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Age-related glutamate and glutamine concentration changes in normal human brain: ^{1}H MR spectroscopy study at 4 T

Lana G. Kaiser*, **Norbert Schuff**, **Nathan Cashdollar**, and **Michael W. Weiner**
Department of Radiology, University of California-San Francisco, MR Unit, DVA Medical Center (114M), 4150 Clement St., San Francisco, CA 94121, USA

Abstract

Proton magnetic resonance spectroscopy was performed at 4 T to determine effects of age, region and gender on glutamate and glutamine in the normal human brain. Furthermore, glutamate and glutamine alterations with age were tested for correlations with other cerebral metabolites. Two 8 cm^3 volumes were selected in corona radiata and mesial motor cortex in normal subjects (N = 24) between 24 and 68 years old. Older subjects had lower glutamate concentration in the motor cortex compared to younger subjects ($p < 0.001$). In corona radiata, older subjects demonstrated a trend in higher glutamine compared to younger subjects ($p = 0.05$). Glutamate in the motor cortex was positively correlated with glutamine, *N*-acetyl aspartate and creatine. Reduced glutamate and *N*-acetyl aspartate in the motor cortex is consistent with neuronal loss/shrinkage with age. In conclusion, different patterns in association with normal aging in these brain regions were detected in this study.

Keywords

Glutamate; Glutamine; Aging; Motor neurons; Spectroscopy; 4 T; Metabolites

1. Introduction

Glutamate (Glu) is the major excitatory neurotransmitter in the brain involved in functions such as motor behavior, cognition and emotion, which are affected in the course of the normal aging process [9,20,34]. Several lines of evidence also implicate glutamate toxicity as a contributing factor in the pathogenesis of age-related neurological conditions, including Parkinson's disease, Alzheimer's dementia, and Amyotrophic lateral sclerosis (for reviews see Refs. [14–16]). Therefore, drugs which counteract glutamate-induced neurotoxicity are being considered for symptomatic and neuroprotective treatment of these disorders [2,26]. Studies of cerebral cortex in rodent brains showed a decrease in glutamate concentration in association with aging [10,40], but there is little information concerning age effects on Glu in human brain.

Proton magnetic resonance spectroscopy (^{1}H MRS) has been used extensively to measure non-invasively metabolic concentrations in human brain. Several ^{1}H MRS studies demonstrated that compared to younger populations, older subjects have reduced cerebral *N*-acetyl aspartate (NAA), a putative neuronal marker [27,42], suggesting neuronal loss or deficit in neuronal metabolism [4,6,35]. Since Glu is also located primarily in neurons [28,29] and its concentration in the brain most likely reflects neuronal integrity, it should also decrease with age, similar to the NAA. In contrast, glutamine (Gln), a precursor of Glu, is thought to be located predominantly in glial cells [21], and may be used to determine changes of glial cells during normal aging.

* Corresponding author. Tel.: +1 415 221 4810x3640; fax: +1 415 668 2864. E-mail address: lkaiser@itsa.ucsf.edu (L.G. Kaiser).

Measurements of Glu and Gln are limited in MRS studies at 1.5 T, due to a significant spectral overlap between the resonances of Glu and Gln. Studies at higher magnetic fields provide increased separation between Glu and Gln resonances, facilitating quantification of both metabolites [18]. In addition, signal to noise ratios of all the metabolites detected by ^{1}H MRS increase linearly with higher field [19]. These effects, combined with advanced spectral processing techniques such as prior knowledge metabolite fitting [32,43], significantly improve accuracy of Glu and Gln measurements with ^{1}H MRS, as previously demonstrated [1,17].

Therefore, the main goal of this study was to determine age-related alterations of Glu and Gln concentrations in the brain, utilizing the improved accuracy of ^{1}H MRS at 4 T. We expected age related decreases of NAA, and concomitant age related decreases of Glu. A second goal was to investigate correlations between Glu and Gln with other metabolites, including NAA. We expected NAA and Glu to correlate, but we did not expect a relationship between NAA and Gln. A third goal was to determine whether there would also be regional and gender variations of Glu and Gln concentrations, independent of age. Finally, the coefficients of variations (CoV) for Glu, Gln and other metabolites were determined to establish a measure of accuracy.

2. Methods

2.1. Human subjects

Twenty-four normal individuals (11 males, 13 females) were recruited from the community for this study. The subjects were dichotomized into a younger group of 11 subjects (mean age = 26 ± 1 years) and an older group of 13 subjects (mean age = 54 ± 6 years). Ten subjects from the younger group were scanned twice within a week to determine reliability and reproducibility of the single voxel ^{1}H MRS measurements. Written informed consent, approved by the committees of human research at the University of California, San Francisco and University of California at Berkeley, was obtained from each subject prior to the measurements.

2.2. MRS acquisition

^{1}H MRS experiments were performed on a 4 T Inova Infinity system (Varian), using a volume head coil provided by the manufacturer. A 3D magnetization prepared Fast Low Angle Shot (FLASH) sequence (TR/TE/TI = 9/5/300 ms) was used to acquire anatomical T_1-weighted images for placement of the MRS voxel in the regions of interest (ROI) and for segmentation of brain tissue. The extent of motion contribution to the spectral quality was monitored via short block acquisition of spectra. Prior to averaging blocks of spectra, phase variations of the NAA signal between the blocks of spectra were determined and used as indicator for subject motion. Older and younger subjects exhibited a similar range of phase variations due to motion, implying that there was no systematic difference between the groups with regard to motion. A ^{1}H MRS Stimulated Echo Acquisition Mode (STEAM) sequence [12] (TE/TM/TR = 15/10/2000 ms, voxel size = 8 mL) was used to acquire MR spectra from two regions of upper motor neurons (corona radiata (COR) and mesial motor cortex (MC)), see Fig. 2. Three sinc pulses optimized for the homogenous excitation profile [23] (4.5 kHz bandwidth, 2 ms duration) were used in combination with 0.53 G/cm magnetic field gradients to excite a region of interest with dimensions of 2 cm × 2 cm × 2 cm. Two thousand and forty-eight spectral points were sampled at 2000 Hz bandwidth, corresponding to a nominal digital resolution of about 1 Hz. Metabolite spectra were obtained from 256 averages and water suppression was achieved using variable pulse power and optimized relaxation delays (VAPOR) scheme [41]. Reference spectra of unsuppressed water were collected from each ROI at TR = 6 s for the purpose of metabolite quantification. In addition, unsuppressed water spectra were collected

from each ROI at 20 different echo times from 15 ms in increments of 15 ms to estimate cerebrospinal fluid (CSF) content of the regions, based on T_2 relaxation difference between tissue and CSF. Spectroscopy data was zero-filled to 4096 points and a 2 Hz Gaussian line broadening filter was applied prior to Fourier transformation. Unsuppressed water signal was used for eddy current correction and to phase metabolite spectra [36].

2.3. Spectral fitting and quantification

Baseline correction and fitting was performed automatically with software, described in detail earlier [43]. Briefly, this software uses a parametric model of known spectral components (metabolites) to fit metabolite resonances and a non-parametric fit of the baseline. A priori spectral information containing the frequencies, phases and relative amplitudes was generated using GAMMA software package [39] for the following metabolites: glutamate, glutamine, *N*-acetyl aspartate, creatine, choline and myoinositol (Glu, Gln, NAA, Cr, Cho and m-Ins, respectively). Prior knowledge of chemical shifts and scalar coupling constants was derived from literature values [13]. Similar to references [7,11], resonance intensities of the metabolites were expressed relative to the concentration of water in the selected ROI, accounting for the compartmental distribution of water in gray matter, white matter and cerebrospinal fluid. Specifically, absolute metabolite concentration can be expressed as the following:

$$C^{\mathrm{met}}=\frac{S^{\mathrm{met}}_{\mathrm{measured}}}{S^{\mathrm{w}}_{\mathrm{measured}}}F\frac{1}{R_1^{\mathrm{met}}} \tag{1}$$

where $S^{\mathrm{w}}_{\mathrm{measured}}$ and $S^{\mathrm{met}}_{\mathrm{measured}}$ are experimentally obtained signal intensities of water and metabolites, respectively, R_1^{met} is the correction factor for the metabolite longitudinal relaxation (T_2 contribution is negligible due to short experimental TE relative to in vivo T_2 values at 4 T [25])

$$R_1^{\mathrm{met}}=(1-\mathrm{e}^{-\mathrm{TR}/T_{1,\mathrm{met}}}) \tag{2}$$

where $T_{1,\mathrm{met}}$ is longitudinal relaxation time constants of metabolites, and F is the factor accounting for compartmentalized water distribution:

$$F=C^{\mathrm{w}}_{\mathrm{tissue}}R^{\mathrm{w}}_{\mathrm{tissue}}+C^{\mathrm{w}}_{\mathrm{CSF}}R^{\mathrm{w}}_{\mathrm{CSF}}\frac{V^{\mathrm{w}}_{\mathrm{CSF}}}{V^{\mathrm{w}}_{\mathrm{tissue}}} \tag{3}$$

where $C^{\mathrm{w}}_{\mathrm{tissue}}$ and $C^{\mathrm{w}}_{\mathrm{CSF}}$ are the concentrations of water in brain tissue and CSF, $V^{\mathrm{w}}_{\mathrm{tissue}}$ and $V^{\mathrm{w}}_{\mathrm{CSF}}$ are the fractional volumes of brain tissue and CSF in the ROI, and $R^{\mathrm{w}}_{\mathrm{tissue}}$ and $R^{\mathrm{w}}_{\mathrm{CSF}}$ are the transverse relaxation rates of water in brain tissue ($R^{\mathrm{w}}_{\mathrm{tissue}}=\mathrm{e}^{-t/T^{\mathrm{w}}_{2,\mathrm{tissue}}}$) and in CSF ($R^{\mathrm{w}}_{\mathrm{CSF}}=\mathrm{e}^{-t/T^{\mathrm{w}}_{2,\mathrm{CSF}}}$). Because reference spectra of water were acquired with a long TR, longitudinal relaxation of water can be ignored. Concentration of water in brain tissue, $C^{\mathrm{w}}_{\mathrm{tissue}}$, was assumed to be 71% in white matter and 81% in gray matter [7]. Biexponential fit of unsuppressed water was used to calculated CSF contribution to the ROI. However, differentiation between gray matter (gm) and white matter (wm) is difficult with this technique, and therefore segmented T_1-weighted MR images were used to estimate wm/gm contribution. T_2 values for water in tissue and CSF and volume correction term in Eq. (3) were calculated using standard Levenberg–Marquardt least square routines for bi-exponential fits (Matlab, The MathWorks, Inc., USA). Metabolite values for age comparison are reported according to Eq. (1), yielding concentrations in units of mM, where R_1^{met} factor is not included. Therefore, calculated concentration represents the lower limit of the metabolite concentration because T_1 time constants of metabolites are not used. Tissue segmentation was performed using Matlab™ software SPM (Wellcome Department of Cognitive Neurology, London, UK).

2.4. Statistical analysis

To determine overall age and regional effects on metabolite variations, repeated measures analysis of variance (rmANOVA) was used with metabolite concentrations as dependent variable and age, regions, and regions × age interaction as independent variables. These tests were performed separately for each metabolite. To avoid false discoveries by chance, we raised the level of significance to $p < 0.01$, according to Bonferroni correction ($p = 0.01 \approx 0.05/6$). For significant effects of region by age interactions, post-hoc Scheffe tests were performed to determine the relationship of metabolite concentrations separately by region with gray/white matter coverage as covariate. In order to test for regional effects, metabolite values for each subject were adjusted for age-variations: linear regressions were used to determine relations between metabolite variations and age for each region. Metabolite variations among subjects were adjusted for differences in age by means of an analysis of covariance approach, according to

$$M_{\text{adj}} = M_{\text{obs}} - \beta(A_i - A_{\text{mean}}) \quad (4)$$

Here M_{adj} and M_{obs} are the adjusted and observed metabolite concentrations in a subject and A_i is the subject's age, β the slope of the regression from group analysis and A_{mean} is the group's mean age. Linear regression analysis that correlated metabolic levels and age was performed for each metabolite. The correction for age variations was applied when significant relationship between metabolite concentration and age was encountered ($p < 0.05$). Effects of gender on metabolite concentrations in each region were tested using two-tailed *t*-tests.

3. Results

Fig. 1 displays representative experimental and baseline corrected ^{1}H MR spectra from mesial motor cortex (MC) (Fig. 1B) and corona radiata (COR) regions (Fig. 1C) and T_1-weighted image for the anatomical selection of the regions of interest. Spectral linewidth (FWHM) of singlet resonance of Cr at 3.0 ppm ranged from 6.4 to 7.9 Hz for all subjects. ^{1}H MR spectrum generated by numerical simulations [38] utilized for prior knowledge spectral fitting of experimental data is shown in Fig. 1A. Fig. 2 displays concentration and intensity ratios of Glu and Gln from each subject as a function of age, together with the mean values and standard deviations. The correlation plots in Fig. 3 depict Glu association with Gln, NAA and Cr in the MC region. Table 1 displays the comparison between metabolic concentrations in younger and older groups in the selected brain regions. Also included in this table are metabolite ratios relative to Cr, which were taken directly from fitted spectral intensities (ratios to creatine are largely used in ^{1}H MRS literature and also provide an alternative method to control for errors introduced in absolute method quantification using cerebral water). Metabolite concentrations in two brain regions corrected for age variations are listed in Table 2. Also listed in Table 2 are the average gray matter indices, defined as the proportion of gray matter to total brain tissue in those regions. These indices indicate that ROI in the COR consisted primarily of white matter, and ROI in the mesial motor cortex contained mainly gray matter (as expected). Results of a test–retests study from 10 subjects to determine reproducibility of short TE single voxel MRS at 4 T using water as internal reference are summarized in Table 3, in terms of values of coefficients of variations (CoV) for each metabolite and region.

3.1. Age group and region effects on metabolites

For Glu, there was strong effect by region ($F[1,44] = 131$; $p < 0.001$) with a higher concentration of Glu in MC than COR. Furthermore, there was an overall effect of age on Glu concentration ($F[1,44] = 9.8$; $p < 0.003$), independent of region and gray/white matter variation. Moreover, there was a significant region by age group interaction ($F[1,44] = 4.85$; $p = 0.03$). Post-hoc analysis of the region by age group interaction revealed a strong effect of age on Glu in MC

(F[1,22] = 3.1; p = 0.02), but no significant effect on Glu in COR. The relationship of Glu of MC with age remained significant after accounting for gray/white matter variations. For Gln, there was strong effect by region (F[1,44] = 43.05, $p < 0.001$) and a trend in age group effect, with higher Gln in COR region of older group (F[1,44] = 3.8, p = 0.05). For NAA, there was strong effect by region (F[1,44] = 11.8; p = 0.001) with a higher concentration of NAA in MC than COR. Furthermore, there was a trend for a relationship with age group (F[1,44] = 3.4; p = 0.06) with lower concentration of NAA in the older group in MC region. Cr showed a marked effect of region (F[1,44] = 16.4, $p < 0.001$), and age group by region interaction (F[1,44] = 9.8, $p < 0.01$), but no overall age effect. Post-hoc analysis of age group by region interaction with gray/white index as covariate, revealed a significant correlation between increased Cr and age group in the COR region ($p < 0.001$). Cho varied by region (F[1,44] = 20.3, $p < 0.001$) and age group (F[1,44] = 4.24, p = 0.04) with higher Cho in COR region in older group compared to younger group, but showed no significant association of age by region interactions. Finally, m-Ins showed a marked age by region interaction (F[1,44] = 3.8, $p < 0.001$), but no significant overall effects of age or region. Post-hoc analysis of age by region interaction with gray/white matter as a covariate, revealed a significant correlation between increased m-Ins and age ($p < 0.01$) in COR region and that m-Ins of MC decreased with increased age ($p < 0.05$).

3.2. Correlations of Glu with Gln and other metabolites (Fig. 3)

In the MC region, the concentration of Glu strongly correlated with the concentrations of Gln (r = 0.52, $p < 0.01$), NAA (r = 0.88, $p < 0.001$), and Cr (r = 0.78, $p < 0.001$). After correction of Glu, NAA, Cr and Gln values in the MC region for gray matter contribution by means of an analysis of covariance approach similar to the one outlined in Eq. (4), the significant positive correlation of Glu and all three metabolites persisted. Glu, Cr and Glu were significantly correlated with gray matter amount in the ROI ($p < 0.05$). In the COR region, however, no correlation of Glu or Gln with other metabolites was detected.

3.3. Gender differences

No significant effect of gender on metabolic differences was detected (all $p > 0.1$).

3.4. Test–retest (Table 3)

For Glu and Gln reproducibility was higher in the MC region compared to COR region (11.9% versus 13.6% for Glu and 21.5% versus 44.0% for Gln, respectively).

4. Discussion

The most important finding of this study was a decrease of Glu concentration with age in the predominantly gray matter motor cortex region and a trend of Gln increase with age in the predominantly white matter corona radiata region. Furthermore, there was a strong positive correlation of Glu with Gln, NAA, and Cr in the motor cortex region. In addition, concentrations of Glu and Gln were higher in motor cortex region compared to the predominantly white matter region of corona radiata.

The finding of reduced Glu with older age in the motor cortex is consistent with histological animal studies of increased deficits of the glutamatergic systems in the aging brain [8,37]. Most of Glu is found in neurons [28,29], with extracellular concentration of Glu normally remaining at relatively low levels (0.1–1 mM), because of its excitotoxicity. In glial cells, the Glu concentration is even lower [38] and thus, contributes virtually no signal to Glu in ^{1}H MRS. Therefore, Glu reduction with age may signify deficits in neuronal metabolism or possible neuronal loss or shrinkage during normal aging. NAA, like Glu, is also localized primarily in neurons and is considered to be a putative neuronal marker [27,42]. Our finding of reduced NAA in the motor cortex region with normal aging is consistent with previous MRS studies

that demonstrated decreased NAA with age in certain regions of the normal brain [4,6,35]. The present study further showed a strong positive correlation between Glu and NAA concentration, which is consistent with the idea that Glu is localized primarily in neurons and that neuronal integrity is vulnerable in the aging process. Detection of differential variations between Glu and NAA concentrations with MRS could potentially provide a better index of neuronal integrity than measurements of each metabolite alone, especially in cases where glutamate excitotoxicity occurs.

In contrast to Glu reduction in motor cortex, Gln concentration was somewhat increased in older subjects in the predominantly white matter region of corona radiata. Gln is localized primarily in astrocytes, where glutamine synthetase considered to be a marker of glial activity [21]. Therefore, the finding of increased Gln in this region could be indicative of glial (astrocytes) proliferation in white matter with normal aging. If neuronal loss is accompanied by glial proliferation in healthy aging, the increase of Gln in the white matter should be occurring concomitantly with decrease of Glu and NAA in the same region. However, we failed to detect decrease of NAA and Glu in COR region.

In addition to Gln, other metabolites such as Cr, Cho, and m-Ins were also elevated in the COR region of older subjects, consistent with findings from other laboratories [31,33]. It should be noted that ^{1}H MRS detects the overall metabolite concentration without differentiation between specific components (i.e. glial versus neuronal), and it is not clear to what extent Cr and Cho represent glial compartments. In contrast, no age-related NAA and Glu changes were detected in the COR region, consistent with other ^{1}H MRS studies that also report stable NAA with aging in white matter [5,6,31,33]. In this context, it is interesting to note that m-Ins concentration decreased in the MC region and increased in the COR region in older subjects compared to the younger group (m-Ins is used predominantly as glial marker in the ^{1}H MRS literature [3]). The increase of m-Ins in white matter is consistent with its role as a glial marker [3] and concomitant Gln increase in the same region, but we have currently no explanation for the reduction of m-Ins in the motor cortex. The observed differential changes of metabolites with age in two regions are most likely affected by white matter/gray matter composition of those regions.

Although a positive correlation between Glu and Gln in the motor cortex seems counter intuitive to our hypothesis of a negative relationship between the two metabolites, with the former representing neuronal pool and the latter, astrocytes/glia, there are at least two possible explanations for a positive correlation. First, since both Glu and Gln have much higher concentrations in gray matter than white matter, partial gray/white matter volumes of voxels in the motor cortex could dominate the relationship. However, after corrections of the metabolites for gray matter composition of the voxels using linear regressions, the positive correlation between Glu and Gln was still significant. Therefore, gray/white matter partial volume effects cannot entirely explain the positive correlation. Second, there are several synthetic pathways for glutamate and glutamine in brain tissue, and some pathways utilize one metabolite as precursor for synthesis of the other. For example one of the pathways for formation of glutamate is via glutamine, which is imported from glia by phosphate-activated glutaminase. Alternatively, glutamine may be formed from glutamate, originating from α-ketoglutarate in glia [38]. Therefore, certain synthetic pathways, which link both metabolites together, could lead to a positive correlation between Glu and Gln, which can only be determined via kinetic studies of specific activities for both metabolites.

Independent of age, levels of Glu, NAA, Gln, and Cr were higher in MC region, compared to COR region. Outside the motor cortex, other MRS studies at 1.5 and 4 T [1,17,24] also reported higher Glu, Gln, and Cr concentrations in gray matter than in white matter. Furthermore, a ^{1}H MRS study on autopsied brains measured higher Glu, NAA, and Cr in gray matter compared

to white matter [30], consistent with the results of this study. However, tissue-related differences of metabolite concentrations may also be regionally dependent, which limits comparisons to other studies that did not specifically measure metabolite concentrations in motor neuron regions.

In this study, cerebral water was used as reference to determine metabolite concentrations in the motor pathways of human brain. The accuracy of this method was assessed with test–retest measurements, which showed coefficient of variations for Glu between 11% and 15%. In contrast, Gln variations were determined to be two to four times larger, reflecting significant limitation in measuring reliably glutamine concentration even at 4 T, presumably due to much lower signal intensity of Gln compared to Glu [22]. Although comparison with another study at 4 T [1] is limited since a different area was studied (thalamus), CV values in this study are similar.

A limitation of the study was that no relaxation measurements of metabolites were obtained for each subject and therefore some regional and age-related changes might be due to relaxation effects (mainly longitudinal relaxation, since the contribution of the transverse relaxation with a very short echo time employed here is negligible). Moreover, different T_1 relaxations for resonances of the same metabolite were not considered. Therefore, metabolite quantification in this study is limited to the degree that resonances of the same metabolite exhibit different T_1 relaxations. However, a differential effect of age or brain region on T_1 relaxations of different functional groups within the metabolite is not expected and therefore observations in this study of age and region related variations of metabolite concentrations should not be confounded by this effect. A second limitation was that we did not correct for partial volumes of gray and white matter when calculating metabolite concentration. Performing this correction is important when interpreting metabolite concentrations of motor cortex, since voxels in those regions contained also substantial amounts of white matter. Another limitation is using cerebral water as a reference without considering potential decrease of water concentration in gray matter and white matter with age, as suggested in Ref. [5]. Although we did not find significant water content correlation with age in our study and therefore did not take this effect into consideration, sensitivity of our methods to detect an effect may have been limited.

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References

1. Bartha R, Drost DJ, Menon RS, Williamson PC. Comparison of the quantification precision of human short echo time ^{1}H spectroscopy at 1.5 and 4.0 Tesla. Magn Reson Med 2000;44:185–92. [PubMed: 10918316]
2. Blandini F, Greenamyre JT. Protective and symptomatic strategies for therapy of Parkinson's disease. Drugs Today (Barc) 1999;35(6):473–83. [PubMed: 12973393]
3. Brand A, Richter-Landsberg C, Leibfritz D. Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. Dev Neurosci 1993;15:289–98. [PubMed: 7805581]
4. Brooks JC, Roberts N, Kemp GJ, Gosney MA, Lye M, White-house GH. A proton magnetic resonance spectroscopy study of age-related changes in frontal lobe metabolite concentrations. Cereb Cortex 2001;11(7):598–605. [PubMed: 11415962]
5. Chang L, Ernst T, Poland RE, Jenden DJ. In vivo proton magnetic resonance spectroscopy of the normal aging human brain. Life Sci 1996;58:2049–56. [PubMed: 8637436]

6. Charles HC, Lazeyras F, Krishnan KRR, Boyko OB, Patterson LJ, Doraiswamy PM, et al. Proton spectroscopy of human brain: effects of age and sex. Prog Neuro Psychopharmacol Biol Psychiatry 1994;18:995–1004.
7. Christiansen P, Henriksen O, Stubgaard M, Gideon P, Larsson HB. In vivo quantification of brain metabolites by ^{1}H-MRS using water as an internal standard. Magn Reson Imaging 1993;11(1):107–18. [PubMed: 8423713]
8. Cobo M, Exposito I, Porras A, Mora F. Release of amino acid neurotransmitters in different cortical areas of conscious adult and aged rats. Neurobiol Aging 1992;13(6):705–9. [PubMed: 1362797]
9. Cotman CW, Monaghan DT, Ottersen OP, Storm-Mathiesen J. Anatomical organization of excitatory amino acids and their pathways. Trends Neurosci 1987;10:273–80.
10. Davis, IM.; Himwich, WA. Neurochemistry of the developing and aging mammalian brain. In: Ordy, JM.; Brizzee, KR., editors. Neurobiology of aging. New York: Plenum; 1975. p. 329-57.
11. Ernst T, Kreis R, Ross BD. Absolute quantitation of water and metabolites in the human brain. Part I Compartments and water. J Magn Reson Series B 1993;102:1–8.
12. Frahm J, Bruhn H, Gyngell ML, Merboldt KD, Hanicke W, Sauter R. Localized high-resolution proton NMR spectroscopy using stimulated echoes: initial applications to human brain in vivo. Magn Reson Med 1989;9:79–93. [PubMed: 2540396]
13. Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain metabolites. NMR Biomed 2000;13(3):129–53. [PubMed: 10861994]
14. Greenamyre JT, Young AB. Excitatory amino acids and Alzheimer's disease. Neurobiol Aging 1989;10:593–602. [PubMed: 2554168]
15. Greenamyre JT. Glutamate–dopamine interactions in the basal ganglia: relationship to Parkinson's disease. J Neural Transm 1993;91:255–69.
16. Heath PR, Shaw PJ. Update on the glutamatergic neurotransmitter system and the role of excitotoxicity in amyotrophic lateral sclerosis. Muscle Nerve 2002;26:438–58. [PubMed: 12362409]
17. Hetherington HP, Mason GF, Pan JW, Ponder SL, Vaughan JT, Twieg DB, et al. Evaluation of cerebral gray and white matter metabolite differences by spectroscopic imaging at 4. 1T Magn Reson Med 1994;32(5):565–71.
18. Hetherington HP, Pan JW, Chu W-J, Mason GF, Newcomer BR. Biological and clinical MRS at ultra-high field. NMR Biomed 1997;10:360–71. [PubMed: 9542734]
19. Hoult DI, Lauterbur PC. The sensitivity of the zeumatographic experiment involving human samples. J Magn Reson 1979;34:425–33.
20. Jolles, J. Progress of brain research. Amsterdam: Elsevier; 1986. Cognitive, emotional and behavioral dysfunctions in aging and dementia. Aging of the brain and Alzheimer's disease; p. 15-39.
21. Martinez-Hernandez A, Bell KP, Norenberg MD. Glutamine synthetase: glial localization in brain. Science 1977;195:1356–8. [PubMed: 14400]
22. Mason GF, Pan JW, Ponder SL, Twieg DB, Pohost GM, Hetherington HP. Detection of brain glutamate and glutamine in spectroscopic imaging at 4. 1 T Magn Reson Med 1994;32:142–5.
23. Matson GB. An integrated program for amplitude-modulated RF pulse generation and re-mapping with shaped gradients. Magn Reson Imaging 1994;12(8):1205–25. [PubMed: 7854027]
24. McLean MA, Woermann FG, Barker GJ, Duncan JS. Quantitative analysis of short echo time ^{1}H-MRSI of cerebral gray and white matter. Magn Reson Med 2000;44(3):401–11. [PubMed: 10975892]
25. Michaeli S, Garwood M, Zhu XH, DelaBarre L, Andersen P, Adriany G, et al. Proton T2 relaxation study of water, N-acetylaspartate, and cratine in human brain using Hahn and Carr-Purcell spin echoes at 4T and 7T. Magn Reson Med 2002;47:629–33. [PubMed: 11948722]
26. Michaelis ML. Drugs targeting Alzheimer's disease: some things old and some things new. J Pharmacol Exp Ther 2003;304:897–904. [PubMed: 12604663]
27. Moffett JR, Namboodiri MA. Differential distribution of N-acetyl-aspartylglutamate and N-acetylaspartate immunoreactivities in rat forebrain. Neurocytology 1995;24:409–33.
28. Ottersen O, Zhang N, Walberg F. Metabolic compartmentation of glutamate and glutamine: morphological evidence obtained by quantitative immunocytochemistry in rat cerebellum. Neuroscience 1992;46:519–34. [PubMed: 1347649]

29. Patel AJ, Hunt A, Gordon RD, Balazs R. The activities in different neural cell types of certain enzymes associated with the metabolic compartmentation glutamate. Brain Res 1982;256:3–11. [PubMed: 6124308]
30. Petroff OAC, Spencer DD, Alger JR, Prichard JW. High field proton magnetic resonance spectroscopy of human cerebellum obtained during surgery for epilepsy. Neurology 1989;39:1197–202. [PubMed: 2771071]
31. Pfefferbaum A, Adalsteinsson E, Spielman D, Sullivan EV, Lim KO. In vivo spectroscopic quantification of the N-acetyl moiety, creatine, and choline from large volumes of brain gray and white matter: effects of normal aging. Magn Reson Med 1999;41:276–84. [PubMed: 10080274]
32. Provencher SW. Automatic quantitation of localized in vivo ^{1}H spectra with LC Model. NMR Biomed 2001;14(4):260–4. [PubMed: 11410943]
33. Saunders DE, Howe FA, van den Boogaart A, Griffiths JR, Brown MM. Aging of the adult human brain: in vivo quantitation of metabolic content with proton magnetic resonance spectroscopy. J Magn Reson Imaging 1999;9:711–6. [PubMed: 10331768]
34. Schmidt WJ, Bubser M, Hauber W. Behavioural pharmacology of glutamate in the basal ganglia. J Neural Transm 1992;38:65–89.
35. Schuff N, Ezekiel F, Gamst AC, Amend DL, Capizzano AA, Maudsley AA, et al. Region and tissue differences of metabolites in normally aged brain using multislice ^{1}H magnetic resonance spectroscopic imaging. Magn Reson Med 2001;45(5):899–907. [PubMed: 11323817]
36. Seeger U, Klose U, Seitz D, Nagele T, Lutz O, Grodd W. Proton spectroscopy of human brain with very short echo time using high gradient amplitudes. Magn Reson Imaging 1998;16(1):55–62. [PubMed: 9436947]
37. Segovia G, Porras A, Del Arco A, Mora F. Glutamatergic neurotransmission in aging: a critical perspective. Mech Ageing Dev 2001;122(1):1–29. [PubMed: 11163621]
38. Siegel, GJ.; Agranoff, BW.; Albers, RW.; Fisher, SK.; Uhler, MD. Cellular and medical aspects. 6. Lippincott Williams & Wilkins; 1999. Basic neurochemistry: molecular; p. 646
39. Smith SA, Levante TO, Meier BH, Ernst RR. Computer simulations in magnetic resonance. An object oriented programming approach. J Magn Reson 1994:75–105.
40. Strolin-Benedetti M, Cini M, Fusi R, Marrari P, Dostert P. The effects of aging on MAO activity and amino acid levels in rat brain. J Neural Transm 1990;29:259–68.
41. Tkac I, Starcuk Z, Choi IY, Gruetter R. In vivo ^{1}H NMR spectroscopy of rat brain at 1 ms echo time. Magn Reson Med 1999;41(4):649–56. [PubMed: 10332839]
42. Urenjak J, Williams SR, Gadian DG, Noble M. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. J Neurosci 1993;13:981–9. [PubMed: 8441018]
43. Young K, Govindaraju V, Soher BJ, Maudsley AA. Automated spectral analysis I: formation of a priori information by spectral simulation. Magn Reson Med 1998;40(6):812–5. [PubMed: 9840824]

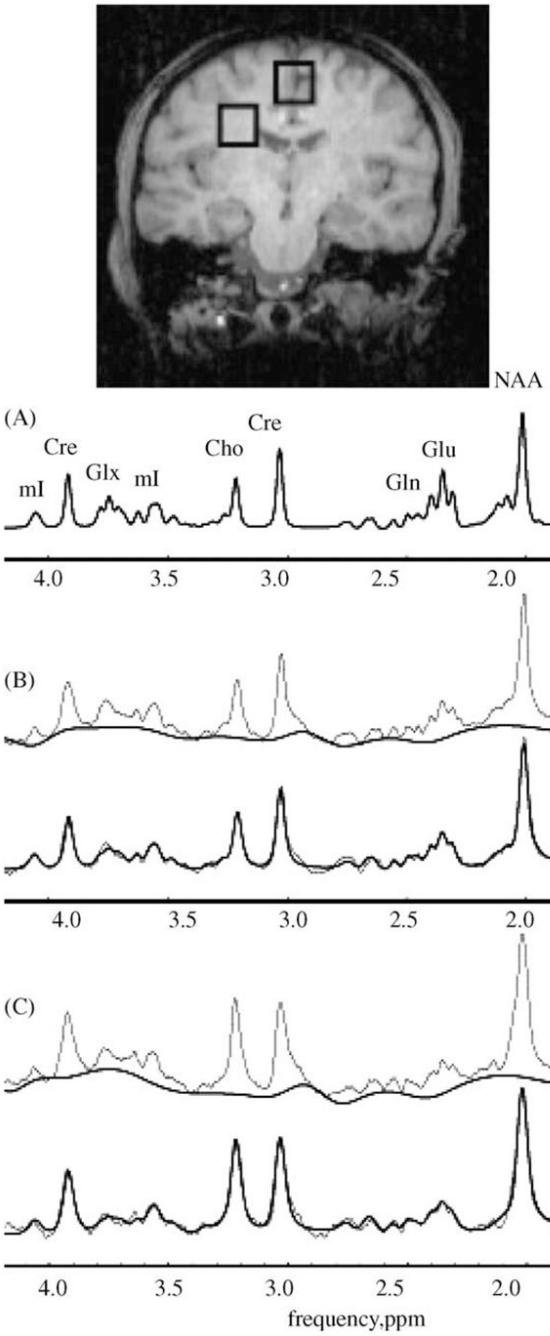

Fig. 1.

Representative T_1-weighted MR image used for the placement of the two regions of interest (8 mL)-mesial motor cortex and corona radiata. (A) Simulated ^{1}H MR spectrum used as a prior knowledge for fitting experimental spectra. (B) Top-representative experimental ^{1}H MR spectrum from mesial motor cortex (thin line) and computed baseline (thick line). Bottom, baseline corrected experimental spectrum from the same region (thin line) with superimposed fitted spectrum (thick line). (C) Top-representative experimental ^{1}H MR spectrum from corona radiata (thin line) and computed baseline (thick line). Bottom, baseline corrected experimental spectrum from the corona radiata (thin line) with superimposed fitted spectrum (thick line).

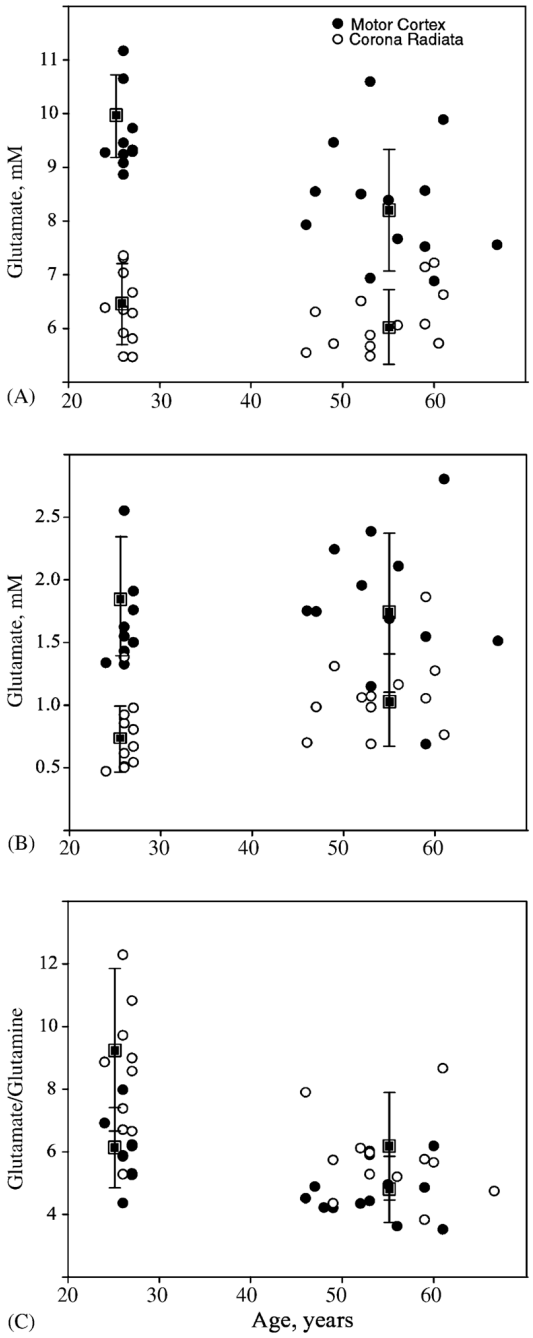

Fig. 2.

Concentrations of glutamate (A) and glutamine (B) as a function of age in corona radiata and mesial motor cortex areas. (A) Glutamate concentration as a function of age. (B) Glutamine concentration as a function of age. (C) The intensity ratio Glu/Gln is calculated directly from the ratio of the respective metabolites areas in the MR spectrum and is shown here as a function of age.

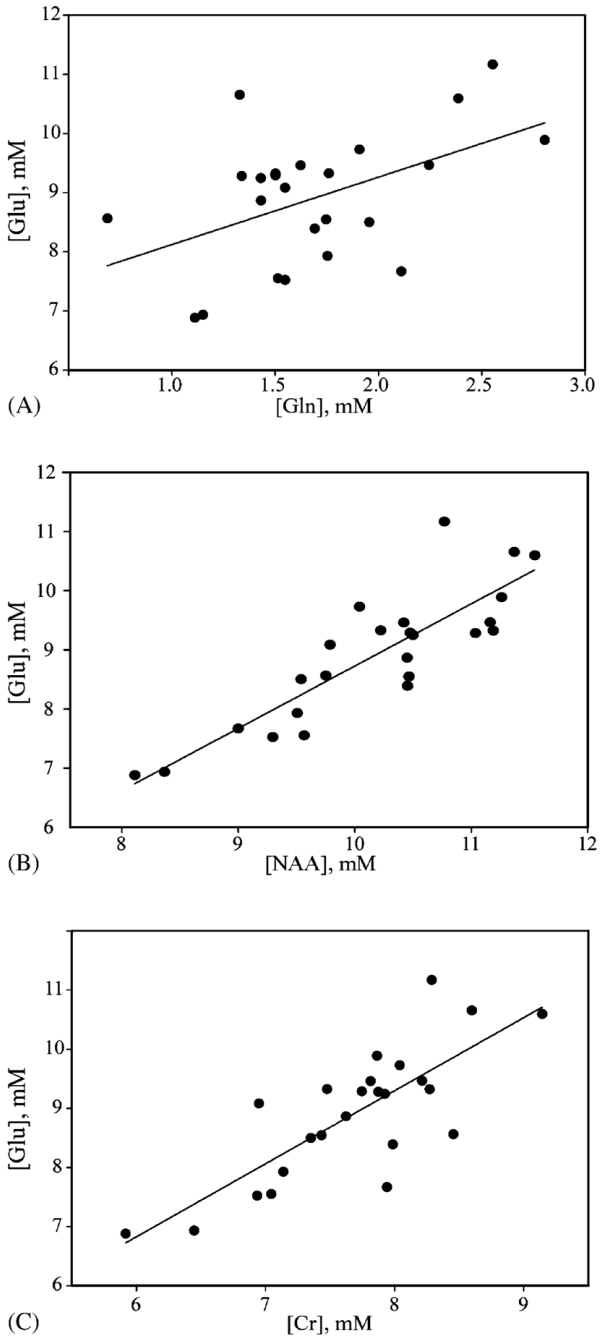

Fig. 3.

Correlations between Glu and other metabolites in the mesial motor cortex area (A) Glu vs. Gln ($r = 0.52$, $p < 0.01$), (B) Glu vs. NAA ($r = 0.86$, $p < 0.001$), (C) Glu vs. Cr ($r = 0.78$, $p < 0.001$).

Table 1

Mean metabolite concentrations (units mM), ratios to creatine[a], and CSF/tissue contribution in corona radiata and mesial motor cortex in younger (mean age = 26 ± 1) and older group (mean age = 54 ± 6)

	Corona radiata		Motor cortex	
	Younger	**Older**	**Younger**	**Older**
GM/(GM + WM)	0.22 ± 0.05	0.22 ± 0.07	0.70 ± 0.10	0.69 ± 0.06
CSF	0.02 ± 0.01	0.03 ± 0.01	0.24 ± 0.08	0.26 ± 0.06
[Glu]	6.37 ± 0.67	6.15 ± 0.57	9.58 ± 0.79	8.34 ± 0.92*
[Gln]	0.75 ± 0.27	1.09 ± 0.30**	1.63 ± 0.36	1.75 ± 0.54
[Cr]	6.63 ± 0.31	7.33 ± 0.46*	7.87 ± 0.44	7.53 ± 0.86
[Cho]	1.83 ± 0.14	2.04 ± 0.17**	1.70 ± 0.14	1.71 ± 0.20
[NAA]	9.47 ± 0.42	9.37 ± 0.74	10.57 ± 0.68	9.85 ± 0.97**
[m-Ins]	4.24 ± 0.49	4.96 ± 0.95*	5.20 ± 0.44	4.15 ± 1.01**
Glu/Cr	0.95 ± 0.09	0.80 ± 0.10	1.23 ± 0.09	1.11 ± 0.07
Gln/Cr	0.11 ± 0.05	0.13 ± 0.07	0.22 ± 0.05	0.23 ± 0.06
Cho/Cr	0.28 ± 0.02	0.28 ± 0.03	0.21 ± 0.02	0.23 ± 0.02
NAA/Cr	1.43 ± 0.09	1.29 ± 0.08	1.35 ± 0.05	1.29 ± 0.08
m-Ins/Cr	0.63 ± 0.08	0.65 ± 0.12	0.67 ± 0.06	0.57 ± 0.13

[a] Ratios to creatine were not tested for statistical significance.

* $p < 0.01$.

** $p = 0.05$.

Table 2

Measured regional metabolite levels (mM) adjusted for age variations between the groups

Region	GM index[a]	NAA	Glu	Cr	Cho	m-Ins	Gln
COR	0.22	9.41 ± 0.65*	6.26 ± 0.62*	7.09 ± 0.44*	1.94 ± 0.18*	4.63 ± 0.85	1.10 ± 0.27*
MC	0.69	10.18 ± 0.82	8.91 ± 0.94	7.68 ± 0.68	1.70 ± 0.18	4.60 ± 0.95	1.70 ± 0.47

[a] Gray matter index is defined as GM/(WM + GM).

* $p < 0.01$.

Table 3

Coefficients of variation (standard deviation to mean ratio in percent) for 10 subjects (scanned twice) compared to literature values in thalamus region [1]

Region	NAA	Glu	Cr	Cho	m-Ins	Gln
COR	5.8	13.4	5.9	7.9	19.7	44.5
MC	7.9	11.6	6.6	10.8	15.3	20.5
Thalamus (from Ref. [1])	7.3	8.9	5.7	8.9	12.4	16.9