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On the relationship between GABA+ and glutamate across the brain

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Abstract

Equilibrium between excitation and inhibition (E/I balance) is key to healthy brain function. Conversely, disruption of normal E/I balance has been implicated in a range of central neurological pathologies. Magnetic resonance spectroscopy (MRS) provides a non-invasive means of quantifying *in vivo* concentrations of excitatory and inhibitory neurotransmitters, which could be used as diagnostic biomarkers. Using the ratio of excitatory and inhibitory neurotransmitters as an index of E/I balance is common practice in MRS work, but recent studies have shown inconsistent evidence for the validity of this proxy. This is underscored by the fact that different measures are often used in calculating E/I balance such as glutamate and Glx (glutamate and glutamine). Here we used a large MRS dataset obtained at ultra-high field (7 T) measured from 193 healthy young adults and focused on two brain regions - prefrontal and occipital cortex - to resolve this inconsistency. We find evidence that there is an inter-individual common ratio between GABA+ (γ -aminobutyric acid and macromolecules) and Glx in occipital, but not prefrontal cortex. We further replicate the prefrontal result in a legacy dataset (n=78) measured at high-field (3 T) strength. By contrast, with ultra-high field MRS data, we find extreme evidence that there is a common ratio between GABA+ and glutamate in both prefrontal and occipital cortices, which cannot be explained by participant demographics, signal quality, fractional tissue volume, or other metabolite concentrations. These results are consistent with previous electrophysiological and theoretical work supporting E/I balance. Our findings indicate that MRS-detected GABA+ and glutamate (but not Glx), are a reliable measure of E/I balance.

Keywords

E/I balance; MRS; glutamate; GABA; 7T; ultra-high field

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INTRODUCTION

Balanced excitatory and inhibitory activity (i.e., E/I balance) is a key feature of healthy brain function, as supported by a large body of theoretical (Marín, 2012; Shadlen & Newsome, 1994; Van Vreeswijk & Sompolinsky, 1996; Vogels & Abbott, 2009) and empirical evidence (Anderson et al., 2000; Poo & Isaacson, 2009; Wehr & Zador, 2003; Wilent & Contreras, 2005). E/I balance sharpens neural tuning (Higley & Contreras, 2006; Wilent & Contreras, 2005) in response to external stimulation and maintains equilibrium throughout the brain during periods of spontaneous activity (Okun & Lampl, 2008). By contrast, E/I imbalance is thought to underlie central pathologies such as epilepsy (Bradford, 1995; Olsen & Avoli, 1997), autism spectrum disorder (Chao et al., 2010; Markram & Markram, 2010; Rubenstein & Merzenich, 2003; Vattikuti & Chow, 2010), and schizophrenia (Kehrer et al., 2008) (for a review see (Sohal & Rubenstein, 2019)).

Magnetic resonance spectroscopy (MRS) can be used to measure *in vivo* concentrations of primary excitatory (glutamate, Glu) and inhibitory (γ -aminobutyric acid, GABA) neurotransmitters within the brain. While empirical evidence supporting E/I balance is based on electrophysiological work, it is common practice in MRS studies to report the ratio of these neurotransmitters as an index of this phenomenon. The ratio of GABA+ (GABA and macromolecules) and Glu or Glx (a complex comprising Glu and glutamine (Gln)) has been used to study a range of healthy brain functions including decision-making (Bezael et al., 2019), visual learning (Shibata et al., 2017), volitional control (Koizumi et al., 2018), memory (Bang et al., 2018; Takei et al., 2016), and visual contrast sensitivity (Betina Ip et al., 2019). It has also been used to investigate other neuroimaging signals, such as default mode network activity (Gu et al., 2019; Kapogiannis et al., 2013), and the effectiveness of non-invasive brain stimulation (Filmer et al., 2019). In the context of theories relating neuropsychiatric conditions to E/I imbalance, these neurotransmitters have been identified as potential biomarkers for autism spectrum disorder and schizophrenia (Brown et al., 2013; Egerton et al., 2012; Horder et al., 2013; Smesny et al., 2015).

Steel et al. (2020) found a positive correlation between inter-individual measurements of GABA+ and Glx measured from a voxel targeting posterior cingulate cortex. This result suggests there may be a common ratio between these neurotransmitters and provides support for the validity of interpreting their relative concentrations as an indicator of E/I balance. That is, the relationship between GABA+ and Glx may reflect the concurrent excitatory and inhibitory activity observed electrophysiologically, e.g., in response to external stimulation (Anderson et al., 2000; Wilent & Contreras, 2005). By contrast, Rideaux (2021) found strong evidence against a positive correlation between GABA+ and Glx in both visual and motor cortices, suggesting that the results of Steel et al. (2020) may be region specific and that the ratio between MRS-detected GABA+ and Glx is unrelated to the E/I balance measured in electrophysiological work.

The discrepancy between these findings may be explained by differences in information processing between medial parietal cortex and visual and motor cortices. The function of the visual and motor cortices targeted by Rideaux (2021) is relatively specialised; these regions

primarily support processing of visual input (Hubel & Wiesel, 1959, 1968) and voluntary hand movement (Hlušík et al., 2001; Penfield & Boldrey, 1937), respectively. By contrast, the medial parietal lobe is functionally complex; it is associated with a diverse range of perceptual and cognitive operations, including (but not limited to) visual scene perception (Epstein et al., 2007; Silson et al., 2019), memory recall (Wagner et al., 2005), future prediction (Szpunar et al., 2007), and heading direction (Baumann & Mattingley, 2010). As such, it may benefit from the maintenance of E/I balance at a temporal and/or spatial scale that is detectable with MRS measurements averaged over a ~10 min period.

Alternatively, it is possible that a brain-wide common ratio of GABA and Glu exists, but its presence is obscured by the influence of Gln in the Glx signal. The previous studies by (Rideaux, 2021) and (Steel et al., 2020) reported the relationship between Glx and GABA+ because the Glu signal cannot be effectively isolated from the Gln signal at the MRI field strength (3 T) used in these experiments. If Gln is negatively correlated with GABA, this could mask a positive relationship between GABA and Glu.

To test these possibilities, here we obtained ultra-high field (7 T) MRS data with two sequences, MEGA-semi-LASER (MEGA-sLASER) followed by semi-LASER (sLASER), to measure the relationship between GABA+ and both Glx and Glu from a voxel targeting (functionally complex) prefrontal cortex in a large sample of participants (n=193) using a pre-registered analytic plan. To preview the results, in line with Rideaux (2021), we found strong evidence that there is no positive relationship between GABA+ and Glx in prefrontal cortex. Further, we replicated this finding using a large sample (n=78) of high field strength (3 T) MRS legacy data targeting the same region. By contrast, we found extreme evidence indicating there is a positive correlation between GABA+ and Glu at 7 T. Our control analyses show that this relationship cannot be explained by participant demographics, voxel tissue composition, signal quality, or other metabolites. Finally, we find extreme evidence that both Glx and Glu are positively correlated with GABA+ in occipital cortex. However, consistent with the results from prefrontal cortex, the relationship between GABA+ and Glu is significantly stronger than that with Glx. These findings indicate that there is a brain-wide common ratio between inhibitory and excitatory neurotransmitters detected with MRS, consistent with electrophysiological work. In order to reliably measure this index of E/I balance, however, it is recommended to measure Glu directly, without the influence of Gln.

METHODS

Participants.

One hundred and ninety-three healthy participants (128 women) were included in the MRS experiment. The mean age was 23.1 yr (range, 18–40 yr). In line with recent expert consensus recommendations (Near et al., 2021), data from 22 participants were omitted from analyses due to the presence of severe spurious echoes in the MRS spectra. Participants were screened for contraindications to MRI before the experiment. All participants gave informed written consent, and the experiment was approved by The University of Queensland Human Research Ethics Committee.

Legacy data.

Legacy data from previous studies (doi.org/10.14264/uql.2019.12; (Filmer et al., 2019; Rideaux et al., 2021) were combined. In these studies, male and female human participants underwent MR spectroscopic acquisition targeting prefrontal cortex (n=78; 42 women; mean age=22 yr).

Data Acquisition.

Magnetic resonance scanning was conducted on a Magnetom 7 T research whole-body MRI scanner (Siemens Healthcare, Erlangen, Germany) equipped with a 32-channel head coil (Nova Medical, USA). Anatomical T₁-weighted images were acquired for voxel placement with an MP2-RAGE sequence. For detection of GABA+ and Glx, spectra were acquired using a MEGA-sLASER sequence (Andreychenko et al., 2012): TE=74 ms, TR=7800 ms, bandwidth=4000 Hz; 64 transients of 4096 data points were acquired, 2 water-unsuppressed transients were additionally acquired; a 23.6 ms Gaussian editing pulse was applied at 1.90 (ON) and 7.46 (OFF) ppm. Water suppression was achieved using variable power with optimized relaxation delays (VAPOR; Tká & Gruetter, 2005) and outer volume suppression. For detection of Glu, spectra were acquired using a sLASER sequence (Scheenen et al., 2008): TE=42 ms, TR=7790 ms, spectral width=4000 Hz; 32 transients of 4096 data points were acquired. Additionally, in line with recent expert consensus (Cudalbu et al., 2021), a single inversion recovery metabolite nulling in sLASER (TI=1135 ms) was performed in two subjects to estimate macromolecule contribution; these results were used to constrain metabolite quantification. Automated shimming with 3D GRE brain, followed by FAST(EST)MAP shimming were run in succession. All MRS data were analysed from their Siemens TWIX format. During the MRS acquisition, participants passively viewed a naturalistic film. The acquisition window for the MRS sequences was 1000 ms; however, the signal-to-noise of the free induction decay after 250 ms is very low, so we only used the first 250 ms of the data for each acquisition.

Spectra were acquired from a location targeting prefrontal cortex (Fig. 1a). The voxel (40×26×26 mm³) was positioned on a slice 1.5 mm above the superior margin of the lateral ventricles, with the centre of the volume placed one third of the total anterior/posterior distance, centred halfway between the left lateral border and the midline. The coordinates of the voxel location were used to draw a mask on the anatomical T₁-weighted image to calculate the fractional volume of grey matter, white matter, and cerebrospinal fluid within each voxel. Tissue segmentation was performed using the Statistical Parametric Mapping toolbox for MATLAB (SPM12, www.fil.ion.ucl.ac.uk/spm; Ashburner & Friston, 2005).

We also analysed legacy MRS data from two previous studies (Filmer et al., 2019; Rideaux et al., 2021). These data were collected at different sites, using a 3 T Magnetom Prisma (Siemens Healthcare, Erlangen, Germany) equipped with either a 32- or a 64-channel receive-only head coil (Nova Medical, USA). For detection of GABA+ and Glx, all data were collected using a MEGA-PRESS sequence (Mescher et al., 1998). The data from Rideaux et al. (2021) were collected using the following parameters: TE=68 ms, TR=3000 ms, spectral width=2400 Hz; 256 transients of 2048 data points were acquired. The data from Filmer et al. (2019) were collected using the following parameters: TE=68 ms,

TR=2000 ms, spectral width=2400 Hz; 144 transients of 2080 data points were acquired. For both datasets, a 14.28 ms Gaussian editing pulse was applied at 1.9 (ON) and 7.5 (OFF) ppm and water-unsuppressed transients were additionally acquired. The voxel size ($40 \times 25 \times 30 \text{ mm}^3$) and positioning used by Filmer et al. (2019) were similar to those used in the current experiment, whereas Rideaux et al. (2021) used a smaller voxel ($25 \times 25 \times 25 \text{ mm}^3$) with similar positioning (left hemisphere, above the superior margin of the lateral ventricles; Fig. 1a).

To test the regional specificity of the results obtained from prefrontal cortex data, we analysed MRS data collected from a voxel targeting occipital cortex (Fig. 2a). These data were collected in the same scanning session as the primary dataset, using the same sequences, from a voxel ($40 \times 26 \times 26 \text{ mm}^3$) positioned centrally across the bilateral midline, above and aligned to the calcarine fissure.

Data processing.

Spectral pre-processing and quantification were conducted in MATLAB (The MathWorks, Inc., Matick, MA) using in-house scripts. Prior to alignment, for consistency with previous work (Rideaux, 2021; Steel et al., 2020) and to improve frequency/phase estimation, all edited (MEGA) subspectra were 3-Hz exponential line-broadened zero-filled to a spectral resolution of 0.061 Hz/point. Unedited (sLASER) data were not line-broadened or zero-filled due to constraints imposed by LCModel (Provencher, 1993). Frequency and phase parameter estimates were obtained by modelling the total creatine (tCr; creatine and phosphocreatine) signal, then these parameters were used to align subspectra to a common frequency and phase, as previous work has shown this is the most effective method of alignment for GABA+ quantification (Rideaux et al., 2021). Estimates of signal area and full width at half maximum (FWHM) were also obtained, and subspectra (and their corresponding ON/OFF subspectra) with parameter estimates >2.5 standard deviations (s.d.) from the mean within a scan were omitted from further analysis. Spectra with standard deviation $>.15$ between frequencies 3.2–3.6 were omitted due to contamination by spurious echoes ($n=22$; (Near et al., 2021); note, however, we found the same pattern of results when these spectra were included in the analyses.

For edited data, tCr, tNAA, and total choline (tCho) signal intensity were determined by fitting a Lorentzian model to the average OFF spectra at 3.0 ppm and 2.0 ppm, respectively. The average ON and OFF spectra were subtracted to produce the edited spectrum (Fig. 1b, 2b), from which GABA+ (3 ppm) and Glx (3.8 ppm) signal intensity were modelled. For 7 T data, GABA+ and Glx were fit using singlet and doublet Lorentzian models, respectively. For consistency with our previous work analysing high field data (Rideaux, 2021), doublet Gaussian models were used to fit GABA+ and Glx signals for the 3 T data. Lorentzian and Gaussian models were used to fit the 7 T and 3 T data, respectively, to account for differences in spectral linewidth (Fig. 1c). Water signal intensity was determined by fitting a Lorentzian model to the average water-unsuppressed spectra at 4.9 ppm. The unedited (sLaser) data, in addition to the macromolecule spectra, were analysed using LCModel (Provencher, 1993) to estimate Glu and Gln signal intensity.

Neurochemical signal intensities were calculated as the area of the fitted peak(s) and expressed in institutional units (i.u.) using the unsuppressed water signal as an internal concentration reference. To ensure relationships between metabolites were not introduced or masked by differences in water quantification, the water estimates described above were also used to scale the Glu signal. Due to differences in metabolite quantification between LCModel and our in-house methods, interpretation of the absolute water-scaled Glu values is limited to the current study. We further applied a tissue-correction method that accounts for differences in relaxation times across the tissue types within a voxel (Gasparovic et al., 2006). The assumed longitudinal relaxation times of water, GABA+, Glx, Glu, Gln, tCr, tNAA, and tCho at 7 T were 1.78 s, 1.33 s, 1.69 s, 1.68 s, 1.69 s, 1.76 s, 1.87 s, and 1.76 s, respectively (Andreychenko et al., 2012; Xin et al., 2013). The assumed transverse relaxation times of these metabolites were 0.05 s, 0.09 s, 0.09 s, 0.09 s, 0.09 s, 0.10 s, 0.09 s, 0.15 s, respectively (Andreychenko et al., 2012; Marja ska et al., 2012). The assumed longitudinal relaxation times of water, GABA+ and Glx at 3 T were 1.10 s, 1.31 s, 1.29 s, respectively (Posse et al., 2007; Puts et al., 2013; Wansapura et al., 1999). The assumed transverse relaxation times of these metabolites were 0.10 s, 0.09 s, 0.18 s, respectively (Edden et al., 2012; Ganji et al., 2012; Wansapura et al., 1999). The assumed relative densities of MR-visible water in grey matter, white matter and cerebral spinal fluid were 0.78, 0.65, and 0.97, respectively (Ernst et al., 1993).

Statistical analyses.

Statistical analyses were conducted in MATLAB. We sought to determine whether the concentration of GABA+ and Glx or Glu were positively correlated across participants. To test this, we computed the Pearson correlation between GABA+ and either Glx or Glu for all spectra. Next, as described in our preregistration (https://osf.io/s3kzy/?view_only=519f07b8ada148fd830aeba5c9a4df08), to test whether the relationship between GABA+ and Glu could be explained by confounding factors, we systematically regressed out the influence of variables that could potentially account for observed relationships using a linear mixed effects model. We then computed the Pearson correlation between the residuals after controlling for the confounding factors. We reasoned that if the residual values remained correlated, the relationship between metabolites would hold when controlling for these confounding variables. To establish the influence of confounding factors, we considered each factor separately and calculated the (two-tailed) significance of the change in correlation by comparing *z*-scored correlation coefficients. Correlations were conducted using the Robust Correlations MATLAB toolbox (Pernet et al., 2013), with the (default) box-plot rule used to omit bivariate outliers. This led to differing numbers of exclusions per analysis denoted as a subscript in the reported statistics and the figure keys. Bayesian analyses were conducted in JASP (www.jasp-stats.org; Wagenmakers et al., 2018). Bayes factors were interpreted according to standard convention (Jeffreys, 1961; Table 1).

During data collection, there was an update to the 7T scanner, which enabled subject-specific B1 calibration, whereas before the upgrade we had used a constant reference voltage of 275V (appropriate for an average subject). We found that this change resulted in different average metabolite values before and after the upgrade. Thus, to control for the associated changes, all 7T metabolite values were first corrected for scanner version prior to analyses,

using the linear mixed effects method described above. Similarly, for the 3T legacy data, we used linear mixed effects modelling to control for differences between studies, prior to conducting analyses.

RESULTS

We found strong evidence against a positive relationship between GABA+ and Glx in prefrontal cortex measured at 7 T ($r_{185}=.05$, $BF_{+0}=0.18$; Fig. 3a). We found a positive correlation between these metabolites measured at 3 T; however, as evidenced by the bimodal distribution of estimates from the two legacy datasets, this was likely due to differences between acquisition parameters. After controlling for differences between datasets, we found anecdotal evidence against a positive relationship between prefrontal GABA+ and Glx measured at 3T ($r_{70}=.15$, $BF_{+0}=0.71$; Fig. 3b). By contrast, we found extreme evidence for a positive relationship between GABA+ and Glu ($r_{174}=.43$, $BF_{+0}=9.71e^6$; Fig. 3c).

There are multiple factors that may influence the quantification of GABA+ and/or Glu with MRS. It is possible that the correlation between these neurotransmitters was produced by an additional moderating factor. To test this possibility, we assessed the relationship between GABA+ and Glu after systematically regressing out a range of possible confounding factors.

Age and gender.

The concentrations of GABA+ and Glx tends to be higher in adult males than females (O’Gorman et al., 2011), and decrease with age (Cassady et al., 2019; Gao et al., 2013; Sailasuta et al., 2008). However, we found no significant difference in the relationship between GABA+ and Glu after controlling for age and gender ($z_{176}=0.09$, $p=.928$).

Spectral quality.

Although GABA+ and Glu signals were quantified from different spectra, signal quality is likely to be correlated between scans, e.g., due to participants’ propensity for head movement. This correlated signal quality may explain the relationship between GABA+ and Glu. That is, low signal quality could result in systematic misestimation of both GABA+ and Glu, producing a spurious positive correlation.

To assess this possibility, we regressed out the differences in four direct measures of signal quality (MEGA-sLASER and sLASER frequency drift, tNAA linewidth, and water linewidth) before re-testing the relationship between GABA+ and Glu. Frequency drift can occur from participant head motion or field gradient heating/cooling and can impact quantification of GABA+ by altering the efficiency with which its signal is edited (Harris et al., 2014; Hui et al., 2021). The editing efficiency of GABA+ decreases (modestly) with frequency drift in either direction (Harris et al., 2014; Hui et al., 2021). Similarly, if not properly corrected for, frequency drift can reduce the amplitude of the Glu signal by blurring the averaged spectra. Thus, frequency drift in both scans could produce a positive correlation between GABA+ and Glu. However, we found no significant difference in the relationship between GABA+ and Glu after controlling for frequency drift in either MEGA-sLASER ($z_{175}=0.08$ $p=.939$) or semi-LASER scans ($z_{175}=0.31$, $p=.756$). The linewidth of metabolite

signals provides an indication of signal quality, as larger linewidths are associated with poorer spectral acquisition, e.g., due to participant head motion/geometry or inefficient shimming (Harris et al., 2014). However, we found no significant change in the correlation between GABA+ and Glu after controlling for either water ($z_{175}=0.02$, $p=.982$) or tNAA linewidth ($z_{175}=0.15$, $p=.878$).

Another (indirect) measure of spectral quality is the residual error associated with fitting models to metabolite signals, i.e., fit error. If the fit error is high, this suggests that the model is poorly fitting the signal and can indicate poor signal quality. Thus, we also included the fit error associated with GABA+ and Glu in our regression analysis. However, we found no significant difference in the correlation between GABA+ and Glu after controlling for either GABA+ ($z_{180}=0.66$, $p=.510$) or Glu fit error ($z_{175}=0.19$, $p=.850$).

Tissue composition.

GABA is approximately twice as highly concentrated in grey matter relative to white matter (Petroff et al., 1988), and negligible in cerebrospinal fluid. Although we corrected for voxel tissue composition (Gasparovic et al., 2006), it is possible that inter-individual differences in the fractional tissue composition within the MRS voxel may still have contributed to the relationship observed between GABA+ and Glu. However, we found no significant change in the correlation between these neurotransmitters after controlling for the proportion of grey matter ($z_{175}<0.01$, $p=.998$).

Other neurochemicals.

Previous work reported that the relationship between GABA+ and Glx is moderated by the concentration of other neurochemicals (Rideaux, 2021; Steel et al., 2020). We found no significant change in the relationship between GABA+ and Glu after controlling for tCr ($z_{179}=0.13$, $p=.899$), tNAA ($z_{179}=0.20$, $p=.841$), or tCho ($z_{177}=0.14$, $p=.887$). Similarly, we found no difference in the relationship between GABA+ and Glu after controlling for the concentration of water ($z_{178}=0.12$, $p=.903$).

Regional specificity.

To test the regional specificity of these results, we ran the same analyses on a dataset collected from the same participants from a voxel targeting occipital cortex (Fig. 2). We had not planned to include this dataset in the current study, because the voxel location was approximately midway between those used by Steel et al. (2020) and Rideaux (2021), who found evidence for and against a relationship between GABA+ and Glx, respectively, and thus we considered the data were unlikely to be diagnostic in relation to the original study aims. However, considering the results found for data collected from prefrontal cortex – namely, no relationship between GABA+ and Glx but a significant relationship between GABA+ and Glu – we reasoned that repeating the same analyses on a dataset collected from a different cortical region would provide a test of the regional specificity of the findings.

In contrast to the results from prefrontal cortex, in occipital cortex we found extreme evidence for a positive relationship between both GABA+ and Glx ($r_{183}=.48$, $BF_{+0}=3.50e^9$; Fig. 4a), and GABA+ and Glu ($r_{176}=.79$, $BF_{+0}=4.83e^{36}$; Fig. 4b); however, the strength

of the relationship between GABA+ and Glx was significantly stronger than that with Glx ($z_{176}=5.26$, $p=1.46e^{-7}$). We found stronger evidence for a positive correlation between GABA+ and Glu in occipital than prefrontal cortices ($z_{174}=5.75$, $p=8.68e^{-9}$). This may be due to a difference in the strength of the coupling between these neurometabolites in prefrontal and occipital regions; however, it may also be due to reduced signal quality in the data from prefrontal cortex, e.g., due to differences in voxel location relative to the receiver coils. Consistent with this, we found that NAA linewidth was broader ($t_{192}=5.93$, $p=1.39e^{-8}$) and GABA+ fit error was higher for the prefrontal than the occipital cortex data ($t_{192}=7.72$, $p=3.77e^{-12}$).

We next measured the relationship between GABA+ and Glx and Glu, after controlling for potential confounding factors (Table 2). This analysis revealed that the factor that reduced the correlation between GABA+ and Glx and Glu in occipital cortex the most was the concentration of water (Glx: $z_{181}=1.68$, $p=.092$; Glu: $z_{177}=2.571$, $p=.01$). Importantly, however, evidence for the relationship between GABA+ and Glx and Glu remained extreme even after controlling for all confounding factors.

Glutamate and Glutamine.

The Glx complex comprises Glu and Gln signals, so given that we found a relationship between GABA+ and Glu, we would expect to find a similar relationship between GABA+ and Glx. A possible explanation for why we did not find this in prefrontal cortex is that the relationship between GABA+ and Glu is obscured by the Gln signal, which may be either negatively correlated or uncorrelated with GABA+. In agreement with this, we found that while in occipital cortex GABA+ and Gln were positively correlated ($r_{178}=.25$, $BF_{\pm 0}=47.95$), in prefrontal cortex these metabolites are not ($r_{174}=-.07$, $BF_{\pm 0}=0.51$). Further, we found that while Glu was positively correlated with Glx in both frontal and occipital cortices, Gln was only positively correlated with Glx in prefrontal cortex (prefrontal, $r_{162}=.14$, $BF_{\pm 0}=0.47$; occipital, $r_{187}=.05$, $BF_{\pm 0}=0.11$) anecdotal and substantial evidence that there is no correlation between GABA+ and Gln in either prefrontal or occipital cortices, respectively (prefrontal, $r_{162}=.14$, $BF_{\pm 0}=0.47$; occipital, $r_{187}=.05$, $BF_{\pm 0}=0.11$).

DISCUSSION

Balanced excitatory and inhibitory activity is considered a canonical feature of healthy brain function (Shadlen & Newsome, 1994; Van Vreeswijk & Sompolinsky, 1996). The ratio between MRS-detected excitatory and inhibitory neurotransmitters is frequently used as an index of this balance. However, previous work testing whether there is a common ratio between these neurotransmitters across individuals has provided conflicting evidence (Rideaux, 2021; Steel et al., 2020), challenging the validity of this ratio as a proxy for E/I balance. To resolve these conflicting findings and test the validity of MRS-detected neurotransmitters as a proxy for E/I balance, here we used a large dataset of ultra-high field (7 T) MRS data from two brain regions to address whether i) a correlation between GABA+ and Glx is observed in other functionally complex cortices and ii) the presence of Gln in the Glx signal obscures a positive relationship between GABA and Glu. We found mixed evidence for a positive correlation between GABA+ and Glx. In prefrontal cortex, we found

evidence against a positive relationship and replicated this result in a separate dataset tested at 3 T field strength, while in occipital cortex we found evidence for a positive relationship. By contrast, we found extreme evidence in favour of a positive correlation between GABA+ and Glu in both prefrontal and occipital cortices.

Previous work yielded inconsistent evidence for a common inter-individual ratio between GABA+ and Glx (Rideaux, 2020, 2021; Rideaux et al., 2019; Steel et al., 2020). Steel et al. (2020) found a positive relationship between GABA+ and Glx in the posterior cingulate gyrus, whereas Rideaux (2021) found evidence against a positive correlation in both visual and motor cortices. The discrepancy might be explained by differences in information processing between parietal, and visual and motor cortices. Visual and motor cortices are functionally specialised; early visual cortex supports processing of visual input from the retina via the lateral geniculate nucleus (Hubel & Wiesel, 1959, 1968) and the targeted motor area 1 (M1) hosts the representation of hand and fingers and supports voluntary hand movement (Hluštík et al., 2001; Penfield & Boldrey, 1937). By contrast, the medial parietal lobe behaves as an information processing hub; it is associated with a variety of perceptual and cognitive functions, including visual scene perception (Epstein et al., 2007; Silson et al., 2019), memory recall (Wagner et al., 2005), future prediction (Szpunar et al., 2007), and heading direction (Baumann & Mattingley, 2010). It is possible that a common ratio between GABA+ and Glx can be detected in functionally diverse, but not sensorimotor, cortices. However, we found evidence against a positive correlation between GABA+ and Glx in a region of prefrontal cortex associated with a diverse range of higher cognitive function.

The majority of published MRS work to date has been performed on high field (3 T) MRI scanners, due in part to hardware availability. As a result, MRS studies assessing E/I balance often use the ratio of GABA+ and Glx as an index, because both can be estimated from 3 T spectra produced by difference-edited sequences such as MEGA-PRESS. The Glx complex contains Glu signal, however, it also comprises Gln and GSH signal. Glu is the primary excitatory neurotransmitter in the central nervous system, whereas Gln and GSH have a variety of metabolic functions, including involvement in the synthesis of other metabolites. Previous (inconsistent) evidence on the validity of MRS-detected E/I balance relied on GABA+ and Glx (Rideaux, 2021; Steel et al., 2020). If there is a relationship between MRS-detected GABA and Glu, this relationship may be obscured when testing GABA and Glx, due to the additional metabolite signals that contribute to the Glx complex. Consistent with this, while we found evidence against a positive relationship between GABA+ and Glx in prefrontal cortex, we found extreme evidence for a common ratio between GABA+ and Glu in both prefrontal and occipital cortices. Further, the relationship between GABA+ and Glx in occipital cortex was significantly weaker than that with Glu. A related explanation is that the Glx signal estimated from MEGA edited sequences does not produce a sufficiently precise measurement of Glu to reliably detect a relationship with GABA+. These sequences have longer TEs (68–80 ms), which are optimized for GABA acquisition, whereas unedited sequences such as sLASER and PRESS use shorter TEs (30–42 ms), which are optimized for Glu or Glx acquisition.

It is possible that unlike GABA+ and Glx, the relationship between GABA+ and Glx is regionally specific. However, given we found a stronger positive correlation between GABA+ and Glu than Glx in occipital cortex, and the relationship between GABA+ and Glu in occipital cortex was also stronger than that the same relationship in prefrontal cortex, this suggests that the signal of the prefrontal data was not sufficiently strong to detect a putative relationship between GABA+ and Glx. Indeed, we found evidence that the linewidth and modelling residuals were both poorer for the prefrontal data. This would explain previous inconsistent findings; that is, if the relationship between GABA+ and Glx is significantly weaker than GABA+ and Glu, for the abovementioned reasons, it may not be a reliable marker of E/I balance.

Previous MRS work on the existence of a common ratio between inhibitory and excitatory neurotransmitters has produced conflicting evidence (Rideaux, 2021; Steel et al., 2020). Here we resolved this issue by demonstrating the existence of a regionally non-specific common ratio between GABA+ and Glu. By contrast and in line with previous inconsistent evidence (Rideaux, 2021; Steel et al., 2020), we found mixed and weaker evidence for a common ratio between GABA+ and Glx across individuals. These results are consistent with previous empirical (electrophysiological) (Anderson et al., 2000; Poo & Isaacson, 2009; Wehr & Zador, 2003; Wilent & Contreras, 2005) and theoretical work (Marín, 2012; Shadlen & Newsome, 1994; Van Vreeswijk & Sompolinsky, 1996; Vogels & Abbott, 2009) supporting E/I balance. Our findings indicate that MRS-detected GABA+ and Glu (but not Glx), can be used as a reliable indicator of E/I balance.

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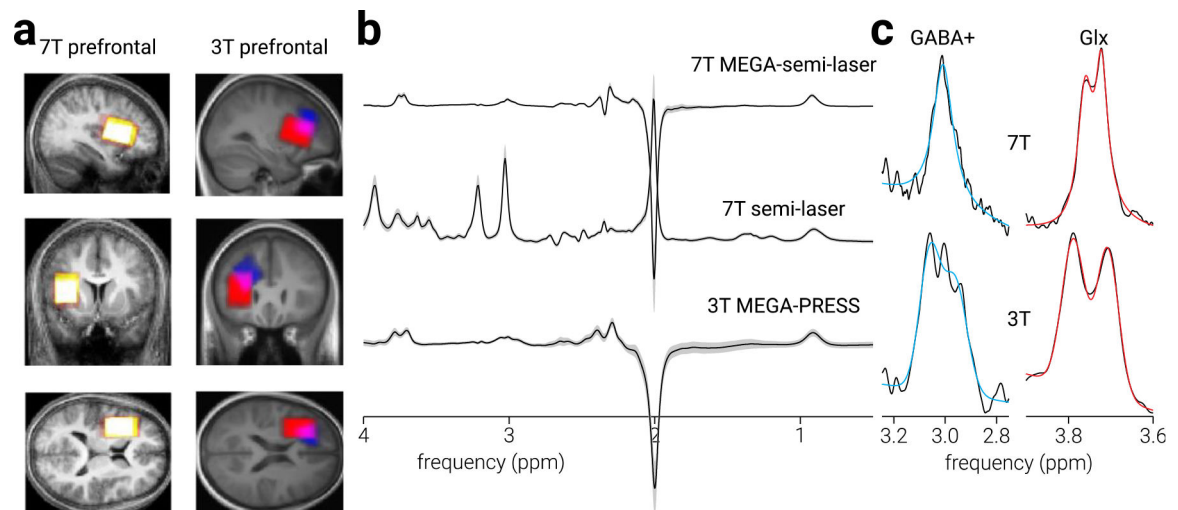


Figure 1. Prefrontal cortex voxel location and spectra.

a) Sagittal, coronal, and axial views of prefrontal cortex MRS voxel placement in the current (7 T) experiment and the legacy experiments, on the average T_1 -weighted structural image in Montreal Neurological Institute (MNI) space. Colour intensity indicates voxel placement overlap across participants; for the 3 T data, red indicates voxels from Filmer et al. (2019) and blue indicates voxels from Rideaux et al. (2021). **b)** Average sLASER, MEGA-sLASER and MEGA-PRESS spectra for all participants. Shaded regions indicate ± 1 standard deviation. **c)** Examples of GABA+ and Glx model fit to the difference-spectra for 7 T and 3 T data.

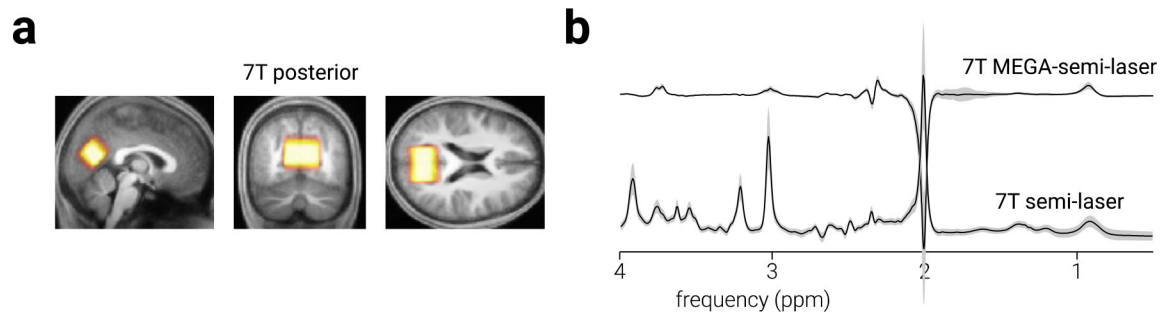


Figure 2. Occipital cortex voxel location and spectra.

a) Sagittal, coronal, and axial views of occipital cortex MRS voxel placement on the average T_1 -weighted structural image in MNI space. Colour intensity indicates voxel placement overlap across participants. **b)** Average sLASER and MEGA-sLASER spectra for all participants. Shaded regions indicate ± 1 standard deviation.

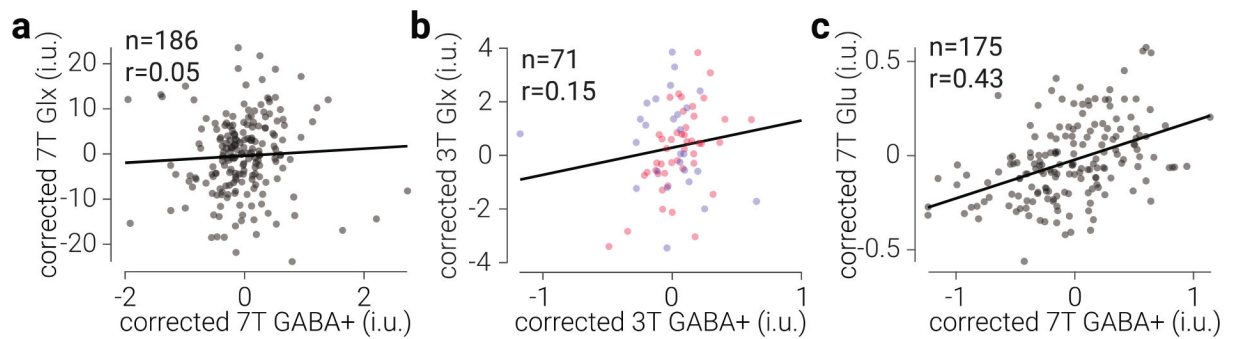


Figure 3. Common ratio between GABA+ and Glu, but not Glx, in prefrontal cortex.

a) Glx concentration as a function of GABA+ concentration in prefrontal cortex, measured at 7 T. **b)** The same as (a), but measured at 3 T. **d)** Glu concentration as a function of GABA+ concentration in prefrontal cortex, measured at 7 T. Glx, Glu, and GABA+ values are tissue-corrected, referenced to water, expressed in institutional units (i.u.), and corrected for changes associated with different scanner software (7T) or studies (3T). Black lines indicate best linear fit; datapoints are semi-transparent, thus darker regions indicate overlap. Note, differences between quantification methods produced differential scaling of GABA+ and Glx relative to Glu concentration.

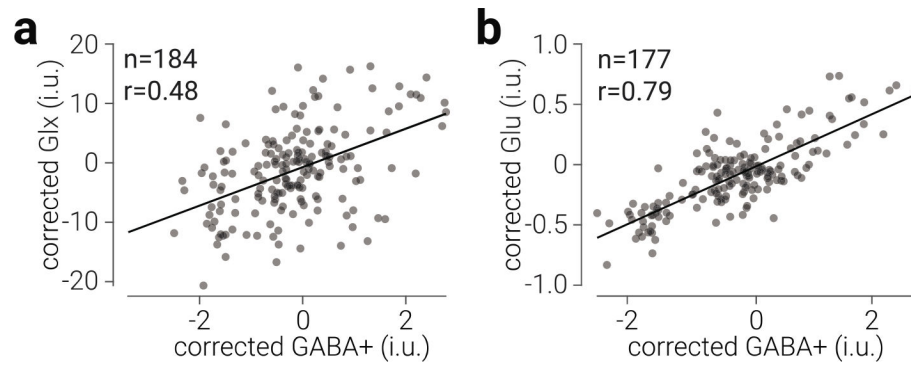


Figure 4. Common ratio between GABA+ and Glu and Glx in occipital cortex.

a) Glx concentration as a function of GABA+ concentration in occipital cortex. **b)** The same as **(a)**, for Glu and GABA+. Glx, Glu, and GABA+ values are tissue-corrected, referenced to water, expressed in institutional units (i.u.), and corrected for changes associated with different scanner software. Black lines indicate best linear fit; datapoints are semi-transparent, thus darker regions indicate overlap.

Table 1.

Evidence categories of Bayes Factors

Bayes factor, BF_{10}	Interpretation
>100	Extreme evidence for H_1
30 – 100	Very strong evidence for H_1
10 – 30	Strong evidence for H_1
3 – 10	Substantial evidence for H_1
1 – 3	Anecdotal evidence for H_1
1	No evidence
1/3 – 1	Anecdotal evidence for H_0
1/10 – 1/3	Substantial evidence for H_0
1/30 – 1/10	Strong evidence for H_0
1/100 – 1/30	Very strong evidence for H_0
<1/100	Extreme evidence for H_0

Table 2.

Probability of positive correlation between GABA+ and Glu and Glx in occipital cortex

Confounding factor	Coefficient (r) (Glx Glu)	Bayes factor BF_{+0} (Glx Glu)
none	.48 .79	3.50e ⁹ 4.83e ³⁶
age and sex	.46 .82	3.07e ⁸ 6.80e ⁴⁰
GABA+ frequency drift	.46 .80	3.00e ⁸ 1.06e ³⁸
Glu frequency drift	.49 .83	1.92e ¹⁰ 5.85e ⁴²
water linewidth	.50 .80	2.67e ¹⁰ 8.84e ³⁷
tNAA linewidth	.46 .80	2.90e ⁸ 2.48e ³⁷
GABA+ fit error	.40 .84	1.07e ⁶ 2.61e ⁴³
Glx/Glu fit error	.54 .81	4.53e ¹² 4.92e ³⁹
grey matter	.47 .76	2.36e ⁹ 3.69e ³³
tCr concentration	.37 .82	7.56e ³ 2.83e ⁴⁰
tNAA concentration	.51 .83	2.50e ¹⁰ 9.69e ⁴¹
Cho concentration	.45 .82	1.13e ⁸ 3.20e ³⁹
water concentration	.33 .67	6.63e ² 4.04e ²¹

Note: BF_{+0} denotes $p(H_1)/p(H_0)$, where the alternate hypothesis (H_1) is that there is a positive correlation between GABA+ and Glu; all Bayes factors indicate extreme evidence for the alternative hypothesis (Jeffreys, 1961).