Simultaneously assessed GABA/Glutamate/Glutamine concentration gender differences at 3.0T

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BACKGROUND: Psychiatric magnetic resonance spectroscopy (MRS) is based on the assumption that controls are sufficiently homogeneous that they can be pooled, and compared as a uniform group to a disordered second group. This assumption is central to the assessment of disorders of inhibition such as depression or obsessive-compulsive disorder, using MRS-obtained estimates of the highly coupled Glx spin system, whose components, γ-Aminobutyric acid (GABA), glutamate (Glu) and glutamine (Gln) are thought to underlie these disorders [1], [2]. It is known, however, that these metabolites show gender differences. The directionality of these differences is complicated. Evidence exists in the literature for GABA concentrations being greater in females than males [3], or for being approximately equal but lesser in females during the menstrual cycle [4]. Gender differences have also been reported for Glu concentrations, with concentrations reported as decreasing across the menstrual cycle in a manner akin to that reported for GABA. While research has been performed on these metabolites separately, to our knowledge no study has focused on their simultaneous quantification *in vivo*, with the intent of assessing gender differences for all Glx metabolites simultaneously. The present investigation seeks to simultaneously assess differences in the concentration of each of the Glx metabolites in the anterior cingulate cortex (ACC) between normal males and females, using a STEAM sequence (TE/TM 72/6) previously optimized for simultaneous Glx resolution. We hypothesis that gender differences will exist between our subject groups in GABA/Glu concentration, at that this difference will indicate the necessity for including gender as a factor in the analysis of psychiatric disorders with hypothesized Glx deficits.

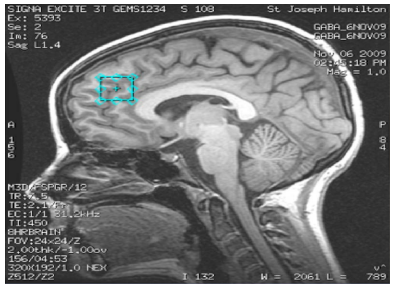

Figure 1: Sagittal fSPGR (flip angle=20, TE=4ms, TR=10ms, FOV = 22mm X 22mm, 120 images, Slice Thickness 20mm, NEX = 2, Frequency Encoding Steps = 256, Phase Encoding Steps = 192) of female subject 1, showing the placement of our voxel in the ACC.

METHODS: Seven male (n=7, mean age 25.7) and five female (n=5, mean age 23.8) subjects were recruited for a study approach by our institutional research ethics board (REB). Subjects were instructed to refrain from fatty foods for 24 hours prior to the scan, and to refrain from drinking alcohol or coffee 12 hours before the scan. A STEAM sequence (TE=72, TM=6, TR=3000, NEX=512, FOV=20 x 20, Slice Thickness = 20, Scan time 25.6min), optimized during a previous investigation for the simultaneous resolving of the Glx components, was used to image the ACC, with the location of this structure determined from a sagittal T1-weighted fSPGR (see Fig.1). An in-house basis set was prepared for spectral deconvolution. Quantization of metabolite concentrations was determined through the use of LCModel's water-scaling algorithm. All scans were conducted using an 8-channel receive-only phased array RF coil on a GE^{TM} Healthcare 3T HD Signa MRI system (GE^{TM} Healthcare, Milwaukee WI). No attempts were made to scan subjects at identical times of day as previous research by our group has indicated no time-of-day effect in Glx metabolite concentration [5].

RESULTS: Cramer-Rao lower bounds (CRLBs) were used to assess the significance of LCModel-obtained metabolite concentrations. Significant levels (defined as SD≤20%) of Glutamate were detected in all male (n=7) and female (n=5) subjects, with significant Gln (n=1) detected in one male subject and one (n=1) female subject. Concentrations trending towards significance (defined as 20>SD%≥30) of GABA were detected in five (n=5) male subjects and one (n=1) female subject, with trending-significant Gln detected in one additional male (n=1) and female (n=1) subject. No significant differences existed within male (F=0.077, p=0.998) or female (F=0.075, p=0.998) subjects' metabolite concentrations. Finally, a Bonferroni-corrected t-test between male and female GABA indicated a significant (p<0.01) difference between male and female GABA concentrations; similarly corrected t-tests of the difference between male and female Glu (p=0.344) and Gln (p=0.85) scores failed to find any significant differences. The mean +/- standard deviation scores for GABA, Glu, and Gln between males and females, as well as the SNR and FWHM of each scan group are reported in Table 1.

Glx Metabolite Concentrations by Gender

Gender	[GABA]	[Glu]	[Gln]	SNR	FWHM
Male	1.987+/- 0.655	10.242+/- 2.901	2.19+/- 1.466	34.571+/- 6.241	0.0476+/- 0.063
Female	1.007+/- 0.362	9.018+/- 1.213	2.331+/- 1.009	37.8+/- 4.438	0.0324+/- 0.010

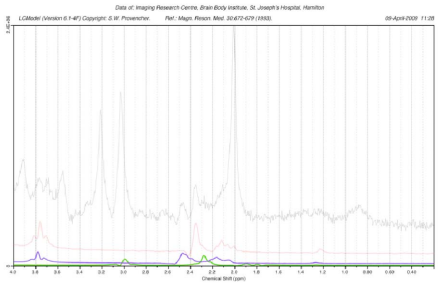

Fig. 2: LCModel output for male subject 7. Glu is reported in red, GABA in green, and Gln in blue. All metabolites of interest are significantly (SD% <20) ([GABA] = 3.94mM, [Glu] = 11.415 mM. [Gln] = 5.768 mM). (FWHM = 0.033ppm, S/N = 35). As can be seen from the overlaid, individual metabolite fits, three unique resonances, one for each metabolite, exist in the 2.2-2.5ppm region and served to enable simultaneous metabolite quantification.

DISCUSSION: Our results demonstrate the necessity for considering the gender of control subjects for Glx metabolite investigations, and for not pooling males and females when developing control groups for psychiatric MRS. Both the male and female metabolite concentrations we have reported here are within accepted norms within the Glx literature; that significant differences exist between genders when this factor is considered supports our argument [5], [6], [7]. A novel finding from our investigation appears to be that the concentration of GABA in women is actually less than that of men, which appears to contradict previously published research. That our study focused on the concentrations within the ACC, and previous investigations focused on quantifying the GABA or Glu signals within the occipital lobe, indicates that this need not be as contentious a point as it may appear. It is possible that GABA concentrations show local versus global menstrual fluctuations. Moreover, our results appear to indicate a reason for the discrepancy seen between mood disorders, which are more prevalent in females and appear to correlate with low cortical GABA, and previous literature reports of increase GABA in women: GABA may be lower in females than in males within higher cognitive structures such as the ACC, a fact that was missed due to previous studys' focus on the occipital cortex. It is also interesting to note the lack of significant difference between Glu concentrations across genders; this is again not in keeping with the broader literature. We hypothesis that this may be due to our optimized sequence design versus that used in traditional scans, where sideband resonances from the GABA triplet overlapping Glu resulted in erroneous concentration estimates; our sequence avoids such issues. Lastly, it should be noted that we did not control for menstrual cycle stage amongst the female subjects scanned during this investigation. While this theoretically could have produced a heterogeneous group, our data did not reflect this; we hypothesis, again, that this may be due to local consistency in Glx concentrations in the ACC versus those in the occipital lobe, which have been demonstrated to fluctuate.

CONCLUSIONS/FUTURE DIRECTIONS: Our results demonstrate that care must be taken at 3.0T in developing control groups for Glx assessment with MRS. The investigators hope to complete their present investigations such that the sample sizes between male and female subjects is identical. We also intend to rescan the female subjects in our study during the luteal and follicular menstrual phases in both the ACC and occipital lobe, with the intent of determining if the stability of the Glx metabolites in the ACC, and the reported lability of these in the occipital lobe, can be verified. Regardless of the subsequent results of this second investigation, however, we are well-justified in arguing that gender must be considered when analyzing Glx-disorders.

References: [1] Ebert D., et al. (1997) Psychiatry Research, Jul 4;74(3):173-6. [2] Block W., et al. (2008) The international journal of neuropsychopharmacology, Oct 10:1-8. [3] Sanacora, G., et al. (1999) Archives of General Psychiatry, 56(11): 1043-1047. [4] Epperson, C.N. (2002Archives of General Psychiatry, 59(9):851-858. [5] Sheffield, P, Noseworthy, M.D., and Bienenstock, J. ISMRM conference. 2009. [6] . Keltner, J. R., et al (1996). Magnetic resonance in medicine 36(3), 458-461. [7] Pouwels, P. J., & Frahm, J. (1998). Magnetic resonance in Medicine 39(1), 53-60. [8] Knorle, R., et al. (1997). Neuroscience letters, 221(2-3), 169-172.