

Monitoring effects of dietary glycine loading on brain glycine levels with TE-Averaged ^1H MRS

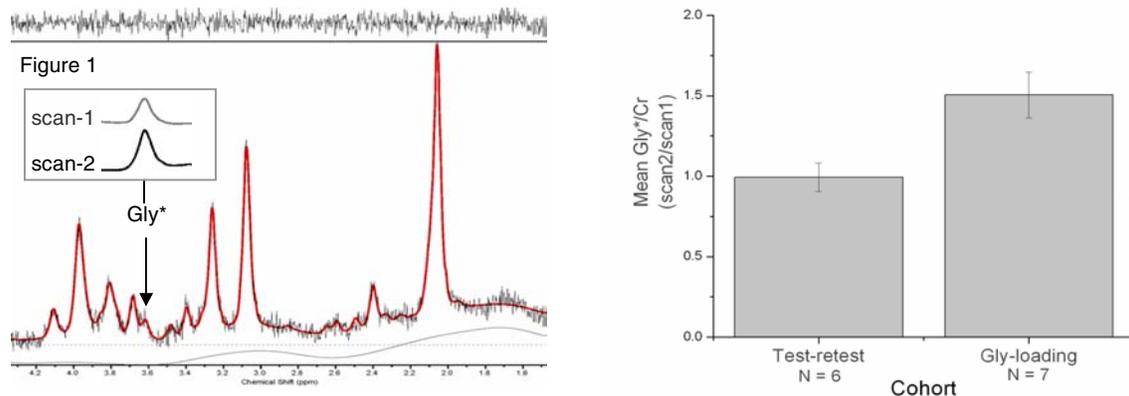
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Introduction: The amino acid glycine (Gly) serves as an essential coagonist for the glutamatergic N-methyl D-aspartate (NMDA) receptor subtype. NMDA receptor hypofunction is implicated in the pathophysiology of schizophrenia (1). Therapeutic interventions for schizophrenia have focused on the development of agents that activate the NMDA Gly receptor site, and several clinical trials have reported that dietary Gly administration, in combination with typical neuroleptic treatments, has promise for reducing negative symptoms in schizophrenia (2, 3). As expected, high-dose dietary Gly therapy increases serum levels in schizophrenics, although clinical responses to Gly loading are highly variable (2, 3). This may be due to variations in the amount of Gly taken up by the brain. A non-invasive method capable of measuring brain Gly levels may help to resolve whether clinical response variability is related to brain Gly uptake variability. To study this issue further, we developed a novel ^1H -MRS method involving TE-averaging to facilitate brain Gly measurements (4). The TE-averaging approach involves the acquisition and averaging of multiple TEs, thereby attenuating *myo*-inositol (mI) resonances that normally overlap and obscure the Gly singlet peak. TE-averaging has minimal effects on the Gly singlet peak, which co-adds constructively between TEs. The resulting Gly peak in TE-averaged ^1H -MR spectra contains a small contribution from residual mI and is thus termed Gly* henceforth. This report describes the acquisition of TE-averaged ^1H -MR spectra from two groups of healthy subjects, one participating in a test-retest (TRT) variability study, upon which we have reported previously (4), and a second group who participated in a high-dose Gly-loading study, to determine whether TE-averaged ^1H -MRS can detect brain Gly increases.

Methods: Seven healthy adult men were recruited for Gly studies (Mean age \pm standard deviation: 32 ± 8 years). Dietary Gly loading was accomplished with a modification of the Gly-loading protocol developed by Evins *et al.* (3), which increases plasma Gly levels (3.5 to 6.5-fold) and improves negative symptoms and cognitive function in schizophrenic subjects (2, 3). Gly loading involved 13 days of twice daily consumption of a Gly-enriched lemon drink, the doses of which were gradually escalated to the peak target dose (0.8 g/Kg/day, ~ 60 g/day). Initial Gly doses were 10g/day for 2 days, followed by 0.2, 0.4, 0.6 g/kg/day each for 2 days, with the terminal 0.8 g/kg/day dose maintained for 5 days. Subjects were scanned prior to Gly-loading (scan-1) and the morning after completing Gly-loading, on day 14 (scan-2). **Data Acquisition:** All measurements were performed on a 4.0 T Varian Unity/INOVA whole body MRI/MRS scanner (Palo Alto, CA) with a transverse electromagnetic (TEM) resonator head coil used for radiofrequency (RF) transmission and reception. A PRESS sequence was modified to enable TE-averaging (TE=30–284 ms, 128 increments, TR = 2000 ms, NEX = 4) and global water suppression was achieved using the WET module (5). TE-averaged ^1H -MRS spectra were recorded from a voxel (8-ml) positioned within the occipital-parietal cortex (predominantly grey matter). **Data Processing:** TE-averaged ^1H -MRS data were transferred offline to a Sun Ultra 10 workstation (Sun Microsystems, Inc., CA) and LC-Model v. 6.0-1 (6) was used to provide an unbiased fitting method for the TE-averaged ^1H -MRS data *via* use of a simulated TE-averaged basis set (4). Spectral fitting was performed between 1.4 and 4.4 ppm, and the raw integrals for the Gly methylene protons (3.55 ppm) and the creatine (Cr) methyl peak (3.0 ppm) were extracted to provide a Gly:Cr ratio for each of the fourteen *in vivo* TE-averaged datasets. Changes in brain Gly were estimated by dividing Gly:Cr calculated at scan-2 by the corresponding value determined at scan-1. These values then were compared to TRT data previously obtained in a separate group of six healthy control subjects (4). Statistical data analyses were performed using Origin v. 7.5 (OriginLab Corporation, MA).

Results: Figure 1 shows a representative fitted TE-averaged ^1H -MR spectrum recorded from a subject who had completed the Gly-loading protocol (scan-2). The Gly* signal is highlighted at 3.55 ppm. The inset shows only the fitted Gly* resonance at scan-1 (grey) and at scan-2 (black), and both peaks are plotted with identical vertical scaling. This particular subject showed a 76% increase in Gly*:Cr following completion of Gly-loading. Figure 2 shows data for all 6 subjects, and compares Gly-loading findings to previously obtained TRT scan data (4). In TRT scans, scan-2 brain Gly levels averaged $99.4\pm 9\%$ (mean \pm SE) of scan-1 levels (see (4)). For the Gly-loading cohort, brain Gly levels increased to $151\pm 14\%$ (mean \pm SE) scan-1 levels. The actual increase in brain Gly levels was found to range between 18 to 125% for the seven subjects who completed the Gly-loading. As expected, we observed a statistically significant increase in brain Gly*:Cr when Gly-loading and TRT data were compared ($*F(1,7)>8.5$, $P<0.02$, versus test-retest variation).



Discussion: These data demonstrate that TE-averaged ^1H -MRS can monitor brain Gly changes following oral high-dose Gly-loading. Intriguingly, the brain Gly increases we measured are significantly lower than previously reported serum Gly increases (3, 4). This may be due to some limitation in brain Gly uptake from blood, could reflect rapid brain elimination of Gly after completion of Gly loading, or may indicate that some fraction of Gly is not detectable with MRS. Regardless of the mechanism, these preliminary findings imply that there is significant variation in the degree of brain Gly increase induced by oral Gly-loading, which may in part account for the variability in the clinical response to Gly adjuvant therapy in schizophrenia. This methodology may be useful for monitoring brain Gly changes following therapeutic treatments that selectively target glycinergic NMDA neurotransmission (e.g. Gly receptor antagonists or Gly transport reuptake inhibitors).

References: ¹Olney JW, *et al.* JPsychiatry Res 1999;33(6):523-533; ²Heresco-Levy U, *et al.*, Arch Gen Psychiatry 1999;56(1):29-36; ³Evins AE, *et al.*, Am J Psychiatry 2000;157(5):826-828; ⁴Prescot AP, *et al.*, Magn Reson Med, 2005:Accepted for publication; ⁵Ogg R, *et al.*, J Magn Reson B 1994;104(1):1-10; ⁶Provencher SW. Magn Reson Med 1993;30(6):672-679.

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