Heart to Heart: Exploring Heart Rate Variability in Insomnia Patient Subtypes

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Preface

This is a thesis by publication. Three manuscripts are presented, each preceded by an explanatory preamble. These are set between the Introduction, to provide the underpinning framework of my research project, and the Conclusion, to synthesise study findings and provide recommendations for future research.

All additional intellectual and written content contained within this thesis is, to the best of my knowledge and belief, my own work unless otherwise acknowledged. I hereby declare that I have not submitted this material for another degree at this or any other institution.

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NAME	SIGNATURE	DATE

Publications, Presentations and Awards

Publications

I. Dodds, K. L., Miller, C. B., Kyle, S. D., Marshall, N. S., & Gordon, C. J. (2016). Heart rate variability in insomnia patients: a critical review of the literature. *Sleep Medicine Reviews*. Advance online publication. doi:http://dx.doi.org/10.1016/j.smrv.2016.06.004

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Presentations at National Meetings

I. Dodds, K. L., Miller, C. B., Kim, J.W., Bartlett, D.J., Grunstein, R.R., & Gordon, C. J. (2015, October). *Heart Rate Variability in Insomnia Patients: A Preliminary Analysis*. Poster at Sleep Down Under (Australasian Sleep Association ASM), Melbourne

II. Dodds, K. L., Miller, C. B., Kyle, S. D., Marshall, N. S., & Gordon, C. J. (2016, October). *The beat up on heart rate variability in insomnia patients: a critical literature review.* Poster session presented at Sleep Down Under (Australasian Sleep Association ASM), Adelaide

Other Presentations

I. 'Heart Rate Variability in patients with Insomnia Disorder' (2015, August)
Poster session presented at the Woolcock Institute Research Symposium, Sydney

II. 'Heart Rate Variability of Patients with Insomnia Disorder' (2015, November)
Sydney Nursing School Research Week talk, Sydney University, Sydney

III. 'The Use of Cardiovascular Markers for Phenotyping Insomnia Patients' (2016, March)
NeuroSleep talk, Woolcock Institute, Sydney

IV. 'Cardiovascular Markers of Autonomic Dysregulation in Insomnia Disorder' (2016, June)
Alertness CRC PhD/Masters 2-day workshop presentation, Melbourne

Awards

I. Best Poster Presentation, Woolcock Institute Research Symposium (2015)
'Heart Rate Variability in patients with Insomnia Disorder'

II. Special Commendation, Alertness CRC PhD/Masters 2-day workshop (2016)
'Cardiovascular Markers of Autonomic Dysregulation in Insomnia Disorder'

III. Oral Poster Presentation Prize, Sleep Down Under (Australasian Sleep Association) (2016)
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a) 2015

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STUDENT SCHOLARSHIP

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Abstract

Insomnia is one of the most common complaints in medical practice and the sleep disorder of highest prevalence. At least 10% of the worldwide population has chronic insomnia, which has been associated with a range of negative health outcomes. Within the clinical setting, patient subtypes have been defined according to symptomology. More recently insomnia researchers have proposed phenotypes based on total sleep time during overnight polysomnography (PSG). Short-sleeping insomnia patients are purported to be a biologically severe phenotype at higher risk of cardiovascular morbidity, poor mental health, and obesity (compared to healthy controls). Heart rate variability (HRV) is an objective marker that provides insight into autonomic nervous system dynamics. The overarching aim of my research was to explore a large clinical sample of patients with Insomnia Disorder to determine whether differences in HRV exist during sleep in empirically-derived insomnia patient subtypes.

The aim of the work presented within Chapter 2 was to identify all previous insomnia-HRV research to determine if HRV was impaired in adult patients with insomnia, and whether treatments altered HRV. A systematic review of five web databases located 22 relevant articles; 17 case-control studies and 5 interventions studies. Results were difficult to synthesise due to incomparable methodology and reporting. There was a high risk of bias in the majority of studies. It was concluded that although HRV impairment in insomnia may be a widely-accepted concept, it is not supported by research nor has it been determined if it varies after treatment or according to patient subtype.

The aim of the first empirical study of the thesis (Chapter 3) was to objectively-derive insomnia patient subtypes and evaluate their physiological signals (HRV and electroencephalography [EEG]) during sleep onset. Patients (n = 96) with clinically-diagnosed Insomnia Disorder underwent overnight PSG to determine sleep metrics for cluster analysis using Ward's method: Total Sleep Time (TST), Wake After Sleep Onset (WASO) and Sleep Onset Latency (SOL). Electrocardiogram (ECG) from the PSG was extracted in the 10 minutes before and after sleep onset. After R-wave detection, the ECG was visually checked and manually corrected as required. Six time and frequency-domain HRV measures were analyzed; heart rate (HR), standard deviation of all N-N intervals (SDNN), root mean square of successive R-R intervals (RMSSD), percentage of successive R-R intervals that differ by > 50 ms (PNN50), high frequency (HF), and low frequency (LF)/HF ratio. Cluster analysis derived two solutions; one comprising two subtypes and another with three subtypes. The two cluster solution consisted of insomnia with short-sleep duration (I-SSD: n = 43) and insomnia with normal objective sleep duration (I-NSD: n = 53). At sleep onset, between-group HRV analysis revealed reduced parasympathetic activity (PNN50 and RMSSD) in the short-sleeping subtype. This was not mirrored by significant increases in HR and/or the LF/HF ratio. These findings suggested that reduced parasympathetic activity during sleep onset might contribute to poor cardiometabolic health outcomes previously reported in short-sleeping insomnia patients.

The final component of this thesis was a case-control study (Chapter 4) which examined whether HRV measures differed between insomnia subtypes across the nocturnal period. It was hypothesized that short-sleeping insomnia patients would have impaired HRV compared to normal-sleep duration insomnia patients, consistent with differences observed

at sleep onset (Chapter 3). Insomnia patients underwent overnight PSG, which provided sleep metrics for cluster analysis and ECG for HRV analysis. ECG was visually checked for accurate R-wave detection, and manually corrected as required. HRV analysis was performed from lights-off to lights-on (and separately by sleep/wake stage) using time and frequency-domain measures. Differences in HRV measures (HR, SDNN, RMSSD, LF, HF, LF/HF) were tested between the subtypes using General Linear Models controlling for age as a core confounder. Short-sleeping insomnia patients (I-SSD: n = 34; 45.5 ± 10.5 years) and normal-sleep duration insomnia patients (I-NSD: n = 41; 37.6 ± 10.9 years) were included in the HRV analysis. There were no statistically significant nocturnal HRV differences between subtypes after controlling for age. As such, short-sleeping insomnia patients did not have statistically significant reductions in HRV measures representative of parasympathetic activity.

In summary, there was a lack of persistent nocturnal HRV disparities (between empirically-derived insomnia patient subtypes) that extended beyond sleep onset in this large clinical sample of patients with Insomnia Disorder. The central tenet of 24-hour hyperarousal amongst short-sleep duration insomnia patients cannot be supported by the combined findings of these two empirical studies. *Post-hoc* calculations revealed larger sample sizes would be required to determine a small to medium effect size difference in nocturnal HRV between insomnia patient subtypes. Until this time, the directional relationship between insomnia, heart rate variability, hyperarousal and cardiovascular disease remains unclear in the heterogeneous insomnia population.

Acknowledgements

After commencing work as a Clinical Trials Coordinator at the Woolcock Institute of Medical Research I was encouraged to undertake a postgraduate research degree. Whilst this facilitated formalised research training, it also provided me with the challenge of independent inquiry. I am indebted to a number of individuals for their personal and professional support throughout this research project -

I am thankful to Brenden Dodds, my husband, for his unwavering belief in my capabilities. Thank you to my family for their love and care, and instilling in me the importance of learning and education. And Dr Julia Chapman, my mentor, whose gentle guidance and constant encouragement is valued beyond words.

As I have been immersed in research groups of the Woolcock Institute of Medical Research, Sydney Nursing School, and Alertness CRC, I was fortunate enough to collaborate with some of the world's best clinicians and researchers including Professor Ron Grunstein, who combines clinical sleep medicine and research better than any other. Working alongside him has been both a privilege and honour.

I am especially grateful to three colleagues who agreed to be my supervisors and have supported my academic journey wholeheartedly; Dr Christopher Gordon, Associate Professor Nathaniel Marshall, and Dr Christopher Miller. Together you have shown me the benefits that arise from academic rigor, divergent opinion and, above all else, collegiality.

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List of Abbreviations

DSM	Diagnostic and Statistical Manual of Mental Disorders
ECG	Electrocardiogram/electrocardiography
EEG	Electroencephalogram/electroencephalography
HF	High frequency power
HFnorm	High frequency power (in normalised units)
HF (nu)	High frequency power (in normalised units)
HR	Heart rate
HRV	Heart rate variability
LF	Low frequency power
LF/HF ratio	Low frequency to high frequency ratio
LFnorm	Low frequency power (in normalised units)
LF (nu)	Low frequency power (in normalised units)
N1	Non Rapid Eye Movement sleep - stage 1
N2	Non Rapid Eye Movement sleep - stage 2
N3	Non Rapid Eye Movement sleep - stage 3 or slow wave sleep
NN interval	Time interval between two successive normal R wave peaks of a QRS complex
NREM	Non Rapid Eye Movement sleep
PNN50	Percentage of number of adjacent NN interval pairs greater than 50ms divided by the total number of NN intervals
PSG	Polysomnogram/polysomnography

REM	Rapid Eye Movement sleep
RMSSD	Square root of the mean of sum of the squares of differences between adjacent NN intervals
RRI	Time interval between two successive R wave peaks of a QRS complex. Synonymous with the NN interval; N denotes a normal, sinus-originated beat
SD	Standard deviation
SDANN	Standard deviation of the average of NN intervals in all 5-minute segments of a 24-hour recording
SDNN	Standard deviation of all NN intervals, typically from a 24-hour recording
SE	Sleep efficiency (%)
SOL	Sleep Onset Latency (minutes)
SAS	Statistical Analysis System
Total power	Variance of all NN intervals, reflects total HRV
VLF	Very low frequency power
TST	Total Sleep Time
WASO	Wake-time After Sleep Onset (minutes)

Glossary of Heart Rate Variability Measures

Time-domain measures			
Measure	Description	Units	Autonomic inference
HRV triangular index	Baseline width of the minimum square difference triangular interpolation of the highest peak of the histogram of all NN intervals, reflects total HRV	ms	-
NN interval	Time between two successive normal R wave peaks (of a QRS complex), the frequency of myocardial contractions	ms	-
PNN50	Percentage of number of adjacent NN interval pairs greater than 50 ms divided by the total number of NN intervals	%	Parasympathetic activity
RMSSD	Square root of the mean of sum of the squares of differences between adjacent NN intervals	ms	Parasympathetic activity
SDANN	Standard deviation of the average of NN intervals in all 5-minute segments of a 24-hour recording, primarily reflects circadian HRV	ms	-
SDNN	Standard deviation of all NN intervals, typically from a 24-hour recording, reflects total HRV	ms	-
Frequency-domain measures			
Measure	Description	Units	Autonomic inference
HFnorm	Similar to HF but the high frequency power is normalised using total power	nu; %	Sympathovagal balance, with higher values representing increases in parasympathetic prevalence
LF	Low frequency power (0.04 - 0.15 Hz), significance is debated	ms^2	Purported to reflect both parasympathetic and sympathetic activity
LF/HF	Ratio of LF to HF, significance is debated	ratio	Purported to reflect sympathovagal balance
LFnorm	Similar to LF but the low frequency power is normalised using total power, significance is debated	nu; %	Sympathovagal balance, higher values may represent increases in sympathetic prevalence
Total power	Variance of all NN intervals, reflects total HRV	ms^2	-
VLF	Very low frequency power (0.003 - 0.04 Hz)	ms^2	Parasympathetic activity and the non-autonomic renin-angiotensin system effects

(Malik, 1998; Malik et al., 1996; Sassi et al., 2015; Stein & Pu, 2012)

Chapter 1: Introduction

Insomnia Disorder is the most prominent sleep disorder with a worldwide prevalence estimate of 10% (Morin et al., 2015; Ohayon, 2002; Ohayon & Reynolds, 2009). Higher prevalence rates are evident with increasing age and also amongst females, who are affected two times more than their male counterparts (Morin et al., 2015; Ohayon, 2002). When chronic, median duration is three years (Morin et al., 2015). It is difficult to report the precise prevalence of Insomnia Disorder in Australia. Over the past 35 years there have been only five Australian prevalence studies, between them using four different definitions of insomnia. A brief overview of Australian studies is presented in Table 1.

Table 1. *Insomnia in Australia*

Prevalence estimate (%)	Reference	n	Year sampled	Location	Details
6	Wilson and Lack (1983)	100	NR	SA	Telephone survey : - 'self-described insomniac?'
5 'insomniac' **13** 'sleeping difficulties'	Lack, Miller, and Turner (1988)	216	NR	SA	Telephone survey : - 'self-described insomniac?' - '*often* experience sleeping difficulties?'
33	Bartlett, Marshall, Williams, and Grunstein (2008)	3400	NR	NSW	Telephone survey, using Athens Insomnia Scale (AIS) and question, 'difficulties falling asleep or staying asleep or a combination of both?'
4	Knox, Harrison, Britt, and Henderson (2008)	9156	2005	National	Family-physician survey, patients that report insomnia syndrome as per ICD-10
6	Bin, Marshall, and Glozier (2012)	8841	2007	National	General household survey, including question on 'sleeping only in short bursts and being awake most of the night?'

Note. ICD-10 = International Classification of Mental and Behavioural Disorders from the International Classification of Diseases, Tenth Revision; n = number sampled; National = Australia-wide; NR = Not Reported; NSW = New South Wales, Australia; SA = South Australia, Australia.

Several classifications exist for the clinical diagnosis of Insomnia Disorder (American Academy of Sleep Medicine, 2005; American Psychiatric Association, 2013; World Health Organization, 1993). According to the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5), Insomnia Disorder occurs when poor sleep at night results in daytime impairment and distress (American Psychiatric Association, 2013). Criteria also require the patient to report difficulty with sleep onset, and/or sleep maintenance, and/or early morning awakening at least three nights per week for three months or more, despite sufficient sleep opportunity (American Psychiatric Association, 2013).

Insomnia Disorder places a significant burden on primary health care (Charles, Harrison, & Britt, 2009; Morin et al., 2015). It is the sleep disorder most commonly seen by Australian family-physicians with 1.7 million encounters per year, 95% of which are managed using pharmacotherapy (Charles et al., 2009). Beyond primary health care and pharmaceuticals, Insomnia Disorder is known to have considerable, yet somewhat immeasurable, indirect costs from occupational (absenteeism/presenteeism), health (medical/psychological morbidity), and safety (accidents/injury) sequelae (Daley, Morin, LeBlanc, Grégoire, & Savard, 2009; Daley, Morin, LeBlanc, Grégoire, Savard, et al., 2009; Deloitte Access Economics, 2011; Léger & Bayon, 2010; Sivertsen, Øverland, Bjorvatn, Mæland, & Mykletun, 2009; Vgontzas, Fernandez-Mendoza, Liao, & Bixler, 2013).

Whilst the impact of Insomnia Disorder is well-recognised, the precise aetiology remains uncertain. Early mechanistic models were based upon psychological paradigms reflecting insomnia as a subjective complaint of sleep loss. The ‘Stimulus Control Model’ suggested by Bootzin in 1972 is centred around the premise of faulty conditioning with maladaptive sleep-wake processes (Bootzin, 1973). The development of chronic insomnia can be explained by Spielman’s ‘Three P Model’ (Spielman, Caruso, & Glovinsky, 1987). This diathesis-stress theory delineates predisposing, precipitating, and perpetuating factors of classic psychophysiological insomnia (American Academy of Sleep Medicine, 2001). From a psychobiological perspective, a number of proposals exist including the ‘Neurocognitive Model’ (Perlis, Giles, Mendelson, Bootzin, & Wyatt, 1997); the ‘Psychobiological Model’ (Espie, 2002; Espie, Broomfield, MacMahon, Macphee, & Taylor, 2006); and the ‘Neurobiological Model’ (Buysse, Germain, Hall, Monk, & Nofzinger, 2011). Taken together, these connect subjectively-reported insomnia to the presence of physiological disruption via

a synergistic cognitive, cortical, and somatic hyperarousal (Bonnet & Arand, 1997; Bonnet & Arand, 2010a, 2010b; Perlis, Gehrman, Pigeon, Findley, & Drummond, 2009; Riemann, 2010; Riemann et al., 2010).

A wide range of objective, physiological measures have been used to support the notion of chronic insomnia as a disorder of 24-hour hyperarousal including, but not limited to, nocturnal and daytime electroencephalography (Edinger, Means, & Krystal, 2013; Freedman, 1986; Freedman & Sattler, 1982), quantitative electroencephalography (Hall et al., 2007), temperature and skin resistance (Monroe, 1967), hormones (including cortisol, ACTH, and catecholamines) (Bonnet & Arand, 1995; Irwin, Clark, Kennedy, Christian Gillin, & Ziegler, 2003), blood pressure (Fernandez-Mendoza et al., 2012; Li et al., 2015), heart rate (HR), and heart rate variability (HRV) (Bonnet & Arand, 1998; Monroe, 1967; Vgontzas & Fernandez-Mendoza, 2013) (see Figure 1).

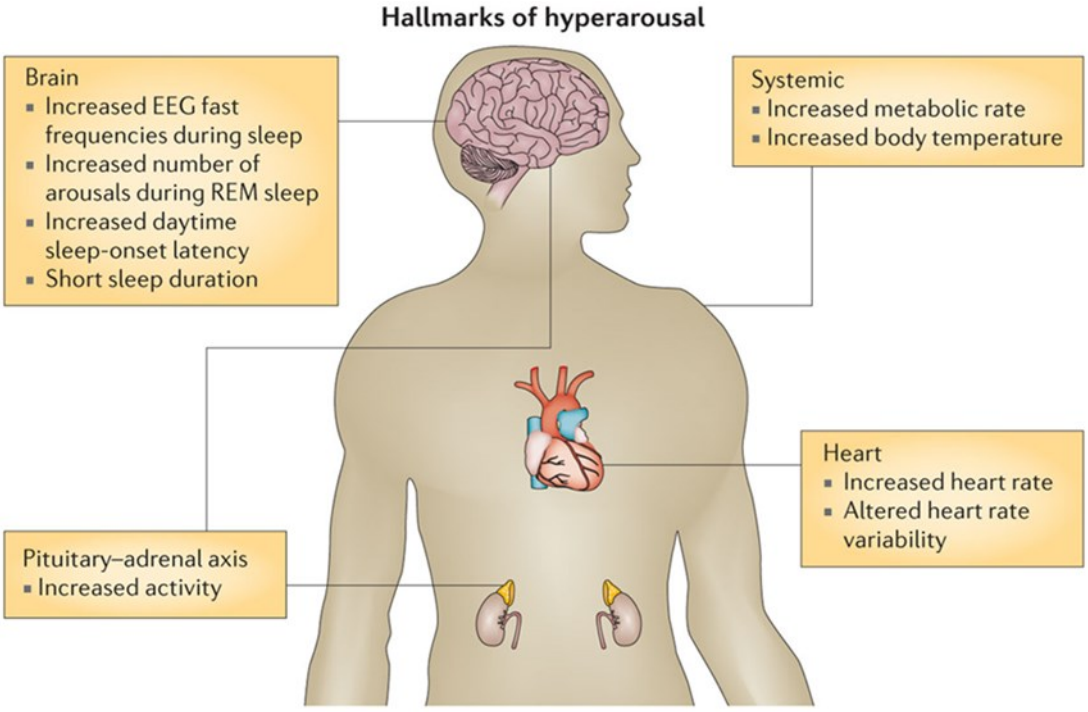

Figure 1. Schematic of systemic physiological hyperarousal. EEG = electroencephalogram. Reprinted from Morin, C. M., Drake, C. L., Harvey, A. G., Krystal, A. D., Manber, R., Riemann, D., & Spiegelhalder, K. (2015). Insomnia disorder. Nature Reviews Disease Primers, 15026. doi: 10.1038/nrdp.2015.26.

Heart rate variability is a common, non-invasive tool for assessing autonomic activity (Malik, 1998). It has been shown to be a predictor of cardiovascular mortality (Kleiger, Miller, Bigger, & Moss, 1987; Kleiger, Stein, & Bigger, 2005), but the connection between HRV impairment and cardiac disease aetiology is yet to be clarified. Nonetheless, HRV has been used broadly in clinical research and specifically as a measure of hyperarousal in a number of insomnia case-control studies (Bonnet & Arand, 1998; Jiang et al., 2015; Spiegelhalder et al., 2011). Although insomnia has been posited as a modifiable cardiovascular risk factor, a direct relationship between insomnia and the pathophysiology of cardiovascular disease has not been established (Grandner et al., 2016; Hertenstein, Johann, Baglioni, Spiegelhalder, & Riemann, 2016; Redline & Foody, 2011; Spiegelhalder, Scholtes, & Riemann, 2010; Strand et al., 2012).

Certain subtypes of the heterogeneous insomnia population may have varied aetiologies and reflect different pathophysiologies. The delineation of insomnia patient subtypes is not an entirely novel paradigm. Long-recognised patient groupings include those based on various diagnostic-criteria subtypes, the timing of symptoms (e.g. sleep onset insomnia vs sleep maintenance insomnia), the presence of comorbid sleep (or other health) disorders, and/or duration of symptoms (e.g. acute vs chronic) (Edinger et al., 2004; Edinger et al., 1996). However, the few studies that have investigated the validity of these subtypes have found them to have somewhat unsatisfactory when compared using quantitative measures and/or empirically derived patient subtypes (Edinger et al., 1996; Pillai, Roth, & Drake, 2015).

Previous associations have been reported between short-sleep duration and poor health outcomes (including obesity, diabetes, cardiovascular morbidity, mortality) although participants of these studies were not pre-screened insomnia patients (Ayas et al., 2003; Gangwisch, Feskanich, Malaspina, Shen, & Forman, 2013; Kjeldsen et al., 2014; Tamakoshi & Ohno, 2004). The concept of short-sleep insomnia was introduced in 2009 by the Vgontzas research group after findings from the Penn State Cohort showed a positive association between objectively-derived sleep duration (of less than six hours) and hypertension (Vgontzas, Liao, Bixler, Chrousos, & Vela-Bueno, 2009). In 2010, they reported objectively-derived short-sleep insomnia patients) had higher risk of mortality and in 2012, incident hypertension (Fernandez-Mendoza et al., 2012; Vgontzas et al., 2010). This group continues

to advocate that insomnia patients with short-sleep duration have greater cognitive and physiological hyperarousal and activation of the stress systems (hypothalamic-pituitary adrenal and sympathetic-adrenal medulla axes) compared to their relatively longer-sleep duration insomnia counterparts who were more likely to have psychological-originated insomnia and less risk of medical sequelae (Vgontzas et al., 2013) (see Figure 2).

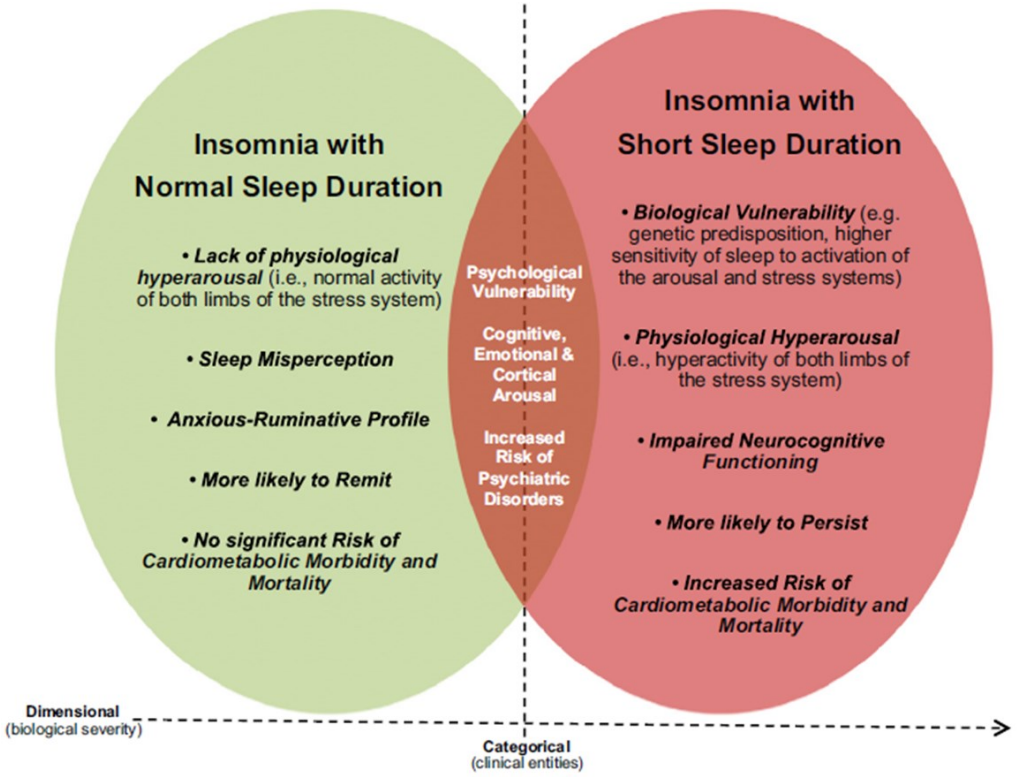

Figure 2. Model of insomnia phenotypes, incorporating pathophysiological mechanisms and clinical sequelae. Reprinted from Vgontzas, A. N., Fernandez-Mendoza, J., Liao, D., & Bixler, E. (2013). Insomnia with objective short sleep duration: The most biologically severe phenotype of the disorder. Sleep Med Rev, 17(4), 241-254. doi:10.1016/j.smrv.2012.09.005.

Therefore, the primary aim of my research project was to explore a large clinical sample of patients with Insomnia Disorder to determine whether differences in HRV exist during sleep in empirically-derived insomnia patient subtypes. This multi-component project includes a critical literature review of all HRV-insomnia research that has been performed to date. Two case-control studies are then presented.

The first compared the HRV of empirically-derived insomnia patient subtypes across the sleep onset period, a time of clinical and physiological significance. The second study compared HRV of previously derived patient subtypes across the whole night. It was hypothesized that insomnia patients with short-sleep duration would have impaired HRV when compared to longer-sleeping insomnia patients. This work is the first to compare directly the HRV of empirically-derived insomnia patient subtypes and contributes significantly to the physiological understanding of short-sleeping insomnia patients.

Chapter 2: Literature Review

Preamble

Insomnia is a highly prevalent and heterogeneous disorder (Edinger et al., 2004; Ohayon, 2002; Ohayon & Reynolds, 2009). Epidemiological studies support the presence of an association with cardiovascular disease yet the mechanistic link remains elusive (Grandner, 2014). Although the aetiology of insomnia is still debated, the hyperarousal hypothesis is currently one of the most prominent models, leading to the exploration of physiological and cognitive hyperarousal using a variety of objective measures.

Cardiovascular autonomic activity can be quantified using HR, HRV, blood pressure, blood pressure variability, baroreflex activity, cardiovascular impedance, muscle sympathetic nerve activity recordings, and measures of catecholamine levels (Trinder, 2007). However, only one – HR – is routinely recorded during overnight polysomnography (PSG) and the interpretation of HRV provides suitable surrogates of autonomic activity.

HR is modulated by the autonomic nervous system and is the basis of HRV. Therefore, if the ability to modulate the HR is impaired this would result in decreased beat-to-beat variability. This phenomenon has been shown in large cardiovascular studies from the 1980s that not only linked impaired HRV to sudden cardiac death, but also demonstrated its ability to be used to risk-stratify patients post-myocardial infarction (Kleiger et al., 1987; Singer et al., 1988). A significant advancement in the technique was introduced by Askelrod (1981) with the introduction spectral analysis of HRV. As parasympathetic and sympathetic branches of the nervous system have different mechanisms that are imposed at different speeds, spectral analysis enabled greater correlation to be made to physiological function and the estimation of sympathovagal balance (Ernst, 2014).

The purpose of this critical literature review (Chapter 2) was to identify all studies that had assessed the HRV of insomnia patients. In regards to HRV, only studies using time and/or frequency domain HRV measures were employed. Whilst a number of advanced

signal analysis methods can now be performed (using nonlinear dynamics, entropy, and fractal signals), traditional methods are still preferred when assessing basic physiological function, and for pathophysiological modelling (Sassi et al., 2015). No studies were excluded on the basis of the classification system used to diagnose insomnia. As such, the review incorporated studies of all patients with insomnia that resulted in sleep-wake impairments.

Since this manuscript was accepted for publication, only one other article has been published that would have otherwise been included. Authored by Jarrin et al (2016), the research studied 65 insomnia patients before and after treatment with six weeks of Cognitive Behavioural Therapy for Insomnia. No changes in HRV (High Frequency [HF] and the Low Frequency/High Frequency ratio [LF/HF]) were found in the sleep stages N2 or REM after treatment, regardless of whether the patient had responded to therapy. Nonetheless, associations were found between HRV measures and PSG-derived WASO, TST, and SE. These findings, however, were only in select HRV parameters and reported inconsistently (i.e. not in both N2 and REM). As such, this study does not alter the findings, practice points or research agenda of the literature review presented below.

Statement of Author Contributions

The literature review that forms Chapter 2 has been accepted and prepared for publication in Sleep Medicine Reviews. It is currently available ahead of press as:

Dodds, K. L., Miller, C. B., Kyle, S. D., Marshall, N. S., & Gordon, C. J. (2016). Heart rate variability in insomnia patients: a critical review of the literature. *Sleep Medicine Reviews*. Advance online publication. doi:http://dx.doi.org/10.1016/j.smrv.2016.06.004

I contributed to the design of the study, independently performed the literature search and bias assessment, synthesized the results, and wrote the manuscript.

Kirsty Lyn Dodds	[redacted]	16th February 2017
NAME	SIGNATURE	DATE

As the primary supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Dr Christopher Gordon	[redacted]	15th February 2017
NAME	SIGNATURE	DATE

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CLINICAL REVIEW

Heart rate variability in insomnia patients: A critical review of the literature

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SUMMARY

Heart rate variability (HRV) is an objective marker that provides insight into autonomic nervous system dynamics. There is conflicting evidence regarding the presence of HRV impairment in insomnia patients. Web-based databases were used to systematically search the literature for all studies that compared the HRV of insomnia patients to controls or reported the HRV of insomnia patients before and after an intervention. 22 relevant papers were identified. Study characteristics were summarised, HRV measures were extracted and a risk of bias assessment for each study was performed. We were limited in our ability to synthesise outcome measures and perform meta-analyses due to considerable differences in patient (and control) selection, study protocols, measurement and processing techniques and outcome reporting. Risk of bias was deemed to be high in the majority of studies. As such, we cannot confirm that HRV is reliably impaired in insomnia patients nor determine the HRV response to interventions. Whilst HRV impairment in insomnia is a widely accepted concept, it is not supported by empirical evidence. Large longitudinal studies incorporating 24-hour recordings are required to elucidate the precise nature of HRV dynamics in insomnia patients.

Introduction

Heart rate variability (HRV) describes the variation in time between consecutive heart beats, which is commonly referred to as the RR (R wave to R wave) or NN (normal beat to normal beat) interval. Pacemaker cells located in the sinoatrial node of the heart possess autorhythmicity to maintain heart rate regularity. The heart rate (HR), however, is modulated by a number of physiological factors which alter autonomic nervous system control and increase variability at various frequencies. There are two prominent approaches for quantifying heart rate variation which use spectral or non-spectral techniques to generate HRV measures.

Non-spectral methods involve mathematical derivations of the NN interval. When taken from an electrocardiogram (ECG), this interval is determined by measuring the length in time between consecutive sinoatrial R wave peaks. As many non-spectral derivations report the HRV in time (milliseconds) or units, they are collectively referred to as time-domain measures. Four time-domain measures are recommended for use by the task force of the European society of cardiology and the North American society of pacing and electrophysiology when assessing HRV for estimation of short term (root mean square of successive differences of NN intervals (RMSSD)), long term (standard deviation of the averages of NN intervals in five minute segments of entire recording (SDANN)) and overall HRV (standard deviation of NN intervals (SDNN), HRV triangular index) [1]. A description of these HRV measures can be found in Table 1.

Spectral analysis of HRV enables the evaluation of frequency-domain measures. As parasympathetic-mediated changes to HR occur more quickly than sympathetic adjustments, the use of spectral analysis to determine frequency can provide insight into autonomic nervous system dynamics. It has been accepted previously that low frequency (LF) activity is a correlate of

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Abbreviations

ECG	electrocardiogram
EEG	electroencephalogram
HF	high frequency power
HFnorm	high frequency power (in normalised units)
HR	heart rate
HRV	heart rate variability
LF	low frequency power
LF/HF	low frequency to high frequency ratio
LFnorm	low frequency power (in normalised units)
NN interval	interval in time between successive normal beats (typically measured from the R wave of a QRS complex)
NREM	non rapid eye movement
pNN50	percent of NN intervals > 50 milliseconds different from previous
PSG	polysomnography
REM	rapid eye movement
RMSSD	root mean square of successive differences of NN intervals for period of interest
RR interval	interval in time between successive R waves (of a QRS complex)
SDANN	standard deviation of the averages of NN intervals in five minute segments of entire recording
SDNN	standard deviation of NN intervals for period of interest
TP	total power

parasympathetic and sympathetic activity whilst high frequency (HF) activity reflects parasympathetic activity only. As such the ratio between these (the LF/HF ratio) provides an estimate of sympathovagal balance which is the putative equilibrium of the sympathetic and parasympathetic systems. However, the physiological significance of the lower frequency bands (including LF and the LF/HF ratio) has been contested and caution is required when interpreting these measures [2–4]. This is further summarised in Table 1. For a more detailed explanation of the physiological correlates of HRV measures refer to the previous review of Stein and Pu [5].

Heart rate variability recordings are employed in a vast range of settings, popular due to the non-invasive methods used for data collection. The clinical utility of HRV was realised in the late 1980s when decreased HRV, defined by a reduction in 24-hour SDNN, was found to be a strong predictor of mortality post myocardial infarct [6]. HRV has since been used to investigate health and disease states for clinical and research purposes.

The incorporation of HRV analysis during sleep has been a logical extension as the ECG is a component of overnight polysomnography (PSG). This has acted as a catalyst for further examination on the bidirectional relationship between autonomic nervous system activity and sleep physiology [7]. It is not surprising that time and frequency-domain HRV measures, primarily from short recordings (i.e., less than 24 h), are increasingly being reported in the sleep literature.

HRV has circadian periodicity with significant alterations during the transition from wake to sleep and across sleep cycles [8,9]. Bonnemeier et al. [10] and Li et al. [11] have shown that vagal HRV measures follow a day-night pattern increasing during the night [12]. Importantly, these changes are correlated with age and sex [10,11]. There is also a shift in sympathovagal balance between non rapid eye movement (NREM) and rapid eye movement (REM) sleep [5,9,13]. Research has now progressed to investigating the associations between HRV, sleep disorders and their subsequent comorbidities and the incorporation of non-traditional HRV techniques [5,13,14].

Given the interplay between HRV and cardiac autonomic activity, the use of HRV in insomnia research may assist further investigation into the pathophysiology and potential health impacts of insomnia. With an estimated worldwide prevalence of 10%, insomnia is the most common sleep disorder [15–17]. Clinical diagnosis in accordance with the most recent diagnostic and

Table 1
Summary of common heart rate variability measures and their physiological correlates.

Measure	Description	Units	Autonomic inference
Time-domain measures			
HRV triangular index	Baseline width of the minimum square difference triangular interpolation of the highest peak of the histogram of all NN intervals, reflects total HRV	ms	–
NN interval	Time between two successive normal R wave peaks of a QRS complex	ms	–
pNN50	Percentage of number of adjacent NN interval pairs greater than 50 ms divided by the total number of NN intervals	%	Parasympathetic activity
RMSSD	Square root of the mean of the squared differences between successive NN intervals	ms	Parasympathetic activity
SDANN	Standard deviation of the average of NN intervals in all 5-minute segments of a 24 h recording, primarily reflects circadian HRV	ms	–
SDNN	Standard deviation of all NN intervals, typically from a 24 h recording, reflects total HRV	ms	–
Frequency-domain measures			
HF	High frequency power (0.15–0.4 Hz)	ms^2	Parasympathetic activity
HFnorm	Similar to HF but the high frequency power is normalised using total power	nu	Sympathovagal balance, with higher values representing increases in parasympathetic prevalence
LF	Low frequency power (0.04–0.15 Hz), significance is debated	ms^2	Purported to reflect both parasympathetic and sympathetic activity
LF/HF	Ratio of LF to HF, significance is debated	ratio	Purported to reflect sympathovagal balance
LFnorm	Similar to LF but the low frequency power is normalised using total power, significance is debated	nu	Sympathovagal balance, higher values may represent increases in sympathetic prevalence
Total power	Variance of all NN intervals, reflects total HRV	ms^2	–
VLF	Very low frequency power (0.003–0.04 Hz)	ms^2	Parasympathetic activity and the non-autonomic renin-angiotensin system effects

Abbreviations: HRV: heart rate variability; ms: milliseconds; nu: normalised units. Refs.: [1–5].

statistical manual of mental disorders requires the patient to report difficulty initiating and/or maintaining sleep with subsequent daytime impairments greater than three nights per week and for at least three months [18]. Insomnia patients also have increased medical and psychological comorbidities [17].

Hyperarousal has been defined as 'a state of increased arousal at the physiological, cortical, cognitive or emotional level' [17] and hypothesised to contribute to the development, maintenance and 24-hour systemic sequelae of insomnia [19]. The notion of physiological hyperarousal has been investigated by measuring HRV in insomnia patients despite the uncertain relationship between autonomic dysfunction, insomnia and cardiovascular disease [19].

Heart rate variability studies of the insomnia population have increased since 1998 after the influential work of Bonnet and Arand [20] which was supportive of the hyperarousal theory. This study revealed altered sympathovagal balance in patients with insomnia compared to controls [20]. However, the extant research literature has not been able to replicate these findings with comparable detail and instead yielded somewhat inconsistent, divergent findings [21].

Whilst two reviews examining HRV, sleep and sleep disorders have been published previously they did not systematically synthesise the findings of the insomnia – HRV literature and we were aware of additional studies that were not included [5,13]. Therefore, the aim of this review was to identify all insomnia related research studies using HRV to determine if HRV was impaired in adult patients with insomnia and whether interventions alter HRV.

Methods

We undertook an extensive search of the literature using CINAHL, Embase, Google Scholar, PubMed, Scopus and Web of Science databases on 25th January 2016. Where possible the search incorporated a subject heading and key word strategy, combining "heart rate variability" or "HRV" and "insomnia". The systematic search strategy was modified as necessary according to the database being searched, as shown in Appendix A. No limitations were used with the exception of Google Scholar where restrictions were used to exclude patents and citations. We had two main questions: 1) whether there were any differences in HRV of insomnia patients compared to controls (observational) and 2) whether treatment interventions resulted in changes to the HRV of insomnia patients (interventional).

In order to be included for review, studies required the following:

1) Participants – humans ≥18 years old (observational) or insomnia patients (interventional)
2) Exposure – diagnosed with insomnia (observational) or a treatment for insomnia (interventional)
3) Comparisons – controls who were deemed to be good sleepers (observational) or the same group of patients compared before and after treatment or an ineffective or placebo treatment (interventional)
4) Outcomes – standard time and/or frequency-domain HRV measures with recordings of any length at any time during sleep and/or wake
5) Study design – case-control studies, observational cohort and cross-sectional studies (observational) and randomised controlled trials, non-randomised controlled trials and uncontrolled trials (before and after studies; interventional).

We included research studies that incorporated HRV measurements as either primary or secondary outcomes. Case studies and publications without original data were excluded from the review. The interventional studies were not restricted to validated insomnia treatments, but rather any interventions that were conducted in insomnia patients and included pre- and post HRV measurements.

On completion of the database searches, duplicates were removed. All remaining studies were reviewed for eligibility by one author (KD) using the study title, abstract and, when required, full text. The reference lists of remaining publications and the bibliographies of relevant review articles were also searched for grey literature and otherwise missed publications.

Study measures were collected through the use of tables that identified study characteristics and standard time and frequency-domain HRV measures. Two tools were used by one author (KD) to assist with assessing the risk of bias within studies. Case-control studies were evaluated using a modified version of the national institute for health and care excellence methodology checklist [22]. This checklist assisted in reviewing studies for internal validity by methodically appraising the selection of cases and controls, confounding factors and statistical methods [22]. Intervention studies were evaluated using the Cochrane collaboration's tool for assessing risk of bias. Designed for randomised controlled studies, this helped to scrutinise the studies for selection, performance, detection, attrition or reporting bias [23].

Findings

Search process

The database search yielded 555 records (CINAHL 9, Embase 182, Google Scholar 21, PubMed 60, Scopus 140, Web of Science 143). After removal of duplicates, 275 publications remained and of these 22 met the inclusion criteria. No additional publications were identified after a manual search of the reference lists. There were 17 case-control studies [20,21,24–38], one of which was a nested case-control study from a larger cohort [36]. We did not identify any cohort or cross-sectional study designs. Five research studies compared insomnia patients before and after an intervention. The types of interventions were cognitive behavioural therapy for insomnia [39], pharmacotherapy (gabapentin) [40], acupuncture [41], acupressure [42] and paced breathing [43]. There were no randomised controlled trials. A diagrammatic schema of the search results is shown in Fig. 1.

Observational studies

Demographics

Participant numbers and demographics of the observational studies are presented in Table 2. The sample size of patients ranged from 8 to 85 (with 8 to 55 controls respectively) [27,29]. Three studies exclusively involved female participants [33,34,36] and one study involved only males [32]. Bonnet and Arand [20] did not report the sex-ratio of their study participants. The age of participants was also variable with the average patient age ranging from 23.0 (SD 2.4) to 53.2 (SD 13.6) years. In accordance with the time span of included studies, the majority of studies involved patients diagnosed according to the fourth edition of the diagnostic and statistical manual of mental disorders [21,26–28,30–33,35,36,38]. In contrast, Varkevisser et al. [37] and Farina et al. [29] utilised the international classification of sleep disorders, whilst two other studies used the research diagnostic criteria for primary insomnia, which set the benchmark at the time of publication [24,25]. Finally, there were two studies that utilised non-standard criterion based upon a combination of subjective symptomology and arbitrary PSG criteria, incorporating sleep efficiency and/or sleep onset latency [20,34].

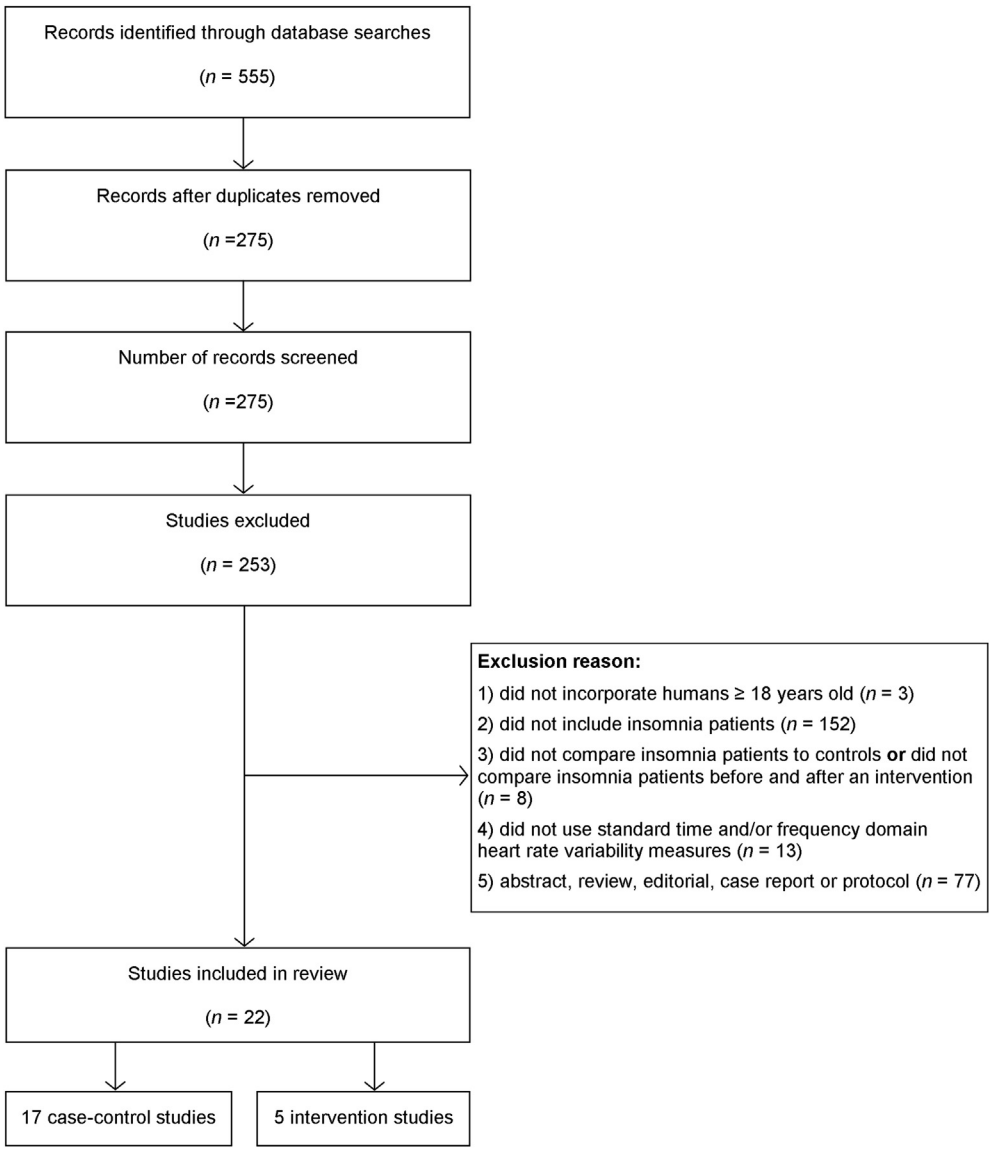

Fig. 1. Modified PRISMA study inclusion flowchart.

Data collection

Most night time HRV data collection was performed in the sleep laboratory, with the exception of four studies that included testing in participants' homes or an alternative health care facility [29,34,36,38]. As shown in Table 2, HR data collection was achieved through the use of Holter monitors, heart monitor belts and PSG-based ECGs, resulting in sampling rates ranging from 125 to 1024 Hz. [30,34,36].

HRV measures

It was difficult to synthesise the findings of HRV measures reported in the observational studies. Total power (TP) was the only HRV measure that provided unequivocal findings across observational studies – no significant differences were reported between insomnia patients and controls (Table 3). The next most consistent finding was that mean HR was found to be higher in insomnia patients compared to controls [20,27,29,38]; nevertheless, others reported no significant group differences in this measure [21,24,26,31,33,37]. Both Bonnet and Arand [20] and Farina et al. [29] found the LF/HF ratio to be increased in insomnia patients compared to controls although 11 other studies did not show this response.

The classic findings of increased sympathetic-related measures (LF/HF ratio) with a reciprocal reduction in parasympathetic measures (HFnorm) in insomnia patients compared to controls [20] has not been replicated comprehensively. Four studies have corroborated some of these findings but not across all HRV measures and sleep stages [21,29,31,38]. We were not able to identify any other study with HRV outcome measures suggestive of both increased sympathetic and decreased parasympathetic activity that would link autonomic dysfunction and physiological hyperarousal [44,45]. Table 3 provides an overview of the HRV outcomes of the case-control studies.

Sleep onset

Heart rate variability during the transition from wake to sleep is of particular interest given the changes in autonomic activity and the reported heightened symptomatology in insomnia patients at

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Table 2
Study characteristics of 17 observational (case-control) studies comparing heart rate variability in insomnia patients and controls.

Author/s, date, reference number	Patient number, sex, age (mean, SD, range)	Diagnostic criteria	Insomnia severity	Comparators number, sex, age (mean, SD, range)	Case-match methodology	Setting	HRV data capture and analysis
Bonnet & Arand, 1998 [20]	12 M and F unknown ratio 31.2 y ± 6.8, 18–50	Subjective report of insomnia ≥four nights per week for one year and EEG SOL >30 min or SE <85% on both PSG nights	Subjective SOL, TST and length of insomnia available for review	12 M and F, unknown ratio 29.1 y ± 5.2, 18–50	Sex, age (within five years), body mass (within 25 pounds) and sleep characteristics	Sleep laboratory 36 h (two nights, one day). Data from second night	ECG sampled at 500 Hz 5 min time periods Automated RRI detection with repeated visual artifact check
McMillan 2001 [34]	*Insomnia*: 13F 46.3 y ± 2.8, 25–60 *Fibromyalgia*: 16F 46.3 y ± 7.0, 25–60	Subjective report of insomnia for at least three months and sleep efficiency index ≤85% on night 2	NS	*From fibromyalgia study* 16F 43.6 ± 6.0, 25–60	Sex, age, BMI (<40 kg/m^2) and SE >85% on night 2	Sleep laboratory and participants' homes >2 nights recorded sleep. Data from second night	ECG sampled at 125–250 Hz 5 min time periods with >90% 'good beats' Visual ECG check Outliers removed from RRI time series
Varkevisser et al., 2005 [37]	6M, 5F 43.8 y ± 8.9, 31–54	ICSD revised edition (with home PSG confirmation prior to testing)	Actigraphy times available for review	7M, 6F 44.9 y ± 7.7, 33–53	Sex and age Controls had actigraphy SE <85% and NS differences in actigraphy TIB, SOL, mean wake time	48 h ambulatory PSG (pre-study) in participants' homes and 26 h sleep deprivation protocol in sleep laboratory	ECG sampled at 1000 Hz 30 min time periods every 3 h (before performance testing) 30 s time periods Outlier IBI values removed (<300 or >1800 ms)
Fang et al., 2008 [28]	6M, 12F 34.16 y ± 14.46, 20–63	DSM IV, Chinese PSQI ≥6 and at least one of the following on three day actigraphy and seven day sleep diary: WASO >30 min, TST ≤6.5 h, SE ≤85%	Chinese PSQI 11.78 ± 2.18, ESS, sleep diary and actigraphy times available for review	7M, 14F 27.81 y ± 8.67, 20–50	Controls were included when Chinese PSQI ≤5, actigraphy and sleep diary confirming WASO ≤30 min, TST >6.5 h and SE >85%	Home actigraphy and sleep diaries followed by laboratory testing. Lab sessions from 14:00–17:00 h 5 min wake ECG Controlled breathing rate	ECG sampled at 500 Hz 5 min time periods Examined for ectopic beats and artifact. Manually adjusted
Jurysta et al., 2009 [32]	14M 42 y ± 12, 16–63	DSM IV and ICSD revised edition	Sleep characteristics available for review	14M 41 y ± 10, 16–55	Age and BMI	Sleep laboratory three consecutive nights. Data from third night	ECG sampled at 500 Hz 2 min time periods Automatic detection and removal ectopics RRI time series corrected with interpolation Visual inspection of detected events and interpolated values
de Zambotti et al., 2011 [27]	4M, 4F 23.25 y ± 2.43, 20–26	PSQI and AIS ≥6 and ISI ≥11 (at screening) and then enrolled according to DSM IV	PSQI 9.62 (1.30), AIS 9.62 (3.16), ISI 13.37 (3.25), further sleep characteristics available for review	3M, 5F 23.25 y ± 3.24, 19–28	Controls were included when PSQI and AIS <6 and ISI <11 (at screening)	Sleep laboratory two consecutive nights in laboratory. Data from second night	ECG sampled at 500 Hz 1 min, 2.5 min, 2.5 min time periods Incorrect interbeat interval detection adjusted manually
Peter et al., 2011 [35]	3M, 18F 48.2 y ± 10.4, 18–75	DSM IV and PSQI >5	PSQI median 13	3M, 18F 48.5 y ± 11.1, 18–75	Sex and age	Interdisciplinary sleep medicine centre -outpatient department Daytime measurement, 45° seated posture, 5–7 min test	ECG sampled at 200 Hz 5–7 min time periods Artifacts were removed NS if any visual inspection performed
Spiegelhalder et al., 2011 [21]	36M, 22F 39.5 y ± 11.8	DSM IV. For further analysis – SE >85% = 'without short sleep' vs SE <85% = 'short sleep duration'	PSQI 11.2 ± 2.8, further sleep characteristics available for review	19M, 27F 37.3 y ± 11.4	No significant difference for sex, age, BMI, BP or smoking status	Sleep laboratory two consecutive nights. Data from second night	ECG sampled at 400 Hz 5 min time periods Visually inspected for artifact or inaccurate R wave detections and corrected with manual editing Ectopics corrected with interpolation Artifact >10 s duration discarded

(*continued on next page*)

Table 2 (*continued*)

Author/s, date, reference number	Patient number, sex, age (mean, SD, range)	Diagnostic criteria	Insomnia severity	Comparators number, sex, age (mean, SD, range)	Case-match methodology	Setting	HRV data capture and analysis
Yang et al., 2011 [38]	16M, 31F 43.9 y ± 10.4, 23–63	DSM IV	PSQI 10.7 ± 3.9	33M, 55F 41.6 y ± 11.7, 22–64	No significant difference for sex ratio, age, BMI	Participants' homes and psychiatric wards 24 h ambulatory ECG	ECG signals were automatically processed and analysed by open source HRV algorithms 2 min time periods Data divided into wake and bed time
Israel et al., 2012 [30]	24M, 30F 34.6 y ± 9.7	DSM IV	Sleep characteristics available for review	3M, 19F 26.5 y ± 7.3	No specific case-matching methodology reported	Sleep laboratory three consecutive nights	ECG sampled at 1024 Hz 2 min time periods Artifacts and ectopics edited manually by interpolating preceding successive beats
de Zambotti et al., 2013 [26]	4M, 5F 23 y ± 2.4, 20–26	DSM IV and insomnia history for ≥1 y	PSQI 9.67 ± 1.22, AIS 10.00 ± 3.16, HS 44.56 ± 4.03, further sleep characteristics available for review	4M, 5F 23.56 y ± 3.17, 19–28	No significant difference for sex, age, BMI	Sleep laboratory two consecutive nights in laboratory. Data from second night	ECG sampled at 500 Hz 2 min time periods R wave detection visually examined Incorrectly detected R-peaks were manually edited
Cellini et al., 2014 [24]	5M, 8F 23.31 y ± 2.50, 20–28	Research diagnostic criteria for primary insomnia, PSQI ≥5 and ISI ≥11	PSQI 10.0 ± 2.0, ISI 15.77 ± 3.27, further sleep characteristics available for review	7M, 6F 24.31 y ± 1.60, 20–28	Controls were included when they met research diagnostic criteria for normal sleepers and PSQI <5 and ISI <11 No significant difference for age, BMI, MEQ	Sleep laboratory two consecutive nights in laboratory. Data from second night HRV recording during evening cognitive testing	ECG sampled at 512 Hz 3 min time periods R wave detection visually examined Incorrectly detected R-peaks were manually edited Missing and ectopic beats were corrected by interpolation
de Zambotti et al., 2014 [25]	5M, 8F 24.4 y ± 1.6, 20–28	Research diagnostic criteria for primary insomnia, PSQI ≥5 and ISI ≥11	PSQI 10.0 ± 2.0, ISI 15.8 ± 3.3, further sleep characteristics available for review	7M, 7F 23.3 y ± 2.5, 23–28	Controls were included when they met research diagnostic criteria for normal sleepers and PSQI <5 and ISI <11	Sleep laboratory two consecutive nights in laboratory, Data from second night	ECG sampled at 512 Hz 2 min periods - frequency domain 5 min periods - time domain Visually inspected to ensure each epoch is of stable sleep state, free of arousal and artifact
Farina et al., 2014 [29]	38M, 47F 53.2 y ± 13.6, 27–81	ICSD 2	NS, sleep characteristics available for review	23M, 32F 54.2 y ± 13.9, 27–76	Sex and age	Participants' homes 24 h home ambulatory PSG	ECG sampled at 256 Hz 5 min time periods, first 5 min of each sleep stage Digital artifact recognition and exclusion
Maes et al., 2014 [33]	17F 36.2 y ± 9.6, 19–53	DSM IV and SOL > 30 min, at least five nights/week	NS, sleep characteristics available for review	11F 37.6 y ± 12.6, 21–59	No significant difference for age, BMI, ESS, caffeine, alcohol or smoking status	Sleep laboratory. Overnight PSG	ECG sampled at 1000 Hz 5 × 5 min time periods Artifacts of the RRI were adjusted manually
Jiang et al., 2015 [31]	M25, F30 30.4 y ± 8.4, 22–38	DSM IV	PSQI 8.6 ± 2.3	M29, F34 31.3 y ± 7.7, 23–39	No significant difference for sex ratio, age, BMI, BP and HR	University laboratory Tested differences in HRV with posture, heart monitor belt	ECG sampled at 1000 Hz 30 s time periods 30 min time periods every 3 h Outlier RRI values removed (<350 or >1500 ms)
Rothenberger et al., 2015 [36]	19F 52.1 y ± 2.1	Insomnia symptom questionnaire (incorporates DSM IV)	NS	146F 52.1 y ± 2.1 (nested case control study within cohort)	Nested case-control study (with no control matching)	Participants' homes Ambulatory EEG - three nights. Data from second or third night	ECG sampled at 1024 Hz 2 min epoch of NREM sleep Automated detection with visual inspection to identify and remove artifact

Note: When case-match methodology was not reported, we have noted demographic results which highlight group characteristics. *Abbreviations*: AIS: Athens insomnia scale; BMI: body mass index; BP: blood pressure, DSM IV: diagnostic and statistical manual of mental disorders, fourth edition; ECG: electrocardiogram; EEG: electroencephalogram; F: female; FFT: fast Fourier transformation; HR: heart rate; HRV: heart rate variability; Hz: hertz; IBI: interbeat interval; ICSD: international classification of sleep disorders; ICSD 2: international classification of sleep disorders, second edition; ISI: insomnia severity index; ISQ: insomnia symptom questionnaire; M: male; MEQ: morningness-eveningness questionnaire; NREM: non rapid eye movement sleep stage; NS: not specified; PSA: power spectral analysis; PSG: polysomnography; PSQI: Pittsburgh sleep quality index; PVC: premature ventricular contraction; RRI: RR interval; SD: standard deviation; SE: sleep efficiency (%); SOL: sleep onset latency; TIB: time in bed; TST: total sleep time; WASO: wake time after sleep onset.

Table 3
Differences in heart rate variability measures in insomnia patients compared to controls from the observational studies.

Author/s, date, reference number	Time-domain measures						Frequency-domain measures						
	Mean HR	RRI	SDNN	SDANN	RMSSD	pNN50	Total power	VLF	LF	LFnorm	HF	HFnorm	LF/HF
Bonnet & Arand 1998 [20] ●	↑	↓	↓									↓	↑
McMillan 2001 [34] ●		ns	↑	↑	↑	↑	ns		ns	ns	ns	ns	ns
Varkevisser et al., 2005 [37] ○	ns				ns								
Fang et al., 2008 [28] ○							ns		ns		ns		ns
Jurysta et al., 2009 [32] ●		ns					ns		ns	ns	ns	ns	ns
de Zambotti et al., 2011 [27]●	↑											ns	
Peter et al., 2011 [35] ○		ns	ns		ns		ns		ns		ns		ns
Spiegelhalder et al., 2011 [21] ●	ns		↓		ns	ns					ns		ns
Short sleepers#	ns		↓		↓	↓					↓		ns
Yang et al., 2011 [38] □	ns w ↑s		↓ w ns s		↓ w ns s	↓ w ns s		↓ w ns s	↓ w, s		↓ w ns s		ns w, s
Israel et al., 2012 [30] ●												ns	ns
de Zambotti et al., 2013 [26] ●	ns						ns		ns		ns	ns	ns
Cellini et al., 2014 [24] ○	ns										↓		
de Zambotti et al., 2014 [25] ●		ns					ns					ns	ns
Farina et al., 2014 [29] □	↑w,s		↑s		↑s	ns			ns	↑w	ns	ns	↑s
Maes et al., 2014 [33] ●	ns								ns		ns		ns
Jiang et al., 2015 [31] ○	ns		↓		↓	ns		ns	↓		↓		ns
Postural change†	ns		ns		ns	ns		ns	↓		↑		ns
Rothenberger et al., 2015 [36] ●												ns	

Key: downwards arrow represent a statistically significant decrease in heart rate variability measure in insomnia patients compared to controls; ns: no significant difference between insomnia patients and controls; s = significant only during sleep; t = significant only during transition from wake to sleep; upwards arrow represent a statistically significant increase in heart rate variability measure in insomnia patients compared to controls; w = significant only during wake; # short sleepers defined according to sleep efficiency; †postural change from sitting to (prolonged) standing; ● predominantly sleep HRV recording; ○ wake HRV recording; □ 24-hour HRV recording.
Abbreviations: HF: high frequency (ms^2); HR: heart rate; norm: power in normalised units; LF: low frequency (ms^2); LF/HF: low frequency (ms^2)/high frequency (ms^2) ratio; pNN50: percent of NN intervals >50 milliseconds different from previous (%); RMSSD: square root of the mean of the sum of the squares of differences between adjacent NN intervals (ms); RRI: RR interval (ms); SDANN: standard deviation of the averages of NN intervals (ms); SDNN: standard deviation of all NN intervals (ms); VLF: very low frequency (ms^2).

this time [46]. We identified several studies that measured HRV during the sleep onset period with differing results. De Zambotti et al. [27] found greater wake to sleep increases in HFnorm (matched by a greater wake to sleep HR reduction) in insomnia patients compared to controls, suggesting a greater increase in vagal control during the sleep onset. Although not technically related to the sleep onset process, Spiegelhalder et al. [21] found a group by stage interaction indicating a dampened HR reduction from wake to (N2) sleep. Maes et al. [33], however, did not find any significant differences between insomnia patients and controls preceding sleep onset in either HR or HRV parameters.

Daytime only

Daytime HRV testing of insomnia patients was undertaken in four studies with contradictory results. Fang et al. [28] reported no significant differences in four frequency-domain HRV measures (TP, LF, HF, LF/HF), whereas Cellini et al. [24] reported an increase in sympathetic activity (based upon impedance cardiography) together with decreased HF, correlating with parasympathetic activity in insomnia patients. Two of the daytime HRV studies involved a physiological challenge – baroreflex sensitivity and postural change. Despite differences in the physiological perturbation, one study did not find any group differences [35], whilst the other reported insomnia patients to have attenuated or absent HRV responses to postural change [31].

Extended recordings

We could only identify three studies with extended HRV recordings. In a constant routine protocol whereby participants remained awake in a semi recumbent position across 24 h, Varkevisser et al. [37] did not find any between-group differences in HR or RMSSD. Both Yang et al. [38] and Farina et al. [29] utilised 24-hour recordings but did not report 24-hour HRV outcomes.

Risk of bias

Risk of bias of the observational studies is presented in Table 4. There was considerable bias in many studies, with six studies not taking cases from the same population as controls [21,29,33–35,37]. This was evident in the absence of a consistent methodology to match cases to controls in Table 2. The selection of controls was problematic with most not abiding to the suggested research diagnostic criteria for selection of insomnia controls [47]. Potential confounders such as age, sex and comorbidities were not considered in HRV analyses. There was a wide age range between, and within studies (Table 2). Spiegelhalder et al. [21] adjusted for covariates and found age to significantly impact on both time and frequency-domain HRV measures (SDNN, RMSSD, pNN50, HF and LF/HF), whilst sex affected time-domain measures (SDNN, RMSSD, pNN50). No studies analysed sex by group interactions or accounted for women's reproductive stage or menstrual cycle phase [48].

Interventional studies

Demographics and data inclusion

All intervention studies were open label, single-arm clinical trials. Participant numbers were mostly small with the largest sample size of 31 patients [42]. The diverse age range is evident in Table 5. Between the five studies, four different diagnostic methods were used for classification of insomnia. Insomnia severity of patients across studies was not able to be compared as one study provided scores for the insomnia severity index [39], two for the Athens insomnia scale [41,42] and two for the Pittsburgh sleep quality index (known to measure sleep quality, not insomnia symptoms) [40,43]. The longest follow-up time post intervention cessation was eight weeks post-therapy [39]. As with the observational studies, there was no uniformity in data capture and analysis methods, with the exception of the acupuncture and acupressure intervention studies as these were performed by the same research group [41,42].

Table 4
Risk of bias assessment of observational studies.

Author/s, date, reference number	Cases and controls are from comparable populations	The same exclusion criteria are used for cases and controls	Participation rate for cases and controls is comparable	Comparison of participants and non-participants	Cases are clearly defined and differentiated from controls	Controls are not cases	Measures taken to prevent knowledge of primary exposure from influencing case ascertainment	Exposure status measured in a standard, valid and reliable way	Potential confounders identified and accounted for in design and analysis
Bonnet & Arand 1998 [20]	?	✓	?	X	✓	✓	?	?	X
McMillan et al. 2001 [34]	X	✓	?	?	X	X	?	X	?
Varkevisser et al. 2005 [37]	X	✓	?	?	✓	X	?	✓	✓
Fang et al. 2008 [28]	✓	✓	✓	?	✓	✓	?	✓	?
Jurysta et al. 2009 [32]	?	✓	?	?	✓	?	?	✓	?
de Zambotti et al. 2011 [27]	✓	✓	?	X	✓	✓	?	✓	?
Peter et al. 2011 [35]	X	✓	?	?	✓	✓	?	✓	?
Spiegelhalder et al. 2011 [21]	X	✓	?	X	✓	X	?	✓	✓
Yang et al. 2011 [38]	?	✓	?	?	✓	?	?	✓	?
Israel et al. 2012 [30]	?	✓	?	?	✓	✓	?	✓	X
de Zambotti et al. 2013 [26]	✓	✓	?	X	✓	✓	?	✓	?
Cellini et al. 2014 [24]	✓	✓	?	?	✓	✓	?	✓	?
de Zambotti et al. 2014 [25]	✓	✓	?	?	✓	✓	?	✓	?
Farina et al. 2014 [29]	X	✓	?	?	✓	✓	?	✓	?
Maes et al. 2014 [33]	X	✓	?	?	✓	?	?	✓	?
Jiang et al. 2015 [31]	?	✓	?	?	✓	?	?	✓	?
Rothenberger et al. 2015 [36]	?	✓	✓	?	✓	✓	?	✓	?

Note: Case-control studies identified by the first author and year of publication. Studies were assessed for risk of bias using a modified version of the national institute for health and care excellence (NICE) methodology checklist for case-control studies. *Legend:* green square with a tick: well covered or adequately addressed; orange square with a question mark: not addressed, not reported or not applicable; red square with a cross: poorly addressed.

HRV measures

All interventions evoked some change in at least one HRV measure in insomnia patients (Table 6). Both acupuncture and acupressure caused stimulation-dependent changes in HRV. Cognitive behavioural therapy (CBT), gabapentin and acupuncture interventions were associated with an increase in parasympathetic activity (pNN50 and HF respectively). Heart rate variability measures that may represent sympathetic activity were reduced after gabapentin (decreased LF, LFnorm and LF/HF ratio) and CBT responders (decreased LF). Interestingly, three (of the five) interventions resulted in TP augmentation which contrasted with findings from the observational studies which found this HRV measure to be equivalent in insomnia patients and controls.

Risk of bias

No randomised control trials of the HRV of insomnia patients were identified. Therefore, no interventional studies achieved random sequence generation, or undertook blinding of participants or study personnel. The possibility of selective reporting was present in all types of studies. Due to the absence of prospective clinical trial registration we could not ascertain whether any outcome switching has occurred except inside the research groups that have maintained their HRV variable list. It was not clear why only some HRV measures were used and if all HRV findings were reported. Statistical under-powering of small studies must also be accounted for when considering the results. Risk of bias assessment for interventional studies is presented in Table 7.

All studies

Technical considerations

Across all studies there was a wide range of data cleaning and processing methods. Some authors used fully automated programs whilst others performed visual inspection of the R wave detection and manual editing for correction when required. There was discrepancy in the handling of HRV time periods that included artifact, sleep-related arousals and/or multiple sleep stages. Not surprisingly, a range of statistical methods were applied, with some but not all studies choosing to normalise the HRV measures.

Time of HRV recording

HRV outcomes reported in Tables 3 and 6 demonstrate a variety of different collection periods. Heart rate variability measurements were taken from participants in wake and/or sleep. Furthermore, sleep-related HRV measures were from various sleep stages, aggregates of sleep stages (e.g., NREM sleep combined), sleep cycles (e.g., early NREM vs late NREM) or time periods (e.g., sleep onset). For example, whilst both Bonnet and Arand [20] and Farina et al. [29] reported an increase in the LF/HF ratio in insomnia patients compared to controls, Bonnet and Arand [20] reported this measure to be increased during stage one, two and REM whereas Farina et al.

Table 5
Study characteristics of five open-label single-arm intervention studies measuring heart rate variability in insomnia patients.

Author/s, date, reference number	Patient number, sex, age (mean, SD, range)	Diagnostic criteria	Insomnia severity (pre-treatment)	Intervention	Follow-up time	Setting	HRV data capture and analysis
Lo et al., 2010 [40]	7M, 11F 43.2 y ± 15.4	Subjective report of difficulty initiating sleep and/or maintaining sleep >3 mo	PSQI 13.54	Gabapentin Mean dose 540 mg Range 200–900 mg	Four weeks after completion of dose titration	Sleep laboratory	ECG sampled at 400 Hz 10 min time periods WASO and movement periods were excluded
Chung et al., 2011 [39]	*Responders* 6M, 10F 57.9 y ± 10.9 *Non responders* 4M, 6F 59.4 y ± 7.4	ICSD 2	*Responders* ISI 19.5 ± 4.0, PSQI - 13.0 ± 4.0 *Non responders* ISI 18.1 ± 4.9, PSQI - 14.9 ± 4.6	Cognitive behavioural therapy four sessions over eight weeks	Eight weeks after commencement of cognitive behavioural therapy	Not specified	5 min time period 'HRV measurements followed standards of the Task Force'
Litscher et al., 2012 [41]	5M, 23F 41.9 y ± 14.6, 22–82	Self-presentation to hospital due to insomnia and AIS (value for inclusion not specified)	AIS 12.4 ± 3.6	Acupuncture Shenmen acupuncture point of wrist	10 min before, 20 min during and 10 min after stimulation	Medical university	ECG sampled at 4096 Hz 8 × 5 min time periods
Wang et al., 2013 [42]	6M, 25F 54.3 y ± 10.6, 29–82	Self-presentation to hospital due to insomnia and AIS ≥7	AIS 14.7 ± 4.4	Acupressure Shenmen accupressure point of ear	10 min before, 20 min during and 10 min after stimulation	Medical university	As above
Tsai et al., 2015 [43]	*Patients* 14, sex not specified 22.50 y ± 1.22, 20–25 *Controls* 14, sex not specified 23.07 y ± 1.64, 20–25	*Patients* DSM IV and PSQI >6 *Controls* Satisfied with sleep quality <one week of sleep disturbance PSQI <5	*Patients* PSQI 11.21 ± 1.97 *Controls* Not applicable	Controlled respiration at a slow frequency rate of 0.1 Hz and a forced rate of 0.2 Hz during daytime rest	Two days after each intervention (one week apart from each other)	Home	ECG sampled at 500 Hz 64s time periods Automatic PVC/noise rejection RR intervals were resampled and linearly interpolated

Abbreviations: AIS: Athens insomnia scale; DSM IV: diagnostic and statistical manual of mental disorders, fourth edition; ECG: electrocardiogram; F: female; Hz: hertz; ICSD 2: international classification of sleep disorders, second edition; ISI: insomnia severity index; ISQ: insomnia symptom questionnaire; M: male; mg: milligrams; min: minute; PSA: power spectral analysis; PSG: polysomnography; PSQI: Pittsburgh sleep quality index; PVC: premature ventricular contraction; RRI: RR interval; SE: sleep efficiency; SOL: sleep onset latency; TST: total sleep time; WASO: wake time after sleep onset.

[29] found this only in early N2. This made direct comparison between studies difficult.

Discussion

There are numerous reports in the literature that HRV is impaired in insomnia patients. These typically draw heavily on the findings of a small number of observational HRV research studies [20,21]. These case-control studies have reported a decrease HRV-related parasympathetic activity in insomnia patients during sleep and these HRV impairments have been used to support a putative causal pathway for physiological hyperarousal in insomnia [49]. This would be a logical inference; however, it is challenging to draw firm conclusions from the studies in our review, with nearly half of the observational studies reporting no significant HRV difference between insomnia patients and controls. In addition, HRV

Table 6
Heart rate variability measures in insomnia patients with an intervention.

Author/s, date, reference number & intervention	Time-domain measures						Frequency-domain measures						
	Mean HR	RRI	SDNN	SDANN	RMSSD	pNN50	Total power	VLF	LF	LFnorm	HF	HFnorm	LF/HF
Lo et al., 2010 [40] ● Gabapentin							↑	↑	↓	↓	↑	↑	↓
Chung et al., 2011 [39] ○ CBT responders	ns	ns	↑		ns	↑	↑	↑	↓		ns		ns
CBT non-responders	ns	ns	ns		ns	ns	ns	ns	ns		ns		ns
Litscher et al., 2012 [41] ○ Acupuncture	↓						ns		↑		↑		ns
Wang et al., 2013 [42] ○ Acupressure	↓						ns						ns
Tsai et al., 2015 [43] ○ At rest		ns					ns			ns	↓		
During paced breathing		↓					↑‡						

Key: ‡ significant only when breathing at a rate of 0.2 Hz; ● predominantly sleep HRV recording; ○ wake HRV recording.
Abbreviations: CBT: cognitive behavioural therapy; HF: high frequency (ms^2); HR: heart rate; norm: power in normalised units; LF: low frequency (ms^2); LF/HF: low frequency (ms^2)/high frequency (ms^2) ratio; ns: no significant difference; pNN50: percent of NN intervals > 50 milliseconds different from previous (%); RMSSD: square root of the mean of the sum of the squares of differences between adjacent NN intervals (ms); RRI: RR interval (ms); SDANN: standard deviation of the averages of NN intervals (ms); SDNN: standard deviation of all NN intervals (ms); VLF: very low frequency (ms^2). *Legend*: downwards arrow: decrease in HRV measure among insomnia patients after intervention; ns: no significant difference in HRV measures among insomnia patients after intervention; upwards arrow: increase in HRV measure among insomnia patients with intervention.

Table 7
Risk of bias assessment of intervention studies.

Author/s, date, reference number	Sequence generation	Allocation concealment	Blinding of participants	Blinding of personnel	Blinding of outcome assessors	Incomplete outcome data (attrition and exclusions from analyses)	Selective reporting	Other sources of bias
Lo et al. 2010 [40]	X	X	X	X	X	?	?	X
Chung et al. 2011 [39]	X	X	X	X	X	X	?	X
Litscher et al. 2012 [41]	X	X	X	X	X	✓	?	X
Wang et al. 2013 [42]	X	X	X	X	X	✓	?	X
Tsai et al. 2015 [43]	X	X	X	X	X	?	?	X

Note: Studies that included an intervention are listed and identified by the first author and year of publication. The cohcrane collaboration's tool for assessing risk of bias was used as a tool to help identify potential areas of bias for each study. *Legend:* green square with a tick: low risk of bias; orange square with a question mark: unclear risk of bias; red square with a cross: high risk of bias.

was measured during a multitude of different states (wake, sleep onset, various sleep stages, post-sleep wake), with a lack of consistent findings across studies. When between-group differences were evident, they were often in dissimilar time and frequency-domain HRV measures and not replicated consistently in other studies. Furthermore, there appears to be insufficient evidence to determine if HRV in insomnia patients is impaired during the day or how it may vary across the 24-hour cycle. We therefore suggest that the evidence for HRV impairment in insomnia is imperfect and that further studies are required.

Only one study examined HRV before and after cognitive behavioural therapy [39], the gold-standard treatment for insomnia patients [50–54]. Interestingly, despite a uniform increase in HR in insomnia patients in most case-control studies (refer to Table 3), HR was only lowered during acupressure and acupuncture. Conversely, whilst there was no difference in TP in case control studies, it increased in three (of five) interventions. HRV changes were not consistent in insomnia patients across interventions studies. This may be related to the intervention modality, for instance, ear accupressure was unlikely to affect insomnia severity [42]. Therefore, the impact of interventions on HRV autonomic characteristics in insomnia patients needs further investigation using well-designed randomised controlled trials to mitigate bias and enhance the clinical application of the findings.

Highly disparate populations were investigated across the studies in our review. In many studies cases and controls were retrospectively selected and despite providing statistical comparison between the groups, there was a lack of case-match methodology reporting. Sample sizes were generally small which limited the generalisability of the findings. The largest of the studies reviewed had 85 cases and 55 controls, derived from non-comparable populations [29]. It is likely that many studies were underpowered and this may have impacted on the findings.

Several diagnostic methods and insomnia severity scales were used, making it difficult to compare populations between studies. An example of this is the use of sleep efficiency for insomnia patient eligibility. Whilst two studies only included insomnia patients when PSG sleep efficiency was 85% or less [20,34], others included insomnia patients with high sleep efficiencies of greater than 90% [26,27]. Only one study classified the severe insomnia phenotype of total sleep time less than 6 h [21,44]. This study quantified short-sleep duration insomnia using PSG-derived sleep efficiency, whereby those with a sleep efficiency of less than 85% were considered to be short-sleepers. This subgroup analysis revealed differences between short-sleeping insomniacs and controls (refer to Table 3); however, no within-group comparisons were made between these insomnia patients i.e., short sleep duration insomnia patients and those with a longer sleep time. Although it was not the focus of our review, we are not aware of any other published research that has used HRV to phenotype insomnia patients.

Sex, sex-ratio and age of participants and controls were diverse. This is of particular concern given the known influence imparted on HRV by sex and age [55,56]. Evidence from a recent meta-analysis found females to have both higher mean HR and greater vagal activity evident in a number of HRV measures [56]. Researchers should be mindful of possible HRV implications from co-morbid sleep disorders (particularly sleep obstructive sleep apnoea) when designing studies particularly given the impact of confounders on HRV [5,57]. Stimulants, medications and medical comorbidities were often unreported and may have influenced HRV findings.

Considering the tremendous amount of inter-individual variability already present in HRV measures, methodological considerations for HRV analysis are of utmost importance [58]. Standards for the measurement and physiological interpretation were developed in 1996 by the task force of the European society of cardiology and the North American society of pacing and electrophysiology, with updated recommendations on the use of novel HRV techniques in the 2015 position statement [1,14]. Despite stating that '…the complete signal should be carefully edited using visual checks and manual corrections of individual RR intervals and QRS complex classifications', many insomnia-HRV studies have not used these standards [1]. Notably, only four case-control studies [21,28,29,32] and three interventional studies [39,40,43] referenced the task force guidelines when describing methodology. Erroneous HRV results may have arisen from physiological artifact (such as ectopic heart beats), technical methods or the algorithms used for R wave detection and editing. Signal length and discontinuity can also easily affect power spectral analysis, obscuring HRV findings [59]. Quintana et al. [60] have recently published guidelines for reporting HRV in psychiatry, specifically outlining the different aspects required when reporting HR collection, RR interval analysis, cleaning methods and HRV calculation techniques. For researchers examining HRV during sleep, a number of additional difficulties must be considered, including the inherent autonomic changes evident in different sleep stages, circadian effect, the influence of comorbid sleep disorders, variable recording lengths and

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the inability to control respiratory rate. Until further clarification is provided about how to handle these factors, researchers should report all aspects of their study protocol, participant demographics, analysis techniques and HRV findings.

The bidirectional relationship between electroencephalogram (EEG) and HRV in insomnia has also been examined to gain a better understanding of insomnia pathophysiology. Jurysta and colleagues [32] found decreased linear coupling between HF HRV power and delta EEG power in insomnia compared to controls. Maes et al. [33] examined the sleep onset period and first NREM sleep cycle and revealed significant associations between EEG microstructure (K-complexes within one second followed by 8–12 Hz EEG activity) and a lowered HF in SWS. Whilst HRV may be similar between insomnia and controls, the relationship between HRV and EEG appears altered and different from controls. Further research is required to elucidate coherence between HRV and EEG frequency domains.

Limitations of our review include the use of different risk of bias assessment tools, determined by only one author. No attempts were made to contact study authors when the method of HRV analysis was unclear. We were unable to perform a meta-analysis due to lack of comparable HRV measures, different study designs, populations, measurement techniques and analyses.

A number of studies in this review have drawn conclusions about the associations between physiological hyperarousal, HRV and cardiovascular disease. It is tempting to deduce that an increase in sympathetic-related HRV measures will be mirrored by a decrease in parasympathetic-related HRV measures and that this explains autonomic dysfunction. However, the traditional notion of an antagonistic autonomic nervous system is now questioned [61]. Bonnet and Arand [20] described an increase in sympathetic and decrease in parasympathetic activity across all reported sleep stages but these findings have not been replicated in the past 18 years. The additional questioning of the correlation between LF and LF/HF with sympathetic activity makes it unlikely that HRV alone can assist in determining causal pathways between autonomic dysfunction and cardiovascular morbidity in the insomnia population. Certainly the findings from this review cannot support this link.

Future research should employ 24-hour HRV recordings. Previous cardiovascular studies have shown associations with impaired HRV in 24-hour and mortality and morbidity and whilst this does not demonstrate causality, it would provide evidence of a link between physiological hyperarousal in insomnia and cardiovascular risk. Moreover, the influence of known confounders, such as age and sex, must be considered in prospective analyses.

In conclusion, our critical review of the insomnia-HRV research studies revealed varied results and a lack of consistent HRV findings in insomnia patients. We suggest that longitudinal studies (of large sample sizes using repeat measures) are required to determine the relationship, if present, between HRV and insomnia.

Practice points

1) There is inconsistent evidence to suggest that heart rate variability is impaired in insomnia.
2) We cannot deduce if or how insomnia interventions alter heart rate variability and autonomic dysfunction.
3) Measurements from short-term heart rate recordings cannot be compared to 24 hour recordings, thus the use of heart rate variability as a prognostic tool for cardiovascular disease in insomnia patients is not yet possible.

Research agenda

1) Develop recommendations for the measurement, analysis and reporting of HRV in sleep and circadian research to aid the interpretation, reproducibility and cross-comparison of results.
2) Perform large longitudinal studies of insomnia patients and controls that incorporate 24-hour recordings to determine the heart rate variability of insomnia patients across the sleep–wake cycle.
3) Consider the retrospective and prospective validation of the cardiovascular disease predictive power of heart rate variability measures in insomnia patients and appropriately matched controls (possibly via a nested case-control study within a cardiovascular disease cohort study).
4) Explore whether heart rate variability can be used to phenotype the heterogeneous insomnia population.

Conflict of interest

The authors do not have any conflicts of interest to disclose.

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Appendix A

Boolean search strategy for each database.
Search performed on 25th January 2016
No limitations on publication date, type or language

CINAHL – 9 results

(MH "Heart Rate Variability" OR "heart rate variability" or HRV) AND (MH "Insomnia" OR insomnia*)

Embase – 182 results

(hrv OR 'heart rate variability'/exp OR 'heart rate variability') AND insomni*

Google Scholar – 21 results

Did not search for patents or citations.
(intitle: "heart rate variability" OR intitle:HRV) AND (intitle:insomnia OR intitle:insomniac OR intitle:insomniacs)

PubMed – 60 results

HRV or "heart rate variability" AND insomni*

Scopus – 140 results

(hrv OR "heart rate variability") AND insomni*

Web of Science – 143 results

(HRV or "heart rate variability") AND insomni*

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Chapter 3: HRV at Sleep Onset

Preamble

Sleep is a dynamic state, consisting of sleep stages and cycles with differing physiology and function. In general, vagal-dominance is expected in healthy sleepers (de Zambotti, Covassin, Tona, Sarlo, & Stegagno, 2011). As many insomnia patients are symptomatic prior to sleep onset, it has been proposed that this is when autonomic dysregulation may be present at this time (de Zambotti et al., 2011).

Although physiological activity during the transition from wake to sleep have long been subject to investigation (Freedman & Sattler, 1982; Lamarche & Ogilvie, 1997; Merica & Gaillard, 1992; Trinder, 2012; Trinder et al., 2001), there are only three studies that have investigated the HRV of insomnia patients around or before/after sleep onset (de Zambotti et al., 2011; Maes et al., 2014; Spiegelhalder et al., 2011).

de Zambotti and colleagues undertook a comprehensive assessment of cardiovascular activity present in a small number of insomnia patients (n = 8) compared to good sleeping controls (n = 8) (2011). Outcomes included HRV and those from impedance cardiography; cardiac output, stroke volume and pre-ejection period. Using their findings from both measures, it was concluded that insomnia patients had continuously high sympathetic activity across the sleep onset period accompanied by gradually increasing parasympathetic activity, compared to good sleeping controls.

In a larger study of 58 insomnia patients and 46 healthy controls, Spiegelhalder et al. (2011) investigated HRV during wake, NREM and REM sleep. Although the sleep onset period was not directly investigated, pre-post comparisons between wake and sleep (REM and NREM) were undertaken. Resultantly, a lower wake-to-sleep (NREM) HR reduction was found amongst insomnia patients when compared to controls.

More recently, Maes et al. (2014) looked at electroencephalography (EEG) and ECG at the sleep onset period exploring whether a correlation between the two domains based

on the presence of systemic hyperarousal. Higher HR and increased beta-EEG power in sleep onset period was found in patients with insomnia (n = 17) but not in the controls (n = 11). This was present despite no statistical significant between-group differences in HRV measures.

The following article comprised the first direct comparison of HRV across the sleep onset period (10 minutes prior and 10 minutes after sleep onset: American Academy of Sleep Medicine-defined) of insomnia patient subtypes, determined from objectively derived sleep-parameters. Although methodology is adequately described in the publication, further details are provided below to describe explicitly the step-by-step process used for HRV analysis.

Sleep onset time was manually extracted from the sleep staging file and imported into Excel. A worksheet was created to determine the sleep onset period in 2 minute windows, from 10 minutes before, to 10 minutes after sleep onset. Individual European Data Files (EDF) containing the nocturnal ECG were exported from the PSG recording program (RemLogic) to Kubios. Using the Excel worksheet to define the sleep onset period, 20 minutes of the recording was segmented into two-minute time samples (10 time samples in total).

Automated QRS detection was visually checked over the entire sample. Any erroneously marked R waves were removed. Any ectopics (atrial or ventricular) were marked as artifact, as was any signal noise that prevented viewing a discernible R wave. Any undetected R waves were checked. Once the 20 minute sleep onset period ECG had been inspected and edited (as required), 'very low' artifact correction was applied. No detrending method was applied.

Frequency bands of 0.04 to 0.15 Hz for low frequency (LF) and 0.15-0.4 Hz for high frequency (HF) were utilised. The interpolation rate was set at 4 Hz with a Fast Fourier Transformation window width of 256 seconds (50% overlap). Values were checked for biological possibility against standards paper and previous research on insomnia patients (Nunan, Sandercock, & Brodie, 2010; Spiegelhalder et al., 2011). Data were then exported from Kubios for statistical analysis.

Limitations not otherwise mentioned within the manuscript (but pertaining specifically to HRV) include not controlling for diet (i.e. stimulants in food and/or drink), circadian periodicity, menstrual cycle, and menopause. Finally, one limitation of HRV analysis over the sleep onset period is that it may not possess stationarity, important for the accurate analysis of the recording. Nonetheless, this was not such a concern in the present study given the observational nature of our study (using subtypes from the same sample).

Statement of Author Contributions

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I extracted the ECGs from the sleep studies, performed the HRV analysis in its entirety, worked with CM on the statistical processing of the HRV data, and wrote the relevant sections of the manuscript. Permission to include the published material has been granted by the corresponding author, Dr Christopher Miller.

Kirsty Lyn Dodds	[redacted]	16th February 2017
NAME	SIGNATURE	DATE

As the primary supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Dr Christopher Gordon	[redacted]	15th February 2017
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INSOMNIA

Clusters of Insomnia Disorder: An Exploratory Cluster Analysis of Objective Sleep Parameters Reveals Differences in Neurocognitive Functioning, Quantitative EEG, and Heart Rate Variability

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Study Objectives: To empirically derive and evaluate potential clusters of Insomnia Disorder through cluster analysis from polysomnography (PSG). We hypothesized that clusters would differ on neurocognitive performance, sleep-onset measures of quantitative (*q*)-EEG and heart rate variability (HRV).
Methods: Research volunteers with Insomnia Disorder (DSM-5) completed a neurocognitive assessment and overnight PSG measures of total sleep time (TST), wake time after sleep onset (WASO), and sleep onset latency (SOL) were used to determine clusters.
Results: From 96 volunteers with Insomnia Disorder, cluster analysis derived at least two clusters from objective sleep parameters: Insomnia with normal objective sleep duration (I-NSD: n = 53) and Insomnia with short sleep duration (I-SSD: n = 43). At sleep onset, differences in HRV between I-NSD and I-SSD clusters suggest attenuated parasympathetic activity in I-SSD ($P < 0.05$). Preliminary work suggested three clusters by retaining the I-NSD and splitting the I-SSD cluster into two: I-SSD A (n = 29): defined by high WASO and I-SSD B (n = 14): a second I-SSD cluster with high SOL and medium WASO. The I-SSD B cluster performed worse than I-SSD A and I-NSD for sustained attention ($P \leq 0.05$). In an exploratory analysis, *q*-EEG revealed reduced spectral power also in I-SSD B before (Delta, Alpha, Beta-1) and after sleep-onset (Beta-2) compared to I-SSD A and I-NSD ($P \leq 0.05$).
Conclusions: Two insomnia clusters derived from cluster analysis differ in sleep onset HRV. Preliminary data suggest evidence for three clusters in insomnia with differences for sustained attention and sleep-onset *q*-EEG.
Clinical Trial Registration: Insomnia 100 sleep study: Australia New Zealand Clinical Trials Registry (ANZCTR) identification number 12612000049875. URL: https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=347742.
Keywords: sleep, Insomnia Disorder, polysomnography, cluster analysis, and phenotyping
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Significance
Using cluster analysis, we derived at least two clusters of Insomnia Disorder, replicating previous findings of long and short objective sleep duration insomnia. Research volunteers with Insomnia Disorder and short objective sleep duration (I-SSD) displayed attenuated sleep-onset heart rate variability relative to those with insomnia and normal sleep duration (I-NSD) and may be at risk of future cardiometabolic ill health. An exploratory 3-cluster solution was uncovered by splitting the short sleep cluster into two: I-SSD A (sleep maintenance difficulties) and I-SSD B (sleep-onset and maintenance difficulties). The 3-cluster solution identified reduced sleep-onset quantitative-EEG power in I-SSD B relative to both I-NSD and I-SSD A. I-SSD B performed worse than I-SSD A and I-NSD for sustained attention.

INTRODUCTION

Insomnia is a prevalent and heterogeneous disorder that has been linked to impaired cardiovascular and neurobiological functioning.[1–6] A number of classical clinical insomnia subtypes have been identified from self-reports of patients and include difficulty with initiating sleep, maintaining sleep, early-morning awakening with an inability to return to sleep, nonrestorative sleep, and paradoxical insomnia among others.[7–12] Two objective subtypes of insomnia have been proposed by dichotomizing total sleep time (TST) derived from first-night polysomnography (PSG), and these groups differ on meaningful clinical outcomes.[5] It remains unclear whether more than two subtypes of insomnia exist, especially under Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5).[7] Uncertainty however in the number of insomnia subtypes may be addressed through an empirical method by cluster analysis. Cluster analysis might be a way to describe meaningful subtypes. No previous cluster analysis in DSM-5 Insomnia Disorder patients used a limited number variables from PSG.[13–16]

Cluster analysis is a data-driven method of generating candidates for meaningful phenotypes.[17,18] A key advantage of this technique is the nonsubjective demarcation of cluster boundaries, which may provide clinicians with data-driven cut-points between phenotypes.[19] Resulting clusters need to be tested for meaningful clinical differences between patients for outcomes that are not used to build the clusters.[20] To do this we identified a number of insomnia based "biomarkers" from previous literature that may be more impaired in one cluster compared to another.[3–6,21] Insomnia Disorder is fundamentally difficulty regulating sleep-wake transitions, and we should test these clusters for both markers of the hypothesized cardiovascular and neurobiological sequelae, and where possible during the process of sleep-wake transition.[3,4]

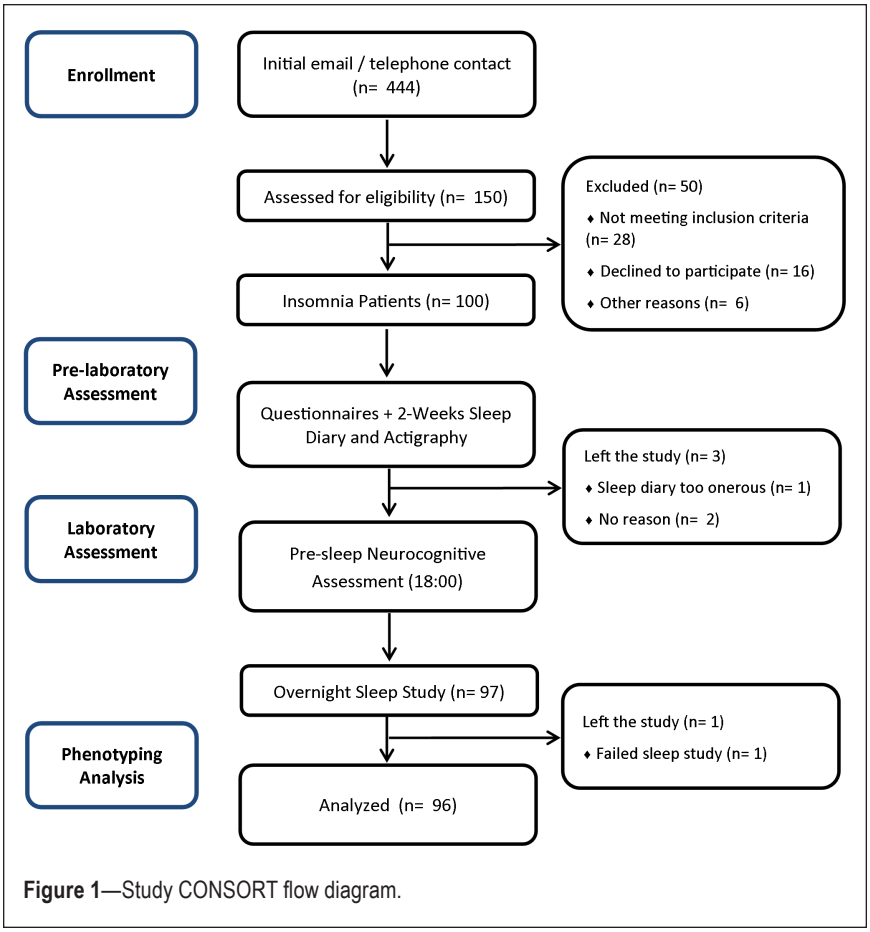

Figure 1—Study CONSORT flow diagram.

The aim of this study was to empirically derive clusters built from first-night PSG-derived sleep parameters (TST, sleep onset latency: SOL, and wake time after sleep onset: WASO) and to test for cluster differences in candidate biomarkers in volunteers with DSM-5 Insomnia Disorder.[7] If meaningful clusters exist they may differ on neurocognitive performance, sleep-onset measures of quantitative (*q*)-EEG and heart rate variability (HRV).

METHODS

Research Volunteers

Research volunteers with Insomnia Disorder were recruited through responses to a database, clinic advertisements, online, and in the local community and were initially screened over the telephone using a standardized assessment tool (based on Morin and Espie).[22] Eligible volunteers (recruited between February 2012 until August 2014) attended the sleep clinic and underwent a comprehensive sleep interview and medical examination by a Sleep Physician or Sleep Psychologist to determine the presence of Insomnia Disorder through the following inclusion criteria: Insomnia Disorder as diagnosed by the DSM-5,[7] specifically: difficulty initiating or maintaining sleep or waking up too early at least 3 nights per week, for at least 3 months, with adequate opportunity and circumstances for sleep and a stable sleep/wake schedule, and a complaint of daytime impairment (e.g., occupational, social, academic settings). In line with research diagnostic criteria for insomnia,[8] exclusion criteria included: illicit substance dependence or alcohol/caffeine, severe or unstable psychiatric disorders, a known sleep disorder other than insomnia, cognitive impairment, and pregnancy or lactation. Volunteers also recorded daily sleep diaries after screening and prior to the laboratory assessment (similar to Morin[22]). All data were collected at the Woolcock Institute of Medical Research, University of Sydney, Australia (see Figure 1 for an overview). This study was reviewed and approved by the Royal Prince Alfred Hospital Ethics Review Committee, Sydney, Australia (Protocol No X11-0392 & HREC/11/RPAH/620); Clinical Trial Registration number: 12612000049875 (ANZCTR). All volunteers gave written informed consent.

Clinical and Demographic Outcomes

Demographic variables included: age, sex, body mass index (BMI kg/m^2), education (highest attained), employment status, ethnicity, medication, alcohol consumption and smoking status and medical comorbidities including: heart disease, cancer, etc.,[23] assessed by a self-report electronic questionnaire prior to the overnight sleep study. Clinical insomnia-related outcomes included: insomnia duration, insomnia as a child, family history of insomnia, Insomnia Severity Index (ISI: Morin),[24] the 21-item version of the Depression Anxiety and Stress Scale (DASS),[25] Epworth Sleepiness Scale (ESS),[26] Flinders Fatigue Scale (FFS),[27] 16-item version of the Dysfunctional Beliefs and Attitudes About Sleep scale (DBAS),[28] and the Ford Insomnia Response to Stress Test (FIRST).[29] Volunteer reported questionnaires were also captured prior to sleep during the in-lab visit by electronic questionnaires. A paper based point in time assessment of the Daytime Insomnia Symptom Scale (DISS) was used prior to lights out to quantify subjective reports of pre- and post-sleep alertness, mood (negative and positive) and sleepiness/fatigue.[30,31]

Sleep

One night of PSG was used to define objective sleep architecture and continuity variables. A specific research montage was applied [EEG: F3, Fz, F4, C3, Cz, C4, Pz, O1, Oz, O2, all signals used ground at FPz and common reference at CPz], electrooculographic (EOG: horizontal and vertical), electrocardiographic (ECG) and electromyographic (EMG: submental) recordings. Data were recorded on the Embla (n = 92: Mortara, Broomfield, CO) and Alice (n = 4: Respironics, Pittsburgh, PA) systems (512 Hz) and scored visually by one experienced sleep scorer, and each study was then independently checked for quality assurance by another independent scorer according to American Academy of Sleep Medicine (AASM) criteria.[32] Each volunteer was set-up between 20:00–21:00 and could select a lights out

time with lights on at 06:00. Blood pressure was captured prior to sleep and on awakening the next morning. To determine sufficient sleep opportunity for all patients, video recording around the time for lights out and lights on was used to verify time in bed prior to initiating sleep (lights out) and leaving the bed the next morning (lights on).

Cluster Formation through Cluster Analysis

A hierarchical cluster analysis (Ward method using the squared Euclidean distance) was used to identify potential insomnia clusters. This technique allows for the formation of subgroups within complex data.[19,33,34] It is recommended for smaller data sets (100 patients) where groupings are examined in successive steps until a number of relatively homogenous subgroups become statistically and clinically meaningful. Here, distinct clustering variables (at least 2), were chosen on theoretical grounds and previous research findings.[5,35] Standardized (converted to z-scores: see Milligan[36]) objective sleep parameters, including TST, SOL, and WASO were used.[35] In order to derive the number of clusters, a dendrogram (hierarchical tree diagram used to represent the distance between cases of cluster groupings) was used in combination with agglomeration findings of the coefficients (see Table S1 in the supplemental material). In the case that a single cluster solution is not entirely clear, all possible cluster solutions were examined against external variables associated with insomnia for validity including: neurocognitive performance,[37] and sleep-onset measures of quantitative EEG (*q*-EEG) and heart rate variability (HRV). The sleep-onset period was selected because of difficulty regulating sleep-wake transitions in insomnia. Entry into sleep may hold unique insights into physiological differences, reflecting cortical and physiological hyperarousal,[3,4,21,38–40] and the perception of sleep during this process[41–43] between insomnia clusters.

Neurocognitive Outcomes

Neurocognitive performance was measured at approximately 18:00 on the night of the sleep study prior to PSG overnight assessment. The tasks lasted approximately 40 min using a web-based platform, and were delivered on a laptop computer with a 17-inch color display, keyboard, and mouse. Volunteers practiced with the researcher until confident with the tasks. Three cognitive tasks of executive functioning previously used in insomnia studies were employed and included: working memory (N-back 2); percentage of total accuracy, total number of incorrect and missed responses,[6] sustained attention (Letter Cancellation Test: LCT); mean correctly marked targets and the trial duration of the second sustained attention and processing speed task,[44] and planning and problem solving (Tower of London: ToL); percentage of errors and mean response latency.[45] Patients did not receive any task-related feedback about their performance.

Quantitative EEG at Sleep Onset

EEG artifact-free epochs, from C3 and C4 (referenced to the right mastoid), were analyzed for power spectra using a previously validated standard fast Fourier transform with a rectangular weighted window for each non-overlapping 5-s epoch.[46] Primarily, C3 was chosen as it is traditionally recommended for use in scoring sleep[47] and has been used previously for spectral analysis in insomnia research.[48,49] A sleep-onset period of 10 min either side of AASM-defined sleep-onset was divided into 1-min intervals for absolute (natural logarithm transformation) spectral bands: delta (0.5–4.5 Hz), theta (4.5–8 Hz), alpha (8–12 Hz), sigma (12–15 Hz), beta-1 (16–24 Hz) and beta-2 (24–32 Hz) frequencies. The 20-min sleep-onset period was selected as it may best reflect difficulty with the wake-to-sleep transition in insomnia. Absolute power was selected as a first principle analysis for two reasons: (1) In absolute power, all frequency bands are independent from one another and this is more advantageous for interpretation as opposed to relative power which is based on a ratio. (2) Individual patient variation may be reduced by using relative as opposed to absolute power, which may result in a source of bias.[42]

HRV at Sleep Onset

A similar approach was employed for HRV obtained from a 2-lead ECG (512 Hz) during PSG assessment for 10 min either side of sleep onset (divided into 2-min intervals). Kubios HRV Version 2.1 was used for HRV analysis.[50] Manual artifact identification and RR data editing was performed prior to analysis. Two-minute intervals that did not contain ≥ 80% normal R-R intervals were rejected and data with numerical values > 4 SD above the mean of the whole sample were excluded as deemed biological implausible.[51] Time-domain measures: heart rate (HR: bpm), standard deviation of all N-N intervals (SDNN: ms), root mean square of successive R-R intervals (rMSSD: ms), percentage (%) of successive R-R intervals that differ by > 50 ms (pNN50); and frequency-domain measures: high frequency (HF: ms^2) and a low frequency (LF)/HF ratio were employed.[52–54]

Statistical Analysis

Differences between clusters in demographic, clinical, sleep and neurocognitive outcome variables were assessed through univariate one-way analysis of variance (ANOVA), Kruskal-Wallis tests or χ^2 tests among the cluster groups. In relation to neurocognitive variables, known confounders (age, gender, and education) were always inserted as covariates (see Table 1 and Table S2 in the supplemental material). For both *q*-EEG and HRV analyzes, linear mixed model analysis, with fixed effects for time (wake-to-sleep) and cluster group, and random intercepts effects for between-subject variation, were implemented for each outcome variable with age and gender as covariates. Least significant differences were employed for post hoc comparisons between cluster groups. Effect size scores were calculated for significant pair-wise group comparisons where appropriate. P was set at < 0.05 for all analyzes. All data were analyzed (by CBM) using SPSS software (IBM v 22.0.0; IBM Corp, Armonk, NY, USA).

RESULTS

Volunteer Characteristics

One hundred volunteers with insomnia consented for this phenotyping experiment and 96 (61 females) were included in the analysis (see Figure 1) with a mean (SD) age of 41.4 years

Table 1—Demographic, sleep parameters (used to cluster individuals), and clinical information between insomnia subgroups in the 2-cluster solution.

Mean (± SD)	Overall (n = 96)	I-NSD Cluster (n = 53)	I-SSD Cluster (n = 43)	ANOVA F (P)	Effect Size (*d*, *r*) I-NSD vs. I-SSD
Age (y), mean (SD), range	41.4 (11.8), 23–75	38.6 (11.6), 23–66	44.8 (11.2), 23–75	**6.97 (0.010)***	0.54
BMI (kg/m^2)	25.0 (4.3)	24.7 (4.2)	25.4 (4.4)	**0.63 (0.429)**	0.16
Total sleep time (min)	346.1 (67.8)	392.3 (35.4)	289.2 (53.4)	**117.97[a] (< 0.001)****	**2.27**
Sleep onset latency (min)	25.3 (23.9)	18.0 (11.7)	34.32 (31.2)	**10.57[a] (< 0.01)***	**0.69**
Wake time after sleep onset (min)	72.5 (58.6)	39.9 (22.6)	112.6 (64.4)	**49.84[a] (< 0.001)****	**1.51**
Insomnia Severity Index	17.3 (4.8)	18.0 (4.0)	16.5 (5.5)	2.07[a] (0.154)	0.31
Insomnia duration (y)	10 (11)	10 (10)	10 (14)	0.03[b] (0.855)	0.02
DASS - Depression	6 (12)	6 (12)	4 (9)	0.42[b] (0.518)	0.07
DASS - Anxiety	4 (6)	4 (7)	4 (7)	0.18[b] (0.669)	0.11
DASS - Stress	15.3 (9.0)	15.8 (8.3)	14.7 (10.0)	0.34 (0.560)	0.12
Epworth Sleepiness Scale	6.4 (4.5)	6.2 (4.5)	6.7 (4.5)	0.38 (0.541)	0.11
Flinders Fatigue Scale	18.0 (6.4)	18.4 (6.2)	17.5 (6.7)	0.43 (0.513)	0.14
Ford Insomnia Response to Stress Test	24.0 (6.1)	24.3 (5.9)	23.7 (6.4)	0.27 (0.608)	0.10
Dysfunctional Beliefs and Attitudes about Sleep scale	6.1 (1.7)	6.2 (1.4)	5.9 (2.0)	0.85 (0.359)	0.17
Pre-sleep DISS - Alert cognition	52.2 (16.0)	52.0 (16.7)	52.4 (15.2)	0.01 (0.917)	0.03
Pre-sleep DISS - Negative mood	27.9 (17.0)	27.7 (16.4)	28.2 (18.8)	0.02 (0.889)	0.03
Pre-sleep DISS - Positive mood	46.0 (12.8)	47.2 (13.4)	44.4 (11.9)	1.08 (0.302)	0.22
Pre-sleep DISS - Sleepiness / fatigue	54.7 (17.7)	55.6 (17.8)	53.6 (17.7)	0.32 (0.576)	0.11
Apnea-hypopnea index	3.1 (5.7)	2.8 (5.1)	3.5 (8.2)	1.91[b] (0.167)	0.14
Evening systolic blood pressure (mm Hg)	115.3 (14.3)	115.4 (15.9)	115.3 (12.3)	0.00 (0.974)	0.00
Evening diastolic blood pressure (mm Hg)	74.5 (9.4)	74.6 (9.5)	74.3 (9.5)	0.04 (0.850)	0.00
Morning systolic blood pressure (mm Hg)	107.0 (13.5)	106.0 (14.8)	108.2 (11.9)	0.61 (0.439)	0.02
Morning diastolic blood pressure (mm Hg)	71.0 (9.1)	69.8 (8.9)	72.5 (9.1)	1.95 (0.166)	0.30
Number of alcoholic drinks per week (standard units)	4.0 (6)	4.0 (5)	3.3 (6)	0.55[b] (0.459)	0.08
Binary Variables	**n (%)**	**n (%)**	**n (%)**	**χ^2 (P)**	**Effect Size (*V*)**
Sex (f)	61 (63.5%)	36 (67.9%)	25 (58.1%)	0.98 (0.322)	0.10
Insomnia as a child	16 (16.7%)	10 (18.9%)	6 (14.0%)	0.45 (0.503)	0.07
Family history	28 (29.2%)	17 (32.1%)	11 (25.6%)	0.02 (0.880)	0.02
Education, highest attained					
High school	12 (12.5%)	8 (15.1%)	4 (9.3%)	0.58 (0.447)	0.08
College graduate	47 (49.0%)	22 (41.5%)	25 (58.1%)	3.05 (0.081)	0.18
Post graduate	32 (33.3%)	21 (39.6%)	11 (25.6%)	1.84 (0.175)	0.14
Ethnicity					
Central South Asian	4 (4.2%)	3 (5.7%)	1 (2.3%)	0.70[c] (0.624)	0.85
East Asian	1 (1.0%)	1 (1.9%)	0 (0%)	0.84[c] (1.00)	0.09
South East Asian	2 (2.1%)	1 (1.9%)	1 (2.3%)	0.02[c] (1.00)	0.01
White Caucasian	88 (91.7%)	47 (88.7%)	41 (95.4%)	0.85[c] (0.457)	0.10
Employment					
Full-time	65 (67.7%)	40 (75.5%)	25 (58.1%)	2.27 (0.132)	0.16
Part-time	16 (16.7%)	8 (15.1%)	8 (18.6%)	0.34 (0.590)	0.06
Unemployed	11 (11.5%)	4 (7.5%)	7 (16.3%)	2.07[c] (0.151)	0.15
Medical comorbidities	35 (36.5%)	15 (28.3%)	20 (46.5%)	2.76 (0.096)	0.17
Mild-to-moderate depression/anxiety	11 (11.5%)	5 (9.4%)	6 (14.0%)	0.12 (0.914)	0.02
Heart disease	0 (0%)	0 (0%)	0 (0%)	0 (0.0)	–
Cancer	0 (0%)	0 (0%)	0 (0%)	0 (0.0)	–
High blood pressure	1 (1.0%)	1 (1.9%)	0 (0%)	1.31[c] (0.441)	0.20
Neurologic	0 (0%)	0 (0%)	0 (0%)	0 (0.0)	–
Breathing	8 (8.3%)	5 (9.4%)	3 (7.0%)	1.43[c] (0.417)	0.21
Urinary	1 (1.0%)	1 (1.9%)	0 (0%)	1.31[c] (0.441)	0.20
Diabetes	1 (1.0%)	0 (0%)	1 (2.3%)	0.81[c] (1.00)	0.16
Chronic pain	8 (8.3%)	3 (5.7%)	5 (11.6%)	0.19[c] (0.709)	0.07
Gastrointestinal	6 (6.3%)	1 (1.9%)	5 (11.6%)	0.03[c] (1.00)	0.03
Thyroid	3 (3.1%)	1 (1.9%)	2 (4.7%)	0.16[c] (1.00)	0.07
Other	11 (11.5%)	5 (9.4%)	6 (14.0%)	0.12 (0.914)	0.02
Current smoker	8 (8.3%)	4 (7.5%)	4 (9.3%)	0.08[c] (0.780)	0.03
Past smoker	29 (30.2%)	11 (20.8%)	18 (41.9%)	**4.76 (0.029)***	**0.22**
Never smoked	58 (60.4%)	37 (69.8%)	21 (48.8%)	**4.93 (0.026)***	**0.23**
Medication	49 (51.0%)	23 (43.4%)	26 (60.5%)	2.32 (0.127)	0.16
Prescription sleep aid	9 (9.4%)	6 (11.3%)	3 (7.0%)	1.56[c] (0.279)	0.18
Over the counter sleep aid	2 (2.1%)	0 (0%)	2 (4.7%)	1.92[c] (0.490)	0.20
Prescription psychiatric medication	5 (5.2%)	3 (5.7%)	2 (4.7%)	0.33[c] (0.660)	0.08
Other medication	31 (32.3%)	13 (24.5%)	18 (41.9%)	1.26 (0.263)	0.16

Means, standard deviations (SD) and effect size (Cohen's *d*, *r* for variables not normally distributed or Cramér *V* for binary variables) for each cluster comparison are provided. [a]Welch statistic correction for violation of homoscedasticity. [b]Kruskal-Wallis test with median (inter-quartile range) for variables not normally distributed. [c]Fisher exact test correction for cells with n < 5. Significant main effects are in bold. *P < 0.05. **P < 0.001. Medical comorbidities: Neurologic (seizures, Parkinson disease), Breathing (asthma, emphysema), Urinary (kidney disease, prostate problems), Chronic pain (arthritis, back pain, gout, migraines) and Gastrointestinal (stomach, irritable bowel syndrome, ulcers). ANOVA, analysis of variance; DASS, Depression, Anxiety, and Stress scales; DISS, Daytime Insomnia Symptom Scale; I-NSD, Insomnia with Normal Sleep Duration; I-SSD, Insomnia with Short Sleep Duration.

Table 2—A 2-cluster solution defined by polysomnography is not associated with any neurocognitive performance measurements.

	Neurocognitive Outcomes				Effect Size (*d*)
Mean (± SE)	**Overall**	**I-NSD Cluster**	**I-SSD Cluster**	**ANCOVA F (P)**	**I-NSD vs. I-SSD**
N-Back 2: Percentage of total accuracy	85.9 (1.3)%	86.6 (1.8)%	85.2 (2.0)%	0.25 (0.622)	0.11
N-Back 2: Total number incorrect	5.3 (0.5)	5.1 (0.8)	5.4 (0.8)	0.05 (0.826)	0.06
N-Back 2: Total number missed	11.2 (1.5)	10.6 (2.1)	11.9 (2.3)	0.17 (0.680)	0.09
LCT: Mean of correctly marked targets	50.7 (1.7)	52.5 (2.1)	51.2 (2.4)	0.14 (0.706)	0.07
LCT: Final trial duration (sec)	321.4 (66.0)	327.3 (90.4)	315.4 (102.9)	0.70 (0.405)	0.21
ToL: Percentage of errors	32.3 (1.9)%	34.1 (2.6)%	30.5 (2.9)%	0.82 (0.369)	0.21
ToL: Mean response latency (sec)	11.4 (65.2)	10.6 (86.7)	12.2 (97.9)	1.42 (0.237)	0.03

Means and standard errors (adjusted for age, gender, and education) and effect size (Cohen's *d*) are provided for each cluster comparison. ANCOVA, analysis of covariance; LCT, Letter Cancellation Test; ToL, Tower of London; I-NSD, Insomnia with Normal Sleep Duration; I-SSD, Insomnia with Short Sleep Duration.

(11.8). Mean (SD) insomnia severity (ISI) was 17.3 (range: 1–28; SD = 4.8): 21 (22%) volunteers experienced insomnia symptoms for the last 5 years, 17 (18%) had symptoms for 5–10 years; 31 (32%) for 10–20 years; 21 (22%) reported symptoms longer than 20 years, with 16 (17%) volunteers reporting symptoms as a child. Using PSG-derived sleep, the average (SD) TST = 346.1 (67.8) mins, SOL = 25.3 (23.9) mins and WASO = 72.5 (58.6) mins (see Table 1).

Cluster Characteristics

Cluster analysis revealed two possible solutions (see Table S1 in the supplemental material). The outcomes for both the 2- and 3-cluster solutions were compared and contrasted in an attempt to solve ambiguity. Solution 1 identified an Insomnia with normal sleep duration (I-NSD) cluster (n = 53) and an Insomnia with short sleep duration (I-SSD) cluster (n = 43). Solution 2 preserves the I-NSD cluster (n = 53) and splits the I-SSD cluster into two further clusters to make three: Insomnia with short sleep duration type-A (I-SSD A: defined by high WASO) (n = 29) and Insomnia with short sleep duration type-B (I-SSD B: defined by high SOL and medium WASO) (n = 14). Table 1 displays the cluster group mean (SD) scores for each of the three sleep variables defining cluster solutions 1 and 2. Between-group differences with estimations of effect size calculations are also listed. Figure 2 displays all volunteers in cluster groups for both possible solutions across the 3-dimensions for PSG-derived TST, SOL, and WASO (see Video 1 and the description of Video 1 in the supplemental material). In the 2-cluster solution, we found I-NSD had significantly higher TST and reduced WASO and SOL compared to the I-SSD cluster. In support of the 3-cluster solution, I-SSD A were significantly higher for WASO than I-NSD and I-SSD B, whereas I-SSD B is significantly higher for SOL than I-SSD A and I-NSD (see Table 1 and Figure 2). There were no differences between clusters for PSG-sleep study recording time (difference between lights-off until lights-on) across solution 1: I-NSD (mean = 450.9; SD = 36.9 mins); I-SSD (mean = 438.4; SD = 40.0 mins) (P > 0.10) or solution 2: I-SSD A (mean = 436.1; SD = 44.6 mins); I-SSD B (mean = 443.1; SD = 29.30 mins) (P > 0.20).

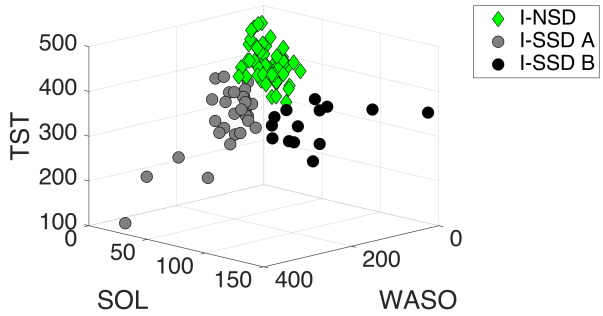

Figure 2—Clusters of all 96 volunteers with insomnia displayed in 3-dimensions according to PSG-defined total sleep time (TST), sleep onset latency (SOL), and wake time after sleep onset (WASO). Cluster solution 1: Insomnia with Normal Sleep Duration (I-NSD: Diamonds; n = 53) vs. Insomnia with Short Sleep Duration (I-SSD: Both circles; n = 43). Cluster solution 2: Insomnia with Normal Sleep Duration (I-NSD: Diamonds; n = 53) vs. Insomnia with Short Sleep Duration type-A (I-SSD A: Gray circles; n = 29) vs. Insomnia with Short Sleep Duration type-B (I-SSD B: Black circles; n = 14).

Neurocognitive Outcomes

For neurocognitive outcome parameters: in solution 1 (2-cluster), the I-NSD and I-SSD clusters did not differ in neurocognitive measures (see Table 2). In solution 2 (3-cluster), LCT sustained attention task scores were better in I-SSD A compared to I-SSD B (P < 0.05, *d* = 0.93) and in I-NSD (better) compared to I-SSD B (P = 0.05, *d* = 0.63: see Table 3).

Quantitative EEG at Sleep Onset

Of the 96 patients, 94 were included for analysis (1 could not be analyzed due to a different sampling rate between C3 and C4 and another had a corrupt data file). For *q*-EEG spectral bands, there were no overall between cluster differences in either cluster solution 1 or 2. At C3, mixed model analysis revealed significant cluster × time (wake-to-sleep) interactions for mean absolute Delta power in both solutions 1 and 2 (P < 0.05). Before sleep-onset, comparisons revealed a significant reduction in solution 2 Delta power in I-SSD B vs. I-SSD A and I-SSD B vs. I-NSD (P < 0.05). The alpha power interaction was significant in both solutions 1 and 2 (P ≤ 0.0001). Before sleep onset, comparisons

Table 3—A 3-cluster solution defined by polysomnography is associated with neurocognitive performance measurements of sustained attention.

	Neurocognitive Outcomes					Effect Size (*d*)		
Mean (± SE)	Overall	I-NSD Cluster	I-SSD A Cluster	I-SSD B Cluster	ANCOVA F (P)	I-NSD vs. I-SSD A	I-NSD vs. I-SSD B	I-SSD A vs. I-SSD B
N-Back 2: Percentage of total accuracy	85.3 (1.5)%	86.5 (1.8)%	87.1 (2.6)%	82.3 (3.3)%	0.73 (0.485)	0.07	0.35	0.41
N-Back 2: Total number incorrect	5.5 (0.6)	5.2 (0.7)	4.4 (1.1)	6.9 (1.3)	1.10 (0.337)	0.13	0.36	0.49
N-Back 2: Total number missed	11.0 (1.7)	10.5 (2.1)	14.3 (2.9)	8.1 (3.7)	0.93 (0.398)	0.26	0.18	0.44
LCT: Mean of correctly marked targets	50.7 (1.7)	52.1 (2.1)	56.3 (3.1)	43.6 (3.8)	3.24 (0.045)*	0.30	**0.63**¥	0.93*
LCT: Final trial duration (sec)	320.9 (75.0)	327.9 (90.8)	307.8 (135.9)	326.9 (167.9)	0.73 (0.488)	0.33	0.02	0.32
ToL: Percentage of errors	31.2 (2.1)%	33.9 (2.6)%	33.3 (3.8)%	26.4 (4.7)%	1.02 (0.367)	0.05	0.45	0.40
ToL: Mean response latency (sec)	11.4 (71.6)	10.5 (0.9)	13.2 (1.3)	10.6 (1.6)	1.52 (0.225)	0.04	0.00	0.04

Means and standard errors (adjusted for age, gender, and education) and effect size scores (Cohen's *d*) are provided for each cluster comparison. Significant effects are in bold. *P < 0.05. ¥P = 0.05. ANCOVA, analysis of covariance; LCT, Letter Cancellation Test; ToL, Tower of London. I-NSD, Insomnia with Normal Sleep Duration; I-SSD A, Insomnia with Short Sleep Duration type-A; I-SSD B, Insomnia with Short Sleep Duration type-B.

revealed I-SSD B to have a lower alpha power compared to I-SSD A ($P < 0.05$) and I-NSD ($P = 0.07$). Interactions were also significant for beta-1 and sigma power in solution 2 only ($P < 0.0001$) with reductions in beta-1 only in I-SSD B vs. I-SSD A ($P < 0.05$) before sleep. A nonsignificant reduction was found for sigma power between I-SSD B vs. I-SSD A before sleep ($P = 0.07$). The beta-2 power interaction was significant in both solutions ($P \leq 0.01$). Before sleep onset, a reduction in beta-2 power was found in I-SSD B vs. I-SSD A ($P = 0.05$). After sleep-onset, I-SSD B showed significantly reduced beta-2 power vs. I-SSD A ($P < 0.05$) and I-NSD ($P = 0.05$). Theta power displayed an interaction for both solutions ($P \leq 0.05$) but no significant comparisons. In solution 1, there were no significant between cluster × time differences for I-NSD vs. I-SSD. Similar results were found at C4 (see Figure 3 and Figure S1 in the supplemental material).

HRV at Sleep Onset

For HRV analysis, out of the 96 volunteers, 1 was left out of the analysis due to a corrupt data file and 9 patients were removed from the analysis for the following reasons: irregular rhythm throughout the recording (n = 3), excessive artifact (n = 3) and unable to visualise the ECG from recording (n = 3). From the 86 volunteers, 93 (10.8%) Two-minute segments were missing or removed due to artifact and 4 were removed due to extreme outliers > 4 SD (0.5%). Mixed model analysis revealed mean cluster differences in solution 1 for both rMSSD and pNN50 ($P < 0.05$) with lower values (attenuated) for the I-SSD cluster (rMSSD (ms): M (SE) = 44.3 (3.1) vs. 34.4 (3.5); pNN50 (ms): M (SE) = 22.4 (2.5) vs. 13.4 (2.7): see Figure 4. HF and the LF/HF ratio approached significance ($P < 0.10$). In solution 2, pNN50 displayed a nonsignificant mean reduction (lower) in I-SSD A vs. I-NSD ($P = 0.07$). There were no significant cluster differences for HR, SDNN, or HF across either solutions 1 or 2. In solution 2 only, the cluster × time (wake-to-sleep) interaction approached for the LF/HF ratio ($P < 0.07$), reduced ($P < 0.05$) in I-NSD (M (SE) = 1.9 (0.3) compared to I-SSD A (M (SE) = 2.8 (0.4) before sleep-onset (see Figure 4).

DISCUSSION

Exploratory cluster analysis of 96 volunteers with Insomnia Disorder derived at least two clusters from objective sleep parameters TST, SOL, and WASO: Insomnia with Normal Sleep Duration (I-NSD: n = 53) and Insomnia with Short Sleep Duration (I-SSD: n = 43). We were unable to rule out a 3-cluster solution that retained the I-NSD cluster and split the I-SSD into two: I-SSD A (n = 29) defined by high WASO; and I-SSD B (n = 14) a second I-SSD cluster with high SOL and medium WASO. It was not possible to evaluate a 4-cluster solution due to a lack of statistical power. More than one cluster solution appears to exist in volunteers who have Insomnia Disorder. We now have a testable model of two cluster solutions to use in analysis of data from existing and future insomnia cohorts. Indeed, we are currently evaluating insomnia cluster response to cognitive behavioural therapy in a new separate sample of patients grouped according to empirical solutions derived here (ANZCTR Clinical Trial Registration Number: 12615000751572).

Three steps are required to evaluate the usefulness of groups derived from cluster analysis. First, both the 2-cluster (I-NSD and I-SSD) and the 3-cluster solutions (I-NSD, I-SSD A and I-SSD B) resulted in groups that were significantly distinguished by the cluster input parameters: TST, SOL, and WASO (see Table 1). Second, when characterizing clusters in terms of clinical and demographic outcomes, there were no differences in traditional self-reported measures of insomnia between clusters for any solution in this select volunteer sample (see Table 1 and Table S2 in the supplemental material). Clusters do not appear to be identifiable from classic measures of insomnia and may offer novel information. This is in line with recent literature where subjective sleep measures are unable to detect insomnia-related morbidity outcomes between I-SSD and I-NSD.[55] The 2-cluster (I-SSD and I-NSD) solution may represent previous findings of long and short objective sleep duration insomnia and the distribution of volunteers across this solution is similar (I-NSD = 53 vs. I-SSD = 43).[5] Third and most importantly, to test the external validity of the clusters we evaluated for between cluster differences in four outcomes that were not used to build the clusters including neurocognitive performance and sleep-onset measures of HRV and *q*EEG.

Sleep Onset Heart Rate Variability

In the 2-cluster (I-SSD and I-NSD) solution only, differences were found for sleep-onset measures of HRV. I-SSD displayed

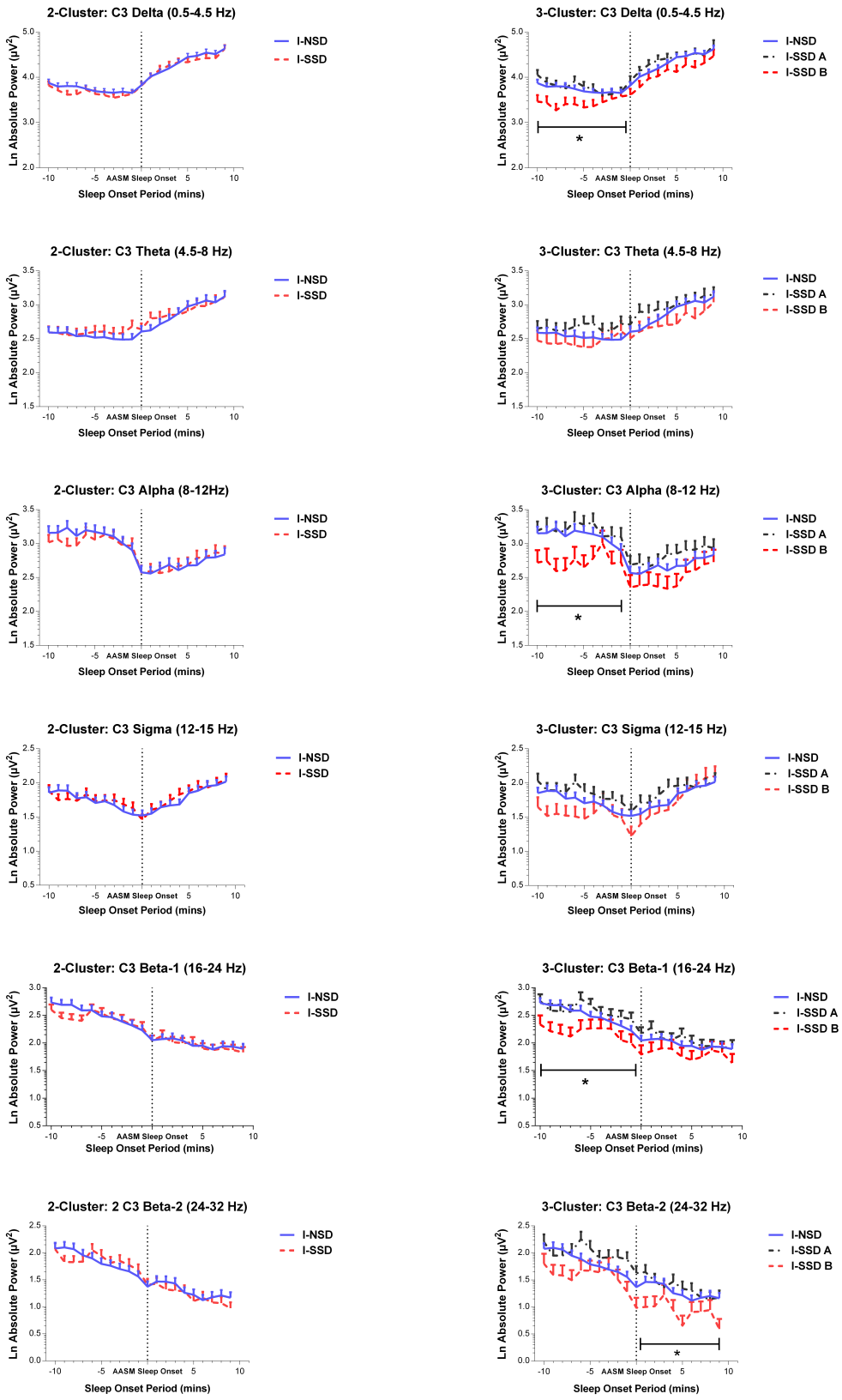

Figure 3—Quantitative analysis of sleep-EEG spectra during sleep-onset at C3 shows reduced Absolute Power (Ln) in the 3-cluster solution only. Quantitative EEG analysis of mean (SE) delta (0.5–4.5 Hz), theta (4.5–8 Hz), alpha (8–12 Hz), sigma (12–15 Hz), beta-1 (16–24 Hz), and beta-2 (16–32 Hz) frequency bands (Ln Absolute Power adjusted for age and gender) for 10 min before-to-after American Academy of Sleep Medicine (AASM) defined sleep onset. I-NSD, Insomnia with Normal Sleep Duration; I-SSD, Insomnia with Short Sleep Duration; I-SSD A, Insomnia with Short Sleep Duration type-A; I-SSD B, Insomnia with Short Sleep Duration type-B. *P < 0.05.

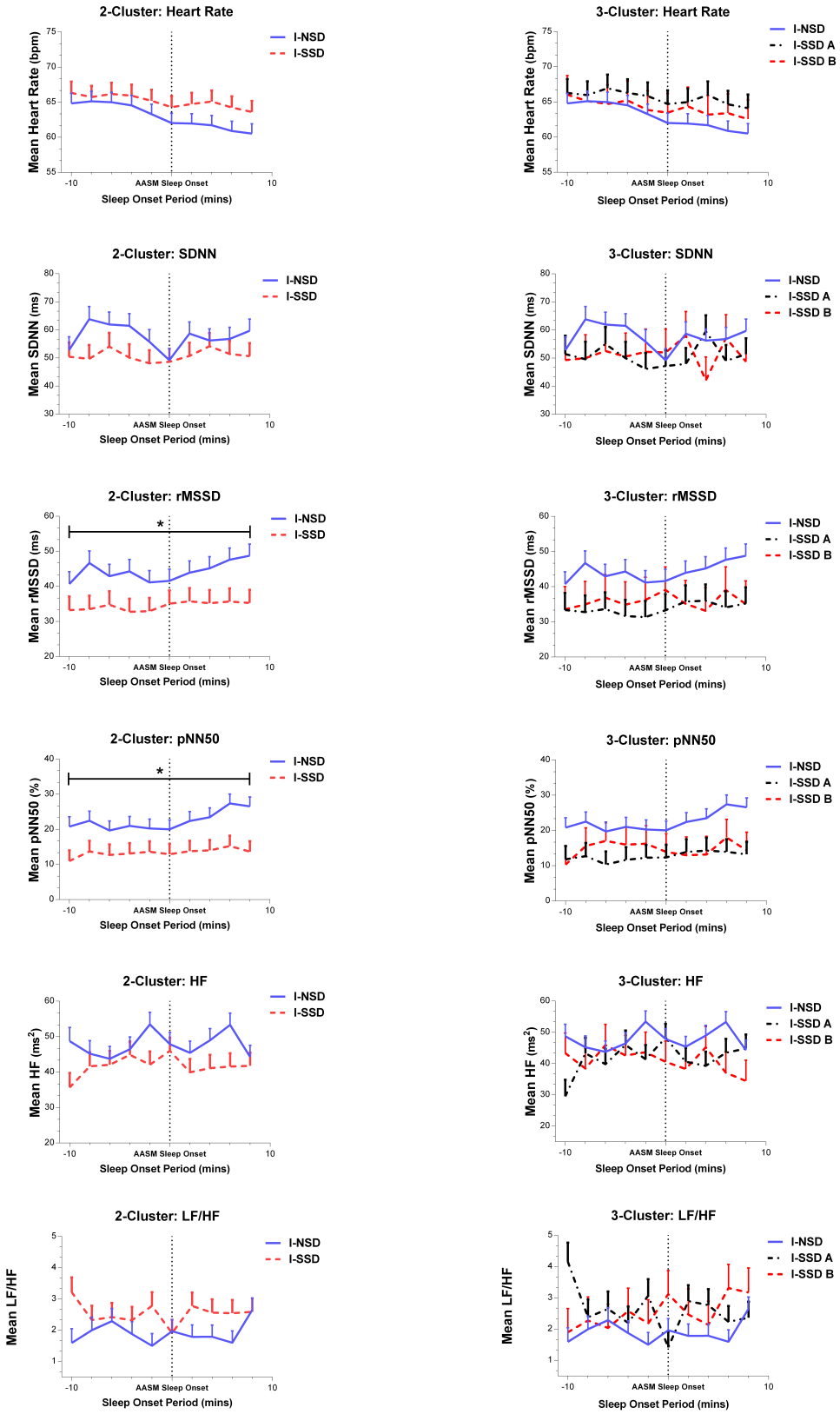

Figure 4—Analysis of heart rate variability during sleep-onset shows impaired variability in the 2-cluster solution only. Mean (SE) Heart rate (HR: bpm), standard deviation of all N-N intervals (SDNN: ms), root mean square of successive R-R Interval differences (rMSSD: ms), percentage (%) of successive R-R Intervals that differ by more than 50 ms (pNN50); high frequency (HF: ms^2) and a low frequency (LF)/HF ratio (adjusted for age and gender) for 10 min before-to-after American Academy of Sleep Medicine (AASM) defined sleep onset for both the 2 and 3-cluster solutions (adjusted for age and gender). I-NSD, Insomnia with Normal Sleep Duration; I-SSD A, Insomnia with Short Sleep Duration type-A; I-SSD B, Insomnia with Short Sleep Duration type-B. *P < 0.05.

attenuated parasympathetic activity (pNN50 and rMSSD) compared to I-NSD. This finding is consistent with previous research where HRV measures were indicative of reduced parasympathetic activity in patients with insomnia.[53] In a clearly defined I-SSD phenotype, reduced parasympathetic activity may contribute to adverse cardiometabolic health outcomes found in I-SSD.[5] Measures of sympathovagal balance between the clusters were not attributed to increased sympathetic activity in the I-SSD group found previously in insomnia compared to controls.[56] Longitudinal follow-up data over several years with large numbers of patients are more appropriate to evaluate risk of cardiometabolic ill-health in I-SSD[57] compared to associations from cross-sectional samples of patients.[58] The 3-cluster solution did not reveal significant differences perhaps due to a lack of statistical power.

Sleep Onset Quantitative EEG

Using *q*-EEG, there were no significant between-cluster differences for solution 1 (I-NSD vs. I-SSD). In the 3-cluster solution only, consistent reductions were found for the I-SSD B (high SOL) cluster in Delta, Alpha and Beta-1 activity before sleep initiation and Beta-2 activity after sleep compared to both I-SSD A and I-NSD clusters. Reduced activation however is not in line with the hyperarousal hypothesis where increased high-frequency beta and gamma activation has been found at sleep-onset and across the night compared to controls.[4,39,42,48] Reduced activation may reflect a previous finding where lower Beta-2 (18–29.75 Hz) power was observed in sleep-onset insomnia compared to sleep maintenance insomnia subtypes during sleep initiation[59] Krystal et al. also showed reduced Beta (16.5–30.0 Hz) power in patients with objective I-SSD compared to I-NSD.[60] Heightened cortical arousal may be higher in those with I-NSD, contribute to subjective insomnia and is linked to nonrestorative sleep.[5,60,61] Here, results suggest a specific impaired short sleep insomnia cluster (I-SSD B), which displayed reduced power in the Beta-1 (16–24 Hz) and Beta-2 (24–32 Hz) frequency range. Figure 3 and Figure S1 in the supplemental material may suggest a 2-cluster solution, not a 3-cluster solution and classification error of volunteers by cluster analysis may have caused this. A control group may have revealed cortical hyperarousal to be present in both the I-NSD and I-SSD groups, in line with the hyperarousal hypothesis and recent findings of adolescent insomnia subtypes.[62] Further investigation of the sleep-onset process between clusters of insomnia is warranted.

Neurocognitive Performance

No differences were found in cognitive performance between I-SSD and I-NSD. This is in contrast to previous findings where I-SSD patients were found to be impaired.[37,63] Lack of differences may be due to the sample of volunteers recruited here who were otherwise healthy apart from Insomnia Disorder and performed well across the range of cognitive domains. In solution 2, the I-SSD B cluster performed worse than both I-SSD A and I-NSD for sustained attention ($P \leq 0.05$). This is very preliminary evidence of neurocognitive impairment within a specific objective PSG-derived cluster of Insomnia Disorder and again may reflect an overall I-SSD group finding because of cluster classification error. Phenotyping studies have found impairments in task processing speed, set-switching attention and visual memory in those with I-SSD.[37,63] Daytime impairments have been found to be of small-to-moderate magnitude in studies of individuals with insomnia compared to controls.[6,64] Variability found in case-control studies may be attributed to certain insomnia clusters performing better than others (i.e., I-NSD / I-SSD A vs. I-SSD B: see Table 3). Combined with reduced *q*-EEG activation at sleep-onset the I-SSD B cluster appears most impaired relative to both I-SSD A and I-NSD.

Limitations

Only three input parameters were used for cluster analysis as these were limited by the sample size of 96 volunteers.[65,66] More clusters may exist with larger samples of volunteers. A lack of variability may have caused a type II statistical error as the sample recruited was somewhat homogeneous (generally healthy, educated, and white) reflecting the local demographics of the one recruitment site at Sydney, Australia. Further testing in more diverse patient groups may establish the external validity of these clusters. Apart from Insomnia Disorder, the sample appeared somewhat healthy and this may have been due to data capture, as our questions on comorbidity were open-ended (i.e., asking the patient to enter the presence of other disease rather than selecting from a list). This may have resulted in underestimation of comorbidity, but the error is unlikely to be large as most of our patients were recruited from our insomnia clinic by referral or by advertisements in clinic. A type I statistical error may have occurred due to the many outcomes that were tested between clusters. Error in cluster classification may have caused a 3-cluster solution when results here may better reflect a 2-cluster (I-NSD and I-SSD) solution with I-SSD B being the most impaired. The lowest score on the ISI in this sample was 1 followed by 7 in the next lowest volunteer with a mean = 17.3 (range: 1–28; SD = 4.8). The collection of the ISI occurred two weeks after consent and may have reflected spontaneous remission. Sensitivity analysis suggested that this did not affect cluster classification or our overall conclusions. As only one night of PSG recorded sleep was measured, it could be argued that night-to-night variability may limit cluster classification. The sleep parameters (TST, SOL, and WASO) have been found to be reliable over several years and I-SSD and I-NSD phenotypes were persistent over three sleep periods, suggesting a single PSG night may be a reliable classification.[35] Poor neurocognitive performance may have affected subsequent PSG sleep in the I-SSD B cluster. However, alert cognition prior to sleep initiation was not different between clusters suggesting against this notion. A protocol limitation is related to time in bed which was compromised, as lights on was at 06:00 for all clinical and research volunteers in our laboratory. Forced awakening may have taken place in some volunteers. There were no significant between cluster differences for total PSG-sleep study recording time (lights-off until lights-on).

CONCLUSIONS

In summary, exploratory cluster analysis of objective sleep parameters (TST, SOL, and WASO) derived at least two

distinct clusters of Insomnia Disorder: Insomnia with Normal Sleep Duration (I-NSD) and Insomnia with Short Sleep Duration (I-SSD) from a volunteer sample. At sleep-onset, I-SSD displayed attenuated parasympathetic activity compared to I-NSD. A second solution suggested preliminary evidence for three clusters including; I-NSD and two groups of I-SSD (I-SSD A and I-SSD B). The I-SSD B cluster appeared impaired on neurocognitive sustained attention (lower compared to both I-NSD and I-SSD A) and *q*-EEG with lower power compared to I-NSD and I-SSD A before and after sleep-onset. Clusters should now be evaluated in further samples of patients for prognosis and treatment response.

ABBREVIATIONS

AASM, American Academy of Sleep Medicine
AHI, apnea-hypopnea index
ANOVA, analysis of variance
ANCOVA, analysis of covariance
BMI, body mass index
bpm, beats per minute
CI, confidence interval
d, Cohen's *d*
DASS, depression anxiety and stress scale
DBAS, dysfunctional beliefs and attitudes about sleep scale
DISS, daytime insomnia symptom scale
DSM, Diagnostic and Statistical Manual of Mental Disorders
ECG, electrocardiography
EEG, electroencephalography
EMG, electromyographic
EOG, electrooculography
ESS, Epworth Sleepiness Scale
FIRST, Ford insomnia response to stress test
FFS, Flinders Fatigue Scale
HF, high frequency
HR, heart rate
HRV, heart rate variability
I-NSD, insomnia with normal sleep duration
ISI, insomnia severity index
I-SSD, insomnia with short sleep duration
I-SSD A, insomnia with short sleep duration type-A
I-SSD B, insomnia with short sleep duration type-B
LCT, letter cancellation test
LF, low frequency
Ln, natural logarithm
M, mean
n, number
pNN50, percentage (%) of successive R-R intervals that differ by more than 50 ms
PSG, polysomnography
q-EEG, quantitative electroencephalography
rMSSD, root mean square of successive R-R intervals
SD, standard deviation
SE, standard error
SDNN, standard deviation of all N-N intervals
SOL, sleep onset latency
ToL, Tower of London
TST, total sleep time
WASO, wake time after sleep onset

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Chapter 4: HRV Across the Night

Preamble

As explained in the Introduction (Chapter 1), it has been posited that physiological hyperarousal may be present particularly amongst short-sleep duration insomnia patients across 24-hours. The literature review (Chapter 2) highlighted that HRV findings from case-control and intervention studies of insomnia patients are inconsistent. Chapter 3 presented the findings from HRV analysis of insomnia subtypes across the sleep onset period, which found evidence of impaired parasympathetic activity marked by a reduction in select HRV measures (PNN50, RMSSD) in the short-sleeping phenotype.

A limited number of studies have used 24-hour recordings to investigate HRV in insomnia patients (compared to controls) despite the advantages of enabling standardised recording length and cross-comparison with large studies from the cardiology domain that have successfully used HRV as a tool for risk stratification (Farina et al., 2014; Kleiger et al., 2005; Yang et al., 2011). Moreover, no research has been performed with the *a priori* aim of investigating the cardiovascular activity of insomnia patient subtypes across the entire night to assess the present of persistent cardiovascular autonomic changes.

This chapter contains a manuscript that extends the work of Chapter 3, with the primary aim of investigating HRV over the entire nocturnal period. As hyperarousal is thought to be a constant phenomenon (Bonnet & Arand, 1997; Bonnet & Arand, 2010a; Riemann, 2010; Riemann et al., 2010), it was hypothesized that HRV changes would extend beyond the sleep onset period.

It is important to note that data are from the same Insomnia 100 patient group as Chapter 2. Furthermore, patient subtypes and nomenclature are consistent; I-SSD (insomnia with short-sleep duration), and I-NSD (insomnia with normal [objective] sleep duration). One final point of clarification; it can be noted that PRANA software program was used for the following manuscript whereas Kubios was employed for the previous sleep onset analysis. A number of software programs are available for HRV analysis; Kubios is freely

available and can be downloaded from the internet. Other sleep researchers have used this program, it is widely accepted within the literature, and has been formally validated (Tarvainen, Niskanen, Lipponen, Ranta-Aho, & Karjalainen, 2014). Kubios is well-suited for short recordings but edits cannot be saved after the outputs have been created. A further challenge with Kubios is that it does not have capability to incorporate the sleep staging file and therefore analysis of HRV according to sleep stage is not automated. PRANA is a more complex program and enables the incorporation of a sleep staging file. Edits can be saved (and returned to at a later time, if required) which is beneficial when checking numerous, extended recordings. Further procedural details on HRV analysis using PRANA are presented in Appendix B.

Statement of Author Contributions

Chapter 4 has been submitted to SLEEP and at the time of writing this thesis is under review as:

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As the lead author, I extracted the ECGs from the sleep studies and was the sole contributor to the HRV analysis. I worked alongside Associate Professor Nathaniel Marshall to perform the statistical processing of the data, and independently wrote the manuscript.

Kirsty Lyn Dodds	[redacted]	16th February 2017
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As the primary supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

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Abstract (250/250)

Study Objectives

Insomnia patients of the Insomnia 100 phenotyping study have previously been placed into one of two subtypes, insomnia with short-sleep duration (I-SSD) or insomnia with normal-sleep duration (I-NSD). This was based on cluster analysis using polysomnography (PSG)-derived sleep metrics: total sleep time, sleep onset time, and wake after sleep onset time. In this case-control study we examined whether nocturnal heart rate variability (HRV) differs between these subtypes. It was hypothesized that I-SSD patients would have impaired HRV compared to I-NSD patients.

Methods

Insomnia Disorder patients (DSM-V) completed overnight PSG, which provided sleep metrics for cluster analysis and electrocardiography (ECG) for HRV analysis. In accordance with gold-standard HRV methodology, each ECG was visually checked for accurate R-wave detection, and manually corrected as required. HRV analysis was performed from lights-off to lights-on (and separately by sleep/wake stage) using time and frequency-domain measures. We tested for differences in HRV measures between the subtypes using General Linear Models where we controlled for age as a core confounder.

Results

34 I-SSD patients (18F, 45.5 ± 10.5 years) and 41 I-NSD patients (21F, 37.6 ± 10.9 years) had ECG suitable for HRV analysis. There were no significant nocturnal HRV differences between subtypes after controlling for age.

Conclusion

I-SSD patients did not have significant (relative) reductions in HRV measures representative of parasympathetic activity. We were therefore unable to demonstrate HRV impairment in this subtype. Larger sample sizes are required to definitively determine a small to medium effect size difference in HRV between insomnia patient subtypes.

Keywords

Cluster Analysis, Electrocardiography, Polysomnography, Sleep, Sleep Initiation and Maintenance Disorders

Details of Clinical Trial

“Insomnia 100 sleep study”

URL: https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=347742

Registration: Australia New Zealand Clinical Trials Registry (ANZCTR)

Registration number: ACTRN12612000049875

Statement of Significance (107/120)

- This is the first study to compare the nocturnal heart rate variability of insomnia patient subtypes that have been empirically-derived using objective sleep parameters.
- We did not find any significant differences in nocturnal heart rate variability (from lights-off to lights-on) between short-sleep duration insomnia patients and normal-sleep duration insomnia patients when controlling for age.
- Heart rate variability measured during nocturnal polysomnography cannot presently be used as evidence of 24-hour cardiovascular autonomic dysregulation in short-sleep duration insomnia patients.
- Future research should aim to use adequately powered sample sizes that can reliably detect small effect sizes and incorporate long-term electrocardiography to investigate around-the-clock cardiovascular activity.

Introduction

Heart rate variability (HRV) provides insight into cardiovascular autonomic activity through the analysis of the beat-to-beat interval.[1] HRV has evolved to become a ubiquitous tool with many applications; 1) for researching autonomic nervous system activity, response to pharmacotherapy, and disease mechanisms 2) for clinical diagnostic purposes, whereby decreased HRV is often indicative of a disease state, particularly those associated with autonomic dysregulation and 3) for risk stratification and prognosis of disease and mortality.[2-4]

Epidemiological studies support an association between insomnia and cardiometabolic disease.[5,6] Although there is heterogeneity in the insomnia population,[7,8] some studies have reported a short-sleeping phenotype, thought to be driven by systemic, 24-hour hyperarousal.[9-12] Investigators of the Penn State Cohort have shown short-sleep (less than six hours) insomnia patients have severe cardiometabolic sequelae, with increased mortality and incident hypertension compared with longer-sleeping insomnia patients.[13-16] As such, it has been hypothesized that cardiovascular autonomic dysfunction is present in short sleep insomnia and reflected in HRV changes in measures representative of reduced parasympathetic activity during sleep .[16]

We previously identified HRV differences in insomnia patient subtypes during sleep onset.[17] Our subtypes were derived through cluster analysis using PSG measures of total sleep time, sleep onset time, and wake after sleep onset instead of a dichotomous cut-point of sleep duration to determine groupings (for example, greater or less than six hours of total sleep time). Given that hyperarousal in insomnia patients is posited to be present throughout the 24-hour sleep-wake cycle,[11] we were interested in extending our initial analysis to investigate the full night.

Previous work has examined HRV of insomnia patients with short-sleep duration (defined as those with a PSG sleep efficiency of less than 85%) finding significant reductions in three HRV measures (HR, PNN50, RMSSD) representative of parasympathetic activity.[18] However, HRV comparisons were made to healthy controls, not insomnia patient subtypes.[18] It is therefore not known whether HRV differences exist according to insomnia subtype as this has not been previously investigated.[19]

In this study, we aim to comprehensively assess nocturnal HRV between our previously determined insomnia patient subtypes. We hypothesize that insomnia patients with short-sleep duration will exhibit impaired HRV with reduced parasympathetic activity across the night when compared to normal sleep-duration insomnia patients.

Methods

This case-control study was a sub-analysis of our previously described exploratory observational study. This was a study of 100 patients, which was a pre-selected sample size (with no formal quantitative power calculation) thought to be of sufficient size to detect clinically meaningful patient subtypes.[17] The current analyses are covered under the original ethical approval of the Royal Prince Alfred Hospital (Protocol Number X11-0392, HREC/11/RPAH/620) and the written informed consent given by patients. This study was prospectively registered with the Australia New Zealand Clinical Trials Registry (ACTRN12612000049875).

Patients

Volunteers responded to advertisements in the community and clinic and were subsequently telephone screened and clinically reviewed by either a sleep physician or sleep psychologist at a screening appointment at the Woolcock Institute of Medical Research Clinic. All included patients met criteria for Insomnia Disorder based on the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders, reporting difficulties with at least one core symptom of sleep initiation, sleep maintenance or early morning awakening for at least three nights per week for at least three months causing significant negative impact upon daytime functioning.[20] Patients were 18+ years of age, and free from illicit substance use, alcohol and caffeine dependence, severe psychiatric disorders, cognitive impairment, and pregnancy or lactation. They were not shift workers nor aware of the presence of a co-morbid sleep disorder. Recruitment occurred between February 2012 and August 2014.[17]

Insomnia subtypes were determined using Ward's hierarchical cluster analysis. This empirically-based approach detected subtypes based on *a priori* selected variables. We chose three sleep parameters derived from polysomnography (PSG); total sleep time (TST), sleep onset latency (SOL), and wake after sleep onset (WASO). As we previously reported, three subtypes may exist, however, only the two subtype solution revealed significant differences in HRV at sleep onset.[17] This solution was also used for the present analysis.

Study protocol

Patients performed a pre-laboratory assessment consisting of a two week sleep diary, concurrent actigraphy, and questionnaires. The laboratory assessment comprised an overnight sleep study at the Woolcock Institute of Medical Research Clinical Sleep Laboratory (New South Wales, Australia) between March 2012 and August 2014. Patients were setup for their study from 20:00. Full PSG was performed and scored according to American Academy of Sleep Medicine criteria guidelines and recommendations.[21] Lights-off time was at the discretion of the patient. Lights-on time was standardized at 06:00. Only the studies recorded with RemLogic software v. 3.2 (Mortara, Milwaukee, USA) were included in the present study.

Electrocardiographic recording and heart rate variability analysis

In order to assess our primary aim of evaluating nocturnal HRV measures of insomnia patient subtypes we included all ECG data from lights-off to lights-on (commonly referred to as 'time in bed'). A modified Lead II Einthoven ECG configuration (torso electrode placement) was used. Studies required continuous sampling at 512Hz to ensure no variations when determining the fiducial point of the QRS complex, the series of deflections of the ECG trace that represent ventricular depolarisation. An ECG filter was applied; low frequency= 0.3Hz, high frequency= 70Hz.

PSG studies were imported into PRANA software v. 12.06.20 (PhiTools, Strasbourg, France) for HRV analysis and R-waves were automatically detected. The ECG signal from the entire overnight

recording was then visually examined by KLD and artifact identification and manual RR interval (the interval between two R waves of neighbouring QRS complexes) correction was performed. Artifact was deemed as any signal noise that prevented viewing a discernible R wave and/or any beat not been preceded by a p wave (deeming it to be of sinus origin). Studies that contained ≥ 20% artifact or cardiac dysrhythmias were excluded (Figure 1).[22] Inter-beat interval analysis of the edited signal (termed the NN interval, where N denotes a normal beat) was performed. For frequency-domain analysis, further resampling of the NN interval occurred using Fast Fourier transform routine (sampling rate= 10Hz), which corresponded to 30-second sleep staging epochs. This enabled estimation of low frequency (LF= 0.04 – 0.15 Hz) and high frequency (HF= 0.14 – 0.40 Hz) power spectra.

Time-domain measures selected for analysis were 1) heart rate (HR), 2) standard deviation of the NN interval (SDNN), and 3) the square root of the mean squared differences of successive NN intervals (RMSSD). RMSSD was selected in preference to NN50 and PNN50 (as per the HRV Standards).[2] Frequency-domain measures included 1) low frequency (LF), 2) high frequency (HF), and 3) the LF to HF ratio (LF/HF). Very low frequency power was not analysed given the need for 24-hour recordings.[2] As recordings were of different durations, frequency-domain measures were reported in normalized units (nu; %).

Data analysis

All statistical analyses were performed using Statistical Analysis System software v. 9.4 (North Carolina, USA) by NSM and KLD. Univariate differences between subtypes were tested using independent samples *t*-tests, Wilcoxon, or chi-square tests, as appropriate. We visually inspected histograms of continuous data and then scatterplots of HRV measures against age to assess normality and identify outliers that may affect statistical test assumptions. Effect size differences between normally distributed outcomes variables are quantified using Cohen's *d*. We tested whether HRV variables were different between the subtypes using General Linear Models (GLM)

where we controlled for age as a core confounder.[23,24] There were no interactions between age and subtype when statistically tested inside the initial GLMs or on visual inspection of scatterplots with regression lines for age for each of the two subtypes in any of the six HRV measures investigated. As such we have reported only the estimates from models without interaction terms. To test differences in whole night measures we set alpha at 0.05 and used 95% confidence limits. Individual sleep stages (wake [W], non-rapid eye movement-stage N1 [N1], non-rapid eye movement-stage N2 [N2], non-rapid eye movement-stage N3 [N3], rapid eye movement sleep [REM]) had a corrected alpha value of 0.01 and confidence limits of 99% for each stage in order to conserve an overall 5% false positive rate within each HRV measure. Post hoc power calculations were undertaken using G*Power v. 3.1 (Dusseldorf, Germany).[25]

Results

Patient progress through the study is outlined in Figure 1. Demographic details of the I-SSD and I-NSD patient subtypes (Table 1). By design, the patient subtypes differed on sleep indices (TST, SOL and WASO) because these were used to define the subtypes.[17] The I-SSD subtype reported greater number of medical comorbidities. The primary publication provides a more comprehensive overview of the patients enrolled.[17]

Insert Table 1 about Figure 1 about here

There were no significant differences in nocturnal HR (from lights-off to lights-on) between insomnia subtypes before and after adjustment for age (Figure 2 & Table 2). After adjusting for age there were no differences between the subtypes in any of the six all-night HRV measures (Table 2). All unadjusted analyses comparing insomnia subtypes for each HRV variable and sleep stages are in supplementary tables (Tables S1-S7).

Insert Table 2 and Figure 2 about here

Discussion

We compared nocturnal HRV (from lights-off to lights-on) between I-SSD and I-NSD patient subtypes. Previous research has shown that HRV is altered in insomnia patients compared to good-sleeping controls, although the evidence is inconsistent.[19] After adjusting for between-group differences in age, we did not find any significant differences in nocturnal HRV across the six measures we calculated. Thus, we did not find support for our hypothesis; I-SSD patients did not have impaired HRV (less parasympathetic activity) compared to the I-NSD subtype.

Most previous studies have compared insomnia patients to good-sleeping controls without examining short-sleepers.[18,26-29] In the only report comparing the HRV of short-sleepers to controls, evidence of decreased parasympathetic-related HRV measures (SDNN, RMSSD, PNN50, and HF) were found in short sleepers (PSG sleep efficiency <85%).[18] We have previously shown lower values for two parasympathetic-related HRV measures (RMSSD and PNN50) during the sleep-onset period in I-SSD patients.[17] Using the entire ECG from the same dataset in this study, I-SSD patients had numerically lower values for three HRV measures of parasympathetic activity (SDNN, RMSSD and HF), although they failed to reach statistical significance. Adjusted analyses using age as a confounder were responsible for this non-significant finding. This is in contrast to Speigelhalder *et al.* however they did not report any HRV measures of the entire recording period of the sleep study.[18]

It was evident from Table S4 that RMMSD (an indicator of parasympathetic activity) was significantly lower during N3 amongst I-SSD patients compared to their longer sleeping counterparts. This was in contrast to the findings in Table S5, where there were no between-group differences in HF nu (also an indicator of parasympathetic activity). Furthermore, whilst RMSSD was lower in N3 compared to Wake and N1 (in both I-SSD and I-NSD), HF nu values for N3 were higher. Several factors may have contributed to these incongruent results.

The time spent in Wake and REM varied according to insomnia patient subtype. As demonstrated in Table 1, significantly different amounts of time were spent in Wake and in REM which may have

influenced the statistical time-domain HRV measures. Accordingly, SDNN also had lower values for N3 than Wake and N1 in both I-SSD and I-NSD patients (refer to Table S3).

RMSSD had very high standard deviations amongst both patient subtypes for Wake, N1 and N3. Similarly elevated standard deviations were evident in Table S3 for SDNN. These deviations were particularly prominent in the I-NSD group. Large inter-individual differences are to be expected within the heterogeneous insomnia population given that homogenous 'healthy' populations can have individual variations of HRV values in excess of 250,000%.[26] Measures of RMSSD are also highly variable under conditions of enhanced vagal outflow due to the rapid speed of parasympathetic nerve activity.[26] Healthier individuals (such as those in the I-NSD group) may therefore have higher vagal activity and increased standard deviations for time-domain measures reflective of parasympathetic activity.[26]

We found no difference in mean HR between I-SSD and I-NSD subtypes. Previous research has shown differences between HR in insomnia and good-sleeping controls,[26,29,30] but others have found no HR differences between these groups.[18,31,32] HR reduction during NREM, which is a consistent finding, and the change in HR between NREM and REM sleep stages in the present study were of similar magnitude to previous research.[18] Our findings do not extend to nocturnal sympathetic activation during wake and sleep and this needs to be explored further in these insomnia subtypes.

An unforeseen consequence was the number of patients excluded from the I-SSD subtype due to dysrhythmia (see Figure 1). The majority of previous HRV-related studies of insomnia patients have not reported whether patients exhibited dysrhythmias during the ECG recording. As HRV is reliant on the calculation of the time interval between R-waves from consecutive QRS complexes, it is imperative that consecutive beats are of sinus origin.[33] We visually inspected the complete ECG recording of every patient and excluded all patients with dysrhythmia. This is both a strength and limitation; although we were confident in the accuracy of our findings, previous studies that have

not followed this stringent process may have included insomnia patients with poorer cardiovascular health resulting in HRV differences between insomnia patients and controls.

As our primary study was intended to be representative of a clinical sample of insomnia patients, it neither excluded those with medical comorbidities or on medication. After reviewing self-reported prescribed medications, we found one I-SSD patient had consumed a class-II antiarrhythmic (atenolol), and one I-NSD patient had consumed a class-IV antiarrhythmic (verapamil) and also digoxin. Both were in sinus rhythm on the night of their sleep study and had HRs within normal ranges for both sleep and wake. These patient's data were included. Confounders for HRV may include caffeine,[34] alcohol,[35,36] and nicotine [37,38] which were not restricted on the day of testing. In addition, our insomnia clinical sample were younger than insomnia subtypes of previous studies (Vgontzas et al.[15] reported their short-sleep group [less than six hours total sleep time] had a median age ~ 54 years). Longitudinal studies of our I-SSD subtype might reveal HRV differences when they are of a similar age to comparative samples. The possibility also remains that both patient subtypes may have impaired HRV relative to controls.

We used time-domain HRV measures (HR, SDNN, RMSSD) despite recordings of non-standardized duration. Historically, 24-hour HRV recordings are used to determine cardiovascular risk.[39,40] Our patient's recordings ranged between 345 to 514.5 minutes, although there were no significant between-group differences in duration (from lights-off to lights-on), suggesting that HRV recording length did not influence the results.

We deliberately did not control for sleep-wake differences between the groups. There were several reasons for this; 1) the short-sleeping phenotype is thought to be driven by persistent hyperarousal,[9-11] 2) in our recent systematic review we were unable to conduct a meta-analysis of previous insomnia-HRV studies due to the heterogeneous reporting of HRV analyses at different times of the night 3) seminal HRV research from the cardiology field linking HRV to incident cardiovascular disease have used long-term recordings.[39,40] Similarly, we chose to present both LF

(nu) and HF (nu) despite their mathematical equivalence in order to facilitate cross-comparison with other studies.[41]

The lack of statistical difference after adjusting for age may have been related to sample size, as we calculated our study to be 80% powered to determine effect sizes over 0.66. The largest effect size we observed was 0.27 for which we were only 21% powered to detect. Case-control studies that are smaller than ours that report statistically significant but small effects, may be reporting false positive findings because they were unable to detect reliably what are probably subtle effects, if they exist at all.[19,42] Future researchers may consider that the 95% confidence limits we report in Table 2 are wide enough that we cannot rule out the possibility that if real effects exist they may be anywhere in the small effect size range (i.e. anywhere up to Cohen's *d*= 0.50 standard deviations but probably not larger). As such, we have calculated sample sizes for effect sizes *d*= 0.50, 0.35, and 0.20 as these represent the largest, middle of the range, and smallest of what are considered 'small' effects. With equal numbers of cases and controls to achieve 80% power, future studies will need 128, 260 or 788 participants to detect effect sizes of 0.5, 0.35, and 0.2 respectively.

In summary, we did not find significant evidence of impaired HRV amongst insomnia patients with short-sleep duration compared to the longer-sleeping subtype across the entire nocturnal sleep period (beyond sleep onset). Whilst there were univariate differences in nocturnal parasympathetic activity in the I-SSD subtype these differences were attenuated significantly and became non-significant when adjusted for age differences. Therefore, we were unable to support the notion that overnight HRV alterations are present in short-sleep insomnia patients compared to the longer-sleeping subtype.

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Insomnia 100 study

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Present study

National Health and Medical Research Council (Australia) NeuroSleep, 1060992

Cooperative Research Centre for Alertness, Safety and Productivity, Australian Commonwealth Government

Figure titles and captions

Figure 1: CONSORT flow diagram

Note: ECG= electrocardiogram, HRV= heart rate variability. Square boxes depict parent study. Round-edge boxes depict present study.

Figure 2: Nocturnal mean heart rate of insomnia patient subtypes from lights-off to lights-on a) unadjusted and b) adjusted for age

Note: Table displays Means and 95% Confidence Intervals. Unadjusted heart rate data are also presented for all patients in both groups highlighted in red circles and black squares. **Abbreviations:** I-SSD= insomnia with short sleep duration, I-NSD= insomnia with normal sleep duration.

Tables

Table 1. Participant demographics

Characteristics	I-SSD *n*= 34 *Mean (SD)*	I-NSD *n*= 41 *Mean (SD)*	*p*
Age, range	**45.5 (10.5),** 26-75	**37.6 (10.9),** 23-65	**0.002**
Female	18 (53%)	28 (68%)	0.17
Insomnia Severity Index	16.0 (5.5)	18.0 (4.3)	0.07
Total sleep time (min)	293.3 (54.9)	393.0 (37.2)	-
Sleep onset latency (min)	34.5 (32.3)	18.6 (12.0)	-
Wake-time after sleep onset (min)	105.5 (59.6)	38.3 (23.2)	-
Duration: lights-off to lights-on (min)	436.9 (41.8)	451.3 (38.3)	0.13
Duration: Wake (mins)	**136.6 (55.6)**	**56.7 (28.3)**	**<0.001**
Duration: N1 (min)	12.6 (7.6)	10.7 (7.7)	0.29
Duration: N2 (min)	**162.8 (45.8)**	**223.6 (38.9)**	**<0.001**
Duration: N3 (min)	69.0 (28.3)	79.4 (27.1)	0.11
Duration: REM (min)	**48.9 (19.9)**	**79.5 (23.8)**	**<0.001**
BMI (kg/m^2)	25.8 (4.7)	24.4 (4.0)	0.16
Self-reported medical comorbidities *(n=70)*	**18 (55%)**	**10 (27%)**	**0.02**
Self-reported heart disease	0 (0%)	0 (0%)	-
On average, more than 2 caffeinated beverages per day	8 (24%)	8 (21%)	0.76
Caffeine on day of study	25 (74%)	30 (79%)	0.59
High risk alcohol consumption	1 (3%)	0 (0%)	0.27
Alcoholic beverages on day of study	4 (12%)	4 (10%)	0.81
Past smoker	**16 (47%)**	**8 (20%)**	**0.01**
Current smoker	3 (9%)	4 (10%)	0.68

Note: Caffeinated beverages were scored using an average caffeine level of 88mg per drink (for coffee) and rounded to 100mg. High risk alcohol consumption was considered ≥36 standard drinks per week, as per the 2001 National Health and Medical Research Council Australian Alcohol Guidelines: Health Risks and Benefits which stipulates different consumption levels according to sex.[43] *p* values <0.05 are bolded. **Abbreviations:** BMI= Body mass index, I-NSD= insomnia with normal sleep duration, I-SSD= insomnia with short sleep duration, kg= kilogram, m= metre, min= minute, n= number, SD= standard deviation.

Table 2. Nocturnal HRV measures (from lights-off to lights-on) of insomnia patient subtypes adjusted for age

HRV measure	I-SSD *n*= 34 Mean age= 45.5 *LSM (SE)*	I-NSD *n*= 41 Mean age= 37.6 *LSM (SE)*	Mean difference [95% CI]	*P*	Effect size [95% CI]
HR (bpm)	62.8 (1.4)	62.9 (1.2)	0.18 [-3.6, 4.0]	0.93	0.02 [-0.47, 0.51]
SDNN (ms)	68.7 (5.2)	71.2 (4.7)	2.44 [-11.9, 16.7]	0.73	0.08 [-0.38, 0.54]
RMSSD (ms)	56.2 (6.8)	64.8 (6.1)	8.67 [-10.1, 27.4]	0.36	0.22 [-0.25, 0.68]
HF (nu)	36.0 (1.8)	39.0 (1.7)	3.01 [-2.1, 8.1]	0.24	0.27 [-0.19, 0.73]
LF (nu)	64.0 (1.8)	61.0 (1.7)	-3.01 [- 8.1, 2.1]	0.24	-0.27 [0.19, -0.73]
LF/HF	16.8 (3.4)	12.6 (3.1)	NC	0.37	NC

Note: Numerical values in this table are derived from generalised linear models. Cohen's *d* effect size with 95% Confidence Intervals. Adjusted mean with unadjusted pooled standard deviation to calculate effect size. **Abbreviations:** bpm= beats per minute, CI= confidence intervals, HF (nu)= high frequency power (normalized units), HR= heart rate, I-NSD= insomnia with normal sleep duration, I-SSD= insomnia with short sleep duration, LF/HF= low frequency power (normalized units) to high frequency power (normalized units) ratio, LF (nu)= low frequency power (normalized units), LSM= least squares mean, ms= millisecond, NC= not calculated, RMSSD= square root of the mean squared differences of successive NN intervals, SDNN= standard deviation of the NN interval.

Supplemental material

Table S1. Nocturnal HRV measures (from lights-off to lights-on) of insomnia patient subtypes (unadjusted)

HRV measure	I-SSD *n*= 34 *Mean (SD)*	I-NSD *n*= 41 *Mean (SD)*	Mean difference [95% CI]	*p*	Effect size [95% CI]
HR (bpm)	63.1 (7.1)	62.7 (8.1)	-0.4 [-3.9, 3.2]	0.83	0.05 [-0.51, 0.42]
SDNN (ms)	64.0 (26.3)	75.1 (34.7)	11.2 [-3.2, 25.6]	0.13	0.36 [-0.10, 0.82]
RMSSD (ms)	**50.8 (31.8)**	**69.3 (45.8)**	**18.5 [0.6, 36.5]**	**0.04**	**0.46 [0.01, 0.91]**
HF (nu)	**34.3 (9.5)**	**40.5 (12.3)**	**6.2 [1.0, 11.3]**	**0.02**	**0.56 [0.09, 1.02]**
LF (nu)	**65.7 (9.5)**	**59.5 (12.3)**	**6.2 [1.0, 11.3]**	**0.02**	**0.56 [0.09, 1.02]**
LF/HF	**8.4 (5.3, 23.8)**	**4.8 (3.0, 16.8)**	**NC**	**0.04**	**NC**

Note: The LF/HF ratio required nonparametric testing and is therefore reported with Means and Inter Quartile Ranges. **Abbreviations:** bpm= beats per minute, CI= confidence intervals, HF (nu)= high frequency power (normalized units), HR= heart rate, I-NSD= insomnia with normal sleep duration, I-SSD= insomnia with short sleep duration, LF/HF= low frequency power (normalized units) to high frequency power (normalized units) ratio, LF (nu)= low frequency power (normalized units), ms= millisecond, NC= not calculated, RMSSD= square root of the mean squared differences of successive NN intervals, SDNN= standard deviation of the NN interval.

Table S2. Heart Rate (HR) of insomnia patient subtypes according to wake or sleep stage (unadjusted)

Stage	I-SSD *n*= 34 *Mean (SD)*	I-NSD *n*= 41 *Mean (SD)*	Mean difference [99% CI]	*p*	Effect size [99% CI]
W	66.5 (7.3)	66.5 (7.6)	0.0 [-4.5, 4.6]	0.98	0.0 [-0.61, 0.62]
N1	63.1 (7.2)	63.9 (7.7)	0.9 [-3.7, 5.4]	0.62	0.12 [-0.49, 0.72]
N2	60.6 (7.2)	61.2 (8.1)	0.6 [-4.1, 5.4]	0.73	0.08 [-0.53, 0.70]
N3	62.2 (7.1)	62.5 (9.1)	0.3 [-4.7, 5.4]	0.87	0.04 [-0.57, 0.65]
REM	63.7 (6.9)	64.2 (8.1)	0.5 [-4.2, 5.2]	0.78	0.07 [-0.55, 0.68]

Abbreviations: CI= confidence intervals, I-NSD= insomnia with normal sleep duration, I-SSD= insomnia with short sleep duration, N1= non-rapid eye movement-stage N1, N2= non-rapid eye movement-stage N2, N3= non-rapid eye movement-stage N3, REM= rapid eye movement, W= wake.

Table S3. SDNN of insomnia patient subtypes according to wake or sleep stage (unadjusted)

Stage	I-SSD *n*= 34 *Mean (SD)*	I-NSD *n*= 41 *Mean (SD)*	Mean difference [99% CI]	*p*	Effect size [99% CI]
W	**81.7 (38.5)**	**135.2 (153.0)**	**53.5 [-13.1, 120.1]**	**0.006**[#]	**0.46 [-0.11, 1.03]**
N1	75.8 (34.8)	121.1 (134.4)	45.3 [-13.3, 103.9]	0.023[#]	1.00 [-0.29, 2.29]
N2	62.7 (30.9)	71.5 (30.5)	8.8 [-10.0, 27.6]	0.22	0.29 [-0.33, 0.90]
N3	40.7 (17.6)	63.5 (69.5)	22.8 [-7.5, 53.0]	0.020[#]	0.43 [-0.14, 1.00]
REM	62.2 (28.2)	68.8 (28.5)	6.6 [-10.8, 24.0]	0.32	0.23 [-0.38, 0.85]

Note: [#]Wilcoxon Test for nonparametric data used to calculate *p* value. **Abbreviations:** CI= confidence intervals, I-NSD= insomnia with normal sleep duration, I-SSD= insomnia with short sleep duration, N1= non-rapid eye movement-stage N1, N2= non-rapid eye movement-stage N2, N3= non-rapid eye movement-stage N3, REM= rapid eye movement, W= wake.

Table S4. RMSSD of insomnia patient subtypes according to wake or sleep stage (unadjusted)

Stage	I-SSD *n*= 34 *Mean (SD)*	I-NSD *n*= 41 *Mean (SD)*	Mean difference [99% CI]	*p*	Effect size [99% CI]
W	63.6 (46.8)	130.2 (225.4)	66.7 [-30.5, 163.9]	0.03[#]	0.39 [-0.18, 0.97]
N1	**49.5 (31.1)**	**115.3 (191.6)**	**65.8** **[-16.1, 147.8]**	**0.007[#]**	**0.46** **[-0.11, 1.03]**
N2	52.0 (41.7)	66.3 (37.2)	14.3 [-9.9, 38.4]	0.12	0.36 [-0.25, 0.98]
N3	**37.5 (19.2)**	**68.5 (95.5)**	**31.0** **[-10.1, 72.1]**	**0.009[#]**	**0.43** **[-0.14, 1.00]**
REM	41.4 (32.8)	55.2 (34.1)	13.8 [-6.8, 34.3]	0.08	0.41 [-0.20, 1.02]

Note: [#]Wilcoxon Test for nonparametric data used to calculate *p* value. **Abbreviations:** CI= confidence intervals, I-NSD= insomnia with normal sleep duration, I-SSD= insomnia with short sleep duration, N1= non-rapid eye movement-stage N1, N2= non-rapid eye movement-stage N2, N3= non-rapid eye movement-stage N3, REM= rapid eye movement, w= wake.

Table S5. High Frequency normalized units (HF nu) of insomnia patient subtypes according to wake or sleep stage (unadjusted)

Stage	I-SSD *n*= 34 *Mean (SD)*	I-NSD *n*= 41 *Mean (SD)*	Mean difference [99% CI]	*p*	Effect size [99% CI]
W	29.9 (9.3)	31.9 (10.7)	2.0 [-4.2, 8.2]	0.40	0.20 [-0.42, 0.81]
N1	32.9 (12.9)	37.8 (12.8)	4.9 [-2.9, 12.8]	0.10	0.38 [-0.23, 1.00]
N2	34.9 (10.8)	41.2 (13.7)	6.3 [-1.4, 13.9]	0.03	0.50 [-0.11, 1.11]
N3	46.2 (13.3)	49.7 (14.8)	3.5 [-5.2, 12.2]	0.29	0.25 [-0.37, 0.86]
REM	**25.8 (9.3)**	**33.1 (13.1)**	**7.3 [0.5, 14.2]**	**0.006**	**0.63 [0.04, 1.23]**

Abbreviations: CI= confidence intervals, I-NSD= insomnia with normal sleep duration, I-SSD= insomnia with short sleep duration, N1= non-rapid eye movement-stage N1, N2= non-rapid eye movement-stage N2, N3= non-rapid eye movement-stage N3, REM= rapid eye movement, w= wake.

Table S6. Low Frequency normalized units (LF nu) of insomnia patient subtypes according to wake or sleep stage (unadjusted)

Stage	I-SSD *n*= 34 *Mean (SD)*	I-NSD *n*= 41 *Mean (SD)*	Mean difference [99% CI]	*p*	Effect size [99% CI]
W	70.1 (9.3)	68.1 (10.7)	-2.0 [-8.2, 4.2]	0.40	-0.20 [-0.81, 0.42]
N1	67.1 (12.9)	62.2 (12.8)	-4.9 [-12.8, 2.9]	0.10	-0.38 [-1.00, 0.23]
N2	65.1 (10.8)	58.8 (13.7)	-6.3 [-13.9, 1.4]	0.03	-0.50 [-1.11, 0.11]
N3	53.8 (13.3)	50.3 (14.8)	-3.5 [-12.2, 5.2]	0.29	-0.25 [-0.86, 0.37]
REM	**74.2 (9.3)**	**66.9 (13.1)**	**-7.3 [-14.2, -0.5]**	**0.006**	**-0.63 [-1.23, -0.04]**

Abbreviations: CI= confidence intervals, I-NSD= insomnia with normal sleep duration, I-SSD= insomnia with short sleep duration, N1= non-rapid eye movement-stage N1, N2= non-rapid eye movement-stage N2, N3= non-rapid eye movement-stage N3, REM= rapid eye movement, w= wake.

Table S7. Low Frequency to High Frequency ratio (LF/HF) of insomnia patient subtypes during overnight polysomnography (unadjusted)

Stage	I-SSD *n*= 34 *Median (IQR)*	I-NSD *n*= 41 *Median (IQR)*	Mean difference [99% CI]	*p*	Effect size [99% CI]
W	15.8 (9.1, 61.5)	18.1 (5.7, 61.4)	N/A	0.72	N/A
N1	3.7 (2.3, 8.6)	3.3 (1.9, 5.5)	N/A	0.30	N/A
N2	3.9 (2.5, 6.5)	3.6 (1.9, 5.5)	N/A	0.57	N/A
N3	2.0 (1.3, 3.1)	1.8 (1.0, 3.0)	N/A	0.57	N/A
REM	5.5 (3.5, 10.9)	3.8 (2.4, 6.2)	N/A	0.05	N/A

Abbreviations: CI= confidence intervals, I-NSD= insomnia with normal sleep duration, I-SSD= insomnia with short sleep duration, N1= non-rapid eye movement-stage N1, N2= non-rapid eye movement-stage N2, N3= non-rapid eye movement-stage N3, REM= rapid eye movement, W= wake.
Note: Because the majority of the ratios in this table required nonparametric testing we decided to test all variables with the Wilcoxon test and report them as Medians and Inter Quartile Ranges (IQR).

Table S8. Primary complaint of insomnia patient subtypes

Characteristic	I-SSD	I-NSD
	n= 34	*n*= 41
Mixed pattern insomnia	16	18
Sleep-onset problems	3	8
Sleep-maintenance problems	8	10
Early morning awakening problems	7	5

Abbreviations: I-NSD= insomnia with normal sleep duration, I-SSD= insomnia with short sleep duration.

Abbreviations

BMI, Body Mass Index

bpm, beats per minute

CI, Confidence Intervals

CONSORT, Consolidated Standards Of Reporting Trials

ECG, Electrocardiographic/electrocardiogram

GLM, General Linear Model

HF, High Frequency

HR, Heart Rate

HRV, Heart Rate Variability

I-NSD, Insomnia with Normal Sleep duration

IQR, Inter Quartile Range

I-SSD, Insomnia with Short Sleep Duration

LF, Low Frequency

LSM, Least Squares Mean

ms, millisecond

M, Mean

Min, minute

N, n, number

NNI, NN interval, where N denotes a normal beat of sinus origin

N1, Non-Rapid Eye Movement Sleep, stage N1

N2, Non-Rapid Eye Movement Sleep, stage N2

N3, Non-Rapid Eye Movement Sleep, stage N3

nu, normalized units

PNN50, percentage (%) of successive NN intervals that differ by more than 50 milliseconds

PSG, Polysomnography

REM, Rapid Eye Movement sleep

RMSSD, Root Mean Square of Successive R-R intervals

RRI, RR interval, where R denotes the R wave of a QRS complex

SD, Standard Deviation

SDNN, Standard Deviation of all N-N intervals

SE, Standard Error

SOL, Sleep Onset Latency

TST, Total Sleep Time

W, Wake

WASO, Wake-time After Sleep Onset

Disclosure Statement

Created via the ICMJE Conflict of Interest form:

A/Prof Marshall reports non-financial support from TevaPharm, and 'other' from Medtronic, outside the submitted work.

Prof Grunstein reports 'other'; he was a Program Leader in the Co-operative Research Centre for Alertness, Safety and Productivity, a collaboration between academia, NGOs and industry supported research. Co-operative Research Centre for Alertness, Safety and Productivity during the conduct of the study.

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Figure 1

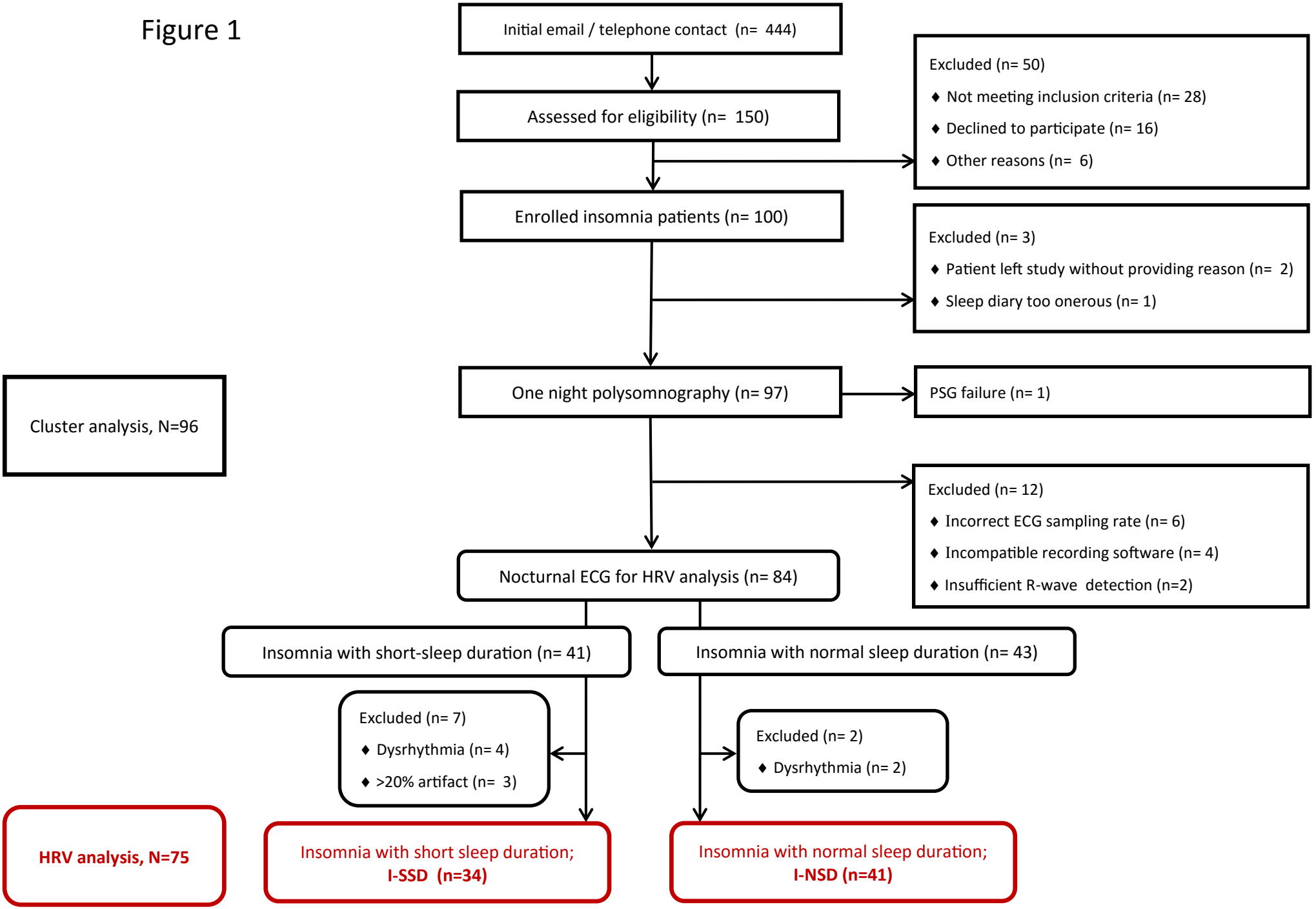
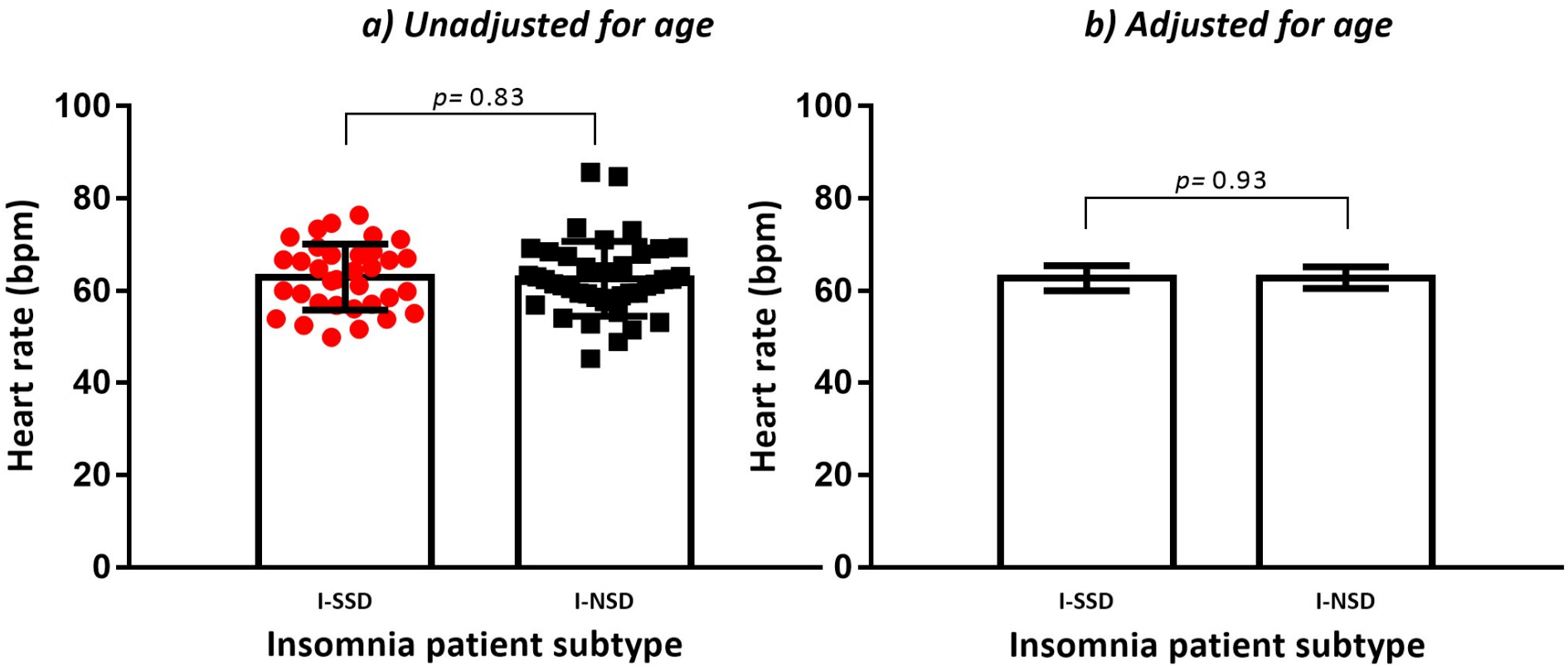

Initial email / telephone contact (n= 444)
Assessed for eligibility (n= 150)
Excluded (n= 50)
♦ Not meeting inclusion criteria (n= 28)
♦ Declined to participate (n= 16)
♦ Other reasons (n= 6)
Enrolled insomnia patients (n= 100)
Excluded (n= 3)
♦ Patient left study without providing reason (n= 2)
♦ Sleep diary too onerous (n= 1)
One night polysomnography (n= 97)
PSG failure (n= 1)
Cluster analysis, N=96
Excluded (n= 12)
♦ Incorrect ECG sampling rate (n= 6)
♦ Incompatible recording software (n= 4)
♦ Insufficient R-wave detection (n=2)
Nocturnal ECG for HRV analysis (n= 84)
Insomnia with short-sleep duration (n= 41)
Insomnia with normal sleep duration (n= 43)
Excluded (n= 7)
♦ Dysrhythmia (n= 4)
♦ >20% artifact (n= 3)
Excluded (n= 2)
♦ Dysrhythmia (n= 2)
HRV analysis, N=75
Insomnia with short sleep duration;
I-SSD (n=34)
Insomnia with normal sleep duration;
I-NSD (n=41)

Figure 2


a) Unadjusted for age
b) Adjusted for age
p= 0.83
p= 0.93
Heart rate (bpm)
100
80
60
40
20
0
I-SSD
I-NSD
Insomnia patient subtype

Chapter 5: Conclusion

Summary and Significance of Findings

The aim of my research was to determine whether differences in HRV exist in objective insomnia patient subtypes. The literature review of Chapter 2 uncovered the discrepancies present in the previous insomnia-HRV literature, underlining the incumbent need to perform research of insomnia patient subtypes, and to do so with stringent methodology.

As the first study to compare the HRV of short and normal sleep-duration insomnia subtypes, the case-control study presented in Chapter 3 found short sleep-duration insomnia patients have significant reductions HRV measures (RMSSD and PNN50) that correlated with parasympathetic activity during the sleep onset period. In one of the largest insomnia-HRV studies to date, this provided evidence that parasympathetic activity was attenuated in insomnia patients with short sleep-duration, compared to the normal sleep-duration subtype, prior to, and following sleep onset. This was consistent with the study hypothesis that the short sleep-duration insomnia subtype would have impaired HRV.

The extent to which HRV was impaired in short-sleep duration insomnia during sleep was explored in Chapter 4, via examination of HRV analysis across the entire nocturnal sleep recording. Preliminary analysis showed that age was a significant confounder between the insomnia subtypes. After controlling for age, there were no statistically significant between-group HRV differences. Therefore, whilst the first study supported the overall hypothesis (of impaired HRV amongst I-SSD patients when compared to I-NSD patients) it was challenged by the findings of the second study.

Discussion

Differences between insomnia patients and controls at selective time points were previously reported by Farina (2014) with reductions to HRV in early wake/sleep stages (wake before sleep and N2), but not during deep SWS (N3), REM sleep, or post-sleep wake. In line with the Psychobiological Model (Espie, 2002; Espie et al., 2006), the failure to inhibit wakefulness (impairing the automaticity of sleep) may conceivable have the greatest ramifications on HRV at sleep onset. Alternatively, it may be that the timing of insomnia

patient symptoms (constituting the clinical subtypes of insomnia, i.e. sleep onset vs sleep maintenance vs early awakening vs mixed-pattern) has more of an influence on psychobiology than acknowledged within this empirically-derived cluster approach. Others have corroborated the results of Chapter 4, showing no HRV differences in insomnia patients (albeit compared to controls) in nocturnal HRV. De Zambotti (2014) failed to find differences in vagal activity between insomnia patients and controls using whole-night recordings when performing an hour-by-hour analysis of HRV commencing from lights-off time.

The presence of constant HRV impairment in insomnia patients is, however, a reasonable deduction. It has previously been established that somatic symptoms, psychiatric illnesses, females, and older individuals have reduced parasympathetic activity evidenced through HRV; each of which is also associated with insomnia (Kemp & Quintana, 2013; Koenig & Thayer, 2016; Nunan et al., 2010; O'Brien, O'Hare, & Corrall, 1986; Thayer, Sollers, Friedman, & Koenig, 2015; Voss, Heitmann, Schroeder, Peters, & Perz, 2012). Similarly, the Neurocognitive Model of insomnia posits a pluralistic perspective of hyperarousal (linking cognitive, cortical, and somatic arousal) (Perlis et al., 2009; Perlis et al., 1997). Whilst HRV is traditionally considered a physiological determinant of autonomic hyperarousal in insomnia, the mind-body (heart) interconnection must not be minimized; HRV may have concurrently been influenced by interplay from cognitive and cortical hyperarousal (Bonnet & Arand, 1997; Bonnet & Arand, 2010a; Riemann, 2010; Riemann et al., 2010).

It is therefore warranted to question whether HRV was indeed reduced across both the sleep onset period and across the whole night even though results of the present study did not show this. Careful review of Table 2 (Chapter 4) suggests a signal may be present with (non-significant) numerical reductions in the parasympathetic related measures analyzed – SDNN, RMSSD and HF. The direction of change in these HRV measures was similar to previous studies comparing short-sleepers to controls [significantly reduced HR, PNN50, RMSSD during wake and sleep] (Spiegelhalder et al., 2011) and the first comparison of subtypes presented in Chapter 3 [significantly reduced RMSSD and PNN50 in I-SSD patients during the sleep onset period]. Additional research into the temporal patterning of HRV across the night is warranted and may offer important insights into the complexity of

cardiac autonomic activity of insomnia patient subtypes. Pertinent questions include whether HRV impairments are restored across the night, and if so, if there is a difference in rate of recovery between insomnia patient subtypes. The addition of Pre-Ejection Period measurements (to complement HRV findings) would allow more precise characterisation of such sympathovagal activity across sleep cycles.

There are a number of factors that need to be considered in relation to these findings -

Sample size

As detailed in the discussion section of Chapter 4, it is quite possible that, despite having one of the largest insomnia patient groups studied to date, it was underpowered. *Post hoc* analysis revealed that for insomnia patient case-control studies to achieve 80% power, they would need to have greater than 128 patients to detect modest effect sizes.

Patient demographics

The clinical sample used for both case-control studies was relatively young (median age = 41.5 years). Even though the I-SSD group was significantly older than the longer-sleeping patients, they were younger than the short-sleep duration group of the USA Penn State cohort (Vgontzas et al., 2010). As the insomnia patients in the current study age, there may be an increase cardiovascular risk, beyond that associated with normal ageing. A repeated-measure study of the Insomnia 100 patients in the future (～ 10 years' time) would be required to test this assumption.

Sleep/wake influences

Sleep generally, is a time of parasympathetic-dominance compared to wake. To test for the possibility of constant physiological hyperarousal, continuous measures are required. The daytime cardiac autonomic activity, which contains greater amounts of sympathetic activity, may have been different between the short-sleep, and normal-sleep duration insomnia subtypes. We did not have the ability to undertake daytime testing in this population. 24-hour HRV during sleep and wake has previously been measured in insomnia patients (and controls), however the 24-hour HRV outcomes were not reported (Farina et

al., 2014; Yang et al., 2011). Furthermore, neither of the empirical studies presented within this thesis accounted for the possibility of circadian influences.

Environmental issues

Given that the studies were performed in a sleep laboratory, a reverse first-night effect phenomenon may have been present. This might result in less psychological distress and greater sleep consolidation. Whether this influences HRV findings is yet to be tested (Hauri & Olmstead, 1989; Riedel, Winfield, & Lichstein, 2001).

It is imperative that the theoretical limitations of HRV are also considered in relationship to the hyperarousal hypothesis. There are no time-domain measures that specifically represent sympathetic activity (Trinder, Waloszek, Woods, & Jordan, 2012). Additionally, there remains uncertainty surrounding the physiological interpretation of both LF (nu) and LF/HF (Billman, 2013), as physiological correlates of sympathetic activity. The only frequency-domain measure that can be confidently associated with autonomic activity is HF, which has been shown to be representative of parasympathetic activity (Malik, 1998; Malik et al., 1996).

Thus, if the hyperarousal model is based upon the classical notion of an increase in sympathetic and concomitant decrease in parasympathetic activity, HRV alone cannot be used to support or refute this concept. This rather simplistic approach does not belie the complex interplay of parasympathetic and sympathetic activity that governs cardiac autonomic control. The possibility of synergistic, rather than antagonistic, activity between the parasympathetic and sympathetic branches of the autonomic nervous system has been largely ignored in the hyperarousal hypothesis of insomnia despite being previously suggested by Bernston (1993) that these divisions can act reciprocally, independently or coactively. Possibly, the use of more invasive autonomic surrogates, such as microneurography, or HRV in combination with other measures (impedance cardiography) would provide greater understanding of parasympathetic/sympathetic balance (Malik, 1998; Zygmunt & Stanczyk, 2010).

Considering HRV within Spielman's Three P model, it is unclear when exactly hyperarousal (with subsequent HRV alterations) develops. This poses the following

questions: Is hyperarousal present in patients who develop insomnia when in a pre-morbid (i.e. pre-insomnia) state? If that is the case, could higher baseline levels of hyperarousal act as a predisposing factor for insomnia? Supporting the notion of pre-morbid vulnerability, a recent meta-analysis of eight studies (n = 21, 988 participants) reported reduced SDNN in healthy people (without known cardiovascular disease) to be predictive of increased risk of a cardiovascular event (Hillebrand et al., 2013). On the contrary, is it only after development of chronic insomnia (with conditioned arousal) that second-order changes arise, marked by objective measures, such as HRV? It may be that HRV impairment is a correlate of the pathophysiological changes that develop due to chronic insomnia. An additional speculation is that the relationship between insomnia and impaired HRV has a bi-directional relationship.

Future Research

HRV measurement is inexpensive, non-invasive, and easily attainable via routine PSG. However, a major caveat of easy data acquisition is the potential for research without clear intention and the subsequent data mining of the many HRV outputs (Ernst, 2014). In order to disentangle the associations between HRV and insomnia aetiology, hyperarousal, and cardiovascular disease, well-designed studies will be required. For this reason, recommendations for further insomnia-HRV research are provided below.

Purposefully-selected participants

Participant samples that are representative of clinical insomnia patients (such as was used in the current studies) are best suited to provide outcomes with immediate clinical relevance. However, studies including patients of narrow age ranges or single sex (see for example de Zambotti et al., 2014; de Zambotti et al., 2013; de Zambotti, Sugarbaker, Trinder, Colrain, & Baker, 2015; de Zambotti, Trinder, Colrain, & Baker, 2017) are better placed to understand underlying physiological mechanisms. All future insomnia-HRV research should endeavour to maximise patient sample size and include comparison between insomnia patient subtypes and also, to well-matched, good sleeping controls.

24-hour recordings

The duration of HRV recordings in the field of sleep research is generally dictated by PSG length. This creates a predicament as a six to eight hour recording cannot be classified as short-term or long-term for HRV purposes, despite the fact that specific HRV measures are best suited to certain recording durations (e.g. time domain measures are best suited for use with 24-hour recordings) (Malik, 1998; Malik et al., 1996). Long-term ECG recordings are necessary for insomnia-HRV research and would improve the accuracy and external validity of future studies. Ideally, 24-hour HRV recordings would be sampled chronologically from insomnia patients to determine if cardiac autonomic activity changes with insomnia duration. This would address allostatic load which may present in insomnia patients and explain the development of co-morbid chronic disease (Chen, Redline, Shields, Williams, & Williams, 2014). HRV recordings from ambulatory monitoring would have the additional advantage of circumventing any confounding effect of the sleep laboratory.

The possibility also remains that there may be differences in insomnia patient subtypes during specific sleep stages and/or cycles. As this has been the first comparison of insomnia subtypes, studies powered to detect differences during sleep stages, would provide evidence of the effect of sleep state on HRV.

Collaboration with neuroscience

Further exploration of the interaction and relationship between physiological and cortical activity has begun (Cervena et al., 2014; Jurysta et al., 2009; Maes et al., 2014; Wei et al., 2016) and is likely to be the direction of future studies. For example, emerging research has investigated the HRV and quantitative EEG coherence in insomnia patients at different times of the night (Jurysta et al., 2009) whilst others have investigated cerebral cortical response to changes to heart beat in insomnia patients, probing for the presence of increased 'interoceptive sensitivity' (Wei et al., 2016). Such research may provide a deeper understanding of the pathophysiology of Insomnia Disorder and differences in patient subtypes (if present).

HRV guidelines for sleep research

Guidelines for HRV use within the field of sleep research are required, and should follow the lead of Quintana, Alvares, and Heathers (2016) who recently published HRV recommendations for use in psychiatry. Consensus on how to handle technical artifact (from the PSG), arousals from sleep, the analysis of HRV during differing sleep stages and cycles, varied recording lengths, and comorbid sleep conditions (such as insomnia and obstructive sleep apnoea) would undoubtedly be of use for all sleep researchers when designing, analyzing, and reporting future studies.

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Appendices

Appendix A: Supplemental Material for Chapter 3

Table S1—Re-formed agglomeration table of cluster coefficients used to demark the optimal number of clusters through the change in coefficients as clustering increases.

No. clusters	*Agglomeration last step*	*Coefficients this step*	*Change*
2	285.000	182.599	102.401
3	182.599	125.046	57.553
4	125.046	87.826	37.220
5	87.826	74.546	13.280
6	74.546	61.930	12.616
7	61.930	53.753	8.177

After 3 clusters, successive clustering adds little to distinguishing between cases. The dotted line denotes the demarcation point (at a 3-cluster solution).

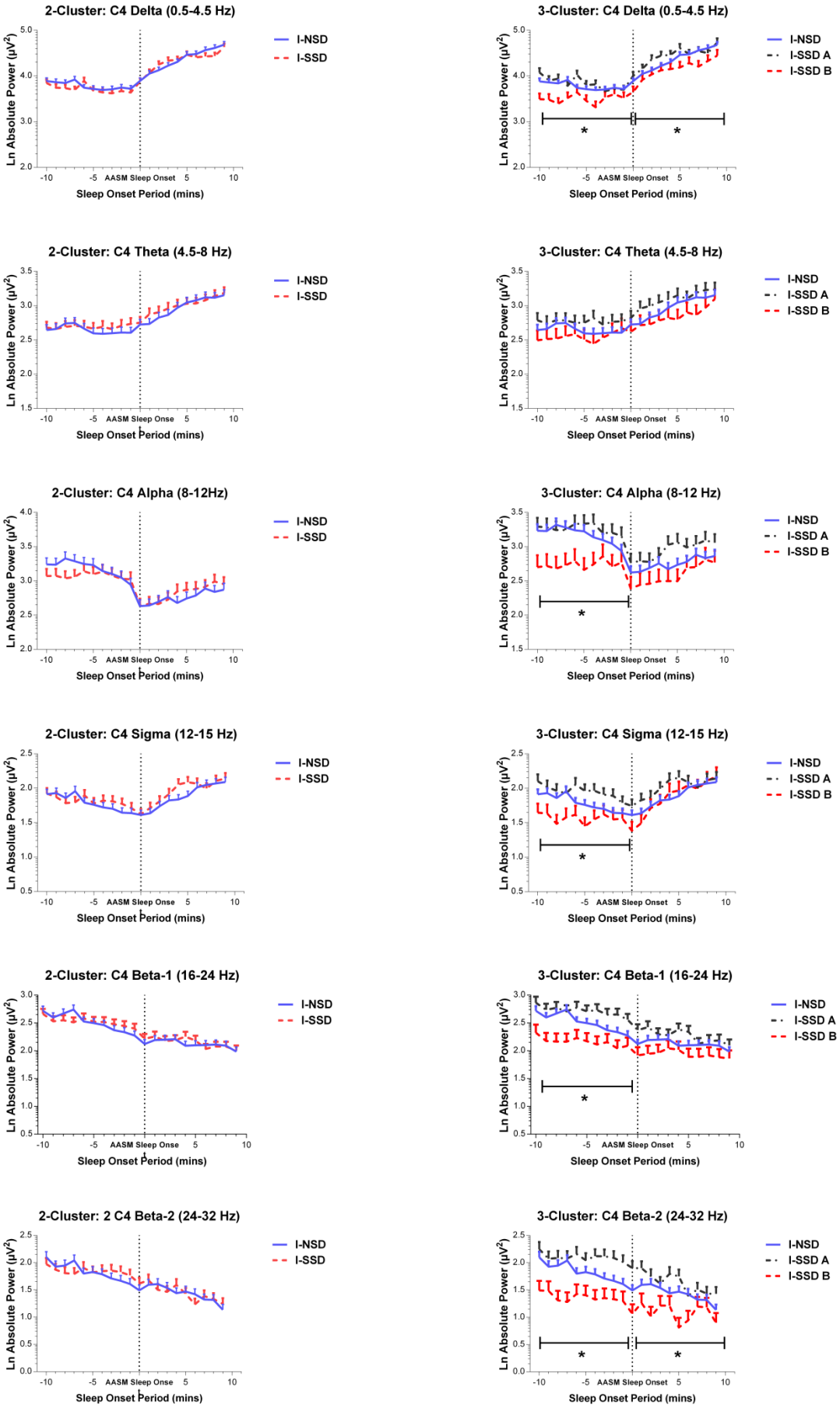
Table S2—Demographic, sleep parameters (used to cluster individuals) and clinical information between insomnia subgroups in the exploratory 3-cluster solution.

						Effect size (d, r)		
Mean (+/- SD)	*Overall (N=96)*	*I-NSD Cluster (N=53)*	*I-SSD A Cluster (N=29)*	*I-SSD B Cluster (N=14)*	*ANOVA F (p)*	*I-NSD vs. I-SSD A*	*I-NSD vs. I-SSD B*	*I-SSD A vs. I-SSD B*
Age (y), mean (SD), range	41.4 (11.8), 23-75	38.6 (11.6), 23-66	46.0 (10.8), 23-75	42.3 (12.3), 26-60	**3.99 (.022)***	**0.66**	0.31	0.33
BMI (kg/m^2)	25.0 (4.3)	24.7 (4.2)	25.0 (3.5)	26.4 (6.0)	**0.87 (.449)**	0.08	0.33	0.29
Total Sleep Time (mins)	346.1 (67.8)	392.3 (35.4)	286.1 (59.2)	295.6 (57.8)	**63.86 (<.001)****	**2.18**	**2.02**	0.16
Sleep Onset Latency (mins)	25.3 (23.9)	18.0 (11.7)	16.4 (11.1)	71.5 (26.0)	**28.86[a] (<.001)****	0.14	**2.65**	**2.76**
Wake-time After Sleep Onset (mins)	72.5 (58.6)	39.9 (22.6)	129.3 (67.5)	78.0 (40.7)	**27.53[a] (<.001)****	**1.78**	**1.16**	**0.92**
ISI	17.3 (4.8)	18.0 (4.0)	16.1 (5.8)	17.5 (5.0)	1.54 (.220)	0.38	0.11	0.26
Insomnia duration (y)	10 (11)	10 (10)	10 (13)	8 (15)	0.04[b] (.980)	0.01	0.03	0.03
DASS - Depression	6 (12)	6 (12)	4 (10)	5 (10)	0.72[b] (.697)	0.09	0.00	0.00
DASS - Anxiety	4 (6)	4 (7)	4 (6)	6 (7)	0.75[b] (.689)	0.16	0.01	0.01
DASS - Stress	15.3 (9.0)	15.8 (8.3)	13.6 (9.3)	16.7 (11.2)	0.71 (.496)	0.25	0.09	0.30
ESS	6.4 (4.5)	6.2 (4.5)	7.2 (4.5)	5.8 (4.4)	0.64 (.530)	0.22	0.09	0.31
FFS	18.0 (6.4)	18.4 (6.2)	17.3 (6.9)	17.9 (6.4)	0.26 (.774)	0.17	0.08	0.09
FIRST	24.0 (6.1)	24.3 (5.9)	23.3 (6.3)	24.4 (6.9)	0.28 (.750)	0.16	0.02	0.17
DBAS	6.1 (1.7)	6.2 (1.4)	5.8 (1.9)	6.1 (2.2)	0.55 (.580)	0.24	0.05	0.15
Pre-sleep DISS - Alert cognition	52.2 (16.0)	52.0 (16.7)	53.3 (15.7)	50.5 (14.6)	0.15 (.865)	0.08	0.10	0.18
Pre-sleep DISS - Negative mood	27.9 (17.0)	27.7 (16.4)	26.1 (16.8)	32.4 (19.7)	0.65 (.525)	0.10	0.26	0.34
Pre-sleep DISS - Positive mood	46.0 (12.8)	47.2 (13.4)	44.8 (13.0)	43.8 (9.7)	0.56 (.574)	0.18	0.29	0.09
Pre-sleep DISS - Sleepiness / fatigue	54.7 (17.7)	55.6 (17.8)	53.5 (19.0)	53.7 (15.3)	0.16 (.855)	0.11	0.11	0.01
AHI	3.1 (5.7)	2.8 (5.1)	3.7 (8.5)	3.3 (6.2)	2.45[b] (.293)	0.17	0.06	0.13
Evening systolic blood pressure (mmHg)	115.3 (14.3)	115.4 (15.9)	115.9 (13.1)	114.0 (10.8)	0.08 (.920)	0.03	0.10	0.16
Evening diastolic blood pressure (mmHg)	74.5 (9.4)	74.6 (9.5)	74.0 (8.8)	74.7 (11.0)	0.04 (.959)	0.07	0.01	0.07
Morning systolic blood pressure (mmHg)	107.0 (13.5)	106.0 (14.8)	107.5 (11.2)	109.4 (13.5)	0.39 (.678)	0.11	0.24	0.15
Morning diastolic blood pressure (mmHg)	71.0 (9.1)	69.8 (8.9)	72.0 (9.1)	73.4 (9.4)	1.09 (.341)	0.24	0.39	0.15
Number of alcoholic drinks per week (standard units)	4.0 (6)	4.0 (5)	4.0 (8)	2.0 (7)	1.94[b] (.380)	0.01	0.17	0.17

Binary Variables	*N (%)*	*N (%)*	*N (%)*	*N (%)*	X^2 *(p)*	*Effect size (V)*		
Sex (f)	61 (63.5%)	36 (67.9%)	15 (51.7%)	10 (71.4%)	2.56 (.278)	0.16	0.03	0.19
Insomnia as a child (y)	16 (16.7%)	10 (18.9%)	2 (6.9%)	4 (28.6%)	3.41[c] (.206)	0.17	0.09	0.28
Family history	28 (29.2%)	17 (32.1%)	9 (31.0%)	2 (14.3%)	0.25[c] (.955)	0.03	0.02	0.05
Education, highest attained								
High school	12 (12.5%)	8 (15.1%)	2 (6.9%)	2 (14.3%)	0.98[c] (.685)	0.11	0.02	0.11
College graduate	47 (49.0%)	22 (41.5%)	14 (48.3%)	11 (78.6%)	5.59 (.061)	0.09	0.29	0.24
Post graduate	32 (33.3%)	21 (39.6%)	10 (34.5%)	1 (7.1%)	**6.12[c] (.048)***	0.03	**0.30**	**0.34**
Ethnicity								
Central South Asian	4 (4.2%)	3 (5.7%)	1 (3.4%)	0 (0%)	0.97[c] (1.00)	0.05	0.11	0.11
East Asian	1 (1.0%)	1 (1.9%)	0 (0%)	0 (0%)	0.84[c] (1.00)	0.08	0.06	.
South East Asian	2 (2.1%)	1 (1.9%)	1 (3.4%)	0 (0%)	0.56[c] (1.00)	0.05	0.06	0.11
White Caucasian	88 (91.7%)	47 (88.7%)	27 (93.1%)	14 (100%)	1.51[c] (.758)	0.05	0.15	0.15
Employment								
Full-time	65 (67.7%)	40 (75.5%)	17 (58.6%)	8 (57.1%)	2.28 (.327)	0.15	0.14	0.01
Part-time	16 (16.7%)	8 (15.1%)	5 (17.2%)	3 (21.4%)	0.46[c] (.799)	0.04	0.08	0.05
Unemployed	11 (11.5%)	4 (7.5%)	5 (17.2%)	2 (14.3%)	2.44[c] (.320)	0.16	0.11	0.04
Medical Comorbidities	35 (36.5%)	15 (28.3%)	15 (51.7%)	5 (35.7%)	4.02 (.134)	0.23	0.05	0.17
Mild-to-moderate depression/anxiety	11 (11.5%)	5 (9.4%)	3 (10.3%)	3 (21.4%)	4.00[c] (.141)	0.15	0.34	0.48
Heart disease	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (.)	.	.	.
Cancer	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (.)	.	.	.
High blood pressure	1 (1.0%)	1 (1.9%)	0 (0%)	0 (0%)	1.78[c] (1.00)	0.19	0.12	.
Neurologic	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (.)	.	.	.
Breathing	8 (8.3%)	5 (9.4%)	2 (6.9%)	1 (7.1%)	1.81[c] (.473)	0.24	0.07	0.13
Urinary	1 (1.0%)	1 (1.9%)	0 (0%)	0 (0%)	1.80[c] (1.00)	0.19	0.12	.
Diabetes	1 (1.0%)	0 (0%)	0 (0%)	1 (7.1%)	4.41[c] (.118)	.	0.46	0.46
Chronic pain	8 (8.3%)	3 (5.7%)	5 (17.2%)	0 (0%)	1.65[c] (.624)	0.15	0.22	0.31
Gastrointestinal	6 (6.3%)	1 (1.9%)	1 (3.4%)	0 (0%)	0.66[c] (1.00)	0.00	0.12	0.12
Thyroid	3 (3.1%)	1 (1.9%)	2 (6.9%)	0 (0%)	0.76[c] (1.00)	0.11	0.12	0.18
Other	11 (11.5%)	5 (9.4%)	5 (17.2%)	1 (7.1%)	0.23[c] (1.00)	0.00	0.07	0.07
Current smoker	8 (8.3%)	4 (7.5%)	3 (10.3%)	1 (7.1%)	0.39[c] (.878)	0.05	0.08	0.05
Past smoker	29 (30.2%)	11 (20.8%)	13 (44.8%)	5 (35.7%)	5.13 (.075)	0.25	0.14	0.09
Never smoked	58 (60.4%)	37 (69.8%)	13 (44.8%)	8 (57.1%)	5.53 (.115)	0.26	0.12	0.12
Medication	49 (51.0%)	23 (43.4%)	17 (58.6%)	9 (64.3%)	2.37 (.317)	0.14	0.15	0.04
Prescription sleep aid	9 (9.4%)	6 (11.3%)	3 (10.3%)	0 (0%)	2.67[c] (.378)	0.10	0.29	0.25
Over the counter sleep aid	2 (2.1%)	0 (0%)	2 (6.9%)	0 (0%)	2.82[c] (.266)	0.27	.	0.20
Prescription psychiatric medication	5 (5.2%)	3 (5.7%)	1 (3.4%)	1 (7.1%)	0.81[c] (.839)	0.12	0.01	0.11
Other medication	31 (32.3%)	13 (24.5%)	11 (37.9%)	7 (50%)	2.35[c] (.312)	0.08	0.28	0.24

Means, standard deviations and effect size (Cohen's *d, r* for variables not normally distributed or Cramér's *V* for binary variables) for each cluster comparison are provided. [a] Welch's statistic correction for violation of homoscedasticity. [b] Kruskal-Wallis test with median (inter-quartile range) for variables not normally distributed. [c] Fisher's exact test correction for cells with $n<5$. Significant effects are in bold. Neurologic disorders (seizures, Parkinson's disease), Breathing disorders (asthma, emphysema), Urinary disorders (kidney disease, prostate problems), Chronic pain (arthritis, back pain, gout, migraines) and Gastrointestinal complaints (stomach, irritable bowel syndrome, ulcers). AHI: Apnea Hypopnea Index; ANOVA: analysis of variance; DASS: Depression, Anxiety, and Stress scales; DBAS: Dysfunctional Beliefs and Attitudes about Sleep scale; DISS: Daytime Insomnia Symptom Scale; ESS: Epworth sleepiness scale; FFS: Flinders Fatigue Scale; FIRST: Ford Insomnia Response to Stress Test; I-NSD: Insomnia with Normal Sleep Duration; I-SSD A: Insomnia with Short Sleep Duration type-A; I-SSD B: Insomnia with Short Sleep Duration type-B. * = $p<.05$. ** = $p<.001$.

Figure S1—Quantitative analysis of sleep-EEG spectra during sleep onset at C4.



Quantitative EEG analysis of Mean (SE) Delta (0.5-4.5 Hz), Theta (4.5-8 Hz), Alpha (8-12 Hz), Sigma (12-15 Hz), Beta-1 (16-24 Hz) and Beta-2 (16-32 Hz) frequency bands (Ln Absolute Power adjusted for age and gender) for 10 minutes before-to-after American Academy of Sleep Medicine (AASM) defined sleep onset. I-NSD: Insomnia with Normal Sleep Duration; I-SSD: Insomnia with Short Sleep Duration; I-SSD A: Insomnia with Short Sleep Duration type-A; I-SSD B: Insomnia with Short Sleep Duration type-B. * = p <.05.

Appendix B: Heart Rate Variability (HRV) analysis using the PRANA® (Polygraphic Recording Analyser) software suite

WOOLCOCK
LEADERS IN BREATHING & SLEEP RESEARCH

STANDARD OPERATING PROCEDURE (SOP)

Heart Rate Variability (HRV) analysis using the PRANA® (Polygraphic Recording Analyser) software suite

Created by:	Kirsty Dodds
Date created:	April 2016
SOP Version:	11
Last amended:	November 2016
Applicable to:	Woolcock Institute of Medical Research

EQUIPMENT

Hardware

- 64 bit Computer
- Wide monitor
- Mouse
- PRANA dongle [must be plugged into computer for HRV plug-in to work]

Software

- PRANA software, version 12.06.20

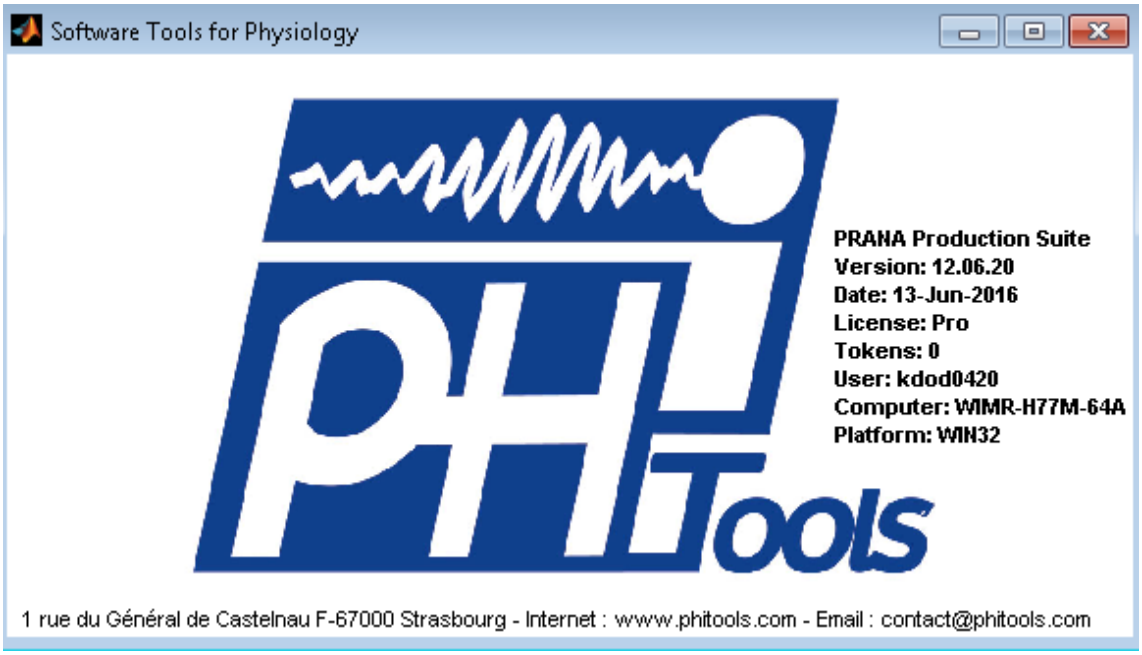


Figure 1. Software licence

FOLDER AND FILE NOMENCLATURE (see page 21 of PRANA User Manual for further details)

Use Setup tab in PRANA manager to arrange PRANA folders prior to your analysis.

Folders must exist within the computer's PRANA folder (or, if desired, on the network drive) before using setup tab in the PRANA manager to map file location.

Once these folders have been created for your specific project, do not change any folder names as this can affect the software's availability to locate the correct files at a later date.

Record folder

Contains EDFs and text files

Report folder

Contains analysis reports,

e.g. event mark reports (evt.*.xls), analysis trend reports (pra.*.xls), and sleep score reports (hpn.*.xls)

Result folder

Contains all the analysis files created using the software plug-ins that can be read by the profiler program,

e.g. PRANA Heart Rate Variables (channel.*.hrv files) and PRANA Spectral Power Array (channel.*.spc files)

Session folder

Session files (data that PRANA creates that creates information with full name of recording file, montage and settings associated with that session),

e.g. continuous (.cnt) and sequential (.spi) files directly loaded by the software

Can be used to open an already existing session

Has same content as PRANA Manager

Setting

Used to store the plug-in settings, montage and calibration files. They may be edited, saved and loaded during reviewing and analysis sessions.

PROCEDURE

Open file

Ensure that the text file is present in the same folder and named the same as the sleep study as this will be required for sleep staging information and the generation of the hypnogram.

Open EDF from personal/shared drive on computer

Click Session → Save

Click Ok, then Yes

Configure montage

HRV analysis in PRANA uses some contextual information derived from other channels, i.e. EMG and EEG for automated artefact detection. These channels and their position on screen can be edited as below. In order to allow batch processing, a common set of settings is required.

Click Review → Montage → Settings

To set up quantified montage, deselect Position checkbox (top left corner) to eliminate all channels and then select:

1. C3 (change to position 2), click on trace, change amplitude to -100 to 100mV so that it doesn't interfere with the ECG channel. Click on trend panel and check that the selected amplitude range is appropriate at other times throughout evening, adjust amplitude accordingly.

Click Next >>

Deselect Position checkbox (top left corner) to eliminate all channels and then select:

2. ECG (change to position 1), click on trace, change amplitude to -1 to 1mV.
3. EMG (change to position 3), click on trace, change amplitude to -100 to 100mV so that it doesn't interfere with the ECG channel.

*If ECG trace is inverted, change amplitude to **+**1mv to **-**1mV.*

Continue to click Next >> and deselect Position checkbox (top left corner) to eliminate all remaining channels

Click Ok

Click Session → Save As

Rename file with extension _HRV

Click Ok, then Yes

Set-up HRV analysis parameters

To enable clear visualisation of the ECG

Ensure window is full-screen

To change HRV settings

Click Analysis → Heart-Rate Variability

Check Heartbeat detection and Interbeat interval analysis settings

Ensure Control checkboxes are unchecked

Control is purely for graphical display (visualisation) of the heart rate detection (for each epoch) and for graphical display of the interbeat interval analysis (again, for each epoch). This doesn't change the algorithm or analysis output.

Ensure Supervise checkbox is checked

This will instruct the plugin to automatically launch event supervision right before the execution of interbeat interval analysis. The usefulness of this is that the current supervision will process all of the event markers or artefacts that have been automatically detected or manually marked on the signal and apply a set of settings that you can visualise in the [Event → Setting] window for each type of event there is a set of supervision attributes which are applied when you check the supervise checkbox in the HRV analysis window before you perform the interbeat interval analysis e.g. the merging of two overlapping but separately marked artifacts.

Click Close

To change the length of epoch (**not recommended** as 30 second windows correlate with sleep staging epochs)

Analysis → Heart-Rate Variability

Change Window length (1200s = 5 minute windows when using a 75% overlap)

Perform analysis

Run analysis for artifact detection and heart-rate variability analysis.

Plug-ins → Launcher → using the Ctrl key select Artifact Detection and Heart-Rate Variability Analysis from Selected Plug-ins window. See Fig. 1 below.

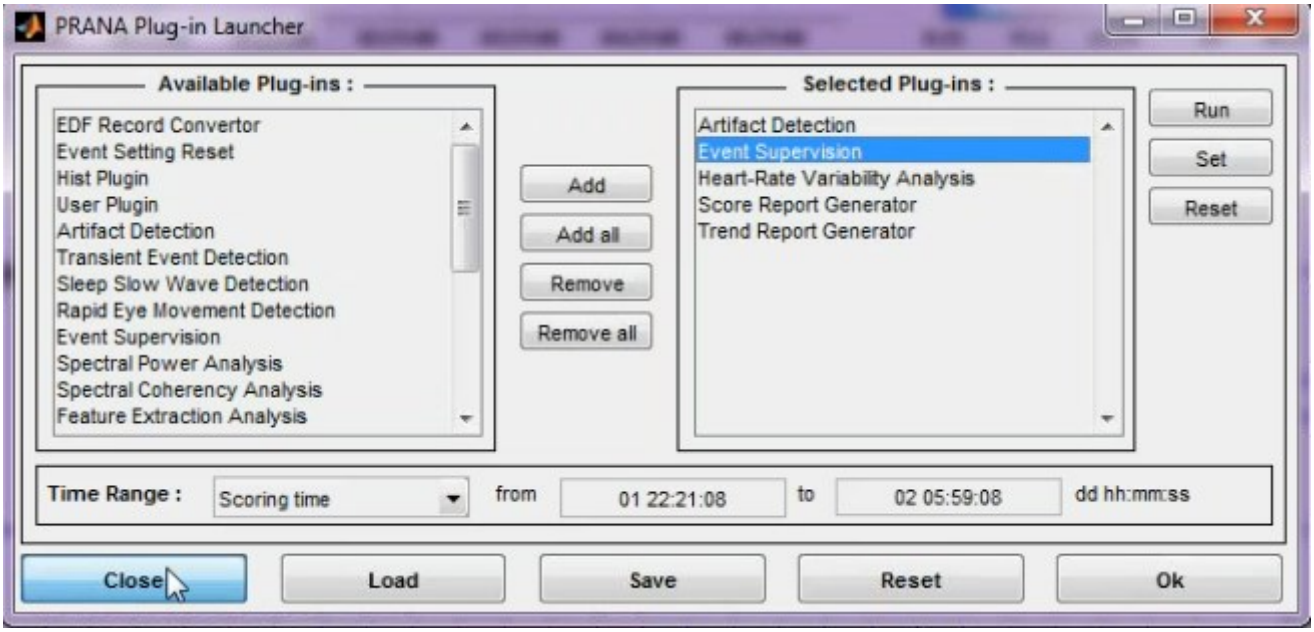


Figure 2. Use of the PRANA Plug-in Launcher

Click Run

This will take approximately 20 minutes depending on the processing speed of the computer that is being used.

Click Close

Generate trend report (automatic analysis) and save. This will enable you to compare the results before and after editing of the ECG.

Click Reports → Trend Report Generator

Results (downwards arrow) → HRV-parameters

Click Styles → State reports

Click ok

Copy data from text file

Open Excel and paste data

Save Excel spreadsheet

Close WordPad and Excel

To change what is on the trend reports, alter the setup of the Trend Report Generator in the Plug-in settings.

Visually inspect ECG and HRV

Use trend panel to show results of HRV analysis

Right click trends panel → Arrays → HRV spectral array (.spc)

Right click trends panel → Features → ECG → HR variability, see Fig. 2 below

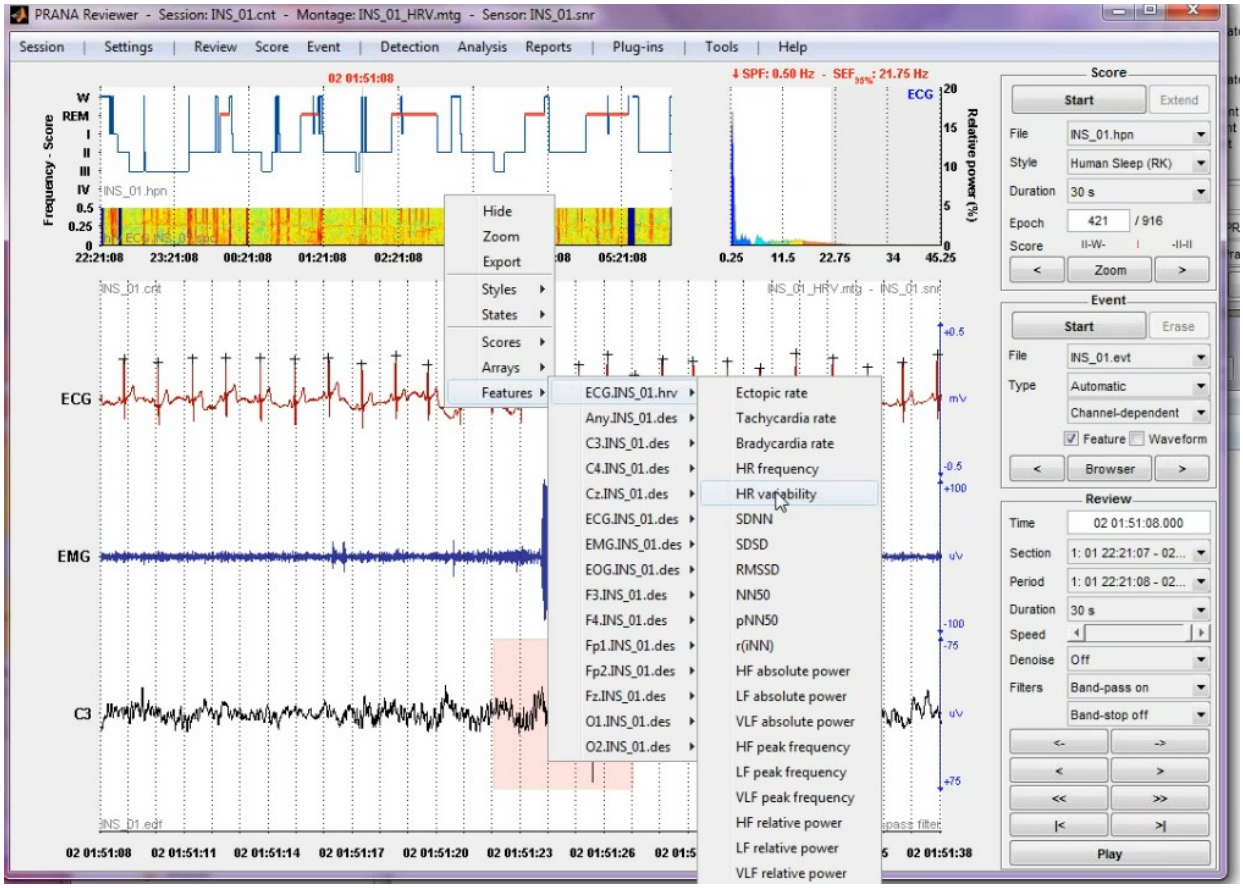


Figure 3. Configuring the trends panel

Visualise and edit heart-beat detection

<u>To change the label for markers (e.g. Ectopic Beats)</u>

This will make it more distinct from the R wave markers. The hot keys can also be changed under settings. Note that the hot keys are case sensitive.

Click Event→ Settings →Markers → Ectopic Beat → Choose Mark (square) and Colour (hot pink) → Ok

<u>To edit the RR interval</u>

Click Event → Start

Look at epoch

Insert a missing R-wave peak marker by maintaining the shift key pressed and mouse-clicking on the peak of a visually identified R-wave that has been missed by automated detection.

Delete an erroneous R-wave peak by selecting the corresponding marker by mouse-click and then pressing the Del key.

Highlight artifact. Click on highlighted segment, use keyboard to type “X” and then use the arrow keys to position.

Artifact is any signal noise that prevents viewing a discernible R wave OR a beat that has not been preceded by a p wave (deeming it to be of sinus origin).

Press Enter after any edits to save changes

Check the edits by clicking on first R wave peak marker present in the viewing pane and using the right sideways arrow in Event viewer to check each beat

When satisfied with ECG, save the file

Click Session → Save → OK

Progress to next epoch and repeat until entire recording has been reviewed

Re-run Interbeat interval analysis

Click Analysis→ Heart-Rate Variability

Deselect heartbeat detection

Click OK → Run

Save outputs

For visualisation

Tools → Profiler

For time series results

Session → Export → Results

Select file Type – PRANA Heart-rate Features (file ending in .hrv)

Double click on desired file

Choose export location

Click Ok

Copy data from text file

Open Excel and paste data

Save spreadsheet

Close WordPad and Excel

The default setting will give output in 30 second epochs

For sleep stages or sleep cycle results

Reports → Trend Report Generator

Results (downwards arrow) → HRV-parameters

Styles → State reports

Click Ok

Open Wordpad

Copy data from text file

Open Excel and paste data

Save spreadsheet

Close WordPad and Excel

To change what is on the trend reports, alter the setup of the Trend Report Generator in the Plug-in settings. Note that all outputs will also be on PRANAs folder of C drive.

Change outputs

Open Profiler

Click Settings → Configure

Select desired HRV measure

Click Add

Use arrows to position HRV measure accordingly (this position will be reflected in the data output)

Analysing outputs

25 HRV measures are available.

The following analysis times may be most beneficial:

- Scoring: All. This is commonly referred to as Time In Bed (lights off to lights on time)
- Scoring: Wake
- Scoring: N1
- Scoring: N2
- Scoring: N3
- Scoring: R
- *Scoring: N2-N3 is classic NREM (N2 is not typically reported)*

Determine/gauge amount of artifact

Method #1

Analysis → Event series → ECG.des → Artifact rate → Generate report

This calculates the Event Temporal Density – the number of events per minute of each event type – as a result file that can then be exported

Method #2

Tools → Profiler → ECG.des → Artifact occurrence or rate → Generate report

Ensure manual edits incorporated

Compare trend reports before and after manual ECG editing

PRANA HRV Training with EGI

Written by Florian Chapiot, software developer

Perform time-varying HRV analysis

PRANA has a dedicated software plug-in featuring a state-of-the-art HRV analysis for long-term recordings including an ECG signal. In contrast with other analysis packages designed for the analysis of short ECG recording sections (such as the traditional 5-min effort/resting test), PRANA offers a streamlined HRV analysis of consecutive ECG segments from fullnight or 24-h PSG recordings and provide all the analysis results as time series.

The algorithms used in this software plug-in are fully configurable through a graphical user interface and come with default settings selected for the analysis of standard human PSG recordings.

The HRV analysis is performed in multiple steps. First, the R-wave peaks are automatically detected. As an option, the result of automated R-wave detection can be manually edited. Finally, the resulting R-R intervals are analyzed resulting in a set of time- and frequency-domain parameters. All the resulting time series from HRV analysis can then be used with the software Export tools, the Trend Report Generators and the Profiler Time Series tools for visualization and further processing.

Automated heartbeat detection

Erroneous R-wave detections may result from an ECG signal contaminated by various kind of artifacts (motion, myographic activity, sensor detachment, etc.). Several available options may improve the R-wave detection:

- ECG signal filtering,
- ECG signal resampling to improve time accuracy,
- Different detection algorithms (R-wave peak default, QRS-complex centroid),
- Selection of time window duration to speed up detection (30-sec default),
- Adjustable heartbeat characteristics for support of multiple species (human default, rodents, etc.),
- Automated detection of artifacts present in the recorded ECG signal (through the Artifact detection plug-in).

Depending on the ECG signal quality and following the Cardiology Task Force recommendations (Circulation 1996), a manual inspection/editing of automatically detected R-waves, may be necessary prior to the analysis of the interbeat intervals.

Manual editing of the heartbeat detection results

After an automated detection, the resulting heartbeat are displayed directly on the ECG signal in the Traces panel of the Reviewer. These markers can be edited by activating the Reviewer Marking mode (Start pushbutton in the Event panel of the Reviewer Toolbar). With the automatic event type marking option selected, the following marking actions are possible:

- Inserting an additional R-wave peak marker by maintaing the shift key pressed and mouse-clicking on the peak of a visually identified R-wave that has been missed by automated detection,

- Deleting an R-wave peak erroneously detected by selecting the corresponding marker by mouse-click and then pressing the Del key,

- Marking an ECG signal artifact by mouse-clicking, dragging and dropping over the corresponding signal segment. If the resulting event is not of the artifact type, select it using the mouse and press the Shift + x keys to change its type to an artifact.

It is important not to re-run an automated heartbeat detection after manual editing as all manually edited R-wave peak markers would be deleted by doing so.

After a manual editing of the markers and artifacts on the ECG signal, the interbeat interval analysis needs to be carried out so that the results of manual editing are taken into account.

Interbeat interval analysis

Optimized for the analysis of PSG recordings usually scored for sleep stages into 30-sec epochs, the interbeat interval analysis of the PRANA HRV analysis plug-in is by default carried out on the whole scoring time range using consecutive 2-min epochs with a 75% overlap, which results in a time resolution identical to that of sleep stage scoring. This facilitates further merging and stage-wise sorting of the HRV time- and frequency-domain parameters obtained from HRV analysis.

Prior to the actual analysis of the interbeat intervals, the software plug-in scans the existing R-wave peak markers to identify events such as ectopic and missing beats, and sequences of tachycardia and bradycardia, according to certain user -selectable criteria. Prior to the interbeat interval analysis, ectopic beats identified from the existing R-wave peak markers are discarded from the R-R interval series in order to deduce the corresponding N-N (normal-to-normal) interval series, which is actually processed for time-and frequency-domain analysis.

In a similar way, and if the corresponding option is selected, R-R intervals overlapping with contaminated segments of ECG signal marked as an artifact, either by automated detection or manual editing, are also discarded prior to the N-N interval analysis.

Then, the actual N-N interval analysis is carried out on all consecutive time-epochs included in the recording under analysis.

Time-domain parameters are extracted directly for each consecutive epoch of the clean N-N interval series.

Except when the Lomb-Scargle method is selected (method designed for the analysis of irregularly-sampled series), frequency-domain analysis includes a further resampling (at 10 Hz by default) of the N-N interval series prior to the estimate of its frequency spectrum using various user-selectable methods (DFT default, Welch, etc.).