

Singlet-filtered 1 H-MRS at 3 T yields phantom-calibrated apparent regional glutamate values in healthy adults and compartmental glutamate alterations in glioma: a prospective observational study

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Abstract

Background

Glutamatergic dysregulation contributes to neurodegenerative and neuro-oncological disease. However, in vivo measurement of glutamate (Glu) at widely available 3 T clinical field strengths is complicated by spectral overlap with glutamine (Gln). We evaluated singlet-filtered proton magnetic resonance spectroscopy with optimal-control pulses (SFOC-MRS) for phantom-calibrated apparent Glu quantification at 3 T, characterised regional values in healthy adults, and applied the workflow in newly diagnosed glioma.

Methods

In this prospective, single-centre observational study, we applied SFOC-MRS to selectively isolate and quantify Glu at 3 T, with conventional short-echo point-resolved spectroscopy (PRESS) acquired in the same session as a clinical reference. Phantom-calibrated apparent [Glu] was derived from a six-point external L-glutamate phantom calibration. Twenty-five healthy adults (sampling three predefined regions: frontal lobe, occipital lobe, and basal ganglia) and 15 patients with treatment-naïve supratentorial glioma (sampling solid tumour, peritumoural oedema, and contralateral frontal lobe) underwent single-voxel acquisitions. Group comparisons used Welch's heteroscedastic analysis of variance (ANOVA) and t-tests with Holm correction; effect sizes are reported as Hedges' g.

Results

SFOC-MRS resolved the Glu triplet at approximately 2.35 ppm with linear phantom calibration ($R^2 = 0.974$). In healthy adults, apparent [Glu] followed a regional hierarchy (frontal lobe 8.50 ± 1.98 mM > occipital lobe 4.79 ± 1.51 mM > basal ganglia 3.24 ± 0.82 mM; Welch's ANOVA $p = 4.2 \times 10^{-12}$; Hedges' g = 3.06 for frontal vs basal ganglia). In glioma, pronounced compartmental heterogeneity emerged (Welch's ANOVA $p = 0.0013$), with peritumoural oedema substantially lower than both solid tumour and the contralateral frontal lobe (both Holm $p = 9.3 \times 10^{-3}$). Glioma-related voxels showed significantly lower [Glu] than frontal- and occipital-lobe voxels in healthy controls (3.75 ± 2.93 vs 7.06 ± 2.56 mM; Welch's $t = 4.14$; $p = 2.8 \times 10^{-4}$; Hedges' g = 1.22).

Conclusions

At 3 T, SFOC-MRS yielded a selective, phantom-calibrated apparent Glu measure and detected regional and glioma-compartment differences. Further test–retest, multi-volume phantom, tissue-correction, and multicentre validation is required before clinical deployment.

Background

Glutamate (Glu) is the principal excitatory neurotransmitter and is coupled to glutamine (Gln) via the astrocyte–neuron glutamate–glutamine cycle that sustains synaptic transmission(1, 2). In Alzheimer’s disease (AD), a meta-analysis of human brain-tissue studies reported lower glutamate levels (standardised mean difference – 0.82; $I^2 = 74.5\%$), whereas a separate 4 T proton magnetic resonance spectroscopy (^1H -MRS) study found lower right-hippocampal Glu in AD than in controls(3, 4). These findings may be compatible with neuronal or synaptic loss but do not establish that mechanism. At 3 T, lower motor-cortex gamma-aminobutyric acid and higher combined Glu + Gln in the riluzole-naïve amyotrophic-lateral-sclerosis subgroup were reported(5). At 7 T, no overall amyotrophic-lateral-sclerosis–control difference in Glu was detected, although motor-cortex Glu varied with diagnostic stage(6). Preclinical glioma studies showed glutamate release and linked it to neuronal hyperexcitability and seizure activity(7, 8). This biology has also motivated positron emission tomography (PET) imaging of metabotropic glutamate receptor 5 (mGluR5) with [^{18}F]PSS232 (9–11) and [^{18}F]FPEB(10).

^1H -MRS provides noninvasive in vivo measurement of brain metabolites, including N-acetylaspartate, choline-containing compounds, creatine, myo-inositol, Glu and Gln(12, 13). At 3 T, substantial overlap and covariance between Glu and Gln complicate their separate estimation using conventional short-echo point-resolved spectroscopy (PRESS), so the combined signal is often reported as Glx (Glu + Gln)(13, 14). However, optimised-echo-time PRESS, spectral editing and prior-knowledge fitting can estimate Glu separately, with method-dependent precision and concordance(13–17). Ultra-high-field MRS improves spectral dispersion but remains less widely available for routine clinical use(13, 14). A clinically practical approach to phantom-calibrated Glu quantification at 3 T, together with regional apparent glutamate values in healthy human brain, is therefore needed for downstream biomarker studies of glutamatergic dysfunction.

Approaches to improve Glu estimation at 3 T include optimised-echo-time PRESS(15) and J-difference editing such as Mescher–Garwood point-resolved spectroscopy (MEGA-PRESS)(16, 17). These approaches may increase acquisition or processing complexity(16, 17). Nuclear spin singlet filtering provides an alternative means of suppressing background signals while retaining selected coupled-spin signals(18, 19). Earlier studies established singlet-state preparation and magnetisation-to-singlet-order transfer(20–22); for the five-spin systems of Glu and Gln, molecule-specific optimal-control pulse design was required(23). We previously demonstrated singlet-filtered ^1H -MRS with optimal-control pulses (SFOC-MRS) in phantoms and 10 healthy volunteers across two 3 T scanners(23); that study established selective in vivo detection but did not provide the six-point phantom-calibrated concentration analysis reported here.

In the present study, we extended SFOC-MRS to phantom-calibrated apparent [Glu] estimation at 3 T, with conventional MRS acquired in the same session as a clinical reference. We first characterised regional apparent [Glu] values in the frontal lobe, occipital lobe, and basal ganglia of 25 healthy adults, and then applied the workflow in 15 patients with untreated supratentorial glioma to characterise compartment-specific apparent [Glu] in solid tumour, peritumoural oedema, and contralateral frontal lobe. Group differences were assessed with Holm-corrected Welch's tests and Hedges' g effect sizes with bootstrap confidence intervals. We hypothesised that SFOC-MRS would (i) yield region-resolved apparent [Glu]

values in healthy adults and (ii) reveal compartment-specific apparent [Glu] differences in untreated glioma, positioning ^1H -MRS as a complementary readout to PET tracers of glutamatergic targets such as mGluR5 (9–11).

Methods

Study design and participants

This prospective, single-centre observational study was reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement, with the checklist and participant flow diagram provided in the Supplementary Materials. The study and analysis of acquisitions obtained from 3 October 2022 through December 2023 were covered by approval from the Ethics Committee of Huashan Hospital, Fudan University (KY2023-022), and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before imaging. Healthy adults and patients with glioma were enrolled at a single 3 T magnetic resonance imaging (MRI) site at Huashan Hospital, Fudan University. As a methodological feasibility study, no formal sample size calculation was performed; the cohort size was determined by the availability of treatment-naïve glioma patients within the recruitment window.

Relationship to prior work. SFOC-MRS was previously demonstrated in phantoms and an earlier, independent cohort of 10 healthy volunteers across two 3 T scanners(23). Those volunteers are distinct from the present 25-participant healthy cohort, and none of their measurements contributes to the present cohort counts, summary statistics, or group comparisons. The illustrative healthy-volunteer imaging and spectra in Fig. 1A and 1C were acquired during the earlier method-development programme and are shown only to demonstrate voxel placement and signal appearance. The present regional phantom-calibrated healthy-cohort analyses and all glioma analyses are reported here for the first time.

Twenty-five healthy adult volunteers (15 women, 10 men; age 36.2 ± 10.5 years) without known neurological or psychiatric disease underwent SFOC-MRS in three predefined single-voxel volumes of interest (VOIs): a frontal-lobe VOI, an occipital-lobe VOI centred near the calcarine region, and a basal-ganglia VOI centred on the putamen/globus pallidus region. Nineteen patients with treatment-naïve supratentorial glioma scheduled for surgery were recruited (age > 18 years; no prior surgery, radiotherapy, or chemotherapy; no major haemorrhage or calcification). Four were excluded for non-diagnostic image or spectral quality, leaving 15 patients (5 women, 10 men; 64.3 ± 8.9 years). All diagnoses were confirmed by histopathology of the surgical specimen; grading and molecular characterisation included isocitrate dehydrogenase (IDH) status and Ki-67 and followed the 2021 World Health Organization (WHO) classification of central nervous system tumours.

In glioma patients, VOIs were placed in the solid-tumour region, peritumoural-oedema region, and contralateral normal-appearing frontal lobe where feasible. The solid-tumour VOI was placed within the radiologically solid component while avoiding necrosis, cystic change, haemorrhage, and calcification. The peritumoural-oedema VOI was placed within T_2 -fluid-attenuated inversion recovery (FLAIR)

hyperintense peritumoural tissue where a voxel could be prescribed without substantial overlap with solid tumour, necrosis, cerebrospinal fluid, or skull-base susceptibility regions. The contralateral normal-appearing frontal-lobe VOI was used as a within-subject reference. Cohort characteristics are summarised in Table 1.

VOI placement was performed by consensus of two board-certified neuroradiologists with more than 20 years of brain MRI/MRS experience, using 3D T_1 -weighted magnetisation-prepared rapid gradient echo (MPRAGE) and T_2 -weighted FLAIR images. Whenever feasible, VOIs were positioned away from air–tissue interfaces and the scalp to reduce susceptibility-related line broadening(24, 25). VOI labels (for example, ‘frontal lobe’ and ‘basal ganglia’) indicate anatomical placement rather than tissue purity; each VOI contains varying mixtures of grey matter, white matter, and cerebrospinal fluid. Disagreements were resolved by discussion until consensus; formal inter-rater agreement statistics were not computed for this consensus-based process.

MRI and MRS acquisition

All in vivo and phantom data were acquired on a 3 T MR system (MAGNETOM Prisma VE11E; Siemens Healthineers, Erlangen, Germany) at Huashan Hospital, Fudan University, using body-coil transmission and a 64-channel head receive array. High-resolution structural reference images were acquired using a 3D T_1 -weighted MPRAGE sequence (repetition time [TR] = 2500 ms, echo time [TE] = 3.3 ms, field of view [FOV] = 200 × 200 mm, acquisition matrix = 256 × 256, in-plane resolution = 0.78 × 0.78 mm, slice thickness = 2 mm) for VOI prescription and anatomical localisation.

The SFOC-MRS method designed for 3 T was applied as previously described(23). Water suppression enhanced through T_1 effects (WET) was used, and PRESS provided voxel selection. Conventional short-echo PRESS spectra were acquired in the same session as a clinical reference. In the present protocol, both SFOC-MRS and conventional PRESS used TR = 1500 ms, TE = 30 ms, spectral bandwidth = 1000 Hz, 512 complex points, 64 averages, and vendor 3D shimming (prespecified target water linewidth ≤ 15 Hz). Relative to the earlier SFOC study (TR = 3000 ms; TE = 35 ms)(23), the present protocol used shorter TR and TE. Differential relaxation was explored with an illustrative sensitivity scenario rather than treated as a validated correction (**Supplementary Table S2**). The optimal-control pulse-I duration was 300 ms for Glu $H^\alpha-H^\beta$, the decoupling pulse was 5 ms, gradient pulses g_1/g_2 were 1 ms each, and optimal-control pulse II matched pulse I in duration. Nominal acquisition time was 1 min 36 s per voxel (1.5 s × 64 averages), excluding preparation, shimming, and voxel prescription; actual examination time varied with setup and the number of voxels and was not analysed. Glu-filtered and conventional spectra were obtained from identical locations. Unsuppressed-water spectra were acquired for quality assessment but were not used to scale the phantom-calibrated SFOC values. The on-scanner workflow is illustrated in **Supplementary Figure S1**; full acquisition and reporting details are provided in **Supplementary Table S1**.

In vivo, examinations followed a fixed order (T_1 -MPRAGE → conventional MRS → SFOC-MRS) with foam padding used to mitigate head motion. Automated 3D shimming was performed before each MRS

acquisition; a water linewidth ≤ 15 Hz was the prespecified acquisition-quality target, and achieved linewidths are reported below. A standard 3.375-mL voxel ($15 \times 15 \times 15$ mm³) was used for healthy frontal lobe, peritumour oedema, and most solid-tumour placements; a larger 8.0-mL voxel ($20 \times 20 \times 20$ mm³) was used for the basal ganglia and occasionally where additional signal was required. Phantom acquisitions used a 6.75-mL voxel ($15 \times 30 \times 15$ mm³) matched to the in vivo SFOC-MRS pulse parameters.

Six aqueous L-glutamate phantoms (6, 8, 10, 12, 14, 16 mM) were prepared by dissolving L-glutamate (99%; Aladdin Industrial Corporation, Shanghai, China) in 50 mL of phosphate-buffered saline (pH 7.2–7.4; Adamas Life, Shanghai, China). The pH of each phantom was titrated to 7.3 ± 0.1 with concentrated NaOH or HCl solution (0.05 mM) and verified with a calibrated pH meter (Jenway-3510, Cole-Parmer, Stone, UK). Phantoms were scanned at room temperature ($\approx 22^\circ\text{C}$) in the same head/neck coil and with identical SFOC-MRS pulse parameters as the in vivo acquisitions, providing a six-point calibration curve for phantom-calibrated Glu quantification (Fig. 1B; six-point linear fit $R^2 = 0.974$). Full acquisition parameters are provided in **Supplementary Table S1** following the MRSinMRS reporting standard(12).

Spectral processing and Glu quantification

Raw free-induction decays (FIDs) were coil-combined by the vendor implementation and corrected for frequency and phase drift by spectral registration(26). Residual water was removed by Hankel–Lanczos singular value decomposition (HLSVD)(27). Spectra were zero-filled to 4096 points and apodised with a 2 Hz Lorentzian window before Fourier transformation and referenced to water at 4.70 ppm. Spectral quality was assessed following consensus recommendations(12, 25, 28). Signal-to-noise ratio (SNR) was defined as the targeted-resonance peak height above the fitted baseline divided by twice the standard deviation of a signal-free region(25); linewidth was the full width at half maximum (FWHM) of the unsuppressed-water peak. The prespecified inclusion criteria were $\text{SNR} \geq 3$ as a signal-presence floor, water linewidth ≤ 15 Hz, no prominent lipid contamination, and acceptable residual-water suppression. The 15 Hz value was an operational study threshold rather than a consensus-derived guarantee of quantitative precision; $\text{SNR} = 3$ likewise indicates signal presence, not reliable quantification(25, 28). For conventional PRESS, archived outputs reported a median water FWHM of 3.9 Hz and median metabolite SNR of 15. No claim of equivalent spectral quality between cohorts was inferred from fitted SFOC component widths.

PRESS spectra were fitted with LCModel(29) using a vendor-matched basis set. The exploratory comparator dataset retained estimates with relative Cramér–Rao lower bounds (CRLBs) $\leq 20\%$; this filter did not affect any primary SFOC endpoint and is acknowledged as a potential source of selection bias because consensus guidance discourages excluding values solely by relative CRLB(28–30). For SFOC-MRS, the targeted Glu-triplet area (A_{Glu}) was integrated over a prespecified 2.20–2.50 ppm window after linear-spline baseline correction. The window was selected after singlet filtering and exceeded twice the fitted triplet FWHM; nevertheless, residual overlap and baseline dependence were not independently quantified, so the output is reported as an apparent rather than tissue-absolute concentration(30, 31). A six-point phantom series (6–16 mM L-glutamate; 6.75-mL voxel, $15 \times 30 \times 15$ mm) yielded $A_{\text{Glu}} = 0.288 \times$

[Glu] + 0.041 ($R^2 = 0.974$). In-vivo values were computed by inversion with voxel-volume normalisation: $[Glu] \text{ (mM)} = (A_{Glu} \times V_{\text{phantom}} / V_{\text{voxel}} - 0.041) / 0.288$, where $V_{\text{phantom}} = 6.75 \text{ mL}$ and V_{voxel} is the prescribed in-vivo voxel volume. Values were recomputed from the archived peak-area workbook using the unrounded calibration coefficients for all tables and figures. No per-voxel internal-water scaling, tissue-fraction correction, or measured metabolite-specific relaxation correction was applied(32).

Matched short-TE acquisitions mitigated but did not remove differential relaxation weighting. Under prespecified illustrative T_1/T_2 assumptions, the calibration slope changed by approximately 18% (**Supplementary Table S2**). The complete set of assumptions was not measured in the phantoms or participants and is not presented as a literature-validated correction factor(32, 33).

Statistical analysis

Continuous variables are summarised as mean $\pm$ standard deviation (SD). Group differences were analysed at the voxel level using heteroscedastic Welch tests (Welch's ANOVA for three-group comparisons) and Holm-corrected pairwise contrasts. Because participants could contribute more than one voxel, analyses were repeated after averaging within each participant–region cell; conclusions were concordant (**Supplementary Table S3**). Effect sizes are Hedges'g with 95% bias-corrected and accelerated bootstrap confidence intervals (10,000 resamples); Mann–Whitney U tests provided a distribution-free robustness check for the smallest subgroups. Brain glutamate has been reported to be lower in older adults(34), and the cohorts differed in age. The cross-cohort comparison was therefore supplemented by an age-restricted sensitivity comparison (**Supplementary Figure S2**); no multivariable age adjustment was performed. Analyses were implemented in R and Python.

Results

Patients

Cohort characteristics are summarised in Table 1; participant flow is shown in **Supplementary Figure S3**. Patients were older than controls (64.3 ± 8.9 vs 36.2 ± 10.5 years; $p < 0.001$) and male-predominant (10/15 vs 10/25 male; Fisher $p = 0.19$). Of the 15 patients, 12 (80%) had WHO grade 4 gliomas and 14 (93%) were IDH-wildtype, with median Ki-67 of 18% (interquartile range [IQR], 14.0–27.5%). Histological and molecular diagnoses were recorded according to the 2021 WHO classification. After spectral quality control, 25 healthy controls contributed 54 voxel-level spectra (frontal lobe/occipital lobe/basal ganglia, $n = 25/16/13$), and 15 patients contributed 25 voxel-level spectra (solid tumour/peritumoural oedema/contralateral frontal lobe, $n = 14/4/7$).

Phantom calibration and illustrative selective Glu detection

We first evaluated the present phantom-calibrated workflow, extending prior selective-detection work(23). An illustrative voxel placement and co-localised spectrum from the earlier, independent method-

development programme are shown in Fig. 1a and 1c; neither contributes to the present cohort-level analyses. Across the 6–16 mM L-glutamate phantom series, the integrated 2.20–2.50 ppm triplet area was linear in nominal [Glu] (Fig. 1B; $A_{\text{Glu}} = 0.288 \times [\text{Glu}] + 0.041$, $R^2 = 0.974$, $p = 3.0 \times 10^{-4}$). In the illustrative in vivo spectrum (Fig. 1C), SFOC-MRS isolated the diagnostic Glu multiplet within the same window, whereas conventional PRESS remained overlapped with neighbouring J-coupled resonances. Non-Glu resonances in the targeted spectrum remained < 15% of baseline. The molecular origin of the approximately 2.35 ppm multiplet (C4 methylene protons H^α and H^β) is shown in **Supplementary Figure S4**.

Selective Glu detection in the brain of glioma patients

The same workflow was applied in patients with glioma. Representative solid-tumour and contralateral frontal-lobe voxels are shown in Fig. 2. Conventional PRESS displayed overlapping Glu/Gln resonances, whereas SFOC-MRS isolated the targeted Glu triplet at approximately 2.35 ppm in the displayed examples. An additional co-localised patient example is shown in **Supplementary Figure S5**. These examples illustrate signal visibility but do not constitute independent validation of specificity.

An exploratory same-session comparison used the creatine-referenced indices SFOC Glu/Cr and LCModel Glu/(Cr + PCr), where Cr and PCr denote creatine and phosphocreatine (**Supplementary Figure S6**). The pooled Pearson correlation was $r = 0.02$. A descriptive difference-versus-mean display yielded a mean difference of -0.32 and an interval of -0.96 to $+0.33$ in ratio units. Because acquisition, quantification, and denominator definitions differed, this display was not interpreted as a formal method-agreement analysis and no mM Bland–Altman inference was made.

Regional and compartmental [Glu]

In controls, [Glu] showed a steep regional [Glu] hierarchy across frontal lobe, occipital lobe, and basal-ganglia VOIs (Fig. 3a): frontal lobe 8.50 ± 1.98 mM > occipital lobe 4.79 ± 1.51 mM > basal ganglia 3.24 ± 0.82 mM; Welch's ANOVA $F(2, 32.5) = 65.26$, $p = 4.2 \times 10^{-12}$. All three pairwise contrasts were Holm-significant, with large effect sizes: frontal lobe vs occipital lobe $g = 2.00$ (1.45–2.86), Holm $p = 1.0 \times 10^{-7}$; frontal lobe vs basal ganglia $g = 3.06$ (2.50–4.12), Holm $p = 6.0 \times 10^{-13}$; and occipital lobe vs basal ganglia $g = 1.21$ (0.63–2.14), Holm $p = 1.7 \times 10^{-3}$. In glioma, [Glu] also differed across the three compartments (Fig. 3b; Welch's ANOVA $F(2, 12.0) = 12.26$, $p = 0.0013$): peritumoural oedema (1.21 ± 1.00 mM) was substantially lower than that of both the solid tumour (4.47 ± 2.91 mM; $g = 1.17$ [0.76–2.08], Holm $p = 9.3 \times 10^{-3}$) and the contralateral frontal lobe (7.52 ± 3.70 mM; $g = -1.88$ [– 4.59 to – 1.13], Holm $p = 9.3 \times 10^{-3}$), whereas the difference between the solid tumour and the contralateral frontal lobe did not reach significance ($g = -0.92$ [– 2.53 to – 0.04], Holm $p = 0.086$). The results of the Mann–Whitney U tests were concordant (Table 2 and Supplementary Table S3).

Cross-cohort comparison of apparent [Glu]

In the primary comparison, apparent [Glu] was lower in glioma-related voxels (solid tumour + peritumoural oedema, $n = 18$; 3.75 ± 2.93 mM) than in healthy frontal- and occipital-lobe voxels ($n = 41$; 7.06 ± 2.56 mM; Fig. 3c; Welch's $t = 4.14$, $p = 2.8 \times 10^{-4}$; Hedges' $g = 1.22$ [0.57–2.05]). Because the cohorts differed in age, a sensitivity analysis compared contralateral frontal-lobe voxels in patients (7.52 ± 3.70 mM, $n = 7$) with frontal-lobe voxels in healthy controls aged ≥ 40 years (9.99 ± 1.07 mM, $n = 8$). The difference did not reach statistical significance ($t = -1.71$, $p = 0.13$; **Supplementary Figure S2**). This small analysis does not establish equivalence or eliminate residual age confounding; the cross-cohort result should therefore be interpreted cautiously. The within-patient compartmental pattern is less directly affected by between-cohort age differences.

Table 1
Demographic, voxel-distribution, and clinicopathologic characteristics of participants included in the final quantitative analysis.

Group	Controls (n = 25)	Patients (n = 15)	p value
Age, y, mean (SD)	36.2 (10.5)	64.3 (8.9)	< 0.001
Sex, n (%)			
Female	15 (60.0%)	5 (33.3%)	0.19
Male	10 (40.0%)	10 (66.7%)	
Voxel distribution, n		-	-
Frontal lobe voxels	25	-	-
Occipital lobe voxels	16	-	-
Basal ganglia voxels	13	-	-
Solid tumour region voxels	-	14	-
Peritumoural oedema voxels	-	4	-
Contralateral frontal lobe voxels	-	7	-
Tumour characteristics			-
WHO grade, n (%)			-
3	-	3 (20.0%)	-
4	-	12 (80.0%)	-
IDH status, n (%)			-
Wild-type	-	14 (93.3%)	-
Mutant	-	1 (6.7%)	-
Ki-67, %, median [IQR]	-	18.0 [14.0–27.5]	-
Ki-67 range, %	-	8–80	-
Notes. Characteristics are shown for participants included in the final quantitative analysis. IDH, isocitrate dehydrogenase; IQR, interquartile range; SD, standard deviation; WHO, World Health Organization.			

Table 2

Summary of the principal quantitative comparisons of apparent glutamate concentration ([Glu], mM).

Analysis	Group / region	n	[Glu] (mM, mean $\pm$ SD)	Test	Statistic	p (Holm)
Healthy control regional gradient	Frontal lobe	25	8.50 $\pm$ 1.98	Welch's ANOVA (3 groups)	F (2, 32.5) = 65.26	4.2 $\times$ 10 ⁻¹²
	Occipital lobe	16	4.79 $\pm$ 1.51	FL vs occipital	g = 2.00 [1.45, 2.86]	1.0 $\times$ 10 ⁻⁷
	Basal ganglia	13	3.24 $\pm$ 0.82	FL vs basal ganglia	g = 3.06 [2.50, 4.12]	6.0 $\times$ 10 ⁻¹³
				OL vs basal ganglia	g = 1.21 [0.63, 2.14]	1.7 $\times$ 10 ⁻³
Glioma patient compartmental ordering	Solid tumour	14	4.47 $\pm$ 2.91	Welch's ANOVA (3 groups)	F (2, 12.0) = 12.26	0.0013
	Peritumoural oedema	4	1.21 $\pm$ 1.00	Solid tumour vs oedema	g = 1.17 [0.76, 2.08]	9.3 $\times$ 10 ⁻³
	Contralateral frontal lobe	7	7.52 $\pm$ 3.70	Oedema vs contralateral FL	g = -1.88 [-4.59, -1.13]	9.3 $\times$ 10 ⁻³
				Solid tumour vs contralateral FL	g = -0.92 [-2.53, -0.04]	0.086
Primary comparison	Healthy control lobe (frontal + occipital)	41	7.06 $\pm$ 2.56	Welch's t-test	t = 4.14; g = 1.22 [0.57, 2.05]	2.8 $\times$ 10 ⁻⁴
	Glioma (solid tumour + peritumoural oedema)	18	3.75 $\pm$ 2.93			
Age-matched sensitivity	Healthy control FL, age $\geq$ 40 y	8	9.99 $\pm$ 1.07	Welch's t-test	t = -1.71	0.13

Notes. Welch's tests were used without assuming equal variances; three-group comparisons used Welch's analysis of variance (ANOVA; R oneway.test, var.equal = FALSE) with Holm-corrected pairwise post-hoc tests. Effect sizes are Hedges'g with 95% bias-corrected bootstrap confidence intervals (CIs; 10,000 resamples; random seed = 42). Bold Holm-adjusted p values indicate significance at $\alpha = 0.05$. Full statistical contrasts and Mann-Whitney U robustness checks are provided in Supplementary Table S3. FL, frontal lobe; OL, occipital lobe; SD, standard deviation.

Analysis	Group / region	n	[Glu] (mM, mean ± SD)	Test	Statistic	p (Holm)
	Glioma contralateral FL	7	7.52 ± 3.70			
Notes. Welch's tests were used without assuming equal variances; three-group comparisons used Welch's analysis of variance (ANOVA; R oneway.test, var.equal = FALSE) with Holm-corrected pairwise post-hoc tests. Effect sizes are Hedges'g with 95% bias-corrected bootstrap confidence intervals (CIs; 10,000 resamples; random seed = 42). Bold Holm-adjusted p values indicate significance at $\alpha = 0.05$. Full statistical contrasts and Mann–Whitney U robustness checks are provided in Supplementary Table S3. FL, frontal lobe; OL, occipital lobe; SD, standard deviation.						

Discussion

Together with the previous proof-of-concept demonstration(23), this study supports the feasibility of acquiring SFOC-MRS at 3 T and deriving phantom-calibrated apparent glutamate values. It does not establish tissue-absolute quantification or clinical utility. We therefore interpret the regional results against prior multi-region MRS, examine the glioma compartmental pattern in the context of 7 T MRSI literature and glutamatergic-dysregulation models, and define the methodological boundaries of the calibration workflow.

Prior studies establish regional and tissue dependence of brain glutamate but do not identify a uniform frontal-to-basal-ganglia hierarchy. At 3 T, whole-brain MRSI showed lower Glu/Cr in the putamen and pallidum than in cortical white matter(35), whereas a 7 T single-voxel study found high Glu in the putamen and an approximately 1.8-fold range across sampled regions(36). The present gradient is therefore not a simple replication of one accepted ordering. Tissue composition is an important contributor: glutamate scales strongly with grey-matter fraction ($r \approx 0.87$), and frontal and occipital glutamate did not differ when tissue fractions were matched(37). Without cohort-wide tissue correction(32), the observed gradient should be interpreted as a combined neurochemical, tissue-composition and acquisition effect. Its magnitude is additionally confounded by region-specific voxel volume, as discussed below; it should not be treated as validation of the method or as a tissue-absolute reference range. The regional distributions are hypothesis-generating apparent values for future validation.

Per-voxel tissue segmentation in one representative participant is shown in **Supplementary Figure S7**. The frontal VOI was grey-matter-predominant (grey matter $\approx 69\%$, white matter $\approx 23\%$), whereas the occipital VOI was white-matter-predominant (grey matter $\approx 17\%$, white matter $\approx 69\%$); this composition difference could contribute to higher apparent glutamate in the frontal voxel. The basal-ganglia VOI had an intermediate grey-matter fraction ($\approx 35\%$) but the lowest apparent [Glu]. Thus, tissue fraction did not vary monotonically with the three-region ranking in this participant. This single example is illustrative only: it neither quantifies cohort-level confounding nor identifies the cause of the basal-ganglia value, and it does not replace systematic cohort-wide segmentation and tissue-fraction correction.

A structural limitation concerns this regional gradient. Frontal and occipital voxels were acquired at 3.375 mL (15 × 15 × 15 mm) whereas basal-ganglia voxels used 8.0 mL (20 × 20 × 20 mm), and apparent

concentrations were derived by normalising the integrated signal to the phantom reference volume. Because the raw integrated glutamate signal was closely similar between frontal and basal-ganglia voxels (mean triplet area 1.32 versus 1.10 a.u.), the ~ 2.6-fold apparent-concentration gradient is generated almost entirely by this volume normalisation, which assumes the targeted signal scales linearly with voxel volume. That assumption was calibrated at a single volume (the 6.75 mL phantom), is perfectly confounded with region, and was not independently validated across voxel sizes. The regional gradient should therefore be regarded as provisional pending a multi-volume phantom validation or acquisition at a common voxel size; absolute concentrations are reported as apparent values.

Within glioma, peritumoural oedema had lower apparent [Glu] than solid tumour and contralateral frontal lobe (both Holm $p = 9.3 \times 10^{-3}$). External literature is mixed. Li et al. reported reduced Glu/tCr in T_2 lesions only in grade 2 glioma(38), whereas Hangel et al. reported higher peritumoural Glu/tCr than tumour and normal-appearing white matter at 7 T(39). Both studies used creatine-referenced ratios and different lesion definitions, so neither directly validates the present phantom-calibrated apparent concentration. Potential contributors include oedema dilution, partial-volume effects, reduced neuronal density and microenvironmental heterogeneity, which this study did not separate(25, 40). Contralateral frontal-lobe VOIs provided within-patient context where available, but the reported Welch compartment tests were voxel-level and unpaired. Preclinical and human studies link glutamatergic pathways to glioma-associated hyperexcitability and seizures(7, 8, 41, 42). ^1H -MRS predominantly reflects the total-tissue Glu pool, whereas excitotoxic signalling concerns a much smaller extracellular pool(43); glioma-driven extracellular release and lower total-tissue Glu can therefore coexist, but the present data do not test that compartmental mechanism. Given the peritumoural sample size ($n = 4$), these findings remain exploratory despite large effect sizes and concordant distribution-free sensitivity results.

Within molecular imaging of glutamatergic dysfunction, ^1H -MRS-derived Glu and PET-derived mGluR5 binding availability provide biologically distinct readouts. [^{18}F]PSS232 PET/MRI showed lower mGluR5 availability in the hippocampus and parahippocampal gyrus in AD(9), consistent with lower hippocampal binding reported with [^{18}F]FPEB in early AD(10) and building on first-in-human [^{18}F]PSS232 evaluation in healthy volunteers(11). These receptor-binding readouts differ from the total-tissue Glu signal measured by ^1H -MRS and may vary partly independently in disease. Complementary neuropathological and autoradiographic work has linked elevated glial fibrillary acidic protein and P2X7 receptor levels to amyloid, tau and synaptic loss in AD tissue(44), illustrating that transmitter, receptor and glial markers reflect distinct biological compartments. SFOC-MRS may therefore complement, rather than duplicate, PET and other molecular biomarkers, but direct multimodal validation is still required.

The phantom calibration supported a linear concentration-dependent response across 6–16 mM. Important limitations remain. Values below 6 mM are linear extrapolations outside the measured calibration range, and the calibration was acquired at one phantom voxel volume; neither a lower limit of quantification nor signal scaling across voxel volumes was independently validated. The approximately 18% relaxation scenario in **Supplementary Table S2** used illustrative assumptions and is not a validated correction factor. No cohort-wide tissue-fraction correction or internal-water scaling was applied(32, 33, 37). The exploratory LCModel comparator used a relative CRLB filter and different creatine denominators,

so it cannot establish convergent validity or formal agreement. The prespecified 15 Hz water-linewidth criterion was more permissive than the 13 Hz upper range cited in an advanced-MRS consensus(28). The study also lacked formal test–retest and multicentre reproducibility assessment(12). Molecular characterisation was limited to IDH status and WHO grade. IDH-mutant gliomas produce 2-hydroxyglutarate by reduction of α -ketoglutarate and may exhibit associated alterations in glutamate/glutamine metabolism(45); the present predominantly IDH-wildtype cohort was not designed to test that pathway. Future studies should use common voxel volumes, measured relaxation and tissue correction, complete per-spectrum quality metrics, same-scale orthogonal comparison, test–retest assessment, and larger histology-matched multicentre cohorts. O6-methylguanine-DNA methyltransferase (MGMT) and 1p/19q status should also be included.

The exploratory comparison yielded a pooled $r = 0.02$ between SFOC Glu/Cr and LCModel Glu/(Cr + PCr). Published same-voxel comparisons of established Glu methods reported correlations of approximately 0.35–0.55(17, 46), so the present association was weaker. Because the two indices used different acquisition, quantification and denominator definitions, their descriptive difference-versus-mean plot cannot establish formal agreement. The near-zero correlation may reflect restricted physiological range, method selectivity, fitting and normalisation differences, but those explanations were not tested here. It is therefore a limitation rather than evidence of concordance, and same-scale orthogonal validation and test–retest assessment are required.

A further quantification caveat concerns the measured calibration range. The 6–16 mM phantom series does not establish a formal lower limit of quantification. Apparent values below 6 mM—seen mainly in basal-ganglia and peritumoural-oedema voxels—are extrapolations and should not be interpreted as validated absolute concentrations.

Conclusions

In summary, singlet-filtered ^1H -MRS with optimal-control pulses yielded phantom-calibrated apparent regional glutamate values in healthy adults and detected compartmental differences in untreated glioma at 3 T. These findings support feasibility and provide hypotheses for further study, but they do not establish tissue-absolute reference values or clinical validity. Cohort-wide tissue correction, multi-volume phantom validation, same-scale orthogonal comparison, test–retest assessment and multicentre replication are required before clinical deployment.

Abbreviations

^1H -MRS

proton magnetic resonance spectroscopy

SFOC-MRS

singlet-filtered proton magnetic resonance spectroscopy with optimal-control pulses

MRI

magnetic resonance imaging

PRESS
point-resolved spectroscopy
MEGA-PRESS
Mescher–Garwood point-resolved spectroscopy
Glu
glutamate
Gln
glutamine
Glx
combined glutamate and glutamine
VOI
volume of interest
STROBE
Strengthening the Reporting of Observational Studies in Epidemiology
MRSinMRS
minimum reporting standards for in vivo MRS
FID
free-induction decay
HLSVD
Hankel–Lanczos singular value decomposition
WET
water suppression enhanced through T_1 effects
FWHM
full width at half maximum
SNR
signal-to-noise ratio
CRLB
Cramér–Rao lower bound
Cr
creatine
PCr
phosphocreatine
tCr
total creatine
TE
echo time
TR
repetition time
FOV
field of view
IDH

isocitrate dehydrogenase
WHO
World Health Organization
FLAIR
fluid-attenuated inversion recovery
MPRAGE
magnetisation-prepared rapid gradient echo
PET
positron emission tomography
mGluR5
metabotropic glutamate receptor 5
AD
Alzheimer's disease
ANOVA
analysis of variance
SD
standard deviation
CI
confidence interval
IQR
interquartile range
MRSI
magnetic resonance spectroscopic imaging
MGMT
O6-methylguanine-DNA methyltransferase
AI
artificial intelligence.

Declarations

Ethics approval and consent to participate

All procedures were conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Huashan Hospital, Fudan University (approval No. KY2023-022). Written informed consent was obtained from all participants (healthy volunteers and patients) prior to MRI/MRS acquisition.

Consent for publication

Written informed consent covered publication of anonymised imaging and spectroscopy examples. No identifiable individual-level information is presented, and all data were anonymised before figure preparation.

Use of generative AI during manuscript preparation

No AI tool was used as a source of scientific evidence or listed as an author. The human authors retain full responsibility for independently reviewing and approving all analyses, numerical results, references, figure content, and manuscript revisions before submission. All authors reviewed and approved this disclosure.

Competing interests

RN is an Editorial Board member of BMC Medicine and of the European Journal of Nuclear Medicine and Molecular Imaging, and AR is an Editorial Board member of the latter; both will recuse themselves from any editorial role in the handling and peer review of this manuscript. The other authors declare no competing interests.

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Author Contribution

Since Yang Yang and Yefeng Yao share the same initials, they are referred to as Y.Yang and Y.Yao, respectively. J.W., J.X., Y.Yao, R.N., and Y.R. conceptualized the study. J.X., Y.Yao, and J.W. developed the methodology and designed the pulse sequence. C.Y., J.X., Y.Yao, and J.W. contributed to methodological validation and interpretation of the phantom and MRS experiments. W.W., R.F., Y.Yang, and Y.L. conducted the clinical investigation and participant recruitment. W.W., Y.Yang, and J.W. acquired the MRS data. J.W., C.Y., and J.X. performed the formal analysis, and J.W. performed the statistical analysis and prepared the figures. J.W. wrote the original draft. Y.R., H.L., Y.Yao, R.N., and A.R. supervised the study. Y.R. and H.L. administered the project, and Y.R. acquired funding. All authors reviewed and edited the manuscript, approved the submitted version, and agreed to be personally accountable for their own contributions and for ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. J.W., C.Y., W.W., R.F., and Y.Yang contributed equally and share first authorship. Y.R., H.L., and R.N. contributed equally as corresponding authors.

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Availability of data and materials

All summary data supporting the findings are provided in the article and its Supplementary Information. Participant-level imaging and spectroscopy files are not publicly posted because of re-identification risk. A de-identified dataset and analysis scripts may be made available by the corresponding author on reasonable request, subject to participant consent, institutional data-governance approval, and a data-use agreement. Editors and reviewers may request access during peer review under the same safeguards. Statistical analyses were implemented in R and Python; the archived scripts and a software-version manifest will accompany any approved data release.

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Figures

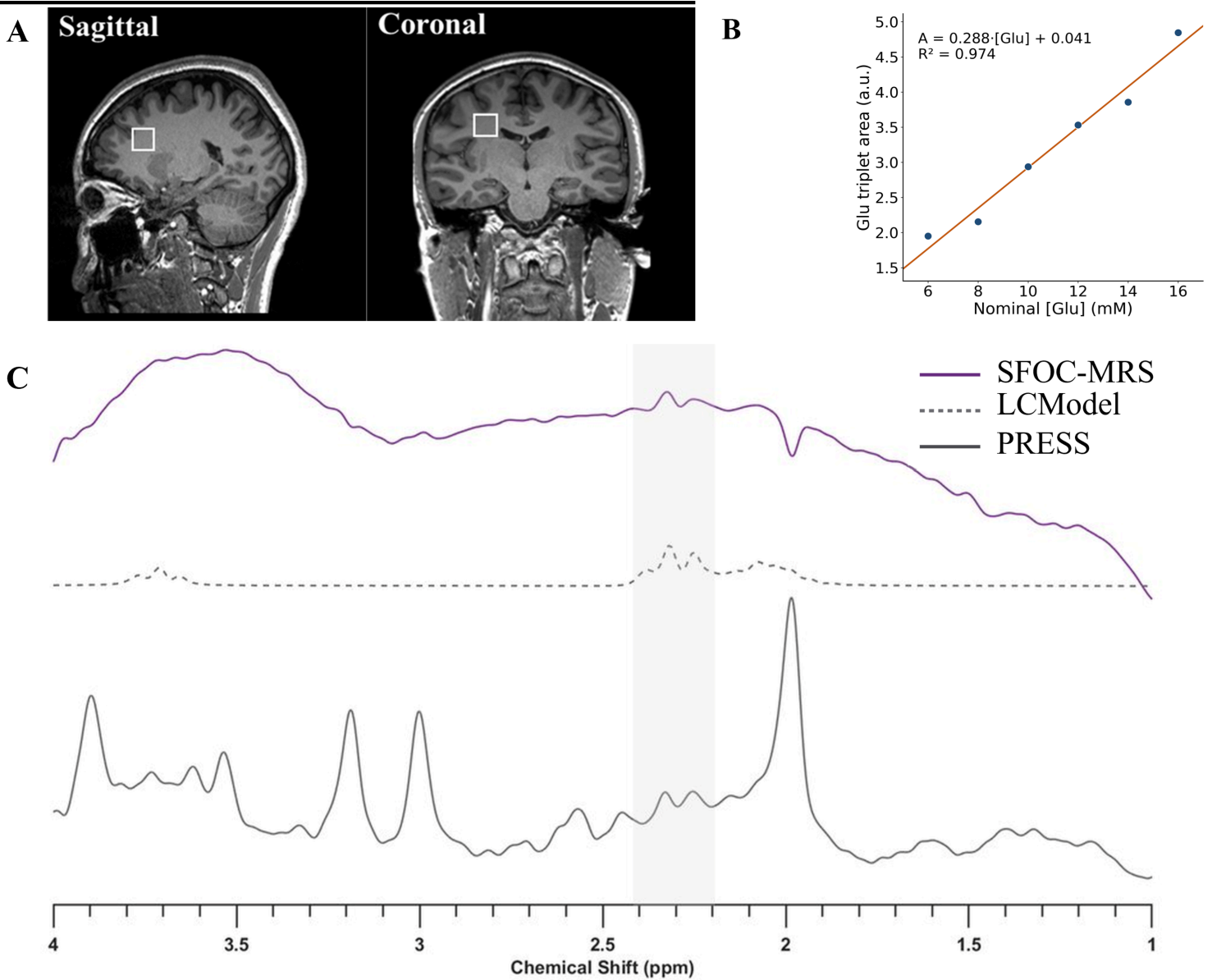


Figure 1

Healthy-volunteer glutamate spectroscopy and phantom calibration at 3 T. Singlet-filtered proton magnetic resonance spectroscopy with optimal-control pulses (SFOC-MRS) was used for the targeted readout. (A) Sagittal and coronal T₁-weighted magnetisation-prepared rapid gradient-echo (MPRAGE) images showing representative voxel placement in the right frontal lobe. (B) Phantom calibration curve

relating integrated 2.20–2.50 ppm glutamate (Glu) triplet area (A_Glu; arbitrary units [a.u.]) to nominal concentration across six L-glutamate solutions (6–16 mM); $A_{\text{Glu}} = 0.288 \times [\text{Glu}] + 0.041$ ($R^2 = 0.974$, $p = 3.0 \times 10^{-4}$). (C) Co-localised spectra from the same voxel: SFOC-MRS (purple), LCModel fit to conventional point-resolved spectroscopy (PRESS; grey dashed), and raw PRESS (dark grey). Panels A and C are an illustrative example acquired during the earlier, independent method-development programme(23) and do not contribute to the present cohort counts or quantitative comparisons. Shading marks the predefined integration window; non-Glu resonances remained below 15 % of baseline. The molecular origin of the approximately 2.35 ppm multiplet is shown in **Supplementary Figure S4**.

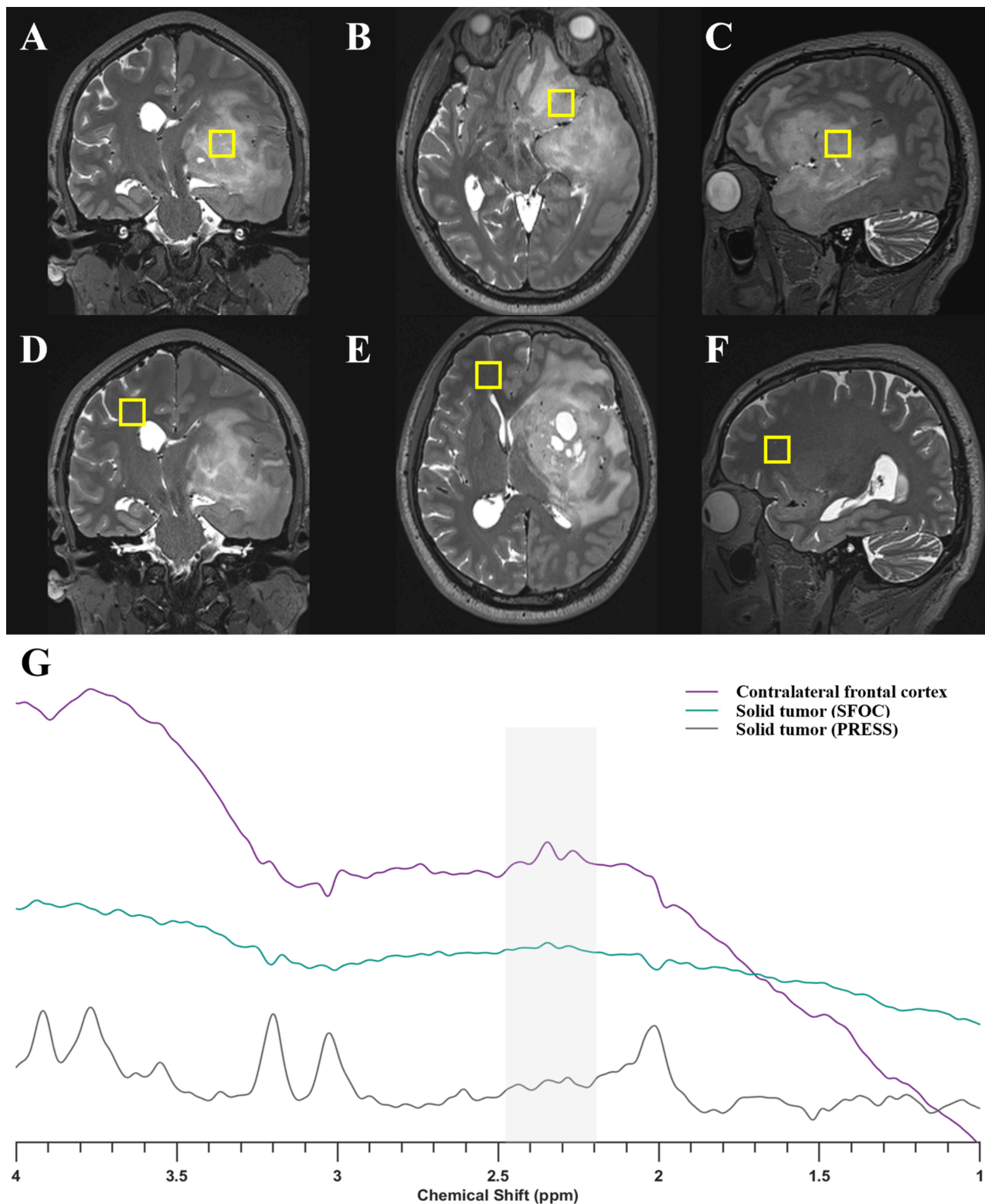


Figure 2

Singlet-filtered proton magnetic resonance spectroscopy with optimal-control pulses (SFOC-MRS) in a patient with treatment-naïve glioma. (A–C) T₂-weighted fluid-attenuated inversion-recovery images showing representative voxel placement in the solid tumour in coronal, axial and sagittal planes. (D–F) Co-localised contralateral frontal-lobe voxel acquired in the same session. (G) Spectra from lesion-related and contralateral voxels: SFOC-MRS from the contralateral frontal lobe (purple), SFOC-MRS from solid tumour

(teal), and conventional point-resolved spectroscopy (PRESS) from solid tumour (dark grey). Shading marks the predefined glutamate (Glu) integration window (2.20–2.50 ppm); non-Glu resonances remained below 15 % of baseline. **Supplementary Figure S5** provides another co-localised patient example.

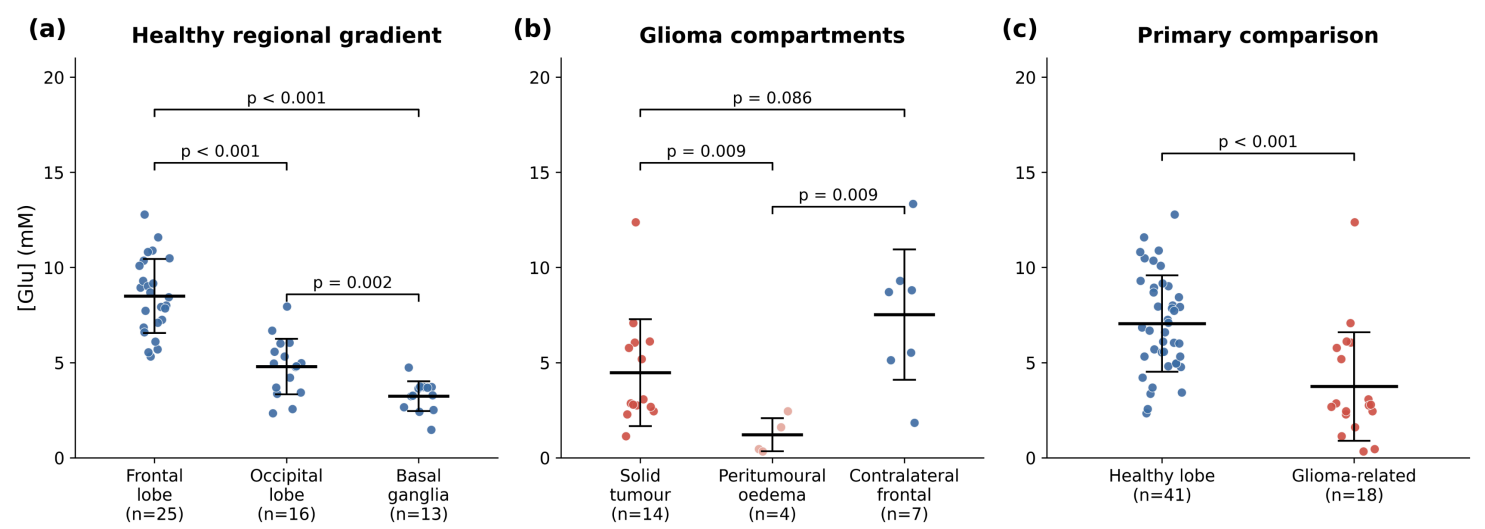


Figure 3

Regional and glioma-compartment apparent glutamate concentrations at 3 T. Apparent glutamate concentration ([Glu], mM) was measured with singlet-filtered proton magnetic resonance spectroscopy with optimal-control pulses (SFOC-MRS).(a) Healthy controls: frontal (n = 25), occipital (n = 16), and basal-ganglia (n = 13) voxels; Welch's analysis of variance (ANOVA) $p = 4.2 \times 10^{-12}$. (b) Glioma compartments: solid tumour (n = 14), peritumoural oedema (n = 4), and contralateral frontal lobe (n = 7); Welch's ANOVA $p = 0.0013$. (c) Primary comparison of healthy frontal- and occipital-lobe voxels (n = 41) with glioma-related voxels (n = 18); Welch's $t = 4.14$, $p = 2.8 \times 10^{-4}$, Hedges' $g = 1.22$ [0.57–2.05]. Dots represent voxel-level estimates; bars and whiskers show mean ± standard deviation. **Supplementary Table S3** gives full contrasts.