# Elevated glutamate concentrations in the visual cortex of migraine without aura detected at 7 Tesla.

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**Introduction:** Migraine is a common, multifactorial, neurovascular disorder. The glutamatergic system is one of the pathogenetic factors. Glutamate is the main excitatory neurotransmitter of the CNS and is synthesized in neurons from glutamine and stored in synaptic vesicles.[1,2] Elevated glutamate concentrations are linked to migraine aura, trigeminovascular activation and central sensitization, mechanisms important in migraine pathophysiology.[1,3,4] The aim was to compare the glutamate concentrations in the visual cortex of interictal (between attacks) migraine patients with and without aura and age and gender matched healthy controls. As separation of glutamate and glutamine is challenging at lower field strengths, we performed this study at 7T.

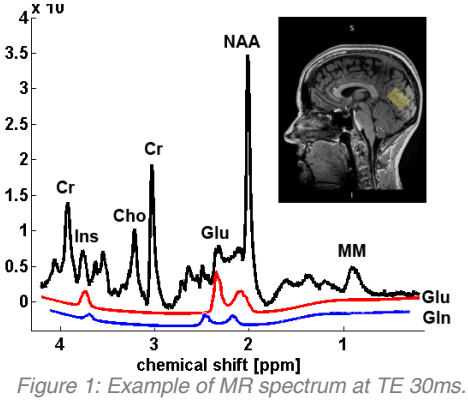


*Figure 1: Example of MR spectrum at TE 30ms. The individual fit of Glu (red) and Gln (blue) is shown below the spectrum. The voxel position is displayed in the inset figure (3x2x2 cm$^3$).*

**Methods:** Migraine patients with and without aura, and age and gender matched healthy control subjects were recruited. Patients were free of a migraine attack at least 3 days before the MRS scan. Patients were classified as strict interictal if they had no migraine attacks until 2 days after the examination and as pre-ictal if they had an attack within 2 days after examination. ***Acquisition:*** Participants were examined at a 7T MR system (Phillips, Cleveland, USA) using a 32 channel Rx and quadrature Tx coil driven by 2 amplifiers with fixed phase (2 times 4 kW) to reach local $B_1$ of 20uT. A 3D T1 weighted scan was used for voxel planning and voxel segmentation. Water suppressed (VAPOR) MR spectra in the visual cortex were acquired (12 cm$^3$) with a semi-LASER sequence. A $B_0$ map was used to optimize up to 2nd order shim gradients for each voxel position. TR/TE was 5000/30 ms, NSA was 32. A water reference scan of each measurement was used (no RF in VAPOR sequence) to correct for receive sensitivity of the coil, eddy current correction and quantification.
***Data analysis***: MR spectra were analyzed with LCModel[5] using a basis set of 22 metabolites and a measured macromolecular profile (Figure 1). The fraction of GM, WM and CSF within the MRS voxel was calculated (Matlab, Mathworks, Inc.). The metabolite levels were corrected for water fraction, water T1 and T2 relaxation and metabolite T2 relaxation using relaxation values from the literature.[6-9]
***Statistical analysis*** was done in SPSS (IBM, Inc). Differences in glutamate concentrations between three groups were evaluated with ANCOVA with the fraction of GM in the voxel as covariate. Post-hoc pairwise comparisons were done with Bonferroni correction to adjust for multiple comparisons.

**Results:** We included 36 migraine without aura patients (age 35 ± 8, 47% male, 27 strict interictal, 11 pre-ictal), 27 migraine with aura patients (age 35 ± 9, 48% male, 23 strict interictal, 4 pre-ictal), and 24 healthy controls (age 34 ± 9, 52% male). Average migraine attack frequency was 2.7 ± 2.0 per month.
Glutamate concentration correlated with the GM fraction in the voxel (Pearson's $r(87)=0.51$, $p<0.001$), therefore the GM fraction was taken as covariate.
In the primary analysis with strict interictal migraine groups and healthy controls there was a difference in glutamate concentration between the groups ($F(2,70)=3.20$, $p=0.047$), with higher concentrations in migraine without aura (mean 7.02 ± 0.50 mM) compared to healthy controls (mean 6.40 ± 0.78 mM, $p=0.042$; Figure 2), with a fold-change of 1.06 after correction for GM fraction. No differences were found for migraine with aura. There was no difference in glutamate concentration between strict interictal migraine patients and pre-ictal migraine patients ($F(1,84)=1.96$, $p=0.16$), and no correlation between glutamate concentration and the time (days) until a next attack (Pearson's $r(36)=-0.18$, $p=0.29$). Pooling both interictal and pre-ictal migraine patients in a secondary analysis also showed higher concentrations in migraine without aura (mean 7.08 ± 0.56 mM) compared to controls (mean 6.40 ± 0.78, $p=0.006$), with a fold-change of 1.07 after correction for GM fraction, but no differences for migraine with aura.

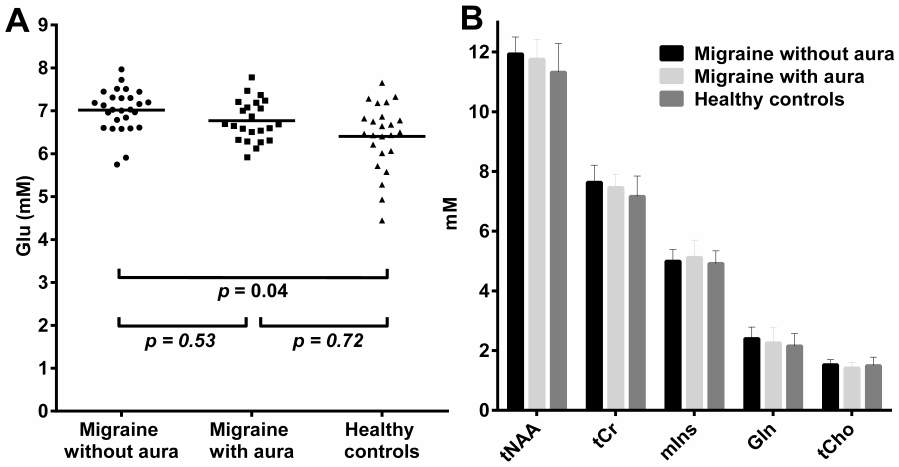


*Figure 2: Comparison of strict interictal metabolite concentration between migraine without (n=27) and with aura (n=23), and healthy controls (n=24). A) Significant differences in Glu concentrations (with Bonferroni correction); B) No significant differences for other metabolites.*

**Discussion and conclusion:** The overall CRLB's of Glu and Gln were 2.8 ± 0.95 and 8.8 ± 2.7 respectively and there were no differences between groups in linewidth, SNR or CRLB's of metabolites, indicating that at ultra-high field, glutamate and glutamine concentrations can be assessed individually. Our results support the concept that the glutamatergic system is involved in migraine. We found that glutamate concentrations in the visual cortex are higher in interictal migraine without aura patients compared to healthy controls, but not higher in migraine with aura patients. The difference is small; however, as glutamate is tightly regulated in the brain large differences are not expected. We found no relation between glutamate and the time until a next attack. The higher glutamate concentration in migraine without aura is more likely a disease trait than a reflection of a disease state (e.g. interictal, or pre-ictal). Future studies should investigate the role of glutamate in migraine in more detail, for example during different phases of a migraine attack with the focus on glutamate dynamics to learn more about the functional role of the glutamatergic system in migraine pathophysiology. Further focus on the pre-aura- and aura-phase of a migraine attack might explain why the current study unexpectedly failed to identify significant differences for the migraine with aura group.

**References:** *[1] Gasparini CF, 2013, IJBS 9 [2] Mckenna MC, 2007, Fates of Glutamate in Brain. [3] Scheller D, 2000, Amino Acids 19 [4] Ramadan N, 2003, CSN Spectro 8 [5] Provencher SW, 1993, MRM 30 [6] Rooney WC, 2007 [7] Bartha R, 2002, MRM 47 [8] Otazo, 2006 [9] Marjanska M, 2012, NBM* 25