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Acute change in anterior cingulate cortex GABA, but not glutamine/glutamate, mediates antidepressant response to citalopram

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ABSTRACT

Little is known about the acute effects of antidepressant treatments on brain glutamate and gamma-aminobutyric acid (GABA) levels, and their association with clinical response. Using proton magnetic resonance spectroscopy (^{1}H-MRS) we examined longitudinally the effects of citalopram on glutamine/glutamate ratios and GABA levels in the pregenual anterior cingulate cortex (pgACC) of individuals with major depressive disorder (MDD). We acquired ^{1}H-MRS scans at baseline and at days 3, 7, and 42 of citalopram treatment in nineteen unmedicated individuals with MDD. Ten age- and sex-matched non-depressed comparison individuals were scanned once. The association between 1) baseline metabolites and 2) change in metabolites from baseline to each time point and clinical response (change in Montgomery-Åsberg Depression Rating Scale (MADRS) score from baseline to day 42) was assessed by longitudinal regression analysis using generalized estimating equations. Contrary to our hypotheses, no significant associations emerged between glutamate metabolites and clinical response; however, greater increases (or smaller decreases) in pgACC GABA levels from baseline to days 3 and 7 of citalopram treatment were significantly associated with clinical response. These findings suggest that an acute change in GABA levels in pgACC predicts, and possibly mediates, later clinical response to citalopram treatment in individuals with MDD.

1. Introduction

Major depressive disorder (MDD) is common, disabling (Hasin et al., 2005; Kessler et al., 2007), and sometimes refractory even to multiple trials of antidepressant medication (Rush et al., 2006). Thus, it is important to better understand the biological mechanisms of action of current treatments in order to identify early biomarkers that predict later clinical response.

Substantial evidence implicates abnormal glutamatergic neurotransmission in MDD, and glutamate-modulating interventions show promising antidepressant effects (Lener et al., 2016). Several classes of antidepressant treatments, including selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants, monoamine oxidase inhibitors, and electroconvulsive therapy (ECT), have been shown to affect the glutamate system (Skolnick et al., 1996). Thus, modulation of glutamatergic neurotransmission might represent a shared biological pathway, as yet largely unexplored, amongst these mechanistically diverse antidepressant treatments (Skolnick, 1999).

Proton magnetic resonance spectroscopy (^{1}H-MRS) enables in vivo measurement of brain glutamate levels, permitting longitudinal examination of changes in glutamatergic activity in individuals receiving antidepressant treatment. Several studies have used ^{1}H-MRS in this context (Croarkin et al., 2016; Dubin et al., 2016; Godlewska et al., 2015; Grimm et al., 2012; Jarnum et al., 2011; Luborzewski et al., 2007; Merkl et al., 2011; Michael et al., 2003; Njau et al., 2016; Pfleiderer et al., 2003; Taylor et al., 2012; Yang et al., 2014; Zhang et al., 2013), but are characterized by important methodological limitations. First, many of these studies measured Glx – a composite measure containing both glutamate and glutamine – making them difficult to interpret. Second, with few exceptions (Njau et al., 2016;

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Taylor et al., 2012; Zhang et al., 2013), these studies acquired only pre- and post-treatment scans after an extended course of treatment, precluding assessment of early glutamatergic changes that might herald later clinical response. Third, few studies have used ^{1}H-MRS sequences capable of simultaneously quantifying glutamate and gamma-aminobutyric acid (GABA) levels, which is critical given the close interplay of these two reciprocal neurotransmitter systems.

To address these limitations, we assessed the acute and subchronic effects of the SSRI citalopram on glutamatergic and GABAergic neurotransmission in patients with MDD. To this end, we used a combined ^{1}H-MRS sequence employing two spectral editing techniques – 2-dimensional *J*-resolved ^{1}H-MRS and MEGAPRESS – to measure levels of glutamate-related compounds (glutamate and glutamine) and GABA, respectively. We focused our investigation on the pregenual anterior cingulate cortex (pgACC) given extensive evidence linking this brain region to treatment response in MDD (Pizzagalli, 2011). In addition to pre- and post-treatment ^{1}H-MRS scans, we performed scans at days 3 and 7 of treatment to probe acute, possibly transient, changes in pgACC glutamate-related metabolites that might represent early biomarkers of treatment response. For comparison, we also obtained single ^{1}H-MRS scans in healthy control individuals without MDD who did not receive citalopram.

Based on our prior work showing a potential association between antidepressant response and an acute increase in the pgACC glutamate/glutamine ratio (an index reflecting the balance of glutamate metabolites) following treatment with the glutamate-modulating drug riluzole (Brennan et al., 2010), we hypothesized that 1) citalopram treatment would be associated with an acute increase in the pgACC glutamine/glutamate ratio and 2) this increase would be associated with clinical improvement on citalopram. Additionally, based on prior studies demonstrating increases in GABA following SSRIs and ECT (Bhagwagar et al., 2004; Sanacora et al., 2003, 2002), we hypothesized that pgACC GABA levels would also increase following citalopram treatment.

2. Methods

2.1. Participant selection

Depressed participants aged 18–65 years, meeting the DSM-IV criteria for current MDD, and scoring ≥ 18 on the 21-item Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960) at screening and baseline, were recruited from the community using radio, print, and Internet advertising. Exclusion criteria included: history of schizophrenia, bipolar disorder, or obsessive-compulsive disorder; active psychosis; suicidal risk defined as a score of ≥ 4 on question #10 on the Montgomery- Åsberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979) or as judged by the study physician (BPB or HGP); alcohol or substance abuse or dependence (other than nicotine) within 3 months of enrollment; history of ECT treatment; positive urine drug screen for substances of abuse; failure to respond to adequate trials of ≥ 2 antidepressants during the current episode; a prior adequate trial of citalopram as judged by the Principal Investigator; current pregnancy or lactation; history of seizure disorder or organic brain disease; clinically significant medical disease; left-handedness or MRI contraindication. Participants were required to be off all psychiatric medications for at least 2 weeks prior to enrollment (5 weeks for fluoxetine), and no new psychiatric medications were permitted during the study. Right-handed, non-depressed, age- and sex-matched comparison participants, with no lifetime DSM-IV diagnoses, no current psychiatric medications, and no history of psychiatric illness amongst first-degree relatives were recruited from the community.

2.2. Clinical and ^{1}H-MRS evaluation

At a screening evaluation, participants signed informed consent as approved by the McLean Hospital Institutional Review Board. We then obtained demographic information, medical/psychiatric history, the Structured Clinical Interview for DSM-IV (SCID), the HAM-D, physical examination, vital signs, electrocardiogram, clinical laboratory tests, and our primary outcome measure, the MADRS. A study physician unblinded to study design (BPB or HGP) obtained all clinical measures.

Eligible participants returned in ~ 1 week for a baseline visit comprising HAM-D, MADRS, their first ^{1}H-MRS scan, and initiation of a 6-week open-label course of citalopram, 20 mg daily. Citalopram could be increased to 40 mg daily at day 14, and if not tolerated, could be reduced back to a minimum of 20 mg daily prior to day 28. On day 3, participants received another ^{1}H-MRS scan after their morning dose of citalopram, but received no depression ratings. On day 7, participants received a third ^{1}H-MRS scan and the MADRS. Participants again received the MADRS at days 14, 21, 28, and 42. At day 42, participants received the fourth ^{1}H-MRS scan and could choose to continue or taper off citalopram.

2.3. Image acquisition

Imaging was conducted on a Siemens Trio (Siemens Medical Solutions USA Inc., Malvern, PA) with a 32 channel Trans-Imaging Matrix (TIM)™ 3-Tesla (3 T) platform upgrade at the McLean Imaging Center. All participants underwent a routine anatomic scan to screen for structural abnormalities, which was evaluated by a board-certified radiologist.

^{1}H-MRS acquisition used a modified protocol similar to that described in our previous studies (Brennan et al., 2010, 2016, 2015; Jensen et al., 2009). Briefly, a 2 × 2 × 2 cm voxel was placed on the pgACC midsagittally, anterior to the genu of the corpus callosum (Fig. 1). Shimming of the voxel was done using a machine automated shimming routine. Following automated optimization of water-suppression, carrier-frequency, tip angles and coil tuning, a modified *J*-resolved protocol (2D-JPRESS) – which was recently found to have good test-retest reliability (Jensen et al., 2017) – was used. The 2D-JPRESS sequence collected 22 TE-stepped spectra with the echo-time ranging from 35 ms to 350 ms in 15 ms increments (TR = 2 s, f1 acquisition bandwidth = 67 Hz, spectral bandwidth = 2 kHz, readout duration = 512 ms, NEX = 16/TE-step, approximate scan duration = 12 min) providing enough *J*-resolved bandwidth (67 Hz) to resolve glutamate and glutamine (Figs. 1 and 2). Additionally, a GABA-optimized difference-edited MEGAPRESS sequence was used immediately following the 2D-JPRESS acquisition with most acquisition parameters being common between both sequences. The MEGAPRESS sequence collected 68 ms spectral pairs with both an “ON” and “OFF” GABA-editing pulse which allows for optimal GABA-edited spectra in the difference spectrum (Fig. 1). This MEGAPRESS sequence collected 256 pairs of “ON” and “OFF” spectra at a TR = 2 s and a spectral bandwidth of 1.2 kHz and the scan duration = 8.5 min. Since the acquisition optimization was already complete after the 2D-JPRESS, no additional time was needed to optimize for MEGAPRESS.

2.4. ^{1}H-MRS data processing and quantification

To quantify glutamate and glutamine with the JPRESS data, the 22 TE-stepped free-induction decay series (FIDS) was zero-filled out to 64 points, Gaussian-filtered, and Fourier-Transformed using GAMMA-simulated *J*-resolved basis sets modeled for 2.89 T. Every *J*-resolved spectral extraction within a bandwidth of 67 Hz was fit with the spectral-fitting package LCModel (Provencher, 1993) and its theoretically-correct template. The integrated area under the entire 2D surface for each metabolite was calculated by summing the raw peak areas across all 64 *J*-resolved extractions for each metabolite as in our prior publications (Brennan et al., 2010, 2016, 2015; Jensen et al., 2009). Our primary ^{1}H-MRS measure, glutamine/glutamate, is expressed as a ratio, while glutamate, glutamine, and GABA levels are expressed as a ratio to total creatine (tCr) – a widely used approach to reduce subject-specific

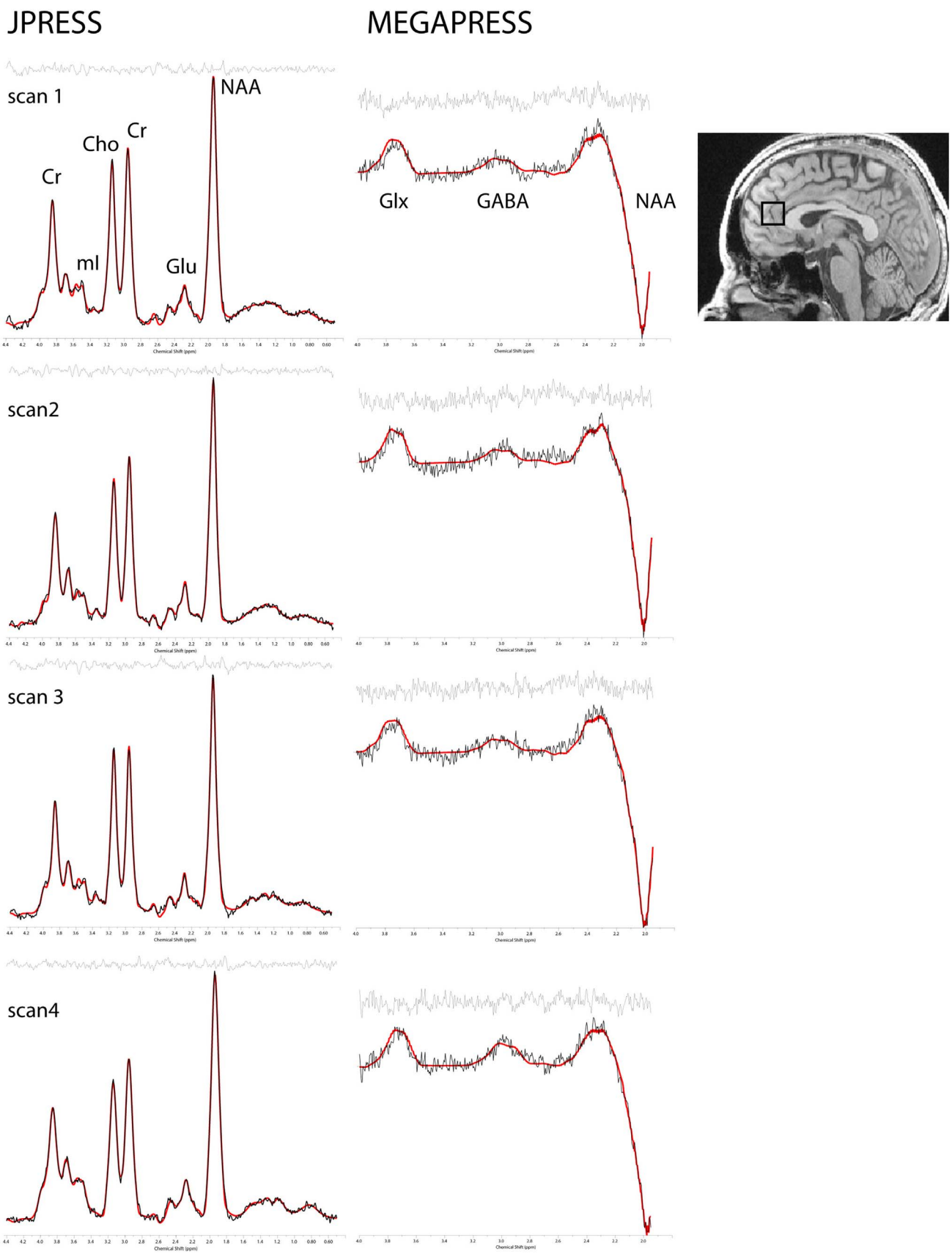

Fig. 1. Sagittal MRI showing placement of magnetic resonance spectroscopy (MRS) voxel in the pregenual anterior cingulate cortex and sample 3 T proton MRS spectra from the pregenual anterior cingulate cortex at baseline (scan 1), day 3 (scan 2), day 7 (scan 3), and day 42 (scan 4) using J-PRESS (extracted from $J = 0.0$ Hz) and MEGAPRESS sequences. Spectra are displayed with LCModel fit and residual. Cho, choline; Cr, creatine; GABA, gamma-amino-butyric acid; Glu, glutamate; mI, myo-inositol; NAA, N-acetylaspartate.

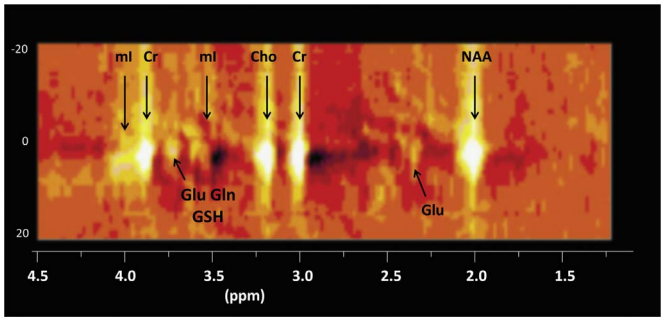

Fig. 2. Two-dimensional spectral plot from the pregenual anterior cingulate cortex showing the J-coupling patterns of the coupled metabolites across J-space. Cho, choline; Cr, creatine; Gln, glutamine; Glu, glutamate; GSH, glutathione; mI, myo-inositol; NAA, N-acetylaspartate.

variance intrinsic to ^{1}H-MRS data (Ongur et al., 2009). To examine potential between-group differences in tCr, we compared the tCr/total signal ratio between groups where total signal is the summation of all fitted raw metabolite peak integrals.

The MEGAPRESS data were first converted into Siemens RDA format and the 68 ms "OFF" subspectrum fitted with a phantom-derived LCModel template acquired under the same in vivo conditions, allowing for quantitation of 68 ms tCr. The difference-edited GABA spectra were fitted with a separate phantom-acquired LCModel difference-edited template allowing for the quantitation of the edited GABA doublet at 3.00 ppm.

For voxel tissue segmentation, 3D mpRAGE axial image data sets were first converted into NIFTI binary image file format using FSL (FMRIB). FMRIB's Automated Segmentation Tool (FAST; Oxford, UK) was used for tissue-segmentation of the T1-weighted image sets into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). Partial tissue fractions were then derived and expressed as a percentage of total tissue contribution for the pgACC voxel using in-house software.

2.5. Statistical analyses

For the primary analysis of 1) change over time in ^{1}H-MRS measures (glutamine/glutamate, glutamate/tCr, glutamine/tCr, GABA/tCr,) and MADRS scores, and 2) the association of change in ^{1}H-MRS measures with change in MADRS, we performed longitudinal analysis with random regression models, which allowed inclusion of all observed data and accounted for the correlation of observations within individuals (Diggle et al., 1994; Fitzmaurice et al., 2004; Gibbons et al., 1993).

For change in MADRS over time, the model included MADRS as the outcome and time (modeled as a continuous variable). The coefficient for the time term in this model quantifies the rate of clinical improvement, which we expressed as the estimated change in the measure at day 42.

For change in ^{1}H-MRS measures over time, our model included the ^{1}H-MRS measure as the outcome and time (modeled as unordered categories) adjusted for age and sex. For the association between change in ^{1}H-MRS measures and change in MADRS, our model included the ^{1}H-MRS measure as the outcome, the day 42 MADRS score, time, and change-by-time interaction adjusted for age, sex, and pre-treatment MADRS score. The coefficient for the interaction term in this model quantifies the association between ^{1}H-MRS measure and day 42 MADRS at the respective time point.

To address the issue of multiple comparisons, we controlled for the false discovery rate (FDR) (Benjamini and Hochberg, 1995) after designating 17 main outcomes of interest. The first of these was decrease in MADRS scores from baseline to week 6 in the citalopram group. Then, for each of the 2 primary metabolites of interest (glutamine/glutamate ratio and GABA/tCr), there were 8 outcomes of interest: 1) the baseline difference between the MDD and the comparison group; 2–4) the change from baseline to each of the 3 post-baseline time points within the citalopram group; 5) the association of clinical response at week 6 with levels of the metabolite at baseline; and 6–8) the 3 post-baseline time points within the citalopram group. Comparisons that were not included among these 17 main outcomes were analyzed descriptively and not inferentially (that is, results given as estimated mean difference and standard error, without calculation of *p*-values).

All analyses were performed using Stata 9.2 software. Alpha was set at 0.05, 2-tailed.

3. Results

3.1. Participants

Thirty-three depressed participants signed informed consent for the study between April 3, 2012 and September 11, 2014. Of these, 13 were withdrawn before receiving treatment (potential MRI contraindications [$N = 3$], withdrew consent [$N = 2$], history of bipolar disorder [$N = 2$], positive drug screens [$N = 2$], lost to follow-up [$N = 1$], abnormal laboratory results [$N = 1$], did not meet criteria for current major depressive episode [$N = 1$], and left-handed [$N = 1$]). All 20 eligible MDD participants completed at least one post-treatment clinical evaluation. Of these, 17 completed 6 weeks of treatment and 3 withdrew due to adverse effects from citalopram. One participant was diagnosed with frontotemporal dementia over a year after completing the study; we excluded this participant's data from analysis. Ten matched non-MDD comparison participants were also included.

Within the MDD group, one participant was taking tramadol and hydrocodone and one participant was taking acetaminophen/hydrocodone for pain secondary to degenerative disc disease. One of these participants was also taking zolpidem as needed for insomnia. Both participants agreed to discontinue use of these medications during the study. One participant with hypothyroidism was taking levothyroxine and had been on a stable dose for 10 years prior to enrollment and one participant was taking omeprazole for gastroesophageal reflux disease. Within the non-MDD group, one participant was taking hydrochlorothiazide and nifedipine for well-controlled hypertension.

Of the 19 MDD participants analyzed, 15 were titrated to 40 mg of citalopram daily; of these, 4 subsequently resumed 20 mg daily because of adverse events at 40 mg. The mean (SD) dose at endpoint was 33.8 (9.6) mg daily. Pre-treatment clinical features are presented in Table 1.

3.2. ^{1}H-MRS

In the MDD group, one participant lacked a day 3 scan, one lacked a day 7 scan (only JPRESS data acquired), and 4 participants lacked a day 42 scan. Additionally, one pre-treatment scan, one day 3 scan, and one day 7 scan were corrupted during data transfer and could not be recovered. After visual inspection of the spectra, one participant's baseline, day 3, and day 42 scans and one participant's day 7 scan (MEGAPRESS data only) were judged unusable due to poor spectral quality and excluded from analysis. Thus, the final sample size for ^{1}H-MRS analyses included 17, 16, 18 (2 participants with JPRESS data only), and 14 MDD participants at baseline, day 3, day 7, and day 42, respectively (Table 2). Of the 10 baseline ^{1}H-MRS scans in non-MDD comparison participants, one was excluded for MEGAPRESS data judged unusable due to poor spectral quality.

Cramer-Rao Lower Bounds (CRLBs) for the final sample ranged from 3% to 11% (glutamate), 5–19% (glutamine), and 4–14% (GABA), and the overall means (SD) did not significantly differ between the groups: glutamate [MDD: 5.6 (1.7), non-MDD: 5.1 (0.7); $t(73) = -1.0$, $p = 0.33$], glutamine [MDD: 12.4 (3.2), non-MDD: 12.5 (2.3); $t(73) = 0.08$, $p = 0.94$], GABA [MDD: 6.7 (2.3), non-MDD: 6 (3.2); $t(70) = -0.8$, $p = 0.45$]. Given the generally accepted upper limit on CRLBs of 20% for reliable fitting using LCModel (Provencher, 2016; Schulte and Boesiger, 2006), we did not exclude any additional ^{1}H-MRS data points on the

Table 1
Pre-treatment demographic and clinical characteristics.

Characteristic	Participants with MDD (*N* = 19)	Participants without MDD (*N* = 10)	*P*
Age, years, mean (SD)	38.5 (12.2)	38.4 (14.1)	0.98[a]
Range	20–56	22–56	
Sex, *N* (%)			0.71[b]
Male	11 (58)	5 (50)	
Female	8 (42)	5 (50)	
Montgomery-Asberg Depression Rating Scale, mean (SD)	26.8 (2.9)	0	
21-Item Hamilton Depression Rating Scale, mean (SD)	21.1 (2.8)	0	
Subtype of depression, *N* (%)			
Atypical	2 (11)	—	
Melancholic	9 (47)	—	
No subtype	8 (42)	—	
DSM-IV comorbidity at time of study, *N* (%)			
None	12 (63)	—	
Dysthymic disorder	4 (21)	—	
Social anxiety disorder	1 (5)	—	
Attention-deficit hyperactivity disorder[c]	1 (5)	—	
Binge-eating disorder	1 (5)	—	
Number of antidepressant trials, lifetime, *N* (%)			
None	11 (58)	—	
1	4 (21)	—	
≥ 2	4 (21)	—	
Duration of MDD, months, mean (SD)	116.5 (139.0)	—	
Range	3–396	—	
Duration of current episode, months, mean (SD)	7.6 (5.7)	—	
Range	3–26	—	
Number of prior depressive episodes, N (%)			
0	7 (37)		
1–5	5 (26)	—	
6–10	2 (11)	—	
11–20	1 (5)	—	
Too many to quantify	4 (21)	—	

MDD, major depressive disorder.
[a] By *t*-test (two-tailed).
[b] By Fisher's exact test (two-tailed).
[c] As per participant report.

Table 2
Mean (SD) metabolite levels in individuals with and without MDD[a].

	Non-MDD	MDD			
	Baseline (*N* = 10)	Baseline (*N* = 17)	Day 3 (*N* = 16)	Day 7 (*N* = 18)	Day 42 (*N* = 14)
Gln/Glu	0.27 (0.04)	0.31 (0.08)	0.33 (0.10)	0.31 (0.12)	0.27 (0.10)
Glu/tCr	0.98 (0.14)	0.89 (0.11)	0.88 (0.17)	0.92 (0.15)	0.91 (0.16)
Gln/tCr	0.27 (0.05)	0.27 (0.05)	0.28 (0.08)	0.27 (0.09)	0.23 (0.06)
GABA/tCr	0.036 (0.010)[b]	0.049 (0.022)	0.034 (0.013)	0.047 (0.020)[c]	0.039 (0.014)

GABA, gamma-amino-butyric acid; Gln, glutamine; Glu, glutamate; MDD, major depressive disorder; tCr, total creatine.
[a] Metabolite levels expressed in arbitrary units.
[b] *N* = 9.
[c] *N* = 16.

basis of CRLBs.

The mean (SD) percentages of GM, WM, and CSF in the pgACC voxel at pre-treatment were 49%(7%), 32%(7%), and 19%(4%) in the non-MDD group and 50%(5%), 29%(8%), and 21%(7%) in the MDD group, respectively. These values showed no significant between-group differences. We found no significant baseline difference in tCr/total signal ratio, $t(25) = 1.2$, $p = 0.26$, between groups. In the MDD group, the percentage of GM in the pgACC was 51%(5%), 50%(4%), and 52%(5%) for day 3, day 7, and day 42, respectively; the percentage of WM was 29%(5%), 28%(7%), and 28%(8%); and CSF was 20%(4%), 22%(8%), and 20%(9%). There were no between-scan differences.

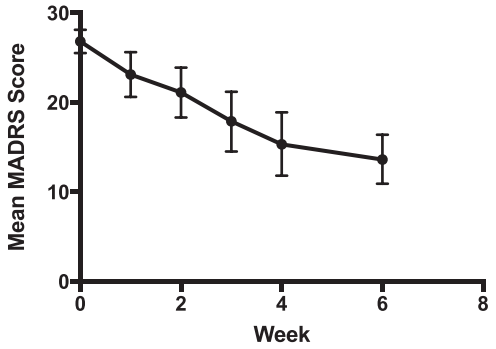

Fig. 3. The mean scores on the Montgomery-Åsberg Depression Rating Scale (MADRS) over 6 weeks of citalopram treatment. Error bars represent 95% confidence interval.

3.3. Calculation of FDR

For the 17 outcomes of interest, the threshold for a 5% FDR was calculated to be 0.015, which yielded 5 *p*-values that were considered statistically significant on this basis (see results in Sections 3.4 and 3.5).

3.4. Clinical measures

In the MDD group, citalopram was associated with significantly decreased MADRS depression scores (estimated mean [SE] change from baseline −13.6 [1.7]; $p < 0.001$; Fig. 3). Eleven of 19 (58%) MDD participants met criteria for response (≤ 50% reduction in MADRS score) (Nierenberg and DeCecco, 2001) and seven of 19 (37%) met criteria for remission (total MADRS score of ≤ 10) (Zimmerman et al., 2004) at day 42 of citalopram treatment.

3.5. ^{1}H-MRS analyses

3.5.1. Between-group analyses

At baseline, there were no significant baseline between-group differences in glutamine/glutamate or GABA/tCr ($p = 0.10$ and 0.15, respectively) (Table 3).

3.5.2. Longitudinal analyses

3.5.2.1. Glutamate-related measures. Contrary to our first hypothesis, we found no significant change in glutamine/glutamate over time with citalopram treatment (Table 3). With regard to our second hypothesis, although the increase in glutamine/glutamate from baseline to day 7 was associated with a numerical decrease from baseline to day 42 in MADRS (β = 0.0094 (0.0047), $p = 0.046$; Table 4), this association failed to be significant upon correction using FDR.

3.5.2.2. GABA/tCr. We found a significant decrease in GABA/tCr levels from baseline to day 3 of citalopram treatment (Table 3). We also observed a significant association between clinical improvement (decrease in MADRS) from baseline to day 42 and increase (or lesser decrease) in GABA/tCr levels from baseline to day 3 and from baseline to day 7. Additionally, lower baseline GABA/tCr levels were associated with greater clinical improvement (Table 4). Although there was an association between clinical improvement at day 42 and increase in GABA/tCr levels from baseline to day 42 (β = 0.0021 (0.00099), $p =$

Table 3
Baseline between-group mean differences in metabolite levels for MDD vs. non-MDD groups and mean differences from baseline to post-baseline time points in metabolite levels within MDD group[a].

	MDD vs. non-MDD group		Within MDD group, Difference from baseline					
	Baseline		Baseline to Day 3		Baseline to Day 7		Baseline to Day 42	
	Difference[b] (SE)	*P*	β[c] (SE)	*P*	β[c] (SE)	*P*	β[c] (SE)	*P*
Gln/Glu	0.039 (0.024)	0.14	0.015 (0.032)	0.64	−0.0025 (0.031)	0.94	−0.039 (0.034)	0.25
Glu/tCr	−0.98 (0.045)	–[d]	−0.0059 (0.047)	–	0.029 (0.046)	–	0.0098 (0.049)	–
Gln/tCr	0.00061 (0.020)	–	0.010 (0.024)	–	0.0017 (0.024)	–	−0.034 (0.025)	–
GABA/tCr	0.013 (0.0074)	0.10	**−0.015 (0.0058)**	**0.0100**	−0.0023 (0.0058)	0.70	−0.011 (0.0060)	0.078

GABA, gamma-amino-butyric acid; Gln, glutamine; Glu, glutamate; MDD, major depressive disorder; tCr, total creatine.
[a] Results that remained statistically significant following control for false discovery rate are bolded (i.e., $p < 0.015$; see Section 3.3 for details).
[b] Estimated mean difference (MDD minus non-MDD), adjusted for age and sex, by linear regression.
[c] Estimated mean difference (post-baseline time point minus baseline), adjusted for age and sex, by random regression, using generalized estimating equations to account for the correlation of observations within individuals (see text).
[d] No *p*-values are reported for Glu/tCr and Gln/tCr because these were secondary measures of glutamate and not part of the set of 17 main outcomes of interest.

0.030; Table 4), this association failed to be significant upon correction using FDR.

We noted upon inspection that 2 MDD participants had much higher pre-treatment pgACC GABA/tCr values (0.10 and 0.096) than the rest of the group ($N = 15$; median [interquartile range] 0.041 [0.033–0.057], with highest value of 0.063). The CRLBs for the outlying GABA measurements were 6% and 8% – well within the generally accepted 20% range for validity for CRLB (Provencher, 2016; Schulte and Boesiger, 2006). Nevertheless, as a sensitivity analysis, we repeated analyses using ranked GABA/tCr data as the outcome. The *p*-values for the 4 previously identified significant *p*-values were somewhat increased, rising to 0.013, 0.010, 0.031, and 0.056 (for the decrease in GABA/tCr from baseline to day 3, and for the 3 associations of GABA/tCr with clinical improvement). Although these differences would not be considered significant using the FDR (which for this analysis is 0.0059), the fact that a clear trend in favor of this set of differences remained intact using this less powerful sensitivity analysis suggests that the findings identified as significant by FDR in the original analysis using non-ranked data are probably not unduly influenced by the 2 high pre-treatment GABA/tCr values in the MDD group.

4. Discussion

The current study revealed several findings. Contrary to our first hypothesis concerning glutamatergic effects, we did not find an acute increase in glutamine/glutamate ratios with citalopram treatment. With regard to our second hypothesis, we found a tentative association between acute increases in glutamine/glutamate ratios (from baseline to day 7 of citalopram treatment) and clinical response at day 42 in the MDD group (nominal *p*-value of 0.046) – but this finding did not survive correction for multiple comparisons using FDR. However, though not hypothesized *a priori*, we observed significant associations between day 42 clinical response in the MDD group and 1) lower GABA/tCr levels at baseline; 2) greater increase (or lesser decrease) in GABA/tCr levels from baseline to day 3 of citalopram treatment; and 3) greater increase (or lesser decrease) in GABA/tCr levels from baseline to day 7 of citalopram treatment.

To our knowledge, this is the first study to use ^{1}H-MRS to investigate both acute and subchronic changes in brain levels of glutamate and glutamine following SSRI treatment in individuals with MDD and to examine their association with clinical response. One prior study examined differences in pgACC Glx and glutamate levels between individuals with MDD who had received one week of treatment with either escitalopram or placebo and a group of non-MDD individuals and found no between-group differences (Taylor et al., 2012). However, this study did not continue escitalopram treatment beyond one week and therefore could not examine the relationship with clinical response nor did it examine pre-treatment between-group differences in Glx or glutamate. Three other longitudinal studies using ^{1}H-MRS failed to detect longer-term changes in glutamate or Glx in MDD individuals following extended antidepressant treatment (Godlewska et al., 2015; Grimm et al., 2012; Jarnum et al., 2011). However, one found an association between clinical response and increased post-treatment glutamate levels in dorsolateral prefrontal cortex (DLPFC) (Grimm et al., 2012). By contrast, increased glutamate and Glx levels in multiple brain regions including pgACC and DLPFC have been associated with response following extended courses of both ECT (Michael et al., 2003; Njau et al., 2016; Pfleiderer et al., 2003; Zhang et al., 2013) and repetitive transcranial magnetic stimulation (rTMS) (Luborzewski et al., 2007; Yang et al., 2014), with two exceptions (Dubin et al., 2016; Merkl et al., 2011). Notably, ECT failed to show acute glutamatergic effects after only two sessions (Njau et al., 2016; Zhang et al., 2013), which may

Table 4
Association of Metabolite Levels at Baseline or Change in Metabolite Levels from Baseline to Time Point with Clinical Improvement at Day 42[a].

	Baseline		Baseline to Day 3		Baseline to Day 7		Baseline to Day 42	
	β[b] (SE)	*P*	β[b] (SE)	*P*	β[b] (SE)	*P*	β[b] (SE)	*P*
Gln/Glu	−0.0043 (0.0037)	0.25	0.0036 (0.0049)	0.47	0.0094 (0.0047)	0.046	−0.0040 (0.0057)	0.48
Glu/tCr	−0.00078 (0.0057)	–[c]	0.0035 (0.0075)	–	−0.0052 (0.0073)	–	0.0042 (0.0088)	–
Gln/tCr	−0.0037 (0.0028)	–	0.0032 (0.0037)	–	0.0062 (0.0036)	–	−0.0019 (0.0043)	–
GABA/tCr	**−0.0021 (0.00064)**	**< 0.001**	**0.0024 (0.00085)**	**0.004**	**0.0033 (0.00096)**	**< 0.001**	0.0021 (0.00099)	0.030

GABA, gamma-amino-butyric acid; Gln, glutamine; Glu, glutamate; MDD, major depressive disorder; tCr, total creatine.
[a] Results that remained statistically significant following control for false discovery rate are bolded (i.e., $p < 0.015$; see Section 3.3 for details).
[b] Estimated mean increase in baseline metabolite level or change in metabolite level for each unit of clinical improvement (specifically, each 1-point decrease in value of day 42 MADRS minus baseline MADRS), adjusted for age and sex, by random regression, using generalized estimating equations to account for the correlation of observations within individuals (see text).
[c] No *p*-values are reported for Glu/tCr and Gln/tCr because these were secondary measures of glutamate and not part of the set of 17 main outcomes of interest.

have been too early to see effects.

Our study failed to support our hypotheses regarding associations between glutamine/glutamate ratios and citalopram treatment or response. Nonetheless, we believe that further studies should still pursue the possibility that a perturbation of the glutamate system in brain regions critically implicated in MDD and treatment changes (Pizzagalli, 2011) may be an essential mechanistic step in antidepressant response across treatment modalities. However, the timing of this perturbation may differ between treatments. Specifically, clinical response to SSRIs may depend on a rapid change in the balance of glutamate metabolites in the pgACC within the first week of treatment. In contrast, response to ECT and rTMS (which are typically administered to more severe and treatment-refractory patients) has largely been associated with subchronic increases in glutamate and Glx following several weeks of treatment perhaps due to the pulsed nature of ECT and rTMS, which may result in more potent cumulative effects on the glutamate system that are detectable weeks after initiation of treatment. Conversely, daily medication administration may result in a less potent initial glutamatergic effect that subsides over time – a phenomenon we have demonstrated previously (Brennan et al., 2010).

Although not hypothesized *a priori*, we found several significant associations between GABA/tCr levels and clinical response to citalopram treatment, as noted above. These findings are consistent with existing evidence of reduced cerebral GABA levels in MDD individuals, which normalizes following treatment with SSRIs and ECT (Sanacora, 2010; Sanacora et al., 2003, 2002) – and even after a single intravenous dose of citalopram (Bhagwagar et al., 2004). We did not find evidence of reduced baseline GABA/tCr levels in our MDD group overall when compared to non-MDD individuals. However, while several groups have demonstrated reduced occipital cortex GABA levels in MDD (Price et al., 2009; Sanacora et al., 2004), studies examining GABA levels specifically in the pgACC of individuals with, compared to individuals without, MDD have found either no difference (Hasler et al., 2007) or deficits only in a treatment-resistant subgroup (Price et al., 2009) suggesting region- and/or subgroup-specificity. Overall, our finding of response-specific increases in GABA/tCr levels in the pgACC after one week of citalopram treatment is consistent with a study demonstrating acute increases in GABA and Glx levels in a similar brain region in MDD patients following ketamine infusion (Milak et al., 2016), suggesting a common mechanistic link between these two distinct antidepressant treatments.

We acknowledge several study limitations. First, our sample size was modest due to participant attrition and the cost and logistical challenges of the study design (which involved four scans per subject), thus limiting statistical power. Second, our open-label design leaves open the possibility of a placebo effect, making it difficult to distinguish biological effects of citalopram from nonspecific effects influencing clinical improvement. Third, we did not assess the menstrual cycle status in female participants, although this has been shown to impact glutamate and GABA levels (Batra et al., 2008; De Bondt et al., 2015). Fourth, with the exception of our primary MRS measure, the glutamine/glutamate ratio, we expressed metabolite levels as ratios to tCr – a method that assumes no between-group differences in tCr (Ongur et al., 2009). However, given the within-subject longitudinal design of this study, and the fact that we found no differences in the tCr/total signal ratio in our MDD and non-MDD groups, this assumption seems reasonable. Fifth, while J-resolved techniques have been shown to improve the specificity of glutamate and glutamine measures in vivo at higher field strength (Jensen et al., 2009), this approach has not been previously demonstrated at 3 T. Therefore, the reduced spectral separation of these complex resonance structures at lower field strength needs to be factored into the interpretation of our findings.

In summary, this study found that clinical response to SSRI treatment in MDD is associated with early changes in GABAergic, but apparently not glutamatergic, activity in the pgACC within the first week of treatment. We believe our findings provide support for: 1) the theory that an acute enhancement of GABAergic activity is a common mechanistic pathway across diverse antidepressant agents; and 2) further investigation of acute increases in glutamine/glutamate ratios and GABA levels in pgACC as potential early biomarkers of response to SSRI treatment in MDD. Overall, this study adds to the existing functional neuroimaging literature (Di Simplicio et al., 2012; Godlewska et al., 2016; Harmer et al., 2009) demonstrating changes in the brain early in antidepressant treatment that may mediate later clinical response – findings that may ultimately be valuable in developing novel depression treatments and informing clinical decision-making.

Trial registration

ClinicalTrials.gov Identifier: NCT01557946

Contributors

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Conflict of interest

Dr. Brennan has received consulting fees from Rugen Therapeutics and research grant support from Eli Lilly and Transcept Pharmaceuticals. Dr. Pizzagalli has received honoraria/consulting fees from Akili Interactive Labs, Black Thorn Therapuetics, Boehringer Ingelheim, Pfizer and Posit Science for activities unrelated to this project. Dr. Hudson has received consulting fees from diaMentis, Shire, and Sunovion; and has received research grant support from Shire and Sunovion. Dr. Pope has received research grant support from Shire and Sunovion. None of the other authors report any potential conflicts of interest.

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