Subjective and Objective Sleepiness in Non-Restorative Sleep, Insomnia Disorder, and Healthy Controls

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Abstract

Non-restorative sleep (NRS) is a condition characterised by subjectively unrefreshing sleep despite normal sleep duration, leading to daytime fatigue and reduced quality of life (Roth et al., 2010). Despite the significant impacts of the disorder, it does not have an established diagnostic criteria, and the characteristics associated with NRS are poorly understood, leading to diminished outcomes for individuals. It has previously been treated as a subtype of insomnia disorder (ID) (American Psychiatric Association, 2013), as daytime impairments are similar despite NRS occurring in the absence of the sleep disruptions associated with ID. This study aimed to examine differences in subjective and objective sleepiness upon awakening in a sample of a sample of 33 age- and sex-matched participants with NRS, ID, and healthy controls.

This study found no significant group differences in self-reported subjective sleepiness upon awakening measured using the Karolinska Sleepiness Scale (KSS). Using high-density electroencephalography (HD-EEG) objective sleepiness was measured through alpha attenuation coefficient (AAC) and slowing ratio (SR), and was not associated with significant group differences. Additionally, there was no significant association between subjective and objective sleepiness across groups, and no significant interaction. These results suggest that within our sample, individuals with NRS and ID do not differ significantly from healthy controls on measures of sleepiness upon awakening, despite daytime impairments.

The absence of significant differences highlights the need to explore other factors contributing to NRS, such as fatigue and subjective sleep quality. Understanding these factors may aid in developing diagnostic criteria and effective treatments for NRS, ultimately improving outcomes for those affected.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

Abbreviation	Full Term
AAC	**A**lpha **A**ttenuation **C**oefficient
AASM	**A**merican **A**cademy of **S**leep **M**edicine
ANOVA	**A**nalysis of **VA**riance
DASS-21	**D**epression **A**nxiety and **S**tress **S**cale - **21**
DSM-5	**D**iagnostic and **S**tatistical **M**anual - **5**
DSM-5-TR	**D**iagnostic and **S**tatistical **M**anual - **5**, **T**ext **R**evision
EEG	**E**lectro**e**ncephalo**g**raphy
EDS	**E**xcessive **D**aytime **S**leepiness
ESS	**E**pworth **S**leepiness **S**cale
FFT	**F**ast **F**ourier **T**ransform
FFS	**F**linders **F**atigue **S**cale
HD-EEG	**H**igh-**D**ensity **E**lectro**e**ncephalo**g**raphy
ICA	**I**ndependent **C**omponent **A**nalysis
ID	**I**nsomnia **D**isorder
IQR	**I**nter**Q**uartile **R**ange
ISI	**I**nsomnia **S**everity **I**ndex
KDT	**K**arolinska **D**rowsiness **T**est
KSS	**K**arolinska **S**leepiness **S**cale
MRI	**M**agnetic **R**esonance **I**maging
MSLT	**M**ultiple **S**leep **L**atency **T**est
MWT	**M**aintenance of **W**akefulness **T**est
N1, N2, N3	Sleep Stages **N**on-REM **1**, **2**, and **3**
NREM	**N**on-**R**apid **E**ye **M**ovement
NRS	**N**on-**R**estorative **S**leep
PALM	**P**ermutation-based **A**nalysis of **L**inear **M**odels
PSG	**P**oly**s**omno**g**raphy
PSQI	**P**ittsburgh **S**leep **Q**uality **I**ndex
PVT	**P**sychomotor **V**igilance **T**ask
REM	**R**apid **E**ye **M**ovement
RSQ-D	**R**estorative **S**leep **Q**uestionnaire - **D**aily Version
SnPM	**S**tatistical **N**on-**P**arametric **M**apping
SOL	**S**leep **O**nset **L**atency
SR	**S**lowing **R**atio
SWA	**S**low **W**ave **A**ctivity
TIB	**T**ime **I**n **B**ed
TST	**T**otal **S**leep **T**ime
WASO	**W**ake **A**fter **S**leep **O**nset

Introduction

Non-restorative sleep (NRS) is a condition characterised by self-reports of unrefreshing sleep despite normal objectively measured sleep duration and architecture, 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 (DSM-5-TR) (American Psychiatric Association, 2022). NRS has previously been clinically managed as a subtype of insomnia disorder (ID) despite patients not exhibiting the symptoms associated with ID, such as difficulty falling asleep, difficulty maintaining sleep, and shortened sleep duration (Roth et al., 2010). ID has received significant attention and research due to being the most common sleep disorder in Australia and the associated health burden (Sweetman et al., 2021). It has been linked to an increased risk of physical and mental health disorders and decreased quality of life (Kyle et al., 2010). In contrast, NRS is poorly understood and treated, largely due to limited available research on the aetiology of the condition. Currently, NRS symptoms are measured through subjective complaints, and it is unclear if the condition is associated with measurable neuropathology that may be different to ID. Identification of measurable changes in brain activity compared to healthy controls and ID could aid in better aetiological classification and diagnosis of the condition.

Both NRS and ID have been consistently associated with increased daytime fatigue (Kim et al., 2019; Zhang et al., 2012), however findings on subjective and objective daytime sleepiness are inconsistent (Hein et al., 2017; Sarsour et al., 2010). While fatigue is a broad construct that refers to feelings of exhaustion or low motivation, sleepiness specifically refers to an individual's sleep propensity, or their drive to sleep (Gradisar et al., 2007). Subjective sleepiness, being an individual's self-assessed perception of their sleep drive at a given moment, and objective sleepiness, being the level of sleep-like brain activity recorded through

electroencephalography (EEG), are correlated and increase in response to prolonged wakefulness and reduce with sleep in healthy populations (Åkerstedt & Gillberg, 1990). Although ID has been associated with decreased subjective and objective measures of sleepiness, it is not known how sleepiness manifests in an NRS population.

EEG recordings provide a spatiotemporally integrated recording of neuronal signals across the cortical surface, allowing for the non-invasive measurement of human brain activity and mental states (Buzsáki et al., 2012). EEG is the gold standard for objectively measuring sleep and wake stages in clinical sleep medicine (Berry et al., 2017). This method enables quantitative identification of intrusions of sleep-like brain activity present during wake, that can occur in localised brain regions rather than entire lobes of the cortex (Siclari & Tononi, 2017). Quantitative analysis of brain activity using high-density electroencephalography (HD-EEG) data provides an accurate method of exploring daytime sleepiness in these populations. HD-EEG enables analysis of cortical activity variations across brain regions with greater spatial resolution than traditional EEG, allowing for neural measures of sleepiness to occur within localised regions of the cortical surface. Therefore, this study will use self-rated sleepiness scores and HD-EEG data to answer the research question of whether there are differences in subjective or objective sleepiness during resting-state wakefulness directly after awakening from overnight sleep between NRS in comparison to ID and healthy controls.

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 linked to detrimental outcomes for individuals, including increased risk of comorbid psychiatric disorders, reduced physical health, diminished quality of life, and significant daytime fatigue (Kim et al., 2019; Kyle et al., 2010; Morin et al., 2015;

Perlis et al., 2022). ID is diagnosed through subjective reports of impaired overnight sleep, difficulty with sleep initiation, frequent overnight awakenings, and/or early morning awakenings without the ability to fall back asleep, leading to clinically significant distress or dysfunction in daily life (American Psychiatric Association, 2022).

Non-Restorative Sleep

NRS is distinct from ID as the primary complaint is unrefreshing sleep despite normal sleep duration of approximately 7-8 hours and no subjective reports of difficulty initiating or maintaining sleep or early morning awakenings (Roth et al., 2010). 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 (Roth et al., 2010). The prevalence of the symptom of unrefreshing sleep upon awakening in Australia is estimated to be between 42-45%, however only approximately 10% of this population receive clinical treatment (Adams et al., 2017; Metse & Bowman, 2020). Despite the negative consequences of the condition, it was removed as a characteristic of ID with the release of the Diagnostic and Statistical Manual-5 (DSM-5) due to its inconsistency with other ID symptoms and lack of validated operational measures (American Psychiatric Association, 2013). Consequently, this population are diagnosed as “other specified insomnia disorder” with no guidelines for diagnosis or treatment (American Psychiatric 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 aetiology to improve outcomes for patients.

Sleep

Sleep is a necessary behaviour for all humans that can be defined as a reversible reduction in responsiveness to external stimuli accompanied by a measurable change in brain activity patterns (Cirelli & Tononi, 2008). Sleep progresses through a series of stages

throughout the night, including rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep, which is further divided into stages N1, N2, and N3, each distinguishable through distinct patterns of brain activity and muscle tone. Sleep is regulated by two interacting systems, with the circadian system operating as a pacemaker that entrains the body to a 24-hour cycle in response to the external environment, while the homeostatic system is responsible for building up sleep pressure that accumulates with wakefulness and dissipates with sleep (Borbély, 1982).

Sleep pressure can only be reduced by sleep (Achermann & Borbély, 2003). High sleep pressure at the start of the night is associated with increased prevalence and amplitude of slow wave activity (SWA) in the EEG, which reduces as sleep pressure dissipates (Vyazovskiy et al., 2011). Slow waves are synchronised oscillations of neuronal membrane potentials between hyperpolarised and depolarised states in the delta frequency range (0.5-4 Hz) that propagate throughout the brain in an antero-posterior cortical progression, with the greatest prevalence in N3 sleep (Achermann & Borbély, 2003; Riedner et al., 2007). SWA has topographic variations throughout the brain, occurring locally and asynchronously across brain regions and most prominently in areas associated with increased activity during wake, suggesting sleep homeostasis is a locally regulated phenomenon (Krueger et al., 2019; Siclari & Tononi, 2017). SWA dissipates with consecutive sleep cycles throughout the night, indicating that homeostatic sleep pressure also dissipates (Dijk, 2009). Emerging evidence suggests that ID is characterised by lower initial SWA at sleep onset (Grimaldi et al., 2021), indicating a deficiency in the homeostatic regulation of sleep.

Sleepiness and Fatigue

Subjective Sleepiness and Fatigue

Increased sleep pressure is perceived subjectively as sleepiness, which is hypothesised to act as a primary motivational drive ensuring organisms sleep regularly despite the risks

arising from reduced consciousness (Axelsson et al., 2020). It 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). Subjective sleepiness can be measured as either trait or state sleepiness, with trait sleepiness being an individual's propensity to fall asleep in a given situation, and state sleepiness being a measure of sleepiness at a point in time (Åkerstedt et al., 2014; Johns, 1991). State sleepiness is most commonly measured using the Karolinska Sleepiness Scale (KSS), a one item nine-point Likert scale correlated with EEG measures of drowsiness in healthy populations (Cluydts et al., 2002; Kaida et al., 2006).

Excessive daytime sleepiness is one of the most common complaints associated with NRS but is inconsistently observed in ID (Hein et al., 2017; Sarsour et al., 2010). Although daytime fatigue is the most prevalent and detrimental complaint observed in ID, the inability to sleep that is characteristic of ID means that subjective sleepiness may not be a sensitive measure of daytime sleepiness in this population (Kyle et al., 2010; Raizen et al., 2023). Therefore, there is a critical need for improved measurement tools that can capture the experiences of ID and NRS.

Objective Sleepiness

Objective sleepiness refers to the quantifiable level of sleep pressure within an individual measurable through behavioural or neurophysiological measures. The most commonly used measures in research and clinical practice sensitive to increased sleepiness are the Multiple Sleep Latency Test (MSLT), which evaluates sleep propensity by measuring how quickly an individual falls asleep; the Maintenance of Wakefulness Test (MWT), which measures the ability to stay awake; and the Psychomotor Vigilance Task (PVT), which assesses sustained attention through reaction time measurements (Basner & Dinges, 2011; Martin et al., 2023). However, these measures do not directly measure the level of

neurobiological sleepiness that can be present in an individual without sleep onset, instead measuring the consequences of increased sleepiness. This limits their use within clinical populations, particularly ID, which is characterised by an inability to fall asleep.

Neurobiological sleepiness can be directly measured through the Karolinska Drowsiness Test (KDT), which uses EEG data to quantify the level of arousal when the eyes are open and closed (Åkerstedt et al., 2014; Åkerstedt & Gillberg, 1990). Increased sleep pressure, such as that caused by experimentally manipulated sleep deprivation, can be observed through a shift of EEG spectral power from fast frequency activity in the alpha (8-12 Hz), beta (15-25 Hz), and gamma (25-40 Hz) frequencies to slower frequency activity in the delta (0.5-4 Hz) and theta (4.5-8 Hz) frequencies (Kaida et al., 2006). This shift is most prominent in the theta frequency range, with increased theta oscillations during eyes-open resting wake mirroring the predicted trajectory of sleep pressure (Snipes et al., 2023). Additionally, this increase occurs in a task-dependent manner within localised cortical regions, mirroring the process observed in sleep deprivation and subsequent recovery SWA during sleep (Huber et al., 2004; Snipes et al., 2022). Theta activity reflects fluctuations in circadian rhythms (Aeschbach et al., 1997; Cajochen et al., 2002), decreases following caffeine consumption (Landolt et al., 1995), and is increased in individuals suffering from excessive daytime sleepiness (EDS) (Melia et al., 2015), meaning it appears to be a marker of sleepiness, rather than just sleep pressure. However, as theta activity is also associated with fatigue, increasing with time-on-task independent of changes in other frequency bands (Li et al., 2020; Tran et al., 2020), it is insufficient as a measure of objective sleepiness alone.

The increase in theta oscillations and shift from fast-frequency to slow-frequency EEG activity can be quantified by the slowing ratio (SR), which measures the ratio of fast frequencies to slow frequencies. Slowing ratio is associated with reduced sleep onset latency (Appleton et al., 2022), impairments to behavioural performance following sleep deprivation

(Gibbings et al., 2022), and can be used to assess treatment efficacy in obstructive sleep apnoea (Tracey et al., 2024). Although this measure is commonly used to measure sleepiness, it does not consider that alpha power manifests differently with increasing sleepiness depending on whether the eyes are open or closed.

Under conditions of low sleepiness, alpha power is most prominent during eyes-closed resting wake EEG recordings, with the greatest power in the occipital region. However, with increasing sleep pressure, alpha oscillations appear with eyes-open resting wake EEG and attenuate when the eyes are closed (Putilov & Donskaya, 2014). Increased eyes-open alpha power is correlated with increased sleep pressure accumulated through sleep deprivation and changes in body temperature occurring in response to circadian influences (Cajochen et al., 2002; Tian et al., 2018). Additionally, decreased eyes-closed alpha power represents the beginnings of sleep onset, as it is replaced by slower mixed-frequency activity in N1 sleep (Berry et al., 2017). This change in spectral power can be quantified as the alpha attenuation coefficient (AAC), which calculates the ratio of eyes-open alpha power to eyes-closed alpha power (Stampi et al., 1995). As alpha attenuation reflects declining alertness and the progression towards sleep, it can be used as an objective measure of sleepiness.

The Association Between Objective and Subjective Sleepiness

Measures of subjective sleepiness were developed and validated in concordance with objective sleepiness outcomes. State subjective sleepiness as measured by the KSS is highly correlated with AAC, alpha, and theta power during the eyes-open KDT in healthy individuals, indicating validity for both measures in measuring the construct (Kaida et al., 2006). KSS additionally correlates with other behavioural measures of sleepiness, including reaction time, sleep latency, and behavioural lapses in a driving task (Baulk et al., 2001; Sandberg et al., 2011; Shin et al., 2024). However, this association is not linear, with associations being strongest at high levels of sleepiness (KSS $\geq$ 7) (Åkerstedt et al., 2014).

This suggests subjective sleepiness is an evolutionary mechanism, alerting individuals to the presence of sleep-like neural activity occurring during wake, signalling that they should go to sleep when possible (Shochat et al., 2021).

This association may also vary significantly within different clinical groups. ID is characterised by cortical hyperarousal, the 24-hour increase of fast-frequency EEG activity occurring during wake and sleep (Colombo et al., 2016; Kao et al., 2021; Riemann et al., 2010). Increased fast-frequency activity in ID has been linked to sleep-state misperception, wherein individuals underreport their subjective sleep time in comparison to PSG-measured sleep (Fasiello, Gorgoni, et al., 2024). Furthermore, this association has not been researched in an NRS sample without a comorbid sleep disorder. Understanding if there are differences in the mechanisms of subjective and objective sleepiness in ID and NRS could provide greater insight into the aetiology and treatment for both disorders.

Aim

This study explored differences in how people with NRS, ID, and healthy controls experience subjective and objective sleepiness, and if differences are associated with topographic differences of spectral power during resting wake. First, we examined if there were group differences in subjective sleepiness levels upon awakening as measured by the KSS administered after habitual wake time, hypothesising that subjective sleepiness would be highest in NRS and lowest in ID, with healthy controls in the middle. Secondly, we examined if there were group differences in HD-EEG measures of objective sleepiness as measured through AAC and SR in the morning KDT. We hypothesised that objective sleepiness would be highest in NRS and lowest in ID, with healthy controls in the middle. Third, we explored if the association between subjective sleepiness as measured by KSS and objective sleepiness as measured by AAC and SR differed across groups, hypothesising there would be a significant main effect for group membership and subjective sleepiness, and a significant interaction.

Method

Participants

The study was approved by the Macquarie University Human Research Ethics Committee (FoRA ID 17112) and all participants provided written informed consent (Appendix A). Participation was voluntary and could be discontinued at any time. Participants were reimbursed for travel costs to and from the laboratory up to the value of $250 and remunerated $100 upon successful completion of the study.

The present study was derived from data captured as part of a larger neuroimaging research study phenotyping individuals with NRS. Due to the complexity of the study and the large number of outcome variables, an a priori power analysis was not performed. A sample size of 12 participants from each population, with a total sample of 36 participants, was proposed due to funding constraints. Participants were sex- and age-matched (with a maximum difference ± 2.5 years) to control for the influence of age and sex on sleep architecture (Mongrain et al., 2005).

Participants were excluded if they had comorbid sleep apnoea, as measured by WristOX pulse oximeter or the STOP-bang sleep apnoea questionnaire (STOP-Bang), which have a high sensitivity of detecting clinically relevant obstructive sleep apnoea syndrome (Chung et al., 2016; Nigro et al., 2009). Participants were additionally excluded if they had clinically significant depression (≥ 10) or anxiety (≥ 7) scores measured through the Depression Anxiety and Stress Scale 21 (DASS-21) (Lovibond & Lovibond, 1995), heavy alcohol use, used medications affecting sleep, or pregnancy. Circadian rhythm disruption was controlled for with an exclusion criteria of recent (≤ 30 days) shift work or international travel, or a natural sleep time outside the hours of 21:30 and 8:00.

ID participants required a clinical diagnosis of ID by a sleep physician following the DSM-5-TR criteria, with difficulty initiating or maintaining sleep persisting for over 1 month,

causing clinically significant distress or impairment in daily life (American Psychiatric Association, 2022). They were additionally required to have a Pittsburgh Sleep Quality Index (PSQI) score ≥ 5 and an Insomnia Severity Index (ISI) score ≥ 15.

NRS participants required a weekly mean Total Sleep Time (TST) ≥ 6 hours as measured by sleep diary and actigraphy, or a mean weekly score ≥ 3 on a 5-point Likert scale of "feeling refreshed upon awakening" measured using the Karolinska Sleep Diary (Åkerstedt et al., 1994). Additional inclusion criteria were a PSQI score ≥ 5, with subcomponent scores ≥ 2 on the PSQI Component 1 and ≥ 10 on PSQI Component 5.

Control participants required a weekly mean TST ≥ 6 hours, PSQI ≤ 4, and ISI ≤ 6.

Procedure

Participants were recruited through referrals to the Woolcock Institute of Medical Research and the Royal Prince Alfred sleep clinics, and via social media advertising (Appendix B). Volunteers completed an online questionnaire to assess eligibility for inclusion in a clinical group (ID, NRS, healthy controls), which was then confirmed through telephone screening by a researcher and an in-person clinical screening by a sleep physician.

Prior to the study, participants attended the Woolcock Institute of Medical Research for initial screening by a sleep physician. Baseline sleep and activity patterns were measured via a Geneactiv Actigraphy watch for 7 days prior, which were validated against self-reported sleep using the Karolinska Sleep Diary (Åkerstedt et al., 1994; Menczel Schrire et al., 2023).

On the day of the study, participants arrived at the laboratory at 17:00 and underwent a final medical screening and a series of cognitive assessments forming part of a larger study. They were then fitted with a high-density electroencephalography (HD-EEG) cap and went to bed at their habitual bedtime. Overnight PSG data were collected using standard American Academy of Sleep Medicine (AASM) clinical practice guidelines (Berry et al., 2017).

Lights were turned on at the participant's habitual wake time. The Karolinska Sleepiness Scale (KSS) and Karolinska Drowsiness Test (KDT) were administered five minutes post habitual wake time (Åkerstedt & Gillberg, 1990). Following the morning KDT, participants completed further cognitive testing and an MRI scan.

Measures

Screening Questionnaire

An online screening questionnaire obtained participants' age, sex, baseline alcohol consumption, absence of pregnancy, suitability for magnetic resonance imaging (MRI), and screened for circadian disruption (Appendix C). Additional questionnaires administered at this stage were the STOP-Bang (Chung et al., 2016), ISI (Bastien et al., 2001), DASS-21 (Lovibond & Lovibond, 1995), and PSQI (Buysse et al., 1989).

Insomnia Severity Index (ISI)

Subjective insomnia symptoms were assessed using the Insomnia Severity Index (ISI), a seven-item self-report measure of subjective insomnia symptoms (Bastien et al., 2001). Items (e.g. "Please rate the CURRENT (i.e. LAST 2 WEEKS) SEVERITY of your insomnia problem(s)") are rated on a 5-point Likert scale ranging from 0 ("none") to 4 ("very severe"). The scale ranges from 0 to 28, with scores of 10 or greater found to have 86.1% sensitivity and 87.7% specificity for detecting ID cases in a community sample, and ISI scores ≥ 15 interpreted as moderate-severe insomnia (Morin et al., 2011). The ISI demonstrated good internal consistency within the current sample with a Cronbach's alpha of .89.

Pittsburgh Sleep Quality Index (PSQI)

Self-assessed sleep quality was measured using the Pittsburgh Sleep Quality Index (PSQI), a 19-item questionnaire assessing sleep quality and disturbance over the past month (Buysse et al., 1989). The PSQI measures a broader construct than insomnia severity as it measures sleep-related disturbances beyond sleep initiation and maintenance. The convergent

validity between the PSQI and ISI within the sample was $r = .79$. The measure produces a global score (PSQI) comprised of seven component scores, relating to subjective sleep quality (PSQI-1; "During the past month, how would you rate your sleep quality overall"), sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances (PSQI-5; ("During the past month, how often have you had trouble sleeping because you…"), use of sleep medication, and daytime dysfunction. Items are rated on a 4-point Likert scale, with greater scores indicating greater impairment. Global PSQI scores range from 0 to 21, with scores ≥ 5 indicating clinically significant poor-quality sleep (Buysse et al., 1989). The PSQI demonstrated acceptable internal consistency ($\alpha = .72$) within the sample, consistent with previously reported values in clinical and non-clinical populations (Mollayeva et al., 2016).

Flinders Fatigue Scale (FFS)

Daytime fatigue impairments was measured using the Flinders Fatigue Scale (FFS), a seven-item measure of fatigue characteristics (e.g. "was fatigue a problem for you") over the previous two weeks (Gradisar et al., 2007). Scores range from 0 to 31, with greater scores indicating greater fatigue. The threshold for clinically significant daytime fatigue is a score of 13-15 for borderline fatigue, 16-20 for moderate fatigue, and ≥ 21 for severe fatigue (Cameron et al., 2017). The scale explicitly defines fatigue as being distinct from sleepiness, stating "We are interested in the extent that you have felt **fatigued** (tired, weary, exhausted) over the last **two weeks**. We **do not** mean feelings of **sleepiness** (the likelihood of falling asleep)." The FFS had good internal consistency within the sample ($\alpha = .86$).

Epworth Sleepiness Scale (ESS)

Trait subjective sleepiness was measured using the Epworth Sleepiness Scale (ESS), an eight-item measure assessing the likelihood of dozing in specific situations (e.g. "sitting and reading") (Johns, 1991). Items are rated on a 4-point Likert scale, ranging from 0 ("would **never** doze") to 3 ("**high** chance of dozing"). Scores range from 0 to 24, with greater ESS

scores reflective of greater sleep propensity and scores ≥ 10 indicating subjective excessive daytime sleepiness (Johns, 1991). The ESS had good internal consistency (α = .85) within the sample within the sample and was not correlated with FFS scores (*r* = .20).

Karolinska Sleepiness Scale (KSS)

State subjective sleepiness was assessed 5 minutes after natural wake time using the Karolinska Sleepiness Scale (KSS), a one-item measure of an individual's perceived sleepiness at a given point, with the instructions "Please measure your sleepiness over the past 5 minutes." It uses a 9-point Likert scale with verbal anchors at every second step ranging from 1 ("Extremely alert") to 9, "Extremely sleepy - fighting sleep" (Åkerstedt & Gillberg, 1990).

Karolinska Drowsiness Test (KDT)

The Karolinska Drowsiness Test (KDT) was administered immediately following the KSS and was used to measure electrophysiological drowsiness using HD-EEG data. 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 minimise 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 approximately 7 minutes long with 3 phases (eyes-open/eyes-closed/eyes-open), each lasting 120 seconds. The eyes-open conditions were concatenated during data analysis.

PSG Sleep Scoring and Sleep Macroarchitecture

Overnight PSG sleep data were recorded and scored in 30-second epochs according to American Academy of Sleep Medicine Manual (AASM) criteria by an experienced rater (Berry et al., 2017). Sleep recordings were evaluated for the following parameters of sleep continuity: time in bed (TIB, measured as total time spent in bed independent from sleep state); total sleep time (TST, defined as time between first sleep onset and final awakening,

excluding periods awake); sleep onset latency (SOL, measured as time from lights out until first epoch of sleep); snooze time (measured from time of final awakening to time out of bed); REM latency (minutes from sleep onset to first epoch of REM sleep); wake after sleep onset (WASO, time spent awake between sleep onset and final awakening); sleep efficiency (ratio of TST to time in bed × 100 %); and total minutes/percentage in N1, N2, N3 and REM sleep (as scored using the AASM criteria).

HD-EEG

High-density EEG data were collected using 256-channel electrode caps and Net Amps 400 amplifiers (MagstimEGI, Eugene, OR, USA) with signals amplified and digitised at 500 Hz referenced to the vertex (CZ). Prior to starting any of the acquisitions, electrode impedance was below 50 kΩ. After acquisition, data were low-pass filtered at 70 Hz, high-pass filtered at 0.3 Hz, and notch filtered at 50 Hz.

Data processing

Visual Inspection and EEG Pre-Processing

All preprocessing was completed using the EEG Processor application for MATLAB (https://eeg-processor.readthedocs.io/). 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 all other good-quality channels using linear mixing, weighted by the squared distance to the poor-quality channel. To enhance the local signal detection of each electrode and minimise the influence of the vertex (Cz) electrode, data were re-referenced to a common average signal (i.e., mean global signal across all EEG channels

Independent Components Analysis

Following visual inspection, independent components analysis (ICA) was used to identify and separate statistically independent components. This was done using a semi-automated process using the MATLAB program ICLabel, which automatically removed

components classified as non-brain activity with a probability ≥ .8 (Pion-Tonachini et al., 2019). Manual inspection was conducted to verify artefact removal and remove components visually identified as non-brain activity not meeting the weighting threshold. The remaining components were back-projected to the EEG data signal, removing channels on the cheek and neck and retaining only cranial channels, resulting in a cleaned EEG time series dataset.

Power Spectra

Power spectral analysis was used to quantify the distribution of EEG data oscillations across frequencies, indicating which frequency components contribute the most to the signal. Power spectra were obtained for each channel using a fast Fourier transform (FFT) to deconstruct the EEG signal from the time domain to the frequency domain. The power spectra were calculated using the Welch method with 6-second windows (50% overlap) and obtained for the eyes-closed condition and the concatenated recording of the two eyes-open conditions. EEG spectral power densities were integrated within the following frequency bands: 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). Absolute EEG spectral power densities were normalised to the grand-total power (average area under the curve between 0.5–40 Hz across all channels) to account for interindividual differences such as head size, scalp tissue, and skull thickness.

Slowing Ratio (SR)

The EEG slowing ratio (SR) is a biomarker of sleepiness reflecting the general slowing of brain activity that appears with increasing sleepiness, with increased slow frequency (delta and theta) activity being indicative of decreased arousal (D'Rozario et al., 2013). SR has been shown to be a valid measure of reduced alertness and increased drowsiness in clinical populations (Sweetman et al., 2021). The SR was calculated for each participant in the eyes-open and eyes-closed conditions using the formula $\log_{10}$(delta + theta)/(alpha + sigma + beta) (Vakulin et al., 2016). A higher SR score indicates increased sleepiness.

Alpha Attenuation Coefficient (AAC)

The alpha attenuation coefficient (AAC) measures alpha frequency power differences between eyes-open and eyes-closed conditions (Stampi et al., 1995). Alpha activity increases with sleepiness when the eyes are open and decreases with sleepiness when the eyes are closed (Putilov & Donskaya, 2014). The AAC is calculated as the log ratio of alpha power in the eyes-closed condition to alpha power in the eyes-open condition, $\log_{10}$(alpha eyes-closed/ alpha eyes-open]. A lower AAC score reflects decreased cortical activity and increased sleepiness.

Statistical Analysis

Statistical analysis of descriptive measures was done using R version 4.3.2 (R Core Team, Vienna, Austria). Analyses involving HD-EEG data were run using the EEG processor application, which makes use of the toolboxes Fieldtrip (Oostenveld et al., 2011) and EEGlab (Delorme & Makeig, 2004). An alpha level of $p = .05$ was used for all analyses.

A one-way analysis of variance (ANOVA) was conducted to examine group differences in continuous variables for demographic variables, survey response measures, and sleep macroarchitecture. Assumptions of normality of the distribution, residuals and outliers was conducted using Q-Q Plots, Shapiro-Wilk normality tests, and visual inspections of histograms, and if violated, values were log-transformed. The assumption of homogeneity of variance was checked using Levene's test, and if violated, Welch's ANOVA was used. Sphericity was checked using Maunchly's test of sphericity, and if violated, a Huynh-Feldt correction was applied. Post-hoc pairwise comparisons using a Bonferroni correction were conducted in the case of a significant result controlling for multiple comparisons.

To compare subjective sleepiness between groups, a one-way ANOVA was conducted with KSS scores as the dependent variable and group membership as the independent

variable. Assumptions were checked as described above. In the case of a significant ANOVA, post-hoc pairwise comparisons with a Bonferroni correction were conducted.

To compare objective sleepiness between groups, separate one-way ANOVAs were applied to test for group differences in AAC, SR eyes-open, and SR eyes-closed at each of the 178 EEG channels. A cluster-mass permutation-based analysis of linear models (PALM) was applied to control for the increase in type-I error rate (Winkler et al., 2014). Clusters were defined as neighbouring EEG channels. Electrodes showing a significant F-statistic for the factor group ($p < .05$), and their mass was derived as the integrated F-statistical value. This cluster mass was compared to an empirical null-distribution. This involved applying the same model to 10, 000 random shuffles of the data, and with each iteration the largest cluster mass was entered to build a reference null-distribution of cluster sizes occurring due to chance, which was then used to compare the found cluster mass size against. Clusters were deemed significant at an alpha threshold of $p < .05$.

To analyse if the association between subjective and objective sleepiness differed across groups, a general linear model was applied with SR and AAC in each EEG channel as the dependent variable and subjective sleepiness and group membership as predictors. Interaction terms between KSS score and group membership were included to test whether the relationship between subjective and objective sleepiness differed across groups. As above, permutation-based analysis was used to control for type-I error rate. Clusters with a p-value < .05 were deemed significant.

Results

Participants

964 participants completed the online expression of interest questionnaire, with 352 (36.5%) meeting eligibility criteria. Of these, 169 participants (17.5%) were unable to be contacted or did not respond to a follow-up email. 180 participants proceeded to pre-

screening, of whom 147 were excluded during the pre-screening and screening visits, with the most common exclusion reasons being medication use (n = 44) or the absence of an age- and sex-matched participant (n = 54). Due to the time constraints of this honours thesis the final sample obtained was 33 participants (13 NRS; 11 ID; 9 Controls; 3% of participants who completed the expression of interest questionnaire). Two control participants were excluded from sleep macroarchitecture analysis due to missing data, as sleep studies could not be scored by an expert sleep technician in time. Participant demographic and survey response details are provided in Table 1, and sleep macroarchitecture in Table 2.

Significant group differences were found for PSQI sleep quality scores, ISI insomnia severity scores, and FFS daytime fatigue scores, with the control group showing the lowest impairment and the ID group reporting the greatest impairment. Due to a violation of the assumption of homogeneity of variances (p = .009), a one-way Welch's ANOVA found significant group differences in PSQI scores, $F(2, 16.32) = 35.99$, p <.001. Post-hoc Games-Howell tests showed the control group had significantly lower PSQI scores than the ID (mean difference = −8.67, p < .001) and NRS groups (mean difference = −4.74, p < .001), and the ID group had significantly higher PSQI scores than the NRS group (mean difference = 3.93, p = .010). A Welch's ANOVA showed a significant group effect for ISI scores, $F(2, 18.96)$ = 112.60, p < .001. Post-hoc analyses revealed the control group had significantly lower scores than the ID (mean difference = -15.2, p <.001) and NRS (mean difference = −12.02, p < .001) groups. Although all groups did not have clinically significant (≥ 13) daytime fatigue as measures by the FFS, a one-way ANOVA found a significant main effect of group, $F(12, 30)$ = 10.56, p < .001. Post-hoc comparisons using a Bonferroni correction showed the control group had significantly lower scores compared to the ID (mean difference = −7.47, p < .001) and NRS groups (mean difference = −5.88, p = .003). No significant difference was found between the ID and NRS groups (mean difference = 1.59, p = .933).

Table 1

Descriptive Measures by Group

Measure	ID		NRS		Control		η^2	*p*
	M	*SD*	*M*	*SD*	*M*	*SD*		
Sex, male/female	4/7		6/7		3/6			
Age	42.22	11.91	40.52	11.59	39.39	13.56	.01	.873
PSQI	12.55	3.45	8.62	1.45	3.88	1.55	.82	<.001 †***
ISI	17.64	3.20	14.46	4.37	2.44	1.59	.92	<.001 †***
FFS	11.36	4.03	9.77	4.04	3.89	2.93	.41	<.001***
ESS	6.67	5.07	5.00	4.22	4.11	3.72	.03	.671
KSS AM	5.09	2.17	5.77	1.92	4.22	1.09	.11	.168

† notes Welch's ANOVA as homogeneity of variances was violated. *** p = <.001.

PSQI = Pittsburgh Sleep Quality Index; ISI = Insomnia Severity Index; FFS = Flinders Fatigue Scale; ESS = Epworth Sleepiness Scale; KSS = Karolinska Sleepiness Scale

Table 2

Sleep Macroarchitecture by Group

Measure	ID		NRS		Control		η^2	*p*
	M	SD	*M*	SD	*M*	SD		
Time in bed	464.1	38.6	491.2	25.8	493.0	31.1	.16	.087
Total sleep time	360.6	87.8	395.3	51.7	415.3	64.5	.10	.246
Sleep onset latency	33.9	36.9	23.7	33.2	20.7	20.9	.02	.773 †
REM latency	95.6	31.9	88.1	29.1	136.4	62.6	.20	.041*
WASO	51.2	33.0	66.4	44.9	56.9	44.1	.03	.637†
Sleep efficiency	77.1	15.5	80.6	10.4	84.0	10.2	.05	.460†
N1 (minutes)	29.5	11.4	34.7	20.7	27.1	9.8	.06	.556‡
N2 (minutes)	181.5	54.7	196.6	37.0	224.8	58.2	.11	.202
N3 (minutes)	66.8	38.6	74.0	24.5	92.6	34.6	.01	.876
REM (minutes)	82.9	34.9	90.0	24.5	92.6	34.6	.02	.777
N1 %	8.1	3.2	8.8	5.0	6.6	2.5	.11	.369‡
N2 %	50.0	9.7	49.8	7.4	53.7	9.6	.04	.608
N3 %	19.8	12.8	18.9	8.8	17.7	7.9	.01	.917
REM %	22.1	5.1	22.5	4.1	22.0	6.7	<.01	.974

† notes variable was log-transformed as assumption of normality was violated. ‡ notes Welch's ANOVA as homogeneity of variances was violated. * p = <.05. WASO = wake after sleep onset. All times reported in minutes. Sleep macroarchitecture variables reported following AASM criteria.

The only significant difference between groups in sleep macroarchitecture was in REM latency, $F(2, 28) = 3.58$, $p = .042$. Post-hoc comparisons using a Bonferroni correction showed the NRS group had significantly reduced REM latency in comparison to healthy controls (mean difference = –48.24 minutes, $p = .044$).

Comparing Subjective Sleepiness Scores Between Groups

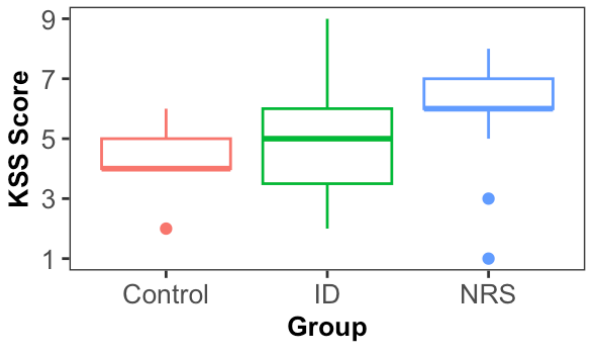
To assess if there were group differences in subjective sleepiness at habitual wake time between groups, a one-way ANOVA was conducted on KSS scores (Table 1). Assumption checks showed that the data met the assumption of homogeneity of variances, $F(2, 30) = 1.77$, $p = .187$, and the assumption of normality, $W = 0.967$, $p = .395$. The ANOVA revealed a medium non-significant effect of group, $F(2, 30) = 1.90$, $p = .168$, $\eta^2 = 0.11$ (Figure 1).

Comparing Objective Sleepiness Between Groups

To investigate if objective sleepiness upon awakening differed between groups, three one-way ANOVAs were conducted using a cluster mass permutation analysis (Figure 2). No significant cluster differences for AAC were detected between groups before or after correcting for multiple comparisons, smallest uncorrected $F_{channel}(2) = 2.66$ $p_{uncorrected} = .080$. For SR in the eyes-open condition, there were no significant cluster differences between

Figure 1

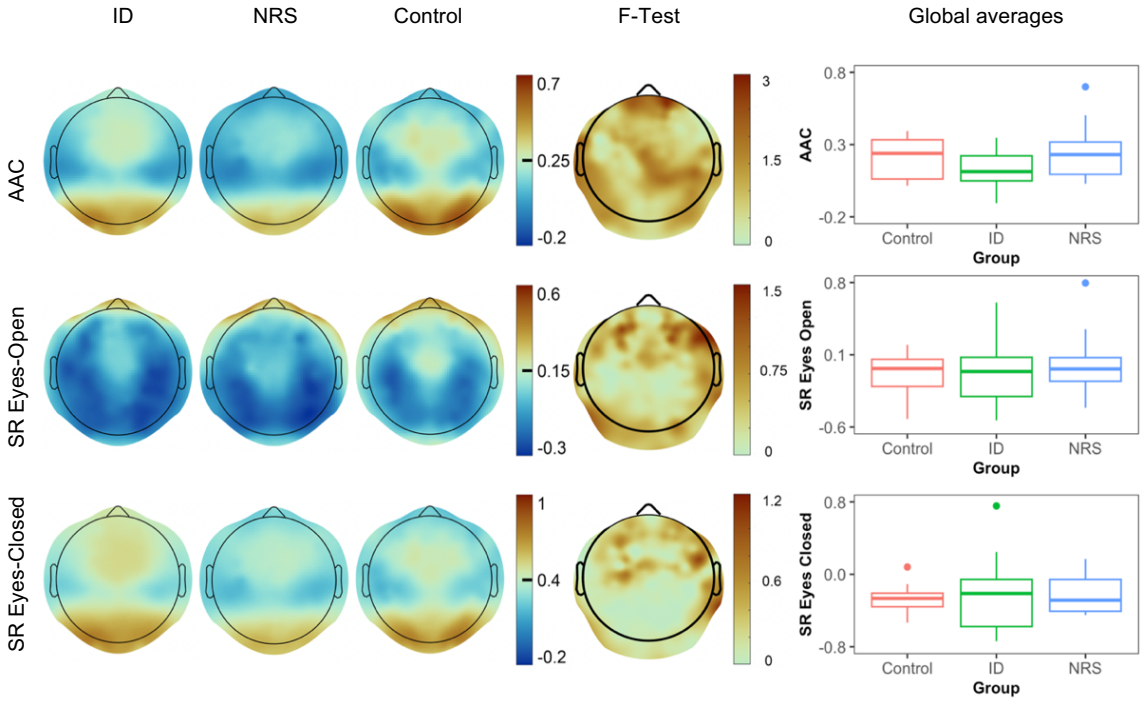
Non-Significant Differences in Subjective Sleepiness Scores by Group



Note. Horizontal lines represent median scores, while boxes show interquartile range (IQR). Whiskers extend to 1.5 times the IQR with outliers shown as dots.

Figure 2

Non-Significant Differences in Objective Sleepiness EEG Measures by Group



Note. Topoplots represent group average AAC and SR values, and F-statistic values. No significant differences were observed between groups in any measures, as shown by F-test maps. All groups showed the greatest AAC values in the occipital regions, with red areas reflecting increased alpha activity when the eyes are closed, and therefore lower objective sleepiness. Blue areas indicate a prevalence of alpha activity when the eyes are open, or the reduction of alpha activity when the eyes are closed, and therefore higher objective sleepiness. Eyes-open SR topoplots indicate the greatest prevalence of slow-frequency activity in the prefrontal regions across groups, while eyes-closed SR topoplots show the greatest prevalence of slow-frequency activity in occipital regions, with the ID group showing non-significantly increased frontal-midline slow-frequency activity. Descriptive box and whisker plots represent global AAC and SR values averaged across all EEG channels per participant across groups and were not statistically analysed.

groups before or after correcting for multiple comparisons, smallest uncorrected $F_{channel}(2) = 1.45$, $p^{uncorrected} = .252$. For the SR in the eyes-closed condition, there were also no significant cluster differences between groups before or after correcting for multiple comparisons, smallest uncorrected $F_{channel}(2) = 1.15$, $p^{uncorrected} = .334$. These findings indicate no group differences in commonly used measures of EEG objective sleepiness within our sample.

The Association Between Subjective and Objective Sleepiness Between Groups

To investigate if there were differences in the association between subjective and objective sleepiness between groups, a general linear model with a cluster mass permutation analysis was conducted (Figure 3). For the AAC, no significant cluster associations were found for the main effect of KSS, smallest uncorrected $T_{channel} = -1.94$, $p_{uncorrected} = .064$. Similarly, although one channel in the left temporal region was significant before correction, $F_{channel}(2) = 4.54$, $p_{uncorrected} = .02$, no significant cluster group differences were observed. A cluster of 5 channels in the central cortical region showed a non-significant interaction effect of group and KSS, $F_{channel}(2) = 10.22$, $p_{cluster} = .164$.

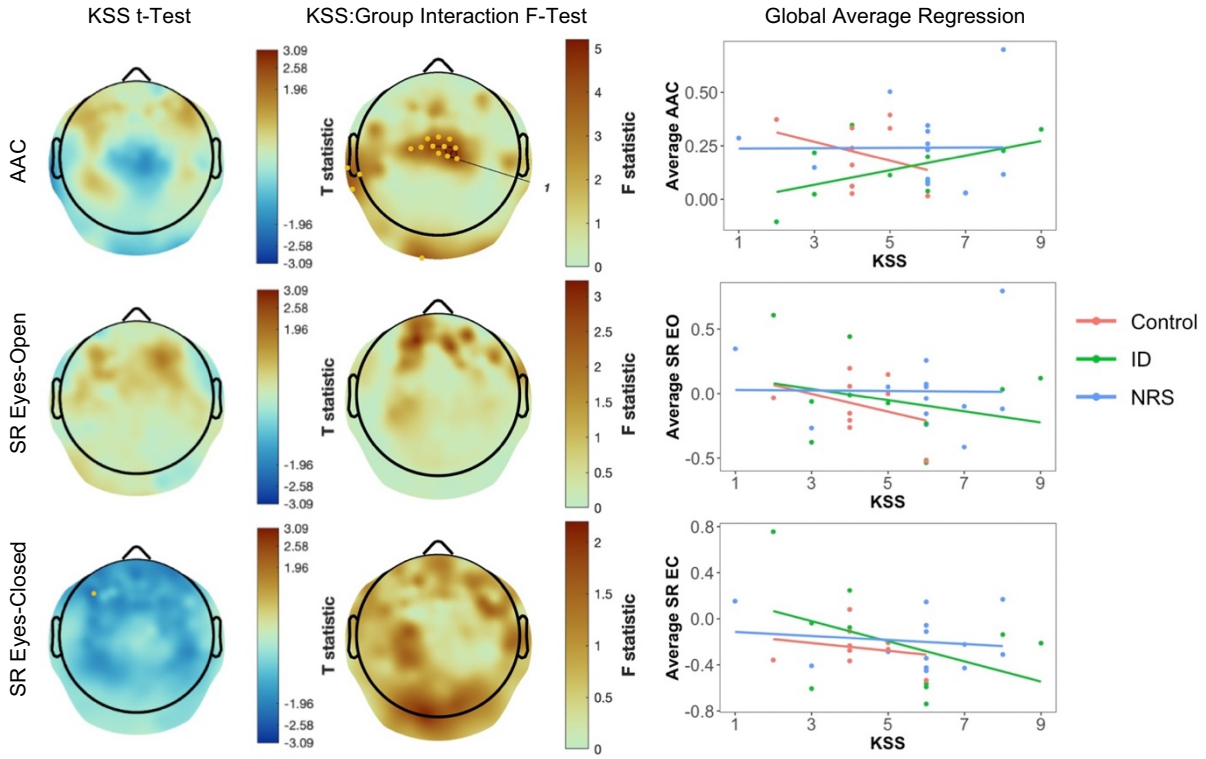
For SR in the eyes-open condition, there we no significant main effects or interaction effect, main effect of KSS smallest uncorrected $T_{channel} = 2.01$, $p_{uncorrected} = .056$; main effect of group smallest uncorrected $F_{channel}(2) = 1.63$, $p_{uncorrected} = .211$; interaction effect smallest uncorrected $p^{channel}$-value $F_{channel}(2) = 3.11$, $p_{uncorrected} = .065$.

Finally, for the SR in the eyes-closed condition, one channel in the left temporal region showed a significant effect of KSS before correction, $T_{channel} = -2.19$, $p_{uncorrected} = .038$, however no significant clusters were observed. No significant cluster differences were observed for the main effect of group, smallest uncorrected $F_{channel}(2) = 1.16$, $p^{uncorrected} = .327$, or interaction effect, smallest uncorrected $F_{channel}(2) = 2.09$, $p^{uncorrected} = .145$.

Overall, these results indicate no significant associations between subjective and objective sleepiness across groups.

Figure 3

Non-Significant Differences in Objective Sleepiness by KSS Score and Group:KSS Interaction



Note. KSS t-test topoplots show regional variance in non-significant correlations between KSS and objective sleepiness, with blue areas indicating negative correlation and brown indicating positive correlation. Lower (blue) AAC values are indicative of increased sleepiness, while higher (brown) SR values are indicative of increased sleepiness. Orange dots show significant channels before correction for multiple comparisons. Regression lines show global values averaged across all EEG channels per participant and are for descriptive purposes and have not been statistically analysed.

Discussion

This study examined if there were differences in subjective or objective measures of sleepiness, or the association between them, in individuals with ID, NRS, and healthy controls. Contrary to our hypothesis, subjective sleepiness upon awakening was non-

significantly higher in NRS and ID in comparison to healthy controls. Furthermore, NRS was not associated with increased EEG measures of objective sleepiness, while ID was not associated with decreased objective sleepiness in comparison to healthy controls. Finally, there were no significant group differences in the association between subjective and objective sleepiness. Neither subjective sleepiness nor group membership significantly predicted measures of objective sleepiness, and there was no significant interaction. These results suggest that although ID and NRS may have different underlying aetiology and causal mechanisms and may be different from healthy controls, we did not find group differences in subjective or objective measures of sleepiness or their association upon awakening.

Subjective Sleepiness

Both ID and NRS groups exhibited a non-significant medium effect of increased state subjective sleepiness upon awakening in comparison to healthy controls. The similarities between groups are further supported by trait sleepiness as measured through ESS scores, which are non-significantly increased in ID and NRS compared to healthy controls and not indicative of excessive daytime sleepiness. This contrasts with prior research which consistently finds lower state and trait subjective daytime sleepiness in ID populations (Fasiello, Mombelli, et al., 2024; Grimaldi et al., 2021; Huang et al., 2012). However, subjective sleepiness within ID has been found to be most pronounced in the early morning (Balter et al., 2024), which may explain this result.

Research has shown increased state and trait subjective sleepiness is associated with NRS in shiftworkers (Gorlova et al., 2019) and individuals with obstructive sleep apnoea (El-Mekkawy et al., 2022), however this study is the first known examination of daytime sleepiness in an NRS-only population. The concordance in daytime impairments between groups is further illustrated by the non-significant differences in fatigue measured by FFS and insomnia symptoms measured by ISI. These results suggest that individuals experiencing

NRS may not have significantly different daytime sleepiness in comparison to ID despite not meeting the DSM-5-TR diagnostic criteria, and the subjective perception of non-restorative sleep may not be associated with sleepiness.

Objective Sleepiness

The results of this study did not support the hypothesis that NRS would be associated with increased measures of objective sleepiness, and ID would exhibit reduced objective sleepiness in comparison to healthy controls. As expected, all groups had the greatest AAC in the occipital region, displaying increased alpha activity when the eyes were closed, typical of low-sleepiness resting state wake (Gibbings et al., 2022; Kaida et al., 2006; Stampi et al., 1995). Contrary to hypotheses, AAC was non-significantly lowest, indicating increased objective sleepiness, in the ID group, which showed decreased alpha activity when the eyes were closed in the occipital region, and increased frontal, central and temporal alpha activity in the eyes-open condition compared to NRS and controls. This contrasts with previously reported findings, which reported increased AAC values, indicating decreased sleepiness upon awakening in ID (Feige et al., 2017). AAC was non-significantly highest in the NRS group, indicating decreased sleepiness.

SR in the eyes-open condition indicated increased slow-frequency (delta and theta) activity in the left frontal, prefrontal, and right frontal areas for all groups. Although non-significant, increased frontal slow-frequency activity was strongest in NRS, and weakest in healthy controls. The dominance of prefrontal slow-frequency activity in all groups is surprising, as frontal regions generally show the greatest increase in fast-frequency activity upon wake (Gorgoni et al., 2015; Marzano et al., 2011). Additionally, the topography of this activity is more frontal than would be expected if it were indicative of sleepiness, as slow-frequency activity in wake that is reflective of increased sleep pressure is primarily in the frontal and central midline regions (Snipes et al., 2023). An alternative possibility for this

activity is artefactual activity from eye-blinks that was not completely removed during ICA. Although data were both automatically and manually scanned, incomplete removal of non-brain components can contaminate signals and limit findings. As such, the prevalence of slow-frequency activity in the prefrontal areas should be interpreted cautiously and may not indicate increased objective sleepiness across all groups. Analysis of the eyes-closed condition shows increased slow-frequency activity in the occipital areas for all groups, following the expected topography of eyes-closed activity upon awakening (Gorgoni et al., 2015). Group differences were most pronounced in both the eyes-open and eyes-closed conditions in the right temporal regions, however did not reach significance.

In contrast to our hypothesis, the ID group did not show global decreases in slow-frequency activity, which would indicate hyperarousal upon awakening. The hyperarousal hypothesis of ID proposes the disorder is associated with an increased fast-frequency neurological activity during both wake and sleep (Dressle & Riemann, 2023), predominantly observed during wake in the beta and gamma spectral bands (Colombo et al., 2016). However, this was not found in our sample in either eyes-open or eyes-closed conditions when measured through the most prevalent way of quantifying SR, which compares the ratio of slow (delta and theta) frequency activity to fast (alpha, sigma, and beta power) frequency activity (D'Rozario et al., 2023; Perrin et al., 2019; Sivam et al., 2020). However, there was a large amount of individual variance within the ID group, suggesting hyperarousal may not manifest uniformly upon awakening within this population.

Furthermore, NRS was not associated with increased objective sleepiness in comparison to ID or controls. If NRS was a result of ineffective reduction of homeostatic sleep pressure during overnight sleep, this would be most noticeable in the wake EEG through increased slow-frequency activity upon awakening. Additionally, time and percentage of sleep spent in N3 sleep, the sleep stage the most significantly associated with reductions in

sleep pressure (Achermann & Borbély, 2003), was not significantly different between groups, indicating similar reductions in sleep pressure in NRS compared to healthy controls.

These findings suggest that either there may be no differences in objective sleepiness between groups, or that the measures used to assess sleepiness may have been inadequate. Looking at measures of sleep macroarchitecture, the ID group showed shorter total sleep time, reduced sleep efficiency, and fewer minutes in N3 sleep in comparison to NRS and healthy controls. The ID total sleep time within our sample was 30 minutes shorter than that reported in a meta-analysis of PSG measured sleep in ID (Baglioni et al., 2014), suggesting the rigorous screening process used to identify a sample of individuals with objective impairments to sleep was effective. However, despite the medium effect size of total sleep time, differences were non-significant. Additionally, group differences in restorative N3 sleep time were non-significant with a small effect size, suggesting similar overnight reductions in sleep pressure. Consequently, it is understandable group differences in objective sleepiness upon awakening were not observed.

Alternatively, the measures used may have been inadequate for capturing group differences. ID is associated with increased fast-frequency activity in the beta and gamma bands (Colombo et al., 2016), which not be noticeable when analysing all fast-frequency bands simultaneously. However, when measuring sleepiness, the ratio of slow to fast frequencies is indicative of sleepiness levels (D'Rozario et al., 2013), while increased beta activity reflects greater cortical arousal (Perlis et al., 2001). Therefore, although there is strong evidence for hyperarousal in ID, it may not result in decreased objective sleepiness.

Association of Objective and Subjective Sleep Measures

This study did not find any significant associations between subjective and objective sleepiness, group differences in objective sleepiness when including subjective sleepiness as a predictor, or interaction effects.

Previous research conducted in healthy populations has consistently found subjective sleepiness is a sensitive and valid indicator of objective sleepiness (Åkerstedt et al., 2014; Kaida et al., 2006; Shin et al., 2024). However, associations between subjective and objective sleepiness are strongest at high levels of sleepiness (KSS values ≥ 7) (Kaida et al., 2006; Manousakis et al., 2021), indicating increased subjective sleepiness is an evolutionary mechanism signalling localised sleep-like brain activity is occurring (Åkerstedt et al., 2014). As such, our sample may not have been sufficiently sleepy to observe a strong relationship. A post-hoc sensitivity analysis (Appendix D) categorising participants by low (≤ 6) or high (≥ 7) sleepiness showed a trend towards significance for AAC (p = .086) and SR in the eyes-open condition (p = .069). This suggests that within our sample subjective and objective sleepiness may be associated at high subjective sleepiness, however repeated measurements taken at increased levels of sleepiness are required to confidently draw conclusions.

Furthermore, validation studies have used experimentally manipulated sleep deprivation to increase homeostatic sleep pressure, limiting ecological validity. Our data is limited due to having a single timepoint at low mean sleepiness levels, and therefore may be inadequate for assessing the association between objective and subjective sleepiness. However, as results indicated no significant group differences or interaction effects, the results of this study suggest that subjective sleepiness upon awakening may not be associated with EEG measures of sleepiness within our sample.

Strengths and Limitations

To the best of our knowledge, this is the first examination of daytime sleepiness using ID, NRS, and healthy control samples that have been extensively screened for comorbid disorders. Previous studies examining the daytime impacts of NRS have examined the symptom as sequelae of other conditions, such as shiftwork or sleep apnoea, limiting ecological validity (El-Mekkawy et al., 2022; Gorlova et al., 2019). The strict inclusion

criteria for the ID group, involving screening via actigraphy, sleep diaries, and clinical diagnosis by a sleep physician, ensured the sample would reflect those with insomnia with objective impairments. Although the intended sample size was unable to be obtained, age- and sex-matching participants ensured the effects of ageing on sleep were controlled for.

Using HD-EEG allowed for topographic analysis of group differences with greater resolution than traditional EEG. This methodology enabled direct analysis of neurobiological daytime sleepiness measures within ID, NRS, and healthy controls, rather than using behavioural measures of objective sleepiness that do not account for localised variations in sleep-like activity. Through using HD-EEG, variations in power spectra were able to be mapped to more precise areas. Additionally, using HD-EEG allowed more accurate detection and correction of artefacts through ICA. The increased sensitivity of HD-EEG increased the resolution of data collection, allowing smaller group differences to be identified.

The study was limited due to low statistical power, as the targeted sample size was not achieved. Post-hoc power and sensitivity analyses conducted using G*power (Faul et al., 2007) indicated the study was only adequately powered to detect large effect sizes (Cohen's f = 0.57), while for the found effect size of group differences in KSS the study achieved power of .39. However, as clinically relevant differences in subjective sleepiness are consistently associated with large effect sizes (Åkerstedt et al., 2014), the achieved sample size was likely sufficient to identify clinically significant group differences.

Additionally, the measures used to assess objective sleepiness may have been inadequate for capturing group differences. The method used to calculate SR in the eyes-open condition categorised alpha as a fast-frequency, low sleepiness measure, while research suggests that alpha activity during eyes-open conditions is indicative of increased sleepiness (Putilov & Donskaya, 2014). This discrepancy may have reduced the sensitivity of the measure to accurately detect sleepiness in the eyes open condition. Additionally, the measures

used may have obscured group differences in individual spectral bands, such as the increased beta and gamma associated with cortical arousal in ID (Colombo et al., 2016).

Practical Implications and Future Directions

The findings of these study suggest that there are no significant differences in subjective or objective sleepiness upon awakening between NRS, ID, and healthy controls. However, NRS is associated with lower subjective sleep quality, increased insomnia symptom severity, and increased daytime fatigue in comparison to healthy controls. The prevalence of NRS in Australia may be as high as 45% (Adams et al., 2017), however is currently no diagnostic criteria or treatment protocol. Although NRS is hypothesised to be caused by deficiencies in slow wave sleep (Gorlova et al., 2019), our study suggests this may not be associated with increased sleepiness upon wake. Future research examining subjective and objective impairments, including analysis in individual spectral bands, is warranted to improve understanding of the aetiology of the condition and improve outcomes for those affected.

Conclusion

In summary, our study did not find significant differences in subjective or objective sleepiness among individuals with NRS, ID, and healthy controls upon awakening. However, questionnaire data showed daytime impairments in NRS were similar to ID, highlighting the need for improved classification and treatment in this population. The finding that NRS may not be characterised by increased sleepiness upon awakening emphasises the need to explore other factors contributing to the condition, such as increased fatigue and low subjective sleep quality.

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Appendix A

Consent Form

CIRUS, Centre for Sleep and Chronobiology

Woolcock Institute of Medical Research

ABN 15 211 513 464

Professor Ron Grunstein

Professor of Sleep Medicine and NHMRC Senior Principal Research Fellow, Senior Specialist Physician; Chief Investigator, NHMRC Centre for Translational Sleep and Circadian Neurobiology, (NeuroSleep CRE) and CIRUS, Sleep and Circadian Group, Woolcock Institute of Medical Research, University of Sydney and Royal Prince Alfred Hospital

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Extreme phenotyping in patients with non-restorative sleep (NRS): is there a "neuromarker" for CFS/SEID

PARTICIPANT CONSENT FORM

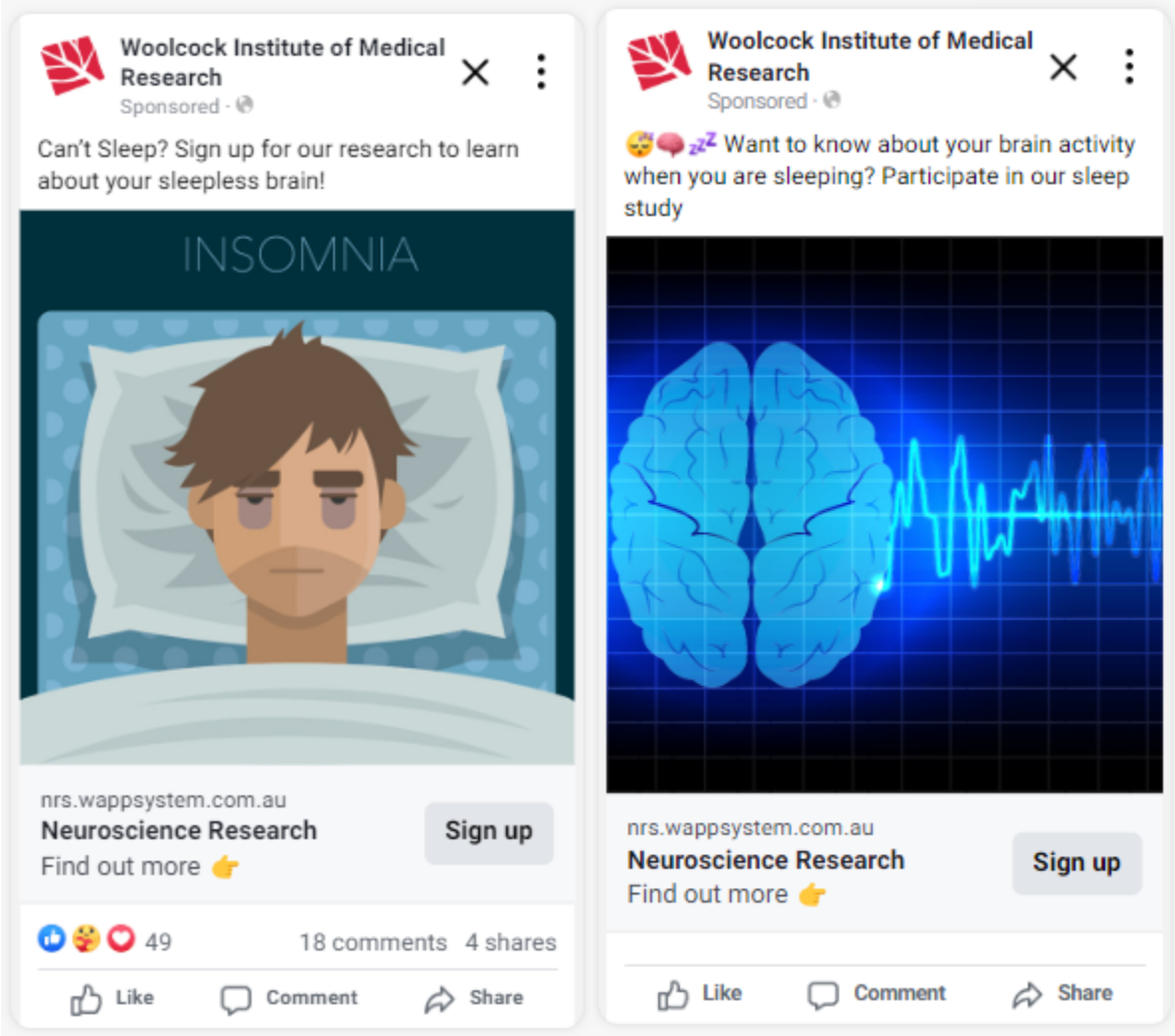
I, ... [PRINT NAME], agree to take part in this research study.

In giving my consent I state that:

- ✓ I understand the purpose of the study, what I will be asked to do, and any risks/benefits involved.
- ✓ I have read the Participant Information Statement and have been able to discuss my involvement in the study with the researchers if I wished to do so.
- ✓ The researchers have answered any questions that I had about the study and I am happy with the answers.
- ✓ I understand that being in this study is completely voluntary and I do not have to take part. My decision whether to be in the study will not affect my relationship with the researchers or anyone else at the Macquarie University, Brain and Mind Centre or Woolcock Institute of Medical Research now or in the future.
- ✓ I understand that I can withdraw my consent from the study completely or my consent on particularly aspects of the study at any time.
- ✓ I understand that personal information about me that is collected over the course of this project will be stored securely and will only be used for purposes that I have agreed to. I understand that information about me will only be told to others with my permission, except as required by law.
- ✓ I understand that the results of this study may be published, and that publications will not contain my name or any identifiable information about me.

Appendix B

Social Media Advertisements



Appendix C

Online Questionnaire to Assess Eligibility

ABOUT YOU

What is your biological sex?

Male Female

Are you between 18 and 65 years of age?

No Yes

On average, do you consume more than 2 standard drinks of alcohol per day?

No Yes

Are you pregnant, or do you expect to be pregnant?

No Yes

Are you a shift-worker or do you have any work schedule that falls outside the hours of 7 am and 6 pm?

No Yes

Next

Thank you for completing these questions. Based on the responses you have provided you may be eligible for the research study. We will need to contact you to discuss this over the telephone. Please provide your phone number and e-mail address and we will contact you shortly.

First name Last name

Email Address

Phone number

XXXX-XXX-XXX

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Appendix D

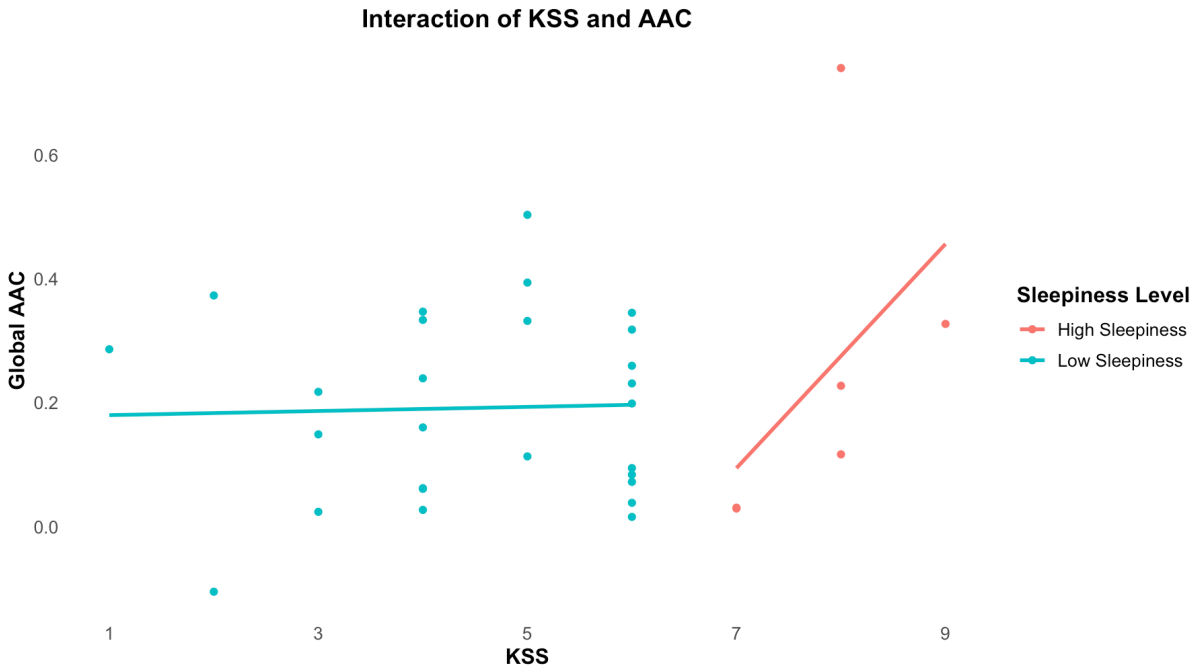
Sensitivity Analysis for KSS and Objective Measures of Sleepiness Split by Low (KSS Values ≤ 6) And High (KSS Values ≥ 7) Subjective Sleepiness

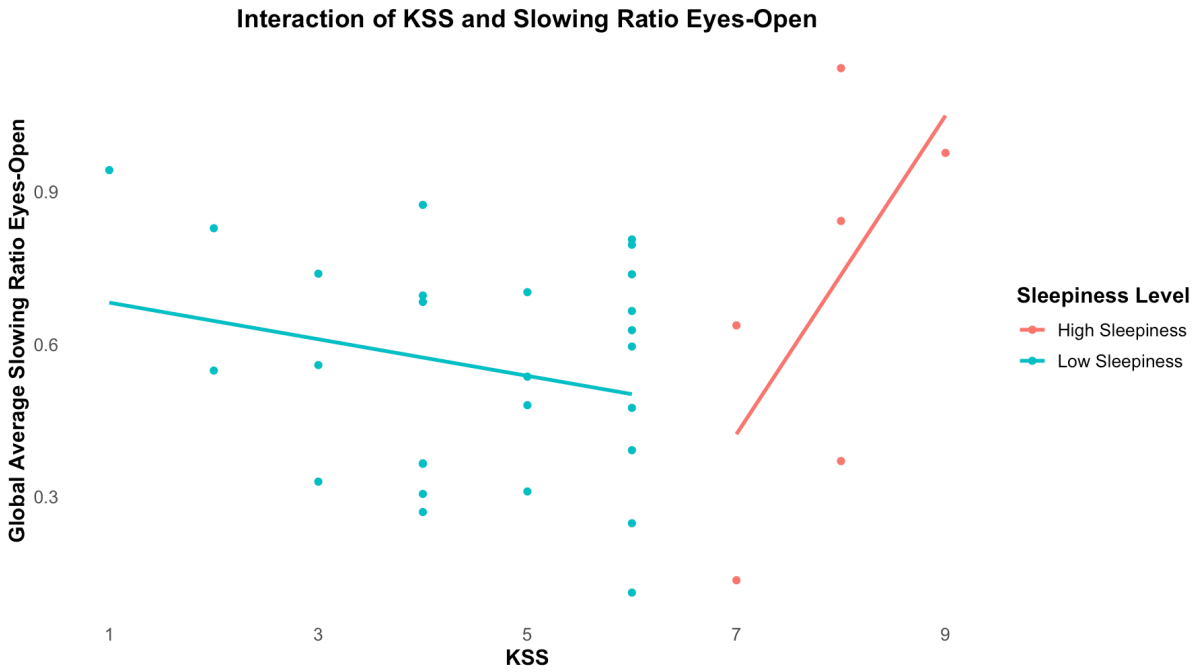
A sensitivity analysis was conducted to examine the interaction between subjective sleepiness (KSS) and group classification when splitting by low (≤ 6) and high (≥ 7) subjective sleepiness on objective sleepiness.

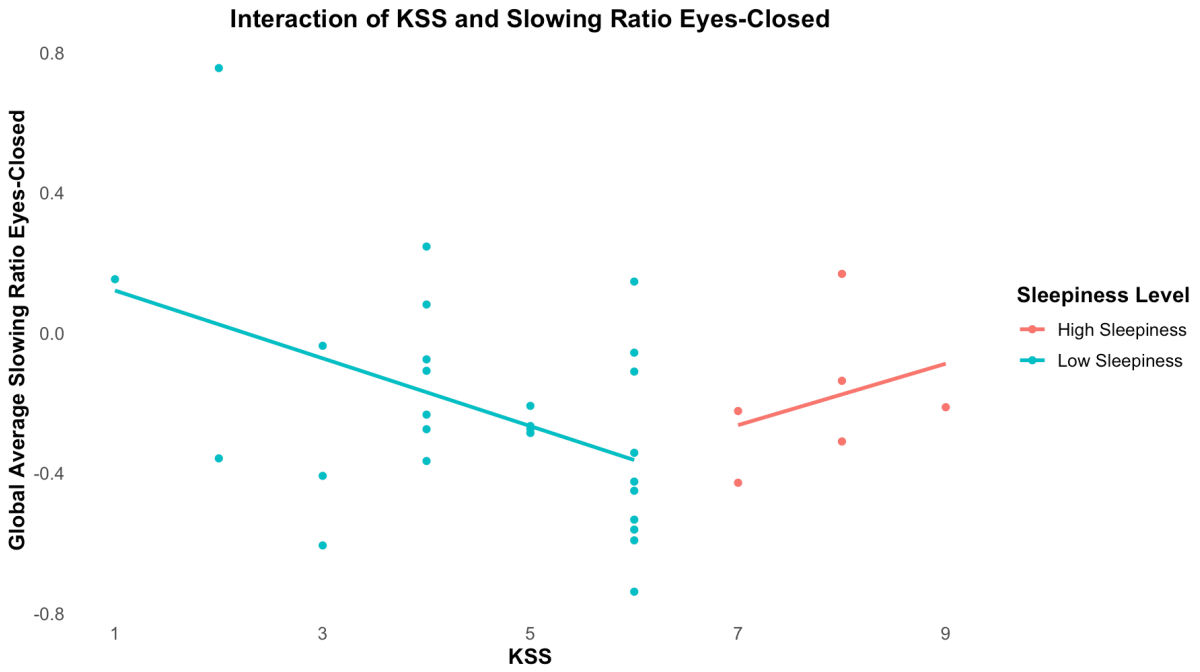
For AAC, the interaction model showed a marginally better fit compared to the original model (AIC = -17.60 vs. -16.41), though the likelihood ratio test was not significant, $\chi^2(1) = 0.085$, $p = .086$. For the slowing ratio in the eyes-open condition, the interaction model showed a modest improvement in fit (AIC = 17.14 vs. 18.70), and the likelihood ratio test approached significance, $\chi^2(1) = 0.273$, $p = .069$. For the slowing ratio in the eyes-closed condition, the interaction model showed no improvement in fit (AIC = 15.17 vs. 14.48), and the likelihood ratio test indicated no significant interaction effect, $\chi^2(1) = 0.092$, $p = .278$.

Bootstrapping indicated moderate variability in the interaction term estimates across models. Bootstrapping was performed with 1,000 replications to assess the stability of the model estimates. For the interaction term of AAC, the original estimate was -0.18, with a bias of -0.01 and a standard error of 0.16, indicating moderate variability. For slowing ratio in the eyes-open condition, the original estimate was -0.32, with a bias of -0.02 and a standard error of 0.27, indicating moderate variability. Finally, for slowing ratio in the eyes-closed condition, the interaction estimate was -0.18, with a bias of -0.01 and a standard error of 0.15, showing relatively low variability.

Overall, the interaction effects were not statistically significant, but improvements in model fit suggests increased subjective sleepiness is more closely associated with objective sleepiness. Further exploration is warranted.


Interaction of KSS and AAC
Global AAC
0.6
0.4
0.2
0.0
1
3
5
7
9
KSS
Sleepiness Level
High Sleepiness
Low Sleepiness


Interaction of KSS and Slowing Ratio Eyes-Open
Global Average Slowing Ratio Eyes-Open
0.9
0.6
0.3
1
3
5
7
9
KSS
Sleepiness Level
High Sleepiness
Low Sleepiness


Interaction of KSS and Slowing Ratio Eyes-Closed
Global Average Slowing Ratio Eyes-Closed
0.8
0.4
0.0
-0.4
-0.8
1
3
5
7
9
KSS
Sleepiness Level
High Sleepiness
Low Sleepiness