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RESEARCH ARTICLE

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Considerations for event-related gamma-aminobutyric acid functional magnetic resonance spectroscopy

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Abstract

The use of sequential proton magnetic resonance spectroscopy (MRS) to follow glutamate and gamma-aminobutyric acid (GABA) changes during functional task-based paradigms, functional MRS (fMRS), has increased. This technique has been used to investigate GABA dynamics during both sensory and behavioural tasks, usually with long 'block design' paradigms. Recently, there has been an increase in interest in the use of short stimuli and 'event-related' tasks. While changes in glutamate can be readily followed by collecting multiple individual transients (or shots), measurement of GABA, especially at 3 T, is usually performed using editing techniques like Mescher–Garwood point-resolved spectroscopy (MEGA-PRESS), which by its nature is a dual shot approach. This poses problems when considering an event-related experiment, where it is unclear when GABA may change, or how this may affect the individual subspectra of the MEGA-PRESS acquisition. To address this issue, MEGA-PRESS data were simulated to reflect the effect of a transient change in GABA concentration due to a short event-related stimulus. The change in GABA was simulated for both the ON and OFF subspectra, and the effect of three different conditions (increase only during ON acquisition, increase during OFF acquisition and increase across both) on the corresponding edited GABA spectrum was modelled. Results show that a transient increase in GABA that only occurs during the ON subspectral acquisition, while not changing the results much from when GABA is changed across both conditions, will give a much larger change in the edited GABA spectrum than a transient increase that occurs only during the OFF subspectral acquisition. These results suggest that researchers should think carefully about the design of any event-related fMRS studies using MEGA-PRESS, as well as the analysis of other functional paradigms where transient changes in GABA may be expected. Experimental design considerations are therefore discussed, and suggestions are made.

KEYWORDS
brain function, functional MRS, GABA, magnetic resonance spectroscopy, MEGA-PRESS, neurotransmitters

Abbreviations used: BOLD, Blood oxygen level dependent; fMRS, functional magnetic resonance spectroscopy; GABA, gamma-aminobutyric acid; Glu, glutamate; Glx, glutamate + glutamine; MEGA-PRESS, Mescher–Garwood point-resolved spectroscopy; ppm, parts per million (chemical shift); PRESS, point-resolved spectroscopy; STEAM, stimulated echo acquisition method; sLASER, semiadiabatic localization by adiabatic selective refocusing; TE, echo time; TR, Repetition time.

1 | INTRODUCTION

There has been an increased interest in the application of functional magnetic resonance spectroscopy (fMRS) to study brain metabolism. fMRS works through the sequential acquisition of magnetic resonance spectroscopy (MRS) during a 'task' driving brain activation. The fMRS data are either collected as sequential blocks of a set number of MRS shots (or transients) that relates to a given task/stimulus block, or in the case of event-related fMRS, as single shots (or transients). Data are then 'binned' in some fashion across the time series, either by stimulus block, or as time periods after stimulus/task onset. Recently, 2D fitting approaches have also become available that model both the expected time series and the spectral fit simultaneously.[1,2] Alterations in the neurotransmitters glutamate and gamma-aminobutyric acid (GABA) have been observed using fMRS techniques with blocked paradigms.[3–13]

Recently, event-related dynamics of glutamate have also been investigated using fMRS, and it has been possible to observe rapid short-lived increases in glutamate as fast as 200 ms after stimulus onset.[9,14–16] While this is still an active field, with researchers still interested in glutamate dynamics, researchers are also wanting to investigate inhibitory processes through GABA dynamics using similar event-related techniques.[6,17,18]

MRS measures of GABA at 3 T are usually performed using edited sequences like Mescher–Garwood point-resolved spectroscopy (MEGA-PRESS).[19,20] MEGA-PRESS is a dual shot technique, whereby one transient/spectrum is acquired with a frequency selective editing pulse applied to the GABA moieties at 1.9 parts per million (ppm) (the EDIT ON transient), and the other transient/spectrum is acquired with the frequency selective pulse applied further upfield, in a region of the spectral range without any coupled moieties (the EDIT OFF transient). The 1.9-ppm selective pulse of the EDIT ON transient has the effect of refocusing J-evolution of the GABA peaks at 3 ppm, such that all the peaks of this triplet are pointing up, in contrast to the OFF transient, when there is no refocusing of J-evolution and the two outer peaks of the triplet are now 180° out of phase with the central peak (at the typical echo time [TE] used in a MEGA-PRESS acquisition of 68–80 ms) (Figure 1). Subtraction of the OFF from the ON spectrum then leads to the central peaks cancelling out, and the two side peaks, producing a pseudo doublet. When this subtraction is applied to in vivo MEGA-PRESS data, peaks from other metabolites that are not affected by the selective excitation pulse are 'edited' out by this subtraction, leaving a much simplified spectrum[19–21] with the GABA peak now visible and able to be fit reliably.[22–24] This dual shot nature of the MEGA-PRESS sequence, and in particular the fact that it involves a subtraction of one of the subspectra, means some thought is required before it can be readily applied to event-related fMRS paradigms.

Of particular interest is what effect a neurological event that causes a short-lived increase (or decrease) in a metabolite, for example GABA, will have on the individual MRS transients. That is, if that increase (or decrease) happened either during the ON acquisition, the OFF acquisition, or lasted across both. From mathematical principles it should be obvious that a change that happens only during the EDIT OFF condition will be reflected differently in the resulting difference spectrum, than one that happens in the EDIT ON condition, as the EDIT OFF subspectra is subtracted from the EDIT ON, such that an increase in signal during the EDIT OFF, with no change in the EDIT ON, would lead to a decrease in the resulting difference spectrum. Basically, $A - B > A - (B + C)$, if C is positive. The opposite would occur for a decrease during the OFF spectrum. This effect can be demonstrated using simulated spectral acquisitions of both the ON and OFF transients. Whereby we can test what the effect of a 10% or 20% increase, or decrease, in the neurotransmitters GABA and glutamate (Glu) during either (or both) transients would be on the resulting 'edited' subtraction spectrum, by performing subtractions for each of three conditions: (i) increases occur during both the EDIT ON and EDIT OFF acquisition; (ii) increases only occur during the EDIT ON acquisition; and (iii) increases only occur during the EDIT OFF acquisition.

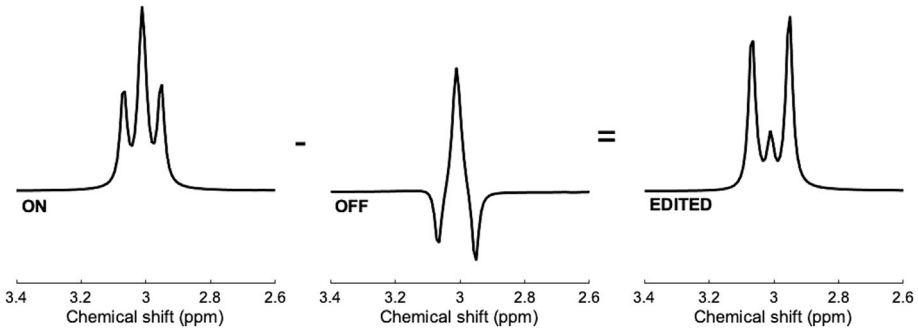

FIGURE 1 Simulated GABA spectrum in the 2.6–3.4 ppm range for the different subspectra (ON and OFF) of the MEGA-PRESS editing sequence, and the edited (subtraction) spectrum. GABA, gamma-aminobutyric acid; MEGA-PRESS, Mescher–Garwood point-resolved spectroscopy;.

2 | METHODS

Simulated spectra were created using the FID-A toolbox[25] in Matlab,[26] and the 'run_simMegaPressShaped_fast' function, creating a typical MEGA-PRESS spectrum for GABA and glutamate, including simulated subspectra for the EDIT ON and EDIT OFF acquisition. The simulation is based on the Siemens implementation of MEGA-PRESS, and simulates the spectrum at 3 T using shaped pulses from a 30 × 30 × 30 mm^3 voxel, accounting for chemical shift effects, by splitting the voxel into an 8 × 8 grid, and simulating each position within that grid, with the EDIT ON frequency set to 1.9 ppm, and EDIT OFF set to 7.5 ppm, with 2048 points across a spectral width of 2000 Hz. MEGA-PRESS simulations were run for both the optimal TE of 68 ms, and the equally common TE of 80 ms (which is often used to allow macromolecular suppression at the same time). Coupling constants used for GABA are those reported by Near et al. in 2013.[27] Then a vector was produced from the Fourier transform of the real part of the spectrum, which had been smoothed by a 12-Hz Gaussian kernel to more closely approximate the line shape experienced in vivo. Each subspectrum was then multiplied by either 1.1 or 1.2 for the 10% and 20% increases for each metabolite, respectively.

Subtraction spectra where then created for both [GABA] and [Glu] at baseline, for a 10%, and a 20% increase from baseline, for three different scenarios:

1. An increase in [GABA] or [Glu] that occurs/persists across both the ON and OFF acquisition;
2. An increase in [GABA] or [Glu] that only occurs/persists during the ON acquisition;
3. An increase in [GABA] or [Glu] that only occurs/persists during the OFF acquisition.

Each resulting edited spectrum was then visually inspected as well as integrated (across the 2.5–3.5 ppm range for GABA and across the 3–4.5 ppm range for Glu) to determine the effect of each proposed scenario on the main peak used for the fitting of each metabolite in the difference spectrum of a typical in vivo MEGA-PRESS experiment.

The simulated data produced, and some of the the Matlab code used to produce it, and the figures for this manuscript, are available at https://osf.io/kyjcf/ and through MRShub.org.

3 | RESULTS

Simulated ON and OFF subspectra for MEGA PRESS acquisition with a TE of 68 ms, with 12-Hz linewidth, and the effect of modelled increases in [GABA], can be seen in Figure 2, with a baseline spectrum in black, and a modelled 10% (dashed) and 20% (dot-dashed) increase in [GABA] overlayed on top.

From a visual inspection of Figure 2, the largest change in both the EDIT ON and EDIT ON spectrum occurs at the middle section around the 3.0-ppm portion of both subspectra. (See supplementary figures S1, S2, showing the impact of linewidth on the appearence of this effect.)

The resulting 3-ppm pseudo-doublet in the MEGA-PRESS difference spectrum (at a TE of 68 ms) for each of the three proposed scenarios for an increase/decrease in [GABA] from an event-related stimulus paradigm, are shown in Figure 3.

From visual inspection it can be seen that change (increase or decrease) in [GABA] that occurs during the ON acquisition alone, or during both the ON and OFF acquisitions, will give rise to an change (increase or decrease) in the area of the GABA peak in the subtraction spectrum (due

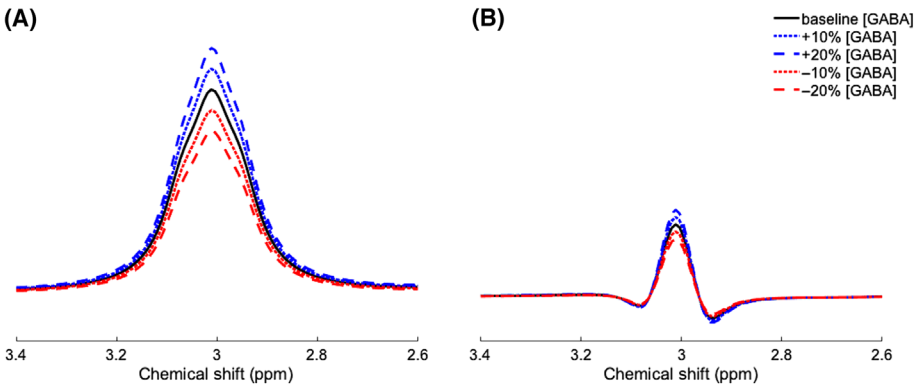

FIGURE 2 Simulated (A) EDIT ON, and (B) EDIT OFF subspectra for MEGA-PRESS (TE = 68 ms, 12-Hz linewidth) for differing values of [GABA] (baseline, solid line; increased by 10%, blue dotted line; decreased by 10%, red dotted line; increased by 20%, blue dashed line; decreased by 20%, red dashed line). Both sets of subspectra are displayed at the same scale, and have been line-broadened by 12 Hz. GABA, gamma-aminobutyric acid; MEGA-PRESS, Mescher–Garwood point-resolved spectroscopy; TE, echo time.

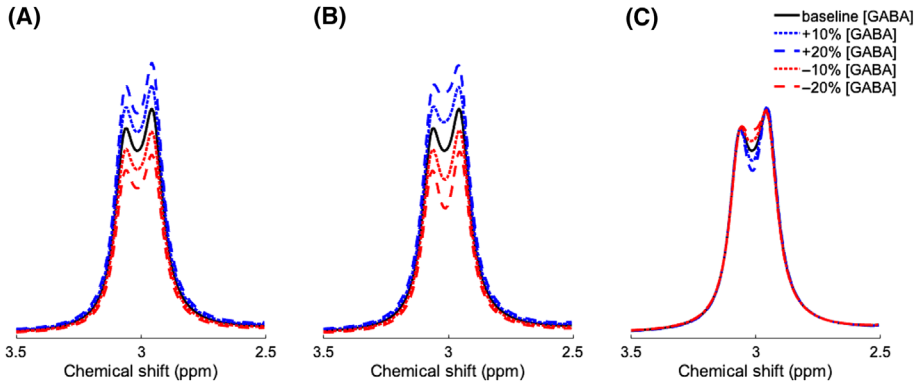

FIGURE 3 Simulated MEGA-PRESS (TE 68 ms, linewidth 12 Hz) edited spectra for [GABA] at baseline (black), a 10%, and a 20% change (increase [blue] or decrease [red]) from baseline for three different scenarios: (A) A change in [GABA] that occurs/persists across both the EDIT ON and EDIT OFF acquisition; (B) A change in [GABA] that only occurs/persists during the EDIT ON acquisition; (C) A change in [GABA] that only occurs/persists during the EDIT OFF acquisition. GABA, gamma-aminobutyric acid; MEGA-PRESS, Mescher–Garwood point-resolved spectroscopy; TE, echo time.

TABLE 1 Integration results, normalised to baseline, for a simulated MEGA-PRESS–edited GABA spectrum (TE 68 ms) for three different conditions in an event-related fMRS paradigm: (A) A change in [GABA] that persists across both EDIT ON and EDIT OFF; (B) A change in [GABA] that occurs for an event during the EDIT ON condition only; and (C) A change in [GABA] that occurs during an event for the EDIT OFF condition only.

	Integrated area of simulated MEGA-PRESS for GABA edited spectrum for three conditions: (normalised to baseline)		
Change from baseline	**(A)**	**(B)**	**(C)**
20% decrease	0.80	0.7847	1.0153
10% decrease	0.90	0.8924	1.0076
10% increase	1.10	1.1076	0.9924
20% increase	1.20	1.2153	0.9847

Abbreviations: fMRS, functional magnetic resonance spectroscopy; GABA, gamma-aminobutyric acid; MEGA-PRESS, Mescher–Garwood point-resolved spectroscopy; TE, echo time.

mostly to changes in the middle of the pseudo-doublet), but a [GABA] change that only occurs during the OFF acquisition leads to smaller changes in the peak from the subtraction spectrum, and in the opposite direction to the concentration change. (Changes occurring again in the middle of the pseudo-doublet.)

Integration of the area under the curve for each condition for each simulated change in GABA is reported in Table 1, and shows that:

1. for the case where [GABA] increases (or decreases) during both the ON and OFF acquisitions, the resulting subtraction peak increases (or decreases) by the same amount (10% or 20%);
2. for the case where [GABA] is only changing during the ON acquisition, the resulting subtraction peak also changes, but by slightly more than the [GABA] (±10.7% and ±21.5% depending on the direction of concentration change);
3. for the case where [GABA] changes only during the OFF acquisition, the resulting subtraction peak does not change much, but changes to the peak are in the opposite direction to the concentration change (±0.7% and ±1.5%).

Repeating the same thought experiment for glutamate, similar results are seen, although the impact of when a change in glutamate occurs is amplified. The effect of a short fast glutamate change on the peak at 3.75 ppm in the subtraction spectra is shown in Figure 4 and integration of the area under the curve (Table S1) shows that:

1. for the case where [Glu] changes during both the ON and OFF acquisitions, the resulting subtraction peak changes by the same amount (±10% or ±20% corresponding to the direction of concentration change);
2. for the case where [Glu] is only increased during the ON acquisition, the resulting subtraction peak also increases, but by slightly more than seen for GABA simulations (±14.8% and ±29.6% corresponding to the direction of concentration change);

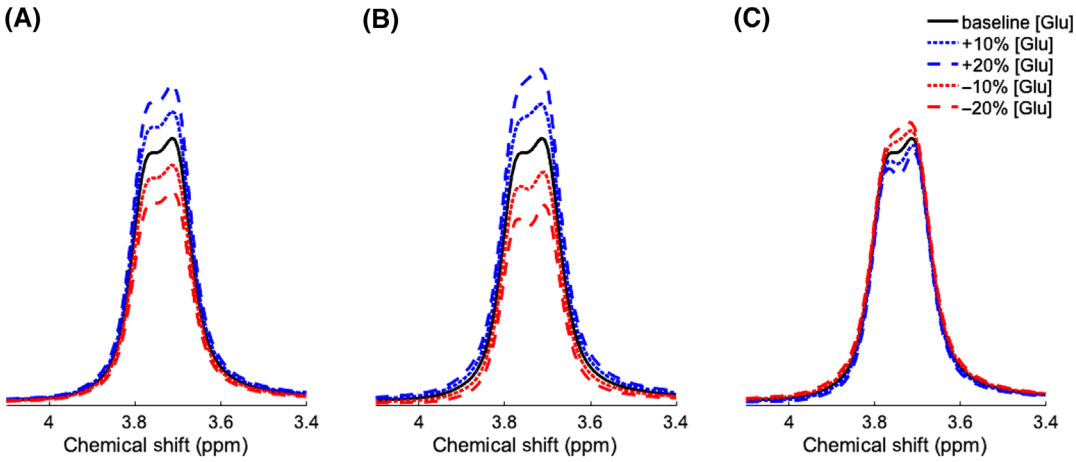

FIGURE 4 Simulated MEGA-PRESS edited spectra for [Glu] (TE 68 ms, linewidth 12 Hz) at baseline, a 10%, and a 20% change (increases in blue, decreases in red) from baseline for three different scenarios: (A) An increase in [Glu] that occurs/persists across both the EDIT ON and EDIT OFF acquisition; (B) An increase in [Glu] that only occurs/persists during the EDIT ON acquisition; (C) An increase in [Glu] that only occurs/persists during the EDIT OFF acquisition. Glu, glutamate; MEGA-PRESS, Mescher–Garwood point-resolved spectroscopy; TE, echo time.

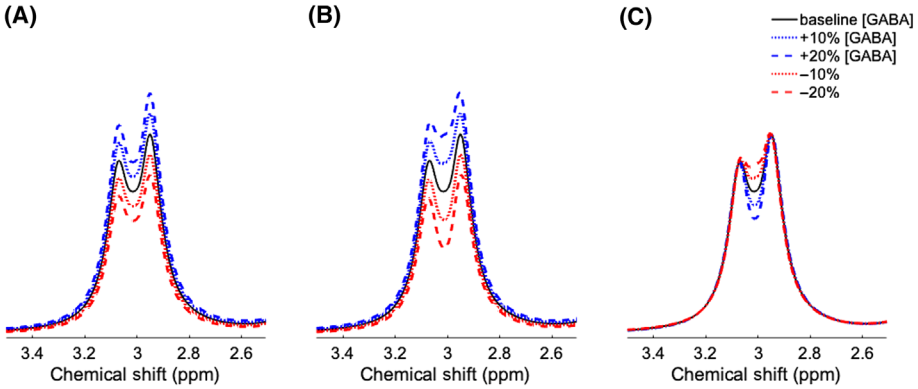

FIGURE 5 Simulated MEGA-PRESS edited spectra for [GABA] (TE 80 ms, linewidth 12 Hz) at baseline, a 10%, and a 20% change (increases in blue, decreases in red) from baseline for three different scenarios: (A) A change in [GABA] that occurs/persists across both the EDIT ON and EDIT OFF acquisition; (B) A change in [GABA] that only occurs/persists during the EDIT ON acquisition; (C) A change in [GABA] that only occurs/persists during the EDIT OFF acquisition. GABA, gamma-aminobutyric acid; MEGA-PRESS, Mescher–Garwood point-resolved spectroscopy; TE, echo time.

3. for the case where [Glu] changes only during the OFF acquisition, the resulting subtraction peak also changes, but this time in the opposite direction to the direction of concentration change (±4.8% and ±10.6%), and by a greater amount than seen for the GABA simulations.

Repeating these simulations and measurements for a MEGA-PRESS experiment with a TE of 80 ms, shows a similar pattern in Figure 5, although the increases (and decreases) are slightly larger (See figure S4 for the corresponding Glutamate simulations). Integration of the area under the curve between 2.5 and 3.5 ppm for the TE = 80 ms case shows that:

1. when [GABA] changes during both the ON and OFF acquisitions, the resulting subtraction peak changes by the same amount (±10% or ±20%, corresponding to the direction of concentration change);
2. when [GABA] is only changing during the ON acquisition, the resulting subtraction peak also changes, but by slightly more than the [GABA] (±11.38% and ±22.75% corresponding to the direction of concentration change);
3. when [GABA] changes only during the OFF acquisition, the resulting subtraction peak changes slightly by a smaller amount, but in the opposite direction to the concentration change (±1.38% and ±2.75% opposite to the direction of concentration change).

Adding noise to any of the simulations performed above does not change the direction of the effects seen, especially the minimal/reversed change in signal for EDIT OFF only conditions, but it does change the absolute value of the changes seen, usually by an amount in line with the

size of the noise signal introduced (see Figure S5 for an example figure with noise, and Table S2 for example integration results when noise is added).

4 | DISCUSSION

These modelled results strongly argue that controlling, or at least being aware of, the timing of stimulus onset in relation to transient acquisition time, is crucial in event-related fMRS studies utilising MEGA-PRESS for data acquisition. While this is especially pertinent when event-related designs are being used, and a 'fast' GABA response is theorised, the impact of the timing of [GABA] changes on the different portions of the MEGA-PRESS acquisition (and by extension other dual/multishot techniques) will impact all MEGA-PRESS–based fMRS studies of GABA, and should be considered when designing both the paradigm, and the acquisition strategy. For example, even for block-related designs, these results suggest that detection of [GABA] increases would be maximised if the on condition for the task and the EDIT ON transients of the MEGA-PRESS acquisition are synchronised and minimised or possibly missed if only occurring during EDIT OFF.

A counterargument to these concerns may be that the MRS-visible GABA changes are too slow and persistent for these concerns to really be an issue. However, in the absence of a known 'GABAergic response function' detailing the response of GABA's rise and fall in response to single stimuli, it would still be prudent to consider the impact of stimulus timing on expected results, and design experiments (stimulation and acquisition timing) accordingly.

Some considerations, and their wider implications, for fMRS experiments using MEGA-PRESS for GABA:

1. If performing an event-related paradigm, controlling the timing of your stimulus/event to start just before the EDIT ON acquisition of a MEGA-PRESS sequence would give the best chance of detecting an increase (or decrease) in GABA. Taking electrophysiological responses into account, 150–300 ms before data acquisition is likely a good estimate[15] of when neural activity occurs. However, further work is required to determine how the GABA response relates to electrophysiologic timings (see Consideration 5), with one report showing an increase between 0.1 and 1.6 s after stimulus onset, and lasting for around 4 s.[28]
2. Consideration 1 is predicated on GABA changes being the sole outcome of interest in such a study; however, it is likely that glutamate (or combined glutamate and glutamine [Glx]) changes may also be of interest. If using the subtraction or difference spectrum to fit the Glu,[29] the same concerns arise (even more so for the case of changes occurring during the EDIT OFF acquisitions), and so again, stimuli occurring just before the EDIT ON acquisitions give the best chance to detect a change. However, if using the EDIT OFF subspectrum to fit for Glu, the experiment should be designed to ensure an equal number of events happen just before an EDIT OFF acquisition. Making sure the number of events/acquisitions is equal, or slightly biased towards the EDIT ON spectra, will give the best chance of detecting a change in GABA, and still allow Glu changes to be detected from the OFF spectrum. Note, allowing the events to occur purely in a random fashion (even if still 'time locked') across a time series may not be appropriate, especially if it leads to a greater number of events occurring before or in-sync with EDIT OFF acquisitions, which could lead to changes being minimised, or even reversed.
3. If a longer block design/task is envisaged, then to maximise the GABA signal, it might appear to be useful to just acquire EDIT ON subspectra across the block and use the EDIT OFF subspectrum from the rest period for subtraction (while also acquiring some EDIT ON spectra during rest as well to provide a baseline). Note that, while this maximises the chance of detection, it will slightly overestimate the size of Δ[GABA] (by 7%–14% of the actual change, depending on the size of change seen).
   However, when considering in vivo data collection, acquiring EDIT ON subspectra only across a task block is not recommended. Best practice for edited MRS recommends the EDIT ON and EDIT OFF acquisitions are collected in a highly interleaved fashion,[30] interleaving EDIT ON and EDIT OFF contiguously as odd/even 'pairs'.[20,31] Doing so means that the two EDIT ON and EDIT OFF subspectra are well matched, with little frequency drift or difference between them, thus reducing the chance for subtraction artifacts. Long blocks of only EDIT ON acquisition, if there is scanner drift or subject motion over the length of the task block, may no longer match the EDIT OFF blocks and so increase the impact of subtraction artifacts. Consideration of blood oxygen level dependent (BOLD) effects reducing linewidth during stimulus/task blocks may also lead to subtraction artifacts if there is a linewidth difference between the ON and OFF subspectra. Therefore, while theoretically acquiring only EDIT ON subspectra during a task should improve detection of GABA dynamics, in practice, the theorised benefits will likely not arise.
4. Sliding window averaging should model the expected effects of increases (or decreases) in [GABA] occurring in mixed fashion across EDIT ON and EDIT OFF acquisitions.
5. Estimating, or measuring, a GABA response function to short events would be useful. This would allow even better modelling of the expected signal response for any given paradigm, and hence allow for improved experimental design. To date, there is only one report of the temporal response of GABA to single events,[28] showing an increase at 1.6 s, lasting until around 4 s. This preliminary datapoint should be followed up with further studies but may provide an indication of the time scales that could be expected. To measure such a response function, an experimental paradigm similar to that employed by Yakovlev et al., with a short stimulus event (800–3000 ms) time-locked to occur before both an EDIT ON, and an EDIT OFF acquisition in equal number allowing for pairing of EDIT ON and EDIT OFF subspectra from similar timepoints

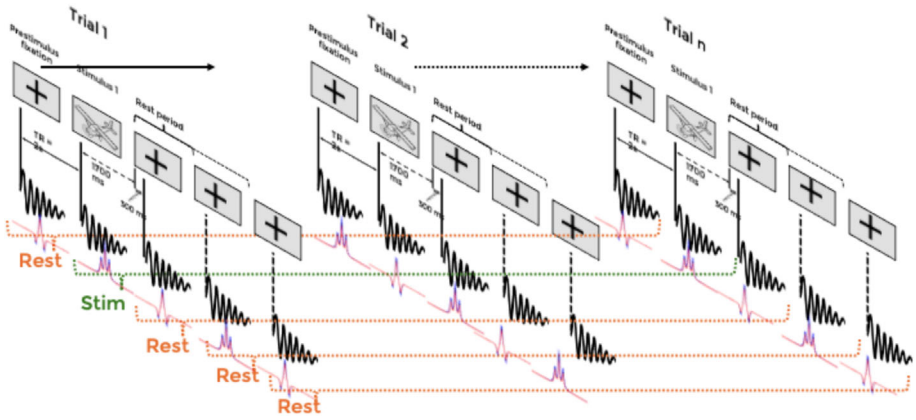

FIGURE 6 Schematic of a proposed paradigm for detection of a GABA response function, across *n* trails. Each trail alternates when the stimulus starts, such that it starts before the EDIT ON acquisition in half the trails, and before the EDIT OFF in the other half. Note, the rest period after stimulus onset should be sufficiently long to allow any momentary GABA increases to return to baseline; an interval of between 10 and 20 s might be a useful starting point. GABA, gamma-aminobutyric acid.

after stimulus onset, followed by longer interstimulus intervals (a value between 10 and 20 s, or varying values between 10 and 20 s), and a relatively normal or short repetition time (TR) (1.5–2 s), might be appropriate. Figure 6 shows a schematic of such a protocol for *n* trials.

In conclusion, these simulations show that the timing of any expected GABA increases in relation to the EDIT ON and EDIT OFF portion of a MEGA-PRESS experiment can impact the amplitude of the resulting GABA peak in the EDITED subtraction spectrum. As a result, controlling, or being aware of the timing of stimulus onset in relation to the specific subspectra, is likely to be crucial in fMRS studies using MEGA-PRESS for GABA, especially for event-related paradigms, or ones where an event-related response could be expected.

CONFLICT OF INTEREST STATEMENT

The author has no financial or other conflicts of interest to report with regards to the content of this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in OSF at https://osf.io/kyjcf/, reference number DOI 10.17605/OSF.IO/KYJCF.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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