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Rostral Anterior Cingulate Glutamine/Glutamate Disbalance in Major Depressive Disorder Depends on Symptom Severity

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ABSTRACT

BACKGROUND: Patients with major depressive disorder (MDD) show glutamatergic deficits in the ventral anterior cingulate cortex. The glutamine/glutamate (Gln/Glu) ratio was proposed to be connected to glutamatergic cycling, which is hypothesized to be dysregulated in MDD. As an indicator of regional metabolite status, this ratio might be a robust state marker sensitive to clinical heterogeneity.

METHODS: Thirty-two MDD patients (mean age 40.88 ± 13.66 years, 19 women) and control subjects (mean age 33.09 ± 8.24 years, 19 women) were compared for pregenual anterior cingulate cortex levels of Gln/Glu, Gln/total creatine (tCr), Glu/tCr, and gamma-aminobutyric acid/tCr as determined by high-field magnetic resonance spectroscopy. We tested if symptom severity (Hamilton Depression Rating Scale) and anhedonia (Snaith-Hamilton Pleasure Scale) influence the relation of metabolites to clinical symptoms.

RESULTS: MDD patients showed higher Gln/Glu. This was driven by marginally higher Gln/tCr and nonsignificantly lower Glu/tCr. Groups defined by severity moderated relationship between Gln/Glu and the Hamilton Depression Rating Scale. Moreover, severe cases differed from both control subjects and moderate cases. Groups defined by the Snaith-Hamilton Pleasure Scale also displayed differential relationship between Gln/Glu and levels of anhedonia, predominantly driven by Gln/tCr.

CONCLUSIONS: We elaborate previous accounts of metabolite deficits in the anterior cingulate cortex toward increased Gln/Glu. There is a moderated relationship between severity and the ratio, which suggests consideration of different mechanisms or disease state for the respective subgroups in future studies.

Keywords: Anhedonia, Gln/Glu ratio, MDD, MRS, pgACC, Severity

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Major depressive disorder (MDD) is a serious disease (1) that shows large heterogeneity in etiopathology, symptomatology, and treatment response (2). The hypothesis of serotonergic-driven pathogenesis has been complemented with evidence for glutamatergic deficits in depression (3). This paradigm shift has opened avenues for new treatment options (4), but the problem of inadequate treatment response and stratification of patients remains unresolved.

To that end, the glutamatergic system was frequently investigated with magnetic resonance spectroscopy (MRS). Most of the spectroscopy studies of MDD have been done with 1.5T or 3T scanners (5). At lower field strengths, Glx, a joint measure of glutamate (Glu) and glutamine (Gln), has been utilized because separation of Glu and Gln signals presents a challenge owing to lower signal dispersion. Glx is thought to account for Glu and Gln, both of which are present in neurons and astrocytes and are available for neurotransmitter and metabolic purposes (6,7).

One finding stands out in terms of meta-analytical reproducibility, namely the reduced levels of Glx in the anterior cingulate cortex (ACC) during a depressive episode (5,8–10). Nevertheless, some studies also found no change of the Glx in MDD (11,12). Using Glx as a measure is moreover inconclusive, as it does not allow for a separate measurement of Glu and Gln, which may change in opposing directions in pathological conditions (13).

In humans, only a handful of MRS studies examined Glu and Gln levels separately, and the results were ambiguous. In a meta-analysis, Luykx *et al.* (8) reported Glu decrease in the ACC in MDD, but with a smaller effect size when compared with the Glx decrease. Other meta-analyses, in contrast, found no changes for the Glu levels (9,10). Although the reviews differed in the methodology and number of studies, they underlined that the central finding of the decreased Glx in the MDD might reflect not only decreased Glu, but also changes in Gln. Measurements at ultra-high field, such as 7T, with an

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optimized sequence (short echo time/mixing time) provide an opportunity to investigate separate Glu and Gln levels in the same measurement (14) and may help to discern contributions of Glu and Gln to the deviant Glx levels.

When investigating these metabolites separately, the Gln-Glu cycling between neurons and astrocytes must be additionally considered. In short, after release to the synaptic cleft from neurons, Glu is taken by the astrocytes and, together with de novo synthetized Glu, converted to Gln by the Gln synthetase. Gln is then released into the extracellular space for uptake into the excitatory and the inhibitory neurons, where it is converted back to Glu via glutaminase (15,16).

Some authors propose that the Gln-Glu cycling is in disbalance in MDD (5,17). Animal models of depression have provided evidence that the cycling might be reduced in the prefrontal cortex (18). The origin of the disbalance, and consequentially depressive behavior, has been connected to astrocyte disturbances, e.g., via toxic glial ablation in animal models (19), or postmortem account of reduction in astrocyte number and density (20) or astrocyte markers (21,22), e.g., cingulate Gln synthetase–positive astrocytes (23). In sum, results hinted toward astrocyte-related reductions in conversion of Glu to Gln, which may lead to downstream Glu reduction (24,25).

The metabolite levels in MDD patients could be an indicator of treatment response to medication targeting glutamatergic receptors (26). Using the *N*-methyl-D-aspartate receptor antagonist ketamine, which was found to exert antidepressant responses at subanesthetic doses (27), our group recently showed an increase in the Gln/Glu ratio 24 hours after infusion in control subjects (28), i.e., at a time point when a maximal antidepressant response was found in patients (29). In another earlier study, baseline Glx/Glu (denoting Gln levels) predicted reduced severity of symptoms in patients after ketamine infusion (30).

This led us to hypothesize that ketamine infusion might also alter Gln/Glu levels in patients (31). This assumption further incorporated findings from Brennan *et al.* (32), who found an increase in the Gln/Glu ratio in the rostral ACC after treatment with another Glu-modulating medication, riluzole. Notwithstanding, it has not yet been demonstrated that MDD patients actually display reduced Gln/Glu, especially in a region that is functionally important for MDD deficits and ketamine's brain response (28,33).

In this study, we assessed changes in levels of Gln/Glu ratio, as a static correlative of cycling disbalance (34,35) and Glu and Gln in MDD. Based on prior reports of a general reduction in the Glx levels, we hypothesized lower levels of Gln/Glu and, to a potentially different degree, Gln and Glu. Moreover, gamma-aminobutyric acid (GABA) was indicated as another target metabolite altered in the MDD pathophysiology (36), and decreased GABA levels were found in the ACC (37–39). The spectroscopic investigation followed the location of prior findings in the rostral ACC. The rostral, pregenual ACC (pgACC) differs from other parts of the ACC cytoarchitectonically (40,41), as well as with regard to neurometabolites (42) and receptor finger printing (43). In congruence with its involvement in processing hedonic information during emotional stimulation (44), it has been characterized as one of the key regions involved in depression and anhedonia (45–47). In our study, we relied on ultra-high-field MRS at 7T, allowing for a small single voxel placement within the anatomical boundaries of the pgACC subregion (14).

Importantly, large trials have shown that baseline clinical features, such as high baseline symptom severity, influence remission rates after selective serotonin reuptake inhibitor treatment (48). Remarkably, patients with high baseline severity have also shown stronger differentiation between active treatment and placebo compared with mild and moderate levels (49). Therefore, associating baseline neurometabolite levels to clinical features might add to these previous findings. So far, two clinical dimensions of MDD, severity and anhedonia, have been attributed to different metabolite status. For severely depressed groups, lower Glx and Glu have been found (12,46,50), although a direct linear relationship between severity scores on depression questionnaires and Glu has been questioned (5,10). In another study, highly anhedonic patients had lower Gln and no changes in Glu levels (51). To address the diversity of the metabolite associations to clinical dimensions, the relationship between metabolites and both the severity and anhedonia levels was evaluated via moderation models.

Finally, some studies have reported that the antidepressant medication selective serotonin reuptake inhibitors increases occipital Glx (52) and GABA (53). We therefore determined whether medication status influences levels of relevant metabolites in the pgACC.

METHODS AND MATERIALS

Subjects

Thirty-two patients (mean age 40.88 ± 13.66 years, 19 women) with an acute major depressive episode were recruited from the inpatient and outpatient clinics of the Psychiatry and Psychotherapy Department, Otto von Guericke University Magdeburg; the Department of Psychosomatics and Psychotherapy, Otto von Guericke University Magdeburg; and the Psychiatry and Psychosomatics Department, AWO Fachkrankenhaus Jerichow. Patients were clinically diagnosed according to the International Classification of Diseases, 10th Revision (54). Exclusion criteria for MDD patients were major medical illness and neurological conditions, e.g., seizures. Additional exclusion criteria were other psychiatric disorders and a history of alcohol or drug abuse or dependence, though smokers could participate. MDD patients were rated with clinical questionnaires assessing symptoms severity and anhedonia. To measure the severity of depressive symptoms at the time of scanning, the German version of the 17-item Hamilton Depression Rating Scale (HDRS) was administered. The German version of the Snaith-Hamilton Pleasure Scale (SHAPS) (55,56) was used to determine the level of anhedonia. Twenty-three patients were under medication (full medication list can be found in Supplemental Table S1), and 9 patients were medication-free.

Healthy control subjects were recruited via public advertisement and 32 subjects were included matching the MDD patient group for sex and age (mean age 33.09 ± 8.24 years, 19 women). Control subjects were without any psychiatric and neurological diseases and were medication free (excluding contraception pills) as determined by

medical history. Control subjects were assessed with the German Version 5.0.0 of the Mini-International Neuropsychiatric Interview (57) to ensure absence of psychiatric conditions according to the DSM-IV. Further exclusion criteria for both groups were MR contraindications. Except for 1 MDD patient, all subjects were right-handed, measured with the short form of the Edinburgh Handedness Inventory (58). Medical history and examination were done and approved by a study physician. Approval was obtained from the Institutional Review Board of the University of Magdeburg, and all subjects provided written informed consent in accordance with the Declaration of Helsinki.

Anatomical Data

MR images were acquired on a 7T scanner with a 32-channel head array coil (Siemens Healthineers, Erlangen, Germany). First, automated shimming was performed. Then, high-resolution T1-weighted anatomical MR images were obtained, using a magnetization prepared rapid gradient-echo (MPRAGE) sequence (echo time = 2.73 ms, repetition time = 2300 ms, inversion time = 1050 ms, flip angle = 5°, bandwidth= 150 Hz/pixel, isotropic voxel size = 0.8 mm). Individual anatomical images were segmented (VBM8 [www.neuro.uni-jena.de/vbm/] in SPM8 [Wellcome Trust Centre for Neuroimaging, London, UK]). Segmented T1 images were used to calculate individual gray matter partial volumes of the pgACC MRS voxel.

MRS Data

The MRS voxel was placed manually in an anatomically defined region following landmark definitions from Dou *et al.* (42): touching the genu of the corpus callosum, while bypassing larger veins. First, a region-specific shimming was done using an optimized vendor-provided, double gradient-echo shim technique, with the following steps: 1) a manually placed B1 map of the voxel, 2) a voxel shim with participant-specific electric tension information, and 3) a field map. Thereafter, a stimulated-echo acquisition mode sequence was applied and ^{1}H spectra were acquired from the pgACC (voxel size = 20 mm^3 × 15 mm^3 × 10 mm^3) (Supplemental Figure S1). Acquisition parameters were the following: number of excitations = 128, echo time = 20 ms, repetition time = 3000 ms, mixing time = 10 ms, bandwidth = 2800 Hz. A single-average water signal served as internal reference for quantification and eddy current correction. Spectral data (0.6–4.0 ppm) were fitted and quantified using LCModel (V6.3.0; Stephen Provencher, Inc., Oakville, Canada) (59,60). The basis set used for fitting was measured in the scanner and included creatine (Cr), Glu, myo-inositol, lactate, *N*-acetylaspartate, phosphocholine, taurine, aspartate, GABA, Gln, glucose, alanine, *N*-acetylaspartylglutamate, phosphocreatine, scyllo-inositol, acetate, succinate, phosphoryl ethanolamine, glutathione, citrate, and glycerophosphocholine. Spectra were excluded based on visual inspection of curve fit, and if one of the following objective criteria were met: Cramér Rao lower bounds >20%, full width at half maximum >24 Hz, or signal-to-noise ratio <20. Glu, Gln (Supplemental Figure S2), GABA, and total Cr (tCr) were used in subsequent analyses.

Statistical Analyses

Based on the MRS quality exclusion criteria and availability of data, the number of subjects per group or MDD subgroup differed between analyses (Supplemental Table S2). All variables were checked for normality with the Kolmogorov-Smirnov test ($p < .05$). For MRS data, groupwise outliers were defined as above 1.5× the interquartile range higher than quartile 3 or 1.5× the interquartile range below quartile 1 and were subsequently removed from analysis.

To compare groups, first, an equality of variances between MDD patients and control subjects was measured using robust Levene's nonparametric test.

Second, group differences for target metabolites Glu/Cr, Gln/tCr, GABA/tCr, and Gln/Glu ratio were calculated. Analyses of covariance were used, with gray matter volume proportion within the voxel, age, and sex as covariates. The significance threshold was Bonferroni adjusted, $\alpha < .0125$, for a target $\alpha < .05$, by four tests.

Third, we focused on metabolites showing differences in MDD compared with control subjects. To assess the relationship between metabolites and clinical questionnaires measuring different aspects of disease, severity, and anhedonia, we conducted a moderation analysis with Gln/Glu levels as predictor variables, questionnaire scores as outcome variables, and subgroups as a moderator variable. Patient subgroups were defined as mild and moderate-severe depression using the HDRS 17-item scale with a clinical cutoff of an HDRS ≥18 (61). For the SHAPS, there is no clinical cutoff to distinguish moderately or highly anhedonic patients. Therefore, we used a mean score (≥5) to define subgroups. Gray matter, age, and sex were used as covariates. Statistical threshold was $p < .025$. The HDRS and SHAPS did not correlate significantly in our sample ($\rho_{26} = 0.03$, $p = .87$), and the severity and anhedonia subgroups did not overlap in subject composition ($\chi^2_1 = 0.49$, $p = .48$). Post hoc moderation analyses were done for separate Gln/tCr and Glu/tCr ratios to discern possible metabolite specificity for clinical dimensions.

As an exploratory follow-up to the moderation analysis of severity of symptoms, MDD patients were further divided to incorporate several intermediate-severity subgroups based on the American Psychiatric Association's Handbook of Psychiatric Measures (49,62): mild (HDRS score 8–13; $n = 10$), moderate (HDRS score 14–18; $n = 14$), and severe and very severe depression (HDRS score >19; $n = 8$, 3 patients had an HDRS score ≥23 [very severe depression]). Four patients had an HDRS score <8 and were added to the mild subgroup. Differences in metabolites were tested with an analysis of covariance. Threshold was set to $\alpha < .0167$ for three comparisons.

Last, we tested if medication affects levels of Gln/Glu, Glu/tCr, and Gln/tCr. We split the MDD patients into two groups, medication-free patients and patients using medication, and applied analysis of covariance with gray matter, age, and sex as covariates. Threshold was set to $\alpha < .0167$. Analyses were done with SPSS version 24.0 (IBM Corp., Armonk, NY), and the extension PROCESS v2.16 was used to calculate moderation (63). Graphs were created with Prism 6 (GraphPad Software, San Diego, CA), and single data points were not corrected for covariates. Graphs depicting adjusted mean ± 95% confidence interval can be found in the Supplement.

RESULTS

Demographics

There were no group differences with respect to demographic variables as tested with Mann-Whitney *U* tests or chi-square tests (Table 1).

Variance Is Equal Between MDD Patients and Control Subjects for the pgACC Metabolites

There was no significant difference between MDD patients and control subjects in the variance of metabolites (Glu/tCr [$Welch_{1,53.755}$ = 0.47, p = .50], Gln/tCr [$Welch_{1,47.351}$ = 1.65, p = .21], Gln/Glu [$Welch_{1,50.895}$ = 0.62, p = .43], GABA/Cr [$Welch_{1,46.846} < 0.001$, p = .98]).

MDD Patients Show Elevated Gln/Glu Ratio

MDD patients displayed significant higher levels of Gln/Glu ($F_{1,47}$ = 7.71, p = .008, η_p^2 = .152) (Figure 1A). This was driven by higher Gln/tCr ($F_{1,48}$ = 4.04, p = .051, η_p^2 = .084) (Figure 1B), which, however, was not significant after correction for multiple comparisons. Glu/tCr ($F_{1,53}$ = 2.41, p = .13, η_p^2 = .047) was not significantly different between groups (Figure 1C). There was no difference in GABA/tCr levels ($F_{1,45}$ = 0.98, p = .33, η_p^2 = .023).

Severity Displays Moderated Relationship With Gln/Glu Levels in the pgACC

Comparing demographic properties, HDRS subgroups differed in age (Supplemental Table S3).

Main moderation analysis for an HDRS score <18 and ≥18 (total model summary [R^2 = .65, $F_{6,19}$ = 5.02, p = .0031]) revealed trend-level interaction effect (R^2 increase = .06, $F_{1,19}$ = 3.68, p = .070) for the relationship of Gln/Glu with HDRS values, with nonsignificant conditional effects for an HDRS score <18 (t_{19} = −1.34, p = .20) and ≥18 (t_{19} = 1.32, p = .20) (Figure 2A, Supplemental Table S4). Post hoc analyses revealed that this interaction pattern was partially reflected for Gln/tCr levels (total model summary [R^2 = .67, $F_{6,18}$ = 5.26, p = .0027]), but the interaction and conditional effects were not significant (R^2 increase = .04, $F_{1,18}$ = 1.44, p = .25; conditional HDRS score <18 [t_{18} = −1.55, p = .14], conditional HDRS score ≥18 [t_{18} = 0.23, p = .82]) (Figure 2B, Supplemental Table S4). Moderation for Glu/tCr showed no significant effects (total model summary [R^2 = .59, $F_{6,19}$ = 6.43, p = .0008]; interaction [R^2 increase = .004, $F_{1,19}$ = 0.50, p = .49]; conditional HDRS score <18 [t_{19} = 0.08, p = .93], conditional HDRS score ≥18 [t_{19} = −0.83, p = .42]) (Figure 2C, Supplemental Table S4).

In a follow-up exploratory analysis, we tested difference between several intermediate-severity groups. There was a main effect of group for Gln/Glu ($F_{3,46}$ = 5.80, p = .002, η_p^2 = .303). Bonferroni-corrected post hoc tests showed a difference between control subjects and mild (p = .044) and severe (p = .005) cases. Additionally, there were differences between moderate and severe cases (p = .040) (Figure 3A). The results were similar for Gln/tCr (main effect of group [$F_{3,48}$ = 5.66, p = .002, η_p^2 = .288]), in which the main difference was between severe cases and control subjects (p = .004) and between severe and moderate cases (p = .008) (Figure 3B). There was no effect for Glu/tCr (main effect of group [$F_{3,51}$ = 1.57, p = .21, η_p^2 = .095]) (Figure 3C).

Difference in Anhedonia-Metabolites Association Is Driven by Gln/tCr Levels

There were no differences in demographic properties between the SHAPS subgroups (Supplemental Table S5).

Main moderation analysis showed a significant interaction effect for Gln/Glu (total model summary [R^2 = .87, $F_{6,16}$ = 9.60, p = .001]; interaction [R^2 increase = .06, $F_{1,16}$ = 5.50, p = .032]), but only on an uncorrected threshold. Conditional effects were not significant (SHAPS score <5 [t_{16} = −1.64, p = .12], SHAPS score ≥5 [t_{16} = 1.53, p = .15]) (Figure 4A, Supplemental Table S6). Corrected significant difference was, however, observed for Gln/tCr (total model summary [R^2 = .90, $F_{6,15}$ = 14.70, $p < .001$]; interaction [R^2 increase = .10, $F_{1,15}$ = 8.55, p = .011]), with trend-level conditional effect for both SHAPS score <5 (t_{15} = −1.93, p = .072) and SHAPS score ≥5 (t_{15} = 1.98, p = .066) (Figure 4B, Supplemental Table S6). In contrast, there was no effect for Glu/tCr (total model summary [R^2 = .80, $F_{6,14}$ = 6.62, p = .0018]; interaction [R^2 increase < .001, $F_{1,14} < 0.001$, p = .98]; conditional effect for SHAPS score <5 [t_{14} = −0.11, p = .91], conditional effect for SHAPS score ≥5 [t_{14} = −0.16, p = .88]) (Figure 4C, Supplemental Table S6).

Table 1. Demographic and Clinical Properties of MDD Patients and Control Subjects

	MDD Group	Control Group	Statistics
Women/Men	19/13	19/13	–
Age, Years	40.88 ± 13.66	33.09 ± 8.24	U = 383.5, p = .084[a]
BMI, kg/m^2 (n = 29 Women/29 Men)	25.33 ± 5.49	23.38 ± 2.80	U = 358, p = .33
Smoking (No/Yes/Quit) (n = 30 Women/32 Men)	13/11/6	11/13 /8	χ^2_2 = 0.56, p = .76
HDRS Score (n = 32 Women/31 Men)	14.97 ± 6.21	0.55 ± 0.81	U = 8.5, $p < .001$[b]
SHAPS Score (n = 30 Women/28 Men)	4.57 ± 3.81	0.37 ± 0.81	U = 97.5, $p < .001$[b]
pgACC gm, % (n = 29 Women/32 Men)	0.601 ± 0.077	0.596 ± 0.078	t_{59} = −0.25, p = .81

Values are n or mean ± SD.

BMI, body mass index; gm, gray matter partial volume of the voxel; HDRS, Hamilton Depression Rating Scale; MDD, major depressive disorder; pgACC, pregenual anterior cingulate cortex; SHAPS, Snaith-Hamilton Pleasure Scale.

[a] $.1 > p > .05$.

[b] $p < .001$.

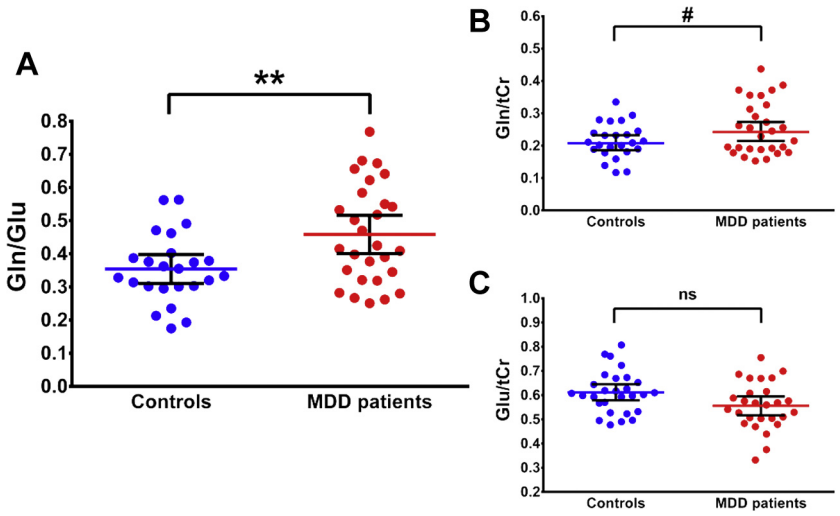

Figure 1. Patients with major depressive disorder (MDD) and control subjects differed in the pregenual anterior cingulate cortex metabolites: **(A)** glutamine/glutamate (Gln/Glu) (**$p < .01$, Bonferroni corrected), **(B)** Gln/total creatine (tCr) ($^{\#}.05 < p < .1$, Bonferroni corrected), and **(C)** Glu/tCr ($^{ns}p > .1$, Bonferroni corrected). Values reported as mean ± 95% confidence interval. ns, not significant.

Gln/Glu Does Not Differ Between Medicated and Medication-Free Patients, but Nonmedicated Patients Differ More From Control Subjects

There were some differences in demographic properties between the medication-free patients and the patients using medication, most notably for body mass index ($U = 24$, $p = .002$) (Supplemental Table S7).

There was a significant main effect of group on Gln/Glu ($F_{2,47} = 5.76$, $p = .006$, $\eta_p^2 = .215$), in which the medication-free patients differed from the control subjects ($p = .006$), but not the patients using medication (compared with control subjects, $p = .27$). The patient groups did not differ between themselves ($p = .27$, all post hoc tests are Bonferroni corrected) (Supplemental Figure S7). Gln/tCr and Glu/tCr were not significant on a corrected threshold (Gln/Cr: main effect of group [$F_{2,48} = 2.58$, $p = .088$, $\eta_p^2 = .107$]; Glu/Cr: main effect of group [$F_{2,53} = 1.23$, $p = .30$, $\eta_p^2 = .049$]).

DISCUSSION

In an age- and sex-matched sample, we observed a general increase in the Gln/Glu ratio in MDD patients, which was driven by marginally higher Gln/tCr and nonsignificantly lower Glu/tCr (Figure 1). The MDD group showed a variation of Gln/Glu levels when it was divided for symptoms severity measured with HDRS (Figures 2 and 3A). Additionally, anhedonia subgroups

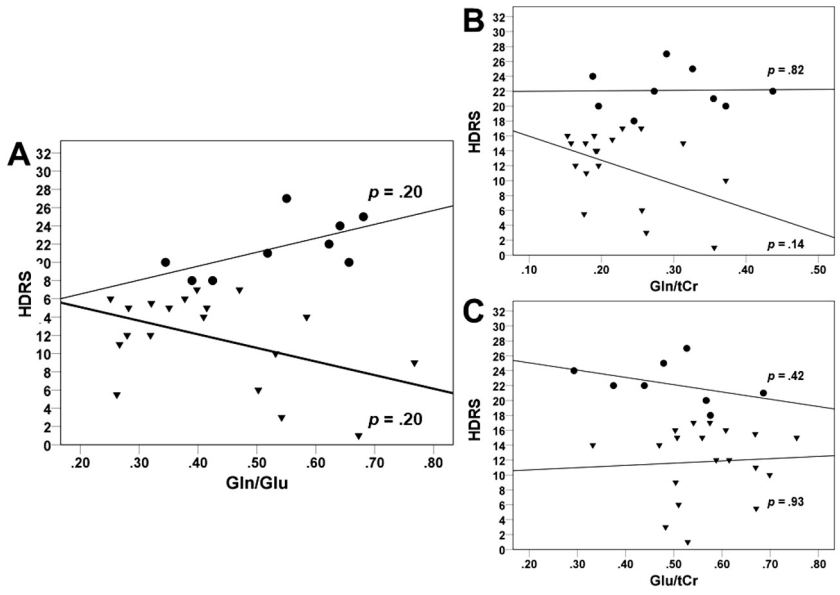

Figure 2. Moderation by Hamilton Depression Rating Scale (HDRS) groups between HDRS scores and metabolites was **(A)** most observable for glutamine/glutamate (Gln/Glu) (interaction; $.05 < p < .1$); follow-up interaction effects for **(B)** Gln/total creatine (tCr) and **(C)** Glu/tCr were not significant ($p > .1$) Values for conditional effects are written on the slopes.

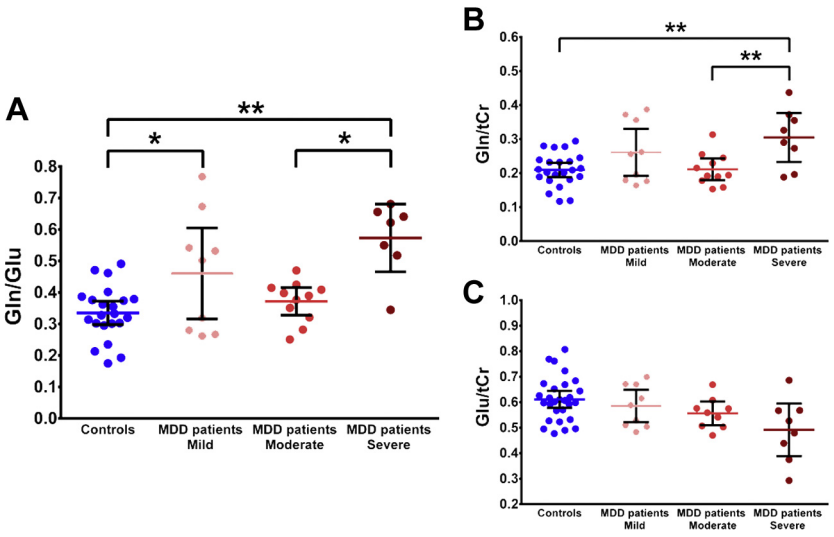

Figure 3. Major depressive disorder (MDD) intermediate-severity groups differ from control subjects but also between themselves. **(A)** Severe cases differed from control subjects and moderate cases for glutamine/glutamate (Gln/Glu); for **(B)** Gln/total creatine (tCr) and **(C)** Glu/tCr, there were no significant differences. * $p < .05$, ** $p < .01$, post hoc Bonferroni corrected (values reported as mean ± 95% confidence interval).

showed differential correlation with Gln/tCr but not with Glu/tCr (Figure 4B, C).

The increase of Gln/Glu and Gln/tCr in MDD patients comes as an unexpected result, contradicting our working hypotheses, which postulated both lower Gln and Glu pool.

The results therefore point to alternative interpretations of ketamine's mechanism of action other than correction of the reduced baseline levels of Gln/Glu in MDD patients. First evidence in patients indicates that, for example, ketamine might not affect Glu or Gln levels in the ventral part of the ACC in MDD (64), thus also limiting the assumptions of earlier studies.

Astrocyte deficits have been found in postmortem samples in depression (21,65,66). Accordingly, our observation might be considered as an indicator of shifted Gln/Glu cycling following a different pathomechanism than the one described in the aforementioned studies.

Further, our results would fit those by Godlewska *et al.* (17), who reported increased Gln/tCr in several other brain regions in MDD. In their report, Gln was increased in the putamen and, on a nonsignificant trend level, also in the ACC.

The very modest decrease of Glu in the MDD is in concordance with the meta-analytical findings of slight and nonsignificant effects as compared with the Glx ones (9,10). Nevertheless, taking into account the abundance of Glu, even a minor decrease could reflect changes in glutamatergic synapses (67,68).

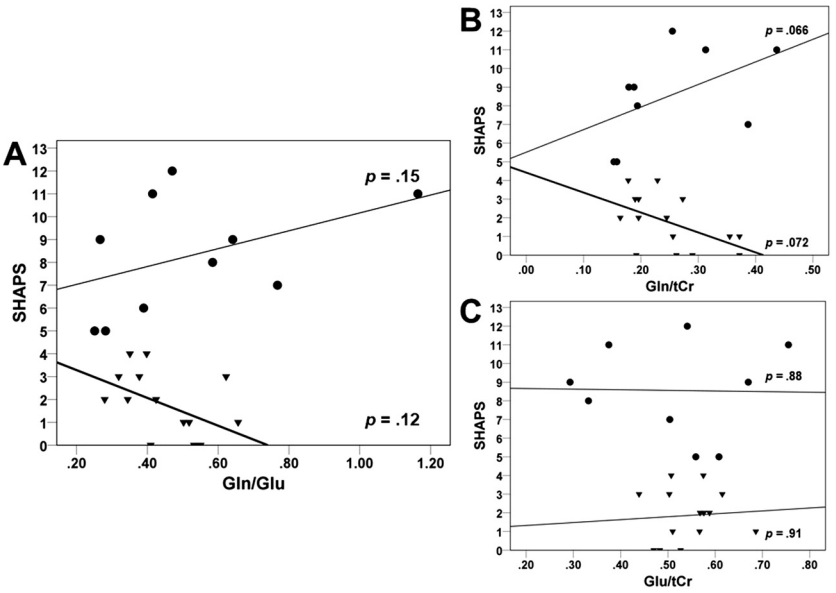

Figure 4. Moderation by Snaith-Hamilton Pleasure Scale (SHAPS) groups between SHAPS scores and metabolites was observable in **(A)** glutamine/glutamate (Gln/Glu) (interaction is uncorrected, $p < .05$) and **(B)** Gln/total creatine (tCr) (interaction is $p < .05$), but not for **(C)** Glu/tCr ($p > .05$). Values for conditional effects are written on the slopes.

Separated by subgroups, the severe and mild groups showed increased Gln/Glu, whereas moderate cases were similar to control subjects. This stands out from the previously observed negative linear relationship between Glx levels and severity (46). However, such simple dependencies have been challenged by negative reports, which seem to outweigh positive findings (5,10). The moderated relationship might thus explain heterogeneity of varying Glu changes and their covariation with clinical severity. The exact mechanisms of symptom severity subgroup differences need to be tested, but we can already detect differential patterns, for example, between the mild and severe subgroups. Here, compared with control subjects, only the severe subgroup shows individually an increase in Gln/tCr and a slight nonsignificant decrease in Glu/tCr, which has been previously seen in chronic disease states (50,69,70). Thus, a possible compensatory mechanism of glial hypertrophy and increase in Gln to Glu astrocyte conversion and Gln/tCr (34) might be happening in severe cases; however, this is only a speculative interpretation lacking direct evidence.

The consequence of assuming different mechanisms across severity would be to expect differences also on the level of treatment success of Glu-based medication. Unfortunately, neither clinical differences in antidepressant efficacy nor metabolite changes have been reported for ketamine across severity subgroups.

For anhedonia, the interaction was driven by the Gln/tCr (Figure 4B), while there was no relationship to Glu/tCr at all (Figure 4C). This contrasts symptoms severity, where Gln/tCr and Glu/tCr contributed to a similar extent (Figure 2B, C). Previously, lower levels of Gln/tCr were found in subgroups of patients with higher anhedonia levels (51). This was not paralleled by the moderation analysis, in which low-anhedonia subgroup showed a trend-negative relationship between Gln/tCr and the SHAPS and a trend-positive slope for the high-anhedonia subgroup. An important limitation in comparing results is the use of different anhedonia questionnaires (Beck Depression Inventory items vs. SHAPS), which tap into distinctive disease categories (amotivation vs. true anhedonia) (71). Moreover, the cutoff used here was made based on the MDD sample mean value and should be taken with caution. Another possible source for discrepancy is different voxel size and position; Walter *et al.*'s (51) voxel was 17.5 mL and spanned different regions: the pgACC and ventromedial prefrontal cortex. In comparison, voxel size in this investigation was 4 mL and was placed in the pgACC (42,72).

Most studies reported no change in the GABA levels in frontal brain regions, in accordance with our results [for a meta-analysis, see Romeo *et al.* (73)]. This might indicate that the ACC GABA deficits in depression could be less prominent than in other conditions such as premenstrual dysphoria disorder (74) or anxiety (75).

Medication-free and patients using medication did not differ between each other significantly for Gln/Glu, speaking for a disease marker. However, the nonmedicated group differed significantly from control subjects, while the medicated one did not (Supplemental Figure S3), indicating some restorative properties of medication. Notably, medicated patients had a higher HDRS score (Supplemental Table S5); therefore, a reduction of the group difference in metabolites cannot be directly interpreted in terms of successfully counteracting depressive symptoms at the time of investigation.

Limitations

There are several limitations of our study that need to be acknowledged, foremost connected to the patient population and study design. MDD patients comprise a heterogeneous group of patients in terms of medication, severity levels, and comorbidities. The generalizability of results thus needs to be taken with caution, as the metabolite levels might change for patients who, for example, have anxiety as a comorbidity (76). The group sizes differed depending on the respective clinical scale, so our conclusions on severity impacts should be tested in a larger sample with equal group sizes for the respective cutoffs. Additionally, 4 patients had a severity score below the clinical threshold set by the HDRS.

Analysis of differences concerning medication intake should be considered as exploratory, as patients were on different medication regimens and we did not account for the pharmacology activity of the medication. One patient was taking lorazepam, a GABAergic medication, which might have influenced the GABA/tCr levels. Both control and MDD groups included smokers, and this should be considered. We used tCr as the denominator, which, on the one hand, corrects for experimental conditions during individual measurements (77), but might, on the other hand, also have an effect on the group variability, as was shown for example in schizophrenia (78). Last, methods such as ^{13}C MRS could be used for a more conclusive outcome on the alteration and directionality of Gln-Glu cycling in the MDD.

Conclusions

We add to previous notions of deviant Gln and Glu mechanisms in a key region involved in MDD. Increased Gln/Glu was accompanied by a modest increase in Gln/tCr and nonsignificant decrease in Glu/tCr. The results indicate the limitations of previous hypotheses on pathomechanisms of shifted cycling in MDD. Most crucially, we have to acknowledge that different mechanisms may apply for different patient subgroups, which can be characterized by symptom severity. Moreover, the absence of group differences at an intermediate severity might help to interpret previous conflicting reports and needs to be addressed during stratification of future patient samples.

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