'Glx' Measured by J-editing/MEGA-PRESS is Primarily 'Pure' Glutamate…Or is it?

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Background: The NMDA receptor hypofunction (NRH) hypothesis of schizophrenia (SZ)[1] predicts that levels of the excitatory amino acid neurotransmitter glutamate (Glu) are elevated in the disorder. While ^{1}H MRS studies measuring frontal lobe "glutamatergic compounds" in medication-naïve, unmedicated or minimally medicated SZ patients have generally found elevations[2-7], several of these studies have reported glutamine (Gln), rather than Glu, elevations[2-4,6]. In one study[8], Gln elevations were observed in healthy subjects in response to the NMDA receptor antagonist ketamine, known to induce symptoms of psychosis that are indistinguishable from primary SZ, and to exacerbate these in patients[1]. These reports of Gln elevations are, however, inconsistent with the strongest available experimental evidence for the NRH hypothesis, notably, rat brain microdialysis studies that found rapid and robust extracellular Glu elevations in response to ketamine[9]. Using the J-editing technique, generally assumed to measure the combined resonances of Glu and Gln, i.e., Glx, our group recently found rapid 'Glx' elevations of up to 60% of baseline following ketamine administration in depressed patients (**Fig. 1**)[10], which we interpreted as Glu elevations based on the rat brain microdialysis studies[9]. Since studies reporting Gln elevations had derived levels of the 'glutamatergic compounds' mathematically from fits of experimental spectra using a prior knowledge approach[11], these discrepancies raise the possibility that the reported Gln increases might be a systematic fitting artifact. To investigate this possibility, we have been acquiring "pure" Glu spectra with CT-PRESS[12,13], and Glx spectra by J-editing in the same setting and in the same subjects to assess the degree to which the two measures correlate. A strong correlation would suggest that 'Glx' likely represents primarily Glu levels, and can be interpreted as such.

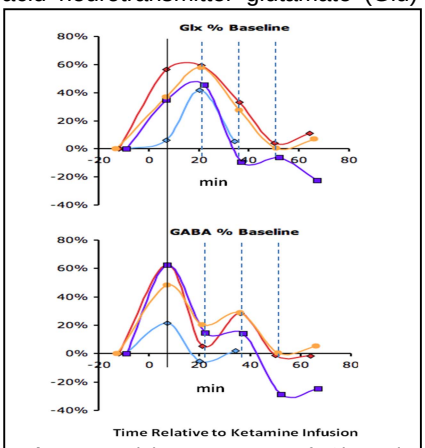


Fig. 1: Real-time responses of Glx and GABA to a single subanesthetic dose of ketamine in major depression *in vivo*.

Methods: The CT-PRESS pulse sequence was implemented to measure, in addition to the presumably Gln-contaminated C2 Glu (i.e., Glx) peak at 3.71 ppm, the C4 Glu at 2.35 ppm uncontaminated by Gln, from voxels in the motor, anterior cingulate or occipital cortices of 45 subjects regardless of age (15-70 yr), sex or diagnosis, which included ALS, major depression, fibromyalgia, and healthy controls. Then, without moving the subjects, the standard J-editing method was used to measure Glx at 3.71 ppm in the same voxel (**Fig. 2**).

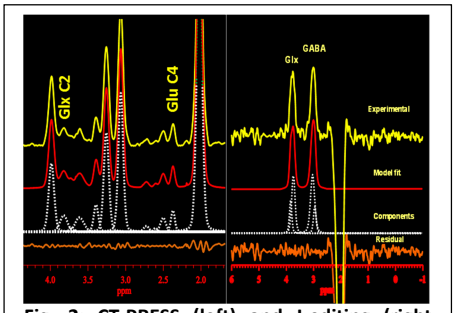
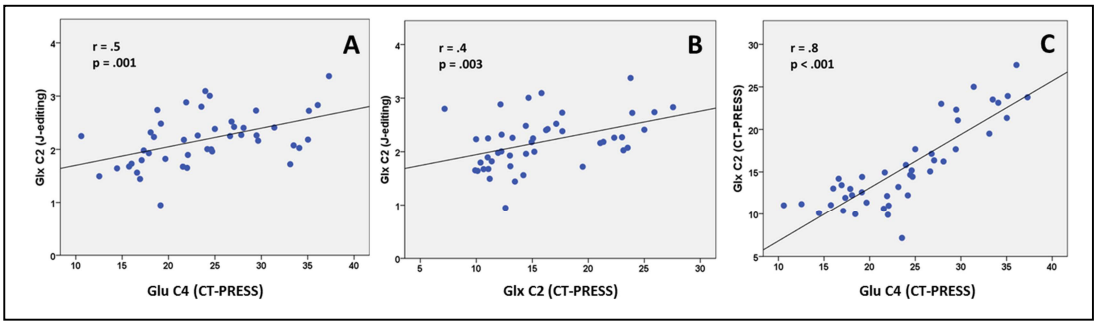


Fig. 2: CT-PRESS (left) and J-editing (right spectra of Glu and Glx, respectively. Also shown are best-fit model spectra.

Results: Figures 3 A-C show the resulting linear regressions: CT-PRESS Glu C4 (**Fig. 3A**) and Glx C2 (**Fig. 3B**) and J-editing Glx C2 were significantly correlated (Pearson's r = 0.5 & 0.4; p = .001 & .003, respectively). Not surprisingly, CT-PRESS Glu C4 and CT-PRESS Glx C2 were also correlated (r = 0.8, p< .001), but even more so since their values were derived from the very same spectra. That the CT-PRESS vs. J-editing comparisons are for methodological and temporally different data may thus explain the larger scatter.

Discussion: The data in **Fig.3**, which show excellent correlations between "pure" Glu and Glx regardless of voxel location and clinical status or diagnosis, strongly support Glu as the primary contributor of signal intensity to the composite Glx peak at 3.71 ppm that is generally assigned to Glu+Gln. Since the aforementioned reports of increased Gln were based on values derived from fitting overlapping Glu and Gln resonances in short TE spectra in which Gln is the minor component, we postulate that Gln levels likely are systematically overestimated. This view is strongly supported by two recent studies. Through simulation and experimental verification, Hancu and Port[14] recently demonstrated that measurements of Gln at 3T using most available methods can have an absolute error range of -50% to +70%. In addition, Kim et al[15] reported the results of a ^{1}H MRS study in rat brain *in vivo* and *ex vivo* that found Glu, but not Gln, increases following a subanesthetic dose of ketamine, which is in excellent agreement (a) with the observation of rapid and robust extracellular Glu surges in rat brain by microdialysis[9], (b) with our demonstration of similarly large and rapid Glx increases in MDD in response to ketamine (**Fig. 1**)[10], (c) with the prediction of the NRH model. In conclusion, definitive studies that cleanly measure both Glu and Gln in larger samples are needed to verify whether the reported Gln elevations are not a spectral fitting artifact.



References: [1] Coyle et al, *Ann N Y Acad Sci* 2003; **1003**:318. [2] Bartha et al, *Arch Gen Psychiatry* 1997; **54**:959. [3] Théberge et al, *Am J Psychiatry* 2002; **159**:1944. [4] Théberge et al, *Br J Psychiatry* 2007; **191**:325. [5] Olbrich et al, *World J Biol Psychiatry* 2008; **9**:59. [6] Bustillo et al, *Mol Psychiatry* 2010; **15**:629. [7] Kegeles LS et al. *Arch Gen Psych* 2012; 69:449. [8] Rowland et al, *Am J Psychiatry* 2005; **162**:394. [9] Moghaddam et al, *J Neurosci* 1997; **17**:2921. [10] Shungu et al, *Proc ISMRM* 2011; 4346. [11] Bartha et al, *Magn Reson Med* 2000; 44:192. [12] Dreher & Leibfritz, *Magn Reson Imaging* 1999; **17**:141. [13] Mayer & Spielman, *Magn Reson Med* 2005; **54**:439. [14] Hancu & Port. NMR Biomed 2011; **24**:526. [15] Kim et al, *NMR in Biomed* 2011; **24**:1235.