Neuropsychopharmacology (2012) 37, 1548–1557

www.neuropsychopharmacology.org

Reduced γ-Aminobutyric Acid in Occipital and Anterior Cingulate Cortices in Primary Insomnia: a Link to Major Depressive Disorder?

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Insomnia is closely related to major depressive disorder (MDD) both cross-sectionally and longitudinally, and as such, offers potential opportunities to refine our understanding of the neurobiology of both sleep and mood disorders. Clinical and basic science data suggest a role for reduced γ-aminobutyric acid (GABA) in both MDD and primary insomnia (PI). Here, we have utilized single-voxel proton magnetic spectroscopy (1H-MRS) at 4 Tesla to examine GABA relative to total creatine (GABA/Cr) in the occipital cortex (OC), anterior cingulate cortex (ACC), and thalamus in 20 non-medicated adults with PI (12 women) and 20 age- and sex-matched healthy sleeper comparison subjects. PI subjects had significantly lower GABA/Cr in the OC ($p = 0.0005$) and ACC ($p = 0.03$) compared with healthy sleepers. There was no significant difference in thalamic GABA/Cr between groups. After correction for multiple comparisons, GABA/Cr did not correlate significantly with insomnia severity measures among PI subjects. This study is the first to demonstrate regional reductions of GABA in PI in the OC and ACC. Reductions in GABA in similar brain regions in MDD using 1H-MRS suggest a common reduction in cortical GABA among PI and mood disorders.
Neuropsychopharmacology (2012) **37,** 1548–1557; doi:10.1038/npp.2012.4; published online 8 February 2012

Keywords: insomnia; magnetic resonance spectroscopy; sleep disorders; GABA; occipital cortex; anterior cingulate cortex

INTRODUCTION

Insomnia is the most common sleep complaint in industrialized countries, affecting roughly one-third of all adults, and resulting in significant daytime consequences in approximately 5–10% of the adult population (Buysse, 2008). As a syndrome, insomnia is a significant public health problem associated with functional impairment, increased health care utilization, and disability (Simon and VonKorff, 1997). In prospective morbidity studies, insomnia is associated with an increased risk of developing major depressive disorder (MDD) (Breslau *et al*, 1996; Chang *et al*, 1997; Buysse *et al*, 2008; Szklo-Coxe *et al*, 2010). In the absence of an adequate understanding of its pathophysiology, insomnia is divided into primary and comorbid forms, the latter diagnosed when coexisting medical, sleep, or psychiatric disorders are present. Roughly 20–25% of those with chronic insomnia are considered to have primary insomnia (PI), in which insomnia occurs in the absence of a related medical or psychiatric illness (Buysse *et al*, 1994).

Gamma aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the central nervous system, and several lines of evidence suggest GABA has a role in the etiology and/or maintenance of insomnia. First, benzodiazepine receptor agonists (BZRAs), that are efficacious in the treatment of insomnia, promote sleep by increasing receptor affinity for GABA (Gottesmann, 2002). Second, physiological, neuroimaging, and cognitive investigations demonstrate hyperarousal in insomnia (Nofzinger *et al*, 2004; Bonnet and Arand, 2010; Riemann *et al*, 2010), which may suggest a relative decrement of inhibitory GABAergic neurotransmission (Klumpers *et al*, 2010). Finally, GABAergic nuclei in the brain, including the ventrolateral preoptic nucleus (VLPO) and thalamic reticular nucleus (TRN), have important roles in sleep initiation and maintenance. The VLPO promotes sleep via suppression of CNS arousal systems in the tuberomammillary nucleus and brainstem monoaminegic systems (Saper *et al*, 2005); the TRN acts as a gating mechanism between the thalamus and cortex, and has a key role in the generation of oscillatory activity

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Received 24 October 2011; revised 4 December 2011; accepted 3 January 2012

during EEG-synchronized sleep (Steriade, 2003; Saper *et al*, 2005).

Preliminary work from our laboratory, using proton magnetic resonance spectroscopy (1H-MRS), demonstrated decreased global brain GABA in PI, the first neurochemical abnormality identified in this disorder (Winkelman *et al*, 2008). This study utilized a 2D chemical shift imaging (CSI) acquisition combined with J-resolved Echo-Planar Spectroscopic Imaging (JEPSI), which yielded a large amount of spectral information with a relatively short scan time. However, because GABA was summed over temporal, parietal, and occipital cortices, as well as subcortical structures including the thalamus and basal ganglia, concentrations of GABA in specific brain regions could not be determined. Thus, the primary aim of this study was to examine brain GABA using single-voxel 1H-MRS at high field (4 Tesla) in specific regions of interest, utilizing J-difference editing using Point Resolved Spectroscopy with MEGA suppression (MEGAPRESS) (Mescher *et al*, 1998), which has evolved into the most commonly used and accurate spectroscopic method for quantifying GABA *in vivo* (Bogner *et al*, 2010; Henry *et al*, 2011). Our hypothesis was that GABA would be reduced in PI in the thalamus, occipital cortex (OC), and anterior cingulate cortex (ACC) relative to healthy controls. Thalamic reductions were hypothesized based on the role of the TRN in sleep (Steriade, 2003; Saper *et al*, 2005), animal and disease models in which damage to the thalamus induces insomnia (Villablanca and Salinas-Zeballos, 1972; Montagna, 2005), and prior spectroscopic studies demonstrating alterations in thalamic GABA from administration of BZRAs (Licata *et al*, 2009). Reductions in occipital GABA were based on anatomical overlap with preliminary 2D-CSI data (Winkelman *et al*, 2008); as well as the aforementioned relationship between insomnia and depression, with several 1H-MRS studies demonstrating decreased occipital GABA in MDD (Sanacora *et al*, 1999; Sanacora *et al*, 2004; Bhagwagar *et al*, 2007; Bhagwagar *et al*, 2008). Hypothesized reductions in GABA in the ACC were based on animal and human investigations of insomnia that demonstrate increased activity in the ACC (Nofzinger *et al*, 2004; Nofzinger *et al*, 2006; Cano *et al*, 2008), as well as 1H-MRS studies demonstrating decrements in GABA in prefrontal regions encompassing the ACC in MDD (Hasler *et al*, 2007; Bhagwagar *et al*, 2008; Price *et al*, 2009).

MATERIALS AND METHODS

Participants

Young adult and middle-aged (18–60 years) subjects were recruited from the greater Boston, MA area from July 2009 to January 2011. PI subjects met DSM-IV diagnostic criteria for PI, with a report of difficulty initiating or maintaining sleep or nonrestorative sleep with resulting daytime distress or dysfunction that was not attributable to another medical or psychiatric disorder. Consistent with recommended research diagnostic criteria (Buysse *et al*, 2006), additional severity criteria included a self-report of typical sleep onset latency (SOL) plus wake after sleep onset (WASO) of at least 30 min, and a duration of insomnia $\geqslant$6 months. Age and sex-matched healthy control subjects without sleep complaints were also recruited. Subjects were not permitted to use CNS active agents for 2 weeks prior to enrollment and for the duration of the study.

All subjects were evaluated with an unstructured clinical interview for history of sleep and medical disorders, and interview for lifetime history of psychiatric disorders with the Structured Clinical Interview for DSM-IV (SCID). Subjects were administered the Insomnia Severity Index (ISI) (Morin, 1993), Pittsburgh Sleep Quality Index (PSQI) (Buysse *et al*, 1989), Dysfunctional Beliefs and Attitudes about Sleep (DBAS-16) (Morin *et al*, 2007), and Beck Depression Inventory (BDI-IA) (Beck and Steer, 1993). Baseline laboratories included urine toxicology and pregnancy testing (for female subjects).

Exclusion criteria for all subjects included clinical evidence of any moderate to severe sleep disorder other than insomnia (eg obstructive sleep apnea, restless legs syndrome, etc.); current or past (within the preceding year) diagnosis of alcohol or drug dependence/abuse; history of significant medical or neurological illness including significant head trauma or loss of consciousness $>$30 min; BMI $>$ 35 kg/m^2; consumption of $>$10 cigarettes per day, $>$2 caffeinated beverages per day, or $>$2 standard alcoholic drinks per day for a period $>$1 month within the preceding year; history of shift-work; peri-menopausal symptoms that disrupted sleep; contraindicated condition for MR scanning; and women who were pregnant, lactating, or planning to become pregnant during the study.

The study was approved by the Institutional Review Board of Partners Healthcare, the parent organization of Brigham and Women's Hospital and McLean Hospital, and carried out in accordance with the Declaration of Helsinki. All subjects received compensation for their participation in this study.

Sleep Diaries and Actigraphy

Following initial evaluation, subjects completed sleep–wake diaries supplemented by wrist-worn actigraphy (Actiwatch AW-64, Minimitter, Bend OR) for 2 weeks. Diaries included self-report of sleep–wake parameters (eg, bedtime and wake-time, estimated SOL, WASO, etc.), caffeine/alcohol/medication consumption, and a visual analog scale (VAS) of subjective sleep quality. Subjects with PI were excluded if their sleep diaries did not demonstrate SOL + WASO $>$30 min on the majority of days during the baseline period. To allow for inter-individual variation of total sleep time, but exclude subjects who were chronically sleep-restricted, sleeping excessive amounts of time, or had substantial night-to-night variation in sleep times, healthy sleeper controls were excluded if they demonstrated $<$7.5 or $>$10 hours of sleep on 10/14 nights during the screening period. Actigraphy corroborated sleep diaries, but was not used as an inclusionary/exclusionary measure *per se*.

MR Imaging

MR imaging was performed at approximately the same time of day (range 0900–1200 hours) and as soon as logistically possible after completion of baseline actigraphy/diaries (1–23 days). Subjects continued sleep diaries and actigraphy until the day of their scans to confirm that typical sleep–wake patterns continued prior to MRS. Subjects were

re-scanned if spectra were not obtained due to movement artifact or technical failure. Consistent with prior studies in MDD (Bhagwagar *et al*, 2008), female subjects were scanned during the follicular phase of their menstrual cycle to control for confounding hormonal effects on GABA.

Imaging and spectroscopy was performed on a whole body 4-Tesla MR scanner (Varian/UNITYInova, Palo Alto, CA) at McLean Hospital in Belmont, MA. Data collection utilized a birdcage-design, radio-frequency (RF) head coil operating at 170.3 MHz for proton (XLR Imaging, London, Canada). Scout images confirmed optimal positioning, and unsuppressed water signal was shimmed to a global water linewidth of less than or equal to 25 Hz. Subsequently, high-contrast T_1-weighted anatomical images were taken in the sagittal and axial planes (echo time/repetition time = 6.2 s/11.4 ms, field-of-view = $24 \times 24 \times 8$ cm (sagittal) and $22 \times 22 \times 16$ cm (axial), readout duration = 4 ms, receive bandwidth = ± 32 kHz, in-plane matrix size = $128 \times 256 \times 16$ (sagittal) and $256 \times 256 \times 64$ (axial), in-plane resolution = 0.94×1.9 mm (sagittal) and 0.94×0.94 mm (axial), readout points = 512, slice thickness = 2.5 mm, flip-angle = 11°) for anatomical landmarking and image segmentation analysis.

Proton MRS

The axial and sagittal high-resolution, T_1-weighted anatomical images were used as a guide to systematically place single voxels in the left thalamic lobe (Thal) ($2 \times 3 \times 2$ cm), bilateral rostral anterior-cingulate cortex (ACC) ($3 \times 2 \times 2$ cm), and bilateral OC ($3 \times 2 \times 2$ cm) (Figure 1). Proton spectroscopy employed a GABA-optimized MEGAPRESS sequence (Mescher *et al*, 1998) for optimal measures of GABA using the difference-editing technique, as well as secondary measures of glutamate (Glu), N-acetylaspartate (NAA), total creatine (Cr) and total choline (Cho) in the 68 ms sub-spectrum. Manual shimming of the magnetic field within each prescribed voxel achieved water linewidths ranging from 7–12 Hz. Following the automated optimization of water suppression power and tip angles, the transmitter frequency was set onto the creatine resonance at 3.00 ppm to minimize chemical-shift displacement artifact for each spectral acquisition. The MEGAPRESS sequence used the following acquisition parameters: TR = 2 s, TE = 68 ms, spectral bandwidth = 2 kHz, readout duration = 512 ms, NEX = 384, total scan duration = 13 min.

Proton MRS Processing

All spectroscopic data processing and analyses were undertaken on a LINUX workstation using reconstruction code written on-site (C-code) and commercial fitting software. In order to quantify difference-edited GABA with MEGAPRESS data, the difference-edited spectra were fitted with LCModel (Provencher, 1993; Provencher, 2001) using basis sets acquired from phantoms at 4T. All phase and frequency-corrected 'ON' and 'OFF' 68 ms sub-spectra were then averaged separately to produce a single 68 ms 'ON' and 'OFF' spectrum, which were then subsequently subtracted to produce the final, optimized, difference-edited GABA spectrum. The appropriate phantom-based LCModel templates were used to fit the 68 ms 'OFF' spectrum for the measurement of creatine, glutamate and other metabolites (Figure 2). The difference-edited GABA resonance area at 3.00 ppm, as well as the 68 ms 'OFF' spectrum metabolite areas, were normalized to the LCModel fitted 68 ms 'OFF' spectrum creatine resonance area and left as simple ratios.

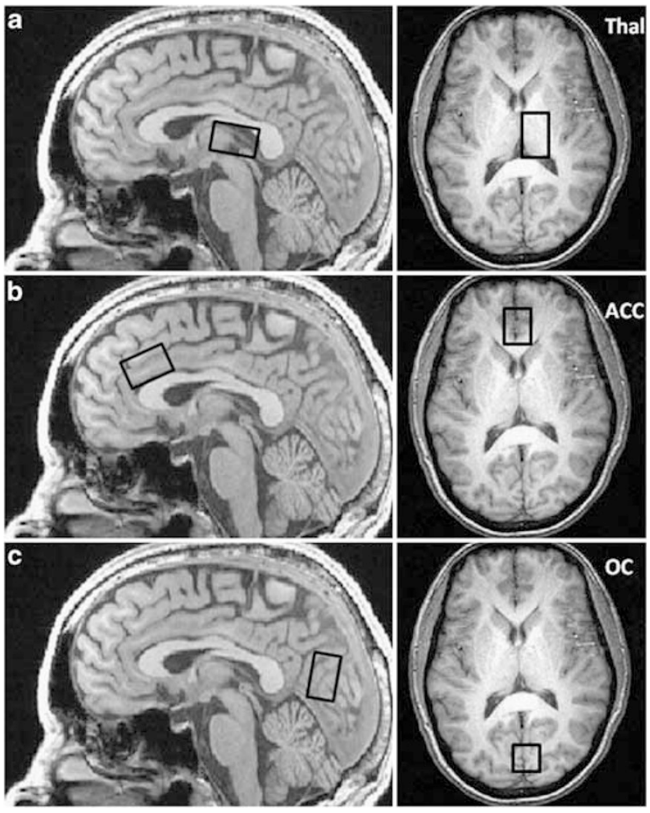

Figure 1 Anatomical placement for thalamic (a), anterior cingulate cortex (b) and occipital cortex (c) proton magnetic spectroscopy (1H-MRS) voxels.

Image Segmentation

To ascertain gray and white matter contribution to each voxel, the axial T1-weighted images were segmented into gray matter, white matter, and cerebrospinal fluid compartments using FSL version 4.1 (FMRIB Software Library; Analysis Group, FMRIB; Oxford, UK) in combination with an in-house automated voxel co-registration and partial-volume analysis program.

Statistics

The *a priori* primary outcome variable was the difference in GABA/Cr between PI and controls in each region of interest, tested with unpaired t-test (two-tailed). Based on previous estimates of global brain GABA/Cr in PI (Winkelman *et al*, 2008), we estimated 80% power to detect a difference between groups at $\alpha = 0.05$, two-sided with $n = 20$ in each group. Voxels were considered outliers if Cr fell outside two standard deviations from the mean for all subjects. GABA/Cr data for individual subjects were excluded if LCModel was unable to fit GABA resonance. Voxel tissue composition, demographics, psychometric scores, and diary/actigraphic

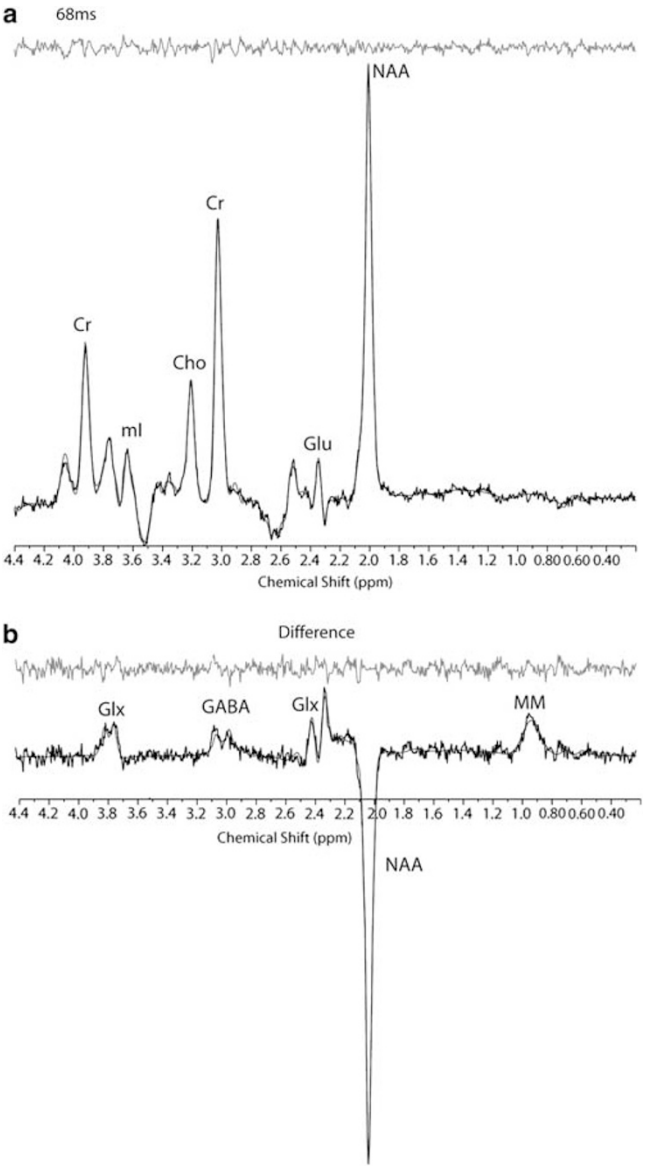

Figure 2 Point Resolved Spectroscopy with MEGA suppression (MEGAPRESS) 68 ms sub-spectrum (a) and γ-aminobutyric acid (GABA) difference-edited spectrum (b) from the occipital cortex (OC) of a study subject. All spectra are displayed with no filtering and show the LCModel fit and residual. Metabolites include: total creatine (Cr), myo-inositol (mI), total choline (Cho), *N*-acetylaspartate (NAA), glutamate (Glu), glutamine plus glutamate (Glx), γ-aminobutyric acid (GABA), and co-edited macromolecule resonance at ~0.9 ppm (MM).

data between groups were compared using unpaired t-tests (two-sided). Differences in other metabolites (NAA/Cr, Cho/Cr, and Glu/Cr) between groups, and Pearson's correlations for insomnia severity measures versus GABA/Cr were examined on an exploratory basis, with Bonferroni correction for multiple comparisons. Statistical analysis was performed using JMP 9 (SAS, Cary, NC).

RESULTS

The PI group ($n = 20$) was comprised of 12 women, with a mean age of 34.3 ± 8.3 (range 22–53) years; the age and sex-matched control group had a mean age of 34.1 ± 9.9 (range 23–55) years (Table 1). All PI subjects had a continuous history of insomnia for at least 1 year, with 16/20 reporting duration of insomnia ⩾5 years (mean 10.1 years). No PI subject had a prior lifetime history of mood or psychotic disorder; one PI subject had a prior history of anxiety disorder not otherwise specified that had been in remission >6 months. Nine PI subjects reported lifetime history of sedative-hypnotic use (4 BZRAs; 5 non-GABAergic hypnotics); five of whom reported non-nightly use within 1 month of enrollment (2 BZRAs; 3 non-GABAergic hypnotics), with the remaining subjects reporting no use of sedative-hypnotics for at least 4 months. Psychometric scales, sleep–wake diaries, and actigraphy corroborated their insomnia complaint (Table 1). BDI scores were significantly higher in PI versus controls, though still far below standard cutoffs for a clinical mood disorder and consistent with typical daytime consequences of insomnia (eg, irritability, fatigue). One male PI subject did not complete the PSQI; two subjects (1 female PI and 1 female control) did not complete the BDI. Baseline actigraphic data for one male PI subject was not available due to device failure.

Two PI subjects required repeat MRS scans due to movement artifact. Voxels excluded from analysis due to Cr outliers were 2 Thal (PI = 1 male, control = 1 male), 2 OC (PI = 1 female, control = 1 male), and 1 ACC (PI = 1 female). One voxel in the ACC (female PI) and two voxels in the OC (2 female controls) were excluded from GABA/Cr analysis due to failure of LCModel to fit the GABA resonance.

PI subjects had significantly reduced GABA/Cr levels compared with controls in the OC (PI = 0.18 ± 0.06; controls = 0.27 ± 0.07, df = 34, t = −3.83, $p = 0.0005$) and ACC (PI = 0.15 ± 0.05; controls = 0.19 ± 0.05, df = 36, $t = -2.32$, $p = 0.03$) (Table 2; Figure 3). Post-hoc analysis to examine possible effects of medications and duration of insomnia revealed that exclusion of PI subjects who had taken sedative-hypnotics (GABAergic or otherwise) within 1 month of enrollment did not substantively alter findings in either the OC (PI = 0.17 ± 0.06; controls = 0.27 ± 0.07, df = 29, $t = -3.95$, $p = 0.0005$) or ACC (PI = 0.15 ± 0.06; controls = 0.19 ± 0.05, df = 32, $t = -2.01$, $p = 0.05$). Similarly, exclusion of subjects whose insomnia was <5 years duration did not alter findings in the OC (PI = 0.18 ± 0.07; controls = 0.27 ± 0.07, df = 30, $t = -3.21$, $p = 0.003$) or ACC (PI = 0.15 ± 0.06; controls = 0.19 ± 0.05, df = 33, $t = -2.06$, $p = 0.05$). In addition, due to prior MRS studies that have demonstrated increases in occipital GABA in the follicular phase in healthy women relative to both men and women with premenstrual dysphoric disorder (Epperson *et al*, 2002; Epperson *et al*, 2005), and aforementioned exclusion of data resulting in loss of sex-matching in the OC and ACC, additional analyses were performed to investigate the effects of sex. Two-way ANOVA demonstrated a significant effect of diagnosis in both the OC ($F_{1,32} = 15.23$, $p = 0.0005$) and ACC ($F_{1,34} = 5.90$, $p = 0.02$), without a significant effect of sex (OC: $F_{1,32} = 0.08$, $p = 0.78$; ACC: $F_{1,34} = 0.05$, $p = 0.83$) or diagnosis x sex interaction (OC: $F_{1,32} = 1.09$, $p = 0.30$; ACC: $F_{1,34} = 0.82$, $p = 0.37$) in either region.

There was no significant difference in GABA/Cr between PI and controls in the thalamus (PI = 0.24 ± 0.07; controls = 0.25 ± 0.05, df = 36, $t = -0.71$, $p = 0.48$) (Table 2;

Table 1 Demographics and Sleep-wake Measures for Primary Insomnia and Controls

	Primary insomnia (mean (SD))	Controls (mean (SD))	*t* ratio	*p*-value (two-tailed)	df
Demographic					
Age, y	34.3 (8.3)	34.1 (9.9)	0.086	0.93	38
Sex (F:M)	12:8	12:8	0.00	1.00	38
BMI, kg/m^2	24.2 (3.2)	23.6 (2.8)	0.583	0.56	38
Rating scales					
ISI	16.0 (2.4)	0.8 (1.1)	25.89	<0.0001	38
PSQI	11.7 (3.0)	1.8 (1.3)	13.59	<0.0001	37
DBAS-16	5.5 (1.5)	3.1 (1.2)	5.47	<0.0001	38
BDI	5.6 (3.3)	2.5 (1.5)	3.66	0.0008	36
BDI−sleep item	4.4 (2.8)	2.4 (1.6)	2.62	0.013	36
Sleep–wake diaries					
SOL, min	40.5 (24.0)	8.9 (4.0)	5.83	<0.0001	38
WASO, min	52.6 (34.3)	6.4 (5.4)	5.97	<0.0001	38
SOL+WASO, min	92.2 (39.8)	15.2 (6.7)	8.52	<0.0001	38
TST, hours	6.36 (1.0)	7.95 (0.6)	−6.04	<0.0001	38
TIB, hours	7.92 (1.1)	8.21 (0.6)	−1.01	0.32	38
SE, %	80.9 (7.4)	96.9 (1.4)	−9.50	<0.0001	38
VAS-sleep quality	0.49 (0.1)	0.80 (0.1)	−8.70	<0.0001	38
Actigraphy					
SOL, min	23.4 (13.9)	13.4 (9.2)	2.64	0.01	37
WASO, min	49.2 (17.6)	40.2 (12.4)	1.85	0.07	37
SOL+WASO, min	72.6 (22.4)	53.7 (18.1)	2.90	0.006	37
TST, hours	6.65 (1.1)	7.33 (0.6)	−2.45	0.02	37
TIB, hours	7.86 (1.1)	8.23 (0.6)	−1.27	0.21	37
SE, %	84.5 (4.8)	89.1 (3.9)	−3.28	0.002	37

Abbreviations: BMI, body mass index; ISI, Insomnia Severity Index; PSQI, Pittsburgh Sleep Quality Index; DBAS-16, Dysfunctional Beliefs and Attitudes about Sleep-sixteen Item; BDI, Beck Depression Inventory IA; BDI−sleep item, BDI score minus sleep-related items; SOL, sleep onset latency; WASO, wake after sleep onset; TST, total sleep time; TIB, time in bed; SE, sleep efficiency; VAS-sleep quality, visual analog scale of sleep quality.

Figure 3). There were no significant differences between PI and controls for Cr, %GM, or %WM in any region of interest (Table 2). Exploratory analyses revealed no significant differences in levels of other metabolites between PI and controls after correcting for multiple comparisons (Table 2). Pearson's correlations of sleep measures and GABA in PI subjects demonstrated a correlation between total sleep time (as measured by actigraphy) and GABA in the ACC ($r = 0.51$, $p = 0.03$), however this correlation did not remain significant after correcting for multiple comparisons.

DISCUSSION

Our findings of reduced brain GABA in unmedicated PI in the OC and ACC, by 33% and 21%, respectively, confirms preliminary data from our laboratory and identifies specific brain regions in which GABA is diminished in PI. These findings provide important insights into the neurobiology of insomnia, and validate the theory of altered GABAergic neurotransmission in PI, which has been suggested by circumstantial anatomical, neurophysiological, and pharmacological evidence, as well as preliminary data from our laboratory (Winkelman *et al*, 2008). Although the mechanisms through which reduced GABA in the ACC and OC relate to the pathophysiology of insomnia are unknown, it is noteworthy that increased activity in the ACC has been implicated in animal models of insomnia (Cano *et al*, 2008) as well as human (^{18}FDG-PET) investigations of PI (Nofzinger *et al*, 2004; Nofzinger *et al*, 2006). Furthermore, altered regional cerebral blood flow (rCBF) in PI as measured by single photon emission computed tomography (SPECT) has been demonstrated during NREM sleep across a number of brain regions, including frontal and occipital cortices (Smith *et al*, 2002), and rCBF in the ACC during wakefulness correlates with insomnia severity in MDD (Périco *et al*, 2005). Although it is not currently clear how MRS-derived measures of GABA directly relate to synaptic activity or cortical excitability, our results are congruent with the hyperarousal model of PI, in which chronic insomnia is due to increased arousal across a range of cognitive and physiological domains, which may be consistent with an imbalance between excitatory and inhibitory neurotransmission in multiple cortical regions (Bonnet and Arand, 2010; Riemann *et al*, 2010).

Beyond the implications for neurobiological research in PI, the findings of this study may have potential implications

Table 2 1H-MRS Measures in Primary Insomnia versus Controls

Region	Metabolite[a]	Primary insomnia (mean (SD))	Controls (mean (SD))	*t* ratio	*p*-value (two-tailed)[b]	df
OC	GABA/Cr	0.18 (0.06)	0.27 (0.07)	−3.83	**0.0005**	34
	Glu/Cr	84.14 (7.8)	83.40 (11.3)	0.23	1.00	36
	NAA/Cr	127.33 (10.4)	125.03 (9.6)	0.71	1.00	36
	Cho/Cr	26.78 (7.9)	23.29 (6.7)	1.47	0.45	36
	Cr	18.51 (3.5)	17.21 (3.2)	1.20	0.24	36
	GM (%)	45.18 (8.2)	47.03 (6.0)	−0.79	0.43	36
	WM (%)	46.24 (9.9)	45.70 (8.2)	0.18	0.86	36
ACC	GABA/Cr	0.15 (0.05)	0.19 (0.05)	−2.33	**0.03**	36
	Glu/Cr	97.91 (13.1)	102.58 (10.3)	−1.24	0.67	37
	NAA/Cr	109.77 (8.8)	117.95 (13.9)	−2.19	0.10	37
	Cho/Cr	42.42 (15.5)	44.59 (12.3)	−0.49	1.00	37
	Cr	17.98 (1.8)	17.15 (2.6)	1.14	0.26	37
	GM (%)	54.21 (4.7)	55.92 (4.1)	−1.22	0.23	37
	WM (%)	34.89 (5.0)	34.06 (4.4)	0.55	0.58	37
Thal	GABA/Cr	0.24 (0.07)	0.25 (0.05)	−0.71	0.48	36
	Glu/Cr	100.68 (20.0)	99.77 (23.0)	0.13	1.00	36
	NAA/Cr	146.70 (18.0)	144.40 (16.7)	0.41	1.00	36
	Cho/Cr	61.13 (26.4)	49.93 (14.4)	1.62	0.34	36
	Cr	16.71 (3.4)	17.67 (2.2)	−1.03	0.30	36
	GM (%)	41.89 (11.4)	42.47 (9.7)	0.17	0.87	36
	WM (%)	54.01 (10.0)	54.46 (11.6)	−0.13	0.90	36

[a]Metabolites/Cr reported × 100. Glu/Cr, NAA/Cr, and Cho/Cr Bonferroni corrected for multiple comparisons.
[b]Values in bold denote significant differences between gruoups.

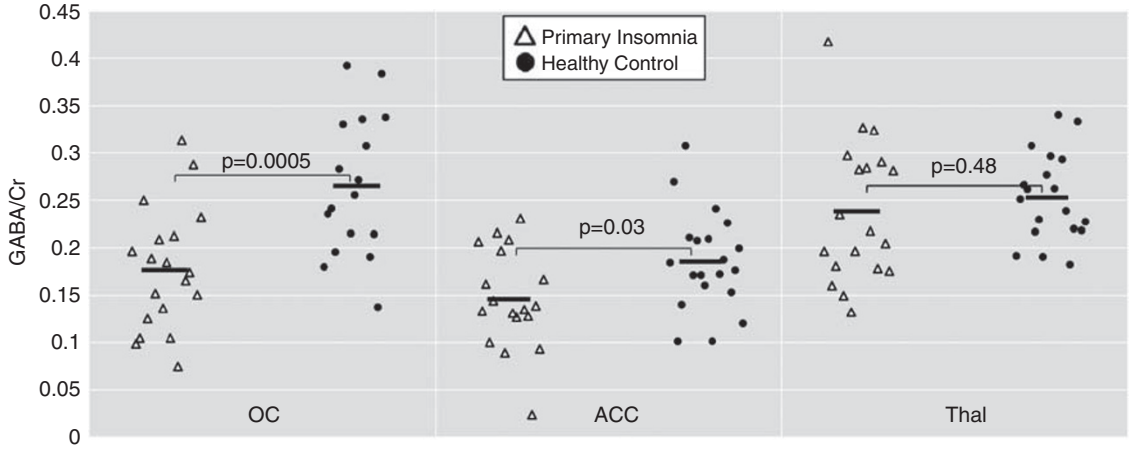

Figure 3 Individual subject GABA/Cr by region of interest. Solid bars denote group means.

for research regarding GABAergic alterations in mood disorders. Over the last 30 years, there has been growing evidence that MDD is associated with altered GABAergic neurotransmission (Sanacora, 2010; Luscher *et al*, 2011). MDD patients have demonstrated low plasma and CSF GABA (Gold *et al*, 1980; Petty and Schlesser, 1981), decreased cell density of GABAergic neurons in both the OC and prefrontal regions in autopsy studies (Rajkowska *et al*, 2007; Maciag *et al*, 2010), decrements of the GABA synthesizing enzyme glutamic acid decarboxylase in the dorsolateral prefrontal cortex (Gos *et al*, 2009; Karolewicz *et al*, 2010), and impaired measures of cortical inhibition using transcranial magnetic stimulation (TMS), consistent with decrements in GABAergic tone (Bajbouj *et al*, 2006; Lefaucheur *et al*, 2008; Levinson *et al*, 2010). However, the most influential data suggesting impaired GABAergic neurotransmission in MDD comes from 1H-MRS studies that directly measure brain GABA *in vivo*. 1H-MRS investigations in MDD demonstrate decrements of GABA in prefrontal regions (in voxels containing the ACC) and the OC (Sanacora *et al*, 1999; Sanacora *et al*, 2004; Hasler *et al*, 2007; Price *et al*, 2009) of similar magnitude to those observed in this study of PI, indicating that decreased GABA in the OC and ACC is a shared neurobiological finding in these disorders, and

although speculative, suggests a physiological connection between the well-established epidemiological relationship between insomnia and MDD (Breslau *et al*, 1996; Chang *et al*, 1997; Ohayon and Roth, 2003; Buysse *et al*, 2008; Szklo-Coxe *et al*, 2010; Baglioni *et al*, 2011).

Insomnia is a risk factor for incident MDD, increasing the risk of depressive illness roughly twofold compared with those without sleep difficulties (Baglioni *et al*, 2011). However, the majority of individuals with insomnia do not go on to develop depression (Chang *et al*, 1997), suggesting PI is not simply a trait-marker for MDD. To develop effective strategies that would prevent the future development of MDD among individuals with PI requires a deeper understanding of the biological factors that convey cumulative risk for the development of depression. To account for heterogeneity among psychiatric disorders, the use of endophenotypes, which are more elementary, state-independent, intermediate phenotypes that bridge genetic variation to complex disease states, is a promising line of research (Gottesman and Gould, 2003). It has been posited that reduced cortical GABA is the most promising imaging endophenotype in MDD (Hasler and Northoff, 2011). However, 1H-MRS measures of GABA do not correlate with depression severity (Sanacora *et al*, 2004; Hasler *et al*, 2007; Price *et al*, 2009) and reductions in brain GABA in the OC have been demonstrated in panic disorder (Goddard *et al*, 2001) and alcohol dependence (Behar *et al*, 1999), suggesting that reduced GABA as an endophenotype is not specific for MDD. However, it is noteworthy that, in addition to increasing risk for incident depression, insomnia increases the risk for, and is a common comorbid symptom of, anxiety and substance use disorders (Ford and Kamerow, 1989; Breslau *et al*, 1996; Weissman *et al*, 1997; Neckelmann *et al*, 2007). Thus, although speculative, it is possible that insomnia may be an important unmeasured covariate in these studies, which could account for reductions observed in cortical GABA in multiple disorders.

The notion that comorbid insomnia is an important consideration in 1H-MRS investigations of GABA in psychiatric disorders is further supported by the fact that atypical depression, which is frequently associated with hypersomnia rather than insomnia, is not associated with reductions in GABA (Sanacora *et al*, 2004). In addition, the literature on reduced cortical GABA as a trait-marker for MDD is inconsistent, with increased, normal, and persistently lowered GABA in remitted MDD subjects reported (Sanacora *et al*, 2003; Hasler *et al*, 2005; Bhagwagar *et al*, 2007; Bhagwagar *et al*, 2008). Because residual insomnia is a common, but not universal symptom among remitted depressed subjects (Nierenberg *et al*, 2010), it is plausible that failure to account for the effects of comorbid insomnia as part of study design may have affected findings in these investigations.

The findings of this study and the aforementioned literature underscore the need for further research to clarify the relationships between clinical sleep disturbance and brain GABA. For PI research, studies utilizing other means to probe GABAergic neurotransmission (eg CSF, autopsy studies) that have already been applied in MDD, could help clarify the role of GABA in the pathophysiology of PI. Similarly, the relationship of brain GABA to other established physiological and cognitive markers of hyperarousal in insomnia may provide deeper insights into that conceptual framework. In addition, future studies that critically examine the role of comorbid sleep disturbance (eg insomnia and/or hypersomnia) in mood disorders and GABAergic neurotransmission are required to clarify whether alterations in GABA are a biomarker for a given mental illness, or an epiphenomenon related to sleep disturbance. Finally, 1H-MRS investigations in both PI and MDD that examine GABA in regions other than occipital and prefrontal cortices and utilize longitudinal designs may help distinguish regional patterns of alterations in GABA that segregate these disorders and clarify whether reduced cortical GABA is a trait-marker for insomnia and/or depressive illness.

There are limitations of this study that merit discussion. Most notably, polysomnography (PSG) was not performed, and thus, correlations between PSG measures of insomnia severity and GABA that have been demonstrated in prior studies cannot be corroborated (Winkelman *et al*, 2008). In addition, subjects in either group may have potentially had occult sleep disorders other than PI that may have affected results. However, PSG is not indicated for the routine clinical evaluation of insomnia, (Littner *et al*, 2003) and has not been used in 1H-MRS studies of MDD, even though sleep-related breathing and movement disorders are frequently associated with psychiatric symptoms (Beebe and Gozal 2002; Winkelman *et al*, 2006). The possibility that occult sleep disorders may have affected results was also minimized by thorough sleep and psychiatric history performed by psychiatrists with fellowship training in sleep medicine (DTP and JWW).

In addition, our findings of reduced GABA in the ACC in PI are not universally comparable to prior MDD 1H-MRS studies that have examined the ACC. Positioning of our voxel in rostral ACC more closely approximates previous studies that have demonstrated significant GABA reductions in MDD in dorsomedial/dorsolateral prefrontal regions (Hasler *et al*, 2007; Bhagwagar *et al*, 2008), and is not directly comparable to voxels positioned in the pregenual/ventromedial prefrontal cortex utilized in other investigations (Hasler *et al*, 2007; Price *et al*, 2009). Moreover, although a recent study has demonstrated the diurnal stability of MEGAPRESS in quantification of brain GABA in healthy individuals (Evans *et al*, 2010), because of recent evidence suggesting the timing of 1H-MRS is important in the study of cortical GABA in shift-work (Kakeda *et al*, 2011), it is plausible that results of this study may have been different had MRS scans been performed at different times of a day. Also, without behavioral or EEG monitoring, it is possible that differences between groups may have been due to either systematic differences in sleep–wake states and/or changes in visual stimulation due to eyes being open or closed at the time of 1H-MRS acquisition. However, it is noteworthy that despite disrupted nocturnal sleep, PI subjects are not more likely to fall asleep during multiple sleep latency testing compared with healthy controls (Edinger *et al*, 2008), and recent data suggest GABA is not altered by visual stimulation in the OC (Lin *et al*, 2011). Finally, this study is not able to determine whether decrements in cortical GABA observed are due to PI itself, or result from chronic partial sleep deprivation associated with the illness.

There are also inherent limitations to MRS that may affect interpretation of this study. First, differences in tissue composition (%GM) or the concentration of an internal standard (Cr) may affect group differences in GABA/Cr (Geramita *et al*, 2011). However, there were no differences between groups in tissue composition or Cr for any voxel, suggesting results are due to differences in GABA concentration, rather than other confounding factors. Additionally, MRS has limited spatial and temporal resolution compared with other neuroimaging modalities, and therefore, only the aggregate quantity of GABA in a relatively large brain region can be measured using 1H-MRS. This may be particularly relevant to our negative findings in the thalamus, as the TRN, which is involved in sleep initiation and maintenance, encapsulates several other nuclei and comprises a relatively small portion of the volume of the thalamus. In addition, our results cannot clarify whether decreased GABA in the ACC or OC in PI reflect decrements in GABAergic cell number or reduced quantity/production in an intact population of neurons. Finally, single voxel 1H-MRS requires the *a priori* selection of specific brain areas of interest, and thus levels of GABA in PI in other brain regions not examined in this study remain unknown.

In summary, our study provides the first evidence of reduced brain GABA in the occipital and anterior cingulate cortices of those with PI compared with healthy sleeper controls. Similar findings of reduced GABA in these brain regions in MDD suggest a common neurochemical alteration in these disorders. Given the strong cross-sectional and longitudinal epidemiological links between insomnia and MDD, further research that examines GABAergic neurotransmission both in PI and insomnia comorbid with MDD may shed further light on the connections between insomnia and mental illness.

ACKNOWLEDGEMENTS

This work was supported in part by the American Sleep Medicine Foundation Physician Scientist Training Award to DTP (#48-PA-09). The funding organizations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript. This work was presented at the meeting of the Associated Professional Sleep Societies, Minneapolis, MN, June 11–15, 2011.

DISCLOSURE

Dr Plante reports having owned stock in Pfizer, and having received honoraria from Oakstone Medical Publishing and royalties from Cambridge University Press. Dr Winkelman reports serving as a consultant or advisory board member for Axon Laboratories, Boehringer-Ingelheim, Covance, GlaxoSmithKline, Impax Laboratories, Jazz Pharmaceuticals, Luitpold Pharmaceuticals, Neurogen, Novadel Pharma, Pfizer, Sunovion, Takeda, UCB, and Zeo; research support from Boehringer-Ingelheim, UCB (Schwarz) Pharma, GlaxoSmithKline, and Sepracor; and stock options in Axon Laboratories. Dr Jensen and Ms Schoerning report no conflict of interest.

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