Cortical GABA levels are impaired after stroke, but may be normalized with rehabilitation

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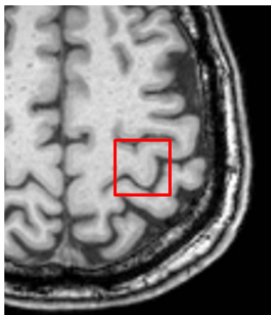
INTRODUCTION: The extent to which GABA levels are changed after stroke and whether the transmitter level changes during recovery is still unknown. GABA levels in stroke have previously been investigated using transcranial magnetic stimulation (TMS) or Flumazenile PET, both sensitive to a combination of postsynaptic receptor levels and the actual amount of GABA. These studies found decreases in inhibitory activity [1,2], though it was unclear whether these results were due to changes in cortical GABA concentrations, or decreases in receptor activity. GABA concentrations can be measured by edited magnetic resonance spectroscopy (MRS) [3]. Such measures of cortical GABA have been correlated with motor learning in healthy subjects [4] and even been shown to change with non-invasive brain stimulation, like transcranial direct current stimulation (tDCS) [4]. In this study, we compared post stroke GABA levels in the motor cortex to those in healthy subjects and investigated the possibility that GABA levels can be modulated after two weeks of intensive hand training.

METHODS: **Participants**: 21 stroke patients were recruited (12 male, mean age 60 (range 36-75)). All patients had suffered from a single stroke 3-12 months earlier and had persisted hand impairment. Patients were included if they fulfilled the criteria for constraint-induced movement therapy (CIMT)[5] and were eligible for MRI, but excluded if they suffered from other neurological diseases. 21 healthy, age and gender matched subjects (12 male, mean age 61 (range 40-75)) were also recruited. All gave informed consent. **Experiment**: Participants were scanned on a 3T (Siemens) MR scanner. A T1-MPRAGE scan was performed and used for voxel placement. MRS MEGA-PRESS: A 2x2x2 cm^3 voxel was placed in the "hand knob" area of the affected hemisphere of the patients (**Fig. 1**) and in the dominant hemisphere of healthy subjects. Baseline edited GABA spectra were acquired. TR/TE=2500/68ms, 186 averages. Editing was achieved with a 14ms dual banded Gaussian pulse with water suppression band centred at 4.7ppm. The GABA editing band alternated between 1.9ppm and 7.5ppm in even and odd acquisitions respectively. **Analyzes**: MRS: A semi-automated preprocessing routine was applied to all spectra to remove motion corrupted averages, and to correct frequency and phase drifts prior to signal averaging. All spectra were then apodized with a 5 Hz filter. Zero and 1st order phase corrections were applied. GABA levels relative to Creatine (Cr) were measured using AMARES within jMRUI. GABA was measured from the difference spectrum (even-odd) (**Fig. 2**), while Cr was measured from summed spectrum (even+odd). An institutional unit (i.u.) GABA:Creatine ratio was determined. **Statistics**: GABA/Cr ratios were compared between healthy subjects and patients at baseline using an unpaired t-test. In patients pre- and post-training GABA/Cr ratios were compared using a paired t-test. **Training**: Patients completed two weeks of constraint-induced movement therapy (CIMT), a well described training method [5], in which patients have their healthy hand immobilised, forcing them to use the impaired hand. The motor function of the patients was assessed using the Wolf Motor Function Test (WMFT) before and after the two weeks of training. One patient dropped out of the study due to study-unrelated disease. In 4 stroke cases, GABA datasets were not collected due to technical issues. Thus a total of 16 complete, pre- and post-training patient datasets were available for data analysis.

RESULTS: In stroke patients, the pre-training GABA/Cr ratio was significantly lower (0.33 (SD 0.06), $p<0.001$) than that of healthy subjects (0.42 (SD 0.08)). After two weeks of training, the patients' GABA/Cr increased significantly to 0.38 (SD 0.08), $p=0.025$, and there was no significant difference in these GABA/Cr ratios compared to that of healthy subjects ($p=0.2$) (**Fig. 3**). Simultaneously, patients showed a significant improvement in hand function after training, based on both the time ($p=0.028$) and quality ($p<0.001$) measures of the WMFT.

Fig. 1: Example of MRS voxel placement, which targeted the hand area of the motor cortex.



DISCUSSION: To our knowledge, this is the first study using GABA-edited MRS to track GABA modulations in stroke patients. The results showed that while GABA/Cr levels were significantly lower in patients post stroke, there appears to be a normalization towards healthy levels after two weeks of motor training, after which WFMT motor scores also improved significantly. Currently we cannot exclude the possibility of changes in GABA/Cr ratios being an effect of spontaneous recovery with time, without scanning patients who did not undergo training. Nonetheless, as the patients were between 3-12 months post stroke, and the two MRS measures were only 2 weeks apart, we find it unlikely that the difference is due to time alone. Several other possible biases should also be considered when interpreting these results. Firstly, the measured GABA peak has a contribution from co-edited macromolecules, and whether the amount of co-edited macromolecules changes after stroke or during rehabilitation is currently unknown. Secondly, a reduction in Creatine in ischemic regions has been reported [6], which could bias our results. However a reduction in Creatine in patients would overestimate GABA/Cr ratios and thus cannot explain our findings of lower GABA/Cr ratios in patients. Finally, differences in the gray matter content between patients and healthy volunteers could also be a factor. The lower GABA/Cr in patients before training could be related to gray matter atrophy, although it is unlikely that the GABA/Cr ratio in patients would normalize during two weeks of training due to gray matter content alone. To address these issues, further analyzes, such as gray matter segmentation are currently being conducted. Bearing in mind the possible biases, our results point towards the explanation that decreases in cortical inhibition after stroke [1,2] could be due to decreased GABA concentrations, rather than decreased receptor activity. Importantly, it appears possible to normalize these GABA levels, as well as hand function with therapy.

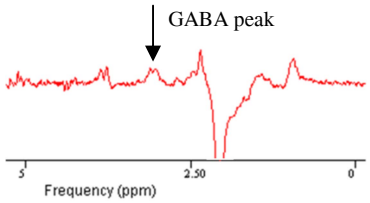


Fig. 2: Example of a filtered, difference spectrum in a patient.

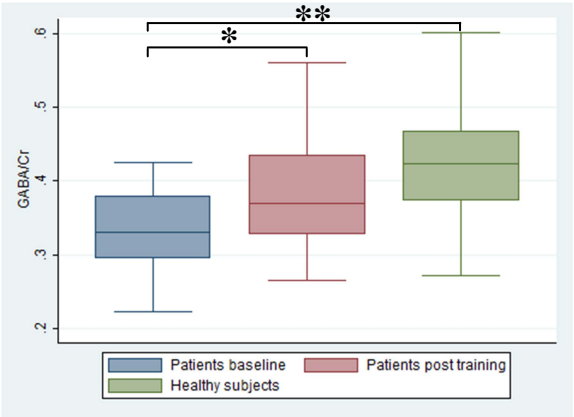


Fig. 3: Prior to training, patient GABA levels were significantly lower than that of healthy subjects, but increased significantly to practically normal levels after training (no significant difference with values from healthy subjects).

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