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Brain Research Bulletin 66 (2005) 9–16

BRAIN RESEARCH BULLETIN

www.elsevier.com/locate/brainresbull

Cognitive correlates of ^{1}H MRS measures in the healthy elderly brain

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Received 14 June 2004; received in revised form 5 November 2004; accepted 13 January 2005
Available online 23 March 2005

Abstract

Ageing is associated with cognitive decline, with some studies indicating that this decline can be mostly accounted for by slowing of information processing speed. Whilst it is likely that this is associated with age-related changes in fronto-subcortical neuronal circuits, such changes are not visible on routine neuroimaging. We examined the integrity of this brain region using proton magnetic resonance spectroscopy (^{1}H MRS) and hypothesised that functional changes measured by ^{1}H MRS would be associated with cognitive performance. Fifty-nine healthy elderly subjects (age 58–85 years) underwent single-voxel ^{1}H MRS in frontal white matter and occipito-parietal gray matter, and a comprehensive neuropsychological battery. The results showed a significant correlation between frontal white matter NAA/H_2O and a composite measure of neuropsychological performance representing speed of information processing, attentional function and visual memory, controlling for age and sex. This research highlights the importance of the relationship between regional brain changes and cognitive function in the ageing brain, and suggests that MRS may be a sensitive marker of subclinical change in cognition.

Keywords: Magnetic resonance spectroscopy; Cognition; *N*-Acetylaspartate; Neuropsychological tests; Ageing

1. Introduction

The neurophysiological basis of cognitive changes with normal ageing is not well understood. According to some studies, much of the age-associated decline in cognition is accounted for by slowing of processing and response speed or reduced information processing ability, such as in working memory and attentional-executive tasks [45]. Underlying these changes is possibly some disruption of fronto-subcortical circuits, but evidence for this is limited. Structural imaging studies indicate that there is loss of cortical volume, enlargement of ventricles [35] and gliosis [31] associated with ageing, but these measures have poor correlation with neuropsychological function. Synapse number and neuronal number have also been found to decline during ageing, although new unbiased stereological methods indicate that previous results are probably due to shrinkage rather than cell loss [26]. Functional imaging studies show that there are relatively slight reductions in total cerebral metabolic rate of oxygen ($CMRO_2$) and brain cerebral blood flow (CBF) with ageing [54], with decreases in rCBF possibly being more pronounced in frontal and parietal regions [23]. During cognitive tasks, ageing brains tend to have reduced activation in the frontal cortex [9], but, improved performance on the cognitive tasks is associated with recruitment of additional brain regions, indicating that there are compensatory mechanisms in operation [8,9,41,42].

One method of determining the functional integrity of brain regions is to examine their neurochemistry. Proton magnetic resonance spectroscopy (^{1}H MRS) allows the non-invasive in vivo analysis of certain neurometabolites that indicate biochemical changes in the brain. The major metabolites detected by this technique are *N*-acetylaspartate (NAA), choline-related compounds (Cho), creatine (Cr) and myo-inositol (mI). While the functional roles of these

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0361-9230/$ – see front matter.
doi:10.1016/j.brainresbull.2005.01.015

metabolites are not fully known, in simple terms NAA may be considered to be a marker of neuronal viability, Cr is involved in energy metabolism, Cho is associated with membrane turnover and mI is a putative glial cell marker [2].

A number of studies have attempted to correlate MRS-visible metabolites with cognitive function. NAA in the occipito-parietal white matter has been shown to be associated with both full-scale intelligence quotient [19] and neuropsychological measures of cognition [20] in young adults, particularly on timed tests. In a study of elderly males, a correlation was observed between NAA/Cr and Cho/Cr in the parietal white matter and various cognitive measures including logical memory and verbal memory factor, possibly accounted for by changes in Cr [18]. In a previous report [56], we observed a correlation between NAA/Cr in the left frontal subcortical white matter and attentional processing ability, which is functionally associated with this region. Verbal fluency and memory were not correlated with NAA/Cr in this area of the brain, nor were there any associations between measures in the occipito-parietal gray matter and cognition in these subjects. This suggests that changes in frontal white matter are related to particular changes in cognition that may be associated with the ageing process. In yet another study, whole brain gray matter NAA was positively correlated with performance in a verbal memory task in elderly healthy controls, but this correlation was not observed in the white matter [36].

We studied a group of 59 healthy elderly subjects in a further attempt to determine the relationship between brain neurometabolites in frontal white matter and occipito-parietal gray matter with cognitive performance. We hypothesised that NAA would be correlated with cognitive performance, particularly in the frontal white matter, and the relationship would be stronger on neuropsychological tests that are timed.

2. Methods

2.1. Subjects

The study sample comprised volunteers recruited from community organizations. They were screened for absence of stroke, cognitive impairment and psychiatric disorder on history and examination. Exclusion criteria included neurological disease known to affect cognition, medical disease that was judged to affect cognition secondarily, mental retardation (DSM-IV diagnosis), alcohol dependence, and contraindications to magnetic resonance imaging (MRI) (pacemaker, metallic foreign bodies, severe claustrophobia).

^{1}H MRS was performed in the left frontal white matter and the midline occipito-parietal gray matter in 86 subjects. The results from 20 of these subjects have been reported in a previous study [56] and are included in the present sample. Usable spectra in either of the voxels were available for 61 subjects. Two subjects were excluded from the study for having cognitive impairment leaving a sample of 59 subjects, with 46 of these subjects with data from the frontal voxel and 56 of these subjects with data from the occipito-parietal voxel. These subjects were 58–85 years old (mean age 70.8 years), with 28 females and 31 males. Reasons for not being able to use spectra included the use of a different acquisition protocol in the first 14 months of the study (data are not included) ($n = 2$), inability of the subject to complete the MR imaging session ($n = 1$), movement during acquisition ($n = 1$), poor quality spectra ($n = 6$), and technical problems with data retrieval from the scanner ($n = 15$). There were no significant differences between those subjects who had ^{1}H MRS and those that did not for age, sex, education, mini-mental state examination (MMSE), or neuropsychological test performance, indicating that there was no selection bias within the ^{1}H MRS sample.

2.2. Neuropsychological assessment

The neuropsychological battery comprised the following tests pertaining to various cognitive domains: verbal memory (Logical Memory I and II from the Wechsler memory scale, revised (WMS-R)) [59]; visual memory (Visual Reproduction I and II from WMS-R) [59]; working memory (digit span backwards, arithmetic from Wechsler adult intelligence scale revised (WAIS-R)) [58]; attention/concentration (digit span forwards (WAIS-R) [58], mental control (WMS-R) [59]); language (15-item Boston Naming Test [27]); information processing speed (Trail Making Test Part A [38] and Symbol Digit Modalities Test [50]); visuoconstruction (Block design (WAIS-R) [58] and copying simple figures); praxis-gnosis (Western Aphasia Battery (WAB) ideomotor apraxia subtest items [22], finger gnosis and stereognosis [6,51]); abstract reasoning (similarities (WAIS-R) [58]); mental flexibility (Colour Form Sorting Test [60] and Trail Making Test Part B [38]); verbal fluency (Controlled Oral Word Association Test [5] and animal naming [30]). Mental flexibility and verbal fluency were together characterised as ‘executive function’. Trained psychologists performed assessments. Subjects were given breaks where appropriate to minimize the effects of fatigue on performance.

2.3. MRI/^{1}H MRS protocol

MRI and ^{1}H MRS were performed on a 1.5 T Signa scanner (GE Medical Systems, Milwaukee, WI, USA). The MRI protocol included a scout mid-sagittal cut for AC–PC plane alignment (2D, repetition time TR 300 ms, echo time TE 14 ms; thickness 5 mm, excitations 1), a 1.5 mm thick T1-weighted contiguous sections through whole brain using a fast spoiled gradient recall (FSPGR) sequence and 3D acquisition (TR 14.3 ms, TE 5.4 ms), and 4 mm thick T2-weighted fluid attenuation inversion recovery (FLAIR) sequence for contiguous coronal slices through the whole brain (TR 8900, TE 145, TI 2200, field of view 25°, 256 × 192). ^{1}H MRS was performed in the left frontal white matter (single voxel 2 cm × 2 cm × 2 cm) and occipito-parietal gray matter (sin-

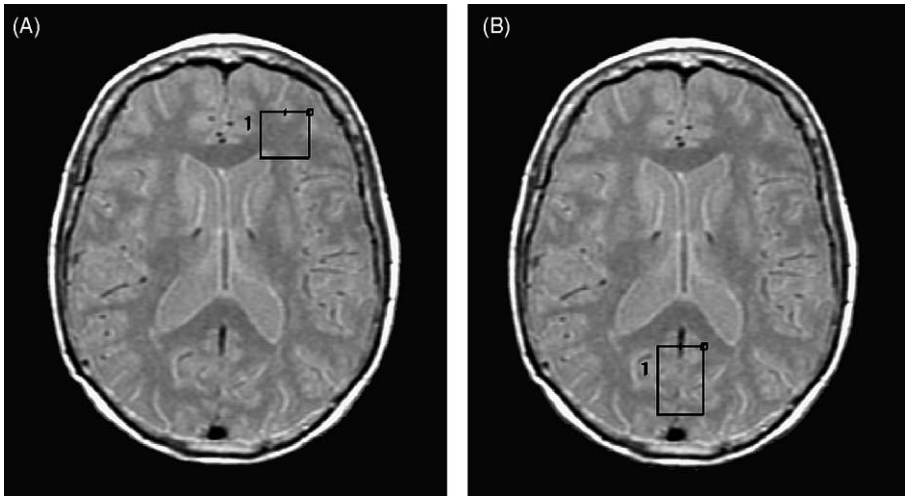

Fig. 1. Localisation of voxels in the frontal white matter and the occipito-parietal gray matter.

gle voxel 2 cm × 2.7 cm × 2 cm). The voxels were localized using the axial plane, with the frontal voxel positioned anterior to the frontal horn of the left lateral ventricle maximizing white matter content, and the occipito-parietal voxel in the posterior midline, anterior to the visual cortex (Fig. 1). Automated shimming was performed before acquisition using the stimulated echo acquisition mode (STEAM) sequence with a 30 ms echo time, 1500 ms repetition time, 13.7 ms mixing time. The number of data acquisitions was 256, averaged across 2048 data points. Typical line-widths for the frontal voxel were 4–10 Hz and for the occipito-parietal voxel were 2.5–6 Hz. Four subjects in the frontal voxel and no subjects in the occipito-parietal voxel had spectral water line-widths greater than 8 Hz and these were excluded from the analysis. The line-width was the full-width of the peak at half height. Fig. 2 shows a representative spectrum from the occipito-parietal voxel.

2.4. MRI/^{1}H MRS analysis

The spectra from the ^{1}H MRS were analyzed using MRUI-99x software [57] on MATLAB 5.3, with the user blind to subject group. Zero-filling of the 2048 acquisition points was performed, with automatic correcting for zero-phasing. The residual water peak was removed using time-domain Hankel Lanczos singular value decomposition (SVD) filtering. Time-domain fitting was then carried out for all peaks using advanced method for accurate, robust and efficient spectral (AMARES) fitting to measure the area under the curve, with input of prior knowledge. The area of the water peak was determined separately from the unsuppressed water scan using SVD. Noise was taken from the last 100 data points. The water peak and the Cr peak were used as internal standards in this study to avoid misinterpreting changes when using Cr as the internal standard, since many ageing studies have observed changes in Cr in various regions of the brain [12,25,37,48].

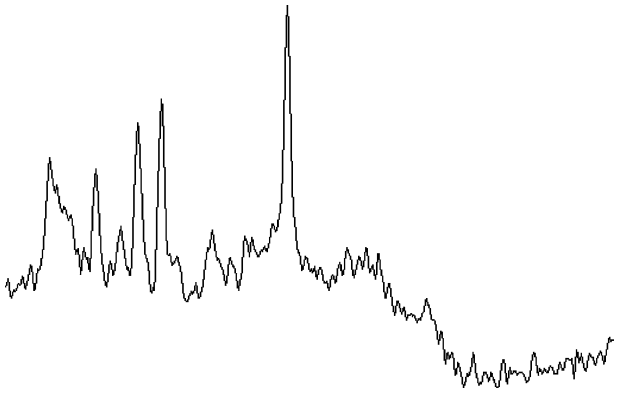

Fig. 2. A representative spectrum from the occipito-parietal voxel.

Structural MRI data were analyzed with ANALYZE® software (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN, USA), by a rater blind to subject group. To correct for partial volume effects within the voxels, the proportion of cerebrospinal fluid (CSF) within the voxel was estimated using an automatic segmentation algorithm developed in-house, with manual input of the threshold for brain tissue, determined individually from T1-weighted structural images. In this program, pixels from within the voxel with an intensity level greater than the level determined by the rater were defined as brain tissue, and those below were defined as CSF. Isolated pixels below the threshold did not contribute to the proportion of brain tissue. The proportion of CSF was multiplied by the unsuppressed water value for each voxel to provide the corrected water value, and then ratio values for NAA, Cr, Cho and mI were calculated with respect to the corrected water value for each voxel.

2.5. Reliability analysis

Reliability analysis was performed in five subjects for the frontal voxel, and six subjects for the occipito-parietal voxel. These subjects were scanned twice on the same day.

Patients were removed from the scanner between scans, and voxel placement was carefully located to the same area. In the frontal voxel, the coefficients of variance were 12.0% for NAA/water, 13.9% for Cr/water, 12.1% for Cho/water and 24.9% for mI/water. In the occipito-parietal voxel the coefficients of variance were 7.1% for NAA/water, 3.9% for Cr/water, 8.3% for Cho/water and 27.5% for mI/water.

2.6. Analysis of cognitive tests

To determine the individual's performance on a test, raw scores were used to create *z*-scores for each test for an individual. Cognitive domain *z*-scores were calculated by combining *z*-scores for all tests assigned to a particular cognitive domain. In a further effort to reduce the number of variables, a principal components analysis (PCA), with varimax rotation, was performed on the *z*-scores of the various tests that had scalar data. The PCA was performed on a larger sample of 100 healthy ageing subjects [43].

2.7. Data analysis

Statistical analysis was performed using SPSS 11.0 for Windows (SPSS Inc., 2001). Exploratory analysis was performed by correlating neuropsychological test domain *z*-scores with the neurometabolite ratios to Cr and H_2O, using partial correlation coefficients, controlling for age and education. Since the correlations were similar for neurometabolite ratios to Cr and H_2O, only the H_2O ratio values were used in further analysis. Stepwise hierarchical linear regression was then performed to assess potential predictors of the first principle component (PC1), with education and age entered as covariates in the first step, and the neurometabolite ratios to water added in the subsequent steps as the independent predictors. This was performed separately for the frontal and occipito-parietal voxels.

3. Results

3.1. The sample

The subjects were 58–85 years old (mean age 70.8 years), with 28 females and 31 males. The range of score on the MMSE score was 24–30 (mean = 28.7), and the average number of years of education was 12 years. Of these subjects, 43.3% were hypertensive and on anti-hypertensive medication and 8.3% were diabetic. A history of myocardial infarction was observed in 5%, hypercholesteremia in 28.3%, alcohol abuse in 8.3% and smoking in 41.7%.

3.2. Neuropsychological tests

Principal components analysis resulted in a four-component solution accounting for 66% of the variance. PC1 was the only component that correlated with neurometabolite ratios. PC1 accounted for 36% of the total variance and was highly loaded with measures of speed of information processing (Trail Making Test A and Symbol Digit Modalities), visuoconstruction (Block design), mental flexibility (Trail Making Test Part B), and visual memory (Visual Reproduction I and II) (Table 1).

Table 1
First principle component structure matrix

Neuropsychological test	Structure matrix coefficient
Block design	0.788
Visual Reproduction I	0.784
Trail Making Test Part A	−0.755
Symbol Digit Modalities Test	0.739
Visual Reproduction II	0.676
Trail Making Test Part B	−0.611
Arithmetic	0.418
Animal naming	0.263
Similarities	0.257
Digit span	0.206
Boston Naming Test	0.187
Logical Memory II	0.186
Mental control	−0.131
Controlled Oral Word Association Test	0.118
Logical Memory I	0.061

3.3. Neuroimaging variables

The neurometabolite ratios and in each voxel are presented in Table 2. There were no significant differences between males and females for any of the neurometabolite ratios. Age was correlated positively with occipito-parietal NAA/H_2O ($r = 0.321$, $p = 0.016$) and Cr/H_2O ($r = 0.285$, $p = 0.033$), but no other neurometabolites.

3.4. Relationship between neuroimaging variables and neuropsychological tests

Age correlated significantly with the cognitive domains of visual memory, executive function, visuoconstruction function and speed of information processing. In the frontal voxel,

Table 2
Mean and standard deviation of ^{1}H MRS results

Voxel	Metabolite	Mean (males)	S.D.	Mean (females)	S.D.
Frontal	*n*	26		20	
	NAA/H_2O	2.05	0.50	1.95	0.44
	Cr/H_2O	1.58	0.37	1.54	0.37
	Cho/H_2O	1.36	0.34	1.27	0.27
	mI/H_2O	0.97	0.27	0.95	0.29
Occipito-parietal	*n*	28		28	
	NAA/H_2O	1.20	0.10	1.22	0.10
	Cr/H_2O	0.94	0.11	0.95	0.07
	Cho/H_2O	0.58	0.10	0.56	0.10
	mI/H_2O	0.55	0.10	0.53	0.08

^{1}H MRS, proton magnetic resonance spectroscopy; S.D., standard deviation; NAA, *N*-acetyl aspartate; H_2O, water; Cr, creatine + phosphocreatine; Cho, choline; mI, myo-inositol; *n*, number of subjects.

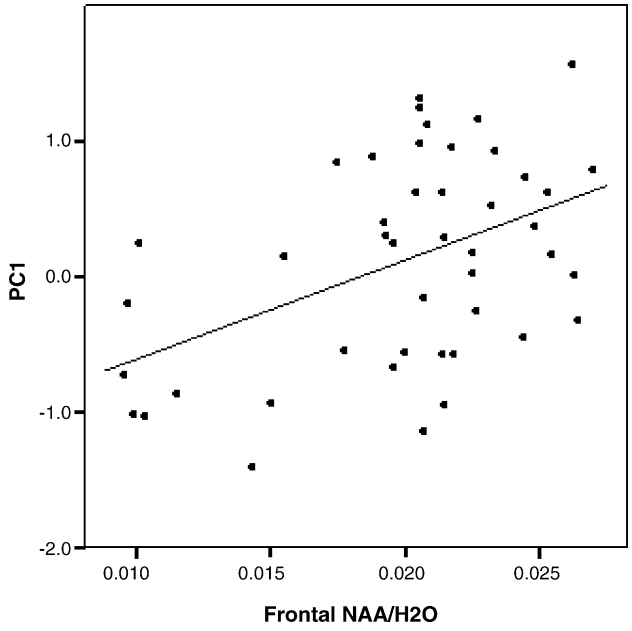

Fig. 3. Correlation between PC1 and frontal white matter NAA/H_2O ($r = 0.316$).

NAA/Cr and NAA/H_2O correlated with speed of information processing ($r = 0.300$, $p = 0.048$, $r = 0.337$, $p = 0.025$, respectively), and Cho/H_2O was correlated with abstraction ($r = -0.347$, $p = 0.032$), controlling for age and education. In the occipito-parietal voxel, NAA/Cr was correlated with working memory ($r = 0.272$, $p = 0.047$) and Cho/Cr and Cho/H_2O with speed of processing ($r = -0.269$, $p = 0.049$, $r = -0.276$, $p = 0.043$, respectively), controlling for age and education. mI/Cr and mI/H_2O were correlated with executive function ($r = -0.329$, $p = 0.15$, $r = -0.325$, $p = 0.017$, respectively), and mI/H_2O was correlated with speed of information processing ($r = -0.280$, $p = 0.040$). These relationships suggested that NAA was lower and Cho and mI were higher in subjects with reduced function in these domains. These correlations are exploratory in nature and were not significant after correction for multiple comparisons.

PC1 correlated with frontal NAA/H_2O ($r = 0.316$, $p = 0.032$) (Fig. 3). The ability of neurometabolites to independently predict PC1 was tested using stepwise hierarchical linear regression, controlling for age and education. The r^2 change values for the frontal voxel indicated that NAA/H_2O and Cr/H_2O together accounted for an additional 16.1% (NAA/H_2O, 6.5%; Cr/H_2O, 9.6%) of the variance in PC1 (r^2 change = 0.161, $p = 0.010$). Neurometabolites from the occipito-parietal voxel did not predict PC1.

4. Discussion

In this study, we report a significant association between neurometabolites visualised on proton MRS and certain cognitive functions in a sample of elderly individuals. The levels of NAA/H_2O in the left frontal white matter were significantly related to the PC1, a measure of cognitive performance, suggesting an overall relationship with cognitive function. Frontal NAA/H_2O and Cr/H_2O independently predicted 16% of the variance in PC1 after controlling for age and education. The levels of metabolites in the occipito-parietal voxel were not so related. When the individual cognitive domains were examined, interesting findings emerged. The frontal NAA/H_2O level was associated with a composite score of speed of information processing. In the occipito-parietal voxel, Cho/H_2O levels correlated with speed of information processing, and mI/H_2O levels correlated with executive function and speed of information processing. These relationships were not significant after correction for the multiple correlations, and are therefore interpreted with caution.

NAA is an amino acid found only in neurons in the adult central nervous system [49,55], and is thought to be a marker of neuronal viability [14,40]. Synthesis of *N*-acetylaspartate (NAA) from acetyl-CoA and aspartate occurs in the mitochondria and has been shown to be almost directly coupled to mitochondrial activity in ATP production and oxygen consumption [4], suggesting an association between NAA concentration and metabolic efficiency [52]. NAA may also be involved in myelin synthesis and/or maintenance of myelin [11], and this is supported by the extremely poor myelination observed in Canavan's disease, which is an inherited disorder of NAA metabolism [29]. The relationship between NAA and cognitive function has been observed in many disorders with associated cognitive dysfunction as well as in normal subjects (reviewed in [39]), with possible mechanisms for reduced NAA levels being: (1) neuronal death; (2) decreased neuronal metabolism; (3) reductions in the area of dendritic arborizations; and (4) reduced myelination.

Previous studies have reported associations between spectroscopic and cognitive measures. NAA in the occipito-parietal white matter has been associated with both intellectual [19] and neuropsychological [20] measures of cognition, particularly on timed tests, in healthy young subjects. In elderly subjects, one large study in males observed positive correlations for parietal white matter NAA/Cr and Cho/Cr and negative correlations for adjusted Cr with various cognitive measures including logical memory and verbal memory factor [18]. The authors concluded that these correlations were probably due to variations in Cr rather than NAA or Cho [18]. This study did not examine speed of processing, however, and the relationship between Cr and verbal memory that they observed has not been replicated in our study. Differences in size and location of the voxel being examined may contribute to this, with the former study applying MRS to a significantly larger area of tissue in the parietal lobe, including some gray matter. A relationship between NAA/Cr and performance on spatial and non-spatial hippocampus-dependent tasks was reported in the hippocampus and the frontal white matter in combined young and elderly subjects ($n = 32$) [17]. In yet another study, whole brain gray matter NAA was positively correlated with perfor-

mance in a verbal memory task in elderly healthy controls, but this correlation was not observed in the white matter [36].

We observed a relationship between frontal NAA/H_2O and a composite measure of neuropsychological function. The tests with high loadings on this component included many timed tests, and tests that use executive function and visual memory. Higher levels of NAA/H_2O were associated with better performance on these tasks. The relationship with speed of information processing was particularly notable, albeit the significance was lost after correcting for multiple comparisons. The theoretical importance of this association suggests that it should be studied in a larger sample. Since speed of information processing is regarded as a fronto-subcortical function, we may be detecting subclinical abnormalities in this pathway. NAA may reflect neuronal or dendritic loss, reduced mitochodrial function in neurons, or reduced myelin. We found no relationship in the frontal voxel between Cho/H_2O and cognitive function, and therefore consider the former two explanations as more likely, since Cho is a marker of membrane turnover.

Old age is also likely to be important in this relationship. Age was correlated with speed of information processing, executive function and verbal memory, which are cognitive functions generally thought to be more selectively affected in normal ageing [44]. This creates greater inter-individual variability in the population, and therefore the relationships are more likely to be detectable in elderly samples. Correlations between Cho/H_2O levels and speed of information processing, and between mI/H_2O levels and executive function and speed of information processing were also observed in the occipito-parietal gray matter. Again, these results were not highly significant, and may be the result of a type I error.

Increasing age was also associated with increased occipito-parietal NAA/H_2O and Cr/H_2O. This may arise due to decreases in brain water [12], however, evidence suggests that brain water is relatively constant in old age [7,13]. A large cross-sectional study of ageing also observed an increase in NAA and Cr in posterior regions only [47]. The increase in NAA may be explained by an increase in neuronal density due to an age-associated loss of neuropil [53]. The reported effects of ageing on neurometabolite levels are variable [34,46,48], therefore we controlled for this variable in the current study.

There are a number of limitations to our study. Firstly, many of our subjects who underwent structural MRI did not have usable MRS spectra. This was related to technical and patient-related factors, as mentioned above. Even though this exclusion was not systematic, to determine the possibility of bias we compared the subjects included in the study with those excluded, and did not find differences between the two groups on age, sex, or neuropsychological test performance. Secondly, technical difficulties must be taken into consideration. The ratios obtained from the frontal voxel showed much greater variability than the occipito-parietal voxel. The quality of the data obtained from the frontal voxel was also poorer, as indicated by the subjects with line-widths greater than 8 Hz. Shimming may be difficult in the frontal area due to susceptibility differences of different tissues, resulting in broader linewidths. This also meant that the sample size in the occipito-parietal voxel was larger than for the frontal voxel. The coefficients of variance that we measured are in line with or better, than other single-voxel studies, in different regions of the brain, and using different methods of acquisition [15,24,28,32,49]. Thirdly, this is a cross-sectional study, which does not necessarily provide insights into the mechanisms by which neurometabolic change may affect cognitive function. While our sample was elderly, there was no significant correlation between the metabolites and age. A younger comparison group may have indicated if the observed associations are only seen in older brains. If this is the case, then these metabolites may serve as markers of decline.

The clinical utility of the findings of this study should be established through studies of populations with mild cognitive impairment (MCI). There have been a few studies investigating MCI that have have observed significantly decreased levels of NAA in white matter [10] and the temporo-parietal area [3,33], compared to controls. NAA/Cr correlated with verbal memory in the temporal lobe of MCI patients, but not in the basal ganglia [33], consistent with localized function of verbal memory to the temporal lobes. mI/Cr may also be increased pre-dementia [21]. The predictive value of NAA for cognitive outcome at one year has also been observed [1,16] indicating a possible prognostic role for ^{1}H MRS in cognitive decline in the elderly.

Acknowledgments

The authors are grateful to Eveline Milne, Annette Koschera, and Lisa Lorentz for their contributions, and to Dr. Ron Shnier and the St. George MRI unit. This work was supported by a grant from the National Health and Medical Research Council of Australia, a scholarship from Pfizer Inc., and the Brain and Ageing Program of UNSW.

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