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Dual Voxel Proton Magnetic Resonance Spectroscopy in the Healthy Elderly: Subcortical-Frontal Axonal *N*-Acetylaspartate Levels Are Correlated with Fluid Cognitive Abilities Independent of Structural Brain Changes

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The published literature suggests that degeneration of the subcorticofrontal networks may underlie cognitive ageing, but appropriate methods to examine this *in vivo* have been lacking. Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) has now been used in a number of clinical studies to assess cerebral pathophysicochemistry and recently has been utilized to examine the relationship between neurochemical markers and cognitive functioning in normal individuals. Results have been somewhat conflicting and difficult to interpret. To further clarify the role of the cognitive spectroscopy technique, we measured *N*-acetylaspartate (NAA) levels in the frontal subcortical white matter and the occipitoparietal grey matter and correlated them with performance in different cognitive domains in a group of twenty healthy elderly individuals. Subjects underwent whole brain T_1- and T_2-weighted magnetic resonance imaging (MRI), dual voxel short echotime ^{1}H-MRS, and a comprehensive neuropsychological assessment. Individual tests of executive and attentional abilities, and a principal components composite score reflecting these skills, but not measures of memory or verbal abilities, were correlated with NAA concentration in the frontal white matter only. These relationships were independent of other neurocognitive predictors of executive impairment such as age, midventricular dilation, frontal white matter disease, and presenescent verbal proficiency. This study suggests the ability of ^{1}H-MRS to differentiate anatomically distinct neurochemical markers related to specific cognitive abilities. In particular, neurometabolic fitness of the frontal subcortical-cortical axonal fibers may be important in mediating fluid intellectual processing. Longitudinal MRS studies are required to determine if the present results reflect different rates of neurocellular degeneration or preexisting individual differences in neuronal density. © 2000 Academic Press

INTRODUCTION

Decline in the fluid intellectual abilities, such as problem solving, attentional switching, conceptual abstraction, working memory and so forth, is a well established and important feature of the ageing process (Baltes *et al.*, 1999; Salthouse, 1985). While the neurofunctional basis of age-related changes in memory has been investigated (Gabrielli, 1998; Schacter, 1997), the neuronal correlates of the executive abilities is less well understood (Smith and Jonides, 1999; Roberts *et al.*, 1998), and research on how these systems change during the human life-span is in its preliminary stages.

Earlier structural–functional studies in this field yielded conflicting results. Attempts to relate whole brain size and regional brain volumes with fluid intelligence, after considering the effects of age, head size, and sex differences, have been inconclusive (Bigler *et al.*, 1995; Wickett *et al.*, 1994; Andreasen *et al.*, 1993; Raz *et al.*, 1993). Whereas lateral ventricular enlargement (Forstl *et al.*, 1996,1995), cortical volume (Obara *et al.*, 1994) and temporal lobe sclerosis (Bobinski *et al.*, 1999; De Leon *et al.*, 1996) have been associated with cognitive decline in Alzheimer's disease, studies are yet to demonstrate a relationship between structural brain changes and normal age-associated cognitive decline after controlling for confounding variables such as head size. Structural explanations for cognitive ageing are also being reevaluated as a result of advances in stereological brain-cell counting research, with a re-

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cent review concluding that neuronal numbers are retained across the human lifespan (Long *et al.*, 1999).

The association between cognition and abnormalities seen on MRI in the elderly have recently been of much interest, with the focus being on white matter lesions (WMLs or leukoaraiosis). At least one-third of elderly patients have deep white matter abnormalities on T_2-weighted MRI (Hunt *et al.*, 1989; Ylikoski *et al.*, 1995), most often in the frontal lobes (Pantoni and Garcia, 1997). Clinicopathological studies have found that these abnormal signals typically coincide with a mixed pattern of lacaunar infarction, demyelination without inflammation, and marked arteriosclerosis (Pantoni and Garcia, 1997). Questions about the over-sensitivity of WMLs seen on MRI (Lopez *et al.*, 1995; Fein *et al.*, 1990) were addressed quantitatively by Boone *et al.* (1992) who showed in otherwise healthy elderly subjects that only those with large WMLs (>10 cm^2 on axial section) demonstrated disturbances in basic attention and frontal lobe skills. Leukoaraiosis has furthermore been found to correlate with slower speed of information processing (Breteler *et al.*, 1994; Schmidt *et al.*, 1993; Ylikoski *et al.*, 1993) and impaired performance in a procedural learning task (Libon *et al.*, 1998). It can therefore be argued that WMLs represent an age-related process that may underlie cognitive decline with age.

Selective disturbance of the frontal lobe during the aging process has been demonstrated more recently by cerebral metabolism studies (Blesa *et al.*, 1997; Petit-Taboue *et al.*, 1998), which have found distinct frontal hypometabolic patterns and blood flow deficits in normal older subjects. Garraux *et al.* (1999) have specifically suggested the development of functional disconnectivity between resting glucose uptake levels in the frontal neocortical and subcortical regions in the healthy elderly.

The above evidence implicates subcorticofrontal networks in the age-related decline of executive and attentional abilities. Until recently, direct methods for assessing the biochemical integrity of the subcorticofrontal white matter tracts *in vivo* were unavailable. Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) has now emerged as a readily accessible tool for the quantification of regional cerebral biochemistry, with reliable detection of metabolite differences in the order of one to two millimoles (Brooks *et al.*, 1999). *N*-Acetylaspartate (NAA), the mobile choline compounds (Cho), myo-Inositol (mI), creatine and phosphocreatine (Cr), glutamate and glutamine (Glx), lactate, the mobile lipids and physiological water can be all measured. Quantification of NAA has been of particular interest, as it is believed to reflect neural density and viability (Ross *et al.*, 1997; Tsai and Coyle, 1995). The application of MRS to the study of neurological and psychiatric disorders has quickly expanded (for reviews see Rudkin and Arnold, 1999; Sanacora *et al.*, 1999; Kegeles *et al.*, 1998; Frangou and Williams, 1996).

The use of MRS as a tool in cognitive neuroscience has recently been advanced. Using *Phosphorous* MRS, Rae *et al.* (1996) demonstrated a moderate association between temporoparietal intracellular pH and verbal intelligence during development ($r = 0.56$), a finding that was not replicated in mature age epileptic patients (Anderson *et al.*, 1998). Rae *et al.* (1998), in a ^{1}H-MRS study of the cerebellar cortex, found a strong correlation between NAA levels and generalized IQ ($r = 0.72$), but control and clinical groups were pooled making clear interpretation of findings difficult. Foong *et al.* (1999) failed to find any association between frontal white matter ^{1}H-MRS metabolite levels and individual executive cognitive tests in either a multiple sclerosis group or middle-aged control group, while Volz *et al.* (1998) found an inverse relationship in control subjects between phosphorous metabolite values and mental flexibility as assessed by the Wisconsin Card Sort Test. Most recently, Jung *et al.* (1999, 2000) has reported and replicated a correlation between occipitoparietal white matter NAA levels and IQ in young adults ($r = 0.52$).

The diversity of MRS protocols, neuroanatomical locations chosen for investigation, neuropsychological tests employed, and subjects in these studies makes a clear assessment of the value of cognitive spectroscopy difficult. Researchers have also not tested for the possible independence of the metabolic covariance from other known predictors of cognition. Furthermore, plausible explanations connecting *local* neurometabolic changes in small areas of the brain to *global* cognitive phenomena have been limited. We believe that for cognitive spectroscopy applications to succeed, multiple regions of interest should be used so as to test the spatial specificity of the findings and attempts should be made to relate biochemical variation in these brain areas to the cognitive structures the putative circuits support.

The aim of this study was to test whether neurochemical changes in the subcorticofrontal white matter in the healthy elderly are directly related to executive cognitive function. We hypothesized that the neural viability marker NAA would covary with cognitive performance, with executive skills showing the strongest relationship. To determine the specificity of this relationship, we chose a volume of interest (VOI) in a brain region not considered to participate in executive function as a control. We further examined whether frontal spectroscopy would predict performance on executive tests after other known determinants, such as age, frontal leukoaraiosis, central atrophy, and verbal ability (Wechsler, 1981), had been accounted for. In this way, we hoped to clarify the interrelationship between ageing, cell loss, white matter lesions, and neurometa-

bolic change in the frontal lobe and age-associated cognitive decline.

MATERIALS AND METHODS

Subjects

Twenty healthy elderly volunteers (11 females, 59–85 years of age, mean 72 years, 2 left handed, median 12 years education) were recruited from community groups. Subjects were excluded if they had an obvious medical or neurological condition known to affect cognition, such as: previous stroke or transient ischaemic attack, Alzheimer's disease, Parkinson's disease, Multiple Sclerosis, learning disability, severe head injury, brain tumor, or alcohol dependency. Written informed consent and institutional ethics approval were obtained before the study. Subjects were interviewed on separate occasions over a 4-week period and underwent medical and neuropsychiatric assessments, physical examination, neuropsychological testing, and combined MRI/^{1}H-MRS. Results of their psychiatric and medical examinations are not reported here.

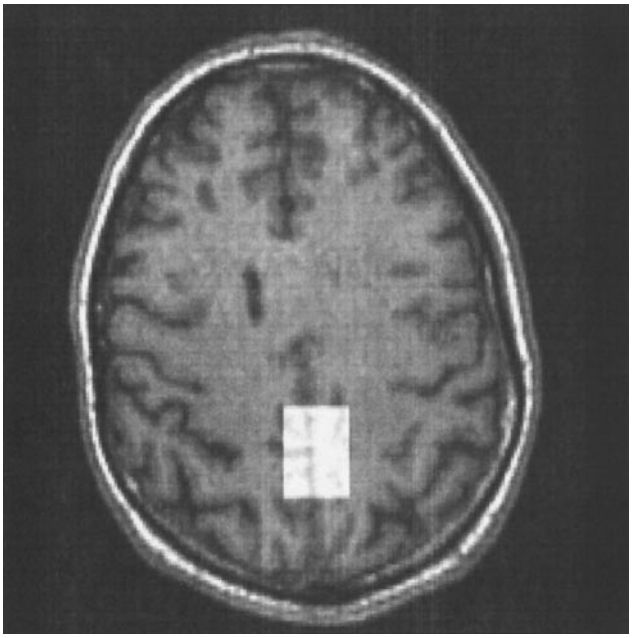

FIG. 1. Axial image of ^{1}H-MRS (STEAM 1500/30) occipitoparietal region of interest (2 × 2.7 × 2 cm).

Neuropsychological Testing

The neuropsychological battery comprised standard clinical tests to assess the major cognitive domains of memory, attention, information processing speed, language, parietal function, and frontal-executive performance (Lezak, 1995). The complete neuropsychological battery included those tests listed in Table 1 in addition to the Boston Naming Test, Token Test, Identities and Oddities, Color Form Sort, Ideomotor Apraxia, Finger Gnosis, Stereognosis, Simple Copying, and Sentence Repetition. The entire battery took approximately 2.5 h to complete. Only those tests that produced scalar information were chosen for data analysis (Table 1). The MOANS norms for the elderly were used when converting between individual raw test scores, aged scaled scores, and percentiles (Ivnik *et al.*, 1996, 1992a,b). The revised National Adult Reading Test (Nelson and Willison, 1991) (NART-R) was also administered to estimate presenescent verbal intelligence (mean NART-R = 113.7, SD = 7.0).

Magnetic Resonance Techniques

^{1}H-MRS was conducted on a GE Signa 1.5T scanner equipped with a spectroscopy package in two neuroanatomical regions. The first was a 10.8 ml mixed grey/white matter VOI in the occipitoparietal region (OPR), an area which has been widely used in spectroscopic studies of Alzheimer's Disease (Shonk *et al.*, 1995) and used in this study as a control (See Fig. 1). The second was an 8-ml VOI in the left frontal white matter (FWM) as shown in Figs. 2a–2c, located anterior to the frontal horn of the left lateral ventricle. Both VOIs were delineated on T_1-weighted axial images and repeatability maximized by using anatomical landmarks. After VOI prescription and shimming, acquisition was with the STEAM sequence, using 30-ms echo time, 1500-ms repetition time, 13.7-ms mixing time, 2048 number of data acquisitions, a bandwidth of 2500 Hz, and phase cycle of 8. Each free induction decay was the result of averaging over 256 excitations. GE PROBE *S/V* software allowed automatic display of spectra after phase correction based on the unsuppressed water signal, residual water signal subtraction, line broadening, fast-fourier transformation (FFT), frequency band extraction, and image scaling (see Fig. 2d). Automatic quantitation of the NAA, Cr, mI, Cho, and free physiological water resonances was possible after linewidth and lineshape apodization enhancement, FFT, baseline-correction, and Marquardt Levenworth curve fitting over the metabolite line region. Ratio metabolite quantitation was compared using both the Cr and internal unsuppressed water signals as reference values. The quality of the MRS signal was adequate with a mean Cr signal to noise ratio of 14.0 in the FWM and 34.6 in the OPR.

Anatomical imaging was conducted using a whole brain T_1-weighted sequence (coronal FSPGR acquisition; 1.5 mm thick, TR 12.2, TE 5.3) for volumetric measurement, and a coronal T_2-weighted FLAIR sequence (4 mm thick, 0 gap, TR 8900, TE 145, IT 2200)

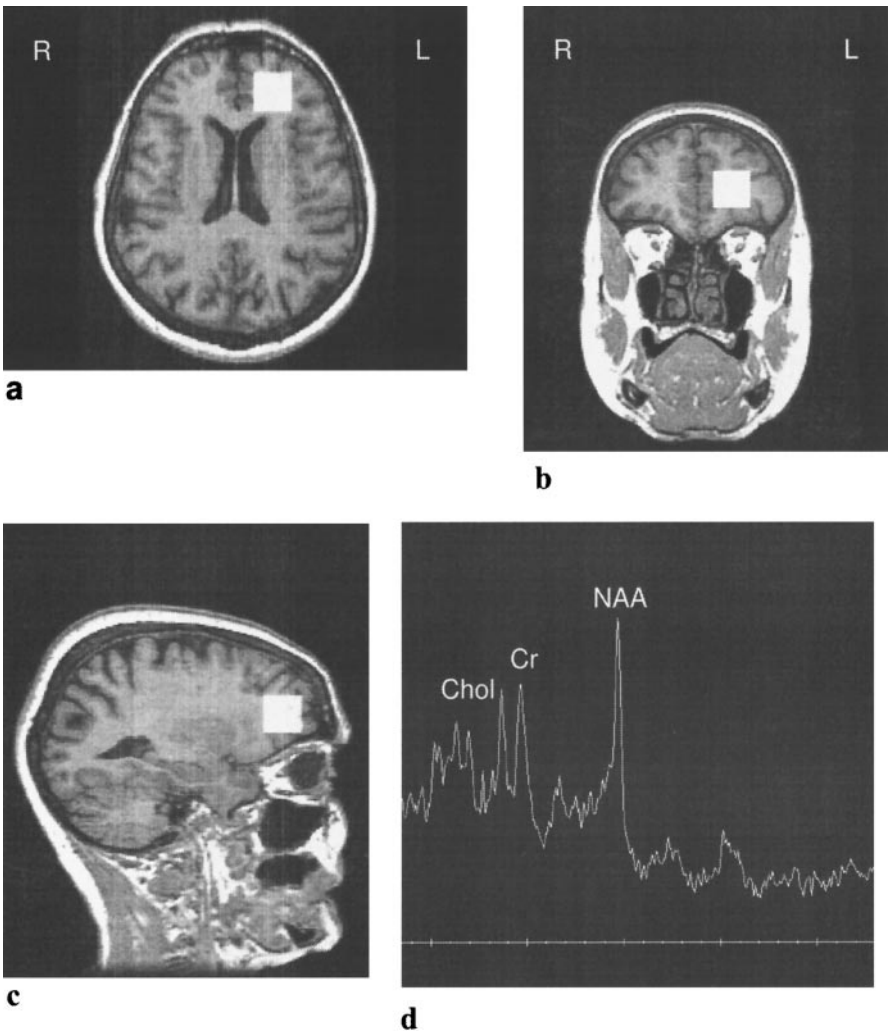

FIG. 2. Orthogonal slices showing (a) axial, (b) coronal, and (c) sagittal localizing images of ^{1}H-MRS (STEAM 1500/30) frontal white matter region of interest (2 × 2 × 2 cm). Figure 1d shows an example of a spectrum acquired from this area in a 72-year-old female. Major metabolites are labeled (*N*-Acetylaspartate, NAA; free cholines, Cho, Creatine plus Phosphocreatine, Cr). The NAA/Cr ratio in this example was 1.81.

to detect abnormal white matter signals. The two ^{1}H-MRS volumes of interest were extracted from these two sets of images and, using an automatic segmentation algorithm, percentage volumes of cerebrospinal fluid (CSF%) and abnormal hyperintense FLAIR signal (HFS%) in each VOI were calculated. Central atrophy was measured using published criteria (Victoroff *et al.*, 1994): a T_1-weighted axial slice through the thalamus and putamen was selected and the ventricle to brain ratio (VBR) at the anterior horn of the lateral ventricles was calculated (VBR_a); a second more superior axial slice at the point of maximum lateral ventricle width was chosen to measure mid-ventricular dilation (VBR_m). All images were transferred to a Windows NT workstation and analysed using the ANALYZE PC Version 3.0 software package (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN).

Data Analysis

Each neuropsychological test was examined for a significant correlation with frontal and occipitoparietal NAA/Cr. To maximize the power of the cognitive testing, reduction of the raw neuropsychological data was performed using oblique rotation Principal Components Analysis (PCA). Each factor was tested for a significant relationship with the variables age, HFS%, VBR, NART, and NAA/Cr using Pearson's product moment coefficient. Linear regression models were then used to assess the independent relationship of each

TABLE 1

Neuropsychological Tests and Structure of First Principal Component

Neuropsychological test	Structure matrix coefficient
Trails making test B (time)	−0.92
Trails making test (difference time)	−0.85
Picture completion (WAIS-R)	0.72
Mental control (WMS-R)	0.69
Trails making test A (time)	−0.68
Arithmetic (WAIS-R)	0.63
Block design (WAIS-R)	0.61
Similarities (WAIS-R)	0.61
Symbol digit modalities test	0.49
Digit span (WAIS-R)	0.48
Visual reproduction I (WMS-R)	0.43
Logical memory I (WMS-R)	−0.17
Visual reproduction II (WMS-R)	0.16
Animal naming	0.15
COWAT	0.13
Logical memory II (WMS-R)	0.11

Note. Listed are clinical subtests taken from the Wechsler Memory Scales-revised (WMS-R), Wechsler Adult Intelligence Scale-revised (WAIS-R), and other stand-alone tests, including the Controlled Oral Word Association Test (COWAT).

predictor variable with the PCA factors. The SPSS for Windows Release 9.0.1 package was used for statistical analysis.

RESULTS

Neuropsychological Variance

Individual neuropsychological test scores were all in the normal range, with mean age-scaled scores varying between the 19th and 97th percentile bands.

A three-factor PCA reduction of the cognitive data accounted for 65.0% of the total variance, with the first PC accounting for 36.6%. The structure matrix of the first principal component (PC1, see Table 1) was highly loaded on tests of attentional switching and working memory (Trail Making Test B), Nonverbal Problem Solving and Spatial Reasoning (Block Design and Picture Completion), speed of information processing (Trail Making Test A and Symbol Digit Modalities), abstraction ability (Similarities), and basic attentional capacity (Arithmetic and Mental Control). PC1 was therefore interpreted as representing executive-attentional capacity. The second and third PCs (not shown) were interpreted as reflecting memory capacity and verbal fluency, respectively.

Brain Volumetry

Ventricle to brain ratios in the anterior and midsection, along with VOI tissue compartment volumes in the frontal and occipitoparietal region are presented in Table 2.

MRS Metabolites

The NAA/Cr ratio varied between 1.00 to 1.86, with a mean of 1.34 (SD = 0.24) in FWM and between 1.13 to 1.56 with a mean of 1.33 (0.09) in the OPR. The other metabolite values are reported in Table 2.

Cognitive-Neuronal Correlations

Table 3 shows the pattern of significant individual neuropsychological test—frontal white matter NAA correlations and nonsignificant OPR NAA correlations. Given that the neurometabolic variation observed may have been due to Cr changes rather than NAA differences (Chang *et al.*, 1996; Oppenheimer *et al.*, 1995), we also used the internal water peak as a reference value after correction for CSF in the VOI (NAA/$H_2O_{CSFcorrected}$; Henriksen, 1995).

VBR_a, VBR_m, age, NART, HFS%, and NAA/Cr in each VOI were individually tested as predictors of PC factors one to three, using Pearson's correlation. Only the first principal component demonstrated a significant correlation with any of the predictor variables; these results are reported in Table 4. PC1 was significantly correlated with the NAA/Cr ratio in the FWM ($r = 0.61$), but was not associated with occipito-parietal NAA levels. $NAA/H_2O_{CSFcorrected}$ in the FWM was also significantly correlated with PC1 ($r = 0.57$, $P < 0.01$, $df = 19$), this being the only relationship that reached significance.

Whether NAA/Cr_{FWM} was associated with PC1 independent of the other predictors was tested using a

TABLE 2

MRS and MRI Results by Region of Interest

Location	MR measure	Mean value	Standard deviation
Anterior horn lateral ventricle	VBR_a	0.31	0.03
Mid lateral ventricle	VBR_m	0.20	0.03
Frontal white matter VOI	CSF%	2.78	0.31
	HFS%	0.28	0.32
	NAA/Cr	1.34	0.24
	Chol/Cr	0.88	0.16
	mI/Cr	0.73	0.21
Occipitoparietal VOI	CSF%	15.92	16.49
	HFS%	0.45	0.69
	NAA/Cr	1.33	0.09
	Chol/Cr	0.61	0.06
	mI/Cr	0.58	0.07

Note. Abbreviations: Ventricle-to-brain ratio (VBR), *N*-acetylaspartate (NAA), mobile choline compounds (Cho), myo-Inositol (mI), creatine and phosphocreatine (Cr), volume of interest (VOI), Percentage volume of cerebrospinal fluid in VOI (CSF%), Percentage volume of FLAIR-weighted hyperintense signal in VOI (HFS%).

TABLE 3

Indicative Individual Neuropsychological Test-NAA Correlations by Region of Interest

^{1}H-MRS measure	TMT-B	TMT-A	Similarities (WAIS-R)	Block design (WAIS-R)	Logical memory I (WMS)	Visual reproduction II (WMS)
NAA/Cr_{FWM}	−0.44	−0.59*	0.54*	0.57*	−0.02	0.19
	(0.05)	(>0.01)	(0.01)	(0.01)	(0.93)	(0.42)
$NAA/H_2O_{CSFcorrected\ (FWM)}$	−0.47*	−0.39	0.51*	0.51*	0.11	0.03
	(0.04)	(0.09)	(0.02)	(0.02)	(0.64)	(0.90)
NAA/Cr_{OPR}	−0.25	−0.16	0.03	0.07	0.07	0.07
	(0.32)	(0.53)	(0.92)	(0.79)	(0.77)	(0.79)

Note. Correlations used Pearson's product moment coefficient procedure (two-tailed, controlling for $\alpha = 0.05$), significant results are marked *. Subtests presented were taken from the Wechsler Memory Scales-revised (WMS-R) or Wechsler Adult Intelligence Scale-revised (WAIS-R) and correlation coefficients are based on age-scaled scores. The Trail Making Test (TMT), Versions A and B, correlations used raw time scores. FWM indicates frontal white mater region of interest and OPR indicates occipitoparietal region of interest.

linear multiple regression model with individuals' age, VBR_m, $HFS\%_{FWM}$, NART, and NAA/Cr_{FWM} scores entered simultaneously. Together these variables accounted for the majority of variance in PC1 (Adjusted R-Square = 0.659, F (5,14) = 8.346, $P < 0.005$); only frontal NAA/Cr was, however, an independent predictor ($\beta = 0.45$, $t = 2.776$, $P < 0.02$). When all other predictors were entered in a first step and NAA/Cr_{FWM} in a second step, this measure independently accounted for 14% of the variance in PC1 (R-Square Change = 0.138, F (1,14) = 7.07, $P < 0.02$). This partial correlation is presented in Fig. 3.

Age, NART, and VBR_m were not significantly correlated with frontal NAA/Cr. There was a nonsignificant trend for a negative association between HFS% and NAA/Cr_{FWM} ($r = -0.395$, $P < 0.09$, $df = 19$). There was also significant difference in NAA/Cr_{FWM} levels between the sexes (male mean was 1.46, female mean 1.25, $t = 2.18$, $P < 0.05$), but no longer so after correcting for age. There was no evidence of spectroscopic differences between right and left handed individuals, although the latter group had only small numbers ($n = 2$). To check that atrophy levels within the frontal VOI may have contributed to performance in tests of frontal lobe function, we used a split-half procedure of PC1 scores to group individuals into high and low cognitive groups. Average percentage of CSF in the FWM was 2.33 (0.22) in the high cognitive group and 3.23 (0.37) in the low cognitive group ($t = -0.65$, $P = 0.52$); using the ^{1}H-MRS unsuppressed water signal for comparison, the high cognitive group H20/Cr ratio mean was 1707.2 (195.0) and was 1652.3 (200.6) in the low cognitive group ($t = -0.62$, $P = 0.54$). Neither method of VOI water quantitation showed significant water content differences between high and low cognitive performers. Our results suggest that NAA levels measured *in vivo* are not an artefact of CSF or creatine reference variation in the region of interest and have no significant relationship with periventricular ischaemic change as measured by white matter hyperintense signal.

DISCUSSION

We conducted whole brain T_1- and T_2-weighted MRI, short-echo proton magnetic resonance spectroscopy in two regions of the brain and comprehensive cognitive testing in a sample of healthy elderly individuals. Levels of the neural metabolite, NAA, in the left subcorticofrontal white matter region were positively correlated with the primary composite neuropsychological measure of executive-attentional ability, but not re-

TABLE 4

Zero Order Correlations between Neurocognitive Predictors and the First Principal Component Representing Executive-Attentional Ability

Predictor		Zero order correlation	P value
VBR_a		−0.17	0.48
VBR_m		−0.52*	0.02
Age		−0.49*	0.03
NART-R		0.14	0.55
Frontal white matter region	HFS%	−0.68*	>0.00
	NAA/Cr	0.61*	>0.01
	Chol/Cr	−0.14	0.57
	mI/Cr	0.47	0.10
Occipitoparietal region	HFS%	0.17	0.51
	NAA/Cr	0.19	0.50
	Chol/Cr	−0.32	0.20
	mI/Cr	0.12	0.66

Note. Abbreviations: Ventricle-to-brain ratio (VBR), National Adult Reading Test-revised (NART-R), *N*-acetylaspartate (NAA), mobile choline compounds (Cho), myo-Inositol (mI), creatine and phosphocreatine (Cr), Volume of Interest (VOI), Percentage volume of cerebrospinal fluid in VOI (CSF%), Percentage volume of FLAIR-weighted hyperintense signal in VOI (HFS%). Significant Pearson correlations (two-tailed, controlling for $\alpha = 0.05$) are marked *.

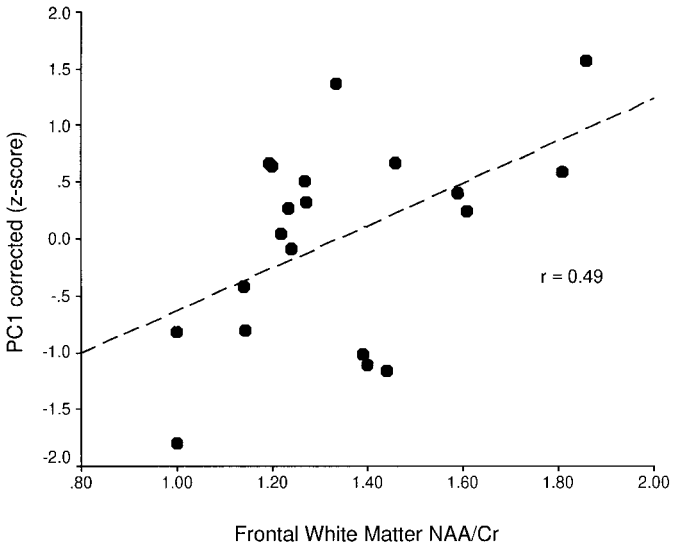

FIG. 3. Scatter plot showing executive-attentional cognitive ability (first principal component, PC1) and frontal white matter NAA/Cr after correction for age, verbal intelligence (NART-R), regional white matter disease (percent hyperintense signal in region of interest), and central atrophy (midventricle to brain ratio).

lated to measures of memory or verbal fluency. Individual cognitive test to NAA correlations confirmed that this relationship was confined to tests requiring higher-order cognitive skills and attentional resources. Also, this relationship was not evident when examining the ^{1}H-MRS results from a mixed occipitoparietal control volume of interest. This finding was independent of other determinants of fluid intellect such as age, presenescent verbal ability, frontal white matter pathology, and ventricular dilation. The results confirmed our original hypothesis.

In the mature brain, MRS-visible NAA occurs most predominately in the neuronal compartment (Simmons *et al.*, 1991), including the axonal and dendritic projections. NAA is thought to be synthesized in the mitochondria, having been found to parallel almost exactly the rate of mitochondrial O_2 consumption and ATP production (Bates *et al.*, 1995). It is preferentially catabolized in the axon rather than the cell body (Goldstein, 1976; Tsai and Coyle, 1995) and has been implicated in a number of neurobiological processes, including osmoregulation (Tsai and Coyle, 1995), the regulatory cycle of the excitatory neurotransmitters *N*-acetylaspartylglutamate and glutamate (Miller, 1991; Tsai and Coyle, 1995), myelination during development (Peden *et al.*, 1990; Grodd *et al.*, 1991), and possibly in adulthood (Bhakoo and Pearce, 2000), and as a carbon donor in a number of critical cellular processes (see Tsai and Coyle, 1995 for a review). Decrements in NAA have been found in a number of neurological conditions known to involve neural loss (Rudkin and Arnold, 1999), so that in MRI-confirmed structurally sound brain tissue, NAA may be a sensitive measure of neurocellular fitness, even though the precise neurobiological function of NAA is currently unknown.

As previously stated, the cell loss theory of brain ageing is undergoing a critical reappraisal. The role of frontal lobe morphological change is receiving similar treatment. O'Donnell *et al.* (1999), for example, showed that behavioural differences between old and young Rhesus monkeys on a delayed response procedure, a test known to require the functional integrity of area 46 of the prefrontal cortex, were not related to prefrontal volume. Even the subset of older monkeys that had performed most poorly had no gross prefrontal cortical atrophy. By contrast, animal and human studies continue to document cerebrometabolic decline with age (see Blass *et al.*, 1997 for a review), with specific decline reported in the frontal lobe (Garraux *et al.*, 1999). Together, these findings implicate connectivity and cell function changes in critical frontal circuits of the brain, rather than neuronal death, with age-associated cognitive impairment.

One interpretation of our findings is that variations in neurometabolic fitness of the frontal subcorticocortical white matter tracts, as revealed by ^{1}H-MRS, are functionally implicated in mediating higher order information processes in the healthy elderly. This relationship may arise from differences in cellular energy degeneration patterns between individuals, such as maximal mitochondrial respiration rate (Benzi and Moretti, 1997) or rate of ATP formation (Hoyer, 1996), the latter having been shown to vary with NAA production *in vitro* and after cognitive activation (Dutschke *et al.*, 1994). Cellular energetic status can have far-reaching influences on neural function. ATP is required for neurotransmitter synthesis and to drive ion pumps necessary for maintaining the resting membrane potential and for propagation of the depolarizing action potential. This explanation is consistent with the research linking mitochondrial dysfunction with neurodegenerative disorders (Benzi and Moretti, 1997; Beal *et al.*, 1993), and the present method may be useful for investigating the transition between normal age-associated cognitive decline and dementing illness.

Alternatively, preexisting individual differences in axonal density, due to neural proliferation and dendritic pruning in development, may have given rise to a higher NAA signal, simply due to the increased number of axons per unit volume. To attempt to address this question we compared high and low PC1 groups for water content in the frontal white matter ROI, using both the gross morphological estimate of CSF in the volume of interest and the unsuppressed water peak from the MRS spectrum as an additional estimator of microscopic atrophy. There were no significant water content differences (or indicative trends) between the two groups of divergent cognitive ability,

using either measure, suggesting that axonal density is possibly not as important as neurocellular fitness when considering executive performance in old age. We must, however, recognize that for comparison purposes our group sizes were small.

Another limitation of this method is that the frontal white matter that we sampled comprises subcorticocortical, corticosubcortical, and corticocortical fibers, and this technique cannot reveal which particular tracts are involved. While MRS technology continues to improve, we used a typical clinical MR scanner which currently limits spatial resolution to between one and eight cubic centimetres. Moreover, the topography of the frontal white matter is poorly understood. Primate and human neurophysiological studies have identified projections that link the lateral and medial prefrontal cortex regions, basal ganglia, thalamus and then feedback to frontal neocortex, completing motivational-executive control circuits (Austin and Mitchell, 1995; Alexander *et al.,* 1986). The NAA variations that were observed may be one measure of the neurometabolic fitness of such circuits.

We report for the first time using MRS, a pattern of neurometabolic change related specifically to executive and attentional function that is circumscribed to a particular region of the normal brain. The lack of an association with occipitoparietal neurometabolites suggests the specificity of this finding. Our results also confirmed the contributions that age, central atrophy, and frontal white matter change make to cognitive function in late life. However, these variables were found to be all highly interrelated. Frontal white matter hyperintensities were in particular related to both cognitive decline and age. Given that the typical distribution of WMLs in the elderly is in the periventricular region (Pantoni and Garcia, 1997), it is likely that sensitive frontocorticostriatal projections are disrupted. Interestingly, increased volumes of hyperintense FLAIR signal were not related to cellular fitness as measured by NAA, although a nonsignificant trend was observed. FLAIR is a long inversion-time, long-echo time, heavily T_2-weighted imaging modality that is particularly sensitive to abnormal water content changes in the periventricular region thought to arise from pathological insult (Alexander, 1996; Scheltens *et al.*, 1995). Since other studies have demonstrated NAA decrements in areas of hyperintense signal (Oppenheimer, 1995), the lack of relationship in our study may be explained by the very small volume of abnormal tissue in the VOI; the frontal region had a mean abnormal tissue content of 0.30% of total volume. Volumes of interest with a larger proportion of hyperintense signal may be more likely to show a reliable relationship. Proton spectroscopy may also have a role in detecting the clinical significance of WMLs in the elderly, having been shown to differentiate innocuous from pernicious lesions of the same size and severity on MRI, on the basis of the lesion's NAA signal intensity (Brooks *et al.*, 1997).

NAA variation in the subcorticofrontal region was, by contrast, significantly independent of these predictors. In our cross-sectional study, while NAA concentration was related to fluid intellectual ability, it was not related to age alone. This may indicate a true age-independent biochemical system involved in cognition, or may reflect the powerful effect of large intersubject variation at one point in time. Longitudinal cognitive spectroscopy studies may be of high value in trying to distinguish the cumulative effects of time and biochemical disruption on human cognition. Further basic research into the role of NAA, the second most prevalent cerebral amino acid and its potential relationship to cognition also seems timely.

We conclude that in healthy individuals NAA in the frontal lobe may be a measure of the metabolic integrity and fitness of frontal-subcortical circuit function. The combined use of MRI and cognitive spectroscopy may find many applications, particularly when multiple regions of interest are contrasted and discrete psychological skills are assessed.

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