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# Temporal Dynamics of Antidepressant Ketamine Effects on Glutamine Cycling Follow Regional Fingerprints of AMPA and NMDA Receptor Densities

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The anterior cingulate cortex (ACC) has shown decreased glutamate levels in patients with major depressive disorder. Subanesthetic doses of ketamine were repeatedly shown to improve depressive symptoms within 24 h after infusion and this antidepressant effect was attributed to increased α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) throughput. To elucidate ketamine's mechanism of action, we tested whether the clinical time course of the improvement is mirrored by the change of glutamine/glutamate ratio and if such effects show a regional and temporal specificity in two distinct subdivisions of ACC with different AMPA/*N*-methyl-D-aspartate receptor profiles. In a double-blind, placebo-controlled intravenous infusion study of ketamine, we measured glutamate and glutamine in the pregenual ACC (pgACC) and the anterior midcingulate cortex at 1 and 24 h post infusion with magnetic resonance spectroscopy at 7 T. A significant interaction of time, region, and treatment was found for the glutamine/glutamate ratios (placebo, $n=14$; ketamine, $n=12$). *Post-hoc* analyses revealed that the glutamine/glutamate ratio increased significantly in the ketamine group, compared with placebo, specifically in the pgACC after 24 h. The glutamine/glutamate increase in the pgACC caused by ketamine at 24 h post infusion was reproduced in an enlarged sample (placebo, $n=24$; ketamine, $n=20$). Our results support a significant temporal and regional response in glutamine/glutamate ratios to a single subanesthetic dose of ketamine, which mirrors the time course of the antidepressant response and reversal of the molecular deficits in patients and which may be associated with the histoarchitectonical receptor fingerprints of the ACC subregions. *Neuropsychopharmacology* (2017) **42,** 1201–1209; doi:10.1038/npp.2016.184; published online 19 October 2016

## INTRODUCTION

The importance of the glutamatergic system in the pathophysiology and therapy of mood disorders (Krystal *et al*, 2002; Sanacora *et al*, 2008) has been recently supported by rapid antidepressant effects following a single intravenous subanesthetic dose of ketamine, a non-selective *N*-methyl-D-aspartate (NMDA) receptor antagonist (Berman *et al*, 2000; Salvadore and Singh, 2013; Zarate *et al*, 2006). Initial evidence suggests that ketamine acutely increases glutamate release, which leads to enhanced glutamatergic transmission (Maeng *et al*, 2008) and ultimately results in increased synaptic plasticity and synaptogenesis (Duman and Li, 2012). However, ketamine's downstream effects have not been thoroughly investigated in humans. It is unknown whether changes in the glutamatergic activity following ketamine administration can be detected using available imaging methods and whether these changes show regional and/or specific temporal dynamics.

The antidepressant response to ketamine administration was associated with the pretreatment activity in the pregenual anterior cingulate cortex (pgACC) in treatment-resistant depressed patients (Salvadore *et al*, 2009; Salvadore and Zarate, 2010). This is in accordance with previous studies, which showed that increased pretreatment activity in the pgACC identifies responders to pharmacological antidepressant interventions (Chen *et al*, 2007a; Mayberg *et al*, 1997; Pizzagalli

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*et al*, 2001). These findings highlight the key role of the pgACC in antidepressant effects and the potential role of its activity as a biomarker of early treatment response in depression.

Studies in depression have reported abnormalities in glutamatergic metabolites in multiple brain regions using $^{1}$H magnetic resonance spectroscopy (MRS) (Luykx *et al*, 2012; Yüksel and Öngür, 2010a). Recently, a study in major depressive disorder (MDD) patients revealed an increase in the glutamate + glutamine (glx) levels in the medial prefrontal cortex during ketamine infusion (Milak *et al*, 2015). In particular, in MDD patients, the pgACC is characterized by reduced glx (Auer *et al*, 2000; Yüksel and Öngür, 2010b), which is potentially reflective of a hypoglutamatergic state. Moreover, evidence from the direct measurements of glutamine concentrations further pointed toward an impaired neuron–glia interactions in the pgACC (Brennan *et al*, 2010; Walter *et al*, 2009). In addition, reduced levels of the astroglial glutamine synthetase have been found in the cingulate cortex of depression patients (Bernstein *et al*, 2015). Furthermore, an MRS study reported metabolic deficits in the pgACC, characterized in a subset of highly anhedonic MDD patients, predominantly showing reduced glutamine but not glutamate concentrations relative to the healthy volunteers or to the non-anhedonic patients (Walter *et al*, 2009). Given that such clinical phenomenology may predispose patients towards treatment resistance (Kautzky *et al*, 2015), a direct reversal of such a glutaminergic deficit was proposed as a putative mechanism of action of novel glutamatergic agents, especially those effective in treatment-resistant patients (Brennan *et al*, 2010; Walter *et al*, 2014).

Glutamine levels have been suggested as a marker for synaptically active glutamate and excitatory neurotransmission (Chowdhury *et al*, 2015; Hancu and Port, 2011). Changes in glutamine levels may be a particularly sensitive measure for detecting acute downstream effects following ketamine administration. Although it is possible to evaluate glutamate and glutamine separately, most early studies primarily focused on one of these two metabolites or pooled as 'glx'. In this study we explicitly focus on their respective ratio and thus follow a protocol, which was previously shown to be able to measure both substances at the same time (Dou *et al*, 2013).

ACC subdivisions can be delineated based on histoarchitectonic and receptor fingerprint markers (Palomero-Gallagher *et al*, 2009; Vogt *et al*, 2003). Although the pgACC has been found to exhibit above average α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptors and below average NMDA receptor densities (compared with whole cingulate cortex), the inverse has been reported for the anterior midcingulate cortex (aMCC) (Palomero-Gallagher *et al*, 2009). In addition, the aMCC and the pgACC have been proposed to have different roles in the regulation of mental and emotional activity interacting with other cortical and subcortical structures (Bush *et al*, 2000; Disner *et al*, 2011). A growing consensus indicates that depression is associated with dysfunction in these two ACC subregions (Chen *et al*, 2007b; Li *et al*, 2014; Pizzagalli, 2011). However, the ketamine effects in the pgACC and in the aMCC have not been directly compared in the scope of ketamine's reported antidepressant efficacy.

Importantly, most studies investigating neuronal and molecular effects of ketamine were performed during ketamine infusion. The transient psychometric effects are frequently found during the administration of ketamine and shortly afterwards (Luckenbaugh *et al*, 2014; Sos *et al*, 2013; Zarate *et al*, 2006). Therefore, measuring ketamine's effects only during or soon after its administration might be misleading, as it is hard to interpret whether findings reflect ketamine's acute dissociative effects or more specifically on antidepressant properties.

Here we assessed whether the reported time course of clinical improvement in patients could be mirrored by increased but delayed glutamine/glutamate ratios in healthy controls after ketamine infusion. We also tested for different pattern of regional and temporal dynamics of glutamine/glutamate ratio.

## MATERIALS AND METHODS

### Study Design

A double-blind, randomized placebo-controlled study was conducted to assess the effects of a single intravenous infusion of ketamine on the brain metabolites in two ACC subregions (the pgACC and the aMCC) at two post infusion timepoints (1 and 24 h). Given the observed ketamine's delayed effects on pgACC resting state activity in healthy individuals (Scheidegger *et al*, 2012, 2016), we focused on the neuronal mechanisms in carefully selected controls, without past or a family history of neuropsychiatric disorders in first-degree relatives.

This study was approved by the Institutional Review Board of the University of Magdeburg. All subjects gave written informed consent to participate after reviewing detailed written information about the study.

### Participants

The study was conducted at the University Clinic of Magdeburg, enrolling a total of 58 healthy subjects, aged 19 to 50 years (mean: $26.48 \pm 5.66$ years, 34 males). The determination of the number of participants and the process of randomization are described in the Supplementary Materials and Methods. All subjects were in good physical state as determined by physical examination, medical history, blood laboratory tests, and electrocardiography. All subjects completed the mini-international neuropsychiatric interview, to ensure the absence of any ICD-10 psychiatric disorders. Subjects were free of comorbid substance abuse or dependence. In addition, all subjects underwent an eye examination to exclude ocular hypertension.

### Study Medication

Study medication was supplied in identical 50 ml syringes containing a clear solution of either 0.9% saline or 0.5 mg/kg body weight racemic ketamine (Ratiopharm GmbH, Ulm, Germany) with added saline to total 50 ml of liquid volume. The infusion was administered continuously over 40 min via an infusion pump (Injectomat 2000, Fresenius Kabi GmbH, Langenhagen, Germany).

### Magnetic Resonance Image Acquisition

*In vivo* magnetic resonance (MR) acquisitions were performed before the infusion (baseline), as well as 1 and 24 h after the start of infusion.

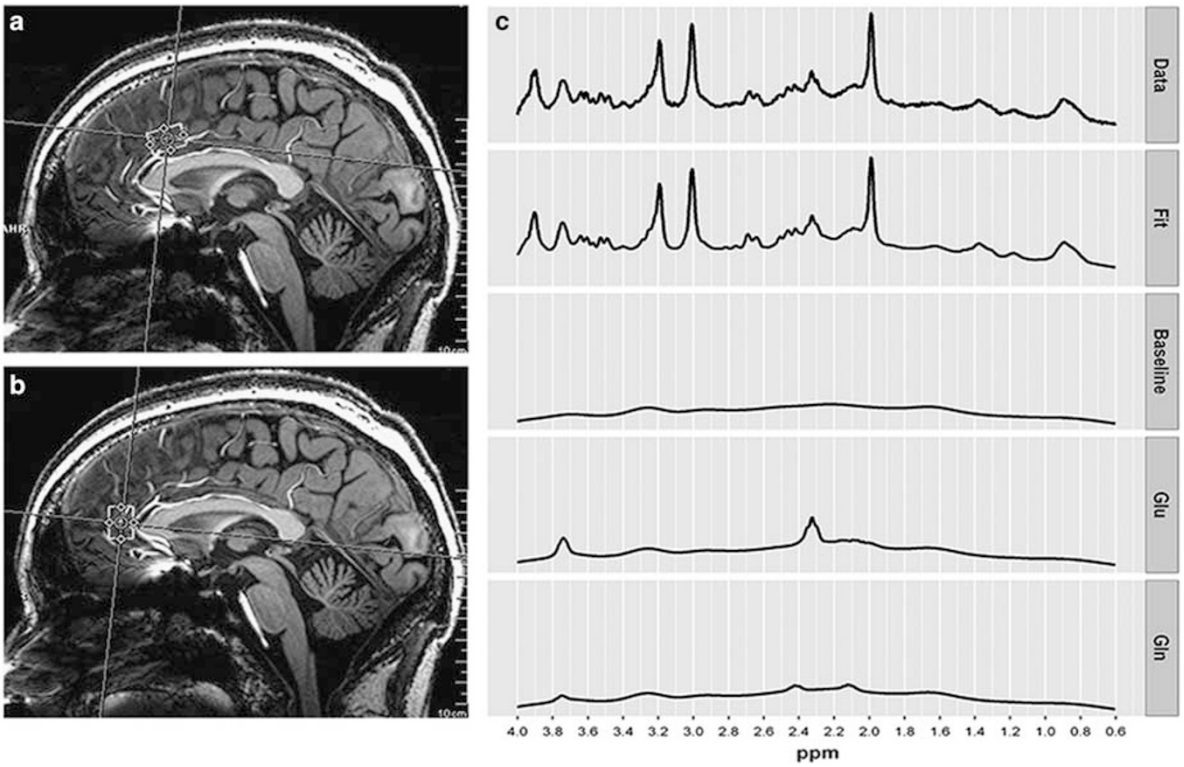


**Figure 1** Magnetic resonance spectroscopy (MRS) voxel locations and exemplary spectrum showing metabolites and the corresponding model fit for our metabolites of interest. The anterior midcingulate cortex (aMCC, a) and pregenual anterior cingulate cortex (pgACC, b) voxel positioning in yellow and the exemplary spectrum (c) acquired from a study participant, showing the raw spectrum, the corresponding model fit, and baseline from LCModel analysis. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

MR images were acquired on a 7 T MR scanner (Siemens Healthcare, Erlangen, Germany) using a 32-channel head array coil. After an automated global shim, high-resolution T1-weighted images were acquired with the following parameters: MPRAGE sequence, echo time (TE) = 2.73 ms, repetition time (TR) = 2300 ms, inversion time (TI) = 1050 ms, flip angle = 5°, bandwidth = 150 Hz/pixel, acquisition matrix = 320 × 320 × 224, and isometric voxel size = 0.8 $mm^3$.

For the MRS acquisition, region-specific shimming was performed using an optimized vendor-provided, double-gradient echo shim technique. Following localized shimming, spectra were acquired from $25 \times 15 \times 10\ mm^3$ voxel containing the aMCC (see Figure 1a) and from $20 \times 15 \times 10\ mm^3$ voxel containing the pgACC (see Figure 1b). As shown in Figure 1a, the location of the aMCC voxel was defined as the center of the voxel projected to the posterior border of the genu of the corpus callosum and the location of the pgACC voxel (Figure 1b) was defined as bordering the lower edge of the genu of the corpus callosum and posteriorly touching the anterior border of the genu of the corpus callosum. Proton spectra were acquired using a stimulated-echo acquisition mode (STEAM) sequence with optimized variable rate selective excitation RF pulses (Elywa *et al*, 2012). The scan parameters included 128 averages, TR = 3000 ms, TE = 20 ms, TM = 10 ms, data size = 2048, and bandwidth = 2800 Hz. It took 6 min and 36 s for each region of interest. Water reference spectra were measured with one average for eddy current correction and served as an internal concentration reference for quantification. In addition, the MRS voxels during all three measurements were positioned in the same place for each subject individually, using a vendor-provided automatic voxel positioning technique (Dou *et al*, 2014).

LCModel (Stephen Provencher, Oakville, ON, Canada, Version 6.3.0) was applied to analyse all spectra (from 0.6 to 4.0 p.p.m.). The sequence-specific basis set for data analysis was measured and described in a previous study (Dou *et al*, 2013). The signals from macromolecules and lipids were simulated in LCModel using the method published by Seeger *et al* (2003). One example of a curve-fitting from a study participant is presented in Figure 1. The absolute concentrations of the target metabolites, ie, glutamine and glutamate, with respective Cramér–Rao Lower Bound (CRLB) and full width at half maximum (FWHM) values for spectral linewidth estimation were obtained. Metabolite concentrations were corrected for cerebrospinal fluid in investigated voxels using the cerebrospinal fluid fractions, obtained by segmenting the T1-weighted images with VBM8 (www.neuro.uni-jena.de/vbm/) in SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK). Metabolite concentrations were expressed in institutional units. A recent study by Kreis (2016) suggested the absolute CRLB, instead of the relative CRLB, is appreciated to serve as an exclusion criterion for spectra analysis. To be specific, after screening the subjects with poor SNR (SNR < 20) and spectral linewidth (FWHM > 24 Hz), the absolute CRLB of each concentration (glutamine or glutamate in both regions at three timepoints) was calculated in each subject. To every single concentration (glutamate or glutamine) in the same MRS voxel (aMCC or pgACC), the measurements at baseline were treated as

control population for absolute CRLB computation. Next, the first and the third quartiles (Q1 and Q3), and the interquartile range (IQR) of absolute CRLB were calculated. Of them, the outliers were detected via boxplot and these subjects were excluded in the following analysis. Meanwhile, the individual absolute CRLB of each concentration at 1 and 24 h post infusion were tested against the threshold, which was defined as Q3 + 1.5 IQR of the corresponding concentration within control population. The subjects would be excluded if its value was larger than the derived threshold. With the resulting concentrations, subjects with abnormal values were detected and removed via boxplot. Our primary analysis only included subjects whose spectra survived in both investigated regions at all three timepoints. To overcome limited generalizability of *post-hoc* findings for this small sample, results were then validated in the region-specific validation data sets from all subjects. In the validation analysis, the spectra were screened from either pgACC or aMCC at all timepoints. In favor of readers interest, we also examined our spectra by replacing absolute CRLB with relative CRLB in the exclusion criteria and reported the results in Supplementary Materials.

### Statistical Analysis

First, the concentration of glutamine and glutamate after partial volume correction for cerebrospinal fluid and the ratios of glutamine to glutamate were calculated for each subject.

Primary outcome measures were the derived glutamine to glutamate ratios at three timepoints in the aMCC and the pgACC. Repeated-measures analyses of variance (ANOVA) was used for the statistical analysis with glutamine/glutamate ratio as the dependent variable, region (aMCC or pgACC) and time (baseline, 1, or 24 h) as the within-subject variables, and drug (ketamine or placebo) as the between-subject variable. The main effects for region, time, and drug, and their interactions are reported. Group comparison on the change of glutamine/glutamate at 1 and 24 h post infusion over baseline in each region was investigated via *post-hoc* two sample *t*-test. Likewise, repeated-measures ANOVA was also applied for the statistical analysis of glutamate and glutamine, respectively.

In the secondary analyses, the change of glutamine/glutamate to its baseline after the infusion in pgACC, as well as the changes in aMCC, was investigated at both timepoints in each group. Meanwhile, the difference of the changes between pgACC and aMCC were also compared.

To confirm the effects revealed in primary analysis, we performed separate analyses of all the data available for the pgACC or the aMCC. The difference of the glutamine/glutamate ratios between the ketamine and the control groups was tested at 1 and 24 h. In the case of such (between subject) effects of drug, paired *t*-tests were used to investigate the (within subject) change of the glutamine/glutamate over baseline at 1 h and 24 h in the respective groups. In addition, the contribution of individual glutamine and glutamate levels to the ratio calculation (glutamine/glutamate) 24 h after ketamine infusion were investigated using a Pearsons product moment correlation.

All reported *p*-values are two-tailed. Results were considered significant at $p<0.05$. All statistical analyses were performed in R version 3.1.2 (www.r-project.org).

## RESULTS

### Spectra Screened by Absolute CRLB

A total of 58 subjects were randomized in the study. Twenty-nine of them failed to meet spectra quality criteria in at least one measurement according to the curve fitting, the SNR, and/or the spectral linewidth. Two subjects were excluded due to abnormal absolute CRLB, compared with the derived value in the control population. One subject was further excluded, because the abnormal concentration in aMCC. After exclusion, 12 subjects ($28.0\pm8.1$ years, 5 female) in the ketamine group and 14 subjects ($27.5\pm6.6$ years, 5 female) in the placebo group were included in the primary analysis. Ketamine and placebo groups did not differ significantly in age ($t(21.3)=-0.198$, $p=0.844$) or sex ($\chi^2(1,n=26)=0$, $p=0.755$). Mean non-CSF tissue proportion, SNR, linewidth, CRLB, levels of glutamine and glutamate (after correction for the cerebrospinal fluid fraction), and the calculated glutamine/glutamate in the aMCC and the pgACC from all three timepoints are listed in Table 1.

*Primary analysis.* A significant interaction of time, region, and treatment was found ($F(2,96)=5.483$, $p=0.005$) on the glutamine/glutamate ratio. Significant main effects was found for time ($F(2,96)=6.307$, $p=0.002$) and region ($F(1,24)=48.072$, $p<0.001$), but not for treatment ($F(1,24)=0.077$, $p=0.784$). The results of the repeated-measurement ANOVA for glutamine and glutamate are reported in the Supplementary Materials.

*Region and time specificity of ketamine effect on the glutamine/glutamate ratio.* In the pgACC, the change in the glutamine/glutamate ratio at 24 h post ketamine infusion compared with baseline was significant ($t(11)=4.136$, $p=0.001$, see Figure 2), which is also significantly larger than the change observed at 24 h in the placebo group ($t(23.3)=2.618$, $p=0.015$, see Figure 2). However, the metabolite levels at 1 h after infusion in the same region in ketamine group was not significant different from the ones at baseline ($t(11)=1.482$, $p=0.166$), as well as the difference of changes between ketamine and placebo at 1 h ($t(22.1))=-0.487$, $p=0.630$), compared with their baseline level. In contrast, the changes between the ketamine and the placebo group in the aMCC were not significant at 1 or 24 h post infusion (1 h: $t(15.9)=0.456$, $p=0.654$; 24 h: $t(22.7)=-0.523$, $p=0.605$). The changes in the glutamine/glutamate ratio in aMCC from baseline in ketamine group (1 h $t(11)=1.237$, $p=0.241$; 24 h, $t(11)=0.978$, $p=0.349$) was not significant.

*Secondary analyses*

*Regional dissociation.* To investigate differential effects of local changes in the glutamine/glutamate ratios in the two subregions, we directly compared relative changes between the two ACC subdivisions. Only after 24 h, the pgACC showed a larger change in the glutamine/glutamate ratio relative to the baseline when compared with the changes in the aMCC. This effect was restricted to the ketamine group only ($t(11)=3.691$, $p=0.003$). After 1 h, this effect had not developed yet in the ketamine group ($t(11)=0.198$, $p=0.846$) and no such effect was observed in the placebo

**Table 1** Mean Tissue Portion, SNR, Linewidth, and CRLB of Gln and Glu in aMCC and pgACC at Three Investigated Timepoints (Placebo: $n = 14$; Ketamine, $n = 12$)

| Group | MRS voxel | Timepoint | Non-CSF tissue portion | SNR | Linewidth (HZ) | Absolute CRLB (i.u.) | | Gln (i.u.) | Glu (i.u.) | Gln/Glu |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Gln | Glu | | | |
| Placebo | aMCC | Baseline | 0.94 (0.05) | 45.21 (4.75) | 4.35 (0.84) | 0.16 (0.02) | 0.15 (0.02) | 1.52 (0.49) | 6.23 (0.49) | 0.24 (0.07) |
| | | 1 h | | 45.14 (5.76) | 4.62 (1.99) | 0.15 (0.02) | 0.14 (0.02) | 1.64 (0.51) | 6.12 (0.42) | 0.26 (0.08) |
| | | 24 h | | 44.85 (7.14) | 4.24 (0.88) | 0.15 (0.02) | 0.13 (0.02) | 1.69 (0.49) | 6.00 (0.55) | 0.28 (0.09) |
| | pgACC | Baseline | 0.92 (0.04) | 46.21 (6.71) | 5.05 (1.40) | 0.16 (0.02) | 0.16 (0.03) | 2.01 (0.86) | 6.22 (0.88) | 0.32 (0.13) |
| | | 1 h | | 45.85 (5.36) | 5.60 (2.76) | 0.16 (0.02) | 0.17 (0.03) | 2.37 (0.63) | 5.99 (0.67) | 0.40 (0.12) |
| | | 24 h | | 45.64 (6.00) | 6.28 (2.39) | 0.16 (0.02) | 0.16 (0.01) | 2.02 (0.78) | 6.50 (0.90) | 0.31 (0.12) |
| Ketamine | aMCC | Baseline | 0.95 (0.02) | 42.58 (5.63) | 4.60 (1.28) | 0.17 (0.03) | 0.16 (0.03) | 1.42 (0.53) | 6.52 (1.05) | 0.22 (0.09) |
| | | 1 h | | 41.33 (3.79) | 4.43 (0.89) | 0.16 (0.01) | 0.16 (0.02) | 1.60 (0.42) | 6.04 (0.64) | 0.26 (0.07) |
| | | 24 h | | 44.33 (5.74) | 4.55 (0.81) | 0.15 (0.02) | 0.14 (0.02) | 1.50 (0.41) | 5.99 (0.39) | 0.25 (0.06) |
| | pgACC | Baseline | 0.94 (0.03) | 48.25 (4.47) | 5.69 (2.55) | 0.15 (0.01) | 0.14 (0.03) | 1.91 (0.47) | 6.04 (0.90) | 0.32 (0.09) |
| | | 1 h | | 45.58 (5.79) | 5.07 (1.64) | 0.16 (0.01) | 0.15 (0.02) | 2.20 (0.88) | 5.89 (0.73) | 0.37 (0.12) |
| | | 24 h | | 46.25 (4.95) | 5.34 (1.95) | 0.15 (0.01) | 0.16 (0.03) | 2.30 (0.42) | 5.23 (0.97) | 0.48 (0.14) |

Abbreviations: aMCC, anterior middle cingulate cortex; CRLB, Cramér–Rao lower bound; Gln, glutamine; Glu, glutamate; i.u., institutional units; pgACC, pregenual anterior cingulate cortex.
All values were expressed using mean ± SD.

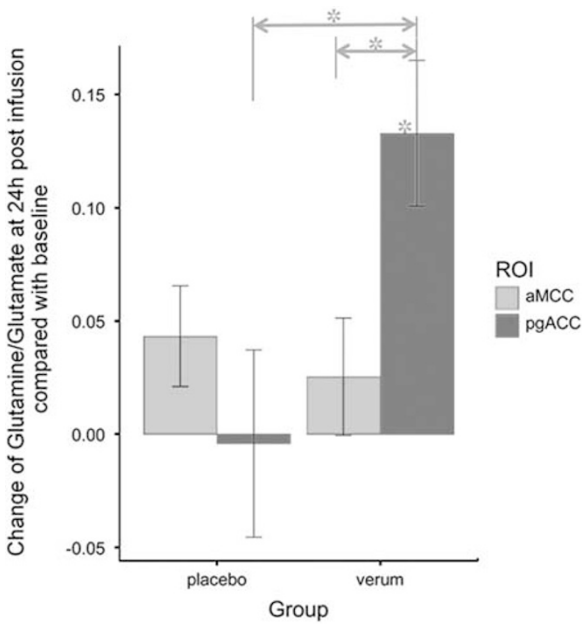


**Figure 2** Results of regional comparison from the primary analysis. The change of glutamine/glutamate ratio at 24 h after intravenous ketamine infusion over baseline in the two anterior cingulate cortex subregions (aMCC, anterior middle cingulate cortex, in red; pgACC, pregenual anterior cingulate cortex, in dark green) in placebo ($n = 14$, saline) and verum groups ($n = 12$, ketamine), respectively. Asterisks denote statistically significant changes in glutamine/glutamine relative to baseline levels. Error bars denote one SEM. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

group (1 h post infusion > baseline: $t(13) = 1.194$, $p = 0.253$; 24 h post infusion > baseline: $t\ (13) = -0.980$, $p = 0.344$).

*Validation analysis.* To confirm the effects revealed in the primary analysis, we performed separate analyses for data acquired in a larger sample. We had comparable datasets with 20 versus 24 subjects for the ketamine and the placebo group in the pgACC and 16 versus 15 subjects in the aMCC in the validation analysis.

The changes of the glutamine/glutamate ratio in the pgACC between ketamine and placebo groups was confirmed, that is significant difference ($t(39.9) = 2.469$, $p = 0.017$, see Figure 3) after 24 h but not in the pgACC after 1 h ($t(41.7) = 0.472$, $p = 0.639$). The difference between the placebo and ketamine after 24 h was driven by increased glutamine/glutamate ratio in the ketamine group ($t(19) = 3.072$, $p < 0.006$; see Figure 3) compared to the baseline and no change in the placebo subjects ($t(23) = -0.238$, $p < 0.813$).

In the ketamine subjects 24 h after infusion, the glutamine/glutamate ratio in the pgACC showed a significant negative correlation with glutamate ($r = -0.751$, $p < 0.001$) and a significant positive correlation with glutamine levels ($r = 0.904$, $p < 0.001$; Figure 4).

In contrast, even with the larger sample size, comprising of all subjects with acceptable spectra in the aMCC, we did not find any effects of ketamine on the glutamine/glutamate ratio (group comparison of the change of the glutamine/glutamate against baseline: 24 h, $t(28.2) = -0.457$, $p < 0.651$; 1 h, $t(24.1) = 0.356$, $p < 0.724$).

## DISCUSSION

In the present study, a significant interaction of time, region, and treatment on the glutamine/glutamate ratio was found after ketamine infusion in healthy subjects. A follow-up analysis revealed significantly increased glutamine/glutamate ratio in the ketamine compared with the placebo group in the pgACC (not the aMCC) and only after 24 h (not after 1 h). This translated into a change of the balance between regions: the glutamine/glutamate ratio in the pgACC *relative* to the levels in the aMCC was significantly increased

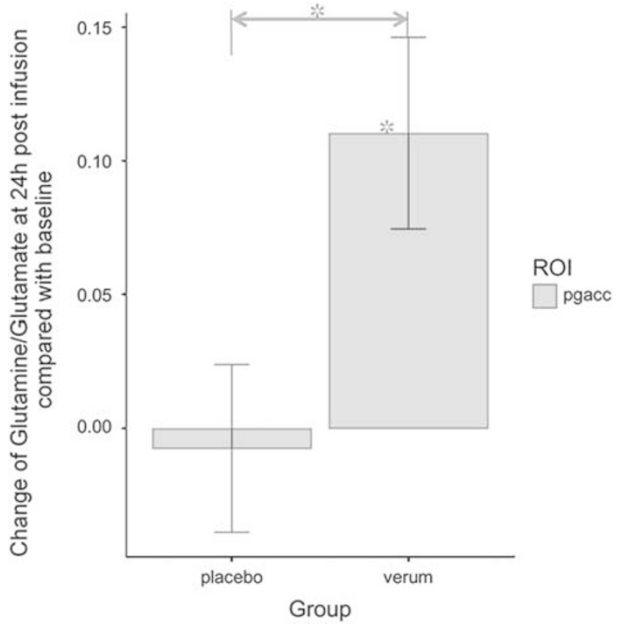


**Figure 3** Validation of the effects in the pregenual anterior cingulate cortex (pgACC): significant increase in change of the glutamine/glutamate ratio after 24 h compared with the baseline in the ketamine group corresponds to a significant difference in the ketamine ($n = 20$) *vs* placebo ($n = 24$) in pgACC. Asterisks denote statistically significant change in the glutamine/glutamate relative to the baseline levels. Error bars denote SEM.

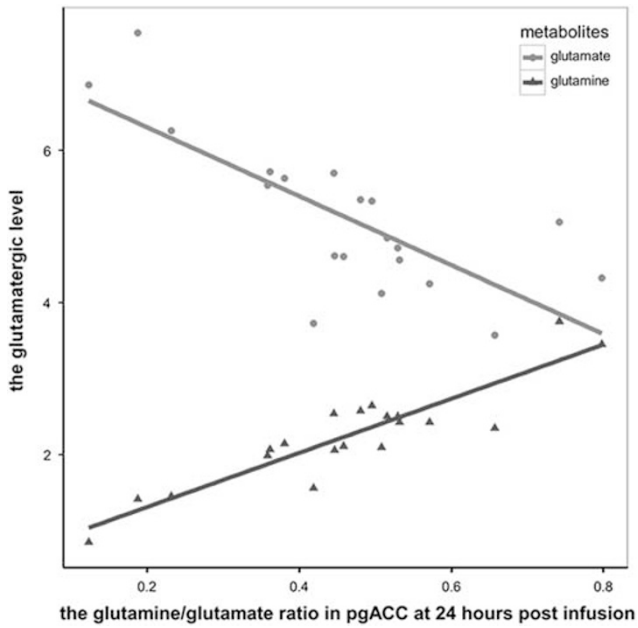


**Figure 4** Correlation of the glutamine/glutamate ratio with the glutamate and the glutamine levels at 24 h post ketamine infusion ($n = 20$) in the validation analysis. The glutamine/glutamate ratios are predicted by the glutamate levels ($r = -0.751$, $p < 0.001$) and the glutamine levels ($r = 0.904$, $p < 0.001$).

only 24 h after the ketamine infusion. This finding largely confirms our hypothesis of a regional specificity of ketamine effects on glutamatergic levels.

In an earlier study, Rowland *et al* (2005) reported evidence for acute glutamine (but not glutamate) changes following ketamine infusion. Rowland's finding was specific to the loading dose of ketamine rather than the maintenance dose afterwards. A recent study by Taylor *et al* (2012) investigating continuous infusion of subanesthetic ketamine dose did not find changes in the cortical glutamate or glx levels relative to baseline, during or at the end of the infusion in healthy volunteers. In contrast to these studies, our findings most closely match previous observations in a temporally similar study by Iltis *et al* (2009) who administered the NMDA antagonist phencyclidine to rats. They found a significant increase in glutamine/glutamate ratio compared with the baseline after phencyclidine infusion. Importantly, this ratio change, in the context of unchanged glx, was related to a strong increase of glutamine levels of 12% and a small decrease of glutamate levels (5%), and increases of glutamine to glutamate ratios correlated with a reduction of glutamate and an increase of glutamine. These studies converge with the postulated reduced conversion of glutamine to glutamate underlying reduced glutamine/glutamate levels in depression (Yüksel and Öngür, 2010b). In an open-label trial, Brennan *et al* (2010) investigated the effects of the glutamate modulating drug Riluzole on the glutamine/glutamate ratio in ACC and parieto-occipital cortex in MDD patients. In their study, they found an increase in the glutamine/glutamate ratio 2 days after treatment. Moreover, this pattern of changes was more pronounced in the ACC than in the parieto-occipital cortex. In our study, using a placebo-controlled double-blind design, we demonstrate—if only for the healthy subjects—regional specificity within the ACC cortex. Our pgACC region not only substantially overlaps with the ACC region reported in Brennan *et al* (2010), but also addresses the margins of underlying histoarchitectonic maps (Palomero-Gallagher *et al*, 2009). Compellingly, these effects are observed both in direct comparisons between ketamine and the placebo as well as in comparison against the baseline.

In line with the importance of regional specificity, we demonstrated a significant difference of changes in glutamine/glutamate ratio in the pgACC relative to the aMCC after ketamine infusion. We hypothesized that this regional specificity is associated with AMPA receptor density and NMDA/AMPA receptor ratios in the respective regions (Walter *et al*, 2014). This hypothesis was based on observations showing that ketamine's antidepressant effects in MDD, which were expected to appear predominantly for the 24 h, might rely on AMPA receptors (Maeng *et al*, 2008). Ketamine's antidepressant effect could be reversed by pretreatment with an AMPA antagonist (Koike *et al*, 2011) and blocked by the co-administration of an AMPA antagonist (Autry *et al*, 2011). A recent study suggested that the antidepressant actions by (R,S)-ketamine are independent of NMDA receptor inhibition but involve early and sustained activation of AMPA receptors (Zanos *et al*, 2016). The reduction of NMDA/AMPA receptor density ratios was further reported in the hippocampus of Wistar–Kyoto rats, a putative animal model of depression, after chronic treatment with an effective dose of ketamine (Tizabi *et al*, 2012). Interestingly, an opposite NMDA/AMPA receptor density ratio was reported in pgACC and aMCC (Palomero-Gallagher *et al*, 2009). Furthermore, regional variations of glutamine and glutamate concentrations have been shown to follow receptor fingerprints of cingulate cortex in the human

brain (Dou *et al*, 2013). Therefore, we were able to directly formulate a hypothesis on the regional specificity of receptor-related drug effects and their temporal profile incorporating mechanisms of homeostatic plasticity. The present data support our hypothesis that the pgACC had decreased glutamine/glutamate ratio in MDD (Walter *et al*, 2009; Yüksel and Öngür, 2010b), as well as high AMPA receptor densities, which resulted in an increase of glutamatergic ratios during antidepressant treatment (Brennan *et al*, 2010). In this view, the capacity of ketamine to reserve depressive symptoms on a similar timescale, ie, after 1–2 days, with efficacy dependent on AMPA-related mechanisms (Maeng *et al*, 2008).

Our findings describe novel neurochemical correlates of a subanesthetic ketamine infusion, which appear as late as 24 h after treatment. Scheidegger *et al* (2012) report the reduction of functional connectivity as a similar mechanism underlying ketamine's antidepressant efficacy, which may counteract the repeatedly observed hyperconnectivity within the default mode network in depressed patients. Although Scheidegger *et al* (2012) did not include earlier timepoints, it is intriguing that the parallel molecular changes seen in our study seem to build up until 24 h and only then do we see effects on cross-regional balance of the metabolite ratios. The present results imply that changes in glutamine/glutamate are different from the acute brain effects during infusion of subanesthetic ketamine. The potential reversal of a pathologically reduced glutamine/glutamate levels as measured in terms of their ratios, as found in MDD, appears to be maximal after 24 h when the acute dissociative effects have vanished, but when the antidepressent effects are at their peak (Caddy *et al*, 2014; Fond *et al*, 2014; McGirr *et al*, 2015; aan het Rot *et al*, 2012).

Consequences for clinical translation need to be considered with caution, given that we only included healthy subjects in our study. Future studies are needed to broaden the time windows of observations especially the acute infusion stages. Moreover, after ketamine infusion in MDD patients, Milak *et al* (2015) have recently reported increased glx and GABA levels over baseline followed by normalization after 1 h in the medial prefrontal cortex. The first part of the acute response within the infusion period needs further confirmation at high field magnetic strength. The ideal next step would be to investigate glutamine/glutamate ratio changes both during and after infusion in patients with depression. Moreover, the fit for very low concentration metabolites such as glutamine via the metabolite basis set fitting program highly depends on what else is included in the basis set. Meanwhile, the ability to achieve reliable spectra quantification might be undermined by baseline distortion, with macromolecules as the main contributors, which is related to the TE values and hence can be a more critical issue at 7T (Jansen *et al*, 2006). Therefore, experimentally determined macromolecules should be applied and the robustness of estimated spectra and their potential overlaps still needs to be assessed in future studies.

In conclusion, we found that ketamine specifically increases glutamine/glutamate ratios in the pgACC, a region previously identified to display a deficit in these ratios in subjects with depression. This effect spatially reflects NMDA/AMPA receptor density profiles within the ACC subregions, which accordingly might be very relevant for ketamine's antidepressant properties. The molecular effects in the pgACC are also consistent with the change of interregional metabolite balance 24 h after ketamine administration, which ultimately may link to previously observed long-term functional network changes.

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