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Regional and tissue-specific differences in brain glutamate concentration measured by in vivo single voxel MRS

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Abstract

Background—There is growing interest in characterizing spatial distribution of glutamate (Glu) in brain disorders. Comparing differences in Glu concentration using magnetic resonance spectroscopy (MRS) is hampered by the confounding effects of different anatomical regions and tissue composition.

New Method—Effect of tissue composition on Glu concentrations was studied by selecting closely adjacent voxels within a designated cortical region. Glu regional differences were assessed using voxels comprising essentially the same tissue composition from different cortical regions.

Results—Using point-resolved-spectroscopy (PRESS)-based averaged echo time method, Glu concentration in the anterior cingulate cortex (ACC) was found to correlate strongly with tissue gray matter (GM) fraction ($r = 0.87$, $p = 10^{-5}$). No significant regional difference in Glu concentration was found between frontal and occipital lobes ($p = 0.23$) when the two measured voxels had essentially the same tissue composition.

Comparison with Existing Methods—The method of the current study is aimed to circumvent the difficulties in differentiating anatomical region from tissue composition, given that both can lead to Glu variations in brain. Glu concentration versus tissue composition was measured in the same anatomical region, while the comparison of regional differences was performed with the two regions that had essentially the same tissue composition.

Conclusions—In brain cortices, Glu level is significantly higher in GM than in WM. Glu level difference between frontal lobe and occipital lobe is insignificant.

Keywords

human brain; glutamate; ¹H MRS; averaged echo time; regional difference; tissue composition; metabolite quantification

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1. INTRODUCTION

Glutamate (Glu) is the principal excitatory neurotransmitter in the central nervous system (CNS). It is involved in glutamatergic neurotransmission through the Glu-glutamine (Glu-Gln) cycle between neurons and surrounding astrocytes (Hertz, 2004). Glutamatergic pathways are involved in diverse processes and brain disorders such as depression, epilepsy, schizophrenia, and ischemic brain damage. In addition to functioning as a neurotransmitter, Glu also serves as a source of energy and ammonia, linking the metabolism of carbon and nitrogen (Erecinska and Silver, 1990). The dual roles of Glu in neurotransmission and metabolism are intricately related. Glu concentrations measured by ^1H magnetic resonance spectroscopy (MRS) result from the interplay between Glu anabolism and catabolism. Differences in Glu concentration across different brain regions and tissues—or lack thereof—provide important insight into alterations in Glu metabolism and neurotransmission.

Glu is very similar to glutamine (Gln) in molecular structure, characterized by the coupled spins of C2–C4 hydrogen nuclei. At prevalent field strength of 1.5 or 3.0 Tesla (T), it is challenging to separate Glu and Gln by conventional one-dimensional spectroscopic methodologies due to the heavy overlap over all their resonance frequencies. The resonances are mostly assigned to a mixture of Glu and Gln, often referred to as Glx. The echo time (TE)-averaged spectroscopy showed the feasibility of measuring Glu without Gln interference (Hurd et al, 2004). Moreover, TE-averaged spectra exhibited simplified resonance patterns and relatively flat baselines, whereby mitigating the statistical bias in quantification of weakly represented metabolites such as Glu (Zhang et al, 2011).

Due to time constraints associated with most clinical studies, the weak Glu signal requires the use of large voxels encompassing both gray matter (GM) and white matter (WM), making it particularly difficult to separate regional differences from those originating from different GM and WM compositions (Kraguljac et al, 2012; Ono et al, 2014; van der Veen and Shen, 2013; Pollack et al, 2008).

In this study, the point-resolved-spectroscopy (PRESS)-based single-voxel TE-averaged spectroscopy method was employed to investigate Glu level differences in the cortices of the human brain. The study was aimed to circumvent the difficulty in differentiating the regional difference from the effect of tissue composition on Glu concentration, given that both could lead to Glu variations in brain. Three closely adjacent voxels in the anterior cingulate cortex (ACC) were used to measure the effect of tissue composition. Two different voxels with essentially the same GM and WM fractions were placed in median prefrontal cortex (MPFC) and occipital cortex (OCC) to assess the regional difference in Glu concentration or lack thereof, without confounding contributions from the tissue type difference.

2. MATERIALS AND METHODS

2.1 Subjects

Twenty healthy volunteers (12 male, eight female; ages 22–55; mean age = 32 years) were recruited for the current study as approved by local institutional review board.

2.2 Data Acquisition

The scans were performed on a 3 T GE whole body scanner (General Electric Medical Systems, Milwaukee, Wisconsin, USA) running the 15M4 software platform. A Medical Advances (Intermedics General Corporation, Milwaukee, Wisconsin, USA) RF quadrature transmit/receive coil was used with an inner diameter of 25 cm and a length of 20 cm. Scan sessions began with a T_1 weighted anatomical scan conducted via three-dimensional spoiled gradient echo sequence (TR = 7.3 ms, TE = 2.7 ms, flip angle 12°; in plane resolution 0.9 mm × 0.9 mm; matrix size 192 × 256; field of view 240 mm × 240 mm; slice thickness 2 mm; total scan time 2 min 32 s). Voxel positioning is shown in Figure 1. The ACC WM voxel was placed in the right frontal WM, directly adjacent to the rostral ACC. The mixed voxel was placed between the GM and WM voxels. The MPFC voxel was placed over the midline in the frontal lobe just anterior to the ventricles. The OCC voxel was placed over the midline in the occipital lobe just posterior to the ventricles. The location of the OCC voxel was chosen to approximate the same amount of GM and WM as the MPFC voxel. All voxels in the ACC region had a volume of $2.0 \times 2.0 \times 4.5 \text{ cm}^3$. The MPFC and OCC voxels had a volume of $3.0 \times 3.0 \times 2.0 \text{ cm}^3$.

TE-averaged spectra were acquired with 32 different echo times. The number of average (NA) is four. TE started at 35 ms (8.5 ms from the excitation pulse to the first refocusing pulse; 17.5 ms from the first refocusing pulse to the second refocusing pulse), and was step-wise increased by 6.0 ms after each echo. All spectra of different echo times were summed to yield a TE-averaged spectrum. Its time domain decay was then approximated by an effective TE of 105 ms. Reference unsuppressed water scans were collected with NA = 2 immediately after the acquisition of spectral data. Spectra were sampled with a repetition time (TR) of two seconds, bandwidth of 5 kHz, and 4096 complex data points.

2.3 Spectral Fitting

Spectral fitting was performed automatically with the software developed in-house using interactive data language (IDL; Research Systems, Inc., Boulder, CO). The number of data points (4096) was expanded to 8192 by zero-filling in the time domain, followed by Fourier transform. Glu and Gln are indistinguishable at the resonance of 3.7 ppm due to the nearly complete overlap. Spectral fitting over the region 1.6–3.4 ppm allows the using of a less complicated model to quantify Glu. As such, the spectral model in the current study consisted of Glu, N-acetylaspartate (NAA), N-acetyl-aspartyl-glutamate (NAAG), creatine (Cr), and choline (Cho)-containing compounds.

The model basis spectra were generated by spin density matrix calculation. The coupling constants were obtained from the literature (Govindaraju et al, 2000). The lineshape was described with the Voigt model (Bruce et al, 2000) in which the Gaussian decay factor was a

common parameter shared by all components in the spectral model. The linewidth of Glu was constrained by the linewidth ratio of Glu-Cr. The frequency of NAAG was constrained to be 4.8 Hz up-field from the peak of NAA at 2.0 ppm, and its linewidth was constrained to the linewidth of NAA. All constraints were enforced in a soft manner (Provencher, 1982 and 1993; Zhang and Shen, 2013). The estimated concentrations of NAA and NAAG were combined to yield a total concentration (tNAA). Because it is essentially impossible to differentiate Cr from phosphocreatine (PCr) at 3 T, the value of total concentration (tCr) was reported. Similarly, choline-containing compounds gave a single concentration (tCho).

2.4 T₁ and T₂ Corrections

The contents of a cortical voxel generally comprise GM, WM, and cerebrospinal fluid (CSF). The anatomical images were segmented with SPM5 (Ashburner and Friston, 1997; Geramita et al, 2011) to determine the fractions of GM, WM, and CSF. An in-house written program in IDL was used to extract the coordinates of the spectroscopy data files and their respective tissue fractions from the segmentation results (Geramita et al, 2011).

The CSF fraction can also be determined by measuring T₂ of unsuppressed water (Ernst et al, 1993; Piechnik et al, 2009). In the current study, the water signal decay with different TEs was fitted with a bi-exponential decay model. T₂ of the longer decay component was fixed at 500 ms (Piechnik et al, 2009) and was ascribed to CSF. The shorter T₂ component accounted for the composite MR-visible signal arising from GM and WM. T₁ attenuation was corrected by the factor $1 - \exp(-TR/T_1)$, assuming T₁ = 1084 ms for WM, and 1820 ms for GM (Stanisz et al, 2005). The values for the water densities of pure GM (43300 mM) and WM (35880 mM) were obtained from the literature (Ernst et al, 1993). The reference water signal intensity after the removal of CSF was corrected by:

$$S_{ref,corr} = \frac{S_{ref}}{f_g W_g + f_w W_w} \left\{ \frac{f_g W_g}{1 - \exp(-TR/T_{1,g})} + \frac{f_w W_w}{1 - \exp(-TR/T_{1,w})} \right\}. \quad [1]$$

where S_{ref} and $S_{ref,corr}$ represent the reference water signal intensities before and after correction for T₁ relaxation, respectively. Note that S_{ref} was extrapolated to TE = 0 with the fitted T₂ as mentioned above. f_g and f_w are the volume fractions for GM and WM within voxels, respectively. They are determined by tissue segmentation. W_g and W_w are the water densities for GM and WM.

The estimated metabolite concentration (denoted by C_m) is the composite concentration of GM (denoted by C_g) and WM (denoted by C_w):

$$C_m = f_g C_g + f_w C_w. \quad [2]$$

where C_m was estimated from the ratio of the fitted metabolite intensity (denoted by S_m) to the reference water intensity:

$$C_m = \frac{\alpha_m S_m}{S_{ref,corr}} (f_g W_g + f_w W_w). \quad [3]$$

where α_m is the factor that accounts for the proton number of the metabolite molecule relative to that of the water molecule. Similar to the reference water signal S_{ref} , S_m should also be corrected for both T_1 and T_2 relaxation decays. Because of T_1 and T_2 heterogeneity between GM and WM, the decay could only be approximately described by a mono-exponential decaying function with an apparent T_1 or T_2 factor. Based on the literature (Ethofer et al, 2003; Traber et al, 2004; Choi et al, 2006; Tsai et al, 2007; Kirov et al, 2010), an apparent T_1 of 1400 ms and 1200 ms were used for NAA and the rest of the metabolite components, respectively, and the apparent T_2 values (ms) were: 200, 260, 160, and 220 for Glu, NAA, Cr, and Cho, respectively.

3. RESULTS

In Figure 1, the anatomic images show the positioning of voxels. The MPFC voxel and the OCC voxel had similar tissue compositions. The GM fractions of the MPFC voxels ranged from 0.47 to 0.63 across all subjects, with an average of 0.53 and a standard deviation of 0.045. For the OCC voxels, the GM fractions ranged from 0.40 to 0.61 with an average of 0.53 and a standard deviation of 0.051. As summarized in Table 1, there were essentially no differences in tissue composition between the MPFC and OCC voxels, with $p = 0.86$ and 0.73 for GM and WM, respectively. Figure 2 shows the comparison of TE-averaged spectra between MPFC and OCC. In contrast, the ratio of GM to WM tissue varied significantly over the three ACC voxels (0.62, 0.26, and 0.41 for GM, WM, and mixed voxels, respectively). Figure 3 shows the three TE-averaged spectra that correspond to GM, WM, and mixed voxels; data were acquired from the three adjacent voxels in ACC (see Figure 1).

Table 2 compares the metabolite concentrations between MPFC and OCC in 15 subjects (aged from 22 to 41; mean age = 29). No difference was observed in Glu concentration between the two voxels ($p = 0.49$). In contrast, tCho levels were significantly higher in MPFC than in the OCC ($p < 10^{-10}$). The differences in tNAA and tCr levels between MPFC and OCC did not reach statistical significance ($p = 0.23$ and 0.069 , respectively). p -values were calculated using student t -statistics with unequal variances.

Figure 4 displays the linear regressions of metabolite concentrations against GM fractions. Data were acquired from three adjacent voxels in the ACC of five subjects (aged from 30 to 55; mean age = 35) with tissue characterized by GM, WM, and mixed composition, respectively. Glu and tCr levels depend significantly on tissue compositions ($r = 0.87$ and 0.65 , $p = 10^{-5}$ and 0.004 , respectively). No significant changes in tNAA or tCho levels were observed over the three different voxels ($r = 0.16$ and -0.17 , $p = 0.28$ and 0.27 , respectively).

4. DISCUSSION

There are several aspects of the current study designed to improve assessing the differences in Glu concentration. First, the effect of tissue composition on Glu concentration was separated from the potential differences associated with region. Secondly, TE-averaged spectroscopy was utilized so that the Gln interference was minimized. Third, a new fitting method was used, which reduced variables and minimized the baseline influences on

metabolite quantification. Notably, we found that Glu concentration varied significantly with tissue composition rather than brain region in the cortices investigated; higher Glu levels were in GM, and lower levels in the WM. In addition, tCr concentrations were also positively correlated with GM fractions. tCho concentrations showed a weakly negative correlation with GM fractions, though they demonstrated the largest regional difference. Cho levels in the frontal lobe were significantly higher than in the occipital lobe. Finally, no region or tissue composition variations were found for the combined concentration of NAA and NAAG.

Inconsistencies from the previous studies are noticeable. Given that both tissue composition and regional differences affect metabolite concentrations, the impetus for this study was born from the assumption that teasing apart these two factors should yield a more reliable assessment of the spatial distribution of metabolite concentrations.

While the intense signals from NAA, Cr, and Cho can be analyzed from a large number of voxels, measuring Glu concentrations is difficult because of the relatively low MRS detection sensitivity and the spectral overlapping from Gln. For these reasons, data in the current study were acquired with PRESS based single-voxel TE-averaged spectroscopy and with relatively larger voxel sizes than typically used in short TE spectroscopy. TE-averaged spectroscopy offers a way to cancel out the C4 signal of Gln (Hurd et al, 2004), whereby yielding unobstructed detection of the remaining Glu signal. In addition, it suppresses macromolecular signals and thus has a flatter baseline than short TE spectroscopy.

Incorporating a baseline into the fitting model is necessary in order to achieve a good fit for in vivo MRS including TE-averaged spectroscopy. However, a good fit of the spectral model, indicated by a minimized least squares difference between the model and data, does not necessarily indicate that metabolite and baseline signal contributions are correctly estimated. An under-smoothed baseline can lead to large errors even though the fitting residuals are minimized. Conversely, an over-smoothed baseline often results in poor fitting, characterized by large fit residuals, because background signals are not well represented. In this study, the spectral baselines were determined by a recently proposed method (Zhang and Shen, in press). This method minimizes the error of both the fit residuals and the uncertainties arising from the interaction between the baseline and metabolite peaks. In Table 2, the mean coefficients of variation were derived from Cramer-Rao Lower Bounds (CRLB) (Cavassila et al, 2001; Zhang and Shen, in press). Note that the MPFC spectra have larger CRLBs than the OCC spectra, echoing that the former have poorer spectral quality than the latter (see Figure 2).

In the present study, no significant differences were detected in Glu levels between the two regions of essentially the same tissue composition in the frontal lobe and the occipital lobe, though Glu in GM is significantly higher than in WM. Higher Glu and/or Glx levels in GM have also been consistently reported in the literature (Kassem and Bartha, 2003; Pan et al, 1996; Sailasuta et al, 2008 McLean et al, 2000; Srinivasan et al, 2006). To the best of our knowledge, this report is the first to control the GW and WM content when comparing the regional difference in Glu concentration. Our results highlight the importance of careful

control of tissue composition when investigating the regional difference in Glu concentration since the difference caused by tissue composition may dominate.

In addition to Glu, we found that tCr concentrations were significantly increased in GM compared to WM (Figure 4c) in agreement with early studies (Pouwels and Frahm, 1998; Schuff et al, 2001; Wiedermann et al, 2001). Baker and colleagues (2008) reported a trend toward decreased tCr levels in WM from anterior to posterior, and that this trend was reversed in GM. The current study agrees with the general consensus established in previous studies that tCr levels are higher in GM than in WM. The present study observed slightly higher Cr concentrations in MPFC than OCC.

The weak association observed in the present study between tCho and GM fractions ($r = -0.17$, $p = 0.27$; see Figure 4d) is in keeping with most previous findings (Schuff et al, 2001; McLean et al, 2000; Wiedermann et al, 2001). tCho levels in the OCC were significantly lower than in the MPFC ($p < 10^{-10}$). Figure 2 clearly shows the difference in the two tCho peak heights. This observation echoes previous MRS studies (Pouwels and Frahm, 1998; Baker et al, 2008), and is supported by a postmortem finding of lower Cho levels in the occipital cortex than in either the frontal or parietal cortices (Nitsch et al, 1993).

As for tNAA, previous MRS studies reported small or no differences (Pouwels and Frahm, 1998; Baker et al, 2008; Maudsley et al, 2009; McLean et al, 2000; Srinivasan et al, 2006). In the present study no significant differences in tNAA levels were observed, regardless of brain region or tissue composition.

A variety of ^1H MRS methods have been proposed for in vivo studies of metabolites. Short TE spectroscopy and short TE-based magnetic resonance spectroscopic imaging (MRSI) are two of the most popular methods. In particular, MRSI can simultaneously detect the metabolites in different regions of interest and has attracted increasing attention (Posse et al, 2013). Separation of Glu from Gln, the aspartyl moiety of NAA and the macromolecule baseline is also feasible at 3 T using short-TE MRSI (Posse et al, 2013). TE-averaged spectroscopy has a long effective TE, and its spectra thus suffer larger signal loss than short TE spectra. Its long TE also makes metabolite quantification more sensitive to T_2 uncertainties. Despite of these disadvantages of TE-averaged spectroscopy, it dramatically simplifies spectral pattern and hence reduces unknown factors. Strong baseline arising from macromolecule and/or lipid signals is known to significantly confound quantification of metabolite concentrations, especially for weakly represented metabolites such as Glu. Thanks to the reduced variables in the spectral model, Glu measured by TE-averaged spectroscopy is less dependent on the fidelity of the model including the baseline.

5. CONCLUSIONS

Glu concentrations were found to vary significantly with tissue composition rather than brain region in the cortices investigated. Higher Glu levels were observed in the GM, and lower levels in the WM. No significant regional differences in Glu levels were observed. As with Glu concentration, tCr concentrations were also positively correlated with GM fractions. tCho concentrations were weakly and negatively correlated with GM fraction,

though they demonstrated the largest regional difference. Cho levels in the frontal lobe were significantly higher than in the occipital lobe. Finally, no region or tissue composition variations were found for the combined concentration of NAA and NAAG. The findings of this study provide reference for patient studies involving MRS of Glu. In particular, it points out the possibility of using OCC Glu as an internal control for monitoring therapies that targeting the frontal region.

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HIGHLIGHTS

- Glutamate levels versus tissue compositions are measured in the same anatomical region of human brain.
- Regional differences in glutamate levels are assessed with regions comprising essentially the same tissue composition.
- Glutamate levels are found to correlate strongly with tissue composition.
- No significant difference of glutamate concentration is found between frontal and occipital regions.

Figure 1.

(a): Locations of the two voxels in the median prefrontal cortex (MPFC) and occipital cortex (OCC). (b): locations of the gray matter (GM) voxel (red), the white matter (WM) voxel (yellow), and the mixed voxel (green) in anterior cingulate cortex (ACC).

Figure 2. Representative echo time (TE)-averaged spectra of the median prefrontal cortex (MPFC) (a) and occipital cortex (OCC) (b). Fitted spectra are displayed in red. Top and bottom traces are fit residuals and baselines, respectively.

Figure 3.

Representative echo time (TE)-averaged spectra with different compositions of gray matter (GM) and white matter (WM) in the anterior cingulate cortex (ACC). GM fractions were 0.62, 0.41, and 0.26, corresponding to (a), (b), and (c), respectively. The spectral data were acquired from the same subject. Fitted spectra are displayed in red. Top and bottom traces are fit residuals and baselines, respectively.

Figure 4.

Pearson's correlations of metabolite concentrations versus fractions of gray matter (GM) in the anterior cingulate cortex (ACC). Data of individual subjects are labeled by square, triangle, plus sign, times sign, and rhombus, respectively.

Table 1

Fractions ($\pm$ standard deviation) of gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) for the voxels of interest.

Region	MPFC	OCC	ACC/GM	ACC/WM	ACC/Mixed
GM	0.53($\pm$ 0.045)	0.53($\pm$ 0.051)	0.62($\pm$ 0.052)	0.26($\pm$ 0.031)	0.41($\pm$ 0.041)
WM	0.37($\pm$ 0.053)	0.38($\pm$ 0.055)	0.27($\pm$ 0.045)	0.71($\pm$ 0.053)	0.53($\pm$ 0.034)
CSF	0.08($\pm$ 0.022)	0.09($\pm$ 0.037)	0.11($\pm$ 0.035)	0.03($\pm$ 0.028)	0.06($\pm$ 0.012)

Table 2

Metabolite concentrations (millimolar (coefficient of variation)^{*}) for 15 subjects.

Region	Glu	tNAA	tCr	tCho
MPFC	10.1(9.2%)	12.5(5.5%)	8.14(6.6%)	2.53(6.4%)
OCC	9.72(5.4%)	12.2(2.6%)	7.67(3.3%)	1.61(4.8%)

^{*} the coefficients of variation were derived from Cramer-Rao Lower Bounds (CRLB).