Reduced hippocampal glutamate in Alzheimer disease

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Abstract

Altered neurometabolic profiles have been detected in Alzheimer disease (AD) using 1H magnetic resonance spectroscopy (MRS), but no definitive biomarker of mild cognitive impairment (MCI) or AD has been established. This study used MRS to compare hippocampal metabolite levels between normal elderly controls (NEC) and subjects with MCI and AD. Short echo-time (TE = 46 ms) 1H spectra were acquired at 4 T from the right hippocampus of 23 subjects with AD, 12 subjects with MCI and 15 NEC. Absolute metabolite levels and metabolite ratios were compared between groups using a multivariate analysis of covariance (covariates: age, sex) followed by post hoc Tukey’s test (p < 0.05 significant). Subjects with AD had decreased glutamate (Glu) as well as decreased Glu/creatine (Cr), Glu/myo-inositol (mI), Glu/N-acetylaspartate (NAA), and NAA/Cr ratios compared to NEC. Subjects with AD also had decreased Glu/mI ratio compared to MCI. There were no differences between subjects with MCI and NEC. Therefore, in addition to NAA/Cr, decreased hippocampal Glu may be an indicator of AD.

Keywords: Alzheimer disease; MCI (mild cognitive impairment); MRS; Spectroscopy; Hippocampus; Glutamate

1. Introduction

Alzheimer disease (AD) is the most common form of dementia, and is characterized by progressive loss of cognitive function as well as a distinct pathological profile of neurofibrillary tangles and amyloid plaques that begins in the mediotemporal lobe (hippocampus and entorhinal cortex) and limbic areas as early as decades before clinical diagnosis (Braak and Braak, 1994). Mild cognitive impairment (MCI) is an intermediate clinical stage along the cognitive spectrum between healthy aging and dementia that many consider to be prodromal AD. Subjects with MCI progress to AD at a rate of up to 15%/year compared to 2%/year for normal elderly to AD (Solfrizzi et al., 2004). The diagnosis of AD and monitoring of disease progression typically involves cognitive assessments to detect changes in memory, language, visuo-spatial and executive function.

The molecular neuropathology of Alzheimer disease is thought to precede structural brain alteration by several years. Hence, measurements of tissue metabolism may be sensitive biomarkers of very early disease processes. Proton magnetic resonance spectroscopy (1H MRS) provides a non-invasive method of assessing brain metabolites in vivo. Short echo-time proton MRS is capable of detecting several metabolic by-products, including N-acetylaspartate (NAA), glutamate (Glu), glutamine (Gln), myo-inositol (mI), choline (Cho) and creatine (Cr). 1H MRS has been applied previously to the study of AD and has most consistently detected decreased NAA or NAA/Cr in the parietal and occipital cortex (Miller et al., 1993), gray matter (Adalsteinsson et al., 2000; Moats et al., 1994), hippocampus (Dixon et al., 2002; Schuff et al., 1997), and posterior cingulate (Kantarci et al., 2000) as well

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as increased mI in parietal and occipital cortex (Miller et al., 1993), gray matter (Moats et al., 1994), and posteriorcingulate (Kantarcı et al., 2000).

Although Glu has been less well studied, it is the principal excitatory neurotransmitter involved in learning, memory and cognition and can be detected directly by short echo-time MRS. Previous MRS studies have reported decreased Glu levels in the cortex and hippocampus of transgenic AD mice (Marjanska et al., 2005) but only relative decreases in the sum of Glu and glutamine over creatine in subjects with AD in the cingulate cortex (Antuono et al., 2001; Hattori et al., 2002) and posterior cingulate gyrus, precuneus, and portions of the cuneus (Hattori et al., 2002).

The purpose of this study was to compare hippocampal metabolite levels measured by high magnetic field MRS, particularly Glu, NAA and mI, in subjects with MCI, AD, and normal elderly controls (NEC). The secondary objective was to correlate these metabolite measures with cognitive test results.

2. Methods

2.1. Subjects

All study participants (30 probable AD, 13 MCI and 17 NEC) were recruited from the Aging Brain and Memory Clinic in London, Ontario, Canada. This study was approved by the University of Western Ontario Health Sciences Research Ethics Board. Informed consent was acquired according to the Declaration of Helsinki. Several subjects did not complete their MRI scan due to discomfort or claustrophobia (2 AD, 1 MCI, 1 NEC) and several datasets were excluded due to poor spectral quality (5 AD, 1 NEC) attributable to a poor shim or patient motion during the scan. The remaining subjects (23 probable AD, 12 MCI and 15 NEC) were included in all statistical analyses.

All subjects with AD had probable Alzheimer disease as diagnosed by the Diagnostic and Statistical Manual for Mental Disorders (DSM) IV criteria for dementia and the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer’s Disease and Related Disorder’s Association (NINCDS/ADRDA) criteria for Alzheimer disease. Subjects with MCI were diagnosed according to the Petersen criteria (Petersen et al., 2001) of: (1) objective memory impairment based on age and education, (2) memory complaint corroborated by a third party, (3) normal general cognitive function, (4) no dementia and (5) intact activities of daily living (ADL). For inclusion in the MCI group, subjects required a global Clinical Dementia Rating (CDR (Morris, 1997)) scale score of 0.5 and intact activities of daily living assessed using the Lawton–Brody Physical Self-Maintenance Scale and Instrumental ADL Scale (Lawton and Brody, 1969). Depression was also ruled out as the cause of memory impairment using the Geriatric Depression Scale-Short Form (GDS-SF (Sheikh et al., 1991)) and the Cornell Scale for Depression in Dementia (Alexopoulos et al., 1988). Cognitive function was assessed in the AD, MCI, and NEC groups using the Mini-Mental State Exam (MMSE (Folstein et al., 1975)) and in the NEC and AD groups using the Dementia Rating Scale-II (DRS-II (Smith et al., 1994)). NEC subjects had: (1) no memory complaints or impairment, (2) normal instrumental ADL, and (3) age and education appropriate scores on the MMSE, and DRS-II.

The exclusion criteria for all subjects included contraindications to MRI, clinical depression, substance abuse, diagnosis of another dementia or the presence of significant vascular disease or cerebrovascular infarcts. NEC and subjects with MCI had no history of medications for Alzheimer disease. All subjects with AD were on a stable dose of a cholinesterase inhibitor (donepezil [10 mg] or galantamine [16 mg]) drug therapy for at least six months prior to data collection, and also had no history of treatment with memantine.

2.2. Data acquisition

Cognitive testing was performed on subjects within two weeks of their MRI scan. The MMSE, scored using WORLD backwards, was conducted on all subjects, while the DRS-II was performed on NEC and subjects with AD only.

To ensure consistency, all data acquisition and post-processing was performed by the same operator (R.R.). MR imaging and spectroscopy were performed on a whole body 4 Tesla Varian (Palo Alto, CA) MRI scanner with a Siemens (Erlangen, Germany) Sonata gradient coil. Prior to imaging, magnetic field homogeneity was optimized using a manual shim over the whole head using linear and Z2 shim coils (FWHM < 24 Hz). Three-dimensional (3D) T1-weighted fast low angle shot (FLASH) (Frahm et al., 1986) volumetric images (TE/TR/TI = 5/9.5/500 ms, FOV = 24 cm, 2.5 mm thickness, 16 slices, 256 × 256 in-plane acquisition matrix) were acquired parallel to the long axis of the hippocampus for voxel positioning (Fig. 1A). Coronal T1-weighted 3D-FLASH volumetric images (TI/TR/TE = 500/510/5.3 ms, FOV = 22 cm × 22 cm × 20 cm, 256 × 256 × 80 acquisition matrix) were also acquired over the whole brain and used for the segmentation of brain tissue components within the spectroscopy voxel.

Short echo-time single voxel spectra were localized by adiabatic selective refocusing (LASER (Garwood and DelaBarre, 2001), 500 μs dwell time, 2 kHz receiver bandwidth, τcp = 6 ms, TE = 46 ms) in the right hippocampus of each patient (Fig. 1A). The acquisition of full spectra (128 averages, TR = 2.2 s), macromolecule spectra (128 averages, T1 = 2.2 s, T2 = 0.7 s, TR = 4.2 s), and water unsuppressed spectra (8 averages, TR = 2.2 s) as well as all spectral post-processing were performed as previously described (Kassem and Bartha, 2003). The acquisition of all spectra took ~15 min out of a total MR scan time of 60 min per subject. Voxels dimensions were chosen to maximize voxel volume while staying largely within the hippocampus of each subject and ranged
from 1.8 to 5.1 cm³ (mean ± S.D., 3.7 ± 0.8 cm³). There was no significant difference in average voxel volumes between the three patient populations.

2.3. Data processing

Spectra were processed by QUALITY deconvolution (de Graaf et al., 1990) and eddy current correction (ECC) (Klose, 1990) with the junction point set at 100 ms (Bartha et al., 2000b). Each macromolecule spectrum was fitted using a fully automatic Hankel singular value decomposition (HSVD) fitting routine that required no prior knowledge (van den Boogaart et al., 1994). The fitted macromolecule spectrum was subtracted from the full spectrum after scaling by a factor of 1.2 to account for $T_1$-saturation (Kassem and Bartha, 2003). Residual water signal was removed from the resulting spectrum by subtracting resonances between 4.1 and 5.1 ppm as determined by HSVD fitting. This final spectrum contained only metabolite information and was fitted in the time domain using a Levenberg–Marquardt minimization algorithm (Marquardt, 1963) that incorporated prior knowledge from 19 metabolite lineshapes. All prior knowledge was acquired at 4 T using the same LASER pulse sequence. The fitting was performed using analysis software and a graphical interface (fitMAN (Bartha et al., 1999)) written in IDL (Version 5.4 Research Systems Inc., Boulder, CO, USA).

Each unsuppressed water spectrum was fitted with a single a priori peak using fitMAN. Voxel water concentration was calculated assuming water content was 81% within hippocampal gray matter (GM), 71% in white matter (WM) and 100% within cerebrospinal fluid (CSF) (Christiansen et al., 1993; Kaiser et al., 2005). The average ratio of gray matter/white matter/CSF was determined for each voxel by segmenting the $T_1$-weighted images into gray matter/white matter/CSF probability maps using Statistical Parametric Mapping (SPM5 (Ashburner and Friston, 1997)) in Matlab (Macintosh v7.3.0.298, The Mathworks, Inc., Natick, MA). To perform the analysis, the $T_1$-weighted images were warped to a standard space and a template of tissue probability was utilized. The contribution of gray matter, white matter, and CSF to each voxel in normal tissue was calculated using a plug-in written in ImageJ (NIH).

The resulting water concentration within the VOI was used to scale metabolite values and calculate an absolute tissue metabolite concentration according to Eq. (1):

$$C_{\text{metab}} = \frac{A_{\text{metab}}}{A_{\text{H}_2\text{O}}} \times \frac{F_{\text{H}_2\text{O}}}{F_{\text{metab}}} \times \frac{P_{\text{H}_2\text{O}}}{P_{\text{metab}}} \times \frac{R_{\text{H}_2\text{O}}}{R_{\text{metab}}}$$

where $C_{\text{metab}}$ is the concentration of the metabolite; $A_{\text{metab}}$ is the area of the metabolite in the suppressed spectrum; $A_{\text{H}_2\text{O}}$ is the area of the water in the unsuppressed spectrum; $F_{\text{metab}}$ is the tissue fraction containing metabolite; $F_{\text{H}_2\text{O}}$ is the tissue fraction containing water; $P_{\text{metab}}$ is the number of protons contributing to the metabolite; $P_{\text{H}_2\text{O}}$ is the number of protons contributing to the water; $R_{\text{metab}}$ is the relaxation correction for the metabolite; $R_{\text{H}_2\text{O}}$ is the relaxation correction for the water.
Specifically, the tissue fractions and relaxation corrections are calculated using Eqs. (2)–(5):

\[
F_{H_2O} = (0.81 \times \%GM) + (0.71 \times \%WM) + \%CSF \quad (2)
\]

\[
F_{\text{metab}} = 1 - \%CSF \quad (3)
\]

\[
R_{H_2O} = \frac{(%GM \times AT_{H_2O,GM}) + (%WM \times AT_{H_2O,WM}) + (%CSF \times AT_{H_2O,CSF})}{1 - \%CSF} \quad (4)
\]

\[
R_{\text{metab}} = \frac{(%GM \times AT_{\text{metab},GM}) + (%WM \times AT_{\text{metab},WM})}{1 - \%CSF} \quad (5)
\]

where \(\%GM\) is the fractional gray matter within the voxel; \(\%WM\) is the fractional white matter within the voxel; \(\%CSF\) is the fractional cerebrospinal fluid within the voxel; \(AT_{\text{compound}, \text{tissue}}\) is the calculated signal attenuation factor for a given compound (\(H_2O\) or metabolite) based on the \(T_1\) and \(T_2\) relaxation time constants of the tissue (GM, WM, or CSF) of interest.

The attenuation factor was calculated using Eq. (6) below:

\[
AT_{\text{compound}, \text{tissue}} = (1 - e^{-TR/T_1^{\text{compound}, \text{tissue}}}) \times e^{-TE/T_2^{\text{compound}, \text{tissue}}} \quad (6)
\]

The concentrations were corrected for \(T_1\) and \(T_2\) relaxation effects (Isobe et al., 2002; Kassem and Bartha, 2003) using values for white and gray matter obtained at 4 T in healthy young subjects taken from Table 1 in Kassem and Bartha (2003). The same \(T_1\) and \(T_2\) values were used in the current study for all groups except that the \(T_2\) of water was adjusted to 86 ms (for all groups) based on a previous study that demonstrated a 7% reduction in the \(T_2\) of water in normal elderly subjects (Haley et al., 2004). The \(T_2\) time constants for metabolites have been shown to be the same in young, old and demented subjects (Moats et al., 1994), hence the same \(T_1\) and \(T_2\) parameter values for metabolites were also used for subjects with MCI, subjects with AD, and NEC. The segmented tissue proportions were used to re-calculate the \(T_1\), \(T_2\) and water concentration scaling factors for each individual dataset.

### 3. Results

Demographic data for each patient population is summarized in Table 1. There was a significant difference in gender between groups (\(\chi^2[2,47] = 8.8, p < 0.01\)), with more males in the MCI and AD groups compared to the NEC group. Subjects with MCI and AD were younger than the NEC group (\(p < 0.05\)). Subjects with MCI had significantly higher education levels than the other groups. The MMSE scores were significantly lower in subjects with AD (\(p < 0.01\)) compared to NEC and subjects with MCI (Table 1).

Fig. 2 depicts a coronal \(T_1\)-weighted MR slice segmented into GM, WM, and CSF, with the VOI shown over the right hippocampus. The voxel clearly contains a contribution from each tissue type, emphasizing the need to correct for such partial volume errors. There was no difference between groups in the average amount of gray matter (~61%) in the VOI. However, the voxels in the AD group contained lower WM (17.2% in subjects with AD compared to 25.5% in NEC).

### 2.4. Statistical analysis

All statistical analyses were performed using SPSS 13. An analysis of variance was conducted using the general linear model (GLM) for selected brain metabolites across the NEC, AD and MCI patient groups. For inclusion in the analyses, metabolites were required to have a coefficient of variation (CV) < 25% within the NEC group. Metabolites meeting this criterion were: NAA, Glu, Cr, mI and Cho. A secondary analysis was performed to compare specific metabolite ratios (NAA/Cr, Glu/Cr, Glu/mI, and Glu/NAA) between groups. An analysis of variance was used to evaluate between-group differences in demographic factors such as age and education, while a Chi-squared test was used to evaluate differences in gender. Demographic factors with significant between-group differences, such as age and gender, were used as covariates in the analysis of variance performed to compare metabolite levels and psychometric test scores between groups. A multivariate analysis of covariance (MANCOVA) was initially used to compare absolute metabolite levels across the NEC, AD and MCI patient groups. This analysis was followed by univariate analyses of covariance (ANCOVA) to determine whether absolute metabolite levels or metabolite ratios were different between groups. Post hoc analysis utilized the Tukey’s test. The Spearman’s rho coefficient was used to test for correlations between metabolite levels, demographic data, and MMSE scores within each group. All statistical tests were two-sided, with significance set at the 0.05 level. Because each statistical test was part of a planned comparison of specific metabolites between groups, no additional correction was made for multiple comparisons. Receiver operating characteristic (ROC) curves were generated between AD and NEC groups based on entering NAA/Cr, Glu, NAA/Cr+Glu+mI, and the MMSE into a logistic regression model.

Table 1

<table>
<thead>
<tr>
<th>Demographic information and cognitive test results.</th>
<th>NEC</th>
<th>MCI</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group size</td>
<td>15</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>(N) males (%)</td>
<td>3 (20)</td>
<td>7 (58)*</td>
<td>15 (65)*</td>
</tr>
<tr>
<td>Age mean (S.D.) years</td>
<td>78.3 (5.9)</td>
<td>71.8 (9.9)*</td>
<td>73.8 (6.9)*</td>
</tr>
<tr>
<td>Education mean (S.D.) years</td>
<td>11.6 (2.6)</td>
<td>18.0 (5.5)**</td>
<td>11.1 (3.6)</td>
</tr>
<tr>
<td>MMSE mean (S.D.) years</td>
<td>29.3 (0.9)</td>
<td>27.6 (1.9)</td>
<td>22.6 (3.7)**</td>
</tr>
<tr>
<td>CDR (Global)</td>
<td>–</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>DRS-II mean (S.D.)</td>
<td>140.3 (2.9)</td>
<td>–</td>
<td>109.1 (13.3)**</td>
</tr>
<tr>
<td>Voxel Volume mean (S.D.)</td>
<td>3.8 (0.7)</td>
<td>3.7 (0.4)</td>
<td>3.7 (0.9)</td>
</tr>
</tbody>
</table>

\* \(p < 0.05\), relative to NEC group.

\** \(p < 0.01\), relative to NEC group.
Coronal T1-weighted volumetric MRI images (3D FLASH, FOV = 22 cm, slice = 2.5 mm, TI/TR/TE = 500/510/5.3 ms) segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using SPM5.

Post hoc analyses (Tukey) showed a significant decrease in Glu (35%, $p < 0.05$) in subjects with AD compared to NEC, and a trend toward higher mI (23%, $p < 0.10$) in subjects with AD compared to subjects with MCI (Fig. 3). Post hoc tests did not reveal specific group differences in Cr.

Differences in metabolite ratios were also observed between groups. Univariate analysis of covariance showed differences in Glu/Cr, Glu/ml, and Glu/NAA between groups ($p < 0.05$), along with a trend toward a difference in NAA/Cr ($p < 0.10$). Post hoc tests (Tukey) revealed decreased NAA/Cr (20%, $p < 0.05$), Glu/Cr (40%, $p < 0.01$), Glu/ml (41%, $p < 0.01$) and Glu/NAA (36%, $p < 0.05$) in subjects with AD compared to NEC. Additionally, Glu/ml was lower (33%, $p < 0.01$) in subjects with AD compared to MCI, and there was a trend toward lower Glu/NAA ($p < 0.10$) in subjects with AD compared to MCI.

Several metabolite measures were correlated with MMSE scores. In the NEC group, NAA/Cr was inversely correlated ($r = -0.76, p < 0.01$) with MMSE. In the MCI group, NAA ($r = 0.64, p < 0.05$), and NAA/Cr ($r = 0.61, p < 0.05$) were positively correlated with MMSE. Finally, in the AD group NAA/Cr was positively correlated with MMSE ($r = 0.46, p < 0.05$).

The ROC curves generated for the MMSE scores as well as the metabolite indicators NAA/Cr, Glu, and NAA/Cr + Glu + mI are shown in Fig. 4. The total area under the curve was 0.98 for the MMSE, 0.71 for NAA/Cr, 0.80 for Glu, and 0.94 for NAA/Cr + Glu + mI.
for Glu, and 0.94 for the combination of NAA/Cr + Glu + mI. The combination of NAA/Cr + Glu + mI gave a significantly higher area under the curve than NAA/Cr alone ($p < 0.05$).

4. Discussion

This study utilized high magnetic field (4 T) short echo-time magnetic resonance spectroscopy to compare metabolite levels in the right hippocampus of subjects with MCI, subjects with AD, and NEC. As expected, decreased levels of NAA/Cr were found in subjects with AD compared to NEC. Additionally, absolute Glu and Glu/Cr levels were reduced in subjects with AD versus NEC. Subjects with MCI showed NAA/Cr and Glu levels that were intermediate to NEC and subjects with AD. There were no changes detected in the absolute levels of Cho in subjects with MCI or AD compared to NEC.

Previous spectroscopy studies comparing subjects with MCI and AD to NEC have found metabolite alterations in the posterior cingulate (Ernst et al., 1997; Kantarci et al., 2000), occipital cortex (Miller et al., 1993; Moats et al., 1994) and the hippocampus (Dixon et al., 2002; Schuff et al., 1997). The hippocampus is generally considered a difficult region to study, due to its proximity to bone and the sinus cavity (Kantarci et al., 2007), however a locally developed protocol has produced consistent results (Kassem and Bartha, 2003). Performing the current study at high magnetic field (4 T), increased the signal-to-noise ratio and spectral resolution compared to lower field and resulted in the reliable quantification of five metabolites, including Glu. The use of high field also enabled relatively small voxels (3.7 ± 0.8 cm$^3$) to be placed around the hippocampus, minimizing the contribution from the surrounding WM and CSF regions. Other studies looking at the effect of magnetic field strength on MRS have generally found that the use of higher magnetic fields improves metabolite measurements (Bartha et al., 2000a; Tkac et al., 2001) due to greater signal-to-noise and spectral dispersion, although one previous study performed without higher order shimming showed no significant benefit of MR spectroscopy at 3 T compared to 1.5 T (Kantarci et al., 2003).

The voxel position within the brain (Fig. 2) did overlap with the parahippocampal gyrus, the temporal horn of the lateral ventricles and the lower portion of the deep white matter (WM) tracts. Interestingly, less WM was found within the VOI of subjects with AD compared to NEC in the current study. Demyelination and the resultant loss of white matter tracts in the hippocampus in AD (Rose et al., 2008) may lead to misclassification of WM as GM in the segmentation, leading to an artificially decreased WM and elevated GM in the subjects with AD. Also, greater average CSF fraction was observed in the voxels of the MCI and AD groups compared to NEC. These results underscore the importance of correcting the measured water concentration for tissue fraction as done in the current study when comparing absolute tissue metabolite levels between groups.

Subjects with AD had lower levels of NAA/Cr in the hippocampus, consistent with previous studies using long echo-time (TE = 135 ms) MR spectroscopic imaging (Dixon et al., 2002; Schuff et al., 1997) and long echo-time (TE = 90 ms) single voxel spectroscopy (Miller et al., 1993). Although previous studies have shown decreased NAA/Cr levels in subjects with MCI compared to NEC (Ackl et al., 2005; Kantarci et al., 2007), decreased NAA/Cr was not found in the hippocampus of subjects with MCI in the current study, possibly due to the small sample size. Decreased NAA or NAA/Cr levels have typically been attributed to a decrease in neuronal density or neuronal function within the VOI, since NAA is almost entirely found within neurons in the CNS. Decreased neuronal density may occur due to neuronal death or increased tissue volume. The link between measures of NAA and neuronal function is further supported by the correlation of NAA and NAA/Cr with MMSE score in the MCI group, and NAA/Cr with MMSE score in the AD group. These correlations are consistent with previous studies (Jessen et al., 2001) and suggest subjects with declining cognitive function have reduced levels of NAA. NAA/Cr levels were inversely correlated with MMSE score in the NEC group, however, this result is difficult to interpret as the range of MMSE scores in the NEC group was very small (28–30).

More interestingly, when compared to NEC, the Glu level detected in the hippocampus was significantly lower in subjects with AD. The ratios of Glu/Cr and Glu/NAA were also significantly lower in subjects with AD compared to NEC. The metabolite resonances of Glu, glutamine (Gln) and GABA all closely overlap in the spectral domain. A systematic fitting bias could unintentionally create the decreased Glu result. However, a post hoc analysis found no significant differences in Gln or GABA between groups. Additionally, Glu + Gln and Glu + Gln + GABA all tended to be lower in subjects with AD compared to NEC, supporting the hypothesis that Glu is different between groups. Previous MRS studies of AD at 1.5 T have reported no change in the sum of Glu and Gln (abbreviated Glx) in the left temporoparietal or midfrontal GM (Ernst et al., 1997), while others found decreases in Glx in the occipital cortex (Moats et al., 1994) and cingulate region (Antuono et al., 2001). One previous study reported a decrease in the level of (Glu + Gln)/Cr in subjects with AD at 3 T (Hattori et al., 2002), which is also consistent with post-mortem results in AD (Sasaki et al., 1986). Glu is involved in several metabolic pathways, including its role as one of the primary excitatory neurotransmitters. The decrease in Glu levels observed in the current study may reflect a loss of glutamatergic neurons or decreased Glu activity (Lin et al., 2003) consistent with the reduced glucose metabolism observed in subjects with AD. A histologic study also found an 80% decrease in the levels of free Glu in the hippocampal formation of subjects with AD compared to NEC (Hyman et al., 1987). There was no correlation between Glu or Glu/Cr and MMSE scores in the current study consistent with Hattori et al. (2002), but in contrast to Antuono et
al. (2001), although the latter study contained a much larger range of MMSE scores than the current study.

A trend was detected toward increased mI in subjects with AD compared to NEC, consistent with earlier studies showing increased mI in subjects with AD (Miller et al., 1993). In addition, the Glu/mI ratio was significantly lower in subjects with AD compared to NEC and compared to subjects with MCI. The precise physiological role of mI is not clearly defined, as mI may act as an intracellular messenger, an osmoregulator, or a marker of glial cell numbers (Brand et al., 1993; Valenzuela and Sachdev, 2001). While many groups have noted elevated mI levels, one of the few MRS studies at a higher field strength (3 T) did not find increased mI/Cr levels in subjects with AD compared to NEC (Hattori et al., 2002), although this study was likely underpowered with only 9 subjects with AD. These differences in mI results may be influenced by variability in the length of illness and/or medication between cohorts (Bartha et al., 2008).

Several MRS studies (Ackl et al., 2005; Frederick et al., 2004) have found NAA/Cr and NAA/mI to be the most robust metabolite ratios for discriminating subjects with AD from NEC. However, the ROC curve areas shown in Fig. 4 demonstrate that Glu increases the area under the curve and the inclusion of Glu and mI improves the ability to discriminate between AD and NEC groups when compared to NAA/Cr alone. The Glu effects may be more pronounced in AD than NAA/Cr alone due to the destruction of synapses with progressive Alzheimer pathology, particularly glutamatergic synapses (Bell et al., 2007; Shankar et al., 2007). Although the MMSE score showed the greatest sensitivity and specificity in the separation of subjects with AD from NEC (Fig. 4), this result was expected as the score was incorporated into the diagnosis of AD in the current study. Metabolite level measures may find greater utility in early disease detection, when the MMSE score has less sensitivity, and in monitoring of disease progression and treatment response.

4.1. Limitations

This study only investigated changes in the hippocampus, and hence cannot comment on regional changes in metabolite levels. The right hippocampus was chosen due to its known involvement in AD. The rate of hippocampal atrophy in subjects with AD has been reported by several groups to show asymmetry when compared to age-matched NEC and longitudinally over time. One study by Barnes et al. (2005) found the atrophy rate of the right hippocampus to be just significantly greater than the left (p = 0.05). Other regions of the brain may show more pronounced changes in some metabolites. For example, the detection of elevated mI/Cr and mI/NAA ratios were found by another group to be significant in posterior cingulate but not in the hippocampus when comparing AD to NEC (Ackl et al., 2005).

The NEC group was significantly older than the other groups. Therefore, the comparison of metabolite levels to this group can be considered conservative since many of the metabolite alterations that occur in Alzheimer disease processes including reductions in Glu are also known to occur with normal aging (Zahr et al., 2008). The advantage of having an older NEC group is that it is more likely that the subjects included in the group are actually healthy controls and not latent subjects with AD. There were also fewer males in the NEC group compared to the MCI and AD group. However, a previous study also performed at 4 T demonstrated no gender based metabolite level differences in the corona radiata and mesial motor cortex (Kaiser et al., 2005). In addition, the statistical analyses in the current study, which incorporated both age and gender as covariates, indicated that neither contributed significantly to the model. Therefore, age and gender differences between groups do not explain the observed metabolite level differences.

The quantification of metabolite concentrations including Glu assumed that the T1 and T2 relaxation time constants of these molecules did not change as a function of disease. It is not possible to measure these time constants for each metabolite in each subject due to time limitations. However, to explain the observed 35% decrease in glutamate in subjects with AD by changes in relaxation would require either a substantial decrease in T2 (from 335 to ~81 ms), or a substantial increase in T1 (from 1.5 to ~3.2 s). Therefore, although we cannot exclude changes in relaxation as contributing factors to the observed metabolite signal changes it is unlikely that the observed decrease in Glu is due only to changes in relaxation.

5. Conclusion

This cross-sectional analysis of absolute metabolite levels and metabolite ratios in the hippocampus of subjects with AD has shown a distinct neurochemical profile that includes decreased Glu, NAA/Cr, Glu/Cr, Glu/mL, and Glu/NAA compared to normal elderly as well as decreased Glu/mL in AD compared to MCI. The MCI metabolite levels and metabolite ratios were generally intermediate to NEC and AD values, supporting the hypothesis of a pathological continuum.

Disclosure

The authors have no actual or potential conflicts of interest and the contents of this manuscript have not been previously published. The authors and their respective institutions have no financial interests conflicting with this work. This study was approved by the University of Western Ontario Health Sciences Research Ethics Board. Informed consent was acquired according to the Declaration of Helsinki.

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