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Glutamatergic Metabolites Are Associated with Visual Plasticity in Humans

S. Andrea Wijtenburg^{a,*}, Jeffrey West^a, Stephanie A. Korenic^a, Franchesca Kuhney^a, Frank E. Gaston^a, Hongji Chen^a, Meredith Roberts^a, Peter Kochunov^{a,b}, L. Elliot Hong^a, and Laura M. Rowland^{a,c,d}

^aMaryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, P.O. Box 21247, Baltimore, MD, USA, 21228

^bDepartment of Physics, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD, USA, 21250

^cRussell H. Morgan Department of Radiology and Radiological Sciences, Johns Hopkins University School of Medicine, 601 N. Caroline Street, Baltimore, MD, USA, 21287

^dDepartment of Psychology, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD, 21250

Abstract

Long-term potentiation (LTP) is a basic cellular mechanism underlying learning and memory. LTP-like plasticity in the visual cortex can be induced by high frequency visual stimulation in rodents and humans. Since glutamate plays a fundamental role in LTP, this study investigated if visual cortical glutamate and glutamine levels, measured by proton magnetic resonance spectroscopy (MRS), relate to visual plasticity in humans. Since plasticity requires a delicate excitation and inhibition balance, GABA was also explored. Eighteen healthy participants completed MRS and a visual fMRI paradigm. Results revealed enhanced fMRI activations after high frequency visual stimulation, suggesting visual plasticity occurred. Higher activations were associated with higher resting glutamine levels after family wise error-correction. Exploratory analyses revealed that higher resting glutamate and GABA levels were associated with visual plasticity, suggesting there may be a critical excitation-inhibition balance necessary for experience dependent plasticity. This is the first empirical evidence that resting glutamine levels and potentially glutamate and GABA levels are associated with visual plasticity in humans.

Keywords

visual plasticity; fMRI; glutamate; GABA; healthy adults; magnetic resonance spectroscopy

*Correspond Author: S. Andrea Wijtenburg, Ph.D., Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, P.O. Box 21247, Baltimore, MD 21228, awijtenburg@mprc.umaryland.edu, Office: (410) 402-6886, Fax: (410) 402-6077.

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1. Introduction

Long-term potentiation (LTP) is a basic cellular plasticity mechanism underlying learning and memory [1]. While LTP is most often studied in the hippocampus [2], LTP-like changes occur in other regions including the somatosensory cortex [3], motor areas [4], and visual cortex [5]. LTP is typically induced by high-frequency electrical stimulation in animals [1, 6]. In addition, recent work indicates that LTP-like plasticity can also occur through training on motor tasks [7, 8] or with high frequency visual stimulation [9, 10]. Clapp et al. developed a translational paradigm known to induce LTP-changes in the visual cortex in rodents, and applied it to humans utilizing EEG and fMRI studies to record LTP-like changes [5, 11, 12]. These studies successfully revealed an increase in fMRI BOLD activation or visual evoked potentials following high frequency stimulation (HFS), similar to previous animal studies [11–13]. Further follow-up studies using EEG demonstrated that the plasticity effect observed was specific to HFS and not due to altered cortical excitability or attention/arousal [14–16].

Human studies of visual plasticity have yielded inconsistent results. Clapp et al. observed increased BOLD response bilaterally in the extrastriate visual cortex whereas Lahr et al. reported decreased activations in primary and secondary visual cortices following HFS [12, 17]. Using EEG, Cavus et al. demonstrated sustained potentiation after HFS in the primary visual cortex and visual association cortices in healthy volunteers, but not in adults with schizophrenia [18]. Porto et al. showed increased amplitudes of early and late N1b components, a type of visual evoked potential generated during an EEG study, post-HFS in healthy elderly adults [19]. However, to date, there have been no human studies that combined assessments of plasticity and the underlying neurochemical environment, which could potentially explain these heterogeneous results.

Proton magnetic resonance spectroscopy (MRS) is a non-invasive method that enables assessment of the neurochemical environment within the brain, including major excitatory and inhibitory neurotransmitters such as glutamate (Glu) and GABA. Glutamate (Glu), the primary excitatory neurotransmitter in the human brain, plays a fundamental role in LTP [20] through binding with AMPA and NMDA receptors [14]. More specifically, coincident depolarization of the both presynaptic and postsynaptic neurons and binding of glutamate to NMDA receptors leads to a cascade of events involving release of calcium, removal of Mg^{2+} ions, and phosphorylation of AMPA receptors [20–23]. Glu that is released into the synapse is quickly transported to astrocytes where it is converted to glutamine (Gln). Then, Gln is transported back to neurons and converted to Glu. Glu, measured by MRS, reflects multiple pools including that involved in neurotransmission, a precursor for GABA and glutathione syntheses, and a building block for protein synthesis [24–27]. While Gln is involved in GABA synthesis, approximately 80% of Gln is derived from Glu involved in neurotransmission [28–30]. Thus, Gln has been suggested as an index of glutamate involved neurotransmission. GABA, the primary inhibitory neurotransmitter in the mammalian brain, is also involved in experience dependent plasticity [31, 32]. Thus, quantifying Glu, Gln, and GABA levels from the same region of experience dependence plasticity may provide insight into the LTP-like mechanisms in humans.

This study investigated visual plasticity with a modified fMRI paradigm based on the work of Clapp et al [12], and examined neurochemistry with MRS in healthy humans. We hypothesized that Glu or Gln levels would modulate the strength of the LTP effect, and this would be reflected as a significant relationship between Glu or Gln and fMRI visual plasticity. We also explored the relationship between GABA and visual plasticity.

2. Material and Methods

All research was conducted at the University of Maryland Center for Brain Imaging Research (CBIR) at the Maryland Psychiatric Research Center. This study was approved by the University of Maryland Institutional Review Board, and all participants provided written, informed consent prior to study initiation. A total of 18 healthy adults (9 males/9 females, mean age of 36.2 ± 16 years, range: 19–62 years) participated. Participants had no current/past psychiatric, neurological, or major medical disorders or substance abuse/dependence.

2.1 Neuroimaging

A Siemens TIM Trio 3T MR system with a 32-channel phased array head coil was utilized for this study. A set of axial T₁-weighted MP-RAGE images were acquired for both MRS voxel and EPI placement. Spectroscopy data were acquired first, followed by fMRI.

2.1.1 MRS—The spectroscopic voxel location was placed along the midline in the occipital cortex in each participant (Figure 1) based on previous significant activations found by Clapp et al. (2005), and the location was consistent for both sequences. Prior to the MRS acquisition, participants were asked to rest but remain awake. Shimming was performed automatically using Siemens advanced shimming algorithm, and further adjustments were made manually. Spectra were acquired with phase rotation STEAM (PR-STEAM) [33–38]: TR/TM/TE = 2000/10/6.5-ms, VOI ~ 3.0x4.0x2.0-cm³, NEX=128, 2.5-kHz spectral width, 2048 complex points, and phases: $\phi_1=135^\circ$, $\phi_2=22.5^\circ$, $\phi_{13}=112.5^\circ$, $\phi_{ADC}=0^\circ$. A water reference (NEX=16) was also acquired for phase and eddy current correction as well as quantification. Data were pre-processed using in-house Matlab code that incorporates automated frequency and phase correction. For further background on the PR-STEAM sequence, please see the following four references [36–39]. Previous studies have shown that the PR-STEAM technique can more precisely detect Glu than other commonly used techniques [33] and that it has good reproducibility when detecting Glu and Gln, separately [34, 35]. For GABA detection, a macromolecule-suppressed MEGA-PRESS sequence was utilized [40]: TR/TE = 2000/68ms, VOI ~ 3.0x4.0x2.0-cm³, NEX=256 (128 ON and 128 OFF), spectral width=1.2 kHz, 1024 complex points, editing pulses of 20.36 ms in length, and 44 Hz bandwidth full width at half maximum editing pulses applied at 1.9 (ON) and 1.5 (OFF) ppm. A water reference was also acquired (NEX=16).

For data acquired using the PR-STEAM sequence, LCModel (6.3-0I) was used for quantification [41] similar to Wijtenburg et al [34]. GAVA [42] was used to create a custom-generated basis set that was used in LCModel. Glu and Gln were the main metabolites of interest. These metabolite levels were reported in institutional units, and only included in statistical analyses if the percent standard deviation Cramer Rao Lower Bounds (CRLBs) were less than 20% for Glu and less than 30% for Gln, which are consistent with published

reports [43, 44]. GABA spectra were processed with Gannet 2.0 software package [45], which incorporates automated frequency and phase correction prior to fitting. GABA levels were reported relative to water and in institutional units (IU). All metabolite levels were corrected for the proportion of the gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) within each spectroscopic voxel using in-house Matlab code based on Gasparovic et al [46].

2.1.2 fMRI—A modified visual plasticity fMRI task based on a study by Clapp et al. (2005) and updated based on more recent studies [5, 18, 19, 47] was created in E-Prime 2.0 (Psychology Software Tools, Inc., Sharpsburg, PA, USA). E-prime was used to display the visual stimulus (a centrally located flashing checkerboard at a visual angle of 10°). The visual checkerboard was presented centrally similar to Cavus et al. [18] and Lahr et al. [47]. Figure 2 illustrates the fMRI block design. The order of events were: (1) two runs of low frequency stimulation (LFS) to assess visual cortex activation, (2) high frequency stimulation (HFS) to induce visual plasticity, (3) 2 minute rest period, and (4) two runs of LFS to assess visual cortex activation. For each LFS run, a checkerboard flashed at a rate of 0.9 Hz for one minute “on”, followed by 30 seconds of rest “off.” Each run consisted of 2 “on” and 2 “off” periods, lasting a total of 3 minutes. The choice of LFS used in this study was motivated by the unpredictable neural response observed by Lahr et al. with a LFS of 0.05–0.23 Hz [47] and the observation of increased neural response after HFS using a LFS of 0.83 Hz or 1 Hz by Cavus et al. [18] and Teyler et al. [5]. For HFS, the checkerboard flashed at a rate of 9 Hz for two minutes. Throughout the study, the participant was asked to fixate on a crosshair except for the 2 minute rest period following HFS during which they were allowed to close their eyes. fMRI was only collected during LFS.

Echo-planar imaging (EPI, TR=2100ms, TE=27ms, Flip Angle=90, FOV=220 x 220mm, matrix size = 128x128, Number of slices=39, Slice thickness=4.0mm, 143 measurements, voxel size=1.7x1.7x4.0mm) with 4 dummy scans were acquired during the LFS blocks to assess visual cortex activation. Data were acquired in the same plane as the T1 images. fMRI data were analyzed using Statistical Parametric Mapping (SPM) 8.0 (Wellcome Trust Centre for Neuroimaging, Department of Cognitive Neurology, Institute of Neurology, London: <http://www.fil.ion.ucl.ac.uk/spm>) and MATLAB 8.1 (R2013a) (The Mathworks Inc., Natick, Massachusetts). Data were preprocessed using head motion correction by coregistration to the first image in each series, correction for differences in slice acquisition times, normalization to a standard Montreal Neurological Institute (MNI) template, and smoothing by convolution with a Gaussian kernel function (FWHM = 8 mm).

2.2 Statistical Analyses

First level models were performed by entering whole run sequences into a single analysis and regressing each sequence against the “on” and “off” condition and pre and post-HFS condition with motion parameters as nuisance regressors. The regressors were convolved with a canonical hemodynamic response function. The double-subtraction contrasts were created as: (post-HFS “on” - “off”) minus (pre-HFS “on” minus “off”), which were used for second level modeling.

Second level analysis tested for visual plasticity via a one sample t-test, appropriate for within subject designs. A mask of the spectroscopic voxel was applied to restrict the analyses to the spectroscopic region. Activations were reported with a threshold at FWE ($p < 0.05$), and a less conservative threshold at $p = 0.001$ uncorrected for continuity with the literature [12, 47] are summarized in the Supplement. Bonferroni-corrected correlations were then conducted using SPSS 23.0 (IBM SPSS Statistics, Armonk, NY, USA) between ROI values extracted from significant activations using MarsBar [34] with 4mm radius and the metabolite levels.

To further explore the relationships between visual plasticity and metabolite values, fMRI regression analyses with the double subtraction contrast and each of the metabolites as a covariate were conducted. Voxels were considered significantly correlated when $k \geq 30$ voxels, where k is the number of voxels, and $T > 3.0$, where T stands for T-statistic and refers to the probability of finding a peak at this or greater height (T-statistic) for a volume [48], at $p = 0.001$ uncorrected voxel level. Scatterplots were created between ROI values, which were extracted from these locations, and metabolite levels in order to better visualize the plasticity-metabolite relationships. In addition, partial correlation analyses were conducted to determine if age was a contributing factor.

3. Results

3.1 MRS

Figure 3a shows a representative spectrum with corresponding LCModel fit acquired from the visual cortex. Figure 3b shows a representative GABA spectrum and Gannet fit. In the supplement, all PR-STEAM spectra are shown in Supplementary Figure 1 and all MEGA-PRESS data are shown in Supplementary Figure 2. Data were of excellent quality with mean linewidth and signal to noise ratio values of 0.41 ± 0.009 ppm and 77.8 ± 7.8 from LCModel and mean fit error of 7.1 ± 5.7 from Gannet. No data sets PR-STEAM were excluded since all datasets had Glutamate CRLB $\leq 20\%$ or Gln CRLB $\leq 30\%$. Mean Glutamate levels were 6.62 ± 0.62 with mean CRLBs of $4 \pm 1\%$ (range: 3–6%). Mean Gln levels were 1.82 ± 0.36 with mean CRLBs of $15 \pm 5\%$ (range: 9–29%). Mean GABA levels were 0.944 ± 0.24 . Two GABA datasets were excluded due to poor data quality with fit error as outputted by Gannet of approximately 17% and 33%.

3.2 fMRI

The one-sample t-test from the double subtraction contrasting post-HFS and pre-HFS showed significant activation in the visual cortex, suggesting a visual plasticity effect (Figure 4). There was one significant ROI (Table 1) after FWE-correction. There were three significant areas of activation at $p = 0.001$, uncorrected, and these results are summarized in Supplementary Figure 3. The opposite contrast of pre-HFS versus post-HFS yielded no significant regions. Correlation analyses between the three metabolites and the one significant ROI from the double subtraction showed a significant correlation with Gln levels ($r = 0.638$, $p = 0.004$, $df = 17$, Figure 4) with significance set to $p = 0.05/3$. Thus, greater activation was associated with higher Gln levels.

Regression analysis revealed significant relationships with Glu, Gln, and GABA and visual plasticity in several regions (summarized in Table 2). None of the areas of activation survived FWE-correction, so those reaching $p = 0.001$ uncorrected were explored. For Glu (Figure 5), there were two significant correlations between Glu and areas located bilaterally (ROI1: $r=0.63$, $p=0.005$; ROI2: $r=0.61$, $p=0.007$), where higher Glu corresponded with larger post-HFS versus pre-HFS activation or higher visual plasticity in these visual cortical areas. For Gln, there were three positively correlated areas with Gln (ROI1: $r=0.75$, $p<0.001$; ROI2: $r=0.72$, $p=0.001$, ROI3: $r=0.69$, $p=0.002$) such that higher Gln correlated with larger post versus pre-HFS activation change (Figure 6). GABA levels were also significantly correlated with two areas (ROI1: $r=0.67$, $p=0.003$, ROI2: $r=0.68$, $p=0.003$), suggesting that higher GABA was associated with higher signal change (Figure 7). Supplementary Figure 4 highlights the overlap between the plasticity activations and the regions that correlated with metabolites. Overall, the metabolite regions overlap with the activations resulting from the double subtraction. In addition, partial correlations between activations and metabolite levels controlling for age remained significant. Thus, the relationships were not influenced by age.

4. Discussion

To our knowledge, this is the first study to investigate visual plasticity using a multimodal neuroimaging approach for function and neurochemistry. For MRS measures, we used a sequence optimized to detect Glu and Gln as well as an optimized technique to quantify GABA without macromolecule contamination. LTP-like visual plasticity was observed, similar to previous animal and human studies [1, 10, 21]. The major finding was that visual plasticity was significantly related to resting visual cortical Gln levels, and that Glu, Gln and GABA levels correlate with visual plasticity ROI signal changes within the spectroscopic voxel. These data show for the first time that resting visual cortical glutamate, glutamine and GABA levels are associated with experience dependent visual plasticity in healthy humans.

Combining MRS measures of Glu, Gln, and GABA with the fMRI measures of visual plasticity provides further insight into the mechanisms underlying experience dependent plasticity in humans. The strong correlations between the fMRI visual plasticity and the glutamatergic metabolites were expected, since Glu is fundamentally involved in LTP [20]. Several preclinical studies support our findings. A study using a glutamate transporter 1 (GLT-1) knockout mouse model recorded excitatory postsynaptic currents post HFS in hippocampal slices and showed that the magnitude of the NMDA synaptic potentiation depended on synaptic glutamate levels [49], and a rat study showed that visual cortex LTP was NMDAR dependent [50]. Similarly in humans, modulation of the glutamate NMDA receptor with d-cycloserine enhanced visual plasticity as assessed with EEG [51], whereas ketamine, an NMDA receptor antagonist, prevented visual plasticity [14]. Our data suggest that optimal ambient levels of glutamatergic metabolites are necessary for visual plasticity to occur in the healthy brain; such that, higher glutamatergic levels relate to enhanced visual plasticity. This concept has been proposed by Krause and Kodosh (2014) within the context of transcranial electrical stimulation induced plasticity [52]. It is also consistent with the idea of “homeostatic plasticity”, whereby a stable foundation is needed to facilitate synaptic plasticity mechanisms like LTP and long-term depression (LTD) [53]. Optimal levels of glutamatergic metabolites may contribute to this stable foundation.

Historically, higher GABA levels have been associated with lower BOLD during visual stimulation [54–57] and motor activity [58]. Yet, two recent studies did not find significant correlations between GABA and BOLD in five brain regions [59] or GABA and magnetoencephalography (MEG) peak gamma levels [60] while two other studies in the insula found a positive correlation between BOLD signal and GABA+ levels [61] and BOLD signal and GABA/NAA levels [62]. In this study, our data revealed a relationship between higher GABA and greater visual plasticity, which may seem counterintuitive. It is important to remember that the visual plasticity measure in this study is the “difference” in visual response (i.e. post versus pre HFS) and not the simple visual response. When comparing baseline visual stimulation (i.e. pre-HFS) and baseline GABA levels, we also found a negative correlation between these two measures, similar to previous studies.

Several studies indicate that a decrease in GABA levels coincides with LTP in rodents [63–65] and motor plasticity through learning and stimulation in humans [58, 66–69]. It is possible that higher baseline GABA levels reflect a greater capacity for GABA to decrease during plasticity. However, future studies are necessary to determine if GABA levels decrease during HFS in this paradigm, and if the baseline GABA levels predict this change. Here, we assessed GABA only at baseline and showed that it positively correlated with visual plasticity; whereas, these other studies did not examine whether baseline GABA predicted the amount of plasticity. Thus, it remains undetermined if higher baseline GABA positively relates to greater plasticity in these other paradigms. The similar relationship patterns between baseline Glu and GABA and visual plasticity likely highlights the delicate excitatory-inhibitory balance critical for experience-dependent plasticity in the visual cortex [70]. GABA is important for “tuning” of pyramidal networks, which includes strengthening of pyramidal circuits, important for experience-dependent plasticity [32, 70]. Optimal levels of GABA are necessary for fine tuning to occur, such as with plasticity mechanisms. Similar to glutamate, the GABA data also fit nicely with the concept of homeostatic plasticity, as previously discussed. In addition, GABA levels are related to glutamate through the glutamate-GABA recycle pathway [30, 71], which may also contribute to the similar positive correlations with visual plasticity.

There are study limitations that deserve mentioning. This study did not assess attention during the visual plasticity paradigm. However, participants were instructed, and intermittently reminded, to fixate on the crosshair during the fMRI runs. Importantly, all participants showed visual cortex activation. Nevertheless, future studies should either assess attention or monitor eye movements. Only one MRS voxel was examined and therefore we cannot comment on regional specificity. Finally, follow-up analyses that examined correlations between metabolite levels and extracted ROI values from previously determined significant regions were performed for visualization purposes, but still could be considered “double dipping.” Given that no other studies have examined whether resting metabolite levels correlate with visual plasticity, we felt exploration was warranted.

5. Conclusion

This multimodal neuroimaging study provides the first empirical evidence that resting visual cortical glutamate, glutamine, and GABA levels correlate with visual plasticity in healthy

humans. This study provides a framework to test the effect of altered resting glutamate or GABA levels on visual plasticity such as in neuropsychiatric disorders known to have glutamatergic or GABAergic alterations [40, 72–74].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- First study of visual plasticity using multi-modal neuroimaging in humans
- Used a fMRI paradigm with high frequency stimulation to study visual plasticity
- Used MRS to measure glutamate (Glu), glutamine (Gln), and GABA
- LTP-like visual plasticity was observed, similar to past animal and human studies
- Resting visual cortical Glu, Gln, and GABA predict visual plasticity in humans

Figure 1.
T1-weighted images showing a 24cm³ voxel placed along the midline of the occipital cortex.

Figure 2.

Schematic diagram of the study. Structural images were acquired first followed by the magnetic resonance spectroscopy data. The fMRI session consisted of the following order of events: (1) Baseline visual activation: 2 runs of low frequency stimulation (LFS) at 0.9 Hz. Each run consisted of 1 minute of a flashing checkerboard stimulus “on” followed by 30 seconds of a fixation point “off”, and there were 2 “on” and 2 “off” within each run for a total of three minutes; (2) 2 minute period of high frequency stimulation (HFS) at 9 Hz with no fMRI collection; (3) 2 minutes of rest with no fMRI collection; and (4) Post-HFS visual activation: 2 runs of LFS identical to #1. Note: fMRI were acquired during the LFS runs only.

Figure 3.

Representative spectrum from the PR-STEAM sequence with the raw data (gray), LCMoel fit (red), and residual shown above (gray) (a). Gln and Glu fits resulting from LCMoel are shown below the representative spectrum. The metabolite spectrum, LCMoel fit, residual, Gln fit, and Glu fit are all scaled identically. Spectra were of excellent quality and the lack of structure in the residue suggests excellent fits. Representative MEGA-PRESS spectrum also from the occipital cortex (b) and the resultant fit in Gannet (c).

Figure 4.

After correcting for multiple comparisons via FWE at $p < 0.05$, activation at $-22, -68, -10$ was still significant. Significant regions within the spectroscopic voxel showing an increase in activations post-HFS versus pre-HFS at $-22, -68, -10; -12, -80, 26$; and $12, -66, 24$ with a $p = 0.001$ uncorrected for multiple comparisons (shown on left). A significant and positive relationship between ROI1 ($-22, -68, -10$) and Gln levels is shown on the right.

Figure 5.

T1-weighted images showing significant regions of interest after examining the contrast of post vs. pre for the condition (on - off) with Glu as a covariate (above). Plots of Glu levels versus change in signal pre to post for two ROIs (diameter 4mm) showing that higher Glu corresponded to higher signal change (shown in a and b).

Figure 6.

T1-weighted images showing significant regions of interest after examining the contrast of post vs. pre for the condition (on - off) with Gln as a covariate (left column). Corresponding plots of Gln levels versus change in signal pre to post for three ROIs (diameter 4mm, ROI1 (a), ROI2 (b), ROI3 (c)) showing that higher Gln corresponded to higher signal change.

Figure 7.

T1-weighted images showing significant regions of interest after examining the contrast of post vs. pre for the condition (on - off) with GABA as a covariate (left column).

Corresponding plots of GABA levels versus change in signal pre to post for two ROIs (diameter 4mm, ROI1(a), ROI2(b)) showing that higher GABA corresponded to higher signal change.

Table 1

Significant activations post-HFS versus pre-HFS

	k	T	Z	x, y, z (region)	Peak-level FWE-corrected p-value	Peak-level Uncorrected p-value
ROI1	265	5.72	4.21	-22, -68, -10 (dorsal V3)	0.047*	<0.001

* significant at p<0.05

Table 2
Significant ROIs from Regression Analyses with the Double Subtraction as the Contrast and Metabolites as Covariates

Metabolites	k	T	Z	x, y, z	Peak-level FWE-corrected p-value	Peak-level uncorrected p-value
Glu ROI1	105	3.72	3.11	24, -70, 16	0.644	0.001
Glu ROI2	49	3.56	3.01	-22, -68, 16	0.731	0.001
Gln ROI1	577	4.82	3.73	-22, -70, -2	0.190	<0.001
Gln ROI2	75	3.98	3.27	10, -64, -10	0.524	0.001
Gln ROI3	149	3.75	3.13	14, -80, 8	0.645	0.001
GABA ROI1	150	3.95	3.22	-8, -86, 4	0.555	0.001
GABA ROI2	33	3.65	3.04	4, -78, 32	0.706	0.001