

## **In vivo $\gamma$ -amino-butyric acid measurement in rats with spectral editing at 4.7T**

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**Running title:** MEGA-PRESS in impulsive rats

## Abstract

### *Purpose*

To evaluate the feasibility of spectral editing for quantification of  $\gamma$ -amino butyric acid (GABA) in the rat brain and to determine whether altered GABA concentration in the ventral striatum is a neural endophenotype associated with trait-like impulsive behaviour.

### *Materials and methods*

Spectra were acquired at 4.7T for twenty-three male Lister-hooded rats that had been previously screened for extremely low and high impulsivity phenotypes on an automated behavioural task ( $n=11$  low-impulsive;  $n=12$  high-impulsive). Voxels of  $3 \times 7 \times 4 \text{ mm}^3$  ( $84 \mu\text{l}$ ) centred bilaterally across the ventral striatum were used to evaluate GABA concentration ratios.

### *Results*

Quantifiable GABA signals in the ventral striatum were obtained for all rats. Mean-edited GABA to n-acetyl aspartate (NAA) ratios in the ventral striatum were 0.22 (95% CI [0.18, 0.25]). Mean GABA/NAA ratios in this region were significantly decreased by 28% in high-impulsive rats compared to low-impulsive rats ( $p = 0.02$ ; 95% CI [-53%, -2%]).

### *Conclusion*

These findings demonstrate that spectral editing at 4.7T is a feasible method to assess *in vivo* GABA concentrations in the rat brain. Results show that diminished GABA content in the ventral striatum may be a neural endophenotype associated with impulsivity. .

**Keywords:** Spectroscopy; behaviour; nucleus accumbens; striatum, GABA



## INTRODUCTION

Gamma-amino-butyric acid (GABA) is a key amino-acid in the brain mediating fast inhibitory neurotransmission (1). Perturbation in GABA-ergic transmission has been widely implicated in epilepsy and other brain disorders (2-4). However, the quantification of GABA by non-invasive MR spectroscopy (MRS) is hampered by its low concentration in the brain and overlapping signals from other metabolites, notably creatine at 3.0ppm with an approximately ten-fold greater abundance in brain tissue (5).

Efforts to measure GABA *in vivo* have included short echo time sequences, two-dimensional *J*-resolved spectroscopy and spectral editing sequences, the most widely used of which is Mescher-Garwood point-resolved spectroscopy, or MEGA-PRESS (5,6). The  $^1\text{H}$  spectrum of GABA ( $\text{H}_3\text{N}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{COO}^-$ ) has three multiplets at approximately 1.9ppm, 2.3ppm and 3.0ppm, which overlap with n-acetylaspartate (NAA), glutamine/glutamate and creatine, respectively. In MEGA-PRESS, selective saturation of the GABA methylene protons at 1.9ppm affects the coupled GABA spins at 3ppm, while the uncoupled creatine resonance at 3ppm is not affected by editing. Thus, subtracting the unedited from the edited spectrum yields a GABA resonance without overlapping creatine signal. For details of MEGA-PRESS acquisition see (6-8).

The present study investigated the feasibility and utility of MEGA-PRESS at 4.7T for resolving regional GABA content in the rat brain.

## MATERIALS AND METHODS

### *Behavioural screening*

Male Lister-hooded rats ( $n = 96$ ) were screened for impulsivity on the five choice serial reaction time task (5-CSRTT), following (9). Briefly, rats were trained in an operant chamber

(Med Associates Ltd. USA) to detect the location of brief (700 ms), spatially-unpredictable, visual stimuli presented on a discrete trial basis in one of five open recesses (10). If the rat selected the correctly illuminated recess, a 45mg food pellet was delivered to a magazine in the chamber. If the rat did not respond or selected an incorrect opening the chamber lights were switched off for 5 seconds and no food pellet was dispensed. A premature response (i.e. a response made before the onset of the imperative signal to respond) triggered the same outcome as an incorrect response with no food pellet delivered on that trial. Once rats had acquired the task over approximately 4-months of training, they were screened for impulsivity over a three-week period. Rats underwent 100 trials in the apparatus on days 1, 2, 4 and 5 with an inter-trial interval (the interval marking the start of the trial and onset of the light stimulus) of 5s. On day 3 the inter-trial interval was extended to 7s as a deliberate challenge to increase the number of premature responses (11). Mean rates of premature responses over the 3 challenge sessions were used to define rats as either highly impulsive (a mean number of premature responses  $\geq 50$ ) or weakly impulsive (average premature responses  $\leq 30$ ). The twelve rats with the highest rates of premature responses and twelve rats with the lowest rates of premature responses were selected as “high impulsivity” and “low impulsivity” groups, respectively.

All experimental procedures were reviewed and approved by a local ethical review committee and were conducted in compliance with appropriate regulatory requirements.

#### *MEGA-PRESS acquisition*

A series of *in vitro* samples containing GABA (Sigma Aldrich) in distilled water (0.5, 1.0, 2.0, 5.0 and 10mM) were scanned to confirm that our implementation of the sequence was correct and gave values corresponding to known concentrations.

Rats were anaesthetised with isoflurane (1.5-2.5% in 1l/min medical O<sub>2</sub>). For physiological monitoring, respiration rates were measured with a pressure sensor, body temperature was measured with a rectal probe and oxygen saturation with pulse oximetry (SA Instruments, Inc., New York, USA). Anaesthetic dose rates were adjusted to keep respiration rates within an appropriate physiological range and a flowing water heating pad was used to maintain body temperature throughout the scan.

A Bruker BioSpec 47/40 4.7T system with ParaVision 5.1 (Bruker Inc., Germany) was used with a 72mm birdcage transmit coil (Bruker model T2000) and manufacturer-provided four-channel receive array for reception (Bruker model T13014). For placement of the voxel, localizer and rapid acquisition with relaxation enhancement images were acquired (repetition time 15.3s, effective echo time 36ms, echo train length 8, field of view 64×64mm<sup>2</sup>, matrix 256×256 for 250µm planar resolution with 250µm slice thickness in 6 minutes 7 seconds).

MEGA pulses were applied at offsets of 1.9ppm and 7.5ppm on alternate acquisitions, based on the MEGA-PRESS sequence with TE<sub>1</sub>=12ms and TE<sub>2</sub>=56ms. Gaussian pulses of 20ms duration were used. Spoiler gradients of 370mT/m were applied for 1ms in the scheme proposed by (6). Water suppression was manually adjusted for each subject using variable power and optimised relaxation delays (VAPOR) (12). The manufacturer-provided map shim method was used to adjust shim currents calculated from a measured field map.

The repetition time was 2s and echo time was 68ms. For acquisition, 2,048 points were acquired in 511ms (bandwidth 4kHz corresponding to 20ppm). A total of 256 transients were obtained (128 'on' and 128 'off') for an acquisition time of 8 minutes 32 seconds per animal. Water reference scans were acquired in a single shot for reference prior to each acquisition to calibrate the phase of the array coil elements.

### *Spectral processing and analysis*

Raw data were processed in Matlab 2011b (Mathworks, Cambridge, MA, USA). A circular shift of 68 points was applied to the raw data to compensate for the digitisation group delay and data were zero-filled to 8,192 points with line-broadening of 3 Hz. To correct frequency and phase drift between transients and identify corruption by motion or physiological disturbance, the creatine peak from each transient was fitted and the parameters used to correct or reject entirely the transient. This was done by first calculating mean 'on' and 'off' spectra to obtain the mean values of frequency and linewidth for the creatine peak at 3.0ppm. These values were then used to initialise linear least squares fitting for the same parameters in each transient separately. Transients were excluded if the frequency of the peak fitted at 3.0ppm differed by more than four standard deviations of the median value, or where the full width half maximum or phase differed by more than two standard deviations of their median values, respectively.

The fitted Lorentzian parameters were used to adjust the frequency and phase of each transient before averaging to produce mean 'on' and 'off' spectra. To obtain GABA/NAA ratios, the NAA peak at 2.0ppm was fitted to a Lorentzian function and the GABA peak at 3.0ppm was fitted to a single Gaussian function, following (13).

### *Statistical analysis*

The fitted Lorentzian peak areas were compared between HI and LI animals using Student's *t*-test. One-sided tests were used to compare the null hypothesis with the a priori hypothesis that HI rats would show reduced GABA ratios compared to LI rats with  $p < 0.05$  used as the threshold for statistical significance.

## **RESULTS**

*In vitro* results are shown in **Error! Reference source not found.**. Good agreement was observed between prepared GABA standards and the signal measured ( $R^2 = 0.998$ ) and the spectra ‘on’, ‘off’, and edited difference spectra have the expected form. Illustrative voxel placement is shown in Figure 2, with *in vivo* acquisition spectra shown in Figure 3. For clearer presentation, line broadening of 7.5 Hz has been applied to the edited spectrum and is shown with an expanded scale. One LI rat did not recover from anaesthesia. As a precaution, data from that subject were not processed or considered further in the analysis.

Good shimming was achieved for all rats with unsuppressed water linewidths below 10 Hz (the mean  $\pm$  standard deviation across all measured spectra here was  $7.5 \pm 1.1$  Hz, full width half maximum). On average, 13 transients were discarded from each series (standard deviation 2.1) due to frequency drifts, excessive linewidth or phase shifts. Typical frequency drift was 3 Hz (0.015ppm). Figure 4 shows typical single transients that were accepted (A), rejected (B) and the mean MEGAPRESS ‘off’ spectrum for comparison (C).

Figure 5A shows the screening data used to segregate LI and HI rats according to the number of premature responses elicited during the three challenge sessions involving an extended waiting interval between the start of the trial and subsequent presentation of the light stimulus. It can be seen that HI rats made consistently more premature responses than LI rats across each of the three challenge days. Group-averaged concentration ratios for LI and HI rats are shown in Figure 5B, with HI rats exhibiting a 28% lower GABA/NAA ratio compared to LI rats ( $p = 0.02$ ; 95% CI [-53%, -2%]). Across all rats, the mean GABA to NAA ratio was 0.22 (95% CI [0.18, 0.25]).

To demonstrate that our findings were not confounded by variation in NAA concentration, we also calculated GABA/Cho and GABA/Cr ratios, which revealed the same significant



contrast between HI and LI rats, specifically a 23% lower GABA/Cho ratio in HI rats ( $p = 0.03$ ; 95% CI [-47%, 0%]) and a 26% lower GABA/Cr ratio ( $p = 0.03$ ; 95% CI [-52%, 0%]).

## DISCUSSION

MEGA-PRESS spectra obtained were of high quality from each rat showing the feasibility of the method in rats at 4.7T.

We chose NAA to normalise the GABA signal for the least error, as it has the strongest signal in the water-suppressed spectra. NAA is usually considered a neuronal marker, and as such is a better normalising metabolite than water in this case due to the likelihood of substantial variability in the fraction of ventricle present in each voxel. This is particularly important for this study due to the impact of variation in ventricle size and anatomy between rats with close proximity to the large voxel used. Small anatomical variations or errors in voxel placement could substantially increase the water signal leading to underestimation of metabolite concentrations. Furthermore, the NAA signal is derived from the MEGAPRESS data and not from a separate water reference scan so may be less susceptible to drift or motion occurring over time. To guard against misinterpretation due to NAA changes themselves, we calculated ratios based on creatine and choline and found no substantial difference in the results.

A criticism of the MEGA-PRESS technique is the possibility of co-edited macromolecule signals that can have a substantial contribution to the measured GABA signal (13), arising from spins at 3.0ppm, which are coupled at 1.7ppm and overlapping with the measured GABA peak. It has been suggested that this can be addressed by metabolite-nulled spectra (e.g. with an inversion time of 1s before acquisition) (14,15) but that was not attempted here. There is some evidence that metabolite-nulling reduces the reliability of GABA measurements as it introduces sensitivity to motion and instabilities as the nulled baseline is not acquired in an interleaved fashion with the MEGAPRESS spectra (16). Despite this, we

cannot be certain that changes in macromolecule concentrations do not contribute to the differences observed here. As GABA is produced from glutamate by glutamate decarboxylase (GAD<sub>65/67</sub>), and this has been shown to be decreased in the ventral striatum of HI rats (9), it is plausible that the lower signals in HI rats were due to lower levels of endogenous GABA.

The measures of GABA concentration in rat brain found here compare well with other MRS measurements made using  $^{13}\text{C}$  doping ( $\sim 2.0\text{mM}$  in (17) at 11.7T),  $^1\text{H}/^{31}\text{P}$  ( $1.9\pm 0.4\text{mM}$  in (14) at 8.4T). From studies primarily of rat cortex, values measured by selective homonuclear polarization transfer in (18) at 11.7T are closer to the values found in human brain,  $1.2\pm 0.1\text{mM}$ , with an *ex vivo*  $^{13}\text{C}$  value of  $1.26\text{mM}$  found by (19). The slightly larger values implied by greater concentration ratios found in the present study may be a reflection of the larger proportion ( $\sim 95\%$ ) of GABAergic neurons of the rodent striatum (20) or contamination by co-edited macromolecules.

Negative associations of GABA concentrations measured by MEGAPRESS have been shown cortically in human studies of impulsivity (21), (see (22) for a more comprehensive review of  $^1\text{H}$ -MRS findings in neuropsychiatric conditions). These common findings reinforce the value of the translational methodology used here.

The limitations of this study are the ambiguity of GABA signals with co-edited macromolecules and the relatively large voxel size compared to the anatomy of the small structures known to be involved in the neurobiology of impulsivity. Furthermore, as concentration ratios rather than absolute GABA quantification was used it is possible that increases in other metabolites could explain the smaller ratios obtained in highly impulsive rats. Although a significant reduction in GABA ratios was shown in highly impulsive animals, there is still some overlap in the values measured in weakly impulsive animals,

indicating that the GABA deficits alone are not a sufficient determinant of impulsive behaviour.

In conclusion, we have demonstrated that MEGA-PRESS in rats is achievable in a reasonable time giving high-quality spectra and further that GABA concentration ratios are significantly reduced in the ventral striatum of highly impulsive rats compared with weakly impulsive rats. It is possible that these findings can be used as the basis of a translatable biomarker to investigate the brain mechanisms of attention deficit/hyperactivity disorder, addiction, and other disorders of impulse control in humans.



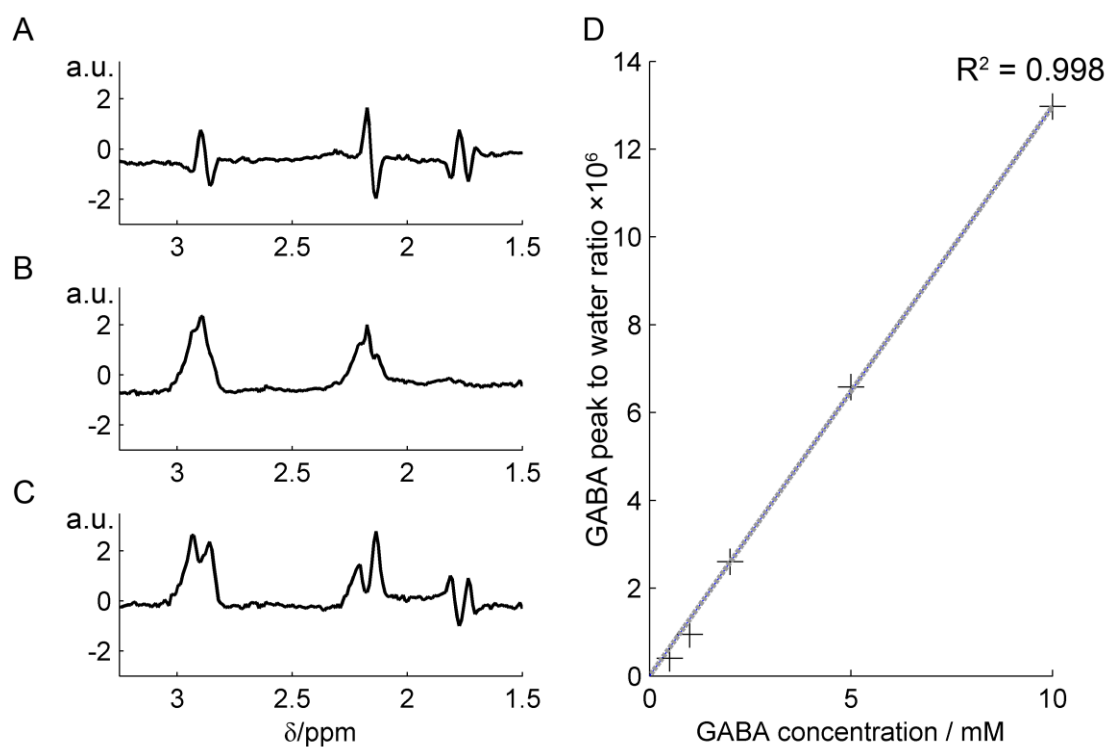
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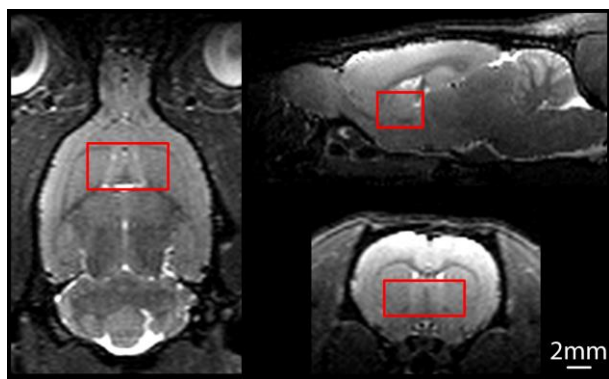
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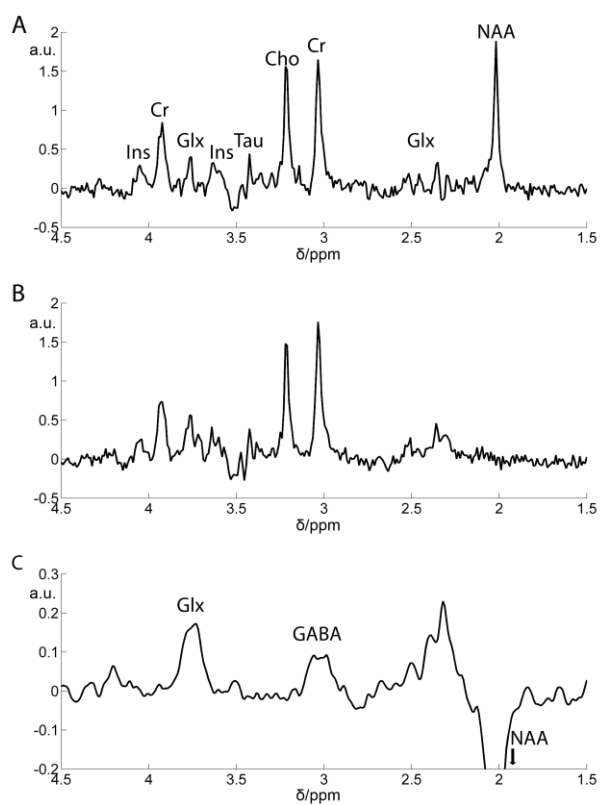
**FIGURE LEGENDS**

**Figure 1.** Typical GABA solution spectra for MEGA-PRESS 'off' (A), MEGA-PRESS 'on' (B), and difference spectrum (C). Plot in (D) shows the ratio of the GABA peak area at 3ppm to the unsuppressed water peak against prepared concentration showing good agreement.

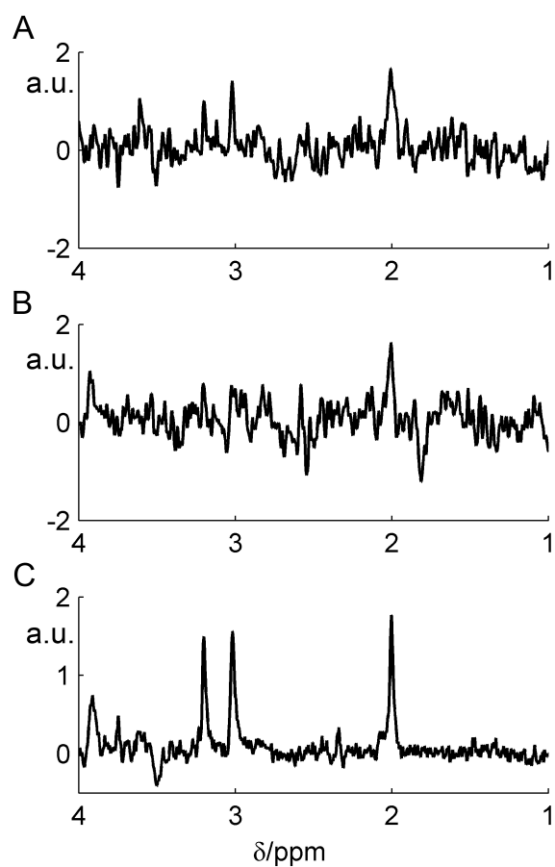




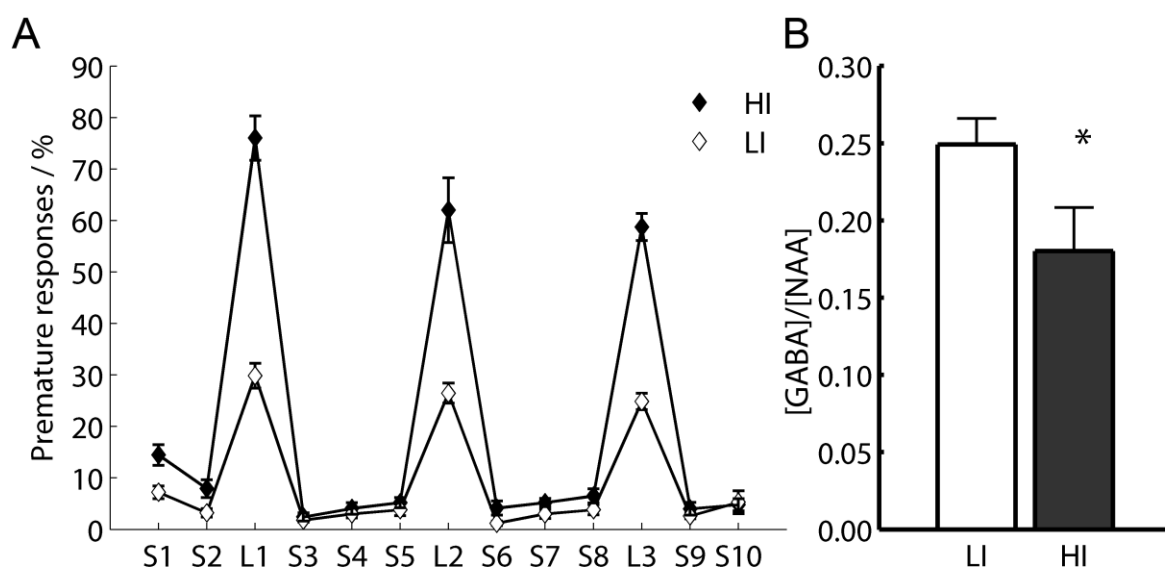
**Figure 2.** Typical voxel placement for the MEGA-PRESS acquisition showing horizontal, sagittal and coronal sections, clockwise from left.



**Figure 3.** Typical spectra seen for MEGA-PRESS ‘off’ (A), MEGA-PRESS ‘on’ (B) and the edited spectrum (C). Note the expanded scale in (C). Line broadening of 7.5Hz has been applied in (C) for clarity. Major peaks in (A) are labelled as Ins (inositol), glutamine/glutamate (Glx), taurine (Tau), choline (Cho), creatine and phosphocreatine (Cr).



**Figure 4.** Illustrative accepted transient (A), rejected transient (B), and the mean spectrum form a typical subject for comparison (MEGAPRESS ‘off’).



**Figure 5.** Impulsivity scores measured in rats (A) showing stability of high premature responding rates over successive long inter-trial interval challenges (L1, L2, and L3) against successive baseline measures (S1, S2, ..., S10). (B) shows GABA to NAA ratios for low-impulsive (LI) and high-impulsive (HI) rats. Asterisk indicates significance at  $p < 0.05$  and error bars show 1SEM.