Investigating GABA Concentrations Measured With Macromolecule Suppressed and Unsuppressed MEGA-PRESS MR Spectroscopy and Their Relationship With BOLD Responses in the Occipital Cortex

Niall W. Duncan, PhD,[1,2,3]* Jianfeng Zhang, MSc,[4] Georg Northoff, PhD,[1,5,6] and Xuchu Weng, PhD[7]

Background: A combination of magnetic resonance spectroscopy (MRS) and functional (f)MRI is a promising method for studying brain activity. Negative results have, however, produced uncertainty as to the validity of the approach. Using a MEGA-PRESS sequence adapted to suppress the macromolecule signal (GABA–) has been suggested as a key methodological improvement, but there is some doubt as to the relationship between such estimates and those from the standard sequence (GABA+), making interpretation difficult.
Purpose: To investigate the relationship between GABA+ and GABA– estimates from the posterior cingulate and occipital cortices. The second aim was to test for a correlation between occipital GABA and blood oxygenation level-dependent (BOLD) responses in the visual cortex to establish which of the two MEGA-PRESS sequences was more related to the functional responses.
Study Type: Prospective.
Subjects: Thirty-one healthy participants.
Field Strength/Sequence: 3T/single-voxel ^{1}H-MRS and gradient-echo echo planar imaging (EPI).
Assessment: GABA estimates were made using the Gannet toolbox. fMRI data were analyzed with FSL and Python scripts.
Statistical Test: Relationships between different variables were tested with Pearson's correlation.
Results: GABA+ and GABA– concentrations were found to be correlated in both regions ($r = 0.52$, 95% confidence interval [CI] = 0.35 0.66, $p_{FDR} = 0.002$). No relationship was found between either the GABA+ or the GABA– concentrations and the amplitude of the BOLD response in the occipital cortex (GABA+, $r = –0.14$, $p_{FDR} > 0.1$; GABA–, $r = –0.29$, $p_{FDR} > 0.1$). However, adding these results to those of prior studies in a meta-analysis of correlation coefficients did provide overall support for a negative correlation between GABA and BOLD response amplitudes ($r = –0.39$, 95% CI = –0.15–0.64).
Data Conclusion: The current findings highlight potential methodological issues that continue to interfere with relating MRS GABA estimates with fMRI responses but, taken in sum, provide support for this general approach.
Level of Evidence: 1
Technical Efficacy: Stage 1

From the [1]Centre for Cognition and Brain Disorders, Hangzhou Normal University, Hangzhou, China; [2]Taipei Medical University Centre for Brain and Consciousness Research, Taipei, Taiwan; [3]Graduate Institute of Humanities in Medicine, Taipei Medical University, Taipei, Taiwan; [4]College of Biomedical Engineering and Instrument Sciences, Zhejiang University, Hangzhou, China; [5]Institute of Mental Health Research, University of Ottawa, Ottawa, Canada; [6]Mental Health Centre, Zhejiang University School of Medicine, Hangzhou, China; and [7]Institute for Brain Research and Rehabilitation, South China Normal University, Guangzhou, China

DETAILING THE PHYSIOLOGICAL PROCESSES underlying the neural activity observed with different neuroimaging techniques is an important step towards understanding brain function in health and disease.[1] Since much of the activity in the human brain reflects the interaction of gamma-aminobutyric acid (GABA)-ergic inhibitory interneurons and glutatmatergic excitatory neurons,[2] these two transmitters are key targets. An apparently important step towards studying the relationship between inhibitory activity and neuroimaging measures was made with the observation that GABA concentrations in the occipital cortex (OC), as measured with magnetic resonance spectroscopy (MRS), correlated with functional magnetic resonance imaging (fMRI) and MEG responses to visual stimuli.[3] The correlation between MRS measures of GABA in this region and fMRI blood oxygen level-dependent (BOLD) responses was subsequently reported in several other studies.[4–6]

The robustness of the association, however, was called into question by a more recent study that found no correlation between GABA concentration estimates and BOLD responses.[7] This was the case not just in the OC with a visual task but also in four other brain regions with tasks specific to each. In addition, that study included a meta-analysis of the correlations between GABA and BOLD previously reported and found no evidence for a relationship.

Each of the prior studies discussed used the MEGA-PRESS method to obtain GABA concentration estimates. This approach attempts to overcome the overlap between the GABA resonances and those of other metabolites by obtaining two subspectra, one of which is tuned to affect only those other metabolites, theoretically leaving the GABA signal in the difference between the two.[8] There is not a complete isolation of the GABA signal, however, as it remains contaminated by the signal from various macromolecules,[9] contributing an estimated 45% of the total GABA plus macromolecule (GABA+) signal.[10]

Although this macromolecule contribution is generally assumed to be irrelevant with regard to functional responses,[7] there does appear to be a reasonable degree of individual variability in macromolecule concentrations.[11] This variation may thus contribute to the reported overall lack of correlation between GABA+ and BOLD responses by introducing additional error into already noisy measurements. This supposition is given some support by the finding that GABA+ concentrations do not correlate well with GABA concentrations obtained using approaches that suppress the macromolecule signal more effectively (GABA–), and so potentially give more accurate estimates.[12,13] In particular, Harris et al[14] report no correlation between GABA+ and GABA– values within the OC (although a correlation was seen when OC values are pooled with those from other brain regions). Given this, it is possible that using a macromolecule-suppressed MEGA-PRESS approach will improve the accuracy of the correlation between GABA estimates and fMRI responses by better representing the true GABA concentration.

The aim of this study was therefore to determine if there is an improvement in the correlation between MRS estimates of GABA and fMRI responses when using a macromolecule-suppressed MEGA-PRESS sequence. It was hypothesized, first, that a correlation between GABA+ and GABA– values would be seen in the OC, in line with previous reports for other brain regions.[13] It was further hypothesized that GABA– values in the OC would correlate with BOLD responses from an fMRI visual task and that this correlation would be stronger than that found using GABA+ estimates.

Materials and Methods

Participants

Thirty-one healthy young adults took part in the study (mean age = 23.0 ± 3.1 SD years; age range = 19–31 years; five female). Note that different numbers of participants were excluded from different aspects of the analysis for data quality reasons (see below for details), and so the numbers included for each statistical test are marked in the relevant places. Participants were screened for current psychiatric or neurological disorders, recent prescription or recreational drug use, and for standard MRI exclusion factors such as claustrophobia or metal implants. All participants had normal or corrected-to-normal vision. Written informed consent was obtained from all participants and the study was approved by the local Ethics Review Board.

Visual Task

The visual task used a grating presented in the lower left visual field, subtending 4° horizontally and vertically.[5] The grating was vertical, stationary, at maximum contrast, with three cycles per degree, and was presented against a mean luminance background. The upper right corner of the grating was located 0.5° horizontally and vertically from a small fixation cross. Each stimulus was presented for 1.5–2 seconds, followed by an 18–20 seconds intertrial interval during which the fixation cross was displayed. Participants were instructed to fixate on the cross and to press a button as quickly as possible after the offset of the grating. A total of 46 trials were presented, split into two runs of approximately equal length.

MRI Data Acquisition

All scanning was done with a 3 T GE MR750 scanner using a body coil for transmission and an 8-channel receive head-coil. Padding was placed around participants heads to limit motion. A high-resolution T_1-weighted anatomical was acquired (FSPGR; resolution = $1 \times 1 \times 1$ mm^3), followed by the four MRS acquisitions and then the two visual task runs. During the visual task, BOLD-sensitive images were acquired using a T_2*-weighted echo planar imaging (EPI) sequence (repetition time [TR] = 1000 msec; echo time [TE] = 30 msec; flip angle = 62°; field of view [FoV] = 220 mm; matrix = 64×64; slice thickness = 4 mm; slice gap = 0 mm; 21 slices). In all, 541 volumes were acquired in the first run and 545 in the second (~9 min per run).

MRS voxels ($30 \times 30 \times 30$ mm^3) were located in the PCC and the OC (Fig. 1a). The OC voxel was located on the midline and rotated to follow the ventral edge of the occipital lobe. The Posterior cingulate cortex (PCC) voxel was also located on the midline, with the rear edge aligned with the splenium and the bottom with the body
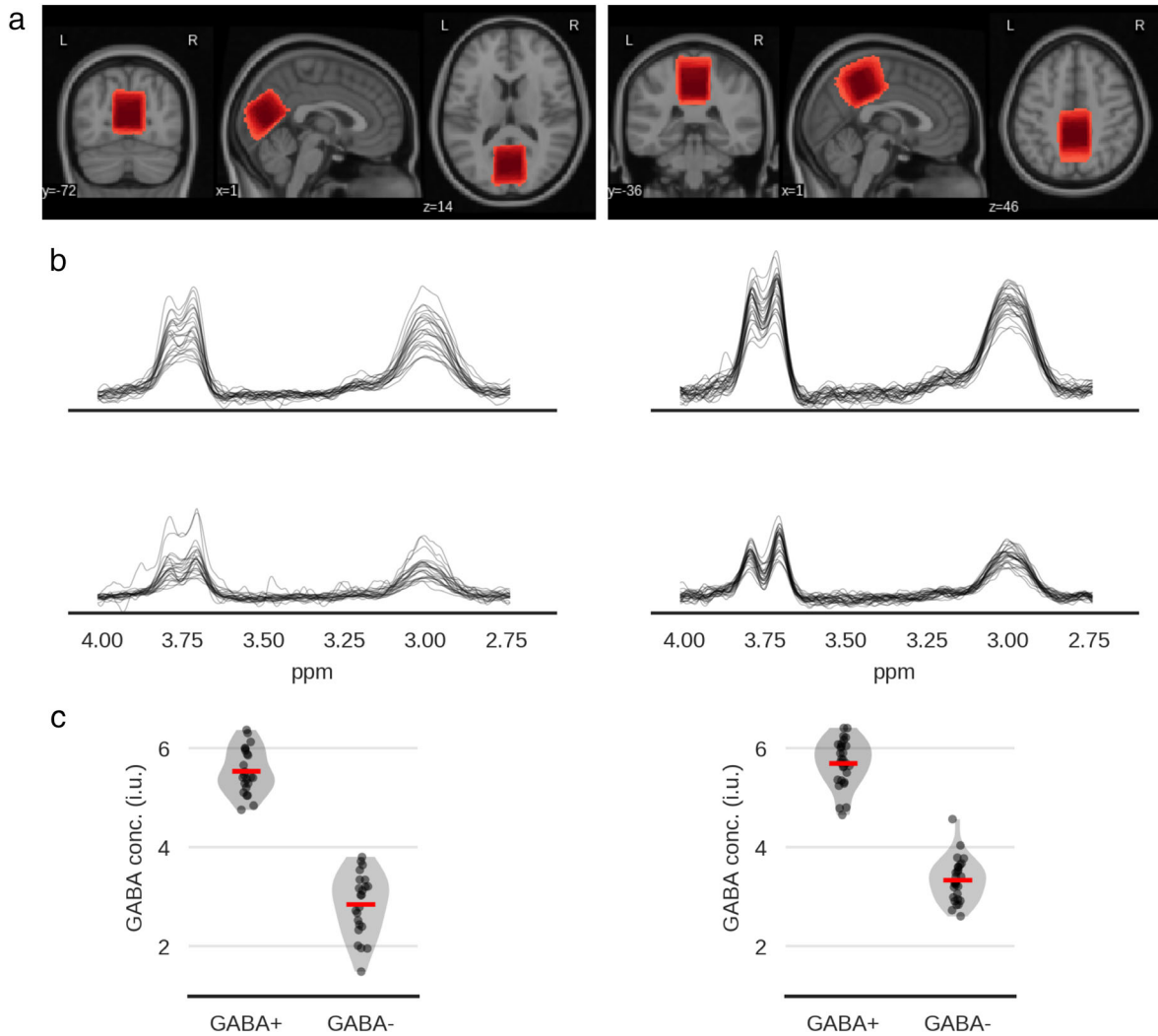
FIGURE 1: (a) Location of the MRS voxels in standard space (OC on left and PCC on right). Darker shading represents greater overlap between participants. (b) Individual spectra from the OC (left) and PCC (right) for GABA+ (upper) and GABA– (lower). (c) Individual estimates for GABA+ and GABA– in the OC (left) and PCC (right). Red lines denote the mean. All brain images were created using the *nilearn* Python toolbox.[40]

of the corpus callosum. The order in which the regions were scanned was counterbalanced across participants, as was the GABA+ and GABA– order for each region.

The following settings were used for both the GABA+ and GABA– MEGA-PRESS acquisitions: TR = 1800 msec; datapoints = 4096; spectral width = 5000 kHz; alternating ON/OFF editing; 192 averages; 8 water unsuppressed acquisitions. GABA+ acquisitions used a TE of 68 msec, with 14 msec editing pulses applied at 1.9 ppm (ON) and 7.46 ppm (OFF). Macromolecule-suppressed GABA– acquisitions used a TE of 80 msec, with 20 msec editing pulses applied at 1.9 ppm (ON) and 1.5 ppm (OFF).[12]

MRS Analysis

MRS data were analyzed using Gannet 3.0.[15] This applies 3 Hz line broadening, phase and frequency correction, outlier rejection, and zero padding as preprocessing steps. The GABA signal at 3 ppm is then modeled as a Gaussian and the concentration estimated relative to water (in institutional units). Following this concentration estimation, the tissue within the MRS voxel is segmented to establish the proportions of gray matter, white matter, and cerebrospinal fluid (CSF). These proportions are then used to correct the GABA concentrations.[16] A macromolecule correction factor of 0.45 is usually applied to the GABA+ estimates[12] but was excluded here. As frequency drift can influence MEGA-PRESS estimates of GABA, this was calculated and an upper threshold of 0.125 ppm applied.[17] One OC GABA– dataset failed to meet this cutoff, leaving 23 with usable OC and 28 with usable PCC data.

fMRI Analysis

Preprocessing of the functional and anatomical data was done using a combination of tools from the FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki) and ANTs (http://stnava.github.io/ANTs) software packages, along with the *nipype* python toolbox (http://nipype.readthedocs.io/en/latest/index.html). Anatomical images were aligned to the ICBM152 MNI template in a multistep process in ANTs (rigid, affine, and SyN registration). For the functional data the preprocessing steps were as follows: brain extraction (BET); slice-time correction (slicetimer); volume registration (MCFLIRT); high-pass filtering with a 100-second cutoff (fslmaths); and smoothing with a 5-mm FWHM

Gaussian kernel (SUSAN). A linear alignment to the participant's anatomical image was calculated (FLIRT), which was then combined with the previously calculated anatomical-to-MNI transform (ANTs) for normalization of the functional images.

Visual stimulus onsets were modeled as a 1.5–2/18–20-second boxcar convolved with a double gamma Haemodynamic response function (HRF), along with head-motion parameters (plus their temporal derivatives and squares), in a first-level general linear model (GLM) analysis. Parameter estimates from each run were then normalized and combined in a second-level fixed-effects analysis for each participant. Finally, group responses were calculated in a mixed effects analysis across all participants (FLAME-12). Images were thresholded at $P < 0.05$, familywise error (FWE) cluster corrected with a voxel threshold of Z > 2.58 ($P < 0.005$).

Stimulus-induced BOLD response parameters were calculated by first identifying the peak voxel within the MRS box for each participant and extracting the timecourse for each run. The peak voxel location was taken from the second-level contrast images and then converted to the original functional image space for each run. In a second step, mean timecourses from all active voxels within the MRS box were extracted. An activation threshold of $p_{FDR} = 0.005$ was used.

These timecourses were converted to percent signal change and a double gamma function fitted to the datapoints resulting from averaging across all trials (stimulus onset +16 sec). The values from the SPM8 canonical HRF were used as the starting values for the fitting (latency 1 = 6 sec, latency 2 = 16 sec, dispersion 1 = 1 sec, dispersion 2 = 1 sec, height ratio = 1:6). Estimates of the BOLD response amplitude were made from these fitted functions. Fitting was done using the *scipy* Python package (https://www.scipy.org). The same procedure was applied to the timecourses from both the peak voxel and all active voxels. Pearson's correlation was used to relate active voxel numbers with the difference in response amplitudes for the peak voxel and active region as the number of voxels that were active within the MRS region varied across participants and so could influence the final value.

Statistical Analysis: GABA+ and GABA– Estimates

Having processed the MRS data and excluded unusable values, data quality measures including frequency drift, the GABA and creatine peak fit error, and the full-width at half-maximum (FWHM) of the GABA and creatine peaks were compared between the GABA+ and GABA– acquisitions using paired-sample *t*-tests. The coefficient of variation was then compared between GABA+ and GABA– measures in each region using a modified signed-likelihood ratio test,[18] implemented in the *cvequality* R package (https://cran.r-project.org/web/packages/cvequality/index.html). Finally, the correlation between GABA+ and GABA– measures was tested with the pooled data and in each region separately using Pearson's correlation. Statistical significance for these and all other tests was set at a $P < 0.05$ threshold. FDR correction, implemented in the *statsmodels* Python package (https://www.statsmodels.org), was used to account for the multiple correlations being performed.[19]

Statistical Analysis: BOLD Responses and GABA

For the correlation between GABA and BOLD response properties within the MRS region, participants were excluded if they did not have activations overlapping the MRS voxel or if there were problems with the HRF fitting procedure. Pearson's correlation was used to relate GABA concentrations to the BOLD response properties amplitude. Correlation strength with BOLD amplitude for GABA+ and GABA– was compared using the *psych* R package (http://personality-project.org/r/html/00Index.html).

In a final step, a meta-analysis of correlation coefficients was performed using the Hunter–Schmidt method,[20] implemented in the *metafor* R package (http://www.metafor-project.org/doku.php/metafor). Included in this were the results from the present study plus the correlation between MEGA-PRESS GABA estimates and OC BOLD responses reported in five previous studies.[3–7] The meta-analysis was conducted with the GABA+ and GABA– correlation results separately.

Data Availability

The data used in this study are available from the authors upon request.

Results

MEGA-PRESS GABA Estimates

The location of the OC and PCC MRS voxels is shown in Fig. 1a, along with the degree of regional overlap across participants. Tissue segmentation showed that the OC voxel contained $65 \pm 3\%$ (SD) gray matter, $25 \pm 3\%$ white matter, and $10 \pm 2\%$ CSF; the PCC voxel contained $58 \pm 4\%$ gray matter, $28 \pm 4\%$ white matter, and $14 \pm 4\%$ CSF.

After visual inspection of the spectra from each region (Fig. 1b), OC data from seven participants and PCC data from three participants were excluded (where data was unusable from one of the GABA+ or GABA– scans, the data from the other for that region was also discarded). As can be seen in Table 1, there was no difference in frequency drift between the GABA+ and GABA– acquisitions in either region. The fit error of the GABA peak was different in each region; however, being higher for the GABA– scans.

Estimated GABA values are given in Table 2, while the values for individual participants are plotted in Fig. 1c. GABA+ values are higher in both regions. The ratio of GABA– to GABA+ was 0.5 ± 0.1 in the OC and 0.6 ± 0.1 in the PCC, which is significantly higher ($t_{(19)} = 3.71$, $p_{unc} = 0.002$, d = 0.7 [0.3 1.0]). The coefficients of variation for the GABA estimates were significantly higher for GABA– than GABA+ in the PCC ($p_{unc} = 0.02$) and OC ($p_{unc} = 0.03$). There was no correlation between voxel gray matter content and either GABA+ (PCC: $r = -0.01$ [−0.31 0.3], $p_{FDR} > 0.1$; OC: $r = -0.06$ [−0.34 0.2], $p_{FDR} > 0.1$) or GABA– (PCC: $r = -0.19$ [−0.13 0.46], $p_{FDR} > 0.1$; OC: $r = 0.05$ [−0.32 0.38], $p_{FDR} > 0.1$) estimates in either region.

Correlation Between GABA+ and GABA–

As shown in Fig. 2, combining the data from both regions a positive correlation between GABA+ and GABA– estimates was found ($r = 0.52$ [0.35 0.66], $p_{FDR} = 0.002$). Taking the regions separately, a positive correlation was found in each

TABLE 1. Data Quality Metrics for GABA Measurements From the OC and PCC Regions (Mean ± SD)

	GABA+	GABA-	t	d
OCC (n = 23)				
Cr FWHM (Hz)	10.59 ± 1.91	10.89 ± 2.53	−0.91	−0.12 [−0.34 0.11]
GABA FWHM (Hz)	17.75 ± 1.31	15.71 ± 1.67	5.27*	1.32 [0.78 1.93]
Cr fit error (%)	5.76 ± 1.41	4.68 ± 1.1	5.35*	0.8 [0.38 1.43]
GABA fit error (%)	5.49 ± 1.34	6.94 ± 1.63	−3.32*	−0.79 [−1.21 −0.24]
Frequency drift (Hz)	4.35 ± 2.26	4.15 ± 1.3	0.49	0.1 [−0.24 0.6]
PCC (n = 28)				
Cr FWHM (Hz)	8.67 ± 0.85	8.88 ± 1.16	−0.83	−0.2 [−0.69 0.19]
GABA FWHM (Hz)	17.17 ± 0.7	15.68 ± 0.95	6.23*	1.77 [1.01 2.5]
Cr fit error (%)	8.72 ± 1.23	7.33 ± 0.98	6.6*	1.2 [0.68 1.83]
GABA fit error (%)	5.69 ± 1.36 SD	6.53 ± 1.11	−2.41*	−0.67 [−1.21 −0.14]
Frequency drift (Hz)	3.98 ± 2.12	3.75 ± 1.56	0.79	0.12 [−0.17 0.37]

Values were compared between acquisitions; t-statistics are given along with Cohen's d (with bootstrapped 95% CI in brackets).
*Significant difference at $P_{unc} < 0.05$.

(OC: r = 0.47 [0.11 0.72], p_{FDR} = 0.02, n = 23; PCC: r = 0.49 [0.2 0.69], p_{FDR} = 0.01, n = 28).

Correlation of GABA and BOLD Responses

The visual task produced responses in the contralateral primary visual cortex. A sample activation map is given in Fig. 3a (individual BOLD response fits for the peak voxel and active region can be seen in Supplementary Figs. 1 and 2, respectively). The difference in amplitude estimates from the peak voxel and the active region was correlated with the proportion of the MRS box that was active (r = −0.56 [−0.8 0.02], p_{unc} = 0.01, n = 19), with an average of 46.6% of the box activated (±29.4 SD).

No significant correlation was found between the BOLD response amplitude at the peak voxel within the MRS region and either the GABA+ (r = −0.14 [−0.54 0.31], p_{FDR} > 0.1, n = 19) or GABA− (r = −0.29 [−0.67 0.39], p_{FDR} > 0.1, n = 19) estimates (Fig. 3b). These correlations were not significantly different (t = 0.57, p_{unc} = 0.58). The BOLD response amplitude within the active region was also

not correlated with either the GABA+ (r = −0.25 [−0.63 0.28], p_{FDR} > 0.1, n = 21) or GABA− (r = 0.15 [−0.38 0.6], p_{FDR} > 0.1, n = 21) estimates (Fig. 3c). There was no significant difference in correlation strength (t = 1.57, p_{unc} = 0.14).

The shape of the brain and the size of the MRS region means that it is not possible to place the MRS voxel directly over the maximal task response region (mean distance between MRS region center of mass and peak response voxel = 63.5 ± 22 mm). To ensure that there was a match in the response properties from these two locations, the relationship between the BOLD properties at the peak voxel within the MRS region and the peak response in the occipital cortex as a whole was tested (BOLD responses from each region are shown in Supplementary Fig. 3). A significant correlation was found between the response amplitudes (r = 0.55 [0.16 0.85], p_{FDR} = 0.02, n = 23), suggesting that the BOLD response in the MRS region reflects the response in V1.

The result of the correlation between GABA estimates and BOLD amplitude was added to a meta-analysis with those of five previous studies investigating GABA+ and

TABLE 2. Mean GABA+ and GABA- Values (i.u.) ± SD for the OC and PCC, Along With the Ratio Between These

	GABA+	GABA-	GABA-/GABA+
OC (n = 23)	5.5 ± 0.4 [7%]	2.9 ± 0.6 [20%]	0.5 ± 0.1
PCC (n = 28)	5.7 ± 0.5 [9%]	3.3 ± 0.4 [12%]	0.6 ± 0.1

Coefficients of variation are given in brackets. Values are corrected for voxel tissue composition.
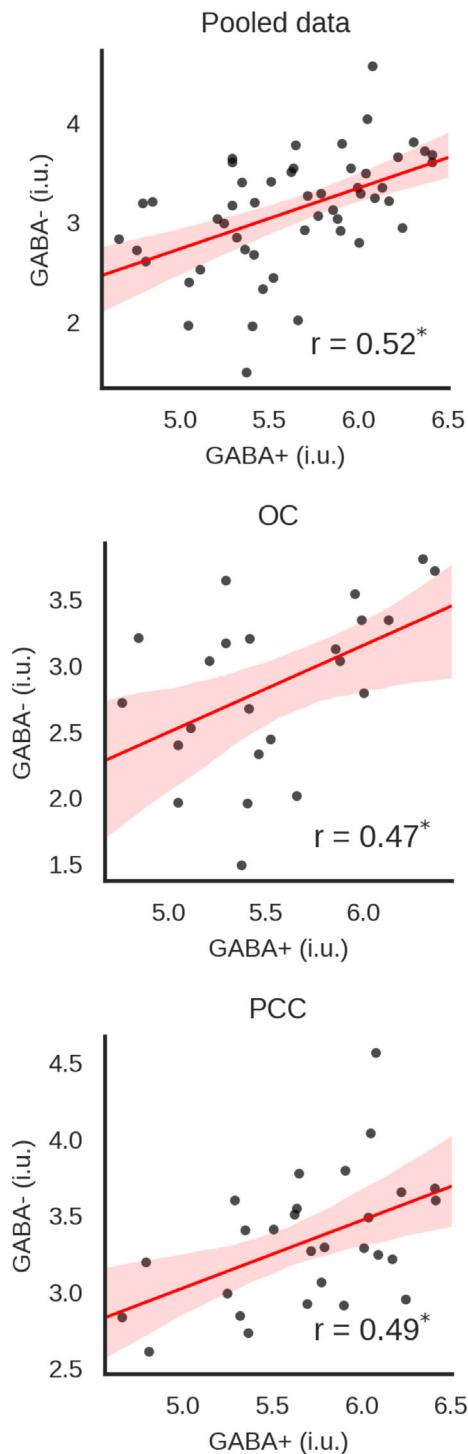
FIGURE 2: Correlation between GABA+ and GABA– for all data pooled together, the OC alone, and the PCC alone. Red lines denote the best straight-line fit with a 95% CI. *Significant correlation at $p_{FDR} < 0.05$.

BOLD responses in the OC. This analysis showed overall evidence in favor of there being a negative correlation when using the peak voxel BOLD response and GABA+ ($r = -0.37$ [–0.12 –0.62]) and GABA– estimates ($r = -0.39$ [–0.15 – 0.64]). This was also the case when the active region response and GABA+ ($r = -0.39$ [–0.14 –0.63]) or GABA– ($r = -0.31$ [–0.01 –0.61]) estimates were used.

Discussion

With GABAergic inhibition playing a central role in brain function, being able to study it in relation to functional responses in vivo in humans will be important for neuroscience research in health and disease. A number of studies pointed to MRS being a useful tool for such investigations[3–7] but the robustness of these results were called into question by more recent findings.[7,14] Based on this prior work, this study set out to provide additional evidence for the efficacy—or otherwise—of the combination of MEGA-PRESS estimates of GABA with functional imaging to understand brain function, focusing on the occipital cortex.

The first question arising is the contribution of macromolecule signals to GABA estimates using the standard MEGA-PRESS sequence. Individual variability in macromolecule concentrations has been reported,[11] which may contribute to the lack of correlation between GABA+ and GABA– estimates in the OC described by Harris et al.[7] Such variability and apparent dissociation in some brain regions between GABA estimates including or suppressing macromolecule signals potentially undermines the interpretation of GABA+ values that see only the GABA component as experimentally relevant, as it could point to individual differences in macromolecules forming an indeterminate part of overall GABA+ differences. In the current work, a correlation between OC GABA+ and GABA– was, however, observed. This was in addition to a positive correlation in the PCC and when combining the data from both regions. The correlation observed is only moderate, similar to that reported by Harris et al,[7] but higher than that reported in a considerably larger multisite study that also compared PCC GABA estimates using the two sequences.[13] There would thus appear to be a consistent relationship between GABA+ and GABA– measures across different brain regions (OC, PCC, and sensorimotor) and across studies. The relatively low correlation strength, however, does point to measurement error having a marked influence. This may stem from reduced measurement accuracy with the GABA– sequence, as indicated by the higher CV seen for these estimates, arising from sources such as eddy currents[21] and the susceptibility of this sequence to changes in the B_0 field.[22] Coefficients of variation are also influenced by the lower signal to be fitted given the reduction in amplitude from that which originated from macromolecules.[10]

No correlation was seen in the current data between BOLD responses in the OC voxel and either GABA+ or GABA– estimates. This was the case for BOLD responses calculated from both the peak active voxel and for those from the total active area within the MRS region. When added to a meta-analysis of previous studies investigating this relationship, evidence for a negative relationship, however, was found. This was the case regardless of which sequence (GABA+ or GABA–) and source region (peak voxel or active region) was used, although there was a difference in the strength of the evidence. A negative correlation within the OC is consistent with
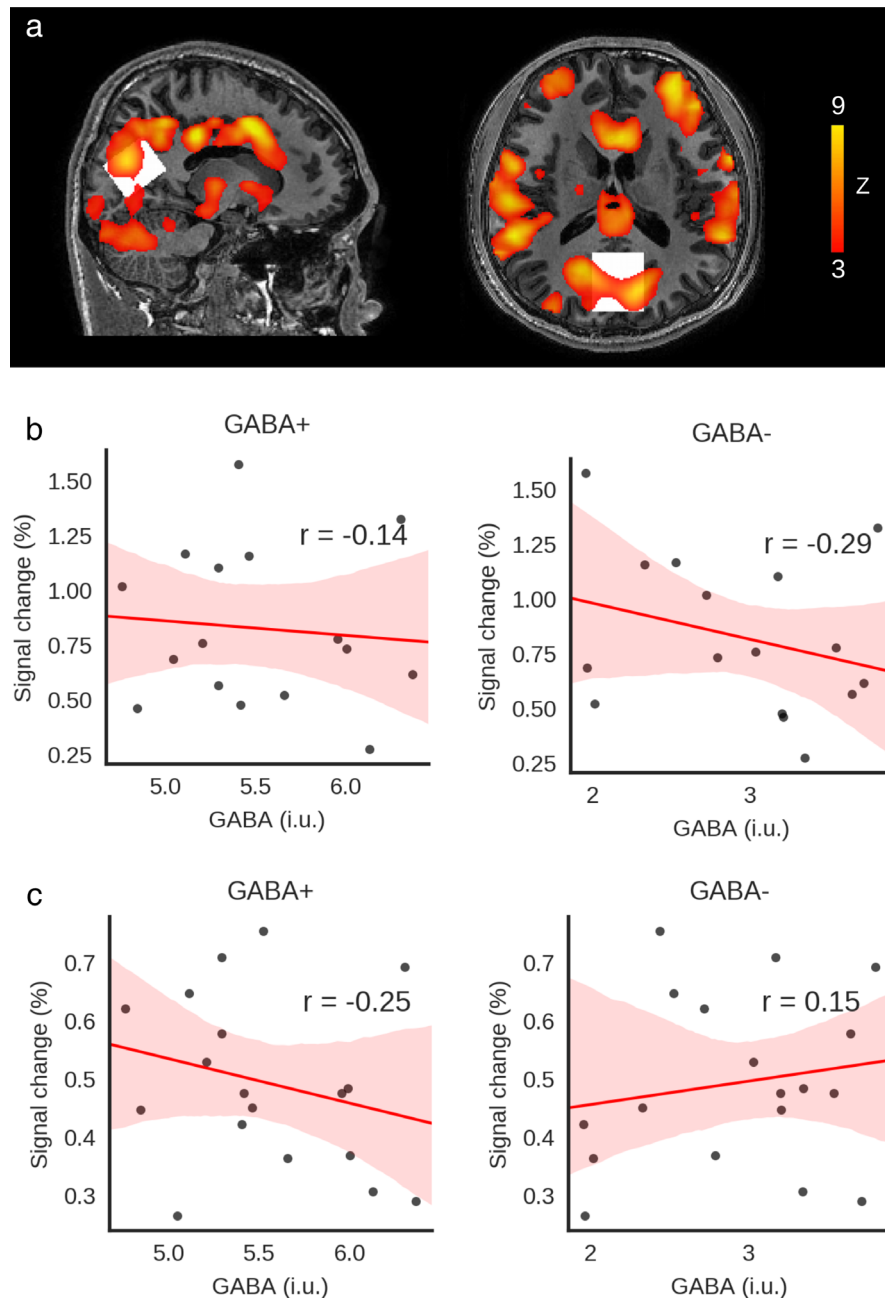
FIGURE 3: (a) Responses to the visual task for one participant (z-scores), shown on their anatomical image. A threshold of *P* = 0.005 (FDR corrected) was applied. The location of the MRS voxel for that participant is shown in white. Scatterplots showing the relationship between BOLD response amplitudes at (b) the peak voxel and (c) the total active area within the MRS region and GABA+ (right) and GABA– (left) estimates. No significant correlations were observed. Red lines denote the best straight-line fit.

findings in other brain regions, such as the cingulate cortex and insula.[23,24] It is also consistent with evidence that challenge with positive GABA modulators, such as vigabatrin and zolpidem, in humans and nonhuman animals induces a reduction in BOLD responses to different stimuli.[25–27] Based on this converging evidence there appears to be good reason to suppose that MRS measures of GABA do provide a useful tool for relating BOLD responses to regional concentrations of that neurotransmitter.

The fact that no correlation was found in this study alone raises the question as to why the relationship that

appears to exist at the population level was not seen in this sample. If we note that the correlation coefficients obtained were negative (other than the GABA– with active region BOLD response amplitude correlation) and in most cases within the confidence interval of the meta-analysis, then the primary explanation to consider is that there was too much measurement noise. The MRS data quality metrics, however, do not point to this being an issue with these measurements per se. Instead, a potential source may be the area covered by the OC MRS voxel often being rather far from the early visual regions where the main response to the Gabor patch

stimulus would be expected. This distance was a result of the relatively small heads of many of the participants,[28,29] making it impossible to locate the voxel any closer to the primary visual regions without including the boundary between the cortex and skull. A good correlation between the BOLD response in the MRS region and in the primary visual cortex was found but a lack of anatomical specificity within the MRS region would still impact the relation of these to biochemical estimates. The issue of MRS region placement is underlined by the observed relationship between the proportion of the MRS region that was active and the mean BOLD response amplitude. Individual variability in the MRS voxel location relative to the active region is thus a potential additional source of measurement noise. Circumventing issues arising from anatomical differences between different participant groups is challenging, but reducing the size of the MRS region would be an important step, something that is becoming possible with higher field strengths and improved sequences. The current results also suggest that future studies should report details about MRS voxel placement relative to active regions and the proportion of the voxel that contains significant activations.

These results also highlight the problems inherent in studying associations between variables derived from low-sensitivity methods, such as fMRI and MRS, in terms of the sample sizes required. For fMRI research, recent work has helped demonstrate the limits of making inferences from small samples, suggesting that larger groups (>100) than have normally been used are necessary for robust and replicable analyses.[30,31] Equivalent studies for MRS are not yet available, but the meta-analysis reported here helps illustrate the problem in this context. Adding the current results to five previous studies produced overall evidence for a negative association between GABA estimates and BOLD amplitudes (total sample size = 100). This can be compared with the previously reported meta-analysis of those five studies, which did not find a statistically significant correlation (95% confidence interval [CI] = [0.09 –0.97]).[7] The average sample size included in the meta-analyses was 17 people (range = 12–26), and so the instability of the outcome between five or six included studies may reflect the lack of power and high measurement variability in these small samples. The need for properly powered studies is thus a fundamental consideration for future research that seeks to relate neural or behavioral features to MRS GABA estimates. One practical approach to achieve this will be collaborative multisite studies that help overcome the inherent financial and logistical barriers involved.[13] It follows also that existing small-sample studies correlating MRS estimates with neural or behavioral measures should be treated with caution.

The indirect nature of the MRS and fMRI measurements introduce further issues that may influence the observed relationship between them. In testing for a linear correlation between GABA estimates and stimulus-induced BOLD responses, it is assumed that the association between these variables is approximately the same in all participants. Intersubject variability may arise within the connection between induced neural activations and the hemodynamic effects of those responses that are then detected in BOLD fMRI. For example, there may be differences in relative stimulus-induced cerebral blood flow versus oxygen metabolism changes[32] and different contributions of nonneural regulation of hemodynamic changes.[33] Importantly, MRS measurements reflect bulk rather than synaptic GABA concentrations,[34] and so likely also reflect transmitter that influences vascular and metabolic responses through routes other than its influence of neuronal signaling[35,36] and for which there may be nonlinearities present.[37] Previous work has provided evidence for a linear relationship between MRS measures of GABA and nonhemodynamic signatures of neural activity, including EEG and MEG,[38] but these results do not provide clear evidence that the MRS measures are related to stimulus-induced responses rather than reflecting structural properties of the local neural networks.[3,39] There is thus a continuing need for detailed studies of what MRS GABA measurements reflect physiologically to allow relations between them and neural measures or behavior to be meaningfully interpreted.

No statistically significant advantage of using the current macromolecule suppressed sequence was seen. This does not rule out there being advantages of using such an approach in the future, however. Future work with larger samples would be warranted to explore this potential. It should also be noted that macromolecule suppressed MEGA-PRESS sequences are, at present, susceptible to sources of noise, such as subject movement and field drift. This can strongly influence the accuracy of the resulting GABA estimates. More sophisticated approaches to macromolecule suppressed GABA measures, such as the use of prospective motion correction,[22] may lead to a significant increase in accuracy and, in turn, improvements in the utility of such estimates in neuroscience research.

In conclusion, the current findings highlight potential methodological issues that continue to interfere with relating MRS GABA estimates with fMRI responses. GABA+ and GABA– measurements were found to be correlated in both the PCC and OC, but the strength of this correlation suggests that improvements in GABA– acquisitions may be required before they can be considered fully reliable. At the same time, an accumulation of evidence points to there being a consistent negative correlation between GABA estimates and BOLD response amplitudes in the OC. The lack of a significant correlation in this individual study does, however, raise questions of robustness. Continuing to improve on these points will be an important step towards effectively combining biochemical and functional imaging approaches to better understand the brain.

Acknowledgments

Conflict of Interest

The authors declare no conflicts of interest.

References

1. Duncan NW, Wiebking C, Munoz-Torres Z, Northoff G, Muñoz-Torres Z. How to investigate neuro-biochemical relationships on a regional level in humans? Methodological considerations for combining functional with biochemical imaging. J Neurosci Methods 2014;221:183–188.

2. Lauritzen M, Mathiesen C, Schaefer K, Thomsen KJ. Neuronal inhibition and excitation, and the dichotomic control of brain hemodynamic and oxygen responses. NeuroImage 2012;62:1–11.

3. Muthukumaraswamy SD, Edden RAE, Jones DK, Swettenham JB, Singh KD. Resting GABA concentration predicts peak gamma frequency and fMRI amplitude in response to visual stimulation in humans. Proc Natl Acad Sci U S A 2009;106:8356–8361.

4. Donahue MJ, Near J, Blicher JU, Jezzard P. Baseline GABA concentration and fMRI response. NeuroImage 2010;53:392–398.

5. Muthukumaraswamy SD, Evans CJ, Edden RAE, Wise RG, Singh KD. Individual variability in the shape and amplitude of the BOLD-HRF correlates with endogenous GABAergic inhibition. Hum Brain Mapp 2012;33:455–465.

6. Violante IR, Ribeiro MJ, Edden RAE, et al. GABA deficit in the visual cortex of patients with neurofibromatosis type 1: Genotype-phenotype correlations and functional impact. Brain 2013;136:918–925.

7. Harris AD, Puts NAJ, Anderson BA, et al. Multi-regional investigation of the relationship between functional MRI blood oxygenation level dependent (BOLD) activation and GABA concentration. PLoS One 2015;10:e0117531.

8. Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R. Simultaneous in vivo spectral editing and water suppression. NMR Biomed 1998;11:266–272.

9. Behar KL, Rothman DL, Spencer DD, Petroff OA. Analysis of macromolecule resonances in 1H NMR spectra of human brain. Magn Reson Med 1994;32:294–302.

10. Mullins PG, McGonigle DJ, Gorman RLO, et al. Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. NeuroImage 2014;86:43–52.

11. Terpstra M, Ugurbil K, Gruetter R. Direct in vivo measurement of human cerebral GABA concentration using MEGA-editing at 7 Tesla. Magn Reson Med 2002;47:1009–1012.

12. Edden RAE, Puts NAJ, Barker PB. Macromolecule-suppressed GABA-edited magnetic resonance spectroscopy at 3T. Magn Reson Med 2012;68:657–661.

13. Mikkelsen M, Barker PB, Bhattacharyya PK, et al. Big GABA: Edited MR spectroscopy at 24 research sites. NeuroImage 2017;159:32–45.

14. Harris AD, Puts NAJ, Barker PB, Edden RAE. Spectral-editing measurements of GABA in the human brain with and without macromolecule suppression. Magn Reson Med 2015;74:1523–1529.

15. Edden RAE, Puts NAJ, Harris AD, Barker PB, Evans JC. Gannet: A batch-processing tool for the quantitative analysis of gamma-aminobutyric acid-edited MR spectroscopy spectra. J Magn Reson Imaging 2013;40:1145–1152.

16. Harris AD, Puts NAJ, Edden RAE. Tissue correction for GABA-edited MRS: Considerations of voxel composition, tissue segmentation, and tissue relaxations. J Magn Reson Imaging 2015;42:1431–1440.

17. Tsai S-Y, Fang C-H, Wu T-Y, Lin Y-R, Barker PB. Effects of frequency drift on the quantification of gamma-aminobutyric acid using MEGA-PRESS. Sci Rep 2016;6:24564.

18. Krishnamoorthy K, Lee M. Improved tests for the equality of normal coefficients of variation. Comput Stat 2014;29:215–232.

19. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Stat Soc Ser B 1995;57:289–300.

20. Hunter JE, Schmidt FL. Methods of meta-analysis: Correcting error and bias in research findings. Newbury Park, CA: Sage; 1990.

21. Oeltzschner G, Snoussi K, Puts NA, et al. Effects of eddy currents on selective spectral editing experiments at 3T. J Magn Reson Imaging 2018;47:673–681.

22. Edden RAE, Oeltzschner G, Harris AD, et al. Prospective frequency correction for macromolecule-suppressed GABA editing at 3T. J Magn Reson Imaging 2016;44:1474–1482.

23. Hu Y, Chen X, Gu H, Yang Y. Resting-state glutamate and GABA concentrations predict task-induced deactivation in the default mode network. J Neurosci 2013;33:18566–18573.

24. Wiebking C, Duncan NW, Tiret B, et al. GABA in the insula — A predictor of the neural response to interoceptive awareness. NeuroImage 2014;86:10–18.

25. Chen Z, Silva AC, Yang J, Shen J. Elevated endogenous GABA level correlates with decreased fMRI signals in the rat brain during acute inhibition of GABA transaminase. J Neurosci Res 2005;79:383–391.

26. Del-Ben CM, Ferreira CAQ, Sanchez TA, et al. Effects of diazepam on BOLD activation during the processing of aversive faces. J Psychopharmacol Oxf Engl 2012;26:443–451.

27. Walter SA, Forsgren M, Lundengård K, et al. Positive allosteric modulator of GABA lowers BOLD responses in the cingulate cortex. PLoS One 2016;11:e0148737.

28. Tang Y, Hojatkashani C, Dinov ID, et al. The construction of a Chinese MRI brain atlas: A morphometric comparison study between Chinese and Caucasian cohorts. NeuroImage 2010;51:33–41.

29. Xing W, Nan C, ZhenTao Z, et al. Probabilistic MRI brain anatomical atlases based on 1,000 Chinese subjects. PLoS One 2013;8:e50939.

30. Turner BO, Paul EJ, Miller MB, Barbey AK. Small sample sizes reduce the replicability of task-based fMRI studies. Commun Biol 2018;1:62.

31. Geuter S, Qi G, Welsh RC, Wager TD, Lindquist MA. Effect size and power in fMRI group analysis. BioRxiv 2018;295048.

32. Buxton RB, Griffeth VEM, Simon AB, Moradi F, Shmuel A. Variability of the coupling of blood flow and oxygen metabolism responses in the brain: A problem for interpreting BOLD studies but potentially a new window on the underlying neural activity. Front Neurosci 2014;8:139.

33. Winder AT, Echagarruga C, Zhang Q, Drew PJ. Weak correlations between hemodynamic signals and ongoing neural activity during the resting state. Nat Neurosci 2017;20:1761–1769.

34. Myers JFM, Evans CJ, Kalk NJ, Edden RAE, Lingford-Hughes AR. Measurement of GABA using J-difference edited 1H-MRS following modulation of synaptic GABA concentration with tiagabine. Synapse 2014;68:355–362.

35. Anenberg E, Chan AW, Xie Y, LeDue JM, Murphy TH. Optogenetic stimulation of GABA neurons can decrease local neuronal activity while increasing cortical blood flow. J Cereb Blood Flow Metab 2015;35:1579–1586.

36. Jessen SB, Brazhe A, Lind BL, et al. GABAA receptor-mediated bidirectional control of synaptic activity, intracellular Ca2+, cerebral blood flow, and oxygen consumption in mouse somatosensory cortex in vivo. Cereb Cortex 2015;25:2594–2609.

37. Blanchard S, Saillet S, Ivanov A, et al. A new computational model for neuro-glio-vascular coupling: Astrocyte activation can explain cerebral

blood flow nonlinear response to interictal events. PLoS One 2016;11: e0147292.

38. Duncan NW, Wiebking C, Northoff G. Associations of regional GABA and glutamate with intrinsic and extrinsic neural activity in humans: A review of multimodal imaging studies. Neurosci Biobehav Rev 2014;47: 36–52.

39. Muthukumaraswamy SD, Myers JFM, Wilson SJ, et al. Elevating endogenous GABA levels with GAT-1 blockade modulates evoked but not induced responses in human visual cortex. Neuropsychopharmacology 2013;1:1–8.

40. Abraham A, Pedregosa F, Eickenberg M, et al. Machine learning for neuroimaging with scikit-learn. Front Neuroinformatics 2014;8:14.