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Proton Magnetic Resonance Spectroscopy (^{1}H MRS) of the Hippocampal Formation in Schizophrenia: A Pilot Study

H. A. NASRALLAH, T. E. SKINNER, P. SCHMALBROCK and P. M. ROBITAILLE

Background. Recent post-mortem and magnetic resonance imaging (MRI) studies strongly suggest a decrease in the volume of the hippocampus and other limbic temporal structures in schizophrenia. Therefore, we hypothesised that N-acetyl aspartate (NAA) which is found mainly in neurons and which can be measured by proton magnetic resonance spectroscopy (^{1}H MRS) would be decreased in the limbic temporal region in schizophrenia.

Method. Consenting subjects fulfilling DSM-III-R criteria for schizophrenia ($n = 11$) and matched healthy volunteers ($n = 11$) who were recruited in a tertiary university referral centre, participated in a ^{1}H MRS brain study. Proton MRS spectra were obtained from a 12 cm^3 voxel ($2 \times 2 \times 3$ cm) in the right and left hippocampus/amygdala region. A researcher blind to the source of the spectra, measured the NAA intensity in all subjects, which were then statistically compared across the two groups.

Results. NAA intensities were significantly reduced in the right hippocampus/amygdala region of schizophrenic patients ($P = 0.038$). The difference of the left side did not reach significance at the 95% confidence level.

Conclusions. The findings of decreased NAA in this study suggest that there may be a decrement in neuronal number or tissue volume of the right hippocampal/amygdala region in schizophrenia. Biochemical alterations in the metabolism of NAA in schizophrenia may be an alternative explanation. The findings are consistent with other types of post-mortem and *in vivo* evidence for hypoplasia of the limbic temporal structures in schizophrenia, postulated to be of neurodevelopmental pathogenesis.

Several lines of evidence point to neurobiological abnormalities of the hippocampus and other limbic (medial) temporal lobe structures (hippocampus, amygdala, parahippocampal gyrus, entorhinal cortex) in schizophrenia (Mednick *et al*, 1991). This includes both post-mortem and imaging data (Nasrallah, 1993). MRI comparison of discordant monozygotic twins (Suddath *et al*, 1990) has pointed to a reduced volume of the hippocampus and other temporal structures in the affected twins. Several histopathological studies have suggested that neuronal cytoarchitecture of the hippocampus in schizophrenia is disrupted and possibly related to impaired migration processes during the second trimester (Conrad & Scheibel, 1987; Nasrallah, 1993). Post-mortem neurochemical studies of the limbic temporal lobe have also reported abnormalities in dopamine and glutamate in that region (Deakin *et al*, 1989; Kerwin *et al*, 1989; Reynolds, 1983).

In vivo studies of brain tissue in the limbic temporal structures may therefore be important for defining the neurobiology of schizophrenia. A potentially useful technique for studying brain biochemistry is proton magnetic resonance spectroscopy (^{1}H MRS), for a 'noninvasive biopsy' of living tissue (Lock *et al*, 1990). Here, we use ^{1}H MRS to investigate the limbic temporal region in schizophrenia.

^{1}H MRS can directly assay such biochemical constituents as choline, creatine, and N-acetyl aspartate (NAA). NAA has been reported to exist mainly intraneuronally (Nadler & Cooper, 1972). A decrease in the amount of NAA in the limbic temporal region in schizophrenia might be expected compared with health controls, given the reduced hippocampal volume in schizophrenia. A reduction in NAA could reflect a decrease in the number of neurons.

Method

Eleven patients who fulfilled DSM-III-R (American Psychiatric Association, 1987) criteria for chronic schizophrenia, and who were clinically stable on a maintenance dose of neuroleptics, and 11 healthy volunteers gave informed consent to participate in the study. Table 1 shows the demographic data of the sample.

All studies were performed on a 1.5 T whole-body NMR imaging/spectroscopy system (General Electric Signa) equipped with actively shielded gradients (1 gauss/cm). Proton spectra were acquired using

Table 1
Demographic data

	Male/Female	Age (years) mean (s.d.)	Education (years) mean (s.d.)	Illness duration (years) mean (s.d.)	Medication status
Control group ($n = 11$)	7/4	28.6 (5.3)	17.3 (2.3)	–	3 birth control 1 tetracycline
Schizophrenia group ($n = 11$)	7/4	32.7 (5.2)	13.4 (2.1)	12.1 (4.9)	All receiving antipsychotic medications

a stimulated echo acquisition mode (STEAM) sequence modified for enhanced water suppression and improved spatial localisation in small volumes (Moonen & van Zijl, 1990). The basic STEAM sequence allows single-step localisation by exciting three intersection slices. Water suppression was achieved by exciting the water resonance using a single Chemical Shift Selective (CHESS) pulse (Haase *et al*, 1985) before the STEAM sequence. A further improvement in the water suppression is achieved by repeating the CHESS pulse in the period between the second and third STEAM localisation pulse in a manner that avoids unwanted echoes (Moonen & van Zijl, 1990). Moonen & van Zijl (1990) report total water suppression factors of as high as 10 000 using this technique. Strong gradients are used in the intervals between the three STEAM localisation pulses to eliminate signals from all echoes but the stimulated echo, thereby improving the localisation.

The three coordinates necessary to locate the volume of interest (VOI) in the limbic temporal lobe were identified in sagittal and coronal slices obtained at the beginning of the procedure. Both imaging and spectroscopy were performed using the standard quadrature head coil for transmission and reception. After determining the 90° pulse amplitude, the field homogeneity was adjusted using the STEAM sequence, without the water suppression pulses. The amplitudes of the CHESS pulses were then adjusted to optimise the water suppression. Proton spectra were acquired from a 12 cm^3 voxel ($2 \times 2 \times 3$ cm) centred on either the left or right hippocampus/amygdala complexes while the subjects were asked to count forward repeatedly to 100, in order to standardise the mental set across subjects. The VOI was then located on the opposite side and the procedure for shimming and water suppression was repeated before obtaining the second spectrum, under identical conditions as for the first spectrum. All spectra were obtained with maximal receiver gain, 1000 Hz sweep width, 1024-point spectral resolution, a 2 s repetition time (TR), a 50 ms echo time (TE), a 60 ms evolution period (TM), and 200 acquisitions. Post-processing of the data included exponential multiplication by 2 Hz and magnitude correction of the spectrum. The data were ported to a SPARC workstation (SUN Microsystems) running Omega software (General Electric) for data analysis.

Spatial localisation was demonstrated by adding phase-encode and read-out gradients to the STEAM sequence to image a $2 \times 2 \times 3$ cm^3 volume in a jelly-filled phantom. Over 90% of the signal came from this volume. Further details can be obtained from the authors.

Results

Figures 1(a) and (b) are coronal and sagittal localiser images acquired using standard T_1-weighted spin echo imaging sequences, and show the position of the voxels from which the spectra were obtained. These voxels are centred on the right and left limbic temporal lobes. Figure 2 displays a typical ^1H spectrum acquired from the right hippocampus/amygdala region of a healthy volunteer. Spectra from the left hippocampus are similar. This spectrum has the expected strong and sharp resonances from NAA at 2.0 ppm, creatine/creatine phosphate at 3.0 ppm, and choline at 3.2 ppm, accompanied by excellent water suppression. Average linewidths of approximately 8 Hz were obtained.

Most of the spectra obtained from the right and left hippocampus region of schizophrenic patients were qualitatively similar to those shown in Figure 2. However, several of the spectra from the right hippocampus in the schizophrenic population exhibited features not seen in any of the controls. As these are possibly susceptibility artefacts, we are not pursuing any scientific implications of these features.

The data were quantified by first-magnitude correcting the spectra to obtain a consistent and objective (operator independent) phasing of the spectra. The area of each peak was obtained using spectral analysis (SPAN) software (General Electric), that performs a least-squares fit to the data using lines of adjustable height, width, and position,

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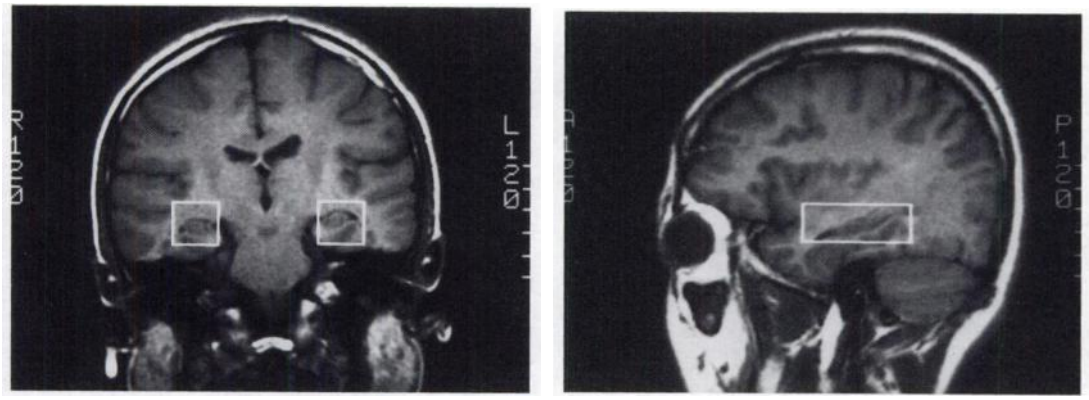

(a) (b)

Fig. 1 Coronal (a) and sagittal (b) localiser images displaying the position of the voxels centred on the right and left hippocampus from which spectra were obtained.

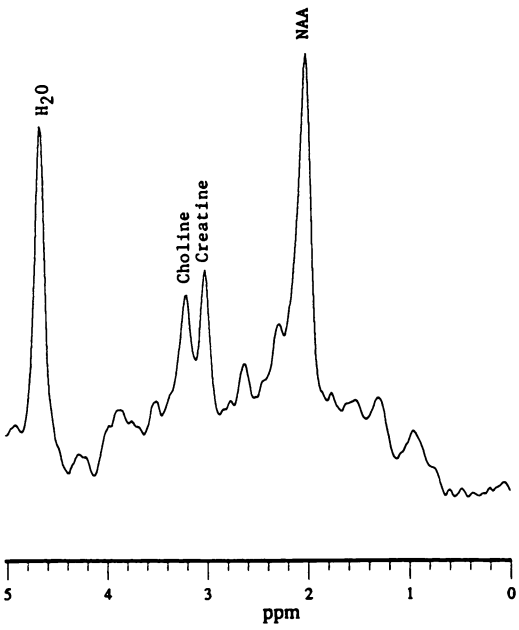

Fig. 2 Typical ^1H MRS spectrum acquired from the right hippocampus of a control subject using the STEAM sequence (TE = 50 ms). Resonances have been assigned to N-acetyl aspartate (NAA) at 2.0 ppm, creatine at 3.0 ppm, and choline at 3.2 ppm.

which are initialised by the operator at the beginning of the program to the peaks in the spectrum. Gaussian line shapes were found to provide the best fit to the data.

To compare spectra obtained from different subjects quantitatively, an unchanging standard is needed as a reference. Changes in the intensity of a given line as a fraction of the standard intensity then accurately reflect real changes in the peak of interest, as opposed to changes in experimental conditions. Since all the spectra had the same acquisition time, a particularly simple standard that can be used is the root mean square (r.m.s.) noise obtained from a given region where there are no peaks. For each spectrum, peak intensities (NAA, choline-containing compounds and creatine) have been obtained as the ratio of the calculated peak area to the r.m.s. noise measured in the region 9–12 ppm. Values of the NAA intensity for the control and schizophrenic populations are listed in Table 2 for the right and left limbic temporal lobe. Nonparametric comparisons (Wilcoxon rank sum test) were applied to the data. A statistically significant reduction of the NAA intensity was observed in the right hippocampus/amygdala complex in the schizophrenia group compared with the controls ($z = 1.78$, $P = 0.038$, one tailed). No statistically significant result was observed for a similar comparison of the left side. Although a statistically significant ($z = 1.84$, $P = 0.033$) reduction in the choline intensity from the right side of the

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Table 2
Measured N-acetyl aspartate intensity (relative to r.m.s. noise) in patients and controls

Diagnosis	Left hippocampus[1]	Right hippocampus[2]
Control 1	2.7	3.9
Control 2	3.3	2.4
Control 3	5.8	4.8
Control 4	3.4	3.2
Control 5	4.7	4.3
Control 6	6.1	2.8
Control 7	6.5	2.9
Control 8	4.0	4.7
Control 9	4.5	4.3
Control 10	4.0	5.6
Control 11	4.7	6.9
Schizophrenic 1	4.0	4.5
Schizophrenic 2	3.8	3.3
Schizophrenic 3	2.8	3.4
Schizophrenic 4	5.5	3.4
Schizophrenic 5	3.6	3.3
Schizophrenic 6	4.6	2.9
Schizophrenic 7	2.0	3.0
Schizophrenic 8	3.6	3.3
Schizophrenic 9	2.6	1.4
Schizophrenic 10	4.8	2.8
Schizophrenic 11	–	2.7

1. Left NAA intensity: Wilcoxon 2-sample test (1-tailed), $P=0.056$; Kruskal–Wallis test (χ^2 approximation), $\chi^2=1.8$, d.f. = 1, $P=0.09$.
2. Right NAA intensity: Wilcoxon 2-sample test (2-tailed), $P=0.044$; Kruskal–Wallis test (χ^2 approximation), $\chi^2=3.0$, d.f. = 1, $P=0.041$.

schizophrenic group compared with the control group was seen, the signal-to-noise ratio (S/N) of the choline peaks (S/N < 3) is too low to make this claim with confidence. However, this tentative result does provide impetus for future investigation.

Two other measures considered less rigorous than the fitting procedure described were also used to analyse the data, for validation. Peak heights (as opposed to peak integrals) were compared, and the integrals were measured without attempting to remove effects from overlapping peaks. In both cases, the results were consistent with those obtained previously, showing a statistically significant ($P<0.04$) reduction in the measured NAA signal from the right side in schizophrenics compared with the control group. The results thus do not have a sensitive dependence on the exact analysis procedure and are fairly robust. However, there was a strong trend for reduced NAA on the left side also in schizophrenia, and when the left and right NAA signals within schizophrenic patients were compared, no significant difference was found (Mann–Whitney $P=0.15$).

Discussion

The primary result of this pilot study using proton MRS to investigate schizophrenia shows a quantitative reduction of NAA intensity in the right limbic temporal lobe in schizophrenia. Lower NAA concentrations in human brain tissue have been shown in stroke studies to reflect neuronal death (Bruhn *et al*, 1989). A reduction in hippocampal volume on the right side in schizophrenia would also be consistent with the result. This *in vivo* MRS study suggests the possibility of neuronal dysfunction or loss of neurons in the hippocampal formation in schizophrenia.

That the schizophrenic patients were medicated may be regarded as a methodological shortcoming. However, there are several indications that the findings are not due to antipsychotic drug treatment. Firstly, there is no evidence in the literature that neuroleptic treatment results in neuronal or membrane dysfunction or neuronal death in the limbic temporal lobe. Secondly, the schizophrenic patients' duration of treatment ranged from 5 to 12 years, and they were medicated with low and high doses of a variety of oral and parenteral neuroleptics, including the atypical neuroleptic clozapine. Variations in NAA among the schizophrenics are not accounted for by a linear regression on either length of treatment or medication dosage (regression coefficient less than 0.1 in both cases). There would therefore appear to be little effect of the treatment on NAA levels among schizophrenics, but future studies of unmedicated and preferably drug-naïve schizophrenics are necessary to resolve this issue completely.

The issue of whether the left or right limbic temporal lobe is more involved in the pathogenesis of schizophrenia remains open. Our findings point to a right-sided asymmetry in NAA levels, contrary to the body of morphological studies that point to a predominantly left-sided pathology in schizophrenia. However, our data also show a strong trend for left-sided pathology, and it is possible that with a larger sample size, both the left and right limbic temporal lobes in schizophrenia will show a significant reduction in NAA. Since the submission of this article, the findings have been replicated on both the right and left sides (Yurgelun-Todd *et al*, 1993).

Further application of ^{1}H MRS to the longitudinal assessment of brain regions in schizophrenia may provide clues as to whether or not progressive changes may occur in schizophrenia consistent with the degenerative v. developmental model of the illness. The combination of structural brain imaging with MRI and chemical/metabolic measures with

MRS may become a powerful method for investigating brain structure and function in schizophrenia.

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Henry A. Nasrallah, MD; **Thomas E. Skinner**, PhD; **Petra Schmalbrock**, PhD; **Pierre-Marie Robitaille**, PhD, Ohio State University, College of Medicine, Columbus, Ohio, USA

Correspondence: Professor Nasrallah, Department of Psychiatry, Ohio State University, College of Medicine, 473 W. 12th Avenue, Columbus, Ohio 43210, USA

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