Thesis

Anastasia Stuart

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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

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, sleepiness, and diminished quality of life (Roth et al., 2010). Despite the impact of this condition, there are no established guidelines for diagnosis or clinical management, and it is not included in the Diagnostic and Statistical Manual-5-TR (Association, 2022). 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 percieved 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 increased fatigue (**cite?**), however this population does not experience increased sleep propensity in comparison to healthy controls (**fasiello2023?**). Although NRS is associated with normal sleep architecture, it may be linked to a reduction in slow wave activity (SWA) (Kao et al., 2021) preventing the dissipation of sleep pressure.

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 (**cite?**). 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). Diagnosis is recommended to be made though subjective self-reporting rather than polysomnographic (PSG) data (American Academy of Sleep Medicine, 2005), as this population often have normal objective sleep paramaters as measured by PSG data (**cite?**). ID is associated with diminished quality of life (**kyle2010?**), increased risk of comorbid psychiatric disorders (Perlis et al., 2022) and increased daytime fatigue (**kim2019?**). These symptoms lead to a significant health impact, however the pathophysiology and etiology of ID remains unclear.

A proposed causal and perpetuating factor in ID is 24-hour hyperarousal, being an increase of physiological, cognitive and cortical activity that contributes to the subjective and objective symptoms of the disorder (Dressle & Riemann, 2023; Riemann et al., 2010).

ID is proposed to be caused and perpetuated by increased physiological and neurobiological arousal, preventing sleep initiation and leading to increased overnight awakenings (Dressle & Riemann, 2023; Riemann et al., 2010). Cortical hyperarousal is present in ID as observed through a 24-hour increase in fast frequency brain activity, particularly in the beta frequency (**shi2022?**). Hyperarousal can prevent daytime sleepiness as measured through sleep latency despite significant fatigue, as the dominance of wake-like neural activity in the beta frequencies prevents a global lapse of consctiousness (**shi2022?**).

Daytime fatigue, being the subjective experience of low energy (**raizen2023?**), is the most prevalent daytime complaint in this population and is associated with the most significant detrimental impact to daily functioning (**kyle2010?**). Severe fatigue is associated with greater insomnia symptom severity, daytime sleepiness, depressive symptoms, and increased habitual sleep duration (**kim2019?**).

Despite the prevalence of fatigue within the population, this population does not consistently exhibit increased measures of sleepiness. The prevalence of excessive daytime sleepiness (EDS) as measured by sleep propensity within ID varies between 10-41.61% and is unrelated to insomnia symptom severity (Hein et al.,

2017; **fasiello2023?**; **seong2022?**). Additionally, despite increased fatigue, ID populations display similar or increased sleep latency in comparison to healthy controls (**cite?**).

The contrasting experiences of increased fatigue with reduced sleepiness may be caused by the competing influences of hyperarousal and sleep pressure (**marques2024?**).

Individuals experiencing a misperception between their subjective and ojective sleep were intitially hypothesised to have an inability to accurately percieve their sleep or wake state leading to a skewed perception of their wake after sleep onset (**dorsey1997?**). However, with the introduction of more refined measurement techniques including HD-EEG and power spectral analysis, research has found that an increase of 'wake-like' brain activity in the alpha, sigma, beta and gamma bands during sleep is associated with increased percieved wakefulness (**andrillon2020?**; **krystal2002?**; **lecci2020?**). These findings have led the suggestion that sleep-state misperception may be due to the inability of current recording and analysis techniques to accurately identify wake-like intrusions into sleep, and the misperception experienced by ID populations possibly being better conceptualised as a mismeasurement instead (Stephan & Siclari, 2023).

As a hallmark of ID is the inability to fall asleep, measuring sleepiness through sleep latency is insufficient for measuring sleepiness. Sleepiness perception, or the subjective evaluation of sleepiness, may provide

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).

decreased sleepiness as measured through sleep latency (Huang et al., 2012; Roehrs et al., 2011).

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.

`is now well established that localised sleep and wake patterns, which are not`

adequately captured by standard sleep recordings (PSG) and scoring methods, can coexist in both physiological and pathological conditions, and likely determine sleep-related conscious experiences [@siclari2017]

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.

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 a 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 flucuates 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). Howecer, sleepiness perception is not experienced uniformly across populations, with the differential influences of factors including fatigue and arousal causing individuals to possibly mispercieve 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 Epsworth 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 sujective 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 approved by the Macquarie University Human Research Ethics Committee (FoRA ID 17112) and all participants provided written informed consent.

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 anaysis of spectral power …

Participants

964 participants completed the online expression of interest questionnaire, 352 found as eligible for participation, and 33 participants were included in the study.

. Of these, 8 were unable to be contacted via email and 161 did not respond to a follow up email. 180 participants proceeded to pre-screening. 145 completers were excluded from participation during the pre-screening and screening visits, with 44 (30%) being excluded for medication use and 54 (15%) being excluded due to having to age or sex match.

Predetermined sample size was 12 participants from each clinical population, determined **how?** Due to the strict exclusion criteria and time constraints, the total sample analysed was 33 participants (13 NRS; 11 ID; 9 Control).

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 ± 2 years.

how many people expressed interest through an online recruitment survey. **how many** were excluded due to **what reasons**

Recruitment was conducted through referrals from the Woolcock Institute and the Royal Prince Alfred sleep clinics, in addition to social media advertising.

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. 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

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 behavioral 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); vandenberg2005] 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`

HD-EEG

High-density EEG data were collected using 256-channel electrode caps (**which one**) and a **which** amplifier and **which** software (digitised?) with electodes referenced to the vertex (CZ) (**cite?**). Electrodes were placed along the scalp, mastiods, **anywhere else?**. Electrooculography (EOG) were recorded using

electrodes placed *where* and electrocardiogram *?* During acquisition, data were low-pass filtered at **70** Hz, high-pass filtered at **0.3** Hz, and notch filtered at **50** Hz (**cite?**). Electrode impedences were below **what** kΩ.

`filter and hanning window`

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% ±

## Data processing

### EEG Preprocessing

All preprocessing was completed using the EEG Processor application ((**wassing2024?**)). Data were visually inspected for artefacts and arousals which were removed across all channels. Poor quality channels were replaced with an interpolated EEG signal from neighbouring channels using linear mixing, weighted by the squared non-linear distance *on average how many per participant, +-SD).*

### Average referencing

To improve the accuracy of recorded signals, data was re-referenced to a common average signal created through finding the mean global signal across all electrodes. This average signal was then subtracted from each individual electrode's signal, reducing the influence of a single electrode that occurs when using the vertex (CZ) signal as a reference. This process enhances the detection of local neuronal activity and enables the rich spatial resolution of HD-EEG data.

### Independent components analysis

Following preprocessing, independent components analysis (ICA) was used to identify and seperate components that are statistically independent from each other in KDT data. This was done using an automated process using the MATLAB program *ICLabel*, removing components with a weighting of .8 or greater for non-brain activity (Pion-Tonachini et al., 2019). Artefact removal of eye, heart, muscle, and
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electrical activity components was conducted, with remaining components being back-projected to the EEG dataset via regression resulting in a cleaned time series signal.

ICA was unable to be applied to PSG data. Although ICA is effective in removing artefacts in short recordings of a stationary subject, it is unable to process PSG recordings as signal sources are variable over the course of the night. Furthermore, the temporal variability of brain activity across sleep stages prevents ICA from being able to reliably differentiate between artefacts and brain activity. As ICA was unable to be applied to PSG, this data is contaminated by non-brain activity, however as the data was visually cleaned for artefacts and interpreted with acknowledgement of artefact contamination, it was still used.

Power spectra

```is this where we excluded non-cranial EEG channels?```

EEG power spectra was obtained for each channel using a fast Fourier transform (FFT) to deconstruct the EEG signal from the time domain to the frequency domain, allowing it to be analysed in power (squared amplitude) in frequency bins (mV2/bin). The power spectra was calculated for 50% overlapping 6-second epochs and obtained for the eyes closed condition and a concatenated recording of the eyes open condition. `with a Hanning window, resulting in a frequency resolution of 0.25 Hz` **boundary clip?** EEG spectral power densities were quantified as: low delta (0.5-1 Hz), 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). These frequency bands were chosen as they reliably identify vigilance states in humans ** paper from Garry?** Power spectral densities represent the distribution of power in a signal across frequencies, allowing analysis of the frequency components that are most significant in each epoch's signal. This allowes the measurement of neuronal activity on vigilance states.

This data was then expressed as both an absolute and normalised **global?** value for all bins.

Slowing ratio

The EEG slowing ratio during each KDT condition was calculated by [(+)/ (+ +)] power.

Alpha attenuation coefficient

The alpha attenuation coefficient (AAC) measures alpha frequency power differences between eyes open and eyes closed conditions. Alpha power is expected to decrease during the eyes closed condition and
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increase during the eyes open condition. A high AAC score reflects high sleepiness.

Statistical analysis

All analyses were performed using MATLAB version r2024a (MathWorks, Natick, MA, USA). The normality of the distribution of dependent variables, demographic variables, and outliers was conducted using Q-Q Plots, Shapiro-Wilk normality tests, and visual inspections of histograms.

A one-way analysis of variance (ANOVA) was conducted to determine if there was a difference in group mean KSS scores. Post-hoc pairwise comparisons were conducted using Tukey's HSD.

Statistical analysis of group-level KDT data was conducted using a one-way ANOVA to assess differences in normalised EEG power spectra across groups and conditions (eyes open/eyes closed). The potential for Type I error during cluster analysis evaluation of EEG data was controlled for using statistical nonparametric mapping (SnPM) to resolve the challenge of multiple comparisons when using a large number of time-frequency comparisons. `clustermass approach` SnPM used 10 000 random permutatuions of the data to establish a distribution of cluster size findings that occur die to chance, which can then be used to compare found cluster sizes to. The cluster alpha was set at .05. Blocks were permuted as whole-blocks and within-blocks.

To account for non-normality, SR and AAC values were log transformed prior to analysis.

Results

Descriptives

33 participants were included, with the sample consisting of 13 individuals with Non-Restorative Sleep (NRS), 11 participants with Insomnia Disorder (ID), and 9 healthy controls (HC). Table 1 summarises the participant demographics and self-report questionnaires.

‘ {r} library(dplyr) descriptives <- data2 %>% group_by(group) %>% summarize(Mean = mean(KSS_AM1) , Median = median(KSS_AM1) , SD = sd(KSS_AM1) , Min = min(KSS_AM1) , Max = max(KSS_AM1)) descriptives[, -1] <- printnum(descriptives[, -1])

apa_table(descriptives , caption = “Descriptive statistics of correct recall by dosage.” , note = “This table was created with apa_table().” , escape = TRUE) ‘

Comparing KSS scores between groups

Analyses were run on R version 4.3.2 (2023-10-31).

A repeated measures ANOVA was conducted to evaluate the effect of group on AM KSS scores. For the KSS_AM1 scores, the mean score for the control group was 4.22 (SD = 1.09), for the ID group was 5.09 (SD = 2.17), and for the NRS group was 5.77 (SD = 1.92). The median scores were 4, 5, and 6, respectively. The minimum and maximum scores were 2 and 6 for CTL, 2 and 9 for GID, and 1 and 8 for NRS. The analysis revealed no significant effect of group, F(2,30)=1.897,p=.168

A post-hoc power analysis conducted in G*power Version 3.1.9.6 reported inadequate power for the given effect size, f=0.356. With a set alpha of 0.05, the power was found to be 0.396.

`huge variance in KSS_AM1 for GID, NRS higher but affected by outliers`

The ANOVA (formula: KSS_AM1 ~ group) suggests that:

- The main effect of group is statistically not significant and medium (F(2, 30) = 1.90, p = 0.168; Eta2 = 0.11, 95% CI [0.00, 1.00])

Effect sizes were labelled following Field’s (2013) recommendations.

Correlation between KSS and slowing ratio scores between groups

Correlation between KSS and AAC between groups

Topography of channel-by-channel comparisons between ID and NRS groups

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