Alterations of Excitatory Neurotransmitter Systems in Brain Regions of Acute Migraine Patients during the Interictal State

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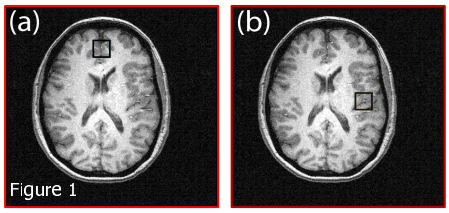
Introduction: Migraine headache is a neurobiologic disorder that affects about 27 million women and 10 million men in the U.S **[1]**. Recent reports suggest alterations in the interictal migraine brain based on changes in cerebral blood flow **[2]** as well as changes in cognitive function in migraineurs with aura **[3]**. In addition, a wealth of evidence, including measurements demonstrating changes in physiological measures (i.e., evoked potentials **[4]**), strongly supports the hypothesis of central neuronal hyperexcitability as playing a major role in the pathogenesis of migraine **[5]**. A potential mechanism for neuronal excitability includes an abnormality of the pre-synaptic release of excitatory amino acid neurotransmitters. Increased synaptic concentrations of excitatory amino acid neurotransmitters may lead to hyperfunction at the N-methyl D-aspartate (NMDA) glutamate (Glu) receptor subtype, which may amplify and reinforce pain transmission in pain and other types of headache. The objective of this work was to employ proton (^{1}H) magnetic resonance spectroscopy (MRS) to assess potential differences in anterior cingulate cortex (ACC) and insula (Ins) metabolite levels in patients with acute migraine compared to control subjects. Two-dimensional (2D) ^{1}H-MRS methods were utilized in attempt to resolve and better characterize an increased number of metabolites including species pertinent to excitatory neurotransmission.

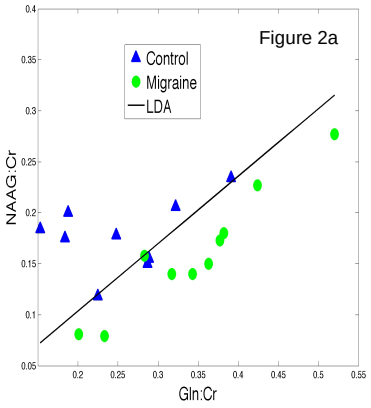
Methods: 10 acute episodic migraine (AEM) patients (7 women, 3 men, age 43±11 years) and 8 age/gender matched healthy controls (HC; 5 women, 3 men, age 41±9 years) participated in this study. **Data Acquisition:** All MRI/MRS measurements were performed using a 4.0 T whole-body Varian Unity/INOVA MRI scanner (Varian Inc., Palo Alto, CA, USA). A birdcage design radiofrequency (RF) head coil tuned to 170.3 MHz was used for RF transmission and reception. High-contrast 3D FLASH T1-weighted axial, coronal and sagital MR images (TE/TR = 6.2/11.4 ms) enabled accurate positioning of the MRS voxel within the region-of-interest (ROI). 2D *J*-resolved ^{1}H-MRS data were localized from 8-cm3 voxels positioned within the midline ACC and left insula (Ins) of each subject (TR = 2000 ms, TE range = 30-260 ms, ΔTE = 10 ms, NEX = 16 per TE). The full width at half maximum (FWHM) of the unsuppressed water resonance was ≤12 Hz for both voxels in all subjects. Total measurement time did not exceed 1 hour. **Data Processing:** Image analyses were performed using the FMRIB Software Library (FSL; **[6]**). Tissue segmentation into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) used FSL's fast automated segmentation tool (FAST; **[7]**). The 2D *J*-resolved ^{1}H-MRS data were quantified using Linear Combination Model (LCModel; **[8]**) using a procedure described elsewhere **[9]**. In brief, the basis function spectra required to fit the entire 2D spectral surface were generated using GAMMA **[10]** for a total of 15 metabolites including aspartate (Asp), choline (Cho), creatine (Cr), g-aminobutyric acid (GABA), glucose (Glc), glutamine (Gln), glutathione (GSH), glycine (Gly), *myo*-inositol (mI), N-acetyl aspartate (NAA), N-acetylaspartyl glutamate (NAAG), phosphocreatine (PCr), *scyllo*-inositol (sI) and taurine (Tau) . Metabolite levels were normalized to total creatine. Standard statistical analyses and linear discriminant analyses (LDA) were performed using Origin version 7.5 (OriginLab Corp., Northampton, MA, USA) and MATLAB (The Mathworks, Natick, MA, USA), respectively.

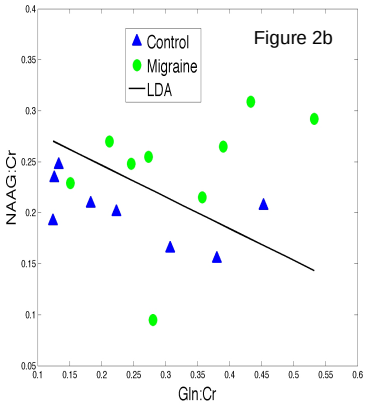
Results and Discussion: Figure 1 shows axial MR images recorded from a 32 year-old female migraine patient and displays positioning of the MRS voxel (black square) within the (a) and (b) left Ins. The results of tissue segmentation analyses are presented in table 1 (values presented as %voxel ±SD). No significant differences existed in tissue content existed between the two groups. However, the WM fraction was significantly different in the Ins voxel compared to the ACC voxel for both subject cohorts (ANOVA: $P < 0.01$). For the control group, the FWHMs (mean ±SD) measured for the unsuppressed water resonance were 9.75 ±1.6 and 9.25 ±1.0 Hz for the ACC and Ins voxels, respectively. For the AEM group, the corresponding values were 10.2 ±2.0 and 10.0 ±1.1 Hz for the ACC and Ins voxels, respectively. Standard statistical analyses showed no significant metabolite differences between the two cohorts. However, by using LDA a clear separation between HC vs. AEM patients is revealed as shown for the ACC and Ins in figure 2a-b. Bootstrap calculations verified the good statistical accuracy of the estimated LDA coefficients (data not shown). The LDA classification is achieved using the metabolites NAAG and Gln for both brain regions, and the within-subject coefficients of variation (CV) for these particular metabolites were 23 and 21%, respectively (CV data taken from **[9]**). NAAG and Gln are linked by the excitatory glutamatergic neurotransmitter system, with NAAG being synthesized in the neurons from NAA and Glu whereas Gln is synthesized exclusively in glial cells from Glu and ammonia. The ACC and Ins LDA plots show oppositely signed gradients, an observation that might be explained by the significant tissue type differences within ACC and Ins voxels and the known uneven distribution of Gln and NAAG throughout the brain and within tissue type. **Conclusion:** These data suggest possible alterations in the glutamatergic and /or glutaminergic pathways in this subgroup of migraine patients. The focus on glutamate and/or glutamine-based therapeutic approaches that could enhance the interictal state may significantly affect migraine frequency, severity or duration.



	ACC (GM)	ACC (WM)	Ins (GM)	Ins (WM)
HC	69±8	16±6	56±5	37±5
AEM	72±6	15±6	59±7	33±5

Table 1





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