

Title: An objective short sleep insomnia disorder subtype is associated with reduced brain metabolite concentrations *in vivo*: a preliminary magnetic resonance spectroscopy assessment

Subtitle: Reduced brain metabolites in short sleep insomnia

Christopher B. Miller, PhD ^{1,2}; Caroline D. Rae, PhD ^{3,4}; Michael A. Green, PhD ³; Brendon J. Yee, MBCHB, PhD ^{1,2,5}; Christopher J. Gordon, PhD ^{1,6}; Angela L. D’Rozario, PhD ^{1,7}; Simon D. Kyle, PhD ⁸ Colin A. Espie, PhD, DSc ⁸; Ronald R. Grunstein, MD, PhD ^{1,2,5}; Delwyn J. Bartlett, PhD ^{1,2}

¹ Woolcock Institute of Medical Research, CIRUS, Centre for Sleep and Chronobiology, Sydney, NSW, Australia; ² Sydney Medical School, The University of Sydney, Australia; ³ Neuroscience Research Australia, Randwick, Australia; ⁴ School of Medical Sciences, The University of New South Wales, Australia; ⁵ Department of Respiratory and Sleep Medicine, Royal Prince Alfred Hospital, Sydney Local Health District, Sydney, NSW, Australia; ⁶ Sydney Nursing School, The University of Sydney, Australia; ⁷ School of Psychology, Faculty of Science, Brain and Mind Centre and Charles Perkins Centre, The University of Sydney, NSW, Australia; ⁸ Nuffield Department of Clinical Neurosciences, The University of Oxford, UK

Corresponding Author: Christopher B. Miller, Woolcock Institute of Medical Research, The University of Sydney, Sydney, Australia. Postal address: PO Box M77, Missenden Road, NSW, 2050
Call: +61 (2)9114 0411; Fax: +61 (2) 9114 0014; Email: chris.miller@sydney.edu.au

Abstract

Objectives: To evaluate brain metabolites in objective insomnia subtypes defined from polysomnography (PSG): insomnia with short sleep duration (I-SSD) and insomnia with normal sleep duration (I-NSD); relative to good sleeping controls.

Methods: PSG empirically grouped insomnia patients into I-SSD ($n=12$: mean (SD) total sleep time (TST) = 294.7min (30.5)) or I-NSD ($n=19$: TST = 394.4min (34.9)). ^1H magnetic resonance spectroscopy acquired in the left occipital cortex (LOCC), left prefrontal cortex and anterior cingulate was used to determine levels of creatine, aspartate, glutamate and glutamine (referenced to water). Glutathione, glycerophosphocholine, lactate, myoinositol and N-acetylaspartate measurements were also obtained. Sixteen good sleeping controls were included for comparison. Multivariate analysis of variance was used to evaluate differences in creatine, aspartate, glutamate, and glutamine.

Results: Aspartate and glutamine concentrations were reduced in the LOCC in I-SSD compared with I-NSD (both $p<.05$, $d = 0.80$ -.99). Creatine displayed a non-significant mean reduction in I-SSD compared with I-NSD ($p = .05$, $d = 0.58$). Glutamine was reduced in I-SSD compared with controls ($p<.05$, $d = 0.93$). There were no differences in metabolites between all (I-SSD plus I-NSD) insomnia patients and controls. In patients with insomnia, LOCC glutamine concentrations were found to be positively correlated with TST ($r = 0.43$, $p<.05$) and negatively correlated with wake-time after sleep onset ($r = -0.40$, $p<.05$).

Conclusions: Results indicate that I-SSD is associated with reduced brain metabolites in the LOCC compared with I-NSD and control concentrations of aspartate, glutamine, and creatine.

Keywords

Sleep and the Brain, Insomnia, Magnetic Resonance Spectroscopy, Brain imaging, and Phenotyping.

Clinical trial registration

Insomnia Magnetic Resonance Spectroscopy (MRS) imaging sleep study: Australia New Zealand

Clinical Trials Registry (ANZCTR) URL:

<https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?ACTRN=12612000050853>

Trial identification number: 12612000050853.

Statement of Significance

This study measured brain metabolites in objectively defined subtypes of short and normal-sleep duration insomnia.

Insomnia patients with short objective sleep duration, defined from polysomnography had reduced concentrations of glutamine, aspartate and creatine compared with insomnia patients with normal sleep duration and good sleeping controls.

Glutamine, a major substrate for GABA synthesis, was reduced in short sleep duration insomnia. In the overall insomnia group, glutamine concentrations were positively correlated with sleep duration and negatively correlated with wake-time after sleep onset.

The overall group of insomnia patients did not have changes in brain metabolites compared with good sleeping controls.

Introduction

Insomnia is associated with increased cognitive and physiological hyperarousal, inhibiting sleep, affecting daytime performance and impairing health-related quality of life.¹⁻³ Two subtypes of insomnia have been proposed from total sleep time (TST) measured by polysomnography (PSG), and these groups differ on clinically meaningful outcomes.⁴ Insomnia with short sleep duration (I-SSD) may be a more biologically severe subtype of insomnia associated with increased cognitive and physiological hyperarousal compared to insomnia with normal sleep duration (I-NSD).^{4, 5} Previously, we used quantitative EEG to investigate cerebral hyperarousal at sleep onset and observed differences between subtypes of patients with insomnia.⁶ As magnetic resonance spectroscopy (MRS) may infer cerebral hyperarousal through measurement of metabolite concentrations across the brain,⁷ insomnia PSG-defined subtypes may differ in brain metabolites using MRS.⁸

In the first use of MRS in insomnia, whole brain levels of γ -aminobutyric acid (GABA) were reduced in 16 insomnia patients (mean (SD) PSG-TST = 354 (59) mins) compared with 16 good sleeping controls (GSC).⁹ Lower GABA levels were correlated with longer time awake during the night from PSG in insomnia. This is in line with the hyperarousal model of insomnia with GABA as the principal inhibitory neurotransmitter promoting sleep.^{9, 10} Consistent with this finding, lower GABA levels (parieto-occipital-cortex) were identified in 27 patients with post-traumatic stress disorder (PTSD) compared with 18 trauma-exposed controls without PTSD. Higher insomnia severity index (ISI) scale scores were associated with lower GABA and higher glutamate (principal excitatory neurotransmitter) levels in these patients.¹¹ However, MRS GABA studies in insomnia have not been consistent. One study quantifying insomnia by symptoms observed reduced GABA in the occipital and anterior cingulate cortices in 20 insomnia patients compared with 20 controls.¹² In contrast, other work identified increased GABA in 16 insomnia patients (mean (SD) ambulatory PSG-TST = 302 (18) mins) compared with 17 controls in the occipital cortex.¹³ Higher GABA levels were associated with lower sleep duration in the insomnia group. Methodological differences between studies were suggested to explain these inconsistent findings.¹⁰ Recent work evaluated diurnal effects (8:00–9:00

AM and 10:00–11:00 PM MRS assessments) of GABA and glutamate/glutamine levels in 20 insomnia patients compared with 20 good sleepers in the anterior cingulate and dorsolateral prefrontal cortices.⁸ GABA levels did not change across the day and were not different compared with controls. However, higher anterior cingulate levels of GABA were associated with increased PSG-sleep duration on the second night suggesting a trait marker of objective sleep disturbance. Importantly, the mean sleep length of the insomnia patients in this study was approximately six hours, suggesting the value of investigating patients with shorter sleep duration insomnia for future MRS work.⁸

Further brain metabolites may also have a role in insomnia pathophysiology.⁹ Phosphorus MRS imaging found reduced phosphocreatine in insomnia ($n=16$) compared with controls ($n=16$) suggesting alteration in the adenosine triphosphate / adenosine diphosphate ratio consistent with hyperarousal.¹⁴ Creatine is a marker of energy metabolism in both neurons and astrocytes^{15, 16} and concentrations have been shown to be reduced in patients with obstructive sleep apnea.¹⁷⁻¹⁹ Glutamine, a major substrate for GABA synthesis,^{20, 21} is increased with benzodiazepine administration²² and decreased in depressive disorder (reflecting glutamatergic dysfunction).²³ Glutamine is synthesized from glutamate and both are correlated, with signal overlaps making them difficult to quantify separately *in vivo*.^{24, 25} Both glutamate and glutamine appear important in insomnia as concentrations have been linked to sleep loss in patients with restless legs syndrome ($n=28$),²⁶ and microinjection of glutamate in rats was associated with enhanced slow wave sleep.^{27,28} Previously, an indirect assessment of GABAergic and glutamatergic activation in insomnia patients ($n=18$) using transcranial magnetic stimulation, found increased activation of glutamatergic but not GABAergic mechanisms compared with controls ($n=10$).²⁹ Other work did not find differences in MRS glutamate or glutamine concentrations when compared with controls. However, these studies were not optimized to detect glutamate and glutamine concentrations.^{9, 12, 13, 30} In this study, we used an asymmetric point-resolved spectroscopy (PRESS) sequence to more specifically measure both glutamate and glutamine concentrations.^{24, 25, 31} Given the clinical differences between short and

normal sleep duration insomnia, we aimed to examine brain concentrations of creatine, aspartate, glutamate and glutamine in these two insomnia subtypes and controls.

Methods

Participants

All participants were recruited from a volunteer database, advertisements in clinic, online and in the local community and were initially screened over the telephone using a standardized assessment tool (based on Morin and Espie, 2003).³² Eligible insomnia patients 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 as specified by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition.³³ This study was part of a larger insomnia phenotyping project (clinical trial registration (ANZCTR): 12612000049875).⁶

Sleep

For insomnia patients only, one night of PSG was used to define sleep and objectively group patients into I-NSD and I-SSD subtypes through an empirical hierarchical cluster analysis. Ward's method (squared Euclidean distance) was used and identified two insomnia subgroups according to standardized (z-scores) clustering inputs of TST, sleep onset latency (SOL) and wake-time after sleep onset (WASO).⁶ These variables (at least two must be used) were chosen based on previous insomnia phenotyping literature.^{5, 34} Cluster analysis is useful as it provides a non-subjective demarcation of patient cut-points from PSG.³⁵ To measure sleep, we applied a specific research montage [EEG: F3, Fz, F4, C3, Cz, C4, Pz, O1, Oz, O2, all signals used ground at FPz and common reference at CPz], electrooculographic (horizontal and vertical), electrocardiographic and electromyographic (submental) recordings. Data were recorded on the Embla (Mortara, Broomfield, CO) system at 512 Hz and scored visually by one experienced sleep scorer to American Academy of Sleep Medicine criteria.³⁶ Each study was also independently checked for quality assurance by another independent expert scorer. Insomnia patients attended the laboratory between

approximately 17:00-18:00, were set-up between 20:00-21:00 and selected when to switch out the lights to initiate sleep. Participants refrained from alcoholic and caffeinated beverages on arrival to the sleep laboratory and lights were turned on at 06:00 which is standard in our laboratory.

Approximately 2-weeks after the overnight PSG, a sub-sample of insomnia patients underwent brain imaging if they met the following specific criteria: males and females aged between 21-55 years with insomnia specifically: difficulty initiating or maintaining sleep or waking up too early for 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). Insomnia patients were included with apnea-hypopnea index (AHI) <12 and periodic limb movement (PLM) arousal index scores of <5 from PSG. In line with research diagnostic criteria for insomnia,³⁷ exclusion criteria included: illicit substance or alcohol/caffeine dependence, severe or unstable psychiatric disorders (including those with major depressive disorder), a known sleep disorder other than insomnia, cognitive impairment, and pregnancy or lactation, overnight shift workers and recent time-zone travel (within the last 2-months), actively treated sleep disorder (e.g., cognitive behavior therapy). Chronic hypnotic use was exclusionary (>3 times per week). Those with occasional hypnotic use (1-3 times per week) at telephone screening were included but asked to refrain from hypnotic medication (under guidance from the Sleep Physician) throughout the study. The insomnia severity index (ISI)³⁸ was used to define insomnia symptom severity.

GSC were recruited to the insomnia patients by gender and age (within +/-3 years). In line with research diagnostic criteria,³⁷ GSC were defined by the absence of a sleep complaint, past diagnosis of a sleep or psychiatric or unstable medical disorder and the endorsement of good sleep quality that was restorative and stable across the night (22:00-08:00 +/- 2 hours) and reported a normal SOL (<15 minutes), WASO (<15 minutes), TST of >6 hours, a high sleep efficiency (%SE >85%) and low amount of night time awakenings (≤2) at telephone screening.³² Any medication known to

impact sleep was exclusionary. If deemed suitable from telephone screening, all participants recorded daily sleep diaries (similar to Morin and Espie, 2003)³² and wore actigraph watches to verify stability of sleep timing (Actiwatch-2, Philips Respironics Mini-Mitter, Bend, OR, USA): see Figure 1 for an overview. All data were collected at the Woolcock Institute of Medical Research, University of Sydney and Neuroscience Research Australia, Australia. All scans were standardized to the afternoon-early evening. This study was reviewed and approved by the Royal Prince Alfred Hospital Ethics Review Committee, Sydney, Australia (Protocol No X11-0392 and HREC/11/RPAH/620); clinical trial registration number: 12612000050853 (ANZCTR). All participants gave written informed consent.

Clinical and demographic outcomes

Demographic variables included: age, sex, body mass index (BMI kg/m²), duration of insomnia symptoms (years) and were assessed by a self-report electronic questionnaire prior to the overnight sleep study. Insomnia-related patient reported questionnaires were also captured prior to sleep during the in-lab visit by electronic questionnaire including: the ISI,³⁸ the 21-item version of the depression anxiety and stress scale (DASS),³⁹ Epworth sleepiness scale (ESS),⁴⁰ Flinders fatigue scale (FFS),⁴¹ 16-item version of the dysfunctional beliefs and attitudes about sleep scale (DBAS),⁴² and the Ford insomnia response to stress test (FIRST).⁴³

Magnetic resonance spectroscopy

Proton ¹H-MRS was used as a measure of relative intracerebral concentrations of metabolites *in vivo*. To measure metabolites, we used an optimized approach for spectral acquisition which allows better discrimination of closely related resonances of glutamine, glutamate, creatine, and aspartate (with relatively small estimation errors).³¹ An asymmetric PRESS sequence (a modification of Snyder & Wilman, 2010²⁵ see Rae et al., 2012)³¹ was used with an echo time (TE) of TE1 = 25ms, TE2 = 85ms and a repetition time of 2 seconds. Alongside this, an additional short echo PRESS sequence was also acquired (TE = 32ms). All spectra were acquired at 3T (Achieva TX, Philips,

Best, The Netherlands) using a 32 channel head coil with concomitant water reference spectra. In line with previous findings, three regions of interest (ROI; 2x2x2cm) were selected *a-priori*. The left occipital cortex (LOCC) was selected as the primary site of interest as differences in GABA were previously detected here in insomnia.^{9, 12, 13} Secondary exploratory locations of interest, including the middle anterior cingulate (ACC) and the left prefrontal (LPFC) cortices, were assessed in line with previous insomnia literature.^{8, 12, 30, 44-48} Acquired T1 images (repetition time, TR = 2530 ms; echo time [TE] = 3.65 ms; flip angle = 7°; slice thickness = 1 mm; 224 slices; field-of-view = 256 × 176 mm²; matrix size = 256 × 256) were used to guide the placement of the ROI for MRS measurements: see Figure 2 (a).

All spectra were visually inspected for quality, including evidence of patient movement and the presence of artifacts⁴⁹, and poor shimming based on line width assessment. Acceptable spectra were fitted by CDR blind to participant group with jMRUI (v 4, build 162) using the QUEST algorithm,⁵⁰ with metabolite basis sets simulated for the appropriate sequence timings with NMR SCOPE, relying on published chemical shift and coupling constant information.⁵¹ Creatine, aspartate, glutamate, and glutamine were acquired and assessed. Glycerophosphocholine, lactate, glutathione, myoinositol, and N-acetylaspartate were also fitted for spectra. All identified metabolites were referenced to water: see Figure 2 (b).⁵² Spectroscopy ROIs were assessed for relative contributions of grey and white matter using a tool for partial volume estimation of Philips data.⁵³ After spectroscopy acquisition, we also obtained high angular resolution diffusion-weighted acquired with diffusion weighting ($B = 2400 \text{ s/mm}^2$) along 61 non-collinear directions for structural white matter assessment, and an echo planar imaging resting state time series.

Brain volumetric assessment

Brain volumes were calculated after being segmented and parcellated using Freesurfer (V5.3.1).^{54, 55} Whole brain volumes were calculated and normalized for brain size using SIENAX,⁵⁶ part of FMRIB's Software Library⁵⁷ and used to calculate relative volumes for ROIs.

Statistical analysis

Differences in demographic, clinical, sleep and brain volumetric variables were assessed through univariate one-way analysis of variance (ANOVA) or chi-squared tests between groups for insomnia subtypes (I-SSD, I-NSD) vs. GSC (Table 1). For brain volumetric variables only, intracranial volume was instated as a covariate. Between subjects one-factor multivariate analysis of variance (MANOVA) was used to evaluate creatine, aspartate, glutamate and glutamine concentrations. MANOVA is the best approach as it allows exploration of complex related data between groups for multi-dependent variables (e.g., Huberty and Olejnik; Meyers et al.).^{58, 59} MANOVA was used for each of the three brain ROIs and compared insomnia subtypes (I-SSD, I-NSD) vs. GSC and insomnia vs. GSC. Follow-up pairwise comparisons using the least significant difference method assessed individual metabolites between groups. Effect size scores (d) were calculated for group comparisons. Exploratory correlations assessed the association between metabolites and objective sleep metrics (TST, SOL, and WASO). Alpha was set at $<.05$. We measured potential confounders including, age, sex, time of scanning, handedness, female menstrual cycle and occasional self-report hypnotic use.⁸ All data were analyzed (by CBM) using Statistical Package for the Social Sciences (SPSS) software (IBM v 22.0.0; IBM Corp, Armonk, NY, USA).

Results

Participant characteristics

Thirty-one patients with insomnia ($n=21$ (68%) female; average age (SD) = 37.5 (9.9)) and 16 GSC ($n=11$ (69%) female; average age (SD) = 37.3 (9.6)) participated in this study. Due to patient-control recruitment, the control group was very similar to the insomnia groups for mean age and sex distribution and BMI (see Table 1). Seven-fourteen day sleep diaries confirmed subjective sleep disturbance in insomnia subtypes with stable sleep timing from actigraphic recordings (Table 2).

Good sleep quality was observed in GSC with significant and healthy scores for the Pittsburgh sleep quality index (PSQI) compared with insomnia patients ($p < .001$). As expected, patients with insomnia had higher ISI and DASS depression, anxiety, and stress scale scores than GSC (see Table 1). Although the DASS depression and anxiety scores were higher compared with controls, they were still in the normal-to-mild range, and the stress scores were clinically elevated. The ISI scores were in the moderate range of insomnia severity. The average duration of insomnia was 11.9 (range 1-20) years. PSG data are presented in Table 3 for the insomnia patients only. Insomnia patients were all grouped into either I-NSD or I-SSD subtypes through an empirical cluster analysis using TST, SOL, and WASO from PSG.⁶

Creatine, aspartate, glutamate and glutamine

For creatine, aspartate, glutamate, and glutamine; we report data from 30 patients with insomnia out of the 31 who consented into the study and 16 GSC. The first patient was treated as a pilot and ROI placement was subsequently improved and so the spectra from this patient could not be included in the analysis. For the LOCC only, on checking the distribution of the dependent variables, a clear outlier (with low values) from the I-SSD group was detected across the four variables (creatine = 8.2; aspartate = 0; glutamate = 7.6; glutamine = 2.3 parts per million: ppm). On inspection of this patient, this was attributed to movement during this acquisition. To be statistically

conservative, the following data reported exclude this patient, however; a sensitivity analysis including this patient did not change the results.

Insomnia subtype analysis

Using data from the asymmetric PRESS acquisitions, the LOCC approached significance (Wilks' $\lambda = .707$, $F(8, 78) = 1.85$; $p = .081$; partial $\eta^2 = .159$) and follow-up individual variable comparisons were undertaken. Creatine displayed a non-significant mean reduction in I-SSD vs. I-NSD only ($p = .05$, $d = 0.58$: see Figure 3 and Table 4). Aspartate and glutamine were reduced in I-SSD vs. I-NSD ($p < .05$, $d = 0.80-.99$). Glutamine was reduced in I-SSD vs. GSC ($p < .05$, $d = 0.93$): see Table 4. For the secondary exploratory ROIs, there were no significant differences from MANOVA between insomnia subtypes (I-NSD vs. I-SSD) and GSC at the LPFC (Wilks' $\lambda = .872$, $F(8, 80) = 0.71$; $p = .682$; partial $\eta^2 = .066$) or ACC (Wilks' $\lambda = .863$, $F(8, 80) = 0.77$; $p = .633$; partial $\eta^2 = .071$).

Potential confounders including time of scanning, handedness, female menstrual cycle and self-reported occasional hypnotic use in insomnia patients were not associated with either insomnia or brain metabolites. Age was associated with brain metabolites and a subsequent sensitivity analysis adjusting for age for the LOCC gave similar results to the unadjusted results presented here (Wilks' $\lambda = .666$, $F(8, 76) = 2.14$; $p = .041$; partial $\eta^2 = .184$), with creatine and glutamine both reduced in I-SSD compared to both I-NSD ($p = .014$ and $p = .028$, respectively) and GSC ($p = .013$ and $.046$). Aspartate was no longer significantly reduced in I-SSD compared to I-NSD ($p = .054$).

There were no differences for any metabolites at any of the ROIs using the standard short PRESS sequence. Figure S1 displays the untested metabolites including N-acetylaspartate, glycerophosphocholine, lactate, and myoinositol acquired at the LOCC between groups. Glutathione was left out as this measure remains to be validated in asymmetric PRESS.

Insomnia vs. good sleeping controls

There were no significant differences between the overall insomnia sample and GSC for creatine, aspartate, glutamate and glutamine in the LOCC using the asymmetric PRESS acquisitions (Wilks' $\lambda = .950$, $F(4, 40) = .528$; $p = .716$; partial $\eta^2 = .050$); LPFC (Wilks' $\lambda = .931$, $F(4, 41) = .561$; $p = .561$; partial $\eta^2 = .069$); and ACC (Wilks' $\lambda = .949$, $F(4, 41) = .550$; $p = .700$; partial $\eta^2 = .051$): see Table 4. There were also no differences for any metabolites at any of the secondary ROIs using the standard PRESS sequence.

Exploratory associations with objective sleep parameters in insomnia

In patients with insomnia, only glutamine concentrations were found to be significantly correlated with sleep parameters at the LOCC. TST was positively correlated ($r = 0.43$, $p < .05$) and WASO was negatively correlated ($r = -0.40$, $p < .05$). SOL did not correlate with glutamine ($p = .704$): see Figure S2.

Voxel volumetric assessment

There were no between-group differences (insomnia vs. GSC) for univariate analyses of variance for grey and white matter volumes in the LOCC, LPFC or ACC ($F(1, 42-43) = 0.00-3.24$, $p = .079-991$). The ACC only displayed a trend for significance in grey matter volume ($p = .079$). Similarly, no differences were found between insomnia subtypes (I-SSD, I-NSD) and GSC ($F(2, 41-42) = 0.01-1.64$, $p = .206-822$).

Discussion

Clinical insomnia research has shown differences between patients with short and normal sleep duration insomnia.⁴ Consistent with these findings, our study shows reduced concentrations of brain metabolites in the left occipital cortex (LOCC) in those with insomnia and short sleep duration compared with normal sleep duration insomnia and good sleeping controls. These findings were most apparent for glutamine concentrations. In the overall insomnia sample, LOCC glutamine concentrations were positively correlated with total sleep time and negatively correlated with wake-time after sleep onset. We also found a reduction in aspartate and a non-significant reduction in creatine in the LOCC for those with short sleep duration insomnia. The LOCC appears to be a key region differentiating GABA concentrations in insomnia and controls in previous studies,^{12, 13} and now for further brain metabolite variables in short sleep duration insomnia. Although we did not find differences in brain metabolites between good sleeping controls and combined normal and short sleep insomnia patients, using MRS in different PSG-determined sleep duration insomnia subtypes may allow us to further understand the pathophysiology of insomnia.⁴

Glutamine is important as it is a major substrate for the synthesis of GABA.^{20, 21} GABA was reported to be reduced in insomnia^{9, 12} but these studies did not detect differences in glutamine.^{9, 12, 13, 30} Glutamine is difficult to measure with standard PRESS sequence timings.^{24, 25, 31} In the present study, metabolite concentrations were measured using a relatively long echo asymmetric PRESS sequence. We did not detect metabolite differences using a short echo PRESS sequence. Glutamine measurement using short echo PRESS is sub-optimal.²⁵ The longer asymmetric PRESS sequence was specifically designed to detect glutamine with improved accuracy^{25, 31} Results are in line with previous work as they suggest glutamine concentrations may only be reduced in a specific insomnia with short sleep duration subtype. Our findings are potentially valuable as they link sleep duration to glutamine concentrations in insomnia. As benzodiazepines are known to increase concentration ratios of glutamine, glutamate, and GABA,²² we speculate that phenotyping insomnia patients using

MRS may help delineate the role of benzodiazepines for the treatment of short sleep duration insomnia.⁵

Previous insomnia MRS studies have shown mixed results, with both higher¹³ and lower¹² LOCC concentrations of GABA using creatine as the reference metabolite. Short echo PRESS measures total creatine which is a composite of phosphocreatine and creatine. At longer echo times, such as with our asymmetric PRESS (TE = 110ms), the creatine signal is more revealing of changes in the ratio of creatine to phosphocreatine due to the relative difference in T2 relaxation times.⁶⁰ Creatine concentration differences in insomnia subtypes may explain these inconsistent findings. Further measurement of these metabolites and GABA, independent of creatine in subtypes of insomnia is required to verify the reliability of these initial findings. Other work also observed increased GABA levels associated with longer PSG-defined sleep duration, suggesting a possible trait marker of sleep length.⁸ These findings were made in the anterior cingulate cortex and the LOCC was not investigated. In our study, the left medial prefrontal cortex, the middle anterior cingulate cortex, and the LOCC were all explored and only between-group differences in the LOCC were identified. However, the LOCC was not associated with grey or white matter differences. This further supports the need to include the LOCC for assessment in relation to insomnia subtypes. Consistent with this study, reduced phosphocreatine levels have previously been identified in insomnia suggesting alteration in the adenosine triphosphate / adenosine diphosphate ratio consistent with hyperarousal.¹⁴ Whether or not these changes occur prior to the development of insomnia or reflect an adaptive response remains unanswered.

There are a number of limitations associated with this study. Causality regarding sleep and brain metabolite concentrations is difficult to determine given the nature of the cross-sectional study design. We identified a relatively small number of patients with I-SSD, based on one night of PSG limiting the generalization of these findings; however, the overall sample size is comparative to previous samples of insomnia patients using MRS. A Type I statistical error is plausible with the use

of multiple exploratory MANOVA's across the three locations of the brain for each of the two hypotheses. A lack of statistical power may have caused a Type II error and results require verification in larger groups of patients with I-SSD. We used multiple follow-up comparisons for each dependent variable without correction on a MANOVA with a non-significant trend ($p = .081$). We believe this is appropriate given the use of MANOVA as a non-specific omnibus test with our *a-priori* hypothesis and the findings of the LOCC in previous insomnia MRS studies. Insomnia patients were grouped using cluster analysis, a nonsubjective data-driven method of generating short and normal sleep duration phenotypes from PSG-derived measures of TST, SOL and WASO.⁶ We did not use a more traditional six-hour cut-point in PSG sleep duration to define subtypes⁵ and cluster classification error may account for findings. A sensitivity analysis using a six-hour cut point in TST to define subtypes, however, suggested similar results as reported here with significant reductions in I-SSD creatine and glutamine concentrations compared to both I-NSD and controls in the LOCC. Aspartate and glutamate were not significantly reduced. Scanning time limitations did not allow the quantification of GABA. Measurement of GABA may have enhanced the interpretation of study findings. Unlike previous MRS insomnia studies we used water as the denominator to infer absolute metabolite concentrations and not creatine.^{8, 9, 12, 13} Use of the water resonance reduces variability in ¹H-MRS measurements.⁵² Previous studies using creatine as a reference assumed it to be stable which may not be the case from results presented here. Uncertainty in the measurement of aspartate may be a source of error and this metabolite should be interpreted with caution.

Insomnia patients were included with AHI <12, AHI was not different between cluster groups, did not correlate with metabolites, and when added to the model as a covariate it did not change our findings. A further limitation was a lack of PSG evaluation in our control group and we are unable to rule out the presence of occult sleep disorders in this group. However, we believe this was mitigated as multiple assessments were undertaken including telephone screening, actigraphy, sleep diaries, and questionnaires. We did not have access to a group of participants who were sleep restricted but otherwise healthy and cannot clarify if results here are due to insomnia or short sleep

duration. Confounders including female menstrual cycle may have affected results. Out of 21 females who reported a regular menstrual cycle, 14 were in the follicular phase and six in the luteal phase (it was not possible to code one response) at the time of scanning; yet brain metabolites were not associated with cycle phase or insomnia. We did not conduct toxicology to rule out medications that may have affected sleep or brain metabolites and relied upon self-report. Five insomnia patients self-reported occasional hypnotic use ($n=2$ from I-SSD and $n=3$ from I-SSD) but this was not associated with insomnia subtype or metabolites, and insertion of this variable into the model produced similar results. Scans were conducted in the early afternoon (12:00) to mid-evening (19:00) and most occurred at about 16:00. Time of scanning was not associated with metabolites, in line with previous findings,^{8, 61} or insomnia and did not change the results of the model.

Conclusion

Brain metabolites appear reduced in a specific subtype of insomnia with PSG-defined short objective sleep duration for measures of aspartate, glutamine, and creatine in the left occipital cortex only. Reduced glutamine was associated with lower total sleep time and increased wake-time after sleep onset from PSG. There were no differences in metabolites between the entire insomnia patient group and good sleeping controls. Future studies evaluating brain metabolites in objective short sleep duration insomnia subtypes will further clarify the role of brain metabolites in the left occipital cortex.

Abbreviation list

ACC, anterior cingulate cortex

AHI, apnea-hypopnea index

ANOVA, analysis of variance

BMI, body mass index

d, Cohen's *d*

DASS, depression anxiety and stress scale

DBAS, dysfunctional beliefs and attitudes about sleep scale

EEG, electroencephalography

ESS, Epworth sleepiness scale

First, Ford insomnia response to stress test

FFS, Flinders fatigue scale

GABA, γ -aminobutyric acid

GCTI, Glasgow content of thoughts inventory

Gln, Glutamine

GSES, Glasgow sleep effort scale

GSC, good sleeping controls

HAS, hyperarousal scale

I-NSD, insomnia with normal sleep duration

ISI, insomnia severity index

I-SSD, insomnia with short sleep duration

LOCC, left occipital cortex

LPFC, left medial prefrontal cortex

M, mean

MANOVA: multivariate analysis of variance

MRS, magnetic resonance spectroscopy

N, n, number

N1: stage 1 of sleep

N2: stage 2 of sleep

NREM, non-rapid eye movement sleep

PLM, periodic limb movement

ppm, parts per million

PRESS, Point-resolved spectroscopy

PSG, polysomnography

PSQI, Pittsburgh sleep quality index

PTSD, post-traumatic stress disorder

REM, rapid eye movement sleep

ROI, region of interest

SD, standard deviation

SE, standard error

SOL, sleep onset latency

SWS, slow wave sleep

TE, echo time

TR, repetition time

TST, total sleep time

WASO, wake-time after sleep onset

Acknowledgements

We wish to thank all of the patients and participants who volunteered for this research. This research was supported by the National Health and Medical Research Council (NHMRC, Australia) Centre for Integrated Research Understanding of Sleep (CIRUS), 571421 (to CBM, DJB, ALD, BJY, CAE and RRG); NeuroSleep, 1060992 (to CBM, DJB, ALD, BJY, CAE and RRG); the Cooperative Research Centre for Alertness, Safety and Productivity, Australian Commonwealth Government (to CBM, DJB, CJG, ALD, BJY and RRG); and the Australian National Imaging Facility (MAG).

We also wish to also acknowledge Drs N. Goulden and P. Mullins of Bangor University for the Partial Volume code for Philips MRS data.

Disclosure Statements

This was not an industry supported study. Dr Espie is a shareholder in Big Health/ Sleepio Ltd, has acted as a consultant for Warnford Wellness Ltd and the American Heart Association, and receives royalties from Little Brown Publishers. None of these are related to the present study and do not represent conflicts of interest. Likewise, all other authors report no conflicts of interest.

References

1. Bonnet MH, Arand DL. Hyperarousal and insomnia: state of the science. *Sleep medicine reviews* 2010;14:9-15.
2. Riedel BW, Lichstein KL. Insomnia and daytime functioning. *Sleep medicine reviews* 2000;4:277-98.
3. Kyle SD, Morgan K, Espie CA. Insomnia and health-related quality of life. *Sleep medicine reviews* 2010;14:69-82.
4. Vgontzas AN, Fernandez-Mendoza J, Liao D, Bixler EO. Insomnia with objective short sleep duration: the most biologically severe phenotype of the disorder. *Sleep medicine reviews* 2013;17:241-54.
5. Fernandez-Mendoza J. The insomnia with short sleep duration phenotype: an update on it's importance for health and prevention. *Curr Opin Psychiatry* 2017;30:56-63.
6. Miller CB, Bartlett DJ, Mullins AE, et al. Clusters of Insomnia Disorder - an exploratory cluster analysis of objective sleep parameters reveals differences in neurocognitive functioning, quantitative EEG and heart rate variability. *Sleep* 2016;39:12.
7. Riemann D, Nissen C, Palagini L, Otte A, Perlis ML, Spiegelhalter K. The neurobiology, investigation, and treatment of chronic insomnia. *The Lancet Neurology* 2015;14:547-58.
8. Spiegelhalter K, Regen W, Nissen C, et al. Magnetic Resonance Spectroscopy in Patients with Insomnia: A Repeated Measurement Study. *PloS one* 2016;11:e0156771.
9. Winkelman JW, Buxton OM, Jensen JE, et al. Reduced brain GABA in primary insomnia: preliminary data from 4T proton magnetic resonance spectroscopy (1H-MRS). *Sleep* 2008;31:1499.
10. Plante DT, Jensen JE, Winkelman JW. The role of GABA in primary insomnia. *Sleep* 2012;35:741.
11. Meyerhoff DJ, Mon A, Metzler T, Neylan TC. Cortical gamma-aminobutyric Acid and glutamate in posttraumatic stress disorder and their relationships to self-reported sleep quality. *Sleep* 2014;37:893-900.
12. Plante DT, Jensen JE, Schoerning L, Winkelman JW. Reduced γ -Aminobutyric Acid in Occipital and Anterior Cingulate Cortices in Primary Insomnia: a Link to Major Depressive Disorder? *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2012;37:1548-57.
13. Morgan PT, Pace-Schott EF, Mason GF, et al. Cortical GABA levels in primary insomnia. *Sleep* 2012;35:807.
14. Harper DG, Plante DT, Jensen JE, et al. Energetic and Cell Membrane Metabolic Products in Patients with Primary Insomnia: A 31-Phosphorus Magnetic Resonance Spectroscopy Study at 4 Tesla. *Sleep* 2013;36:493-500.
15. Lin A, Ross BD, Harris K, Wong W. Efficacy of proton magnetic resonance spectroscopy in neurological diagnosis and neurotherapeutic decision making. *NeuroRx* 2005;2:197-214.
16. Rae CD, Bröer S. Creatine as a booster for human brain function. How might it work? *Neurochemistry International* 2015;89:249-59.
17. Bartlett DJ, Rae C, Thompson CH, et al. Hippocampal area metabolites relate to severity and cognitive function in obstructive sleep apnea. *Sleep Med* 2004;5:593-6.

18. Sarchielli P, Presciutti O, Alberti A, et al. A ¹H magnetic resonance spectroscopy study in patients with obstructive sleep apnea. *European journal of neurology* 2008;15:1058-64.
19. Rae C, Bartlett DJ, Yang Q, et al. Dynamic changes in brain bioenergetics during obstructive sleep apnea. *J Cerebr Blood F Met* 2009;29:1421-8.
20. Peng L, Hertz L, Huang R, et al. Utilization of glutamine and of TCA cycle constituents as precursors for transmitter glutamate and GABA. *Developmental neuroscience* 1993;15:367-77.
21. Tapia R, Gonzalez RM. Glutamine and glutamate as precursors of the releasable pool of gaba in brain cortex slices. *Neurosci Lett* 1978;10:165-9.
22. Henry ME, Jensen JE, Licata SC, et al. The acute and late CNS glutamine response to benzodiazepine challenge: a pilot pharmacokinetic study using proton magnetic resonance spectroscopy. *Psychiatry research* 2010;184:171-6.
23. Walter M, Henning A, Grimm S, et al. The relationship between aberrant neuronal activation in the pregenual anterior cingulate, altered glutamatergic metabolism, and anhedonia in major depression. *Arch Gen Psychiatry* 2009;66:478-86.
24. Rae CD. A Guide to the Metabolic Pathways and Function of Metabolites Observed in Human Brain H-1 Magnetic Resonance Spectra. *Neurochem Res* 2014;39:1-36.
25. Snyder J, Wilman A. Field strength dependence of PRESS timings for simultaneous detection of glutamate and glutamine from 1.5 to 7T. *Journal of Magnetic Resonance* 2010;203:66-72.
26. Allen RP, Barker PB, Horská A, Earley CJ. Thalamic glutamate/glutamine in restless legs syndrome Increased and related to disturbed sleep. *Neurology* 2013;80:2028-34.
27. Kaushik MK, Kumar VM, Mallick HN. Glutamate microinjection at the medial preoptic area enhances slow wave sleep in rats. *Behavioural brain research* 2011;217:240-3.
28. Stutzmann JM, Lucas M, Blanchard JC, Laduron PM. Riluzole, a glutamate antagonist, enhances slow wave and REM sleep in rats. *Neurosci Lett* 1988;88:195-200.
29. Salas R, Galea J, Gamaldo A, et al. Increased use-dependent plasticity in chronic insomnia. *Sleep* 2014;37:535-44.
30. Spiegelhalter K, Regen W, Baglioni C, Riemann D, Winkelmann JW. Neuroimaging Studies in Insomnia. *Curr Psychiat Rep* 2013;15.
31. Rae C, Geng J, Williams SR. Going for glutamine: evaluation of asymmetric PRESS approaches. *Proc. Intl. Soc. Mag. Reson. Med.* 2012;20.
32. Morin CM, Espie CA. *Insomnia: A clinician's guide to assessment and treatment*: Springer, 2003.
33. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed: American Psychiatric Pub, 2013.
34. Gaines J, Vgontzas AN, Fernandez-Mendoza J, et al. Short- and Long-Term Sleep Stability in Insomniacs and Healthy Controls. *Sleep* 2015;38:1727-34.
35. Norusis MJ. Chapter 16: Cluster analysis. *PASW Statistics 18 Statistical Procedures Companion*. Upper Saddle River, NJ: Prentice Hall, 2010.
36. Iber C, Ancoli-Israel, S., Chesson, A., Quan SF. *The AASM manual for the scoring of sleep and associated events: rules, terminology, and technical specification*. For the American Academy of Sleep Medicine 1st ed. . 2007.

37. Edinger JD, Bonnet MH, Bootzin RR, et al. Derivation of research diagnostic criteria for insomnia: report of an American Academy of Sleep Medicine Work Group. *Sleep* 2004;27:1567-96.
38. Morin CM. *Insomnia: Psychological assessment and management*. New York, NY: Guilford Press, 1993.
39. Lovibond PF, Lovibond SH. The structure of negative emotional states: Comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. *Behaviour research and therapy* 1995;33:335-43.
40. Johns MW. Reliability and Factor-Analysis of the Epworth Sleepiness Scale. *Sleep* 1992;15:376-81.
41. Gradisar M, Lack L, Richards H, et al. The Flinders Fatigue Scale: preliminary psychometric properties and clinical sensitivity of a new scale for measuring daytime fatigue associated with insomnia. *JCSM* 2007;3:722-8.
42. Morin CM, Vallières A, Ivers H. Dysfunctional beliefs and attitudes about sleep (DBAS): validation of a brief version (DBAS-16). *Sleep* 2007;30:1547.
43. Drake C, Richardson G, Roehrs T, Scofield H, Roth T. Vulnerability to stress-related sleep disturbance and hyperarousal. *Sleep* 2004;27:285-91.
44. Nofzinger EA, Buysse DJ, Germain A, Price JC, Miewald JM, Kupfer DJ. Functional neuroimaging evidence for hyperarousal in insomnia. *Am J Psychiat* 2004;161:2126-9.
45. Winkelman JW, Plante DT, Schoerning L, et al. Increased rostral anterior cingulate cortex volume in chronic primary insomnia. *Sleep* 2013;36:991.
46. Spiegelhalder K, Regen W, Baglioni C, Nissen C, Riemann D, Kyle SD. Neuroimaging insights into insomnia. *Curr Neurol Neurosci Rep* 2015;15:9.
47. Nofzinger EA, Nissen C, Germain A, et al. Regional cerebral metabolic correlates of WASO during NREM sleep in insomnia. *J Clin Sleep Med* 2006;2:316-22.
48. Altena E, Van Der Werf YD, Sanz-Arigita EJ, et al. Prefrontal hypoactivation and recovery in insomnia. *Sleep* 2008;31:1271-6.
49. Kreis R. Issues of spectral quality in clinical 1H-magnetic resonance spectroscopy and a gallery of artifacts. *NMR in Biomedicine* 2004;17:361-81.
50. Ratiney H, Sdika M, Coenradie Y, Cavassila S, van Ormondt D, Graveron-Demilly D. Time-domain semi-parametric estimation based on a metabolite basis set. *NMR Biomed* 2005;18:1-13.
51. Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR Biomed* 2000;13:129-53.
52. Li BS, Wang H, Gonen O. Metabolite ratios to assumed stable creatine level may confound the quantification of proton brain MR spectroscopy. *Magn Reson Imaging* 2003;21:923-8.
53. Gasparovic C, Song T, Devier D, et al. Use of tissue water as a concentration reference for proton spectroscopic imaging. *Magnetic resonance in medicine* 2006;55:1219-26.
54. Fischl B, Salat DH, Busa E, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002;33:341-55.
55. Fischl B, van der Kouwe A, Destrieux C, et al. Automatically parcellating the human cerebral cortex. *Cereb Cortex* 2004;14:11-22.
56. Smith SM, Zhang Y, Jenkinson M, et al. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. *NeuroImage* 2002;17:479-89.

57. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage* 2004;23:S208-S19.
58. Huberty CJ, Olejnik S. *Applied MANOVA and discriminant analysis*: John Wiley & Sons, 2006.
59. Meyers LS, Gamst GC, Guarino A. *Performing data analysis using IBM SPSS*: John Wiley & Sons, 2013.
60. Ke Y, Cohen B, Lowen S, Hirashima F, Nassar L, Renshaw P. Biexponential transverse relaxation (T2) of the proton MRS creatine resonance in human brain. *Magnetic resonance in medicine* 2002;47:232-8.
61. Evans CJ, McGonigle DJ, Edden RAE. Diurnal stability of γ -aminobutyric acid concentration in visual and sensorimotor cortex. *Journal of Magnetic Resonance Imaging* 2010;31:204-9.

Figure captions

Figure 1 Title: Overview of study procedures and timing.

Figure 1 Caption: I-NSD: insomnia with normal sleep duration; I-SSD: insomnia with short sleep duration.

Figure 2 (a) Title: Regions of interest across the brain.

Figure 2 (a) Caption: ACC: middle anterior cingulate cortex; LOCC: left occipital cortex; LPFC: left medial prefrontal cortex.

Figure 2 (b) Title: Acquired spectrum.

Figure 2 (b) Caption: Representation of a typical spectrum acquired *in vivo* from magnetic resonance spectroscopy. Metabolites fitted included aspartate, creatine, glutamate, glutamine, glutathione, glycerophosphocholine, lactate, myoinositol and N-acetylaspartate. Chemical shift δ : parts per million (ppm).

Figure 3 Title: Reduced brain metabolites in insomnia with short sleep duration compared with insomnia and normal sleep duration and good sleeping controls in the left occipital cortex.

Figure 3 Caption: Means and standard errors (SE) for creatine, aspartate, glutamate and glutamine (parts per million). Control: good sleeping controls ($n=16$); I-NSD: insomnia with normal sleep duration ($n=18$); I-SSD: insomnia with short sleep duration ($n=11$); [#] = $p = .05$; * = $p < .05$.

Figure S1 Title: Further brain metabolites acquired at the left occipital cortex.

Figure S1 Caption: Control: good sleeping controls ($n=16$); I-NSD: insomnia with normal sleep duration ($n=18$); I-SSD: insomnia with short sleep duration ($n=11$).

Figure S2 Title: Brain glutamine concentrations are positively associated with total sleep time and negatively associated with wake-time after sleep onset.

Figure S2 Caption: Scatter graphs of Glutamine (Gln) with total sleep time (TST) ($r = 0.43, p < .05$), wake-time after sleep onset (WASO) ($r = -0.40, p < .05$) and sleep onset latency (SOL) ($r = -0.07, p = .704$) in the left occipital cortex (\pm 95% confidence bands for the line of best fit) for all patients with insomnia ($n=29$).

Tables

Table 1: Differences for demographic and clinical outcomes between insomnia subtypes built from polysomnography and controls.

| Mean scores (+/- SD) | I-NSD (n=19) | I-SSD (n=12) | GSC (n=16) | ANOVA F (p) | I-NSD vs. I-SSD | I-SSD vs. GSC | I-NSD vs. GSC |
|-----------------------------|----------------------|-----------------------|----------------------|--|--------------------|------------------|------------------|
| Age (y), range | 35.7 (9.6), 23-56 | 40.2 (10.1), 23-51 | 37.3 (9.6), 24-54 | 0.8 (.470) | 0.46 | 0.29 | 0.17 |
| BMI (kg/m ²) | 24.9 (4.7) | 24.2 (3.4) | 22.4 (2.1) | 2.2 (.128) | 0.17 | 0.64 | 0.69 |
| Insm duration (y), range | 12.4 (7.3), 2-25 | 11.1 (13.7), 1-49 | . | 0.9 (.770) | 0.12 | . | . |
| ISI | 18.3 (4.5) | 18.2 (4.2) | 2.0 (1.8) | 100.7^a (<.001)*** | 0.02 | 5.01 | 4.76 |
| PSQI | 12.1 (5.0) | 13.6 (4.6) | 3.1 (2.1) | 25.8^a (<.001)*** | 0.31 | 2.94 | 2.35 |
| ESS | 5.8 (4.5) | 5.9 (2.6) | 4.8 (2.9) | 0.5 (.607) | 0.03 | 0.40 | 0.26 |
| FFS | 18.3 (7.1) | 19.8 (5.7) | 6.5 (4.1) | 30.0 (<.001)*** | 0.23 | 2.68 | 2.04 |
| GSES | 7.2 (3.7) | 6.5 (4.0) | 0.9 (1.1) | 18.1^a (<.001)*** | 0.18 | 1.91 | 2.31 |
| HAS | 42.0 (10.3) | 37.7 (7.2) | 29.4 (8.4) | 8.7 (.001)* | 0.48 | 1.01 | 1.35 |
| GCTI | 59.3 (12.1) | 52.8 (12.6) | 38.8 (17.1) | 9.1 (<.001)*** | 0.53 | 0.93 | 1.38 |
| DBAS | 5.9 (1.5) | 6.0 (0.9) | 3.0 (1.3) | 26.0 (<.001)*** | 0.08 | 2.68 | 2.10 |
| DASS – D | 7.4 (6.2) | 5.7 (5.9) | 2.3 (3.0) | 26.0^a (.009)** | 0.28 | 0.73 | 1.05 |
| DASS – A | 5.6 (5.9) | 4.5 (3.6) | 1.0 (2.5) | 5.3^a (.003)** | 0.23 | 1.13 | 1.02 |
| DASS – S | 16.1 (8.9) | 15.7 (9.1) | 4.8 (3.9) | 11.4^a (<.001)*** | 0.04 | 1.56 | 1.65 |
| Binary Variable | I-NSD (n=19) | I-SSD (n=12) | GSC (n=16) | χ^2 (p) | I-NSD vs. I-SSD | I-SSD vs. GSC | I-NSD vs. GSC |
| Females = n (%) | 14 (74%) | 7 (58%) | 11 (69%) | 0.80 (.669) | 0.16 | 0.05 | 0.11 |

Table 1 Caption: Means and standard deviations are provided for each comparison with effect size (Cohen's *d* or Cramér's *V* for binary variables). Significant main effects are in bold. ^a Welch's statistic correction for violation of homoscedasticity. ANOVA: analysis of variance; BMI: body mass index; DASS: depression (D), anxiety (A), and stress (S) scale; DBAS: dysfunctional beliefs and attitudes about sleep scale; ESS: Epworth sleepiness scale; FFS: Flinders fatigue scale; GCTI:

Glasgow content of thoughts inventory; GSC: good sleeping controls; GSES: Glasgow sleep effort scale; HAS: hyperarousal scale; I-NSD: Insomnia with normal sleep duration; I-SSD: Insomnia with short sleep duration; PSQI: Pittsburgh sleep quality index; ISI: insomnia severity index. * = $p < .05$; ** = $p < .01$; *** = $p < .001$.

Table 2: Sleep diary (a) and actigraphic (b) outcomes between insomnia subtypes built from polysomnography and controls.

(a) Sleep diary outcomes

| Mean scores (+/- SD) | I-NSD (n=17) | I-SSD (n=12) | GSC (n=16) | ANOVA F (p) | I-NSD vs. I-SSD | I-SSD vs. GSC | I-NSD vs. GSC |
|-----------------------------------|---------------|---------------|---------------|---|--------------------|------------------|------------------|
| TST (min) | 343.5 (75.0) | 331.9 (65.5) | 454.1 (35.0) | 18.7 (<.001)*** | 0.16 | 1.89 | 2.33 |
| SOL (min) | 37.8 (34.3) | 39.7 (27.0) | 10.3 (5.4) | 11.5^a (.004)** | 0.06 | 1.12 | 1.51 |
| WASO (min) | 33.5 (18.3) | 61.5 (54.7) | 5.9 (7.4) | 21.2^a (<.001)*** | 0.69 | 1.98 | 1.42 |
| TIB (min) | 478.2 (52.8) | 511.6 (59.3) | 508.2 (43.2) | 2.0 (.151) | 0.59 | 0.62 | 0.07 |
| Number of awakenings | 1.7 (1.1) | 2.0 (1.3) | 0.6 (0.6) | 8.4 (.001)** | 0.25 | 1.24 | 1.38 |
| %SE | 71.3 (14.3) | 66.2 (13.4) | 89.0 (5.0) | 15.9 (<.001)*** | 0.37 | 1.65 | 2.25 |
| Ratings of sleep quality (0-2) | 0.8 (0.5) | 0.8 (0.5) | 1.5 (0.4) | 11.0 (<.001)*** | 0.00 | 1.55 | 1.55 |
| Bedtime (hh:mm) | 23:07 (00:49) | 23:14 (01:28) | 22:56 (00:59) | 0.3 (.761) | 0.08 | 0.22 | 0.24 |
| Wake-time (hh:mm) | 06:21 (00:59) | 06:38 (01:19) | 07:14 (00:40) | 3.3 (.048)* | 0.26 | 1.04 | 0.57 |

(b) Actigraphic outcomes

| <i>Mean scores (+/- SD)</i> | <i>I-NSD (n=14)</i> | <i>I-SSD (n=10)</i> | <i>GSC (n=14)</i> | <i>ANOVA F (p)</i> | <i>I-NSD vs. I-SSD</i> | <i>I-SSD vs. GSC</i> | <i>I-NSD vs. GSC</i> |
|---------------------------------|---------------------|---------------------|-------------------|--------------------|----------------------------|--------------------------|--------------------------|
| TST (min) | 413.3 (51.1) | 444.7 (39.3) | 424.7 (38.1) | 1.5 (.234) | 0.69 | 0.25 | 0.52 |
| SOL (min) | 11.2 (9.6) | 12.3 (6.4) | 8.9 (7.2) | 0.6 (.563) | 0.13 | 0.27 | 0.50 |
| WASO (min) | 37.6 (15.4) | 44.7 (17.3) | 38.0 (11.2) | 0.8 (.442) | 0.43 | 0.03 | 0.46 |
| TIB (min) | 450.9 (59.1) | 489.4 (48.9) | 462.7 (36.7) | 1.8 (.177) | 0.71 | 0.24 | 0.62 |
| %SE | 87.5 (3.8) | 86.8 (4.1) | 88.8 (3.0) | 0.9 (.402) | 0.18 | 0.38 | 0.56 |
| Bedtime (hh:mm) | 23:33 (01:14) | 22:57 (00:50) | 23:30 (01:08) | 1.0 (.392) | 0.55 | 0.04 | 0.55 |
| Wake-time (hh:mm) | 07:02 (00:52) | 07:06 (01:05) | 07:12 (00:51) | 0.1 (.900) | 0.07 | 0.21 | 0.12 |

Table 2 Caption: Means and standard deviations for each comparison with effect size (Cohen's *d*). Significant main effects are in bold. ^a Welch's statistic correction for violation of homoscedasticity. ANOVA: analysis of variance; TIB: time in bed; TST: total sleep time; SOL: sleep onset latency; WASO: wake-time after sleep onset; %SE: percentage sleep efficiency. * = $p < .05$; ** = $p < .01$; * = $p < .001$.**

Table 3 Title: Differences for overnight sleep parameters between Insomnia subtypes built from polysomnography.

| <i>Mean scores (+/- SD)</i> | <i>I-NSD (n=19)</i> | <i>I-SSD (n=12)</i> | <i>ANOVA F (p)</i> | <i>I-NSD vs. I-SSD</i> |
|---------------------------------|---------------------|---------------------|---------------------------|------------------------|
| TST (min) | 394.4 (34.9) | 294.7 (30.5) | . | . |
| SOL (min) | 18.0 (12.8) | 36.2 (25.7) | . | . |
| WASO (min) | 35.3 (20.7) | 105.67 (47.8) | . | . |
| SE (%) | 88.0 (5.3) | 67.5 (8.5) | 68.7 (<.001)*** | 2.89 |
| NREM sleep (min) | 306.4 (33.1) | 248.8 (27.8) | 25.1 (<.001)*** | 1.89 |
| REM sleep (min) | 88.0 (21.9) | 46.0 (14.1) | 34.8 (<.001)*** | 2.28 |
| REM latency (min) | 120.4 (42.2) | 189.9 (68.0) | 12.4 (.001)** | 1.23 |
| N1 (min) | 13.1 (4.2) | 14.0 (10.1) | 0.13 (.726) | 0.12 |
| N2 (min) | 217.1 (44.7) | 159.9 (29.8) | 15.6 (<.001)*** | 1.51 |
| SWS (min) | 75.5 (28.9) | 74.9 (27.2) | 0.0 (.951) | 0.02 |
| AHI (/hr) | 1.9 (4.0) | 2.8 (3.6) | .727 ^a (.394) | 0.15 |
| PLM arousal index | 0.0 (0.1) | 0.0 (0.0) | 1.4 ^a (.240) | 0.21 |

Table 3 Caption: Means and standard deviations are provided for each comparison with effect size (Cohen's *d* or *r* for variables not normally distributed). ^a Kruskal-Wallis test with median (inter-quartile range) for variables not normally distributed. Significant main effects are in bold. Measures of TST, SOL and WASO were used to build the I-NSD and I-SSD subtypes and not tested for differences. ANOVA: analysis of variance; AHI: apnea-hypopnea index; I-NSD: Insomnia with normal sleep duration; I-SSD: Insomnia with short sleep duration; N1: stage 1 of sleep; N2: stage 2 of sleep; NREM: non-rapid eye movement; PLM: periodic limb movement; REM: rapid eye movement; SE: sleep efficiency (%); SOL: sleep onset latency; SWS: slow wave sleep; TST: total sleep time; WASO: wake-time after sleep onset. * = $p < .05$; ** = $p = .01$; * = $p < .001$.**

Table 4: Displays brain metabolite concentrations between insomnia subtypes and controls (a) and overall insomnia and controls (b) in the left occipital cortex, left medial prefrontal cortex and middle anterior cingulate cortex.

| <i>Spectra</i> | <i>Group</i> | <i>Left occipital cortex</i> | | <i>Left medial prefrontal cortex</i> | | <i>Middle anterior cingulate cortex</i> | |
|------------------|--------------|------------------------------|-----------|--------------------------------------|-----------|---|-----------|
| | | <i>Mean</i> | <i>SD</i> | <i>Mean</i> | <i>SD</i> | <i>Mean</i> | <i>SD</i> |
| <i>Creatine</i> | I-NSD | 18.8 | 1.4 | 19.6 | 2.9 | 15.3 | 2.9 |
| | I-SSD | 17.9 | 1.7 | 18.6 | 2.0 | 15.8 | 3.0 |
| | INS | 18.5 | 1.6 | 19.2 | 2.6 | 15.5 | 2.9 |
| | GSC | 19.0 | 1.2 | 19.1 | 2.7 | 15.9 | 1.4 |
| <i>Aspartate</i> | I-NSD | 5.1 | 2.8 | 4.4 | 6.0 | 2.3 | 2.5 |
| | I-SSD | 2.9 | 2.7 | 6.4 | 6.6 | 3.9 | 3.2 |
| | INS | 4.2 | 2.9 | 5.2 | 6.2 | 3.0 | 2.9 |
| | GSC | 4.7 | 2.9 | 4.5 | 4.4 | 2.9 | 2.9 |
| <i>Glutamate</i> | I-NSD | 15.1 | 2.2 | 16.8 | 3.4 | 15.0 | 3.4 |
| | I-SSD | 13.6 | 3.1 | 14.9 | 3.0 | 14.3 | 3.9 |
| | INS | 14.5 | 2.6 | 16.0 | 3.3 | 14.8 | 3.5 |
| | GSC | 14.7 | 1.6 | 16.5 | 5.0 | 14.2 | 3.1 |
| <i>Glutamine</i> | I-NSD | 7.0 | 1.9 | 8.7 | 3.3 | 6.6 | 1.9 |
| | I-SSD | 5.5 | 1.0 | 8.1 | 3.1 | 6.2 | 2.5 |
| | INS | 6.4 | 1.8 | 8.4 | 3.2 | 6.4 | 2.1 |
| | GSC | 6.8 | 1.7 | 7.2 | 2.3 | 5.9 | 2.3 |

Table 4 Caption: Mean and standard deviation (SD) for aspartate, creatine, glutamate and glutamine (parts per million). I-NSD: insomnia with normal sleep duration ($n=18$); I-SSD: insomnia with short sleep duration ($n=12$: apart from the left occipital cortex where $n=11$); INS: overall insomnia group including both I-NSD & I-SSD patients ($n=30$); GSC: good sleeping controls ($n=16$).