, 145, 1–10. <https://doi.org/10.1016/j.neuroimage.2016.09.027>

- Gillberg, M., Kecklund, G., & Akerstedt, T. (1994). Relations between performance and subjective ratings of sleepiness during a night awake. *Sleep*, 17(3), 236–241. <https://doi.org/10.1093/sleep/17.3.236>
- Hein, M., Lanquart, J.-P., Loas, G., Hubain, P., & Linkowski, P. (2017). Prevalence and risk factors of excessive daytime sleepiness in insomnia sufferers: A study with 1311 individuals. *Journal of Psychosomatic Research*, 103, 63–69. <https://doi.org/10.1016/j.jpsychores.2017.10.004>
- Hogan, S. E., Delgado, G. M., Hall, M. H., Nimgaonkar, V. L., Germain, A., Buysse, D. J., & Wilckens, K. A. (2020). Slow-oscillation activity is reduced and high frequency activity is elevated in older adults with insomnia. *Journal of Clinical Sleep Medicine*, 16(9), 1445–1454. <https://doi.org/10.5664/jcsm.8568>
- Huang, L., Zhou, J., Li, Z., Lei, F., & Tang, X. (2012). Sleep perception and the multiple sleep latency test in patients with primary insomnia. *Journal of Sleep Research*, 21(6), 684–692. <https://doi.org/10.1111/j.1365-2869.2012.01028.x>
- Huber, R., Deboer, T., & Tobler, I. (2000). Topography of EEG Dynamics After Sleep Deprivation in Mice. *Journal of Neurophysiology*, 84(4), 1888–1893. <https://doi.org/10.1152/jn.2000.84.4.1888>
- Huber, R., Felice Ghilardi, M., Massimini, M., & Tononi, G. (2004). Local sleep and learning. *Nature*, 430(6995), 78–81. <https://doi.org/10.1038/nature02663>
- Hung, C.-S., Sarasso, S., Ferrarelli, F., Riedner, B., Ghilardi, M. F., Cirelli, C., & Tononi, G. (2013). Local Experience-Dependent Changes in the Wake EEG after Prolonged Wakefulness. *Sleep*, 36(1), 59–72. <https://doi.org/10.5665/sleep.2302>
- Johns, M. W. (1991). A new method for measuring daytime sleepiness: The Epworth sleepiness scale. *Sleep*, 14(6), 540–545. <https://doi.org/10.1093/sleep/14.6.540>
- Johns, M. W. (2009). What is excessive daytime sleepiness !!@cite fix this citation!! In *Sleep deprivation: Causes, effects and treatment* (pp. 59–94). Nova Science Publishers Inc.
- Kaida, K., Takahashi, M., Akerstedt, T., Nakata, A., Otsuka, Y., Haratani, T., & Fukasawa, K. (2006). Validation of the Karolinska sleepiness scale against performance and EEG variables. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, 117(7), 1574–1581. <https://doi.org/10.1016/j.clinph.2006.03.011>
- Kao, C.-H., D’Rozario, A. L., Lovato, N., Wassing, R., Bartlett, D., Memarian, N., Espinel, P., Kim, J.-W., Grunstein, R. R., & Gordon, C. J. (2021). Insomnia subtypes characterised by objective sleep duration and NREM spectral power and the effect of acute sleep restriction: An exploratory analysis. *Scientific Reports*, 11(1), 24331. <https://doi.org/10.1038/s41598-021-03564-6>
- Krueger, J. M., Nguyen, J. T., Dykstra-Aiello, C. J., & Taishi, P. (2019). Local sleep. *Sleep Medicine Reviews*, 43, 14–21. <https://doi.org/10.1016/j.smrv.2018.10.001>
- Krueger, J. M., & Obäl Jr., F. (1993). A neuronal group theory of sleep function. *Journal of Sleep Research*, 2(2), 63–69. <https://doi.org/10.1111/j.1365-2869.1993.tb00064.x>
- Lunsford-Avery, J. R., Edinger, J. D., & Krystal, A. D. (2021). Optimizing computation of overnight decline in delta power: Evidence for slower rate of decline in delta power in insomnia patients. *Clinical Neurophysiology*, 132(2), 545–553. <https://doi.org/10.1016/j.clinph.2020.12.004>
- Marques, D. R., Gomes, A. A., & de Azevedo, M. H. P. (2019). DSDS-4: A Brief Measure of Perceived Daytime Sleepiness. *Current Psychology*, 38(2), 579–588. <https://doi.org/10.1007/s12144-017-9638-0>
- Martin, V. P., Lopez, R., Dauvilliers, Y., Rouas, J.-L., Philip, P., & Micoulaud-Franchi, J.-A. (2023). Sleepiness in adults: An umbrella review of a complex construct. *Sleep Medicine Reviews*, 67, 101718. <https://doi.org/10.1016/j.smrv.2022.101718>
- Merica, H., Blois, R., & Gaillard, J.-M. (1998). Spectral characteristics of sleep EEG in chronic insomnia. *European Journal of Neuroscience*, 10(5), 1826–1834. <https://doi.org/10.1046/j.1460-9568.1998.00189.x>
- Mongrain, V., Carrier, J., & Dumont, M. (2005). Chronotype and sex effects on sleep architecture and quantitative sleep EEG

- in healthy young adults. *Sleep*, 28(7), 819–827. <https://doi.org/10.1093/sleep/28.7.819>
- Neu, D., Mairesse, O., Verbanck, P., & Le Bon, O. (2015). Slow wave sleep in the chronically fatigued: Power spectra distribution patterns in chronic fatigue syndrome and primary insomnia. *Clinical Neurophysiology*, 126(10), 1926–1933. <https://doi.org/10.1016/j.clinph.2014.12.016>
- Nigro, C. A., Aimaretti, S., Gonzalez, S., & Rhodius, E. (2009). Validation of the WristOx 3100™ oximeter for the diagnosis of sleep apnea/hypopnea syndrome. *Sleep and Breathing*, 13(2), 127–136. <https://doi.org/10.1007/s11325-008-0217-3>
- Perlis, M. L., Smith, M. T., Andrews, P. J., Orff, H., & Giles, D. E. (2001). Beta/Gamma EEG activity in patients with primary and secondary insomnia and good sleeper controls. *Sleep*, 24(1), 110–117. <https://doi.org/10.1093/sleep/24.1.110>
- Pigeon, W. R., & Perlis, M. L. (2006). Sleep homeostasis in primary insomnia. *Sleep Medicine Reviews*, 10(4), 247–254. <https://doi.org/10.1016/j.smr.2005.09.002>
- Plante, D. T., Goldstein, M. R., Cook, J. D., Smith, R., Riedner, B. A., Rumble, M. E., Jelenchick, L., Roth, A., Tononi, G., Benca, R. M., & Peterson, M. J. (2016). Effects of partial sleep deprivation on slow waves during non-rapid eye movement sleep: A high density EEG investigation. *Clinical Neurophysiology*, 127(2), 1436–1444. <https://doi.org/10.1016/j.clinph.2015.10.040>
- Riemann, D., Spiegelhalter, K., Feige, B., Voderholzer, U., Berger, M., Perlis, M., & Nissen, C. (2010). The hyperarousal model of insomnia: A review of the concept and its evidence. *Sleep Medicine Reviews*, 14(1), 19–31. <https://doi.org/10.1016/j.smr.2009.04.002>
- Roehrs, T. A., Randall, S., Harris, E., Maan, R., & Roth, T. (2011). MSLT in Primary Insomnia: Stability and Relation to Nocturnal Sleep. *Sleep*, 34(12), 1647–1652. <https://doi.org/10.5665/sleep.1426>
- Roth, T., Zammit, G., Lankford, A., Mayleben, D., Stern, T., Pitman, V., Clark, D., & Werth, J. L. (2010). Nonrestorative sleep as a distinct component of insomnia. *Sleep*, 33(4), 449–458. <https://doi.org/10.1093/sleep/33.4.449>
- Sarsour, K., Van Brunt, D. L., Johnston, J. A., Foley, K. A., Morin, C. M., & Walsh, J. K. (2010). Associations of nonrestorative sleep with insomnia, depression, and daytime function. *Sleep Medicine*, 11(10), 965–972. <https://doi.org/10.1016/j.sleep.2010.08.007>
- Siclari, F., & Tononi, G. (2017). Local aspects of sleep and wakefulness. *Current Opinion in Neurobiology*, 44, 222–227. <https://doi.org/10.1016/j.conb.2017.05.008>
- Stephan, A. M., & Siclari, F. (2023). Reconsidering sleep perception in insomnia: From misperception to mismeasurement. *Journal of Sleep Research*, 32(6), e14028. <https://doi.org/10.1111/jsr.14028>
- Steriade, M., Timofeev, I., & Grenier, F. (2001). Natural waking and sleep states: A view from inside neocortical neurons. *Journal of Neurophysiology*, 85(5), 1969–1985. <https://doi.org/10.1152/jn.2001.85.5.1969>
- Stone, K. C., Taylor, D. J., McCrae, C. S., Kalsekar, A., & Lichstein, K. L. (2008). Nonrestorative sleep. *Sleep Medicine Reviews*, 12(4), 275–288. <https://doi.org/10.1016/j.smr.2007.12.002>
- Tinajero, R., Williams, P. G., Cribbet, M. R., Rau, H. K., Bride, D. L., & Suchy, Y. (2018). Nonrestorative sleep in healthy, young adults without insomnia: Associations with executive functioning, fatigue, and pre-sleep arousal. *Sleep Health*, 4(3), 284–291. <https://doi.org/10.1016/j.sleh.2018.02.006>
- Tononi, G., & Cirelli, C. (2006). Sleep function and synaptic homeostasis. *Sleep Medicine Reviews*, 10(1), 49–62. <https://doi.org/10.1016/j.smr.2005.05.002>
- Vyazovskiy, V. V., Cirelli, C., Pfister-Genskow, M., Faraguna, U., & Tononi, G. (2008). Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. *Nature Neuroscience*, 11(2), 200–208. <https://doi.org/10.1038/nn2035>
- Vyazovskiy, V. V., & Harris, K. D. (2013). Sleep and the single neuron: The role of global slow oscillations in individual cell

- rest. *Nature Reviews Neuroscience*, 14(6), 443–451. <https://doi.org/10.1038/nrn3494>
- Vyazovskiy, V. V., Olcese, U., Hanlon, E. C., Nir, Y., Cirelli, C., & Tononi, G. (2011). Local sleep in awake rats. *Nature*, 472(7344), 443–447. <https://doi.org/10.1038/nature10009>
- Werth, E., Achermann, P., & Borbély, A. A. (1996). Brain topography of the human sleep EEG: Antero-posterior shifts of spectral power. *NeuroReport*, 8(1), 123.
- Zhang, J., Lam, S.-P., Li, S. X., Li, A. M., & Wing, Y.-K. (2012). The longitudinal course and impact of non-restorative sleep: A five-year community-based follow-up study. *Sleep Medicine*, 13(6), 570–576. <https://doi.org/10.1016/j.sleep.2011.12.012>
- Zhao, W., Van Someren, E. J. W., Li, C., Chen, X., Gui, W., Tian, Y., Liu, Y., & Lei, X. (2021). EEG spectral analysis in insomnia disorder: A systematic review and meta-analysis. *Sleep Medicine Reviews*, 59, 101457. <https://doi.org/10.1016/j.smr.2021.101457>