Hasler et al: Effect of Acute Psychological Stress on Prefrontal Gamma-Aminobutyric Acid Concentration Determined by Proton Magnetic Resonance Spectroscopy

Supplemental Material

Magnetic Resonance Spectroscopy

Subjects were scanned in a single session on a 3 Tesla GE MRI scanner using an RF body coil for transmit and an eight channel NOVA receive coil (Nova Medical, Inc. Wakefield MA, USA). The body RF coil provides the homogeneous RF excitation field over the brain including the prefrontal cortex; the 8 channel receive coil was used to improve the signal to noise ratio. The signals of the eight receive coils were combined using the water reference signal (1). Based on previous imaging studies on anxiety (2), proton MRS spectra were acquired from a voxel that extended 3 cm x 3 cm x 2 cm and was positioned with the posterior edge located 1 mm anterior to the rostrum of the corpus callosum, and was centered on the midline in horizontal planes and on the bicommissural line in sagittal planes. This voxel included portions of the perigenual anterior cingulate gyrus, and the adjacent frontal polar cortex (i.e., portions of BA 24, 32, and 10 (3)).

GABA was measured using an interleaved PRESS-based J editing method (4,5). This method uses PRESS for spatial localization. GABA H-3 at 1.9 ppm is inverted by an editing pulse in alternating scans. When GABA H-3 is inverted, the J evolution between GABA H-3 and GABA H-4 is refocused and the two outer resonance lines of GABA H-4 at 3.0 ppm are in phase with respect to its central resonance line and the creatine methyl proton signal. When GABA H-3 is not inverted during the control scan, the two outer resonance lines of GABA H-4 are in antiphase with respect to its central resonance line and the creatine methyl proton signal. Subtracting the two scans reveal the two outer resonance lines of GABA H-4 but eliminates the creatine methyl proton signal at 3.0 ppm because it is not affected by the editing pulse. The frequency profile of the editing pulse is flat over the frequency range of the GABA H-3 at 1.9 ppm resonance. This characteristic rendered the method insensitive to small

variations in frequency during the scan, i.e., the editing efficiency was not changed by small variations in frequency making the method robust for scanning *in vivo*. The echo time was 68 ms, the repetition time was 1.5 seconds, the number of excitations (NEX) was two and the number of acquisition points was 2048 with a sample frequency/spectral width of 5000 Hz. The scan time was 32 minutes for a total of 1280 averages.

Individual peak areas were fitted using a non-linear fitting program written in IDL (Research Systems Incorporated, Boulder, CO), which performed time domain spectral analysis to determine the amplitude of the spectroscopic peaks in a fully automated manner (eliminating the possibility of rater bias). The concentrations of GABA, choline, N-acetyl-aspartate and co-edited glutamate/glutamine were expressed in mmol/liter (mM) referenced to concentration of creatine that was set to 7.1 mmol/L because this values represents an average concentration from literature reports of creatine in gray and white matter (6,7). The creatine reference method is well-suited for the current study because the rate of creatine turnover is approximately 0.04%/hr (8). In particular, mammalian brains lack the enzyme machinery for creatine biosynthesis. Therefore, practically no change in brain total creatine concentration is possible as a result of acute psychological stress over a relatively short duration. .

The spectroscopy data were processed in two steps. First the unedited spectra were fitted for the amplitudes of choline, creatine, and N-acetyl-aspartate. Secondly the GABA at 3.0 ppm and co-edited glutamate/glutamine-2 at around 3.8 ppm were extracted from the edited spectra and fitted accordingly. The GABA intensity then was corrected for contamination from co-edited macromolecules (4,9). At experimental conditions optimized for GABA editing, a fraction of glutamate/glutamine-2 at 3.8 ppm and glutamate/glutamine-4 at 2.4 ppm were co-edited because of their J couplings to the glutamate/glutamine-3 multiplets at 2.1 ppm. The cleanly co-edited glutamate/glutamine-2 signal was used for measurement of glutamate/glutamine as its intensity is proportional to the total concentration of

glutamate/glutamine (5,10). The GABA multiplets closest to the glutamate/glutamine-2 peaks resonates at 3.0 ppm, which does not overlap with glutamate/glutamine-2.

The current study used a subtraction-based MRS methodology to detect GABA in the brain. As such, it may be susceptible to subtraction errors due to subject movement and/or scanner instability. As shown in a recent study of glutathione in stroke patients using a similar MRS method (11), when subtraction errors are present, an error signal at 3.2 ppm originated from imperfect subtraction of the intense choline signal can be detected. No subtraction errors were detected in the current study. In addition, interleaved acquisition and careful in vivo and in vitro quality control procedures were employed to ensure the quality of the data.

The macromolecule multiplets are contributed by a very large number of different proteins and its contribution to the edited signal at 3.0 ppm should be considered unchanged by the employed task. Due to their diverse sources, the contribution of the macrolecule multiplets to the edited signal at 3.0 T is dependent on many factors including the relaxation delay of the MRS measurement. Using pulse sequence and experimental parameters very similar to those of the current study, Kegeles et al (12) reported a co-edited macromolecule fraction of 41-49% at 3 Tesla, similar to that reported for 2.1 Tesla by Rothman et al (13). At substantially longer relaxation delay which allows inclusion of macromolecular components with long relaxation times, a higher (~56%) macromolecule fraction was found (9). Because macromolecule contribution has been subtracted during quantification of GABA (5,10), the GABA concentration reported here should be interpreted as GABA instead of GABA+macromolecules.

Legends to Additional Figures

Figure 4. Display of spectroscopy voxel

Figure 5. Four GABA editing spectra obtained at 3 Tesla from a single shock experiment. Period 0, eight minutes before the threat-of-shock period, period 1, eight minutes where shocks may occur, period 2 and 3, a total of 16 minutes of rest. The dotted line is the fit to the data. Glx=glutamate/glutamine, GABA=gamma-aminobutyric acid, NAA=N-acetyl-aspartate.

Figure 6. Scatterplots of the individual gamma-aminobutyric acid concentrations (upper row) and glutamate/glutamine concentrations (lower row) in the threat-of-shock session (left, “threat”) and in the no threat-of-shock session (right, “no threat”) from 10 healthy volunteers. The x-axis indicates the concentration of the chemical (mmol/L for gamma-aminobutyric acid, arbitrary unit for glutamate/glutamine), the y-axis indicates the period of the scan (see legend to Figure 5). GABA=gamma-aminobutyric acid, Glx=glutamate/glutamine.

References (Supplement)

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