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 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 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 ≤ 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-NSD and I-SSD (P ≤ 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.

Keywords: sleep, Insomnia Disorder, polysomnography, cluster analysis, and phenotyping


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.1–4 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.1–4,6,21 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
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. 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). 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, 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, 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). 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: 1261200049875 (ANZCTR). All volunteers gave written informed consent.

**Clinical and Demographic Outcomes**

Demographic variables included: age, sex, body mass index (BMI kg/m²), education (highest attained), employment status, ethnicity, medication, alcohol consumption and smoking status and medical comorbidities including: heart disease, cancer, etc., 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), the 21-item version of the Depression Anxiety and Stress Scale (DASS), Epworth Sleepiness Scale (ESS), Flinders Fatigue Scale (FFS), 16-item version of the Dysfunctional Beliefs and Attitudes About Sleep scale (DBAS), and the Ford Insomnia Response to Stress Test (FIRST). 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.

**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 Embia (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. 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. It is recommended for smaller data
sets (100 patients) where groupings are examined in succes-
sive 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. Standardized (con-
verted to z-scores: see Milligan) objective sleep parameters,
including TST, SOL, and WASO were used. In order to derive
the number of clusters, a dendrogram (hierarchical tree dia-
gram used to represent the distance between cases of cluster
groupings) was used in combination with agglomeration find-
ings of the coefficients (see Table S1 in the supplemental mate-
rial). 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: neu-
rocognitive performance, and sleep-onset measures of quan-
titative EEG ($g$-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 cor-
tical and physiological hyperarousal, and the perception of
sleep during this process 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; sustained attention (Letter
Cancellation Test: LCT); mean correctly marked targets and
the trial duration of the second sustained attention and pro-
cessing speed task, and planning and problem solving (Tower
of London: ToL); percentage of errors and mean response la-
tency. 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 previ-
ously validated standard fast Fourier transform with a rectan-
gular weighted window for each non-overlapping 5-s epoch.
Primarily, C3 was chosen as it is traditionally recommended
for use in scoring sleep and has been used previously for
spectral analysis in insomnia research. A sleep-onset pe-
riod 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.

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. Manual artifact iden-
tification 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
biologically implausible. 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: ms²) and a low frequency (LF)/HF ratio were
employed.

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 $\chi^2$ 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 $g$-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 imple-
mented for each outcome variable with age and gender as co-
variates. 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.

<table>
<thead>
<tr>
<th>Table 1—Demographic, sleep parameters (used to cluster individuals), and clinical information between insomnia subgroups in the 2-cluster solution.</th>
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</thead>
<tbody>
<tr>
<td>Mean (± SD)</td>
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<tr>
<td>Age (y), mean (SD), range</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Total sleep time (min)</td>
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<td>Sleep onset latency (min)</td>
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<td>Wake time after sleep onset (min)</td>
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<td>Insomnia Severity Index</td>
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<td>Insomnia duration (y)</td>
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<tr>
<td>DASS - Depression</td>
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<tr>
<td>DASS - Anxiety</td>
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<td>DASS - Stress</td>
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<td>Flinders Fatigue Scale</td>
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<td>Epworth Sleepiness Scale</td>
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<tr>
<td>Pre-sleep DISS - Alert cognition</td>
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<tr>
<td>Pre-sleep DISS - Negative mood</td>
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<tr>
<td>Pre-sleep DISS - Positive mood</td>
</tr>
<tr>
<td>Pre-sleep DISS - Sleepiness / fatigue</td>
</tr>
<tr>
<td>Apnea-hypopnea index</td>
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<tr>
<td>Evening systolic blood pressure (mm Hg)</td>
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<tr>
<td>Morning systolic blood pressure (mm Hg)</td>
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<tr>
<td>Smoking status</td>
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<tr>
<td>Past smoker</td>
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<tr>
<td>Never smoker</td>
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<tr>
<td>Employment</td>
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<td>Full-time</td>
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<td>Part-time</td>
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<tr>
<td>Unemployed</td>
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<tr>
<td>Medical comorbidities</td>
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<tr>
<td>Cancer</td>
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<tr>
<td>Cancer</td>
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<td>High blood pressure</td>
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<tr>
<td>Neurologic</td>
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<td>Urinary</td>
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<td>Diabetes</td>
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<td>Chronic pain</td>
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<td>Gastrointestinal</td>
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<td>Thyroid</td>
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<td>Hypertension</td>
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<tr>
<td>Past smoker</td>
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<tr>
<td>Never smoker</td>
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<tr>
<td>Medication</td>
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<tr>
<td>Prescription sleep aid</td>
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<tr>
<td>Over the counter sleep aid</td>
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<tr>
<td>Prescription psychiatric medication</td>
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<tr>
<td>Other medication</td>
</tr>
</tbody>
</table>

Means, standard deviations (SD) and effect size (Cohen’s d, r for variables normally distributed or Cramer V for binary variables) for each cluster comparison are provided. *Welch statistic correction for violation of homoscedasticity. **Kruskal-Wallis test with median (inter-quartile range) for variables not normally distributed. 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.
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).

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

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

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.
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 ≤ 0.01). Before sleep onset, a reduction in beta-2 power was found in I-SSD B vs. I-SSD A (P = 0.07). 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 ≤ 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 difference clusters for the two 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). Third and most importantly, to test the external validity of the clusters we evaluated for between cluster differences in functional outcomes that were not used to build the clusters including neurocognitive performance and sleep-onset measures of HRV and qEEG.

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

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

<table>
<thead>
<tr>
<th>Mean (± SE)</th>
<th>Overall</th>
<th>I-NSD Cluster</th>
<th>I-SSD A Cluster</th>
<th>I-SSD B Cluster</th>
<th>ANCOVA F (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Back 2: Percentage of total accuracy</td>
<td>85.3 (1.5)%</td>
<td>86.5 (1.8)%</td>
<td>87.1 (2.6)%</td>
<td>82.3 (3.3)%</td>
<td>0.73 (0.485)</td>
</tr>
<tr>
<td>N-Back 2: Total number incorrect</td>
<td>5.5 (0.6)</td>
<td>5.2 (0.7)</td>
<td>4.4 (1.1)</td>
<td>6.9 (1.3)</td>
<td>1.10 (0.337)</td>
</tr>
<tr>
<td>N-Back 2: Total number missed</td>
<td>11.0 (1.7)</td>
<td>10.5 (2.1)</td>
<td>14.3 (2.9)</td>
<td>8.1 (3.7)</td>
<td>0.93 (0.398)</td>
</tr>
<tr>
<td>LCT: Mean of correctly marked targets</td>
<td>50.7 (1.7)</td>
<td>52.1 (2.1)</td>
<td>56.3 (3.1)</td>
<td>43.6 (3.8)</td>
<td>3.24 (0.045)*</td>
</tr>
<tr>
<td>LCT: Final trial duration (sec)</td>
<td>320.9 (75.0)</td>
<td>327.9 (90.8)</td>
<td>307.8 (135.9)</td>
<td>326.9 (167.9)</td>
<td>0.73 (0.488)</td>
</tr>
<tr>
<td>ToL: Percentage of errors</td>
<td>31.2 (2.1)%</td>
<td>33.9 (2.6)%</td>
<td>33.3 (3.6)%</td>
<td>26.4 (4.7)%</td>
<td>1.02 (0.367)</td>
</tr>
<tr>
<td>ToL: Mean response latency (sec)</td>
<td>11.4 (71.6)</td>
<td>10.5 (0.9)</td>
<td>13.2 (1.3)</td>
<td>10.6 (1.6)</td>
<td>1.52 (0.225)</td>
</tr>
</tbody>
</table>

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.
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: ms²) 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. In a clearly defined I-SSD phenotype, reduced parasympathetic activity may contribute to adverse cardiometabolic health outcomes found in I-SSD. 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. 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 compared to associations from cross-sectional samples of patients. 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. 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 Krystal et al. also showed reduced Beta (16.5–30.0 Hz) power in patients with objective I-SSD compared to I-NSD. Heightened cortical arousal may be higher in those with I-NSD, contribute to subjective insomnia and is linked to nonrestorative sleep. 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. 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. 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 ≤ 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. Daytime impairments have been found to be of small-to-moderate magnitude in studies of individuals with insomnia compared to controls. 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. 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. 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.

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