Research Paper

Decreased GABA+ ratios referenced to creatine and phosphocreatine in the left dorsolateral prefrontal cortex of females of reproductive age with major depression

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Background: It has been suggested that the dorsolateral prefrontal cortex (DLPFC), especially the left DLPFC, has an important role in the pathophysiology and the treatment of major depressive disorder (MDD); furthermore, the contributory and antidepressant role of γ-aminobutyric acid (GABA) is increasingly recognized. Given that most female patients with MDD are of reproductive age, we sought to assess in vivo baseline GABA levels in the left DLPFC among unmedicated females of reproductive age with depression. **Methods:** We compared healthy females and females with MDD. Both groups were of reproductive age. We confirmed absence of current or past psychiatric diagnosis among healthy controls or a current diagnosis of MDD via a structured interview. We measured GABA+ (including homocarnosine and macromolecules), referenced to creatine and phosphocreatine, via magnetic resonance spectroscopy using a 3 Tesla magnet. **Results:** We included 20 healthy controls and 13 participants with MDD. All participants were unmedicated at the time of the study. All females were scanned during the early follicular phase of the menstrual cycle. Levels of GABA+ in the left DLPFC were significantly lower among participants with MDD (median 0.08) than healthy controls (median 0.10; $U = 66.0$, $p = 0.02$, $r = 0.41$). **Limitations:** When we adjusted for fit error as a covariate, we lost statistical significance for left DLPFC GABA+. However, when we adjusted for signal-to-noise ratio, statistical significance was maintained. **Conclusion:** Our results suggest that GABA+ levels in the left DLPFC may vary by depression status and should be examined as a possible treatment target.

Introduction

It has been suggested that the dorsolateral prefrontal cortex (DLPFC), especially the left DLPFC, has a role in the pathophysiology and the treatment of major depressive disorder (MDD). Positron emission tomography and functional magnetic resonance imaging studies have shown that the left DLPFC is hypoactive in patients suffering from MDD.[1,2] The best evidence for that major role of the left DLPFC in MDD is that applying transcranial magnetic stimulation (TMS) to the left DLPFC is a well-established treatment of MDD.[3]

The importance of γ-aminobutyric acid (GABA) in the pathophysiology of MDD has gained increased recognition.[4,5] Recent data show that medications that directly modulate the GABA receptors display rapid antidepressant activity. For example, allopregnanolone is a metabolite of progesterone and a potent positive allosteric modulator of the $GABA_A$ receptor.[6] Zuranolone is an exogenous formulation of allopregnanolone and is effective in treating MDD.[7]

A more specific role of GABA in the DLPFC of people with MDD has also been suggested in postmortem studies. For example, Yin and colleagues[8] found lower gene-level expression of a protein-coding transcript of the $GABA_A$ receptor γ-2 subnit in the DLPFC of 9 participants with MDD. Furthermore, the expression of the neuropeptide somatostatin, a specific GABA interneuron subtype, is dysregulated in the DLPFC of people with MDD.[9] Decreases in the density and size of GABAergic interneurons, and in levels of glutamic acid decarboxylase-67 in the DLPFC, have also been observed among people with MDD.[5]

Magnetic resonance spectroscopy (MRS) is the sole noninvasive neuroimaging technique that enables in vivo detection and measurement of brain metabolite concentrations, such as GABA, in localized brain regions.[10] Quantification of a metabolite using MRS is made in reference to another metabolite; results are therefore reported as ratios. Most MRS investigations of MDD have used creatine and phosphocreatine as measurements of

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reference.[11,12] Homocarnosine and macromolecules are neurometabolites that share similar chemical shifts as GABA in the human brain. Mescher–Garwood point resolved spectroscopy (MEGA-PRESS) is the spectral difference method used to isolate GABA resonance. Since it is difficult to obtain GABA resonance without contamination from macromolecules and homocarnosine, we refer to GABA as GABA+.[9,13] Given that glutamate and GABA are the main contributors to the excitation–inhibition balance in the brain, it is also important to report on glutamate in the investigation of left DLPFC GABA+.

Measuring GABA in the prefrontal cortex using MRS is not new.[14] However, a very limited number of MRS investigations have compared baseline levels of GABA+ in the left DLPFC of unmedicated people currently experiencing MDD with healthy controls. A recently published investigation by Ritter and colleagues[15] reported reduced levels of GABA+ in a group of patients with past and current MDD (some of whom were medicated), compared with healthy controls, in a large prefrontal brain area centred on the left DLPFC. On the contrary, another recent MRS investigation did not find differences in left DLPFC GABA levels among unmedicated young adult females with current or past MDD, compared with healthy controls.[16]

The importance of left DLPFC GABA in MDD is also evidenced by 2 recent investigations that showed that treatment with TMS, applied to the left DLPFC of patients experiencing MDD, resulted in increased levels of GABA+.[17,18] Both of these investigations also found that the antidepressant response to TMS, determined by a percentage of improvement on the depression scale, was associated with a greater increase in GABA+ levels in the left DLPFC. As there were no healthy control groups in these studies, it was not clear if the increase in GABA+ levels in the left DLPFC among patients with MDD was a normalization of abnormally decreased GABA+ levels in the left DLPFC in MDD or a marker of clinical response to TMS treatment.[17,18] Of note, a very small study (6 patients with MDD) that was recently published could not replicate these results.[19]

We have shown that brain GABA+ levels can be affected by a woman's reproductive status. For example, we have found that GABA+ levels are decreased in the medial prefrontal cortex of healthy perimenopausal females.[20] Similarly, Epperson and colleagues[21] reported that healthy menstruating females had a significant decrease in occipital GABA levels from the follicular phase to the late luteal phase. It is therefore important to control for reproductive status and hormonal environment when investigating GABA+ levels among people with MDD.

The objective of the study was to investigate left DLPFC GABA+ levels (referenced to creatine and phosphocreatine) during the follicular phase of the menstrual cycle among females of reproductive age with MDD that was not treated with medication. We hypothesized that GABA+ levels and the ratio of GABA+ to glutamate in the left DLPFC would be lower among those with MDD compared with healthy controls.

Methods

Participants

We recruited participants without psychopathology (healthy controls) and those with MDD for the study. We recruited physically healthy females of reproductive age (aged ≥ 18 yr) with regular occurrence of their menstrual cycle who used a method of birth control that did not deliver female hormones. Only sex at birth was assessed; we did not collect information on gender identity, race or ethnicity. The healthy controls of this investigation were the same as previous publications.[12,20]

For participants with MDD, we confirmed the presence of a current MDD episode using the Mini-International Neuropsychiatric Interview (MINI version 7.0.2),[22] based on criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*. We excluded those with current comorbid psychiatric disorders, with the exception of anxiety disorders such as social anxiety disorder, panic disorder and generalized anxiety disorder. These anxiety disorders are frequently comorbid with MDD episodes.[23] For healthy controls, we excluded those with current or lifetime history of any confirmed psychiatric illness, using the Mini-International Neuropsychiatric Interview (MINI version 7.0.2).[22]

For both participant groups, we excluded those with any contraindications to magnetic resonance imaging (MRI), pregnant females, those using birth control methods that deliver female hormone, those with any medical condition that would interfere with the study (e.g., endocrine or neurologic conditions),[24] those taking medications that may affect brain GABA function at any time while participating in the study[25] and those currently using antidepressants.

Study protocol

We first conducted a prescreening telephone interview. Participants who appeared to be eligible for the study were scheduled for a screening session. Written informed consent was collected, after which participants took part in the screening interview. During this interview, participants underwent a complete medical and psychiatric history. The MINI was used to screen for psychiatric illnesses. Participants who met the inclusion criteria were then booked for a scanning visit. Participants completed the scanning visit between day 2 and 6 of the follicular phase of the menstrual cycle.

All participants underwent an MRS scan and completed the Beck Depression Inventory (BDI), which has an internal consistency (Cronbach α) of 0.86 for psychiatric patients and 0.81 for nonpsychiatric patients.[26] We collected blood samples to measure plasma estradiol and progesterone. We used a third-generation Elecsys immunoassay (Roche Diagnostics) to measure plasma estradiol, and an Access Progesterone assay (Beckman Coulter) to measure plasma progesterone.

Magnetic resonance spectroscopy and imaging

We collected magnetic resonance data at the Peter S. Allen MR Research Centre, University of Alberta, using a Siemens

Prisma 3 T scanner equipped with a 64-channel head-neck coil for signal reception. We acquired anatomic images using sagittal 3-dimensional T_1-weighted magnetization-prepared rapid gradient-echo (MP-RAGE) sequence with an acquisition time of 3 minutes, 39 seconds (repetition time 1800 ms, echo time 2.37 ms, inversion time 900 ms, flip angle 8°, field of view 250 × 250 mm, image matrix 288 × 288, slice thickness 0.85 mm, number of slices 208, resolution 0.87 × 0.87 × 0.85 mm, parallel acceleration factor 3). We used these images for planning the position of spectroscopy voxels, as well as for volumetric and segmentation analysis.

Voxel position and orientation were positioned so that the voxel was perpendicular to the midline in transverse and coronal views, and parallel to the corpus callosum line in the sagittal view; the voxel was then placed, using all 3 planes of the anatomic image, above the horn of the lateral ventricles, anterior and as far lateral as possible while remaining in the cortex. Care was taken to avoid any lipid or skull contamination (Figure 1). We used an identical voxel size (20 × 20 × 20 mm^3) and position for both PRESS and MEGA-PRESS acquisitions.

We used a customized version of the PRESS sequence, allowing for asymmetric echo times to measure spectra for glutamate and for creatine and phosphocreatine. Data were acquired summing 64 averages, repeated twice, in 5 minutes, 45 seconds (repetition time 2500 ms, echo time 1 = 90 ms, echo time 2 = 18 ms, bandwidth 2000 Hz, δ frequency –2.4 ppm, 2048 spectral data points). A sample PRESS spectrum is shown in Figure 2. Automated metabolite quantification of the proton MR spectra was performed using LCModel (version 6.3–1L).[27]

We used MEGA-PRESS, implemented as Siemens' work in progress WIP859F, to measure the spectra for GABA and for creatine and phosphocreatine.[27,28] A sample MEGA-PRESS spectrum is shown in Figure 3A and 3B. We obtained an adequate signal-to-noise ratio (SNR) by summing 320 averages composed of 160 pairs, whereby the editing pulses were either on or off, acquired in 10 minutes, 56 seconds (MEGA-PRESS repetition time 2000 ms, echo time 68 ms, bandwidth 2000 Hz, editing pulse frequency 1.9 ppm and 1.1 ppm, δ frequency 1.7 ppm, 2048 spectral data points). We performed automated metabolite quantification of the proton magnetic resonance spectra using Gannet software (version 3.0, http://www.gabamrs.com) on saved raw data (.DAT files), providing relative concentrations of GABA+ and creatine and phosphocreatine. Mikkelsen and colleagues[29] have shown that, within site, the coefficient of variation of the GABA+ data referenced to creatine is typically 10%. No unsuppressed water spectra were acquired.

We conducted manual and quantitative inspection on each spectrum, fitted by both LCModel and Gannet to assure quality with respect to line shape, line width (full width at half maximum [FWHM]), SNR and quality of fit (fit error). The FWHM is a rough estimate of the line width in the in vivo spectrum, whereas SNR is a metric used to describe the performance of the MRI system and the overall data quality. We excluded spectra that did not meet quality check criteria from further analysis. We accepted spectra with a standard deviation of 15% or less on LCModel fit; we accepted Gannet data with an SNR above 80 and a FWHM less than 10 Hz.

We used statistical parametric mapping with SPM12[30] for T_1 image volumetric and segmentation analysis. Included in Gannet package pipeline were steps to generate a mask of the MRS voxel in T_1 image space and to use the SPM12 segmentation function to calculate relative fractions of grey matter, white matter and cerebrospinal fluid (CSF) within the left DLPFC voxel. The methods outlined here were also used in our previous studies.[12,20]

Statistical analysis

We analyzed all data using IBM SPSS software (version 26.0), reported as means and standard deviations (SDs) for parametric tests and medians for nonparametric tests. We

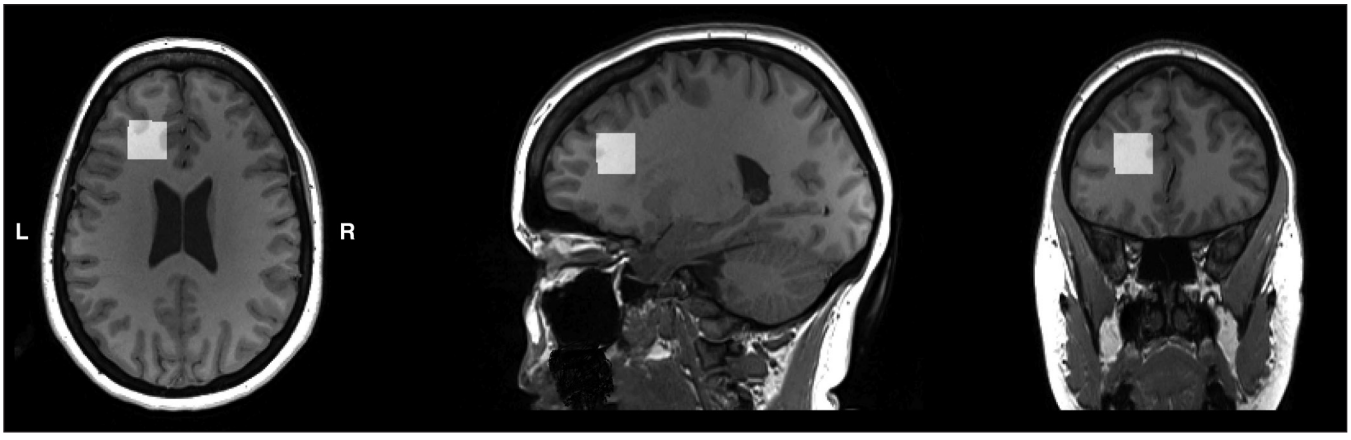

Figure 1: From left to right, transverse, sagittal and frontal T_1-weighted images for a sample participant (depressed, aged 32 yr) with overlaid voxel of magnetic resonance spectroscopy of the left dorsolateral prefrontal cortex. Voxel position and orientation were prescribed so that the voxel was perpendicular to the midline in transverse and coronal views and parallel to the corpus callosum line in a sagittal view; the voxel was then placed, using all 3 planes of the anatomic image, above the horn of the lateral ventricles, anterior and as far lateral as possible while remaining in the cortex. Care was taken to avoid any lipid or skull contamination.

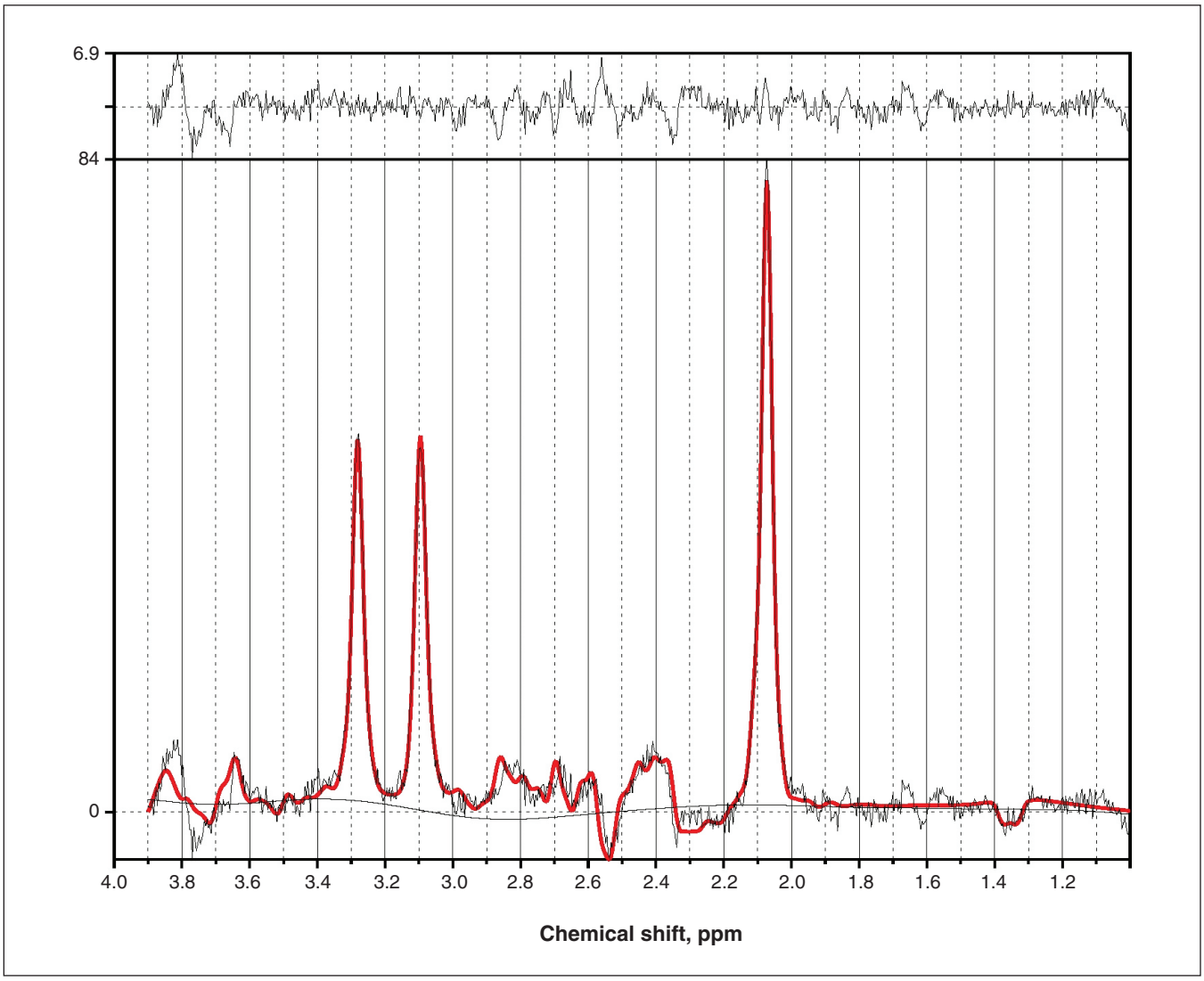

Figure 2: A sample point resolved spectroscopy (PRESS) spectrum with sequence timings optimized for recovering signal from glutamate (PRESS echo time 1 = 90 ms, echo time 2 = 18 ms), from the same participant as Figure 1. The spectrum illustrates the unfiltered data, superimposed with the LCModel (version 6.3–1L) fit in red. The residual noise is shown at the top.

assessed the normality of variables using the Shapiro–Wilk test instead of the Kolmogorov–Smirnov test because our sample size was less than 50. In cases of variables for which the Shapiro–Wilk test was significant ($p \leq 0.05$), we used a nonparametric test (Mann–Whitney *U* test), and in cases of variables for which the Shapiro–Wilk test was nonsignificant ($p > 0.05$), we applied a parametric test (independent sample *t* test).

Overall, the level of glutamate and GABA+ in the left DLPFC, the ratio of GABA+ to glutamate in the left DLPFC, the proportion of CSF in the left DLPFC, BDI score, estradiol, progesterone, SNR, fit error and level of education had a non-normal distribution. Age, the proportion of grey matter, and white matter in the left DLPFC and FWHM had a normal distribution.

We calculated an effect size (*r*) for nonparametric tests, and a 95% confidence interval (CI) for the parametric tests to illustrate the precision of the data.[31] We analyzed the correlation between 2 variables using the Spearman rank-order correlation (nonparametric test) or Pearson correlation tests (parametric test).

We conducted a mediation model for left DLPFC GABA+ levels, left DLPFC glutamate levels and BDI scores using the PROCESS macro extension on SPSS.[32] We conducted separate mediation models of these variables for the full sample size and for participants with MDD only. In these models, the independent variable was the GABA+ level of the left DLPFC, dependent variable was the BDI score and the mediator variable was the glutamate level of the left DLPFC. The proposed mediation is that glutamate in the left DLPFC will mediate the association between GABA+ in the left DLPFC and BDI score. We considered the indirect effect of left DLPFC GABA+ on BDI scores via left DLPFC glutamate statistically significant only if its bias-corrected 95% CI excluded zero.[32]

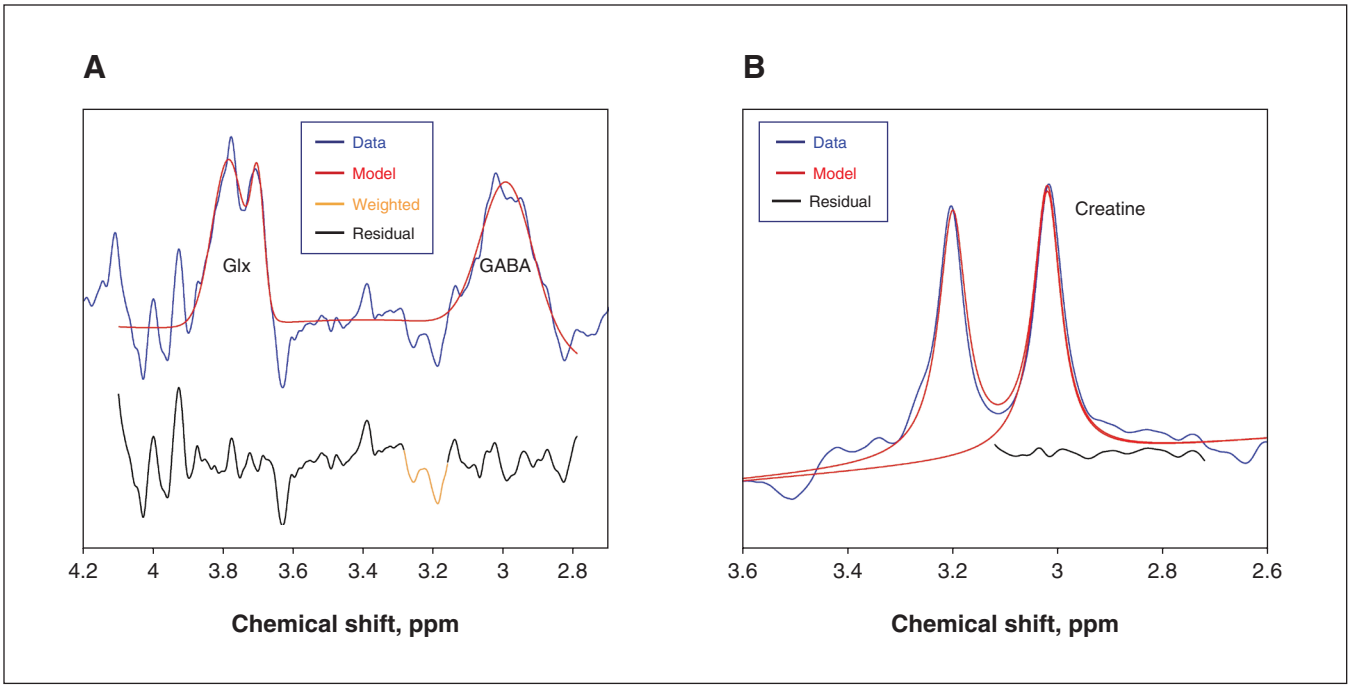

Figure 3: (A) Sample spectra of γ-aminobutyric acid, homocarnosine and macromolecules (GABA+) and glutamate–glutamine (Glx, not analyzed here), with fit from Gannet (version 3.0), from the same participant as Figures 1 and 2. (B) Sample creatine spectrum, with fit from Gannet.

We used Quade nonparametric analysis of covariance (ANCOVA) to adjust for covariates with nonnormal distribution. We analyzed metabolite data using creatine and phosphocreatine as a reference molecule. For all statistical tests, the level of significance was defined to be p less than 0.05.

Ethics approval

The study protocol was approved by the Health Research Ethics Board (Pro00079226) of the University of Alberta and conducted in accordance with the Declaration of Helsinki.

Results

Participants

We recruited 20 females without psychopathology (healthy controls) and 13 with MDD. All participants were of reproductive age and older than 18 years. Of note, no participants were taking any medications for the duration of the study. Of the 33 participants, 32 (96.9%) were enrolled in postsecondary education or had graduated, with no significant differences between controls (median 1.0) and participants with MDD (median 1.0; $U = 120.00$, $p = 0.73$, $r = 0.22$). There was no age difference between the 2 groups, as healthy controls had a mean age of 31.46 (SD 9.66, range 18–47) years and participants with MDD had a mean age of 31.55 (SD 8.90, range 18–48) years ($t_3 = –0.03$, 95% CI –6.90 to 6.72).

The mean duration of illness for our participants with MDD was 16.69 (SD 21.13) weeks. Scores on the BDI were significantly different between healthy controls (median 1.0) and participants with MDD (median 27.0; $U = 260.00$, $p = 0.00$, $r = 0.84$), reflecting that participants with MDD had moderate-to-severe depression on the BDI scale.

We did not remove any data points based on FWHM, fit error and SNR. The quality of MRS is particularly sensitive to participant motion; the Gannet pipeline automatically excludes samples for which the frequency offset deviates sufficiently to affect the spectrum quality (i.e., it excludes metabolite data that are unusable because of brief movements during the scanning period). The number of averages that were automatically excluded were similar between the 2 groups with a mean of 1.51% (SD 1.61%) excluded averages for participants with MDD and 1.30% (SD 1.49%) for healthy controls, with a maximum of 17 excluded averages for 2 participants, of 320 averages acquired ($p = 0.70$). For 1 healthy control, MRS data were severely affected by motion; we scanned this participant again between day 2 and 6 of her follicular phase, consistent with study protocol.

Brain composition and MRS quality control parameters

There were no significant differences in the fraction of grey ($p = 0.83$) or white matter ($p = 0.89$) in the left DLPFC between the 2 groups (Table 1). The proportion of CSF in the left DLPFC was not significantly different between healthy controls (median 1.79%) and participants with MDD (median 1.06%; $U = 120.00$, $p = 0.73$, $r = 0.06$). The FWHM was not significantly different between controls and participants with MDD ($t_{31} = –1.10$, 95% CI –0.38 to 0.11). The SNR was significantly different between controls (median 110.70) and

Table 1: Summary of group differences using independent samples *t* test

Variable	Mean ± SD: Healthy controls *n* = 20	Mean ± SD: Participants with MDD *n* = 13	*p* value	*t*	95% CI
Brain tissue					
Grey matter, %	29.36 ± 4.34	29.00 ± 5.45	0.83	0.21	–3.12 to 3.85
White matter, %	68.86 ± 4.88	69.14 ± 6.29	0.89	–0.14	–4.25 to 3.69
MRS parameter					
FWHM, Hz	7.64 ± 0.34	7.78 ± 0.34	0.28	–1.10	–0.38 to 0.11

CI = confidence interval; FWHM = full-width height maximum; MDD = major depressive disorder; MRS = magnetic resonance spectroscopy; SD = standard deviation.

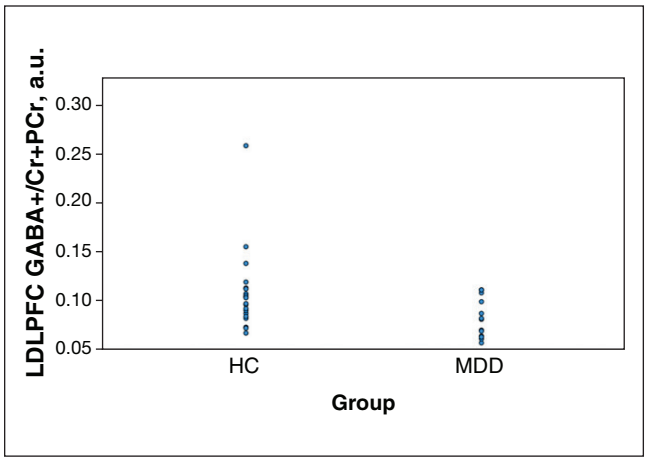

Figure 4: Comparison of left dorsolateral prefrontal cortex (LDLPFC) creatine- and phosphocreatine-referenced (Cr+PCr) γ-aminobutyric acid, homocarnosine and macromolecules (GABA+) levels in healthy controls (HC) and participants with major depressive disorder (MDD).

participants with MDD (median 95.38; $U = 62.00$, $p = 0.01$, $r = 0.44$). Similarly, the fit error was significantly different between controls (median 8.66) and participants with MDD (median 12.48; $U = 213.00$, $p = 0.00$, $r = 0.53$).

Neurometabolites

Levels of GABA+ in the left DLPFC were significantly lower among participants with MDD (median 0.08) than among healthy controls (median 0.10; $U = 66.0$, $p = 0.02$, $r = 0.41$) (Figure 4). There was one outlier among healthy controls (the data point was > 1.5 times the upper quartile). Upon removing this outlier, participants with MDD still had significantly lower levels of GABA+ in the left DLPFC (median 0.08) than healthy controls (median 0.10; $U = 66.0$, $p = 0.03$, $r = 0.39$). Glutamate levels in the left DLPFC were not significantly different between healthy controls (median 0.57) and participants with MDD (median 0.57; $U = 145.00$, $p = 0.60$, $r = 0.10$) (Table 2).

Interestingly, the ratios of GABA+ to glutamate in the left DLPFC were significantly different between healthy controls (median 0.17) and participants with MDD (median 0.13; $U = 70.0$, $p = 0.03$, $r = 0.38$). We noted 2 outliers (1 for the control group and 1 for the MDD group; these data points were > 1.5 times the upper quartile). When removing these outliers, participants with MDD still had significantly lower ratios (median 0.17) than healthy controls (median 0.13; $U = 50.0$, $p = 0.01$, $r = 0.47$).

Hormone levels

Estradiol levels were not significantly different between healthy controls (median 144.0) and participants with MDD (median 154.0; $U = 139.50$, $p = 0.73$, $r = 0.06$). Progesterone levels were also not significantly different between controls (median 1.20) and participants with MDD (median 1.60; $U = 150.00$, $p = 0.48$, $r = 0.13$).

Correlational analysis

Age was not significantly correlated with the proportion of grey matter ($r = –0.24$, $p = 0.18$), white matter ($r = 0.23$, $p = 0.20$) or CSF ($r_s = –0.05$, $p = 0.80$) in the left DLPFC. Similarly, age was not significantly correlated with levels of GABA+ ($r_s = –0.28$, $p = 0.12$) or glutamate ($r_s = –0.14$, $p = 0.44$) in the left DLPFC (for the full sample). When only participants with MDD were analyzed, age and GABA levels in the left DLPFC were not significantly correlated ($r_s = –0.07$, $p = 0.82$). Similarly, age and glutamate levels in the left DLPFC were not significantly correlated for participants with MDD ($r_s = 0.07$, $p = 0.80$). However, when only healthy controls were analyzed, age and GABA+ levels in the left DLPFC were significantly correlated ($r_s = –0.45$, $p = 0.04$) but glutamate levels were not correlated with age ($r_s = –0.28$, $p = 0.23$).

We did not observe a significant correlation between the ratio of GABA+ to glutamate in the left DLPFC and age for the full sample ($r_s = –0.18$, $p = 0.33$) or for subgroups (participants with MDD: $r_s = –0.03$, $p = 0.92$; healthy controls: $r_s = –0.32$, $p = 0.18$).

Levels of GABA+ in the left DLPFC were not significantly associated with glutamate ($r_s = 0.17$, $p = 0.34$). Among participants with MDD, the association between left DLPFC GABA+ and left DLPFC glutamate remained statistically insignificant ($r_s = –0.14$, $p = 0.65$). Interestingly, for the full sample, GABA+ levels in the left DLPFC were significantly associated with BDI scores ($r_s = –0.34$, $p = 0.05$) (Figure 5A). However, when we removed an outlier (1 left DLPFC GABA+ value was > 1.5 times the upper quartile), GABA+

levels in the left DLPFC were no longer significantly associated with BDI scores (r_s = –0.33, p = 0.07) (Figure 5B). Similarly, among participants with MDD only, GABA+ levels in the left DLPFC were not significantly correlated with their BDI scores (r_s = –0.15, p = 0.62) (Figure 5C). Glutamate levels in the left DLPFC were not significantly associated with BDI scores among the full sample (r_s = 0.32, p = 0.07) or among only participants with MDD (r_s = –0.12, p = 0.71).

Age was not significantly associated with estradiol (r_s = 0.00, p = 1.00). Conversely, age was significantly associated with progesterone (r_s = –0.37, p = 0.03).

Mediation model

When analyzing the mediating role of left DLPFC glutamate on the relationship between left DLPFC GABA+ and BDI score, there was no statistically significant indirect effect of GABA+ levels in the left DLPFC on BDI score (–1.84, t = –1.944, bootstrap 95% CI –16.32 to 68.06). Furthermore, the direct effect of left DLPFC GABA+ on BDI score in the presence of the mediator, left DLPFC glutamate, was found to be insignificant (–140.46, p = 0.06). Therefore, left DLPFC glutamate did not mediate the relationship between left DLPFC GABA+ and BDI score (Table 3).

When only participants with MDD were analyzed, there was also no statistically significant indirect effect of GABA+ levels in the left DLPFC on BDI score (15.20, t = 0.459, bootstrap 95% CI –77.80 to 160.62). The direct effect of left DLPFC GABA+ on BDI score in the presence of the mediator, left DLPFC glutamate, was also insignificant (–68.20, p = 0.66). Therefore, left DLPFC glutamate did not mediate the relationship between left DLPFC GABA+ and BDI score among participants with MDD (Table 3).

Quade nonparametric ANCOVA

Fit error and SNR were significantly different between healthy controls and participants with MDD. Consequently, we used Quade ANCOVA to adjust for these covariates. When SNR was adjusted for, left DLPFC GABA+ remained significantly different between groups ($F_{1, 31}$ = 4.57, p = 0.04). However, when fit error was adjusted for, left DLPFC GABA+ was no longer significantly different between groups ($F_{1, 31}$ = 2.78, p = 0.11). Given that age was significantly correlated with left DLPFC GABA+ for healthy controls, we adjusted for age as a covariate. Even after adjustment, GABA+ levels in the left DLPFC remained significantly different between groups ($F_{1, 31}$ = 7.05, p = 0.01).

Discussion

Our study suggests that GABA+ levels in the left DLPFC are decreased during the follicular phase among females of reproductive age with MDD.

These results replicate, to a certain degree, recently published results by Ritter and colleagues,[15] who found lower levels of GABA+ among females and males with MDD in a voxel encompassing the left DLPFC. Alternatively, Ironside and colleagues[16] found no difference in GABA+ levels in the left DLPFC among female adults with current MDD, compared with healthy controls. The clinical relevance of these

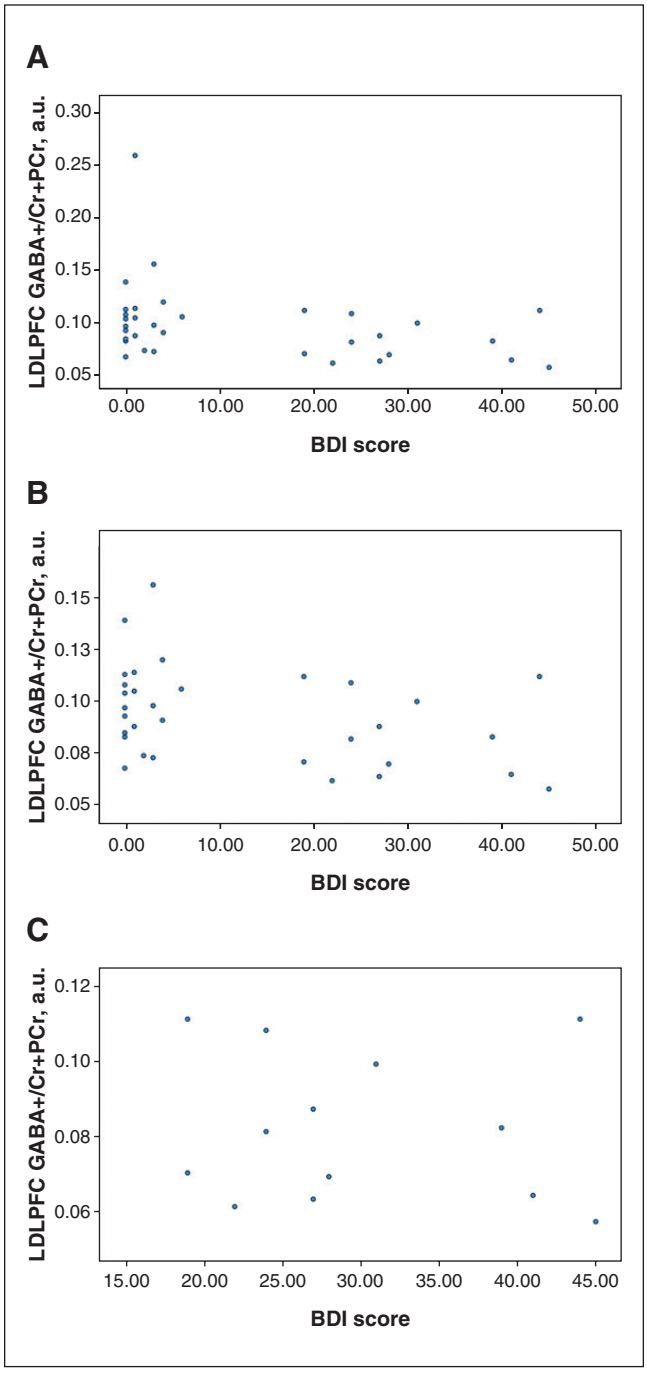

Figure 5: Correlational analysis between score on the Beck Depression Inventory (BDI) and levels of γ-aminobutyric acid, homocarnosine and macromolecules (GABA+) in the left dorsolateral prefrontal cortex (LDLPFC), referenced to creatine and phosphocreatine (Cr+PCr) for (A) the full sample size, (B) for the full sample size excluding 1 outlier and (C) for participants with major depressive disorder.

Table 2: Summary of group differences using Mann–Whitney *U* test

Variable	Mean rank: Healthy controls *n* = 20	Mean rank: Participants with MDD *n* = 13	*U*
Neurometabolite			
LDLPFC GABA+/Cr+PCr	20.20	12.08	66.00*
LDLPFC GABA+/Cr+PCr†	19.53	12.08	66.00*
LDLPFC Glu/Cr+PCr	16.25	18.15	145.00
LDLPFC GABA+/Glu	20.00	12.38	70.00*
LDLPFC GABA+/Glu†	19.37	10.67	50.00*
Brain tissue			
LDLPFC % CSF	17.50	16.23	120.00
MRS parameter			
SNR	20.40	11.77	62.00*
Fit error	12.85	23.38	213.00*
Hormone			
Estradiol	16.53	17.73	139.50
Progesterone	16.00	18.54	150.00
Depression scale			
BDI	10.50	27.00	260.00*

BDI = Beck Depression Inventory; Cr = creatine; CSF = cerebrospinal fluid; GABA = γ-aminobutyric acid; Glu = glutamate; LDLPFC = left dorsolateral prefrontal cortex; MDD = major depressive disorder; PCr = phosphocreatine; SNR = signal-to-noise ratio.
*$p \leq 0.05$.
†Data reported exclude outliers.

Table 3: Summary of mediation analysis

Model*	Total effect (*p* value)	Direct effect (*p* value)	Indirect effect	95% CI	*t*	Conclusion
LDLPFC GABA+ → LDLPFC Glu → BDI	–142.30 (0.05)	–140.46 (0.06)	–1.84	–16.32 to 68.06	–1.94	No mediation
LDLPFC GABA+ → LDLPFC Glu → BDI†	–52.99 (0.71)	–68.20 (0.66)	15.20	–77.80 to 160.62	–0.46	No mediation

BDI = Beck Depression Inventory; CI = confidence interval, Cr = creatine; GABA = γ-aminobutyric acid; Glu = glutamate; LDLPFC = left dorsolateral prefrontal cortex; PCr = phosphocreatine.
*GABA+ and Glu are referenced to Cr+PCr.
†Participants with major depressive disorder only.

results is supported by investigations showing that antidepressant response to treatment with TMS over the left DLPFC is associated with increased GABA+ levels in the left DLPFC of patients with MDD.[17,18] These results from studies involving only participants with MDD, combined with reports of lower levels of GABA+ in the left DLPFC among people with MDD at baseline versus healthy controls, suggest that a normalization of GABA in the left DLPFC of patients with MDD may be a critical contributor to the improvement of depressive symptomatology, at least with TMS. The critical role of GABA in the treatment of MDD is also strongly supported by the demonstration of the rapid antidepressant effect of zuranolone, an exogenous formulation of allopregnanolone, a potent allosteric modulator of GABA receptors.[7]

The balance of inhibition–excitation effects are controlled mainly by GABA and glutamate as the inhibitory and excitatory neurotransmitters, respectively.[33] Interestingly, glutamate levels in the left DLPFC were not different between participants with MDD and healthy controls in our study. However, the ratio of GABA+ to glutamate in the left DLPFC was significantly lower among participants with MDD than healthy controls, suggesting an imbalance toward excitatory activity for the former group.

It is important to note that our results may have been different if our MRS scan had taken place during the luteal phase. Indeed, occipital levels of GABA+ have been shown to decrease from the follicular phase to the late luteal phase among healthy controls.[21] Our results may also have been different if participants had been scanned at a different stage of their reproductive life, such as during perimenopause. Indeed, perimenopause is a period of increased risk for an episode of MDD.[34] The fact that a substantial number of females with no previous history of MDD develop their first episode of MDD during perimenopause has suggested a possible different pathophysiology of perimenopausal depression.[35] However, this stringent control of reproductive status and the menstrual cycle phase is a strength of our study, especially considering the interpretation of the

GABA–glutamate inhibitory–excitatory balance.[36] Indeed, we have previously shown that glutamate levels in the medial prefrontal cortex decreased from the follicular phase to the luteal phase.[37]

Two other investigations have compared GABA+ levels in the left DLPFC among people MDD and healthy controls, with some methodological differences. The voxel of interest investigated by Ritter and colleagues[15] was a large voxel of 25 × 40 × 30 mm^3, encompassing the left DLPFC. The left DLPFC voxel used by Ironside and colleagues[16] was also larger than our study (25 × 30 × 25 mm^3 v. 20 × 20 × 20 mm^3, respectively). Ritter and colleagues[15] and Ironside and colleagues[16] used water as a reference in their MRS investigation.[15,16] Ironside and colleagues[16] included only females in their investigation. They were of reproductive age and imaging took place during the follicular phase of the menstrual cycle. Of note, Ritter and colleagues[15] found a difference by sex in their report of decreased levels of GABA+ in the left DLPFC among participants with MDD.[15] However, Ritter and colleagues[15] did not report on the reproductive status (reproductive, perimenopausal or menopausal) of females with current MDD for whom they obtained data on left DLPFC GABA+. We assume that there were females of reproductive age in this sample, but the authors did not report on or control for the phase of the menstrual cycle when measurements of left DLPFC GABA+ were obtained.[15] Another strength of our study is that the participants with current MDD were unmedicated, whereas 10 participants with MDD were taking psychotropics at the time of scanning in the study by Ritter and colleagues.[15] Ritter and colleagues[15] found similar results to ours using a larger sample size. They compared 47 participants with current MDD with healthy controls, including 30 females, some of whom were medicated.[15] The number of unmedicated females with current MDD in the study by Ironside and colleagues[16] was 19. Our sample size of 13 females with MDD is in the range of previous investigations that have measured brain GABA+ with MEGA PRESS at 3 Tesla among participants with current or past MDD.[38] Our investigation, as with the studies by Ritter and colleagues[15] and Ironside and colleagues,[16] have the limitation of relying only on sex at birth.

Limitations

It is important to note that when we adjusted for fit error as a covariate, we lost statistical significance for left DLPFC GABA+. However, when we adjusted for SNR, statistical significance was maintained. This may indicate that our ability to model the data is influencing our results, warranting the need for further analysis or replication with a larger sample size. However, the effect was not lost when including SNR which provides value to our study as SNR is a metric describing the performance of the MRI system and overall data quality. Other limitations are classical limitations associated with MRS investigations, which do not measure GABA+ levels directly but rather a ratio relative to a reference molecule (in our case, creatine and phosphocreatine). An increase in creatine and phosphocreatine among participants with MDD could appear as lower levels of GABA+. However, creatine and phosphocreatine has been shown to be a stable and appropriate reference in MRS investigations of people with MDD.[11,30,37] Furthermore, a recent investigation has confirmed that the variation in water-referenced GABA+ measurements is similar to referencing against creatine and phosphocreatine.[39] Combined with our results, it suggests that difference in our metabolite of reference (creatine and phosphocreatine) was not responsible for our findings of decreased levels of GABA+ in the left DLPFC of people with MDD. The other classical limitations are the contamination of our GABA measurements by macromolecules and that only carbon-13 (^{13}C) MRS allows for assessment of whether the measurements of neurotransmitters, such as GABA, are relevant to neurotransmission or cell-specific neuroenergetics.[40]

Conclusion

We observed lower levels of GABA+ in the left DLPFC during the follicular phase among females of reproductive age with MDD, compared with healthy controls. These results, combined with those of previous investigations showing that antidepressant response to TMS is associated with an increase in GABA+ levels in the left DLPFC, suggest a possible role of left DLPFC GABA in the pathophysiology and the treatment of MDD.

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