

## **Activation induced changes in GABA: functional MRS at 7 T with MEGA-sLASER**

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**ABSTRACT**

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## **INTRODUCTION**

Functional magnetic resonance spectroscopy (fMRS) is a powerful tool for probing human brain metabolism non-invasively by investigating the metabolic change in response to a physiological stimulus. With the benefits of increased SNR and spectral dispersion, recent fMRS studies at ultra-high field (7 T) have measured activation-induced neurochemical responses occurred during the stimulation of the human visual cortex (1–7) and motor cortex (8). Increases in glutamate (Glu) and lactate (Lac) concentration observed upon visual or motor activation were consistent across these recent studies, despite of the variation in the percentage of changes. Some studies also reported an increase in aspartate (Asp) and a tendency for a decrease in glucose (Glc) level during visual stimulation (1; 3; 5). These observed changes of metabolite concentrations imply an overall rise in oxidative metabolism and the malate-aspartate shuttle activity during neuronal activation. However, the functional response of  $\gamma$ -aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the brain, is still not well characterised dynamically. Lin et al. (3) has reported a tendency for an increase in GABA levels during visual activation compared to the initial resting state level, while a more recent study by Mekle et al. (7) detected a significant decrease in steady-state GABA levels during visual activation when comparing the activation voxel with a control voxel.

Previous fMRS studies have commonly utilized  $^1\text{H}$  direct detection methods with short TE at ultra-high field, which allows detecting a wide range of brain metabolites simultaneously with the benefits of enhanced signal-to-noise ratio (SNR) and spectral resolution (2; 9–11). However, the unambiguous detection and quantification of GABA levels in human brain with the direct detection approach is still challenging, due to the signal overlap with other more concentrated compounds, such as creatine (Cr), phosphocreatine (PCr), Glu, N-acetylaspartate (NAA) and several macromolecules (MMs) (12–15). The J-difference spectral editing techniques have enabled the direct observation of GABA signal by the cancellation of non-coupled overlapping signals (16–18). This is based on the subtraction of two scans: one scan with frequency-selective editing pulses applied at 1.9 ppm GABA resonance to refocus the J-coupling evolution of the 3.0 ppm GABA resonance ('ON'); and one scan without editing at 1.9 ppm so that the 3.0 ppm GABA resonance undergo undisturbed J-coupling evolution ('OFF'). Combining the Mescher-Garwood (MEGA)-editing scheme (16) with a localisation method, the MEGA-PRESS (point-resolved spectroscopy) has been commonly used for selective GABA detection at 3 T (19–22) and recently the MEGA-sLASER (semi-localised by adiabatic selective refocusing) has shown to be highly efficient for GABA editing at 7 T (14; 18; 23). To further improve the

accuracy of GABA quantification by minimising the macromolecular (MM) contamination in the edited signal, the Henry method (14; 24), a T<sub>1</sub>-insensitive MM-eliminating approach, is favoured and incorporated into the MEGA-sLASER sequence used in this study. It eliminates the co-edited MM signal at 3.0 ppm by applying editing pulses at 1.5 ppm for OFF scans, which are symmetrically around the 1.7 ppm MM signal as compared to ones applied at 1.9 ppm for ON scans. To the best of our knowledge, no previous fMRS study has investigated the feasibility of using MM-eliminated MEGA-sLASER sequence to reveal the dynamic changes in GABA at 7 T.

GABAergic system is involved in modulation of many important physiological processes, such as motor control and pain (25–27), and dysfunction in the system has been implicated in pathological processes underlying many neurological and psychiatric conditions, such as autism, Tourette syndrome, and schizophrenia (28). One <sup>1</sup>H MRS study reported lowered resting state GABA concentration in the auditory cortex of autism patients (29). Inconsistent findings have been reported for the resting state GABA level measured using <sup>1</sup>H MRS in Tourette syndrome patients (30–32) and schizophrenia patients (33; 34) compared to healthy controls. Some argue that <sup>1</sup>H MRS-measured GABA is a better marker of GABAergic tone (i.e. levels of 'tonic' inhibition) rather than of inhibitory activity (i.e. levels of 'phasic' inhibition) (28; 35; 36). Recently, an fMRS study has reported weaker glutamatergic responses in the anterior cingulate cortex of schizophrenia patients to a colour-word Stroop Task (37). The dynamic measurement of GABA levels with fMRS may have the potential to provide unique information on the dynamic nature of GABAergic activities or abnormalities.

Therefore, this study aims to determine the dynamic changes in GABA levels in response to motor activation in healthy subjects using the enhanced sensitivity of a MM-eliminated MEGA-sLASER sequence at 7 T.

## **METHODS**

Sixteen healthy volunteers (age: 26±3 years, 8 males) participated in this study. Written informed consent was given by all subjects prior to MR measurements. This study was approved by the Medical School Ethical Committee of the University of Nottingham.

### **Stimulation paradigm**

During the fMRI and fMRS scans, the participant was asked to perform hand clenching task by following the instructions created using PsychoPy2 (38) and presented on the projector screen with grey background. Participants were instructed to clench both hands simultaneously at 1 Hz

when intermittently flashing green circle (visible for 0.7 s and invisible for 32 s) presented on the screen (task-period), and to relax their hands when stationary red circle appeared (rest-period). The functional paradigm for fMRI scan consisted of eight cycles of rest-period (32 s) followed by task-period (8 s). For the fMRS scans, the rest-period of 5 min was followed by a task-period of 10 min and then a rest-period of 5 min (Rest-Task-Rest). To guarantee the good understanding of the functional paradigm, a practice session outside the scanner was carried out beforehand. To reduce the noise and minimize motion, foam padding was placed between participants' heads and the head coil.

### **MR measurements**

All MR measurements were performed on a 7 T Philips Achieva scanner (Philips Medical Systems, Best, Netherland) equipped with a 32-channel receive head coil and a surrounding volume transmit (maximum  $B_1 = 15 \mu\text{T}$ ). The BOLD-fMRI  $T_2^*$ -weighted images were acquired using a single shot gradient echo EPI sequence (TE/TR = 25/2000 ms, matrix size =  $128 \times 128$ , FOV =  $208 \times 192 \text{ mm}^3$ , 30 slices, slice thickness = 3 mm, 160 dynamics). BOLD responses were analysed online to localise the volume with the optimal response in the left primary motor cortex for placement of the spectroscopic voxel in the functional MRS scans. High resolution 3D  $T_1$ -weighted MPRAGE images ( $1 \text{ mm}^3$  isotropic resolution, matrix size =  $256 \times 256 \times 180$ , TE/TR/TI = 3.5/7.3/987 ms, FA =  $8^\circ$ , shot interval = 2 s, SENSE factor = 2) were acquired for tissue segmentation and for checking the position of the spectroscopic voxel based on anatomical structure.  $^1\text{H}$  spectra were continuously acquired during the functional paradigm using the MM-eliminated MEGA-sLASER sequence (TR/TE = 5000/72 ms, voxel size =  $15 \times 20 \times 30 \text{ mm}^3$ , spectral bandwidth = 4000 Hz, samples = 4096, phase cycle step = 2, 240 scans, 20 min). VAPOR (variable pulse power and optimised relaxation delay) scheme (39) was used for water suppression, and  $B_0$  shimming of the voxel with second-order shim fields was performed automatically by the Philips pencil beam volume algorithm, similar to the FASTMAP method (40).

The MEGA-sLASER sequence used for GABA editing is shown in Figure 1. The sLASER localisation sequence consists of an asymmetric excitation pulse (BW of 4.7 kHz, 8.8 ms), and two pairs of the offset independent trapezoid (OIT) pulses (BW of 4.8 kHz, 5.2 ms). These adiabatic full passage (AFP) refocusing pulses with large bandwidth were used to reduce chemical shift displacement artefacts (CSDA) and improve editing efficiency. The MEGA editing pulse (SINC envelope with duration of 8.3 ms, maximum amplitude of  $2.5 \mu\text{T}$ ) with narrow band (full width at half maximum (FWHM) = 120 Hz, full width at 95% maximum (FW95%M) = 30 Hz)

was centred at 1.9 ppm and 1.5 ppm, respectively, for the ON and the OFF scan, in order to obtain GABA signals in the edited spectra (ON – OFF scans) with MM contamination eliminated. The ON and OFF scans were performed in an interleaved manner to minimise the impact of the phase and frequency instability on the edited spectra. The editing efficiency of this MEGA-sLASER was assessed based on the in vitro spectra (TR = 10 s, 16 scans) acquired from an isotropic volume of 27 ml in the centre of a sphere phantom with 200 mM GABA. To demonstrate the performance of MM-elimination, edited spectra were acquired from the same motor voxel ( $15 \times 20 \times 30 \text{ mm}^3$ ) in one subject using MEGA-sLASER (TR = 5 s, 60 scans, 5 min) with and without the MM-elimination scheme for comparison.

### **Data analysis**

The spectral editing data was processed using an in-house-developed routine built based on a MATLAB (The MathWorks Inc., Natick, MA, USA) data analysis toolbox (available at [https://github.com/chenkonturek/MRS\\_MRI\\_libs](https://github.com/chenkonturek/MRS_MRI_libs)), prior to metabolite quantification using LCModel (41). The raw signals from the multiple channels were combined based on an optimised weighting scheme (42) and phase corrected. Spectra were frequency aligned with the choline signal at 3.2 ppm, before the subtraction and the sum of the OFF scans from the ON scans, which results in the edited spectra and sum spectra, respectively. The motion-corrupted data were identified and discarded. For individual analysis, after performing a moving average with a window size of 40 scans (200 s) and step size of 20 scans (100 s), 11 data points for each individual subject were obtained for the metabolite quantification. For group analysis, after frequency alignment across subjects (number of subjects = 14), the group-mean spectra with the same temporal resolution were also obtained for the metabolite quantification. Data from two subjects were discarded for analysis, due to artefacts possibly induced by poor water suppression. Data from one additional subject was discarded for individual analysis, due to low SNR (mean SNR of tCr < 30), which substantially increased the quantification uncertainty (mean CRLB is > 40%) for GABA time course for that subject.

For quantification of GABA and Glx, the edited spectra were fitted in LCModel with a basis set of simulated spectra of five metabolites, including GABA, Glu, Gln, glutathione, and NAA. This basis set was simulated with jMRUI-NMRscope tool (43) based on the published values for chemical shift and J-coupling (12). The spectral range for LCModel analysis was set to 1.95 ppm - 4.0 ppm. To improve the baseline fitting consistency, the hidden LCModel control parameter DKNTMN, controlling the knot spacing for the spline baseline fitting, was set to 4,

since the edited spectrum had flat baseline. The total creatine (tCr, i.e. PCr + Cr) was chosen as an internal reference for quantification, due to its relatively high and stable concentration in the human brain (44). The tCr was quantified from the sum spectra using LCModel with a basis set of simulated spectra of five metabolites (Cr, PCr, choline (Cho), phosphocholine (PCho) and NAA) and default parameter settings. In addition, the GABA/tCr ratios quantified using this approach was also compared to ones determined using the AMARES algorithm (45) in the jMRUI software, where the 3.0 ppm GABA resonance in edited spectra and the 3.0 ppm tCr singlet in the sum spectra were simply fitted as single Lorentzian peaks (46).

The relative changes in GABA/tCr, Glx/tCr, NAA/tCr signal ratios with respect to the baseline (mean of the first two time points) were calculated for individual and group metabolite time courses. Statistical significance of metabolite changes from the baseline was inferred by two-tailed paired t-tests based on all individuals' metabolite time courses. For each time point in the metabolite group time course, Monte Carlo simulation was performed based on the corresponding quantification error, estimated by Cramér–Rao lower bounds (CRLBs), to statistically test the significance of the change relative to the baseline.

## **RESULTS**

Figure 2[A] shows the in vitro edited spectrum of GABA, resulting from the subtraction of the acquired OFF spectrum (J-modulation undisturbed) from the ON spectrum (3.0 ppm GABA pseudo-triplet is J-refocused). Based on these phantom data, the editing efficiency of the MEGA-sLASER sequence for GABA measurement was 0.48, determined as a peak area ratio of the edited and J-coupling refocused 3.0 ppm GABA signals per unit time. Figure 2[B] shows that the 3.0 ppm GABA signal with MM-contamination in the edited spectrum is about 1.6-fold larger than the one with MM-elimination, indicating that the undesired MM contribution (~38%) at 3.0 ppm was successfully corrected. The absence of residual signal from Cho resonance at 3.2 ppm indicated good singlet elimination and thus a reliable editing performance.

Regarding to the quality of the in vivo  $^1\text{H}$  spectra obtained in the fMRS experiments, the average linewidth of the tCr singlets in a sum spectrum (40 scans, 3.3 min) was measured to be  $9.9 \pm 0.6$  Hz, indicating good performance of shim. Figure 3[A] shows that the spectroscopic voxel for the 3.0 ppm GABA resonance, consisting of  $37 \pm 5\%$  gray matter (GM),  $56 \pm 5\%$  white matter (WM) and  $7 \pm 3\%$  cerebrospinal fluid (CSF), has been positioned in the motor cortex consistently across subjects. Figure 3[B] illustrates the good quality of the overall fit of an edited spectrum (40 scans, 3.3 min) from a representative subject based on LCModel analysis. Across all

individuals' time points (11 points × 13 subjects), GABA, Glx, and NAA were quantified from the edited spectra with mean CRLBs of 17%, 9% and 1%, respectively. The group-mean edited spectra allowed the quantification of GABA and Glx with CRLBs of 7% and 3%, respectively.

Figure 4 shows the time course of an individual's edited spectra and the group-mean spectra obtained during the functional paradigm, which were used for metabolite quantification. The clean baseline and well-resolved 3.0 ppm GABA signal in each edited spectrum (3.3 min) imply the consistent and highly efficient performance of the MEGA-sLASER editing sequence. The coefficient of variation (CV) of GABA/tCr measured at baseline (first two time points, 13 subjects) across subjects was 18% using LCModel analysis and 20% using AMARES algorithm, indicating good reproducibility of both methods. In addition, the GABA/tCr levels (11 time points, 13 subjects) quantified with these two approaches were highly correlated ( $P = 7e-16$ ), indicating the good consistency in quantification. The baseline GABA/tCr concentration ratio ( $[GABA]/[tCr]$ ) was calculated to be  $0.11 \pm 0.02$ , based on the 3.0 ppm GABA/tCr signal ratios obtained using AMARES approach and the correction of editing efficiency as described in Terpstra et al. (47). Assuming a creatine concentration of 6.7 mM, an estimate based on voxel tissue composition and its concentration in GM and WM (48), the average baseline, resting GABA concentration ( $[GABA]$ ) would be  $0.72 \pm 0.14$  mM and  $0.82 \pm 0.16$  mM, prior to and after the relaxation correction with  $T_1$  and  $T_2$  values at 7 T (49), respectively.

Figure 5[A] shows the mean of individual time courses of changes in metabolite levels with respect to the baseline. Based on the time points with statistically significant change ( $P < 0.05$ ) upon activation, an average decrease of  $12 \pm 5\%$  in GABA/tCr levels and an average increase of  $11 \pm 5\%$  in Glx/tCr levels from the baseline were found in the motor cortex during a 10-min hand-clenching task. In contrast, no statistically significant change was observed in NAA/tCr and tCr levels. Consistent with LCModel results, a significant decrease of  $13 \pm 7\%$  was found in GABA/tCr using AMARES approach. In addition, no statistically significant change in linewidth or peak height of tCr resonance at 3.0 ppm were found between activation and baseline (%change in linewidth  $\sim 0.4\%$ ,  $P = 0.7$ ; %change in height  $\sim 0.5\%$ ,  $P = 0.5$ ).

Figure 5[B] shows the time course of relative changes in metabolite levels quantified from the group-mean spectra (group analysis), which exhibits a similar behaviour as the averaged individual metabolite time courses. The GABA/tCr slowly decreased ( $-15 \pm 2\%$ ) to a stable level while Glx/tCr increased ( $8 \pm 2\%$ ) more rapidly during the activation, and then both recovered towards baseline after motor stimulation. This dynamic change in GABA levels is also reflected

in the time course of the edited GABA signals at 3.0 ppm in Figure 4[B], since there is no significant change in tCr signals from baseline.

## **DISCUSSION**

This study has shown the efficiency and consistent performance of the MM-eliminated MEGA-sLASER sequence implemented for selective detection of GABA at 7 T. With the benefits of using broadband slice-selective pulses and ultra-high field, the GABA editing efficiency of this developed MEGA-sLASER sequence ( $E_{\text{eff}} = 0.48$ ) is higher than the MEGA-PRESS sequence at 7 T ( $E_{\text{eff}} = 0.36$ ) (47) and MEGA-SPECIAL at 3T ( $E_{\text{eff}} = 0.45$ ) (46), although it is lower than the other group's MEGA-sLASER sequence ( $E_{\text{eff}} = 0.52$ ) used on a 7 T system with higher RF power capacity (20  $\mu$ T) (14). The high efficiency of this spectral editing sequence has allowed the use of a relatively small voxel size (9 ml) to increase the overlapping volume with the activated region in the motor cortex, as well as a reasonable time resolution (moving step of 100 s) for a functional study. The editing performance was shown to be quite consistent over the scan time of 20 mins, evidenced by the clean baseline and the stable NAA signals in the edited spectra, indicating relatively good frequency stability. Different from using dual-band pulses for MEGA-editing (14), the bandwidth and off-resonance frequency of the single-band MEGA-editing pulses in this MEGA-sLASER sequence can be adjusted flexibly to perform spectral editing for another type of metabolite, such as Lac and GSH, without hard-coding a new pulse into the system.

The MM contribution to the 3.0 ppm resonance in the MM-uncorrected edited spectrum was found to be  $\sim 38\%$  for a 9 ml voxel in motor cortex ( $N = 1$ , GM%  $\sim 34\%$ , CSF%  $\sim 5\%$ ) in this study, and to be  $\sim 55\%$  for a 22 ml voxel in occipital lobe ( $N = 5$ , GM%  $\sim 66\%$ , CSF%  $\sim 7\%$ ) with the same editing sequence (50). With differences in tissue composition, voxel size and position, sequence and field strengths, the MM contribution previously reported has ranged from 34% (51) and 46% (46) to 50% (14; 52) and 60% (53) for occipital lobe. MM level has been reported to be about 49% higher in GM than in WM (54). The [GABA]/[tCr] ratio measured from the primary motor cortex in this study ( $0.11 \pm 0.02$ ) is lower than the previously reported [GABA<sub>+</sub>]/[tCr] ratio of  $0.16 \pm 0.04$  (55) and  $0.20 \pm 0.06$  (56) obtained from sensorimotor cortex without MM-elimination. The GABA concentration ([GABA]) in the motor region determined by this study ( $0.72 \pm 0.14$  mM) was in agreement with Kalra et al. ( $0.72 \pm 0.11$  mM) (57), but was in general lower than  $1.2 \pm 0.1$  mM (53) and  $1.1 \pm 0.1$  mM (49; 51) measured from the occipital regions. This is possibly resulted from the variation of [GABA] in different tissue types and brain regions. Previous  $^1\text{H}$  MRS

studies have reported higher [GABA] in GM than in WM from sensorimotor regions and frontal lobes (55; 58).

Among different metabolite quantification tools, such as jMRUI, LCModel, TARQUIN (59) and Gannet (60), LCModel has previously shown to provide the best reproducibility for GABA quantification with MEGA-PRESS data (20; 21). This study has shown that the GABA levels quantified using jMRUI and LCModel were highly correlated, while LCModel offers slightly lower inter-subject CV%. However, the benefits of using jMRUI (AMARES algorithm) are the transparency and simplicity. In addition, several previous short TE fMRS studies have observed a BOLD effect on the linewidth and thus the peak height of tCr of 3% (61), 2% (4; 8), and more recently 0.5% (7) during the activation period, whereas the BOLD-related change found in this long TE fMRS study was small and statistically insignificant (~ 0.5% for tCr peak height), despite of good shimming.

This study has shown the feasibility of the detection of dynamic changes in GABA/tCr during a functional paradigm using a spectral editing sequence with high efficiency and sensitivity at 7 T. A significant decrease of  $12\pm 5\%$  in GABA/tCr from the baseline was found during the 10 min task-period from the resting baseline level. In most previous fMRS studies, which use the conventional short TE direct detection methods, no significant alternation in GABA levels have been reported upon visual or motor activation (1–6). One study found a reduction in the steady-state GABA concentration of 5% in response to visual stimulation when compared to a control voxel (7). In addition, despite that the sequence used in this study was not optimal for the detection of Glu and Gln, an activation-induced increase in Glx/tCr ( $11\pm 5\%$ ) was observed, which is higher but consistent with those previously reported for Glu/tCr ( $5\pm 1\%$  (6),  $4\pm 1\%$  (61),  $3\pm 1\%$  (5),  $2\pm 1\%$  (8)). [Stephen/Hilmar: add a bit more about the possible interpretation] The observed xxx can be interpreted xxx

Along with the advantage of providing well-resolved GABA signals, which allows the direct visual inspection of dynamic changes in GABA levels, there are also a few drawbacks associated with spectral editing methods for fMRS studies. Firstly, the number of metabolites that can be simultaneously edited is limited. Recently, Saleh et al. has demonstrated the possibility to co-editing multiple metabolites by using the Hadamard Encoding and Reconstruction of MEGA-Edited Spectroscopy (HERMES) (62). Secondly, the unavoidable penalty on SNR, due to the use of two acquisitions and a long TE to achieve spectral editing, would limit the temporal resolution or voxel size that can be achieved. Lastly, the subtraction

scheme involved in spectral editing makes it susceptible to motion-induced artefacts. To reduce the number of spectra to be discarded due to motion-corruption, the ON and OFF scans were implemented in an interleaved manner and small but sufficient steps of phase cycling were used.

### **CONCLUSION**

This study has demonstrated that it is possible to investigate the activation-induced changes in cerebral GABA levels dynamically, with the high sensitivity of GABA detection using the MEGA-sLASER sequence at 7 T. This approach is promising for the investigation of the abnormalities in GABA dynamic changes in future, in order to gain better understanding of the underlying pathology of disorders or disease, which may be associated with the abnormalities of GABAergic system.

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**FIGURE LEGENDS**

**Figure 1.**

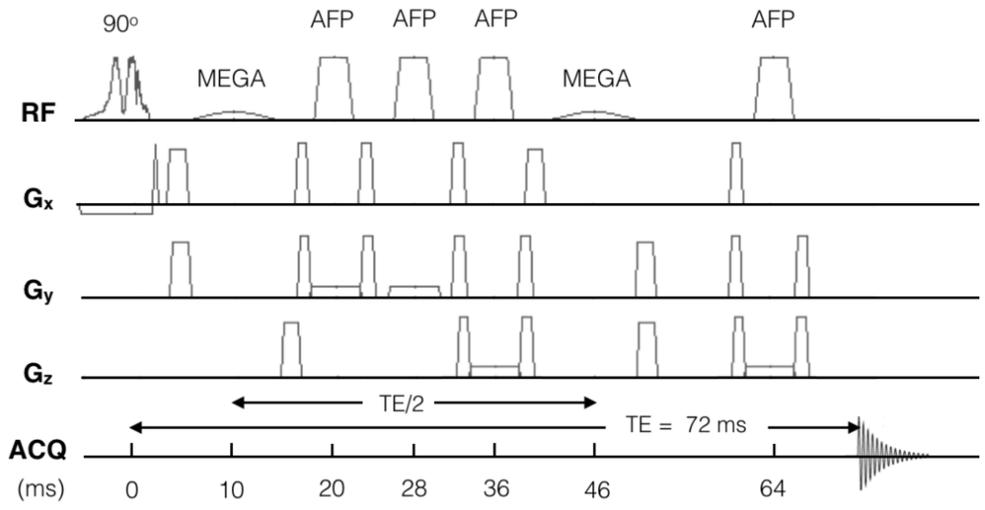


Figure 2.

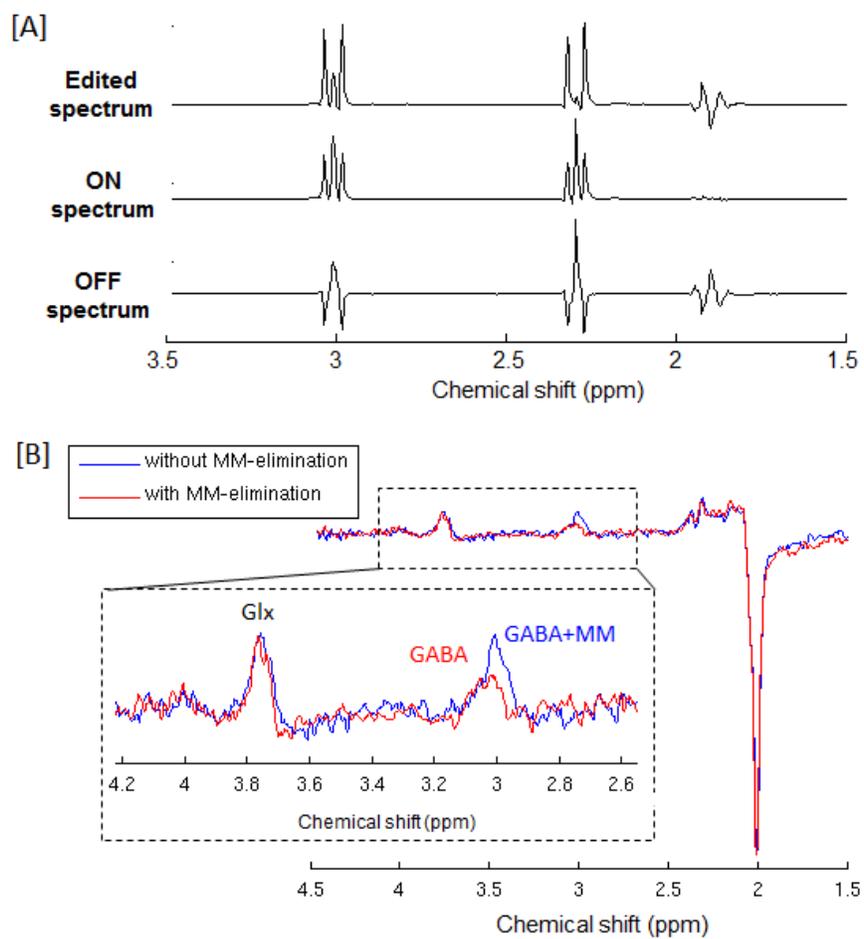
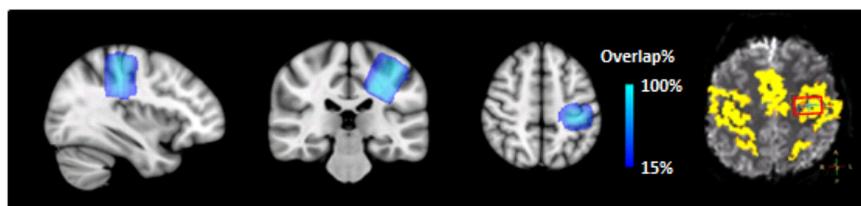


Figure 3.

[A]



[B]

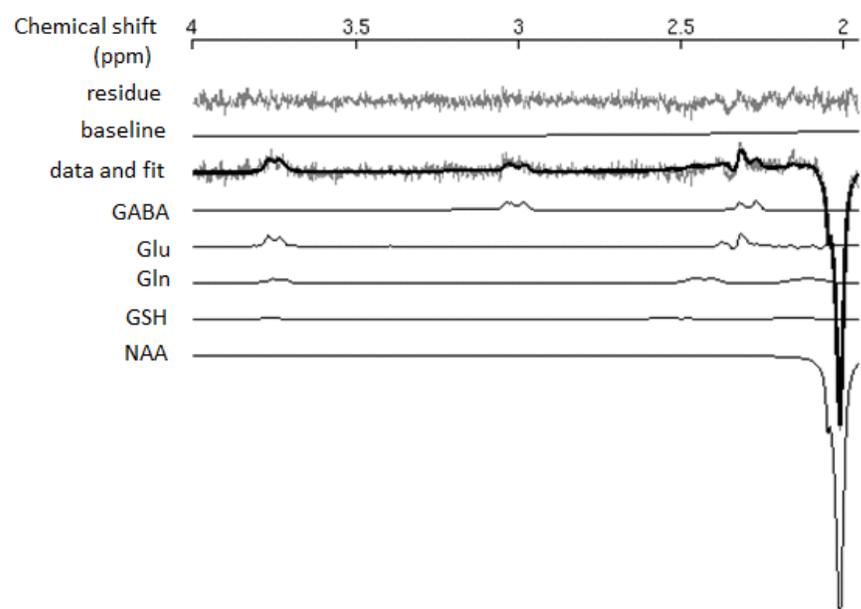


Figure 4.

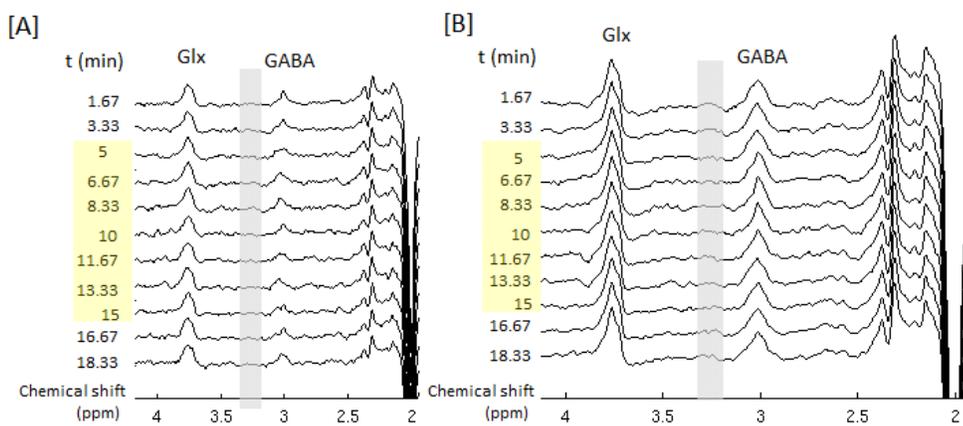


Figure 5.

