Ketamine effects on brain GABA and glutamate levels with 1H-MRS: relationship to ketamine-induced psychopathology

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Supplementary materials

Methods

The study was approved by the East London Research Ethics Committee, UK. Healthy male volunteers were recruited by advertising. After the purpose of the study was explained to them verbally and in writing, they were invited to come for a screening assessment where, after giving written informed consent to be included in the study, they were assessed for their suitability for the study according to the following inclusion and exclusion criteria:

Inclusion criteria:

1. Healthy right−handed male subjects between the ages of 18 and 50 years, inclusive (healthy is defined as no clinically relevant abnormalities identified by a detailed medical history and full physical examination, including blood pressure, pulse rate measurement and 12−lead ECG).
2. Body Mass Index (BMI) of 18 to 30 Kg/m^2; and a total body weight 50−100 kg.

Exclusion criteria:

1. Evidence or history of clinically significant haematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurological, or allergic disease (including drug allergies, but excluding untreated, asymptomatic, seasonal allergies at time of dosing). This also includes subjects with previous history of epilepsy or seizures, psychiatric illness, attempted suicide or suicidal ideation, or glaucoma.
2. Use of prescription or non−prescription drugs within 7 days or 5 half−lives with the exception of paracetamol (acetaminophen).
3. History of sensitivity to ketamine.
4. History of febrile illness within 5 days prior to the first dose.
5. 12−lead ECG demonstrating QTc >430 msec at screening.
6. Fulfillment of any of the MRI contraindications on the standard radiography screening questionnaire.

7. History of regular alcohol consumption exceeding 28 drinks/week (1 drink = 5 ounces (150 mL) of wine or 12 ounces (360 mL) of beer or 1.5 ounces (45 mL) of spirits) within 6 months of screening.
8. Use of tobacco− or nicotine−containing products in excess of the equivalent of 5 cigarettes per day.
9. A positive urine drug screen for drugs of abuse.

Thirteen male volunteers were included in the study. In order to assess their eligibility for the study, they underwent psychiatric and medical history, mental state, and physical examination (by clinical psychiatrists - JS or CD). Investigations included electrocardiogram (ECG), urine drug screen, weight, temperature and blood pressure. They were cannulated in the left antecubital fossa, and connected via a venous line to a computer-controlled pump-driver, with a 50ml syringe containing 4mg/ml racemic ketamine.

All volunteers underwent MRI imaging on a General Electric (Milwaukee, Wisconsin) 3-Tesla HDx magnetic resonance system. Subjects underwent an initial localizer scan followed by acquisition of structural images including an axial 2D T2-weighted fast spin echo scan and an axial fast fluid-attenuated inversion recovery scan (total scan time = 5 min). These were followed by a whole-brain three-dimensional coronal inversion recovery prepared spoiled gradient echo (IR-SPGR) scan, giving isotropic 1.1-mm voxels in a scan time of approximately 6 min (echo time (TE) = 2.82 msec; repetition time (TR) = 6.96 msec; inversion time = 450 msec; excitation flip angle = 20°).

GABA-edited proton magnetic resonance spectroscopy (1H-MRS) was performed using the MEGA-PRESS method,[1, 2] from a 30×30×30mm volume positioned medially over the thalami (bilaterally) and surrounding subcortical structures. This method has been shown to have good test-retest reliability for measuring GABA (R^2=0.999),[3] with a coefficient of variance of 7%.[4] To

visualise the thalami, the IR-SPGR scan was reformatted into an orientation orthogonal to the posterior border of the pons. The MEGA-PRESS voxel was then prescribed on the slice of this reformatted image in which the thalamus was widest, with its left/right position centred on the midline of the brain and its anterior/posterior position determined by the thalami (Figure S1). MEGA-PRESS parameters were: TE=68ms; TR= 1800ms; 16ms Gaussian editing pulse applied at 1.9ppm (ON scans) and 7.5ppm (OFF scans) in an interleaved fashion (332 averages). Four-hertz exponential line broadening was used and the MEGA-PRESS difference spectrum produced. Finally, a high-pass water filter was applied. As the unsuppressed water was small in the difference spectra, applying this filter did not substantially disturb the baseline at 3ppm.

Following this, all subjects underwent PRESS (Point RESolved Spectroscopy) 1H-MRS – a method with with good test-retest reliability (R^2=0.987) for measuring glutamate.[3]. PRESS data (TE = 30 msec; TR = 3000 msec; 96 averages) were acquired using the standard GE PROBE (proton brain examination) sequence, which uses a standardized chemically selective suppression (CHESS) water suppression routine. Shimming (using first order shims only) was optimized, with auto-prescan performed twice before each scan. The anterior cingulate ROI was prescribed from the midline sagittal localiser, and the centre of the 20mm x 20mm x 20mm ROI was placed 13mm above the anterior section of the Genu of Corpus Callosum at 90^o to the AC-PC line (Figure S2).

Unsuppressed water reference spectra (16 averages) were acquired from the same brain regions as part of the standard PRESS and MEGA-PRESS acquisitions.

A dynamically modeled intravenous infusion of ketamine was commenced (target plasma level: 150ng/mL). The rate of infusion was controlled over the full period of infusion by a laptop computer running Stanpump software [available free of charge from Dr S. Shafer MD at http://anesthesia.stanford.edu/pkpd/ (accessed September 2011)] driving a Graseby 3400 syringe-

driver. The rate was determined based on the pharmacodynamic properties of ketamine from the "Clements 250 model".[5, 6] In practice, this translated to a rapid bolus over 20 seconds of approx. 0.26mg/Kg followed by a slow infusion of approx. 0.42mg/Kg/Hr (Figure S3).

The MEGA-PRESS and PRESS 1H-MRS acquisitions were repeated at 25 and 35 minutes after the start of the infusion respectively. The time points and sequence of the PRESS and MEGA-PRESS acquisitions were chosen on the basis of microdialysis data [7], which demonstrated that changes in cortical extracellular glutamate levels were relatively slow, increasing at 20-40 minutes post-injection, and peaking around 120 minutes. We hypothesized that, if changes in GABA gave rise to downstream effects on glutamate transmission, they would be detectable before glutamate increases, and so positioned the MEGA-PRESS acquisition just before the PRESS.

Each scanning session concluded with the collection of a PRESS spectrum from a phantom containing standard concentrations of brain metabolites to allow monitoring of the effect of scanner drift on 1H-MRS metabolite estimates. There was no significant effect of scanner drift for the duration of the study, and therefore, no corrections were required.

Rating Scales

After completing the scan, the ketamine infusion was stopped, and volunteers were interviewed in an adjoining room about their experiences during the infusion. Subjective descriptions of the strongest effects during the infusion were elicited. Ketamine-induced effects were measured using the positive and negative syndrome scale (PANSS),[8] by JS.

1H-MRS quantification

MEGA-PRESS data were analysed with in-house software developed using Matlab (Figure S4). The edited GABA signal at 3 ppm in the MEGA PRESS difference spectrum, and the unsuppressed

water signal from the same region were integrated. The integral of the GABA peak was calculated automatically using a linear fit of the baseline and a Gaussian fit to the peak itself , as we have used previously.[9-13] The water lineshape was modeled as a mixed Gaussian-Lorentzian lineshape.[14] Adequate MEGA-PRESS spectrum quality was confirmed by visual inspection. A concentration measurement in institutional units was derived by accounting for the editing efficiency and the T1 and T2 relaxation times of water and GABA, using published values for the metabolite relaxation times.[15-19]

PRESS spectra (Figure S5) were analyzed using LCModel version 6.1-4 F.[20] The raw spectral data were read into LCMgui, the graphical user interface for LCModel, which automatically combined the data from the eight-channel coil with a weighted coherent average over the eight receive channels using the intensity of the first point of the Free Induction Decay of the unsuppressed water reference from each coil. A standard basis set of 16 metabolites (L-alanine, aspartate, creatine, phosphocreatine, GABA, glucose, glutamine, glutamate, glycerophosphocholine, glycine, myo-inositol, L-lactate, N-acetylaspartate, N-acetylaspartylglutamate, phosphocholine, taurine), included as part of LCModel and acquired with the same field strength (3-T), localization sequence (PRESS), and echo time (30 msec) as our study was used. Model metabolites and concentrations employed in the basis set are fully detailed in the LCModel manual (http://s-provencher.com/pages/lcm-manual.shtml). For all metabolites we used the recommended cut-off of Cramer-Rao minimum variance bounds (CRMVB) of 20% to exclude poorly fitted metabolite peaks.

The IR-SPGR scans were used for localization of the spectroscopy voxels and were segmented into grey matter, white matter and cerebrospinal fluid (CSF) using Statistical Parametric Mapping 8 software (SPM8; Wellcome Trust Centre for Neuroimaging, Institute of Neurology, University College London, United Kingdom) to allow correction of water-scaled metabolite values for partial volume CSF contamination. Voxel CSF, grey- and white-matter content was determined from the

voxel location using in-house software. Metabolite values were corrected for the presence of CSF in the voxel using the formula Mcorr = M/(wm +gm), where M is the uncorrected metabolite value, and wm and gm are the white and grey matter fractions from the segmented images (ranging between 0 and 1) for the spectroscopy voxel. Since the metabolite measures were derived with water-scaling, a further correction was applied to correct the estimated water concentration of the voxel for partial volume CSF contamination. We used the same default CSF and brain water concentrations employed by LCModel (55,556 mol/m^3 and 35,880 mol/m^3 respectively; http://s-provencher.com/pages/lcm-manual.shtml). For practical purposes, these two correction factors were combined into a single equation (Mcorr = M / (35,880 * (wm+gm)/(35,880 * (wm+gm) + 55,556 * CSF)), where CSF is the fraction of the voxel filled with cerebrospinal fluid, which simplifies to Mcorr = M*(wm+gm + 1.55CSF) / (wm+gm).

Statistics

Normality of data was checked using the Shapiro-Wilk test. Changes in metabolite levels with ketamine administration were analysed using paired t-tests. Correlations between metabolite levels and PANSS scores were analysed using Pearson's product moment correlation coefficient.

Figure S1: Position of MEGA-PRESS voxel

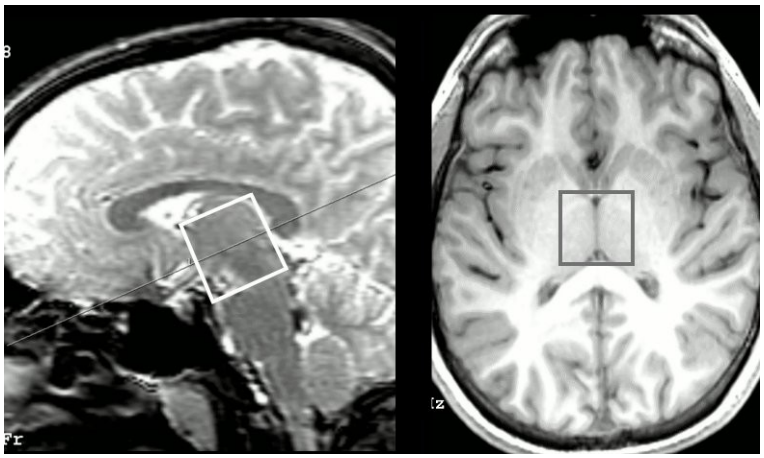

Figure S2: Position of PRESS voxel.

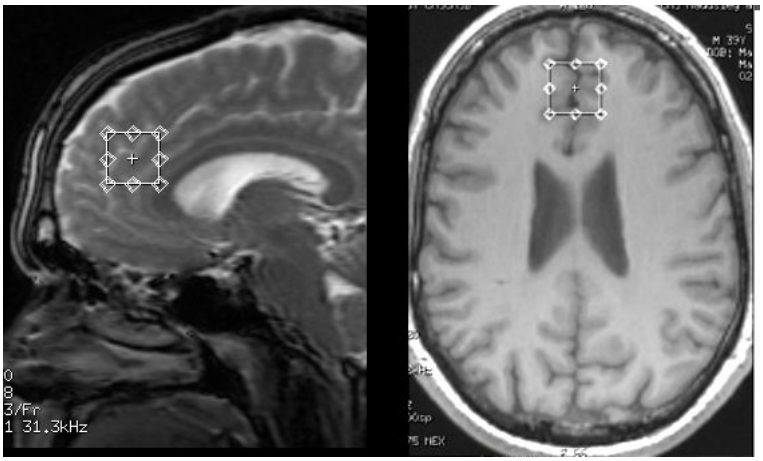

Figure S3: Representative plot of volume infused (mL) vs. time (10s increments). 4mg/mL racemic ketamine.

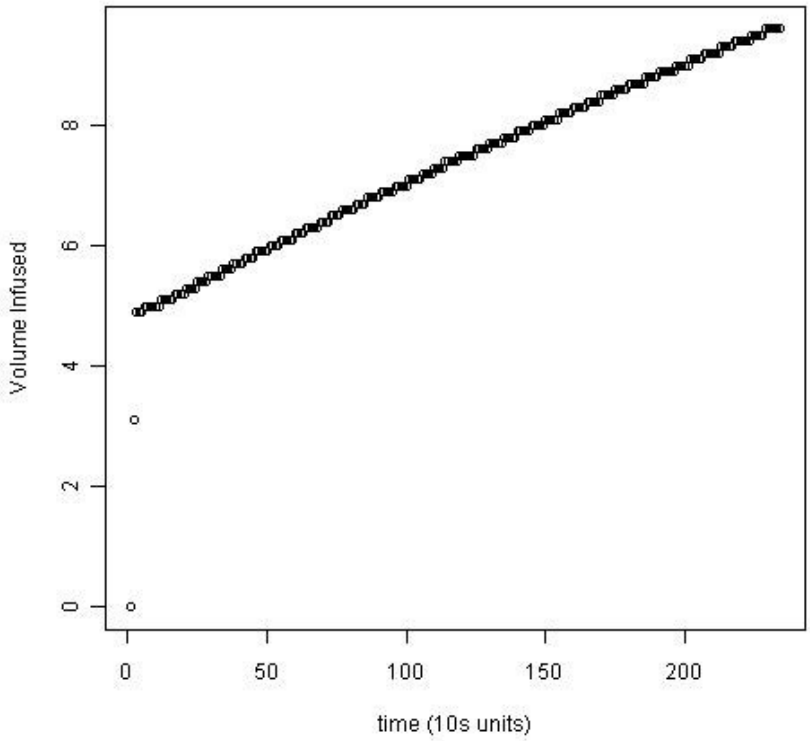

Figure S4: Sample pre- and post-ketamine (left and right column respectively) MEGA-PRESS spectra from 3 participants.

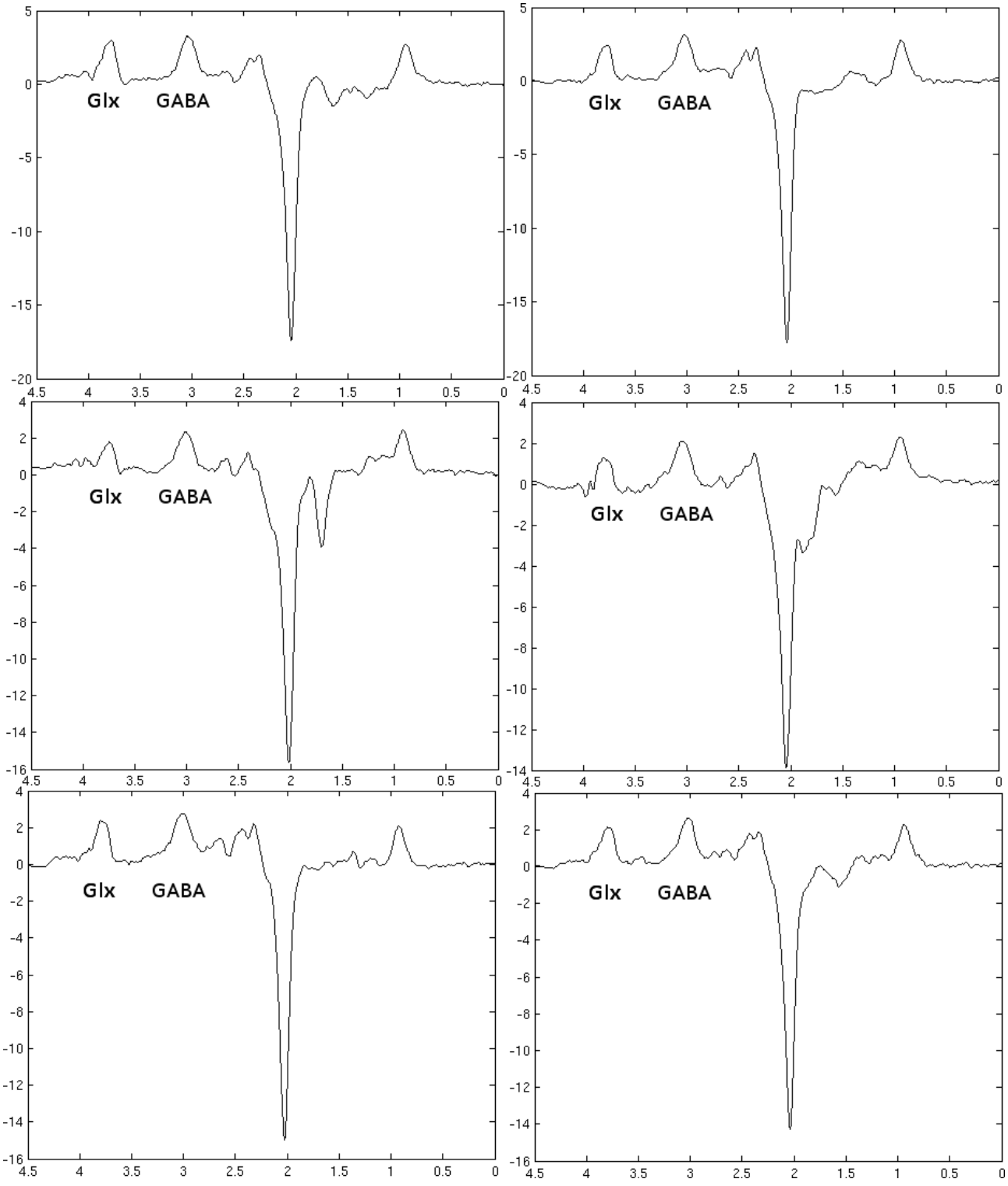

Figure S5: Sample pre- and post-ketamine (left and right column respectively) PRESS spectra (LCModel output) from 3 participants.

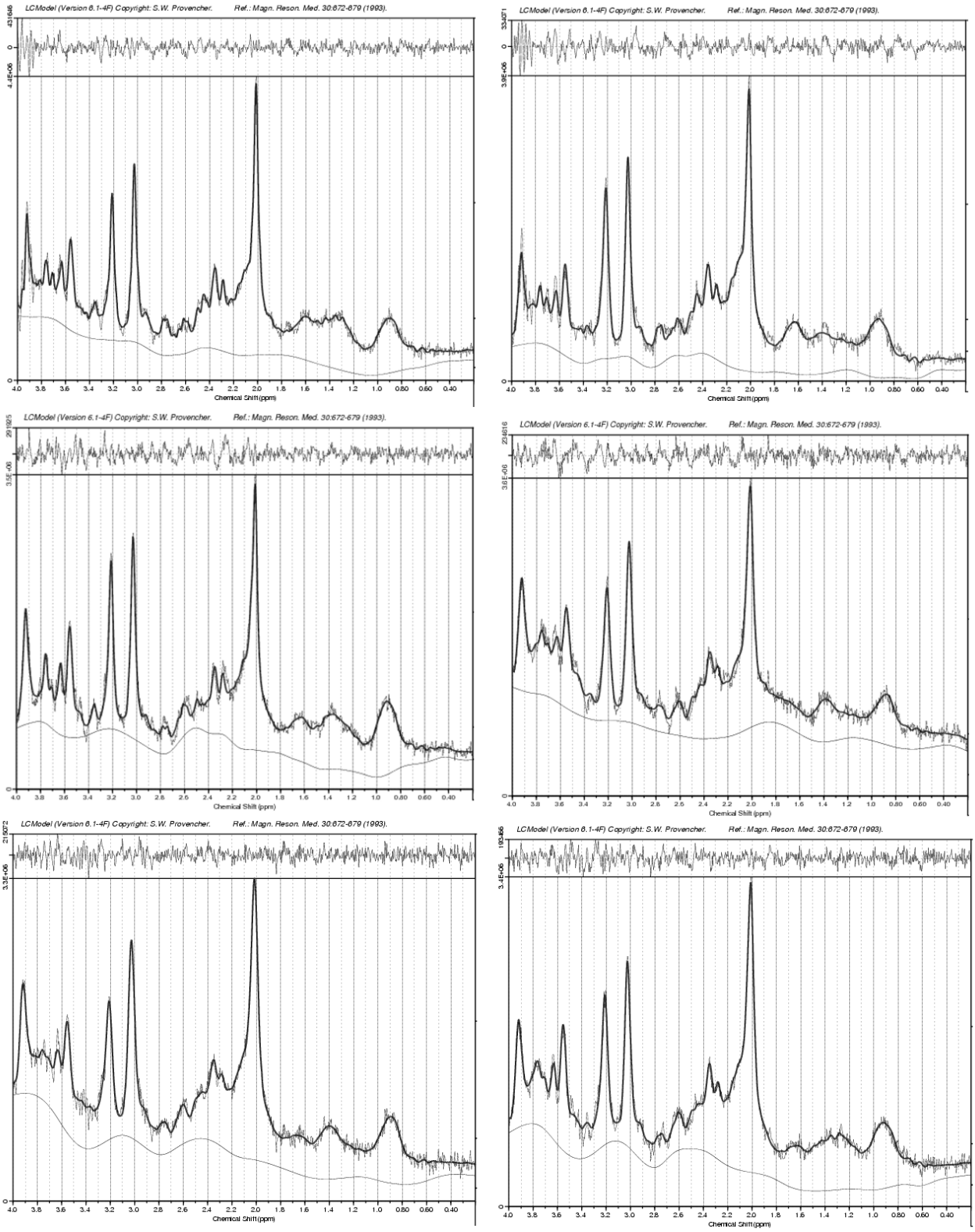

Table S1: Cramer-Rao Mean Variance Bounds (%CRMVB) in anterior cingulate (glutamate, glutamine) and thalamus (GABA) for all subjects. Brain metabolite levels (mean(SD) including data where individual CRMVB<20%) before and after ketamine administration (* paired p<0.01) are also presented. All values are reported as i.u.

	Pre-ketamine			Post-ketamine		
	Mean(SD) %CRMVB reported by LCModel	Mean(SD) linewidth of water reported by scanner (Hz)	Mean(SD) metabolite levels	Mean(SD) %CRMVB reported by LCModel	Mean(SD) linewidth of water reported by scanner (Hz)	Mean(SD) metabolite levels
Anterior Cingulate Glutamate	9.8(1.5) n=13	5.7(0.8) n=13	12.4(1.78) n=13	8.2(2.5) n=13	5.53(0.52) n=13	14.0(2.31) * n=13
Anterior Cinglulate Glutamine	34.3(24.1) n=12	5.7(0.8) n=13	9.3(1.51) n=3	26.9(15.0) n=12	5.53(0.52) n=13	9.69(0.94) n=4
Anterior Cingulate Glutamate+Glutamine	10(1.8) n=13	5.7(0.8) n=13	17.5(4.1) n=13	11.3(1.7) n=13	5.53(0.52) n=13	19.7(3.86) n=13
Thalamus GABA	N/A	11.1(2.23) n=13	33.7(4.8) n=13	N/A	11.5(3.0) n=13	34.0(6.1) n=13

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