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Measurement of GABA and glutamate-glutamine levels with ¹H-magnetic resonance spectroscopy in the neocortex of healthy subjects: the influence of age, sex, and education.

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ABSTRACT

INTRODUCTION: The two main neurotransmitters in the human brain are excitatory glutamate and inhibitory gamma-aminobutyric acid (GABA). Measuring their levels in the living brain is an important readout for studies investigating the pathophysiology of psychiatric disorders. However, numerous factors may influence neurotransmitter levels; therefore, it is important to determine their effects. Here, we studied the effects of age, sex, and education level on glutamate and GABA levels.

METHODS: A total of 69 healthy subjects, aged 18-49 years were investigated, with a sex ratio of 29 males / 40 females. GABA+ and glutamate + glutamine (Glx) levels were measured using a MEGA-PRESS sequence at 3T in the anterior cingulate cortex (ACC). Water- and creatine-referenced GABA+ and Glx measurements were performed to compare different referencing and correction approaches and examine the influence of demographic factors.

RESULTS: We found a significant influence of sex, age, and education level on neurotransmitter levels. Glx levels were higher in the ACC in men than in women, whereas GABA+ levels were similar between the sexes. Age was positively correlated with GABA+ levels in the ACC in the entire cohort. In contrast, Glx levels were negatively correlated with age in the ACC of the entire cohort and in women only. Education level was negatively correlated with Glx levels in the ACC in men.

CONCLUSION: Our data support the notion that both age and sex affect glutamate and GABA levels in the human brain. Furthermore, our results imply that MRS studies should also consider the education level of subjects.

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Keywords: anterior cingulate cortex; cognition; Glx; MEGA-PRESS; MRS; γ -aminobutyric acid

1. INTRODUCTION

Normal functioning of the human brain is based on the fine-tuned balance between the two major neurotransmitters, excitatory glutamate (Glu) and inhibitory gamma aminobutyric acid (GABA). Glutamate is an immediate precursor of GABA, and Glu is synthesized from glutamine (Gln). These three metabolites participate in complex neurotransmitter cycling involving numerous enzymes and transporters in both neurons and astrocytes (Bak et al. 2006). Both GABA and Glu play multiple roles. Among others, Glu is critically involved in neuroplasticity (Mattson 2008) and neurotoxicity (Choi 1988), whereas the GABAergic system plays a key role in cognitive functions, such as learning and memory (Koh et al. 2023) and orchestrating neuronal networks (Buzsáki & Chrobak 1995). Disruption of this excitatory/inhibitory balance has been implicated in numerous neuropsychiatric disorders, including schizophrenia-spectrum disorders (Merritt et al. 2021a; Nakahara et al. 2021), depressive and anxiety disorders (Pollack et al. 2008; Duman et al. 2019; Sarawagi et al. 2021; Tao et al. 2025), autism spectrum disorders (Horder et al. 2018), epilepsy (Sarlo & Holton 2021), dementia (Ebrahimi et al. 2025), and others (Maddock & Buonocore 2011; Zahid et al. 2023; Zhang et al. 2024). Therefore, measuring Glu and GABA levels in vivo is an important readout for studies investigating the physiology of neurocognitive and psychiatric disorders and the effectiveness of therapeutic strategies.

Proton magnetic resonance spectroscopy (^1H -MRS) is a powerful, non-invasive method that enables the simultaneous detection of in vivo metabolites in the brain including GABA and Glu. As Glu and Gln peaks partially overlap in the MR spectrum, the sum of Glu and Gln (Glx) is often reported as a measure of glutamatergic metabolite concentrations. Compared to other brain metabolites, the concentration of GABA in the human brain is relatively low ($\sim 1\text{--}2\text{ mM}$); therefore, conventional MR spectroscopy (MRS) techniques only allow the measurement of macromolecules and metabolites that are present in much higher concentrations in the brain, such as Glx, N-acetyl aspartate (NAA), choline (Cho), and creatine (Cr). The measurement of GABA is particularly demanding, not only because of its low concentration but also because the GABA peak in the spectra overlaps with neighboring metabolites with much higher concentrations, such as NAA or Cr (Puts & Edden 2012; Kanagasabai et al. 2024). Several strategies have been developed for selectively editing GABA resonance from the complicated spectral background, but currently, probably the most widely used technique for measuring GABA is the MESHcher-GARwood Point RESolved Spectroscopy (MEGA-PRESS) sequence which uses J-editing pulses and is specifically designed for the detection of low-concentration

J-coupled metabolites (Mescher et al. 1998)(Mullins et al. 2014). In the MEGA-PRESS sequence, edit-ON pulses are applied at 1.9 ppm to refocus GABA coupling, while edit-OFF acquisitions (usually applied at 7.5 ppm) allow the spin system to evolve freely. Subtracting the edit-OFF from the edit-ON spectra isolates the GABA signal at 3 ppm, but also co-edits macromolecular resonances (which can contribute up to ~50% of the measured peak), producing the composite GABA+ signal. Incorporating a 1.5 ppm pulse in the edit-OFF condition can theoretically co-edit macromolecules equally in both halves of the experiment, allowing their subtraction; however, this approach increases sensitivity to experimental instability (Mikkelsen et al. 2017; Mikkelsen et al. 2019; Peek et al. 2023). While the primary target of MEGA-PRESS techniques is GABA, co-edited signals from Glu and Gln can also be detected, allowing for the estimation of a combined Glx signal as a secondary outcome in the same acquisition. As both metabolites are increasingly examined under different conditions, their measurement reliability represents a critical prerequisite for meaningful interpretation (Sanaei Nezhad et al. 2018; Maddock et al. 2018).

Fundamental observations made it clear that the living human brain is chemically heterogeneous and that brain metabolite concentrations, including Glu and GABA, are age- and sex-dependent, with additional variability across brain regions (Grachev & Apkarian 2000; Gao et al. 2013a; Grachev & Vania Apkarian 2001; Zuppichini et al. 2024; Porges et al. 2021; Hädel et al. 2013; Aufhaus et al. 2013; Epperson et al. 2005; O’Gorman et al. 2011; Simpson et al. 2012). The chemical heterogeneity of the brain may be further influenced by the subtle effects of cognition on GABA and glutamatergic metabolism, emphasizing that brain neurochemistry is shaped by numerous interacting factors (Zahr et al. 2012; Li et al. 2024). However, the findings remain controversial, as others have reported that while uncorrected GABA levels are significantly lower in older adults, this age-related effect is absent when GABA levels are corrected for voxel composition (Maes et al. 2018). This result implies that age-related differences in GABA levels are, at least partly, driven by age-related gray matter (GM) loss. Consequently, different tissue correction methods are essential for the accurate quantification of GABA and Glx levels, as they account for the varying contributions of GM, white matter (WM), and cerebrospinal fluid (CSF) within the MRS voxel (Harris et al. 2015). More sophisticated correction strategies that utilize unsuppressed water as a reference signal acquired from a separate acquisition enable further adjustments to metabolite signals (Harris et al. 2015; Gasparovic et al. 2006). Conversely, employing an endogenous metabolite signal, such as Cr, as a reference for determining metabolite ratios eliminates the necessity for separate water

acquisition and may mitigate error propagation associated with more complex signal-scaling procedures. However, this approach may compromise signal stability, particularly in the context of neurological or psychiatric conditions (Mikkelsen et al. 2019).

Building on these considerations, and to further elucidate methodological aspects, this study was designed to address two objectives. First, we evaluated how different referencing and correction approaches, including unsuppressed water-based correction strategies, influenced the quantification of GABA+ and Glx levels in the ACC region. Second, we examined the influence of demographic factors, specifically age, sex, and years of education on the creatine-referenced GABA+ and Glx levels in the ACC.

2. MATERIALS AND METHODS

2.1. Subjects

A total of sixty-nine healthy adults (40 females; mean age = 25.67 ± 6.75 ; age range 18-49 years) were recruited for this study. The cohort was divided into two study groups to explore the two research questions.

- 1) Thirty volunteers participated in the first experiment, with 21 females; mean age = 27.07 ± 7.84 ; age range 18-49 years. Both water- and creatine-reference signals were examined in the ACC to assess how different referencing and correction approaches influenced the quantification of GABA+ and Glx metabolite levels.
- 2) The second investigation involved the complete cohort, i.e. sixty-nine healthy adults, including participants from the first study group. Here, we evaluated the influence of sex, age, and years of education using Cr as the sole reference signal and examined the correlations between these demographic parameters and GABA+/Cr and Glx/Cr ratios.

The following exclusion criteria for participation were applied: head injury, current substance use, history of internal medical or neurological disorders, and any MR scanning issues (e.g., claustrophobia or metal objects in the body). Scans were scheduled at the same time of day for each participant to minimize the diurnal variability. Participants provided information on their demographic characteristics and education level (Table 1) and were instructed to refrain from caffeine and alcohol consumption for 12 h before the examination. All participants were Caucasian, native Hungarian speakers, and lived in the urban and suburban areas of Pécs. Participants provided written informed consent after being fully informed about the study objectives, procedures, potential risks and benefits, and their right to withdraw at any time

without any consequences. The study design and protocol were performed in accordance with the Declaration of Helsinki and approved by the Regional Research Ethics Committee of the University of Pécs (Ethical Approval No. 2018/7371).

Table 1.

Demographic characteristics of the participants

	First study group (<i>n</i> = 30)	Second study group (<i>n</i> = 69)
Age (years)	27.07 ± 7.84 (18-49)	25.67 ± 6.75 (18-49)
Females (%)	70	58
Education (years)	13.70 ± 3.00 (8-19)	13.45 ± 3.09 (8-21)

Data are expressed as mean ± SD (range).

2.2. *Magnetic resonance imaging*

Magnetic resonance imaging data were collected using a 3T Siemens Magnetom Prisma Fit (Siemens Healthineers, Erlangen, Germany) scanner with a 64-channel receive-only head and neck coil. The scanning protocol included an isotropic T1-weighted 3D magnetization prepared rapid gradient echo (MPRAGE) sequence with the following parameters: repetition time/inversion time/echo time (TR/TI/TE) = 2530/1100/3.37 ms; flip angle = 7°; 176 sagittal slices with a thickness of 1 mm; field of view (FOV) = 256 mm × 256 mm²; matrix size = 256 × 256; receiver bandwidth = 200 Hz/pixel; number of averages = 1. Proton GABA+ and Glx measurements were acquired with a MEGA-PRESS sequence using the following parameters: TE = 68ms, TR = 1500 ms, spectral bandwidth = 1200 Hz, 512 data points, total number of signal averages = 128, FOV: 30 × 30 × 25 mm³, total scan duration = 6 min 30 s. The editing pulse (duration: 13.8 ms; bandwidth: 49 Hz; delta frequency: -2.8ppm) was applied at 1.9 ppm for the edit-ON condition and 7.5 ppm for the edit-OFF condition. Each reported spectrum was obtained by subtracting the edit-OFF data from the edit-ON data. A modified Water suppression Enhanced through T₁ effects (WET) approach was applied using a CHEMical Shift Selective (CHESS) pulse to achieve water suppression. Unsuppressed water reference spectra were collected for eddy current correction and quantification of the absolute concentrations. Supplementary Table 1 summarizes the complete set of parameters used for the MRS acquisition.

2.3. Spectral Processing

MEGA-PRESS spectral preprocessing and quantification were performed by S. A. N and Zs. K. using the Gannet 3.1.5 software pipeline (Edden et al. 2014), both with 7 years of experience in MRS data acquisition and evaluation. The data were first loaded, eddy-current corrected, zero-order phase corrected, 3 Hz exponential line-broadened, and zero-filled to a spectral resolution of 0.061 Hz/point. Frequency and phase alignment of acquisitions (Mikkelsen et al. 2020) was performed, followed by the subtraction of the ON and OFF series to create difference (DIFF) spectra. Subsequently, GABA+ and Glx in the DIFF spectra, Cr in the edit-OFF spectra, and unsuppressed water peak signals (if available) were fitted to the data. The GABA+ and Glx signals at ≈ 3.0 and ≈ 3.75 ppm were fitted using a three-Gaussian model with a linear slope and nonlinear baseline. When modeling the GABA+ peak, the effects of Cho subtraction artifacts were reduced by down-weighting the signal between 3.16 and 3.285 ppm. For each participant, the percentage fit errors (computed as the combined quadrature sum of the metabolite and reference signal model fit errors [GABA+,water; GABA+,Cr; Glx,water; and Glx,Cr]), the linewidth of water and Cr, the signal-to-noise ratio (SNR, defined as the signal model amplitude divided by twice the standard deviation of noise signal) for GABA+ and Glx and the water frequency full width at half maximum (FWHM) during manual shimming before acquisition were determined and used for evaluating the quality of the spectra. Participants were excluded if the percentage fit errors of GABA+ or Glx exceeded 20% or if the linewidths of Cr (>20 Hz) or water (>25 Hz) exceeded the established cut-off values. The voxels were superimposed on the 3D T1-weighted MPRAGE images by Gannet using modules from Statistical Parametric Mapping (SPM12), and tissue segmentation of the spectroscopy voxels was performed based on the MPRAGE images. Thereafter, the fractions of GM (f_{GM}), WM (f_{WM}), and CSF (f_{CSF}) were determined (Ashburner & Friston 2005). Uncorrected signal integral ratios ($c_{uncorrected}$) of GABA+ and Glx (I_M) and the metabolite reference signals (I_{Ref}) water or Cr in all tissue compartment (f_{GM}, f_{WM}, f_{CSF}) were derived from Eq. 1.

$$c_{uncorrected} = \frac{I_M}{I_{Ref}} \quad \text{Eq. 1.}$$

The CSF-corrected GABA+ and Glx metrics (c_a) relative to water (if available) and creatine (Cr) were estimated using the following equations:

$$c_a = \frac{c_{uncorrected}}{f_{GM} + f_{WM}} \quad \text{Eq. 2.}$$

The GABA+ and Glx measures using $c_{uncorrected}$ (see Eq. 1.) are defined as GABA+/Water uncorrected or GABA+/Cr uncorrected and Glx/Water uncorrected or Glx/Cr uncorrected, whereas the metrics derived from Eq. 2. are denoted as GABA+/Water_a or GABA+/Cr_a and Glx/Water_a or Glx/Cr_a, respectively (Harris et al. 2015).

2.4. Quantification of brain metabolites

GABA+ and Glx levels were quantified in Gannet using three approaches, each employing the unsuppressed water signal as a reference.

In the first approach, metabolite concentrations were estimated while correcting for the partial volume effects of water. This correction followed the method proposed by Gasparovic et al., as detailed in Eq. 3. (Gasparovic et al. 2006).

$$C_b = \frac{I_M}{I_W} \cdot \frac{H_W}{H_M} \cdot \frac{MM}{\kappa} \cdot C_W \cdot \frac{\sum_i^{GM,WM,CSF} f_i \beta_i \exp\left(-\frac{TE_W}{T_{2W,i}}\right) \left[1 - \exp\left(-\frac{TR_W}{T_{1W,i}}\right)\right]}{(1-f_{CSF}) \exp\left(-\frac{TE_M}{T_{2M}}\right) \left[1 - \exp\left(-\frac{TR_M}{T_{1M}}\right)\right]}, \quad \text{Eq.3.}$$

where C_b is the estimated metabolite concentration in institutional units corrected for the partial volume effects of water, I_M and I_W are the metabolite and water signal integrals, respectively, H_M and H_W are the number of ^1H protons that give rise to the metabolite and water signals, respectively, MM is the correction factor for the contribution of the co-edited macromolecule signal in the metabolite signal (0.45 for GABA+ and 1 for other metabolites), κ is the editing efficiency, C_W is the molecular concentration of pure water (55.51 mol/kg), f_i is the voxel volume fraction of GM, WM, or CSF, β_i is the relative MR visibility of water in GM, WM, or CSF (0.78, 0.65, and 0.97, respectively), TE_W and TE_M are the echo times of the water reference and metabolite acquisitions, respectively, TR_W and TR_M are the repetition times of the water reference and metabolite acquisitions, respectively, $T_{2W,i}$ (0.110, 0.0792, and 0.503 s) and $T_{1W,i}$ (1.331, 0.832, and 3.817 s) are the transverse and longitudinal relaxation times in GM, WM, or CSF, respectively, and T_{2M} and T_{1M} are the transverse and longitudinal relaxation times of the metabolite. The GABA+ and Glx concentrations were calculated using Eq. 3. denote GABA+/Water_b and Glx/Water_b, respectively.

In the second approach, metabolite concentrations were estimated with corrections for both water and metabolite partial volume effects, following the method proposed by Harris et al., as described in Eq. 4. (Harris et al. 2015).

$$C_c = \frac{I_M}{I_W} \cdot \frac{H_W}{H_M} \cdot \frac{MM}{\kappa} \cdot \frac{\sum_i^{GM,WM,CSF} f_i C_{W,i} \exp\left(-\frac{TE_W}{T_{2W,i}}\right) \left[1 - \exp\left(-\frac{TR_W}{T_{1W,i}}\right)\right]}{\exp\left(-\frac{TE_M}{T_{2M}}\right) \left[1 - \exp\left(-\frac{TR_M}{T_{1M}}\right)\right]} \cdot \frac{1}{f_{GM} + \alpha f_{WM}}, \quad \text{Eq.4.}$$

where C_c is the estimated metabolite concentration in institutional units corrected for the partial volume effects of water and metabolite, $C_{W,i}$ is the molal concentration of water in GM, WM, or CSF (43.30, 36.08, and 53.84 mol/kg, respectively), and α is the ratio of intrinsic WM:GM metabolite concentrations. The GABA+ and Glx concentrations were calculated using Eq. 4. and are denoted as GABA+/Water_c and Glx/Water_c, respectively.

In the third approach, metabolite concentrations were estimated with corrections for both water and metabolite partial volume effects and were additionally adjusted to the average GM and WM composition derived from the included participants, as described in Eq. 5. (Harris et al. 2015).

$$C_d = \frac{I_M}{I_W} \cdot \frac{H_W}{H_M} \cdot \frac{MM}{\kappa} \cdot \frac{\sum_i^{GM,WM,CSF} f_i C_{W,i} \exp\left(-\frac{TE_W}{T_{2W,i}}\right) [1 - \exp\left(-\frac{TR_W}{T_{1W,i}}\right)]}{\exp\left(-\frac{TE_M}{T_{2M}}\right) [1 - \exp\left(-\frac{TR_M}{T_{1M}}\right)]} \cdot \frac{\mu_{GM} + \alpha \mu_{WM}}{(f_{GM} + \alpha f_{WM})(\mu_{GM} + \mu_{WM})}, \quad \text{Eq.5.}$$

where C_d is the estimated metabolite concentration in institutional units corrected for the partial volume effects of water and metabolite and adjusted to the average voxel GM and WM composition across our group, and μ_{GM} and μ_{WM} are the group-averaged voxel volume fractions of GM and WM derived from the included participants. The GABA+ and Glx concentrations were calculated using Eq. 5. denote GABA+/Water_d and Glx/Water_d, respectively.

2.5. Statistics

Statistical analyses were performed using SPSS software (version 29.0.2.0, IBM Inc., Armonk, NY, USA Armonk, NY). For the first research question, Spearman's rank-order correlations were conducted to assess the correlation of uncorrected and CSF-corrected metabolite ratios referenced to water versus Cr (e.g., GABA+/Water vs. GABA+/Cr; Glx/Water vs. Glx/Cr), as well as to evaluate the correspondence among water-referenced metrics obtained using uncorrected, CSF-corrected, and quantitative approaches for GABA+ and Glx.

Thereafter, for the second research question, the potential effects of age and years of education on creatine-referenced metabolite ratios (i.e., GABA+/Cr and Glx/Cr with and without CSF correction) were tested using stepwise multiple linear regression analyses. In cases where no significant independent variables were identified by the stepwise procedure, p-values and standardized beta coefficients were derived using the enter method with a single predictor (either age or years of education). The assumptions of multiple linear regression were satisfied, as judged by checking for independence of errors, linearity, homoscedasticity, multicollinearity, outliers, and residue normality. Potential sex differences in creatine-referenced metabolites

were evaluated using the Mann–Whitney U test for both uncorrected and CSF-corrected measurements. For all statistical procedures, results were considered statistically significant at $p < 0.05$.

2.6. Data availability statement

All data generated or analysed during this study are included in this published article [and its supplementary information files]."

3. RESULTS

3.1. Participants of the two studies

In the first study, four participants were excluded due to inadequate reference signal fitting, and one was excluded because the linewidth of Cr exceeded the cut-off of 20 Hz. The final dataset included 25 participants (17 females; mean age = 27.72 ± 7.89 ; range = 18–49 years), for whom creatine- and water-referenced data from the ACC were analyzed and correlated.

In the second study, four participants were excluded due to inadequate reference signal fitting, two participants were excluded owing to severe motion artifacts affecting the 3D T1-weighted MPRAGE images, three subjects were excluded because the percentage fit error of GABA+ and Glx exceeded 20%, and one was excluded because the linewidth of Cr exceeded the cut-off of 20 Hz. The final sample comprised 59 participants (35 females; mean age = 25.25 ± 6.68 ; range = 18–49 years), and their creatine-referenced metabolite data from the ACC were analyzed.

No structural abnormalities or potential pathological lesions were observed on structural MRI scans.

3.2. Spectral quality control

Representative examples of reference signal fitting (water and creatine) and spectral processing of GABA and the combined glutamate and glutamine (Glx) signal are displayed on Figure 1.

In the first study, the FWHM values in Hz were 12.57 (11.69–14.16) for water and 12.12 (10.96–13.48) for creatine, while the water FWHM obtained during manual shimming was 18.00 (16.85–19.95) Hz. The percentage fitting errors were as follows: GABA+,water = 4.93 (4.17–5.59), GABA+,Cr = 5.05 (4.34–5.90), Glx,water = 5.51 (3.63–7.58), and Glx,Cr = 5.62 (3.91–7.78). Tissue fractions for GM, WM, and CSF were 0.56 (0.52–0.58), 0.28 (0.26–0.31),

and 0.13 (0.15–0.18), respectively. The SNR for GABA+ and Glx were 28 (21–34) and 25 (19–31.5), respectively.

In the second study, the FWHM values in Hz were 11.40 (10.61–12.30) for creatine, whereas the water FWHM obtained during manual shimming was 16.90 (15.58–18.70) Hz. The percentage fitting errors were as follows: GABA+,Cr = 5.03 (4.30–6.80) and Glx,Cr = 5.58 (3.80–7.36). Tissue fractions for GM, WM, and CSF were 0.55 (0.53–0.57), 0.29 (0.27–0.32), and 0.15 (0.14–0.17), respectively. The SNR for GABA+ and Glx were 28 (23–33) and 28 (23–33), respectively. All values are reported as medians with interquartile ranges (25th–75th percentiles).

3.3. First study: Water and creatine reference consistency

The water-referenced signal integral ratios of both GABA+ and Glx exhibited robust positive correlations with their creatine-referenced counterparts in the ACC ($p < 0.001$, $n=25$; see Table 2).

Table 2.

Correlations between water- and creatine-referenced measures of GABA+ and Glx in ACC, evaluated with and without corrections for cerebrospinal fluid.

Metabolite	Data n=25	Spearman's correlation p-value (rho)
GABA+/Water uncorrected	0.7819 (0.6630-1.0248)	p<0.001 (0.8775)
GABA+/Cr uncorrected	0.1088 (0.0970-0.1340)	
GABA+/Water_a	0.9150 (0.7864-1.2060)	p<0.001 (0.8962)
GABA+/Cr_a	0.1280 (0.1161-0.1569)	
Glx/Water uncorrected	1.6194 (1.4834-2.0753)	p<0.001 (0.8202)
Glx/Cr uncorrected	0.0708 (0.0580-0.0780)	
Glx/Water_a	1.9683 (1.7959-2.4712)	p<0.001 (0.7862)
Glx/Cr_a	0.0824 (0.0694-0.0934)	

Data is presented in median (interquartile range). Water- and creatine-referenced metabolite measurements are signal integral ratios.

_a Estimated metabolite ratio corrected for cerebrospinal fluid fraction.

Abbreviations: GABA+: gamma Aminobutyric acid plus co-edited macromolecules; Glx: combined glutamate and glutamine signal; ACC: anterior cingulate cortex; Cr: Creatine

Within the same subject cohort and across all water-referenced correction methods, GABA+ and Glx exhibited strong intercorrelations ($\rho > 0.97$; $p < 0.001$; Table 3), reflecting the high consistency between the different water-based correction methods used.

Table 3.

Correlations between corrected and uncorrected water-referenced measures of GABA+ and Glx in the ACC.

Metabolite	Spearman's correlation p-value (rho) n=25			
	GABA+/Water _a	GABA+/Water _b	GABA+/Water _c	GABA+/Water _d
GABA+/Water uncorrected	p<0.001 (0.9946)	p<0.001 (0.9946)	p<0.001 (0.9931)	p<0.001 (0.9931)
GABA+/Water_a		p<0.001 (1.0000)	p<0.001 (0.9977)	p<0.001 (0.9977)
GABA+/Water_b			p<0.001 (0.9977)	p<0.001 (0.9977)
GABA+/Water_c				p<0.001 (1.0000)
	Glx/Water_a	Glx/Water_b	Glx/Water_c	Glx/Water_d
Glx/Water uncorrected	p<0.001 (0.9808)	p<0.001 (0.9762)	p<0.001 (0.9777)	p<0.001 (0.9777)
Glx/Water_a		p<0.001 (0.9992)	p<0.001 (0.9923)	p<0.001 (0.9923)
Glx/Water_b			p<0.001 (0.9931)	p<0.001 (0.9931)
Glx/Water_c				p<0.001 (1.0000)

_a Estimated metabolite ratio corrected for cerebrospinal fluid fraction.

_b Estimated metabolite concentration corrected for partial volume effects of water.

_c Estimated metabolite concentration corrected for partial volume effects of water and metabolite.

_d Estimated metabolite concentration corrected for partial volume effects of water and metabolite and adjusted to the average voxel gray matter and white matter composition across our final study sample of 59 healthy subjects.

Abbreviations: GABA+: gamma Aminobutyric acid plus co-edited macromolecules; Glx: combined glutamate and glutamine signal; ACC: anterior cingulate cortex

3.4. Second study: The influence of demographic parameters on GABA+ and Glx levels

No significant sex $\times$ age or sex $\times$ years of education interactions were observed for either the uncorrected or CSF-corrected GABA+/Cr and Glx/Cr values. Age and years of education were positively correlated in the third study sample ($p < 0.001$, $n = 59$) and within both men ($p = 0.0007$, $n = 24$) and women ($p = 0.0098$, $n = 35$) according to Spearman's correlation (Table 4). Among females, age and years of education ranged from 18 to 49 years and 8 to 19 years, respectively, whereas among males, the corresponding ranges were 18–40 years and 8–21 years. Neither the uncorrected nor CSF-corrected GABA+/Cr levels showed significant sex differences. In contrast, Glx/Cr levels showed significant sex differences, with males exhibiting higher ratios than females. No significant sex differences were observed in age or years of education (Table 4).

3.4.1. The effect of age

GABA+/Cr exhibited only weak associations with age in the whole population and in females, irrespective of the correction method, whereas no age-related effects were found in males (Table 4). In detail, GABA+/Cr_a showed a significant positive association with age in the whole population after adjusting for years of education ($p = 0.0467$). Using the enter method, slightly positive associations with age were also observed for uncorrected GABA+/Cr in the whole sample ($p = 0.0629$) and for both uncorrected and CSF-corrected measures in females ($p = 0.0683$ and $p = 0.0635$, respectively), whereas no such age-related associations were observed in males (Table 4).

Glx/Cr showed robust negative associations with age in the total sample for both uncorrected and CSF-corrected measures ($p = 0.0071$ and $p = 0.0126$, respectively) after controlling for years of education. When the analyses were stratified by sex, females showed significantly negative age associations across both metrics after adjusting for years of education (Glx/Cr uncorrected $p = 0.0152$, Glx/Cr_a $p = 0.0183$), whereas no comparable associations were observed in males (Table 4).

3.4.2. The effect of years of education

GABA+/Cr levels generally showed no significant associations with years of education across the total sample or within males and females, regardless of the correction method applied.

In contrast, uncorrected Glx/Cr exhibited a significant negative association with years of education corrected for age in men ($p = 0.0388$), whereas no significant associations were

observed in women or in the total sample. In males, Glx/Cr_a demonstrated a marginally negative association with years of education using the enter method ($p=0.0622$). See details in Table 4.

Table 4. Creatine-referenced GABA+ and Glx measures in the ACC with and without cerebrospinal fluid correction, and their associations with age, education level, and sex.

Data				Association with age p-value (standardized beta)			Association with years of education p-value (standardized beta)			Sex difference (p-value)
metabolite	All (n=59)	male (n=24)	female (n=35)	All (n=59)	male (n=24)	female (n=35)	All (n=59)	male (n=24)	female (n=35)	
GABA+/Cr uncorrected	0.1010 (0.0879-0.1137)	0.0922 (0.0837-0.1088)	0.1015 (0.0928-0.1200)	0.0629 (0.2437)	0.6488 (0.0980)	0.0683 (0.3117)	0.5600(0.0774)	0.7261 (-0.0754)	0.3549 (0.1612)	0.1820
GABA+/Cr_a	0.1165 (0.1045-0.1342)	0.1160 (0.0988-0.1295)	0.1186 (0.1066-0.1415)	<i>0.0467 (0.2601)*</i>	0.5350 (0.1332)	0.0635 (0.3171)	<i>0.7015 (-0.0578)</i>	0.8520 (-0.0402)	0.3597 (0.1596)	0.3234
Glx/Cr uncorrected	0.0748 (0.0630-0.0903)	0.0822 (0.0692-0.0989)	0.0708 (0.0587-0.0826)	<i>0.0071(-0.3468)**</i>	<i>0.9030 (-0.0317)</i>	<i>0.0152 (-0.4073)*</i>	<i>0.5051(-0.0975)</i>	<i>0.0388 (-0.4242)*</i>	<i>0.6290 (0.0870)</i>	0.0338*
Glx/Cr_a	0.0897 (0.0740-0.1060)	0.1003 (0.0837-0.1152)	0.0828 (0.0697-0.0959)	<i>0.0126 (-0.3229)*</i>	0.2176 (-0.2612)	<i>0.0183 (-0.3967)*</i>	<i>0.5414 (-0.0902)</i>	0.0622 (-0.3864)	<i>0.6269 (0.0879)</i>	0.0124*
Age (years)	23 (20-30)	23.50 (20-30.75)	23 (20-30)	-	-	-	<0.001 (0.5120)**	0.0007 (0.6386)**	0.0098 (0.4307)**	0.9691
Years of education	12 (12-15)	12 (11.25-15)	12 (12-16)	<0.001 (0.5120)**	0.0007 (0.6386)**	0.0098 (0.4307)**	-	-	-	0.7050

Data is presented in median (interquartile range). Creatine-referenced values represent signal integral ratios. For metabolite related associations, italic values indicate parameters retained in the stepwise linear regression model, while plain text values were analyzed using the enter method.

_a Estimated metabolite ratio corrected for cerebrospinal fluid fraction.

Abbreviations: GABA+: gamma Aminobutyric acid plus co-edited macromolecules; Glx: combined glutamate and glutamine signal; ACC: anterior cingulate cortex; Cr: Creatine

* Significant at the 0.05 level (2-tailed).

** Significant at the 0.01 level (2-tailed).

4. DISCUSSION

The present study had the following main findings: Our test-retest experiment demonstrated that Glx measures generally outperformed GABA+ metrics, with lower CVs and higher ICCs values. The water-referenced GABA+ and Glx ratios showed strong correlations with their creatine-referenced counterparts in the ACC, underscoring the consistency of the referencing approaches. Furthermore, the different water-based correction methods yielded highly intercorrelated results, indicating the methodological robustness of the correction strategies. Although no significant sex $\times$ age or sex $\times$ years of education interactions were observed for either the uncorrected or CSF-corrected GABA+/Cr and Glx/Cr measures in the ACC, separate analyses were conducted for each sex to account for the known age- and sex-related associations of the investigated metabolites (Huang et al. 2016, O’Gorman et al. 2011). Consistent with the findings of (O’Gorman et al. 2011), our cohort exhibited sex differences in Glx/Cr levels, with males displaying higher Glx/Cr ratios than females using both uncorrected and CSF-corrected methods. However, the GABA+/Cr levels did not differ significantly between the sexes. An age-related effect was also observed in females; GABA+/Cr levels showed slightly positive associations with age, whereas Glx/Cr levels were negatively related to age in the ACC. These female-specific associations likely account for the marginally significant and significant age effects observed in the entire sample. Surprisingly, the level of education also had an impact on brain metabolites, as we found a negative association between Glx/Cr levels and years of education in the ACC of men, whereas no such association was found in women.

4.1. *Effect of sex, age, and correction strategies on GABA+ and Glx*

In the present study, we found significantly higher Glx/Cr levels in the ACC of men than in women, whereas the GABA+/Cr levels were similar between the sexes. Comparable data were documented by (Tayoshi et al. 2009). They measured Glu and Gln levels separately and found sex differences only for Gln, but not for Glu, in the ACC. Notably, several studies have reported negative results. For instance, a point-resolved spectroscopy study involving 118 healthy participants determined the absolute concentrations of Glu and Gln in the hippocampus and ACC. This study identified a statistically significant sex-related difference in Glu levels in the hippocampus but not in the ACC (Hädel et al. 2013). Another study that focused on the pregenual ACC (pACC) and the left cerebellar hemisphere found no sex-related differences in Glx levels in the pACC (Endres et al. 2016). A study focusing only on GABA concentrations in the ACC found no sex differences (Aufhaus et al. 2013).

In the present study, we found that age had an impact on females; moderate positive associations were observed between age and GABA+/Cr levels, while age was negatively related to Glx/Cr levels in the ACC of female participants. Comparable data within a similar age range have been reported by Ghisleni et al., who found an age-related increase in GABA+ levels in the frontal cortex of young adults (Ghisleni et al. 2015). In contrast, another study reported an age-dependent decline in GABA+ levels in the frontal region of healthy subjects aged 20–76 years, which was more rapid in females than in males (Gao et al. 2013b). Age-related decline of GABA and Glu levels has been reported in the dorsolateral prefrontal cortex of healthy middle-aged subjects (Grachev & Vania Apkarian 2001), while another study involving 77 control subjects reported only a tendency for age-related decline of GABA levels in the medial frontal/ACC, but this decline was statistically non-significant (Rowland et al. 2015). Another study reported significantly lower GABA levels in older adults; however, this age effect disappeared when GABA levels were corrected for voxel composition, suggesting that age-related differences in GABA levels may be caused by age-related GM loss (Maes et al. 2018). Others found an age-related decline in GABA+ levels in the frontal region when no tissue correction was used and also when CSF correction was applied; however, they examined healthy subjects with more advanced ages, that is, aged between 44 and 92 years (Porges et al. 2017). However, when they applied the α -correction, wherein GABA+ levels in the GM are assumed to be twice those in the WM (analogous to our second MRS quantification approach), no correlation was observed between age and GABA+ levels (Porges et al. 2017). A more recent longitudinal study reported a within-person age-related decline in cortical GABA levels at two time points a few years apart; however, when a cross-sectional design was applied to the same cohort, the effect of age on GABA levels disappeared (Zuppichini et al. 2024). A meta-analytic study that created a model of the pattern of frontal GABA estimates reported an asymmetric lifespan trajectory characterized by a fairly quick increase in GABA during development, followed by a flattening during adolescence, and a subsequent slow decrease with aging (Porges et al. 2021). The considerable variability in GABA levels can be attributed to its relatively low concentration in the brain (~1–2 mM) and the overlap of its signal with metabolites present at higher concentrations (Puts & Edden 2011). Additionally, normal diurnal fluctuations during the light-dark cycle (Evans et al. 2010), as well as confounding factors, including smoking, menstrual cycle phase (Epperson et al. 2005), dietary conditions (van Nieuwenhuizen et al. 2025), and cannabis use (Kirkland et al. 2025), may contribute to this variability. The effect of age on Glu and Glx levels appears to be more consistent. An age-related decline in Glu and Glx levels has been reported in the ACC and medial prefrontal cortex during the lifetime period

(Huang et al. 2016; Merritt et al. 2021). Reduced Glu concentrations have also been reported in the motor cortex of older adults (Kaiser et al. 2005), but negative findings also exist (Aufhaus et al. 2013). However, it is worth mentioning that age-related metabolite changes are often associated with a reduction in GM and an increase in CSF (Maes et al. 2018). Consequently, the tissue composition of the MRS voxels has a significant impact on the metabolite levels (and reference signals), as they naturally differ between the CSF and brain tissues (GM and WM). When tissue correction is not applied, metabolite estimation is conducted without accounting for the relative proportions of GM, WM, and CSF within the voxel. When tissue correction is applied, the process typically refers to accounting for the CSF fraction within the voxel by normalizing the voxel with the non-CSF voxel fraction ($f_{GM} + f_{WM}$), with the implicit assumption that the metabolites in question are negligible in the CSF or, if present, do not contribute to the MRS signal. Among tissue correction strategies, GM correction (which considers only the GM fraction) also exists, but it increases the dependence of GABA measurements on the GM/WM ratio and can lead to an overestimation of GABA by a factor of 1.5 (Harris et al. 2015). Consequently, this correction approach is not frequently used in MRS studies. When a water-reference signal is available, more sophisticated tissue corrections are available that correct for partial volume effects that attenuate the observed water signal; or correct for partial volume effects that attenuate the observed water and metabolite signals, accounting for the intrinsic differences in metabolite concentrations in the GM and WM (where GM:WM is 2:1 estimated). This could be further refined if the average voxel GM and WM compositions are calculated from the studied group and represent the examined conditions of the study.

4.2. *The relationship between education and neurotransmitters*

Research indicates complex, mostly positive associations between education level and cognitive performance across the lifespan of healthy subjects in both males and females (Lövdén et al. 2020). Most studies examining cognition, learning, age, and sex dependencies on brain metabolites usually control for education level (Treacy, Andrews, and Levenstein 2025; Kantarci et al. 2011), but its effect on Glx and Cr has rarely been investigated, even though Cr levels in the brain are associated with performance in memory and intelligence tasks (Avgerinos et al. 2018) and are also associated with benefits for cognition in healthy older adults (Marshall et al. 2025). Sex-specific alterations may also exist, as Glx was found to be related to a reversal learning task in male marmosets but not in female specimens, reflecting potential sex-dependent glutamatergic metabolism (Lacreuse et al. 2018). Glutamatergic metabolism in females may

appears less influenced by educational exposure, likely due to hormonal, metabolic, or executive control differences (Knouse et al. 2022; Goyette et al. 2023; Barth, Villringer, and Sacher 2015). We found a negative association between Glx/Cr ratios and years of education in males, while this relationship was absent in females and in the overall cohort. In a study by Borgan et al., no associations were found between Glu levels and cognitive function or between Glu levels and years of education following compulsory education in the ACC of healthy young adults with a male-to-female ratio of 2:1. However, water was used as the reference signal, and direct sex-specific associations were not examined (Borgan et al. 2019). The sex-specific effect observed in our study indicates differential neurochemical adaptations to educational exposure, with men potentially exhibiting greater glutamatergic efficiency, reflected in reduced Glx, or enhanced creatine-linked metabolic regulation in response to prolonged educational engagement.

4.3. Limitations

This study, like any other investigation, has limitations that should be considered when interpreting its results. Macromolecule contamination of the edited GABA signal should be acknowledged as a methodological consideration, as it may affect the specificity and precision of GABA measurements, thereby influencing the detectability of true neurochemical effects and the interpretation of the observed associations. Additionally, participants were instructed to refrain from caffeine and alcohol consumption for at least 12 h prior to the examination. Despite these controls, the inherent biological variability and low sensitivity of MRS in detecting subtle changes in GABA levels may limit the ability to fully characterize age-related effects. Furthermore, the analyses of demographic effects were limited to creatine-referenced GABA+ and Glx metabolites, whereas water-referenced and tissue-corrected estimates were not included in the analyses. Despite this, strong correlations were observed between water- and creatine-referenced measures, as well as among various water-referencing strategies, indicating that both referencing approaches provide reliable estimates suitable for addressing the specific research questions of this study.

4.5. Conclusions

Creatine- and water-referenced metabolite measures in the ACC exhibited strong correlations, indicating consistency between the two referencing strategies; therefore, the use of Cr as an internal reference was considered appropriate for quantifying GABA+ and Glx levels and examining the influence of sex, age, and education in our cohort. Sex differences were observed

in Glx/Cr ratios, with males exhibiting higher ratios, whereas GABA+/Cr levels remained comparable between the sexes. Age-related effects were specific to females, indicating distinct neurochemical trajectories, with GABA+ being positively and Glx negatively associated with age in females. A novel negative association between Glx/Cr levels and years of education was identified in males, suggesting a sex-specific neurochemical adaptation related to the educational level. These findings highlight the importance of considering sex, age, and education level in MRS studies and emphasize the effectiveness of Cr referencing under appropriate conditions. However, in multicenter studies or in patients with neurological conditions, water-referenced quantification using unsuppressed water scans may be preferable, as it enables the application of more advanced correction methods for assessing GABA and Glx alterations.

FIGURE LEGEND

Figure 1.

Reference signal fitting (water and creatine) and spectral processing of GABA and the combined glutamate and glutamine (Glx) signal were performed, with down-weighting of the residual choline signal between 3.16 and 3.285 ppm. Data derived from voxels in the anterior cingulate cortex (A).

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR'S CONTRIBUTION

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Zsófia Kürtös, Szilvia Anett Nagy, Gergely Orsi, and Gábor Perlaki. The first draft of the manuscript was written by Zsófia Kürtös, Szilvia Anett Nagy, and Boldizsár Czéh and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ABBREVIATIONS

ACC: anterior cingulate cortex;

Cho: choline;

Cr: creatine;

CSF: cerebrospinal fluid;

CV: coefficients of variation within subjects;

FWHM: full width at half maximum

GABA: gamma-aminobutyric acid;

GABA+: gamma-aminobutyric acid plus co-edited macromolecules

Gln: glutamine;

GM: gray matter;

Glu: glutamate;

Glx: combined glutamate and glutamine signal in the MR spectrum;

ICC: intra-class correlation coefficient;

MEGA-PRESS: MESHcher-GARwood Point RESolved Spectroscopy;

MR: magnetic resonance;

MRS: magnetic resonance spectroscopy;

NAA: N-acetyl aspartate;

OCC : occipital cortex;

PrC : precuneus cortex;

WM: white matter.

REFERENCES

- Ashburner J, Friston K J (2005). Unified segmentation. *NeuroImage*, 26(3), 839–851. <https://doi.org/10.1016/J.NEUROIMAGE.2005.02.018>
- Aufhaus E, Weber-Fahr W, Sack M, Tunc-Skarka N, Oberthuer G, Hoerst M, Meyer-Lindenberg A, Boettcher U, Ende G (2013). Absence of changes in GABA concentrations with age and gender in the human anterior cingulate cortex: A MEGA-PRESS study with symmetric editing pulse frequencies for macromolecule suppression. *Magnetic Resonance in Medicine*, 69(2), 317–320. <https://doi.org/10.1002/MRM.24257>
- Avgerinos K I, Spyrou N, Bougioukas K I, Kapogiannis D (2018). Effects of creatine supplementation on cognitive function of healthy individuals: A systematic review of randomized controlled trials. *Experimental Gerontology*, 108, 166. <https://doi.org/10.1016/J.EXGER.2018.04.013>
- Bak L K, Schousboe A, Waagepetersen H S (2006). The glutamate/GABA-glutamine cycle: Aspects of transport, neurotransmitter homeostasis and ammonia transfer. *Journal of Neurochemistry*, 98(3), 641–653. <https://doi.org/10.1111/J.1471-4159.2006.03913.X>
- Barth C, Villringer A, Sacher J (2015). Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. *Frontiers in Neuroscience*, 9(FEB), 113668. <https://doi.org/10.3389/FNINS.2015.00037>
- Borgan F R, Jauhar S, McCutcheon R A, Pepper F S, Rogdaki M, Lythgoe D J, Howes O D (2019). Glutamate levels in the anterior cingulate cortex in un-medicated first episode psychosis: a proton magnetic resonance spectroscopy study. *Scientific Reports 2019 9:1*, 9(1), 8685-. <https://doi.org/10.1038/s41598-019-45018-0>
- Buzsáki G, Chrobak J J (1995). Temporal structure in spatially organized neuronal ensembles: a role for interneuronal networks. *Current Opinion in Neurobiology*, 5(4), 504–510. [https://doi.org/10.1016/0959-4388\(95\)80012-3](https://doi.org/10.1016/0959-4388(95)80012-3)
- Choi D W (1988). Glutamate neurotoxicity and diseases of the nervous system. *Neuron*, 1(8), 623–634. [https://doi.org/10.1016/0896-6273\(88\)90162-6](https://doi.org/10.1016/0896-6273(88)90162-6)
- Duman R S, Sanacora G, Krystal J H (2019). Altered Connectivity in Depression: GABA and Glutamate Neurotransmitter Deficits and Reversal by Novel Treatments. *Neuron*, 102(1), 75–90. <https://doi.org/10.1016/J.NEURON.2019.03.013>
- Ebrahimi R, Mohammad Soltani S, Masouri M M, Seif M, Ghafourian K, Noori S (2025). Glutamatergic and GABAergic metabolite levels in Alzheimer’s disease: a systematic review and meta-analysis. *BMC Neurology 2025 25:1*, 25(1), 344-. <https://doi.org/10.1186/S12883-025-04375-2>
- Edden R A E, Puts N A J, Harris A D, Barker P B, Evans C J (2014). Gannet: A batch-processing tool for the quantitative analysis of gamma-aminobutyric acid-edited MR spectroscopy spectra. *Journal of Magnetic Resonance Imaging*, 40(6), 1445–1452. <https://doi.org/10.1002/JMRI.24478>
- Endres D, van Elst L T, Feige B, Backenecker S, Nickel K, Bubl A, Lange T, Mader I, Maier S, Perlov E (2016). On the effect of sex on prefrontal and cerebellar neurometabolites in healthy adults: An MRS study. *Frontiers in Human Neuroscience*, 10, 197013. <https://doi.org/10.3389/FNHUM.2016.00367>
- Epperson C N, O’Malley S, Czarkowski K A, Gueorguieva R, Jatlow P, Sanacora G,

- Rothman D L, Krystal J H, Mason G F (2005). Sex, GABA, and nicotine: The impact of smoking on cortical GABA levels across the menstrual cycle as measured with proton magnetic resonance spectroscopy. *Biological Psychiatry*, 57(1), 44–48. <https://doi.org/10.1016/j.biopsych.2004.09.021>
- Evans C J, McGonigle D J, Edden R A E (2010). Diurnal stability of γ -aminobutyric acid concentration in visual and sensorimotor cortex. *Journal of Magnetic Resonance Imaging*, 31(1), 204–209. <https://doi.org/10.1002/JMRI.21996>
- Gao F, Edden R A E, Li M, Puts N A J, Wang G, Liu C, Zhao B, Wang H, Bai X, Zhao C, Wang X, Barker P B (2013a). Edited magnetic resonance spectroscopy detects an age-related decline in brain GABA levels. *NeuroImage*, 78, 75–82. <https://doi.org/10.1016/J.NEUROIMAGE.2013.04.012>
- Gasparovic C, Song T, Devier D, Bockholt H J, Caprihan A, Mullins P G, Posse S, Jung R E, Morrison L A (2006). Use of tissue water as a concentration reference for proton spectroscopic imaging. *Magnetic Resonance in Medicine*, 55(6), 1219–1226. <https://doi.org/10.1002/MRM.20901>
- Ghisleni C, Bollmann S, Poil S S, Brandeis D, Martin E, Michels L, O’Gorman R L, Klaver P (2015). Subcortical Glutamate Mediates the Reduction of Short-Range Functional Connectivity with Age in a Developmental Cohort. *Journal of Neuroscience*, 35(22), 8433–8441. <https://doi.org/10.1523/JNEUROSCI.4375-14.2015>
- Goyette M J, Murray S L, Saldanha C J, Holton K (2023). Sex Hormones, Neurosteroids, and Glutamatergic Neurotransmission: A Review of the Literature. *Neuroendocrinology*, 113(9), 905–914. <https://doi.org/10.1159/000531148>
- Grachev I D, Apkarian A V (2000). Chemical Heterogeneity of the Living Human Brain: A Proton MR Spectroscopy Study on the Effects of Sex, Age, and Brain Region. *NeuroImage*, 11(5), 554–563. <https://doi.org/10.1006/NIMG.2000.0557>
- Grachev I D, Vania Apkarian A (2001). Aging alters regional multichemical profile of the human brain: An in vivo ^1H -MRS study of young versus middle-aged subjects. *Journal of Neurochemistry*, 76(2), 582–593. <https://doi.org/10.1046/J.1471-4159.2001.00026.X>
- Hädel S, Wirth C, Rapp M, Gallinat J, Schubert F (2013). Effects of age and sex on the concentrations of glutamate and glutamine in the human brain. *Journal of Magnetic Resonance Imaging*, 38(6), 1480–1487. <https://doi.org/10.1002/JMRI.24123>
- Harris A D, Puts N A J, Edden R A E (2015). Tissue correction for GABA-edited MRS: Considerations of voxel composition, tissue segmentation, and tissue relaxations. *Journal of Magnetic Resonance Imaging*, 42(5), 1431–1440. <https://doi.org/10.1002/JMRI.24903>
- Horder J, Petrinovic M M, Mendez M A, Bruns A, Takumi T, Spooren W, Barker G J, Künnecke B, Murphy D G (2018). Glutamate and GABA in autism spectrum disorder—a translational magnetic resonance spectroscopy study in man and rodent models. *Translational Psychiatry* 2018 8:1, 8(1), 106-. <https://doi.org/10.1038/s41398-018-0155-1>
- Huang D, Liu D, Yin J, Qian T, Shrestha S, Ni H (2016). Glutamate-glutamine and GABA in brain of normal aged and patients with cognitive impairment. *European Radiology* 2017 27:7, 27(7), 2698–2705. <https://doi.org/10.1007/S00330-016-4669-8>

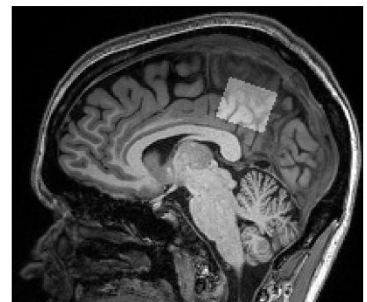
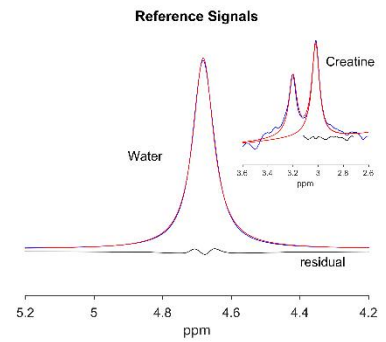
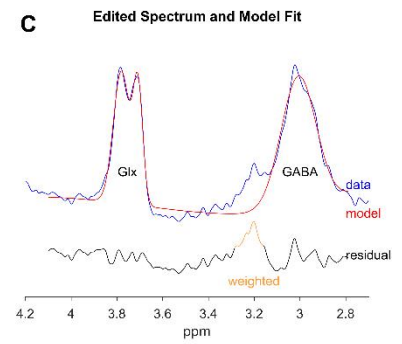
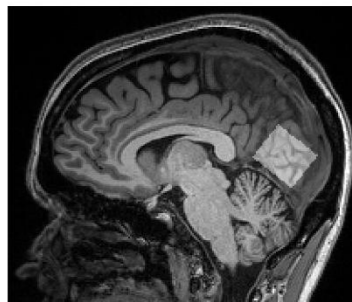
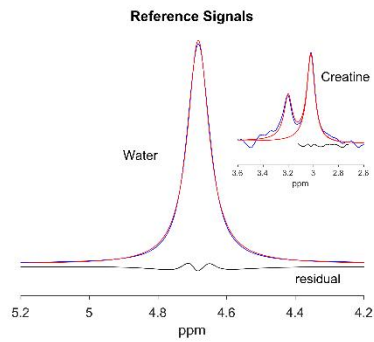
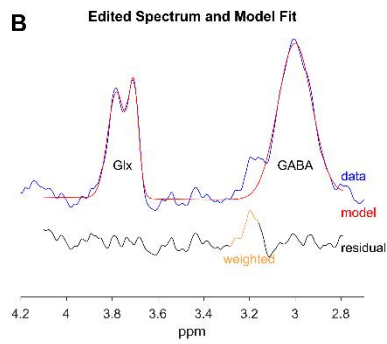
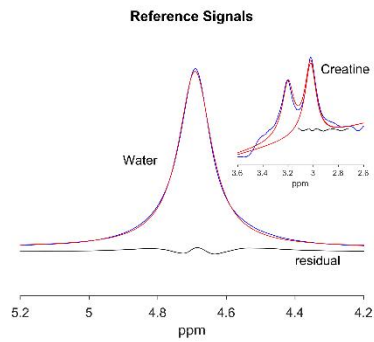
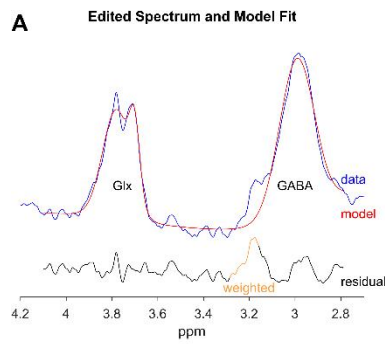
- Kaiser L G, Schuff N, Cashdollar N, Weiner M W (2005). Age-related glutamate and glutamine concentration changes in normal human brain: ^1H MR spectroscopy study at 4 T. *Neurobiology of Aging*, 26(5), 665–672.
<https://doi.org/10.1016/J.NEUROBIOLAGING.2004.07.001>
- Kanagasabai K, Palaniyappan L, Théberge J (2024). Precision of metabolite-selective MRS measurements of glutamate, GABA and glutathione: A review of human brain studies. *NMR in Biomedicine*, 37(3), e5071. <https://doi.org/10.1002/NBM.5071>
- Kantarci K, Lowe V, Przybelski S A, Senjem M L, Weigand S D, Ivnik R J, Roberts R, Geda Y E, Boeve B F, Knopman D S, Petersen R C, Jack C R (2011). Magnetic resonance spectroscopy, β -amyloid load, and cognition in a population-based sample of cognitively normal older adults. *Neurology*, 77(10), 951–958.
<https://doi.org/10.1212/WNL.0B013E31822DC7E1>
- Kirkland A E, Browning B D, Green R J, Agbeh S O, Squeglia L M (2025). Neurometabolite Alterations Associated With Cannabis Use: A Proton Magnetic Resonance Spectroscopy Meta-Analysis. *Human Brain Mapping*, 46(8), e70236.
<https://doi.org/10.1002/HBM.70236>
- Knouse M C, McGrath A G, Deutschmann A U, Rich M T, Zallar L J, Rajadhyaksha A M, Briand L A (2022). Sex differences in the medial prefrontal cortical glutamate system. *Biology of Sex Differences*, 13(1), 66. <https://doi.org/10.1186/S13293-022-00468-6>
- Koh W, Kwak H, Cheong E, Lee C J (2023). GABA tone regulation and its cognitive functions in the brain. *Nature Reviews Neuroscience* 2023 24:9, 24(9), 523–539.
<https://doi.org/10.1038/s41583-023-00724-7>
- Lacreuse A, Moore C M, LaClair M, Payne L, King J A (2018). Glutamine/glutamate (Glx) concentration in prefrontal cortex predicts reversal learning performance in the marmoset. *Behavioural Brain Research*, 346, 11–15.
<https://doi.org/10.1016/J.BBR.2018.01.025>
- Li H, Rodríguez-Nieto G, Chalavi S, Seer C, Mikkelsen M, Edden R A E, Swinnen S P (2024). MRS-assessed brain GABA modulation in response to task performance and learning. *Behavioral and Brain Functions : BBF*, 20(1). <https://doi.org/10.1186/S12993-024-00248-9>
- Lövdén M, Fratiglioni L, Glymour M M, Lindenberger U, Tucker-Drob E M (2020). Education and Cognitive Functioning Across the Life Span. *Psychological Science in the Public Interest*, 21(1), 6–41. <https://doi.org/10.1177/1529100620920576>
- Maddock R J, Buonocore M H (2011). MR Spectroscopic Studies of the Brain in Psychiatric Disorders. *Current Topics in Behavioral Neurosciences*, 11, 199–251.
https://doi.org/10.1007/7854_2011_197
- Maddock R J, Caton M D, Ragland J D (2018). Estimating glutamate and Glx from GABA-optimized MEGA-PRESS: Off-resonance but not difference spectra values correspond to PRESS values. *Psychiatry Research: Neuroimaging*, 279, 22–30.
<https://doi.org/10.1016/J.PSCYCHRESNS.2018.07.003>
- Maes C, Hermans L, Pauwels L, Chalavi S, Leunissen I, Levin O, Cuypers K, Peeters R, Sunaert S, Mantini D, Puts N A J, Edden R A E, Swinnen S P (2018). Age-related differences in GABA levels are driven by bulk tissue changes. *Human Brain Mapping*, 39(9), 3652–3662. <https://doi.org/10.1002/HBM.24201>

- Marshall S, Kitzan A, Wright J, Bocicariu L, Lindsay, Nagamatsu S (2025). Creatine and Cognition in Aging: A Systematic Review of Evidence in Older Adults. *Nutrition Reviews*. <https://doi.org/10.1093/NUTRIT/NUAF135>
- Mattson M P (2008). Glutamate and neurotrophic factors in neuronal plasticity and disease. *Annals of the New York Academy of Sciences*, 1144(1), 97–112. <https://doi.org/10.1196/ANNALS.1418.005>
- Merritt K, McGuire P K, Egerton A, Aleman A, Block W, Bloemen O J N, Borgan F, Bustillo J R, Capizzano A A, Coughlin J M, De La Fuente-Sandoval C, Demjaha A, Dempster K, Do K Q, Du F, Falkai P, Galinska-Skok B, Gallinat J, Gasparovic C, Yamasue H (2021a). Association of Age, Antipsychotic Medication, and Symptom Severity in Schizophrenia With Proton Magnetic Resonance Spectroscopy Brain Glutamate Level: A Mega-analysis of Individual Participant-Level Data. *JAMA Psychiatry*, 78(6), 667–681. <https://doi.org/10.1001/JAMAPSYCHIATRY.2021.0380>
- Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R (1998). Simultaneous in vivo spectral editing and water suppression. *NMR in Biomedicine*, 11(6), 266–272. [https://doi.org/10.1002/\(SICI\)1099-1492\(199810\)11:6<266::AID-NBM530>3.0.CO;2-J](https://doi.org/10.1002/(SICI)1099-1492(199810)11:6<266::AID-NBM530>3.0.CO;2-J)
- Mikkelsen M, Barker P B, Bhattacharyya P K, Brix M K, Buur P F, Cecil K M, Chan K L, Chen D Y T, Craven A R, Cuypers K, Dacko M, Duncan N W, Dydak U, Edmondson D A, Ende G, Ersland L, Gao F, Greenhouse I, Harris A D, Edden R A E (2017). Big GABA: Edited MR Spectroscopy at 24 Research Sites. *NeuroImage*, 159, 32. <https://doi.org/10.1016/J.NEUROIMAGE.2017.07.021>
- Mikkelsen M, Rimbault D L, Barker P B, Bhattacharyya P K, Brix M K, Buur P F, Cecil K M, Chan K L, Chen D Y T, Craven A R, Cuypers K, Dacko M, Duncan N W, Dydak U, Edmondson D A, Ende G, Ersland L, Forbes M A, Gao F, Edden R A E (2019). Big GABA II: Water-referenced edited MR spectroscopy at 25 research sites. *NeuroImage*, 191, 537–548. <https://doi.org/10.1016/j.neuroimage.2019.02.059>
- Mikkelsen M, Singh K D, Sumner P, Evans C J (2016). Comparison of the repeatability of GABA-edited magnetic resonance spectroscopy with and without macromolecule suppression. *Magnetic Resonance in Medicine*, 75(3), 946–953. <https://doi.org/10.1002/MRM.25699>
- Mikkelsen M, Tapper S, Near J, Mostofsky S H, Puts N A J, Edden R A E (2020). Correcting frequency and phase offsets in MRS data using robust spectral registration. *NMR in Biomedicine*, 33(10). <https://doi.org/10.1002/NBM.4368>
- Mullins P G, McGonigle D J, O’Gorman R L, Puts N A J, Vidyasagar R, Evans C J, Edden R A E, Brookes M J, Garcia A, Foerster B R, Petrou M, Price D, Solanky B S, Violante I R, Williams S, Wilson M (2014). Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. *NeuroImage*, 86, 43–52. <https://doi.org/10.1016/J.NEUROIMAGE.2012.12.004>
- Nakahara T, Tsugawa S, Noda Y, Ueno F, Honda S, Kinjo M, Segawa H, Hondo N, Mori Y, Watanabe H, Nakahara K, Yoshida K, Wada M, Tarumi R, Iwata Y, Plitman E, Moriguchi S, de la Fuente-Sandoval C, Uchida H, Nakajima S (2021). Glutamatergic and GABAergic metabolite levels in schizophrenia-spectrum disorders: a meta-analysis of 1H-magnetic resonance spectroscopy studies. *Molecular Psychiatry* 2021 27:1, 27(1), 744–757. <https://doi.org/10.1038/s41380-021-01297-6>

- O’Gorman R L, Michels L, Edden R A, Murdoch J B, Martin E (2011). In vivo detection of GABA and glutamate with MEGA-PRESS: Reproducibility and gender effects. *Journal of Magnetic Resonance Imaging*, 33(5), 1262–1267. <https://doi.org/10.1002/JMRI.22520>
- Peek A L, Rebbbeck T J, Leaver A M, Foster S L, Refshauge K M, Puts N A, Oeltzschner G, Andronesi O C, Barker P B, Bogner W, Cecil K M, Choi I Y, Deelchand D K, de Graaf R A, Dydak U, Edden R A, Emir U E, Harris A D, Lin A P, Wilson M (2023). A comprehensive guide to MEGA-PRESS for GABA measurement. *Analytical Biochemistry*, 669. <https://doi.org/10.1016/j.ab.2023.115113>
- Pollack M H, Jensen J E, Simon N M, Kaufman R E, Renshaw P F (2008). High-field MRS study of GABA, glutamate and glutamine in social anxiety disorder: Response to treatment with levetiracetam. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 32(3), 739–743. <https://doi.org/10.1016/J.PNPBP.2007.11.023>
- Porges E C, Jensen G, Foster B, Edden R A E, Puts N A J (2021). The trajectory of cortical gaba across the lifespan, an individual participant data meta-analysis of edited mrs studies. *ELife*, 10. <https://doi.org/10.7554/ELIFE.62575>
- Porges E C, Woods A J, Lamb D G, Williamson J B, Cohen R A, Edden R A E, Harris A D (2017). Impact of tissue correction strategy on GABA-edited MRS findings. *NeuroImage*, 162, 249–256. <https://doi.org/10.1016/J.NEUROIMAGE.2017.08.073>
- Puts N A J, Edden R A E (2011). In vivo magnetic resonance spectroscopy of GABA: A methodological review. *Progress in Nuclear Magnetic Resonance Spectroscopy*, 60, 29. <https://doi.org/10.1016/J.PNMRS.2011.06.001>
- Rowland L M, Krause B W, Wijtenburg S A, McMahon R P, Chiappelli J, Nugent K L, Nisonger S J, Korenic S A, Kochunov P, Hong L E (2015). Medial frontal GABA is lower in older schizophrenia: a MEGA-PRESS with macromolecule suppression study. *Molecular Psychiatry* 2016 21:2, 21(2), 198–204. <https://doi.org/10.1038/mp.2015.34>
- Sanaei Nezhad F, Anton A, Michou E, Jung J Y, Parkes L M, Williams S R (2018). Quantification of GABA, glutamate and glutamine in a single measurement at 3 T using GABA-edited MEGA-PRESS. *NMR in Biomedicine*, 31(1), e3847. <https://doi.org/10.1002/NBM.3847>
- Sarawagi A, Soni N D, Patel A B (2021). Glutamate and GABA Homeostasis and Neurometabolism in Major Depressive Disorder. *Frontiers in Psychiatry*, 12, 637863. <https://doi.org/10.3389/FPSYT.2021.637863>
- Sarlo G L, Holton K F (2021). Brain concentrations of glutamate and GABA in human epilepsy: A review. *Seizure*, 91, 213–227. <https://doi.org/10.1016/j.seizure.2021.06.028>
- Simpson H B, Shungu D C, Bender J, Mao X, Xu X, Slifstein M, Kegeles L S (2012). Investigation of Cortical Glutamate–Glutamine and γ -Aminobutyric Acid in Obsessive–Compulsive Disorder by Proton Magnetic Resonance Spectroscopy. *Neuropsychopharmacology* 2012 37:12, 37(12), 2684–2692. <https://doi.org/10.1038/npp.2012.132>
- Tao S, Deng R, Wei M, Huang Y, Sun H, Yang S, Li S, Xiao C, Li M (2025). A Meta-Analysis of Magnetic Resonance Spectroscopy Studies on Glutamatergic Neurometabolite Levels in Major Depressive Disorder. *Depression and Anxiety*, 2025(1), 5180077. <https://doi.org/10.1155/DA/5180077>

- Tayoshi S, Sumitani S, Taniguchi K, Shibuya-Tayoshi S, Numata S, Iga J ichi, Nakataki M, Ueno S ichi, Harada M, Ohmori T (2009). Metabolite changes and gender differences in schizophrenia using 3-Tesla proton magnetic resonance spectroscopy (1H-MRS). *Schizophrenia Research*, 108(1–3), 69–77. <https://doi.org/10.1016/J.SCHRES.2008.11.014>
- Treacy C, Andrews S C, Levenstein J M (2025). Relationships between GABA + and Glx concentrations with age and inhibition in healthy older adults. *Brain Structure & Function*, 230(8), 149. <https://doi.org/10.1007/S00429-025-03017-0>
- van Nieuwenhuizen H, Antal B B, Hone-Blanchet A, Lithen A, McMahon L, Nikolaidou S, Kuang Z, Clarke K, Jenkins B G, Rothman D L, Mujica-Parodi L R, Ratai E M (2025). Ketosis Elevates Antioxidants and Markers of Energy Metabolism: A Proton Magnetic Resonance Spectroscopy Study. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, 10(11), 1186–1196. <https://doi.org/10.1016/J.BPSC.2025.06.009>
- Zahid U, Onwordi E C, Hedges E P, Wall M B, Modinos G, Murray R M, Egerton A (2023). Neurofunctional correlates of glutamate and GABA imbalance in psychosis: A systematic review. *Neuroscience & Biobehavioral Reviews*, 144, 105010. <https://doi.org/10.1016/J.NEUBIOREV.2022.105010>
- Zahr N M, Mayer D, Rohlfing T, Chanraud S, Gu M, Sullivan E V, Pfefferbaum A (2012). In Vivo Glutamate Measured with MR Spectroscopy: Behavioral Correlates in Aging. *Neurobiology of Aging*, 34(4), 1265. <https://doi.org/10.1016/J.NEUROBIOLAGING.2012.09.014>
- Zhang J, Touloupoulou T, Li Q, Niu L, Peng L, Dai H, Chen K, Wang X, Huang R, Wei X, Zhang R (2024). Charting brain GABA and glutamate levels across psychiatric disorders by quantitative analysis of 121 1H-MRS studies. *Psychological Medicine*, 54(15), 4071–4082. <https://doi.org/10.1017/S0033291724001673>
- Zuppichini M D, Hamlin A M, Zhou Q, Kim E, Rajagopal S, Beltz A M, Polk T A (2024). GABA levels decline with age: A longitudinal study. *Imaging Neuroscience*, 2, 1–15. https://doi.org/10.1162/IMAG_A_00224/123270

Figure 1



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