HHS Public Access

Author manuscript

J Affect Disord. Author manuscript; available in PMC 2023 November 21.

Published in final edited form as:

J Affect Disord. 2020 September 01; 274: 624–631. doi:10.1016/j.jad.2020.05.026.

^{1}H MRS Measurement of Cortical GABA and Glutamate in Primary Insomnia and Major Depressive Disorder: Relationship to Sleep Quality and Depression Severity

Kathleen L. Benson[a,b], **Ryan Bottary**[c], **Laura Schoerning**[d], **Lee Baer**[b,†], **Atilla Gonenc**[a,b], **J. Eric Jensen**[a,b,†], **John W. Winkelman**[b,e,*]

[a] McLean Imaging Center, McLean Hospital, Belmont, MA, USA

[b] Department of Psychiatry, Harvard Medical School, Boston, MA, USA

[c] Cognitive and Affective Neuroscience Laboratory, Department of Psychology, Boston College, Chestnut Hill, MA, USA

[d] University of Massachusetts School of Medicine, Worcester, MA, USA

[e] Departments of Psychiatry and Neurology, Massachusetts General Hospital, Boston, MA, USA

Abstract

Background: Both Major Depressive Disorder (MDD) and Primary Insomnia (PI) have been linked to deficiencies in cortical γ-aminobutyric acid (GABA) and glutamate (Glu) thus suggesting a shared neurobiological link between these two conditions. The extent to which comorbid insomnia contributes to GABAergic or glutamatergic deficiencies in MDD remains unclear.

Methods: We used single-voxel proton magnetic resonance spectroscopy (^{1}H MRS) at 4 Tesla to examine GABA+ and Glu relative to creatine (Cr) in the dorsal anterior cingulate cortex (dACC) and in the parieto-occipital cortex (POC) of 51 non-medicated adults with MDD, 24 adults with Primary Insomnia (PI), and 25 age- and sex-matched good sleeper controls (HC). Measures of depression severity and subjective and objective sleep quality were compared with ^{1}H MRS metabolite measures.

Results: MDD subjects exhibited a 15% decrease in Glu/Cr in the dACC compared to HC. Within the MDD group, there was a trend inverse correlation between dACC Glu/Cr and anhedonia ratings. We observed no significant association between measures of sleep quality with dACC Glu/Cr in those with MDD.

*Corresponding author at: John W. Winkelman, M.D. Ph.D. Sleep Disorders Clinical Research Program, Massachusetts General Hospital, 1 Bowdoin Square, 9th Floor, Boston, MA 02114, jwwinkelman@partners.org.

†Deceased

Limitations: The protocol and data interpretation would have been enhanced by the recruitment of MDD subjects with a broader range of affect severity and a more comprehensive assessment of clinical features.

Conclusions: These findings support the role of cortical glutamatergic mechanisms in the pathophysiology of MDD. Insomnia severity did not further contribute to the relative deficiency of glutamatergic measures in MDD.

Keywords

GABA; Glutamate; Magnetic Resonance Spectroscopy; Depression; Insomnia; Sleep

INTRODUCTION

Recent advances in neuroimaging techniques, such as the increasing use of proton magnetic resonance spectroscopy (^{1}H MRS), have made it possible to examine *in vivo* an array of major CNS neurochemicals including gamma-amino-butyric acid (GABA), glutamate (Glu), and a composite measure (Glx) of glutamate (Glu) and glutamine (Gln). Many of these ^{1}H MRS studies of GABA and Glu have suggested that both neurotransmitters are involved in the neurobiology of PI and MDD. There is both indirect and direct evidence consistent with this conclusion.

With respect to indirect lines of evidence, GABA and Glu neurons discharge maximally in association with discrete sleep-wake states (Jones, 2017). From a treatment perspective, broad support for GABA's role in sleep regulation derives from the observation that benzodiazepine receptor agonists, which are efficacious in the treatment of insomnia, increase activity at GABA neurons (Gottesmann, 2002). In an analogous manner, the role of Glu in the neurobiology of MDD is advanced by the therapeutic benefit of ketamine, an NMDA Glu receptor antagonist whose mechanism of action remains obscure (Lener et al., 2017; Zanos and Gould, 2018).

Direct evidence of GABA's role in the neurobiology of PI is suggested by previous work in our laboratory. Using ^{1}H MRS, we demonstrated global brain reductions in GABA (Winkelman et al., 2008) and locally specific reductions in the occipital (OC) and dorsal anterior cingulate cortices (dACC) in subjects with PI (Plante et al., 2012). Moreover, within those with PI, reductions in GABA were inversely correlated with the severity of insomnia as indexed by polysomnographic wake after sleep onset (WASO). In a more recent study, exploratory data suggested that GABA levels in the dACC were associated with habitual sleep duration (Spiegelhalder et al., 2016). In addition to GABAergic abnormalities in PI, sleep impairment has also been associated with abnormalities in glutamatergic systems. Relative to healthy controls, ^{1}H MRS measures of Glu were reduced in the occipital cortex of patients with insomnia notable for short sleep durations (Miller et al., 2017).

In addition to studies of the neurobiology of PI, a range of reports over the past two decades directly documented abnormalities in ^{1}H MRS-detected GABA and Glu in MDD. A recent meta-analysis of ^{1}H MRS studies in MDD found that MDD is associated with reductions in GABA, especially in occipital brain regions, and that these reductions are state-dependent;

depressed individuals show significantly lower levels of GABA compared to those with remitted MDD (Schur et al., 2016). With respect to glutamatergic abnormalities, reviews and meta-analyses are largely in agreement that Glu and/or Glx is decreased in MDD (Yuksel and Ongur, 2010; Luykx et al., 2012; Moriguchi et al., 2019), particularly in anterior brain regions such as the prefrontal cortex (Michael et al., 2003; Hasler et al., 2007) and ACC (Auer et al., 2000; Zhang et al., 2013).

Broadly stated, these studies of ^{1}H MRS-detected abnormalities in both GABAergic and glutamatergic brain chemistry suggest some overlap in the underlying neurobiology of PI and MDD. This suggestion is reinforced by the interrelationship of PI and MDD on the clinical level. Sleep disturbance is a diagnostic feature of MDD with insomnia occurring in nearly 75% of patients with depression (Nutt et al., 2008). Furthermore, the relationship between PI and MDD appears to be bidirectional. For example, pre-morbid insomnia increases the risk of subsequently developing MDD (Chang et al., 1997; Riemann and Voderholzer, 2003). Furthermore, residual insomnia following resolution of a major depressive episode increases the risk of depressive relapse (Dombrovski et al., 2007; Cho et al., 2008).

These clinical observations coupled with overlapping GABA and Glu abnormalities in both PI and MDD suggest that the GABAergic and glutamatergic abnormalities observed in MDD might better reflect the co-morbid sleep disturbance in this disorder rather than core mood or affective symptoms. The objective of the present study was to compare regional ^{1}H MRS-derived measures of GABA and Glu in three groups: healthy, good sleeping controls (HCs), subjects with PI, and subjects with MDD. We hypothesized that GABA and Glu levels would be reduced in both patient groups relative to controls. Furthermore, we hypothesized that increasing levels of sleep disturbance in MDD would correspond with decreased ^{1}H MRS-measured cortical GABA and Glu levels, independent of the degree of mood disturbance. The primary outcome measures included ^{1}H MRS-derived measures of GABA+ and Glu relative to creatine (Cr), in the parieto-occipital cortex (POC) and dorsal anterior cingulate cortex (dACC). Secondary outcome measures included questionnaire assessments of sleep quality and depressive affect as well as measures of sleep quality derived from subjects' diaries and overnight polysomnography.

METHODS

Participants

One hundred adult (ages 18–70 years) subjects were recruited from the greater Boston, MA area from February 2013 to September 2016, primarily using online and poster advertisements as well as hospital-based recruitment tools available through the Depression Clinical Research Program at Massachusetts General Hospital. Efforts were made to match subjects by age and sex across MDD, PI and HC groups. All subjects were evaluated with an unstructured clinical interview for history of sleep and medical disorders. Lifetime history of Axis I and II psychiatric disorders was evaluated using the Structured Clinical Interview for DSM-IV (SCID-I/SCID-II) (First et al., 2002; First et al., 1997). Subjects were also evaluated with the 17-item Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1960), the Insomnia Severity Index (ISI) (Morin, 1993), Beck Depression Inventory (BDI-

IA) (Beck and Steer, 1993), Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989), Dysfunctional Beliefs and Attitudes about Sleep (DBAS-16) (Morin et al., 2007), Perceived Stress Scale (PSS) (Levenstein et al., 1993), and the Fatigue Severity Scale (FSS) (Krupp et al., 1989). A measure of Anhedonia was derived by summing items 4, 12, 15 and 21 from the BDI-IA.

Major Depressive Disorder subjects met DSM-IV criteria for MDD (296) and required a HAM-D score ≥ 11 (not including insomnia items). Primary Insomnia subjects met DSM-IV criteria for PI (307.42). Inclusion criteria for PI also required an ISI ≥14, a HAM-D score ≤8 (excluding 3 insomnia items), and a BDI-IA ≤10. Age and sex-matched healthy control subjects (HC) without sleep complaints or history of psychiatric conditions were also recruited. Inclusion criteria for HC subjects specified an ISI ≤ 4, a HAM-D ≤ 8, and a BDI-IA ≤ 10.

All subjects were required to be free of CNS active agents for two weeks (or 5 half-lives) prior to the first study visit or willing to taper off CNS active agents prior to beginning the study. Use of CNS active agents was prohibited for the duration of the study. Moderate consumption of alcohol (no more than 2 standard alcoholic drinks per day for a period > 1 month in the preceding year) with no history of alcohol abuse was permitted. Subjects were excluded if caffeinated beverage/pill consumption was estimated to contain more than 300 mg of caffeine daily (approximately 3 standard cups of coffee). Subjects were also excluded for current smoking of more than 10 cigarettes per day. A total of 5 smokers were included in the study: 1 of 25 controls, 4 of 51 in the MDD group, and 0 in the PI group. Of these 5 smokers, only 2 smoked at least one cigarette per day; both were in the MDD group (2 of 51 Ss). Proscriptions against CNS agents were assessed using urine toxic screens at baseline and end of study which tested for benzodiazepines, opioids, THC, and amphetamines. Use of other CNS medications was assessed through interview at each of the study visits and through daily sleep diary reports of tobacco, caffeine, alcohol, or medication consumption. Exclusion criteria for all subjects included clinical evidence of any moderate to severe sleep disorder other than insomnia (e.g. obstructive sleep apnea, restless legs syndrome, narcolepsy, etc.) within the preceding year; apnea + hypopnea index (AHI) > 25 during the first night of polysomnographically-recorded home sleep testing; lifetime history of Axis I DSM-IV schizophrenia, psychotic disorders, or panic disorder, or significant Personality Disorder (PD); lifetime history of Axis I DSM-IV alcohol or illicit substance dependence; history of significant medical or neurologic conditions that may exacerbate or cause depressive symptoms or insomnia (including menopause); and women who were pregnant, lactating, or planning to become pregnant during the study. Baseline laboratories included a urine pregnancy test (for female subjects).

The study was approved by the Institutional Review Board of Partner's Healthcare, the parent organization of Massachusetts General Hospital and McLean Hospital, and carried out in accordance with the Declaration of Helsinki. All subjects provided written informed consent and were compensated for their participation in this study.

Actigraphy and Sleep Diaries

Following initial evaluation, subjects wore a wrist actigraph (Actiwatch AW-64, Minimitter, Bend OR) and completed sleep-wake diaries for two weeks. Wrist actigraphy provided a continuous record of sleep patterns in the home environment while sleep diaries reported sleep-related parameters such as bed- and rise-time, estimated Sleep Onset Latency (SOL), Wake After Sleep Onset (WASO), Total Sleep Time (TST), and Sleep Efficiency (SE%), as well as caffeine/alcohol/medication consumption and a visual analog scale (VAS) of subjective sleep quality. HC subjects were excluded if they reported ≤7or ≥10 hours of sleep or SOL or WASO >30 minutes on ≥ 5/14 nights of sleep diaries. To be included in the PI group, subjects had to report ≤ 7 hours of sleep and SOL or WASO>45 minutes or SOL+WASO>60 minutes on at least 7/14 nights of sleep diaries. Actigraphy corroborated sleep diaries but were not used to determine subject inclusion/exclusion.

Polysomnography

Subjects completed two consecutive nights of home polysomnography (PSG) using an ambulatory Somte PSG device (Compumedics, Charlotte, NC). The first night was an acclimation night to assess for comorbid sleep disordered breathing and periodic limb movements of sleep. On the second night of home sleep recording, subjects were equipped with an abbreviated recording montage, to assess sleep architecture. All sleep recordings were scored using the American Academy of Sleep Medicine sleep scoring criteria (Iber et al., 2007) by the same experienced, registered PSG technologist, blind to subject diagnostic group.

Magnetic Resonance Imaging

MR imaging was performed as soon as logistically possible after completion of actigraphy/diaries and the second home PSG (average = 2 days; range = 0–21 days). 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 menstruation cycle to control for confounding hormonal effects on GABA.

For those MDD subjects who underwent a 2-week washout of CNS active agents prior to initial evaluation, the completion of actigraphy/diaries and 2 home PSGs extended the washout interval prior to MR imaging to a minimum of 4 weeks. Subjects continued sleep diaries and actigraphy until the day of their scans to confirm that typical sleep-wake patterns continued prior to MRS. The sleep diary data shown in Table 1 constitute the within-subject average of the final 7 days of diary documentation.

All MRS data were collected at McLean Hospital in Belmont, MA. We utilized a whole body 4-Tesla MR scanner (Agilent Technologies; Santa Clara, CA) using a 16-rung, volumetric birdcage design RF head coil (XLR Imaging, London, Canada) operating at 170.3MHz for proton imaging and spectroscopy. The axial and sagittal high-resolution, T_1-weighted anatomical images were utilized to systematically place single voxels in the bilateral dorsal anterior-cingulate cortex (dACC) (35 × 25 × 20 mm) and the bilateral

parieto-occipital cortex (POC) (30 × 25 × 25 mm) (See Figure 1). Voxel placement was visually checked by an experienced user (JEJ).

Proton spectroscopy employed a GABA-optimized MEGAPRESS sequence (Mescher et al., 1998) for measures of GABA using difference-editing as well as measures of glutamate using the 68ms sub-spectrum. 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 = 2s, TE = 68ms, spectral bandwidth = 2 kHz, readout duration = 512 ms, NEX = 384, total scan duration = 13min.

Subsequent data processing and analyses were conducted on a LINUX workstation using in-house software as well as commercial fitting software. To quantify difference-edited GABA and glutamate with the MEGAPRESS data, the difference-edited spectra were fitted with LCModel (Provencher et al., 1993; Provencher et al., 2001). All phase and frequency-corrected ‘ON’ and ‘OFF’ spectra were averaged separately to produce a single 68 ms ‘ON’ and ‘OFF’ spectrum, which were subsequently subtracted to produce the final, optimized, difference-edited GABA spectrum. The appropriate LCModel templates were used to fit the 68ms ‘OFF’ spectrum for the measurement of glutamate and the difference-edited spectrum for GABA measurement. The difference-edited GABA resonance area at 3.00ppm, as well as the fitted 68 ms glutamate resonance were normalized to the LCModel fitted 68 ms ‘OFF’ spectrum creatine resonance area and left as simple ratios. As the edited GABA signal includes contributions from GABA as well as macromolecules, we refer to the signal obtained as GABA+.

The high resolution T_1-weighted axial images were segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) compartments employing FSL 4.1 (FMRIB Software Library; Analysis Group, FMRIB; Oxford, UK). Subsequently, segmented images were input to an automated voxel co-registration and partial-volume analysis program to calculate volumetric contributions of GM, WM and CSF.

Statistical Analysis

The primary outcome variables were the GABA+/Cr and Glu/Cr metabolite concentrations in the two regions of interest (dACC, POC). HC, PI, and MDD group differences were tested with one-way ANOVA. Post-hoc contrasts were assessed by the Tukey-Kramer (T-K) test. Voxels exhibiting a Cramér-Rao index >20% or data values falling 3 standard deviations outside of the group mean were excluded from analyses. Between group differences in our secondary outcome variables i.e., psychometric scores and PSG/sleep-wake diary variables, were also compared using one-way ANOVA with post-hoc contrasts assessed by T-K tests. Finally, the relationship of our secondary outcome variables to MRS metabolite levels were explored using Pearson’s correlations. Statistical analyses were performed using R (Version 3.6.0), Excel (Version 2013, Microsoft Corp, Redmond, WA), SPSS (Version 2016, IBM Corp, Armonk, N.Y.).

RESULTS

Table 1 reports demographic, psychometric, and sleep-wake measures for HC, PI, and MDD groups. The MDD group (n=51) included 33 women. The mean age of the MDD group was 33.2 ± 14.4 (range = 18–70) years. Comorbid diagnoses included anxiety disorders (n=23), dysthymia (n=6), eating disorders (n=5), personality disorders (n=9), and ADHD (n=1). The PI group (n=24) included 17 women, with a mean age of 39.5±14.2 (range = 21–69) years. The age and sex-matched HC group (n=25, 68% female) had a mean age of 33.9 ± 14.6 (range = 19–68) years.

ANOVA documented significant ($p<0.001$) between-group differences for all rating scale variables shown in Table 1. Post-hoc tests revealed that subjects with MDD, compared to HCs, reported significantly greater levels of depression on the HAM-D and BDI, greater levels of Anhedonia as derived from the BDI, and increased stress, as measured by the PSS ($p<0.001$). Compared to HCs, subjects with MDD also reported significantly worse sleep quality as measured by the ISI and PSQI, greater dysfunctional beliefs and attitudes about sleep as measured by the DBAS-16, and increased fatigue as measured by the FSS ($p<0.001$). Relative to HCs, subjects with PI reported significantly worse sleep quality measured by the ISI and PSQI, greater dysfunctional beliefs and attitudes about sleep reflected by the DBAS-16, and increased fatigue as measured by the FSS ($p<.001$). Finally, relative to those with PI, subjects with MDD reported greater levels of depression measured by the HAM-D, BDI, and Anhedonia subscale, as well as higher levels of stress (PSS) ($p<.001$). Notably, MDD and PI subjects did not differ on ratings of sleep quality by ISI or PSQI.

ANOVA of all measures derived from the sleep-wake diaries also documented significant between group differences ($p<0.001$). Post-hoc tests revealed that both MDD and PI subjects, compared to HCs, reported significantly greater sleep disturbance, i.e., longer SOL, less TST, poorer SE% and reduced sleep quality ($p<0.001$); MDD subjects also reported more WASO than HCs ($p<0.05$). Relative to those with MDD, subjects with PI reported even greater sleep disturbance, i.e., more WASO, less TST, and poorer SE% ($p<0.001$). In large part, objective sleep quality, measured by PSG and subjected to ANOVA, corroborated many of the diary entries. Compared to HCs, MDD exhibited increased SOL ($p<0.01$), decreased TST ($p<0.05$), and poorer SE% ($p<0.05$) whereas subjects with PI exhibited significantly more WASO ($p<0.05$), less TST ($p<0.001$), and poorer SE% ($p<0.01$). However, latency to the first epoch of sleep was significantly longer for those with MDD relative to those with PI ($p<0.05$).

Voxels excluded from analysis due to the Cramér-Rao index exceeding 20% included 18 for GABA+/Cr in the dACC (4 HC subjects, 10 MDD subjects, and 4 PI subjects), 2 for Glu/Cr in the POC (2 PI subjects), and 3 for Glu/Cr in the dACC (1 HC subject, 2 MDD subjects). Additional data excluded from analysis due to falling 3 standard deviations outside of the mean included 1 for GABA+/Cr in the dACC (1 HC subject), 1 for Glu/Cr in the dACC (1 MDD subject), and 1 for Glu/Cr in the POC (1 MDD subject). We observed no significant between-group differences for creatine, or grey or white matter (as percent of grey matter plus white matter) in either POC or dACC.

Our primary outcome measures are shown in Table 2 and Figure 2a–c. A significant between-group difference was observed for Glu/Cr in the dACC ($F=4.97$, $df=95$, $p<.009$). Post-hoc Tukey-Kramer contrasts determined that dACC Glu/Cr was significantly lower (~15%) in MDD subjects than in HCs ($q=4.41$, $p<.01$). No significant between group differences were observed for GABA+/Cr in the dACC nor for GABA+/Cr or Glu/Cr in the POC. However, there was a trend in the POC suggesting a modest reduction in Glu/Cr in those with PI relative to healthy controls and those with MDD.

To further explore the significant decrement in dACC Glu/Cr levels in MDD relative to controls, we examined the relationship between the rating scale measures and dACC Glu/Cr levels within the MDD sample. In the dACC, Glu/Cr was not significantly associated with age, sex, ISI, PSQI, HAM-D, BDI, or PSS. There was, however, a trend negative correlation between dACC Glu/Cr and Anhedonia ($r=-0.24$, $p=0.10$). Also, of note, no sleep diary or PSG measure was significantly correlated with MDD dACC Glu/Cr. Within the PI sample, we partially confirmed our previous finding (Winkelman et al., 2008) of an inverse relationship between GABA/Cr levels and PSG measures of WASO; there was a strong trend towards an inverse relationship between dACC GABA+/Cr and WASO ($r=-0.43$ $p=0.06$). However, we failed to demonstrate a significant inverse relationship between POC GABA+/Cr and WASO ($r=0.05$, $p=0.82$). Finally, we observed a significant, but positive correlation ($r=0.47$, $p<0.01$) between POC GABA+/Cr and WASO in the MDD sample.

DISCUSSION

In this study, we demonstrated glutamatergic abnormalities in the dACC of subjects with diagnosed MDD. Relative to controls, Glu/Cr was significantly reduced by about 15 percent; this decrement did not occur in those with PI. Broadly stated, our observations concur with most (Yuksel and Ongur, 2010; Moriguchi et al., 2019), but not all (Sanacora et al., 2004; Godlewska et al., 2017) ^{1}H MRS investigations of Glu and/or Glx concentrations in those with MDD. Commonly noted are reports of Glx deficits in the dACC and several neighboring loci in the prefrontal cortex in unmedicated MDD subjects (Pfleiderer et al., 2003; Hasler et al., 2007; Chen et al., 2014). Glu and Glx deficits have also been observed in the ACC in symptomatic but medicated patients with MDD (Auer et al., 2000; Horn et al., 2010). This report of elevated levels of glutamine in the putamen of those with MDD, and no decrement in either the OC or dACC (Godlewska et al., 2017), argue for a broadened inquiry into potential regional glutaminergic abnormalities.

Our demonstration of Glu/Cr deficits in the dACC represents only a partial confirmation of the hypotheses we articulated in the introduction. A secondary hypothesis suggested that Glu/Cr decrements in MDD would be related to the extent of sleep disturbance in that group. As shown in Table 1, subjects with MDD were clearly characterized by poor sleep quality, a diagnostic feature of MDD. Despite this demonstration of disordered sleep in those with MDD and, contrary to our secondary hypothesis, dACC Glu/Cr was not significantly correlated with any subjective or objective measure of sleep disturbance.

Although sleep quality was not a significant covariate of Glu/Cr decrements in the dACC, the extent of depressive symptoms remains a potential alternative covariate contributing to

the Glu and Glx values in those with MDD. A recent meta-analysis including over 1000 MDD patients determined that Glx deficits in patients relative to controls could not be explained by symptom severity (Moriguchi et al., 2019). This conclusion was based on several studies. Glu and Glx levels in the ACC did not correlate with HAMD ratings (Auer et al., 2000). Also, in MDD patients, Glx deficits in prefrontal areas did not correlate with MADRS (Montgomery-Asberg Rating Scale) scores (Hasler et al., 2007). Likewise, Glx deficits in the ACC of MDD patients did not correlate with HAMD ratings (Chen et al., 2014). Our data provides mixed support for these conclusions as we observed no association between Glu/Cr levels with neither the HAMD nor the BDI rating scales in our MDD sample. In contrast, the role of the dACC in mood per se (Stan et al., 2014) is modestly supported by our findings. Not only were Glu/Cr levels in the dACC significantly reduced in our MDD subjects relative to controls, but Glu/Cr levels, as a trend, were inversely correlated with levels of anhedonia, a defining feature of MDD. This latter finding is consistent with the observation that anhedonia in MDD is associated with decreased Glu in the pregenual ACC (Walter et al., 2009).

We observed no differences in GABA+/Cr levels in either the POC or the dACC between MDD relative to HCs. These findings are consistent with other reports documenting no GABA differences in the OC (Godlewska et al., 2017) or in the dACC (Walter et al., 2009; Brennan et al., 2017) in MDD relative to controls. These reports stand in contrast to other MRS studies documenting reduced GABA levels in OC (Sanacora et al., 1999; Sanacora et al., 2004; Price et al., 2009), prefrontal loci (Hasler et al., 2007), and dACC (Gabbay et al., 2012) in actively symptomatic, unmedicated MDD subjects.

Among these studies, discrepant GABA findings might be attributed to differences in MRS data acquisition and processing or to heterogeneity in the MDD samples. Within our study and the eight studies cited above, acquisition and processing of MRS data varied in several respects including magnet strength, dimension and placement of voxels in the ROIs (OC, dACC, and prefrontal cortex), as well as referencing of GABA or GABA+ levels either to creatine or to unsuppressed voxel tissue water. Broadly speaking, none of these acquisition and/or processing factors consistently differentiated positive from negative GABA findings. A more compelling explanation of disparate findings might be found in the heterogeneity of the MDD samples, in particular, illness severity and symptom profile. Cogent evidence for the impact of illness severity and symptom profile on GABA is suggested by studies of GABA levels in the OC. In our protocol, MDD subjects were largely recruited through community advertisements, were characterized by mild to moderate depression severity, and few had a history of treatment resistance. In Sanacora's seminal study (Sanacora et al., 1999), GABA levels in the OC were reduced by 52% in the MDD sample compared to controls. The depressives in that sample were markedly ill and were largely recruited from an inpatient psychiatric unit. In a subsequent study from the same group, GABA levels in the OC were reduced by an average of only 15% in the MDD sample relative to controls (Sanacora et al, 2004). That sample, like ours, was recruited through advertisements and community referrals. The combined studies suggested that GABA deficits were particularly prevalent in those with more severe depression.

The role of GABA in sleep regulation is supported by a range of evidence. Through direct study, GABAergic neurons, notably in the ventrolateral preoptic nucleus, have been shown to suppress CNS arousal (Saper et al., 2005). Indirect confirmation of the involvement of GABAergic mechanisms in the pathophysiology of insomnia derives from the efficacy of benzodiazepine and nonbenzodiazepine hypnotics in the treatment of insomnia. These observations are consistent with our previous MRS studies of disordered sleep in PI. These studies revealed a significant inverse relationship between sleep disturbance, notably the amount of polysomnographically-recorded WASO, and GABA/Cr levels both globally (Winkelman et al., 2008) as well as in both the OC and dACC (Plante et al., 2012). In contrast to these previous findings, our current study did not demonstrate a POC or dACC GABA+/Cr deficit in our PI subjects or in our MDD subjects although both groups demonstrated significant comorbid sleep disturbance. We observed a trend inverse association between dACC GABA+/Cr and WASO in the PI group. However, within our MDD sample, we did not observe an inverse correlation between dACC or POC GABA+/Cr and either subjectively or objectively recorded sleep disturbance. We cannot readily explain our finding of a positive association between PSG WASO measures of sleep disturbance and GABA+/Cr levels in the POC.

Across psychiatric conditions, a cohesive picture of the relationship of disordered sleep to GABA mechanisms has failed to emerge. In a study contrasting subjects with PI to good sleeper controls, sleep disturbance in the insomniacs, quantified by PSG WASO, was inversely correlated with OC GABA despite the observation that GABA levels were higher in the insomniacs than in the controls (Morgan et al., 2012). In a more recent study comparing patients with PI to good sleeper controls, GABA and Glx were measured in dACC and dorsolateral prefrontal cortex (DLPFC). GABA levels did not differentiate insomniacs from controls; however, GABA levels in dACC were positively associated with sleep duration suggesting that GABA deficits in the dACC might herald objective sleep disturbances (Spiegelhalder et al., 2016). Two studies of patients with PTSD, a diagnosis characterized by heightened levels of disordered sleep, have also corroborated an association between GABA deficits and poor sleep quality. In a comparison of those with PTSD to trauma-exposed controls without PTSD, GABA levels in the parieto-occipital cortex were significantly reduced in those with PTSD, and, in this patient group, these reductions were associated with poor sleep quality as measured by the Insomnia Severity Index (Meyerhoff et al., 2014). Finally, PTSD subjects have demonstrated not only a lower dACC GABA/H_2O level relative to controls, but also an inverse correlation between these GABA deficits and subjective measures of sleep disturbance (Sheth et al., 2019).

In contrast to the well-studied role of GABA in sleep regulation, our understanding of the role of Glu and Gln in sleep regulation is less well advanced, which is puzzling given that Gln is a major substrate for the synthesis of both Glu and GABA and for the maintenance of their neurotransmitter pools. However, in a recent MRS study of insomnia patients with objectively verified short sleep duration, in contrast to insomnia patients with normal sleep duration and to good sleeper controls, Glu levels in the left OC were significantly reduced in the short sleep duration insomniacs relative to controls and relative to the insomnia group with normal sleep duration. In addition, Glu concentration in the left OC was significantly correlated with total sleep duration and negatively correlated with WASO (Miller et al.,

2017). These findings are consistent with our hypothesis (although not supported in our current data) that GABAergic and potentially glutamatergic mechanisms might mediate disordered sleep in MDD.

Our study design reflected several important strengths. We employed ^{1}H MRS to assess *in vivo* potential GABAergic and glutamatergic abnormalities in both POC and dACC while utilizing a 4 Tesla MRS scanner with greater spectral signal dispersion compared to lower field strengths. Relative to other ^{1}H MRS studies of GABA and Glu abnormalities in depression, our protocol involved one of the largest MDD sample sizes to date (51 depressed). They were age and gender-matched to 25 healthy controls, and 24 enrollees with PI. All subjects with MDD were free from psychoactive medications for a minimum of 4 weeks at the point of MRS scanning, and most for more than a year (44 of 51 participants). Sleep quality was extensively assessed utilizing both subjective and objective measures: ISI and PSQI questionnaires, a minimum of 2 weeks of sleep diaries, 2 weeks of actigraphy monitoring, and 2 overnight polysomnograms which included an assessment of comorbid disordered breathing and periodic limb movements.

In contrast to the strengths of our study design, we also acknowledge the following limitation. Adherence to exclusion criteria proscribing CNS active agents was assessed by urine toxic screens at baseline and end of study, by interview at each study visit, and through daily sleep diary self-report. Use of CNS agents was not confirmed through any other means. The protocol and attendant analytic capabilities would have been enhanced by a more comprehensive assessment of the clinical features of the MDD sample as well as by the recruitment of MDD subjects with a broader range of depression severity.

Summary

This study documented a 15% reduction in Glu/Cr levels in the dACC in MDD compared to healthy controls. In those with PI, there was a trend reduction in Glu/Cr levels in the POC. We did not observe GABA+/Cr decrements in either the POC or the dACC in either patient group compared to controls or to each other.

Although glutamatergic abnormalities may lack diagnostic validity for MDD, they may track specific symptoms or comorbidities both within and across this and other psychiatric disorders. In our protocol, Glu/Cr decrements in the dACC suggested greater ratings of anhedonia, a core feature of MDD. Because of our previous findings linking GABA/Cr deficits in the OC and dACC to disordered sleep in PI, we hypothesized that GABAergic and glutamatergic deficits in unipolar depression might reflect comorbid sleep disturbance rather than the affective component of depressive illness. The results of our current protocol did not confirm this hypothesis. However, coupling our previous findings to subsequent reports of an inverse relationship between occipital GABA levels and PSG measures of WASO (Morgan et al., 2012), as well as self-rated sleep quality (Meyerhoff et al., 2014), suggests that our hypothesis warrants further investigation.

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Highlights:

- ^{1}H MRS was used to examine GABA+ and Glu relative to creatine (Cr) in the dorsal anterior cingulate cortex (dACC) and parieto-occipital cortex (POC) in those with Major Depressive Disorder (MDD), Primary Insomnia (PI), and Healthy Controls (HC).
- Relative to HCs, subjects with MDD exhibited decreased Glu/Cr in the dACC.
- In those with MDD, Glu/Cr levels in the dACC were inversely correlated with anhedonia ratings.
- In those with MDD, sleep quality was not associated with Glu/Cr in the dACC.
- Relative to HCs, subjects with PI exhibited no significant differences in GABA+/Cr or Glu/Cr.

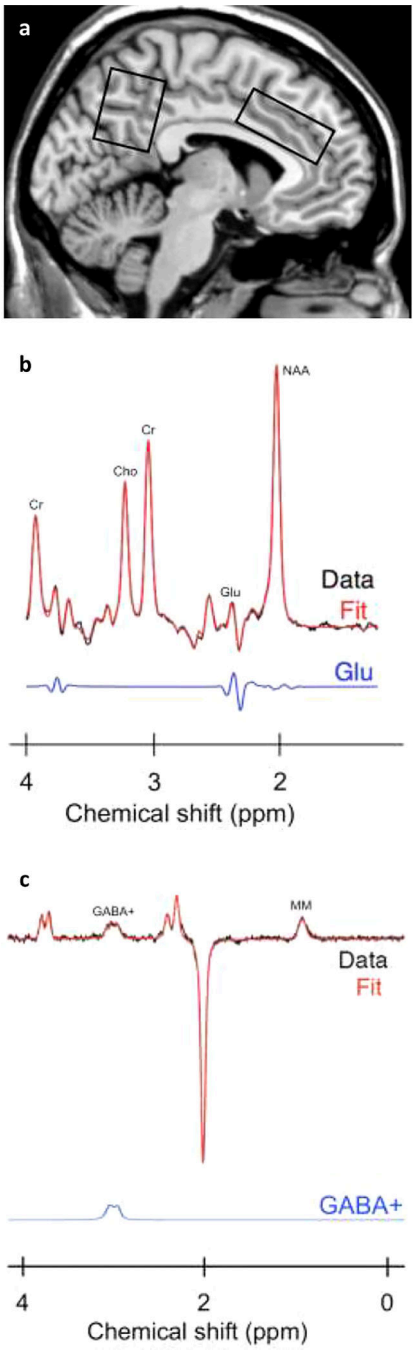

Figure 1.

(a) Mid-sagittal slice showing anatomical placement of MRS voxels in the parieto-occipital cortex and anterior cingulate cortex from a single subject. “Off” spectrum (b) and difference-edited spectrum (c) from a study subject. The two spectra are displayed with no filtering. Metabolites shown include: total creatine (Cr). choline (Cho). glutamate (Glu), N-acetylaspartate (NAA), γ-ami-nobutyric acid plus co-edited macromolecule resonance (GABA +), and co-edited macromolecule resonance at −0.9 ppm (MM).

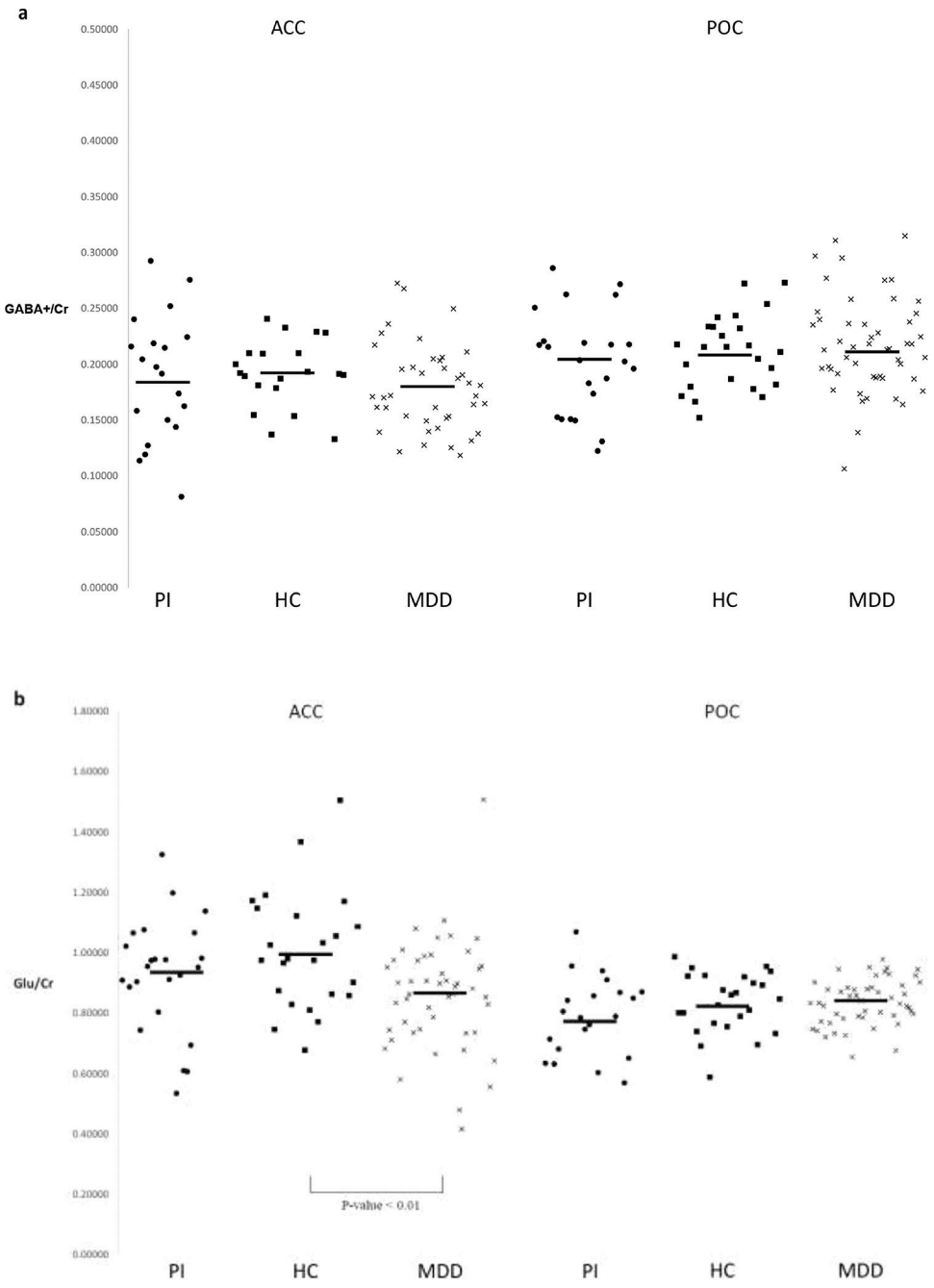

Figure 2.

Individual subject GABA+/Cr (a) and Glu/Cr (b) by brain region of interest. Horizontal bars denote group means.

Table 1

Demographics, Rating Scales, and Sleep-Wake Measures in Healthy Controls, Primary Insomnia, and Major Depression.

	Healthy Controls (Mean (SD)) (n = 25)	Primary Insomnia (Mean (SD)) (n = 24)	Mayor Depressive Disorder (Mean (SD)) (n = 51)
Demographics			
Age, y	33.9 (14.6)	39.5 (14.2)	33.2 (14.4)
Sex (F:M)	17:8	17:7	33:18
BMI, kg/m	22.7 (3.2)	24.4 (4)	23.6 (4.1)
Rating Scales			
Insomnia Severity Index	1.32 (1.57)	17.58 (2.80)	14.86 (5.86)
HAM-D (without sleep items)	0.44 (0.71)	1.92 (1.74)	16.49 (4.07)
Beck Depression Inventory[1]	2.21 (0.98)	5.13 (2.80)	23.55 (7.68)
Anhedonia	0.16 (0.37)	0.54 (0.88)	4.37 (1.91)
Pittsburgh Sleep Quality Index	1.56 (1.33)	10.58 (2.10)	9.67 (3.56)
DBAS 16[2]	2.73 (1.26)	4.70 (1.22)	5.14 (1.47)
Perceived Stress Scale[3]	18.5 (2.47)	18.61 (3.46)	22.43 (4.07)
Fatigue Severity Scale[4]	2.12(0.85)	3.48 (1.31)	4.57 (1.19)
Sleep-Wake Diaries			
Sleep Onset Latency, min	11.62 (6.27)	38.37(21.23)	37.98 (30.81)
Wake After Sleep Onset, min	9.18(7.41)	68.29 (39.40)	31.16(30.75)
Total Sleep Time, hours	7.80 (0.54)	5.55 (0.88)	6.52 (1.21)
Sleep Efficiency. %	93.44 (2.68)	69.65 (9.27)	80.87(11.65)
VAS SIecp Quality	73.57(11.62)	38.70 (10.03)	47.98 (15.68)
Polysomnography			
Apoca/Hypopnea Index	0.94 (1.43)	3.23 (9.49)	2.68 (5.51)
LPS, min	14.22 (19.21)	26.23 (29.22)	41.23(38.89)
SOI min*	11.20(19.48)	18.38 (22.41)	3S.37 (32.49)
Wake After Sleep Onset, min	44.16 (36.62)	87.50 (88.55)	61.48 (57.30)
Total Sleep Time, hours[5]	7.59 (0.72)	6.09 (1.72)	6.79 (1.18)
Sleep Efficiency, %	89.47 (7.26)	78.17 (17.72)	81.18(10.18)

Table 2

^{1}H MRS Measures in Healthy Controls, Primary Insomnia, and Major Depression.

REGION OF INTEREST	^{1}H MRS Measures[a]	Healthy Controls (Mean, SD)	Primary Insomnia (Mean, SD)	Major Depressive Disorder (Mean, SD)	F	P-value (two tailed)	df	Tukey Kramer Post-Hoc Contrasts
Anterior Cingulate Cortex	GABA + /Cr	0.19 (0.03)	0.19 (0.06)	0.18 (0.04)	0.75	0.474	80	
	Glu/Cr	1.00 (0.20)	0.93 (0.19)	0.86 (0.18)	4.97	0.009	95	MDD<HC <.01
	Creatine	161.48 (42.25)	170.93 (42.34)	165.97 (37.72)	0.34	0.71	99	
Parieto-Occipital Cortex	GABA + /Cr	0.21 (0.03)	0.20 (0.05)	0.22 (0.04)	1.21	0.302	98	
	Gtu/Cr	0.83 (0.10)	0.79 (0.13)	0.84 (0.08)	2.60	0.080	95	
	Creatine	219.2 (40.78)	214.83 (45.27)	217.44 (34.02)	0.08	0.93	98	

[a]Values reportd only include subjects with Cramér-Rao < 20%.