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ATTN:	SUBMITTED: 2002-08-29 17:22:16
PHONE: 301-594-7305	PRINTED: 2002-08-31 12:19:02
FAX: -	REQUEST NO.: NIH-10286771
E-MAIL:	SENT VIA: LOAN DOC 7967407

NIH	Fiche to Paper	Journal
TITLE:	AMERICAN JOURNAL OF PSYCHIATRY	
PUBLISHER/PLACE:	American Psychiatric Association Washington Dc	
VOLUME/ISSUE/PAGES:	1996 Dec;153(12):1554-63	1554-63
DATE:	1996	
AUTHOR OF ARTICLE:	Bertolino A; Nawroz S; Mattay VS; Barnett AS; Duyn JH; Moone	
TITLE OF ARTICLE:	Regionally specific pattern of neurochemical patho	
ISSN:	0002-953X	
OTHER NOS/LETTERS:	Library reports holding volume or year 0370512 8942451	
SOURCE:	PubMed	
CALL NUMBER:	W1 AM513	
REQUESTER INFO:	JEFFDUYN	
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Regionally Specific Pattern of Neurochemical Pathology in Schizophrenia as Assessed by Multislice Proton Magnetic Resonance Spectroscopic Imaging

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***Objective:** Several single-voxel proton magnetic resonance spectroscopy (¹H-MRS) studies of patients with schizophrenia have found evidence of reductions of N-acetyl-aspartate (NAA) concentrations in the temporal lobes. Multislice proton magnetic resonance spectroscopy imaging (¹H-MRSI) permits simultaneous acquisition and mapping of NAA, choline-containing compounds (CHO), and creatine/phosphocreatine (CRE) signal intensities from multiple whole brain slices consisting of 1.4-ml single-volume elements. We have used ¹H-MRSI to assess the regional specificity of previously reported changes of metabolite signal intensities in schizophrenia. Hippocampal volume was also measured to test the relationship between ¹H-MRSI findings and tissue volume in this region. **Method:** Ratios of areas under the metabolite peaks of the proton spectra were determined (i.e., NAA/CRE, NAA/CHO, CHO/CRE) for multiple cortical and subcortical regions in 10 inpatients with schizophrenia. **Results:** Patients showed significant reductions of NAA/CRE and NAA/CHO bilaterally in the hippocampal region and in the dorsolateral prefrontal cortex. There were no significant changes in CHO/CRE or in NAA ratios in any other area sampled. No significant correlation was found between metabolite ratios in the hippocampal region and its volume. **Conclusions:** NAA-relative signal intensity reductions in schizophrenia appear to be remarkably localized, involving primarily the hippocampal region and the dorsolateral prefrontal cortex, two regions implicated prominently in the pathophysiology of this disorder.*

(Am J Psychiatry 1996; 153:1554-1563)

Several neuroimaging and neuropathological findings have been associated with schizophrenia. Post-mortem studies (1-7) have shown abnormalities in several brain regions, particularly in the hippocampal region and the prefrontal cortex. The most often observed change is a reduction in cortical volume. In these same anatomical regions, reactive gliosis, dying neurons, inclusion bodies, or other signs of inflammation or degeneration tend not to be found.

Anatomical studies with magnetic resonance imaging

(MRI) have produced results consistent with those from postmortem examinations, revealing most often bilateral reduction of the volume of the hippocampal region and of the frontal lobe (8-13), although negative reports have also appeared (14).

Another in vivo approach that has provided evidence of brain pathology in schizophrenia is proton magnetic resonance spectroscopy (¹H-MRS). At a relatively long echo time, ¹H-MRS detects strong signals from N-acetyl-containing chemical moieties (mainly N-acetyl-aspartate [NAA]), choline-containing compounds (CHO), creatine/phosphocreatine (CRE), and lactate. NAA, the most prominent peak in the proton spectrum other than water, is found largely, if not entirely, within neurons (15), but its intracellular function is unclear. The CHO signal reflects brain choline stores, with major contributions from glycerophosphocholine and phosphocholine (16, 17). The CRE signal measures both creatine and phosphocreatine, metabolites involved in cellular energy metabolism. Lactate is produced whenever the glycolytic rate exceeds the capacity of oxidative metabolism.

Received Oct. 16, 1995; revisions received Feb. 8 and June 4, 1996; accepted July 1, 1996. From the Clinical Brain Disorders Branch, Intramural Research Programs, NIMH Neurosciences Center at Saint Elizabeths, the Neuroimaging Branch, National Institute of Neurological and Communicative Disorders and Stroke, the Laboratory of Diagnostic Radiology Research, the Office of the Director, and the In Vivo NMR Center, NIH. Address reprint requests to Dr. Weinberger, Clinical Brain Disorders Branch, Intramural Research Programs, NIMH Neurosciences Center at Saint Elizabeths, 2700 Martin Luther King Jr. Ave., S.E., Washington, DC 20032.

The authors thank Dr. Alan McLaughlin for his help with analysis of the data and Dr. Alan Breier and Dr. David Pickar for letting the authors study one of their patients.

TABLE 1. Clinical Information for 10 Patients With Schizophrenia Assessed by Multislice Proton Magnetic Resonance Spectroscopic Imaging

Patient	Age (years)	Medication	Daily Dose (mg)	Diagnosis	Handedness	Illness Duration (years)	Number of Hospitalizations	Duration of Neuroleptic Exposure (years)
1	33	Risperidone	4	Paranoid schizophrenia	Right	8	2	5
2	29	Clozapine	350	Paranoid schizophrenia	Right	14	4	13
3	51	Risperidone	6	Undifferentiated schizophrenia	Right	33	3	20
4	22	Clozapine	600	Paranoid schizophrenia	Right	4	3	4
5	41	Risperidone	7	Undifferentiated schizophrenia	Right	18	5	15
6	37	Haloperidol	8	Undifferentiated schizophrenia	Right	17	11	15
7	41	Haloperidol	20	Paranoid schizophrenia	Right	20	9	20
8	39	Haloperidol	10	Undifferentiated schizophrenia	Left	25	22	17
9	33	Risperidone	6	Undifferentiated schizophrenia	Right	10	3	8
10	48	Haloperidol	10	Paranoid schizophrenia	Right	30	5	30

Previous single-voxel ^1H -MRS studies of patients with schizophrenia have reported reductions of NAA signal intensity or concentration in the temporal lobes (18, 19), in the region of the hippocampus (20, 21), and in the frontal lobe (22). Other studies, however, have failed to show changes in the frontal lobe (23–25). A recently introduced ^1H -MRS imaging (^1H -MRSI) technique permits the acquisition of proton spectra signals from a great number of small volume elements (nominal volume resolution of 0.84 ml) within whole brain slices (26). The acquired data can be displayed in a tomographic image format, thus providing a brain map of metabolite signals. The ^1H -MRSI technique has been used to study several CNS disorders (27–31) but has yet to be applied to schizophrenia.

The purposes of the present ^1H -MRSI study were 1) to assess specific chemical aspects of the hippocampal region and dorsolateral prefrontal cortex as well as of several other relevant brain structures in patients with schizophrenia and 2) to explore whether the ^1H -MRSI findings are dependent on the hippocampal region tissue volume.

METHOD

Subjects

Ten inpatients (eight men and two women with a mean age of 37.4 years, $SD=8.6$) from the National Institute of Mental Health Neuropsychiatric Research Hospital at St. Elizabeths Hospital in Washington, D.C., volunteered and gave their written informed consent to participate in this study. All patients fulfilled DSM-IV criteria for chronic schizophrenia, based on a diagnostic interview (the Structured Clinical Interview for DSM-IV) and review of medical records. They were receiving neuroleptic drugs at the time of the study. Nine of the 10 subjects were right-handed (table 1). The comparison group was composed of 10 healthy volunteers matched for sex, age (mean=33.1, $SD=5.45$), and handedness; all were National Institutes of Health (NIH) employees. Subjects with a history of alcohol or drug abuse, brain injury, or any diagnosable systemic or neurological condition were excluded from the study.

^1H -MRSI Procedure

Multiple-slice ^1H -MRSI was performed on a conventional GE-SIGNA 1.5 Tesla MR imaging system (GE Medical Systems, Milwaukee, Wis.) equipped with self-shielded gradients according to

the method of Duyn et al. (26). The standard quadrature head coil was used in all cases. A sagittal localizer scan was first obtained (fast spin echo repetition time [TR] of 3500 msec, echo time [TE] of 102 msec) followed by a scan consisting of T_1 -weighted, 3-mm thick oblique axial slices (spin echo TR of 500 msec, TE of 12 msec) acquired in a plane parallel to the angle of the Sylvian fissure. From this scan, five slices were chosen in which, according to the consensus of two investigators (A.B. and S.N.), the hippocampal region was most clearly visualized. These five MRIs represented the volume from which the first of the four spectroscopic images was obtained. The other three contiguous ^1H -MRSI slices were acquired parallel and superior to the first.

Phase-encoding procedures were used to obtain a 32×32 array of spectra from volume elements (voxels) in the selected slice (240-mm field of view). Each voxel had nominal dimensions of 7.5 mm \times 7.5 mm \times 15 mm (0.84 ml). Actual volume, based on full width at half maximum after filtering of k-space to shape the point spread function, was 1.4 ml (26). The ^1H -MRSI sequence involves a spin echo slice selection with TR of 2200 msec and TE of 272 msec and includes suppression of water and most of the signal arising from lipids in skull marrow and in surface tissues. To suppress lipid signals from the skull and scalp, the ^1H -MRSI sequence included an outer-volume saturation pulse. This technique does not significantly affect the metabolite signals within the brain regions studied.

After acquiring the spectroscopic data, we obtained another T_1 -weighted scan at the same location as the first T_1 scan to visually inspect for subject movement. Any visually apparent shift in the location of the hippocampal region from one scan to the next was considered evidence for movement and was grounds for exclusion from the study. After the second set of T_1 -weighted images, a 3-D MRI dataset was acquired as 124 conventional sagittal slices (SPGR, TR of 24 msec, TE of 5 msec). The entire examination was completed in about 1 hour.

The raw ^1H -MRSI data were processed on Unix workstations (Sun Microsystems, Mountain View, Calif.) using proprietary software. First, NAA, CHO, CRE, and lactate peaks were identified in the spectra of each voxel. Voxels in which these metabolite signals could not be recognized (e.g., voxels located outside the head and on or near the skull's surface) were manually nulled. On a voxel-by-voxel basis, the signal strength in a range of 0.1 ppm around the NAA, CHO, CRE, and lactate signal positions was integrated for each peak. The average number of brain voxels in the four spectroscopic images was about 700. Metabolite signals are reported as ratios of the integrals of the area under each peak: NAA/CRE, NAA/CHO and CHO/CRE.

A rater blind to diagnosis drew regions of interest on the T_1 -weighted coaxial MR images. This procedure used proprietary software to manually draw regions of interest on the T_1 -weighted images and then transfer these regions of interest to the exact same location on the metabolite maps. Voxels that were contained within the anatomical region of interest but not present on the metabolite maps were removed. Thus, the final calculations were performed only on voxels containing ^1H spectra. The program computed the average value of the integral of the area under each peak in all voxels within the regions of interest on the metabolite maps.

Regions of interest were drawn bilaterally in the hippocampal region

FIGURE 1. Metabolite Signal Intensity Maps of *N*-Acetyl-Containing Compounds (NAA), Choline-Containing Compounds (CHO), and Creatine/Phosphocreatine (CRE) of a Patient With Schizophrenia

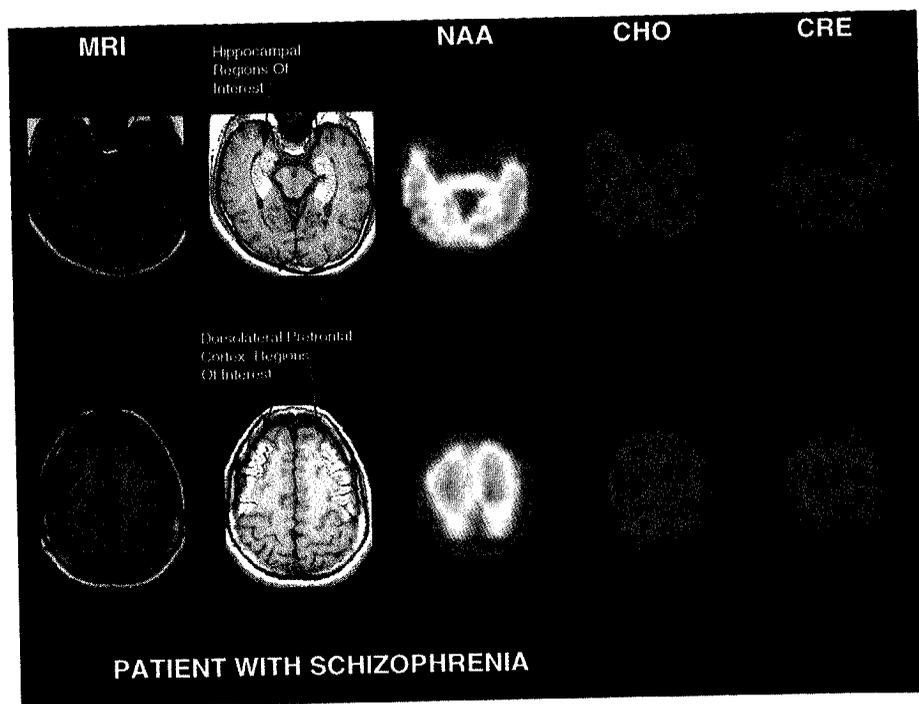
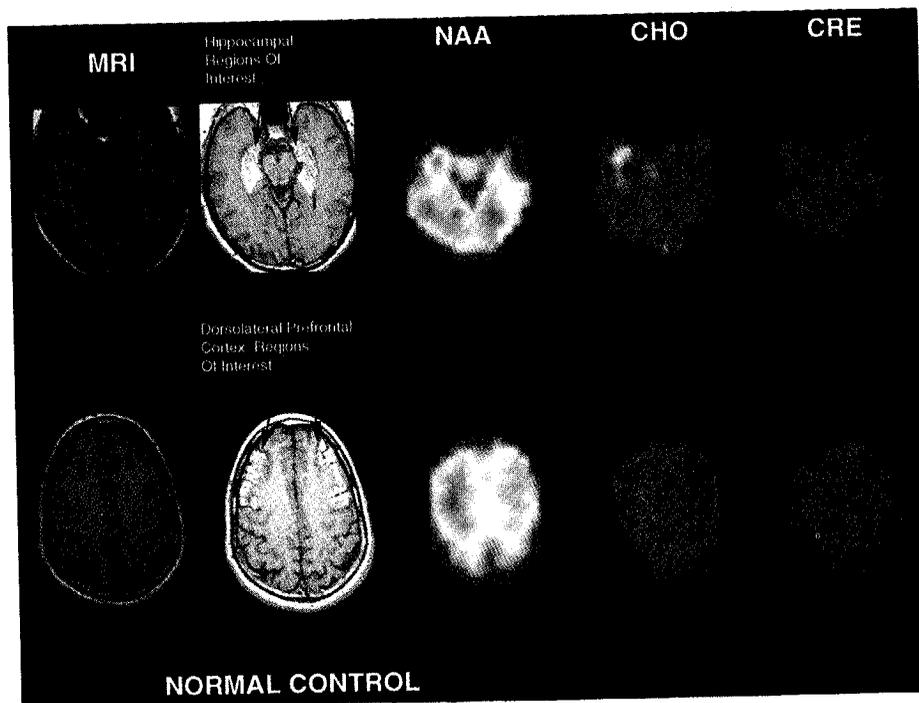


FIGURE 2. Metabolite Signal Intensity Maps of NAA, CHO, and CRE of a Normal Comparison Subject



(for patients the right mean=10.4 voxels, SD=1.7, and the left mean=10.2 voxels, SD=1.8; for the comparison subjects the right mean=10.4 voxels, SD=1.6, and the left mean=10.1 voxels, SD=2.4) and the dorsolateral prefrontal cortex (for patients the right mean=14.2 voxels, SD=2.4, and the left mean=14 voxels, SD=2.3; for comparison subjects the

right mean=12.3 voxels, SD=2.7, and the left mean=12.6 voxels, SD=2.8), as well as in the superior temporal gyrus, orbitofrontal cortex, occipital cortex, anterior and posterior cingulate, centrum semiovale, prefrontal white matter, thalamus, and putamen. There were no significant differences in the sizes of the regions of interest between patients and comparison subjects. A second rater (S.N.), also blind to the diagnosis, drew regions of interest in 10 randomly selected cases to determine interrater reliability of the region of interest metabolite ratios. The mean intraclass correlation coefficient (ICC) for all metabolite ratios in the hippocampal region and the dorsolateral prefrontal cortex was 0.80.

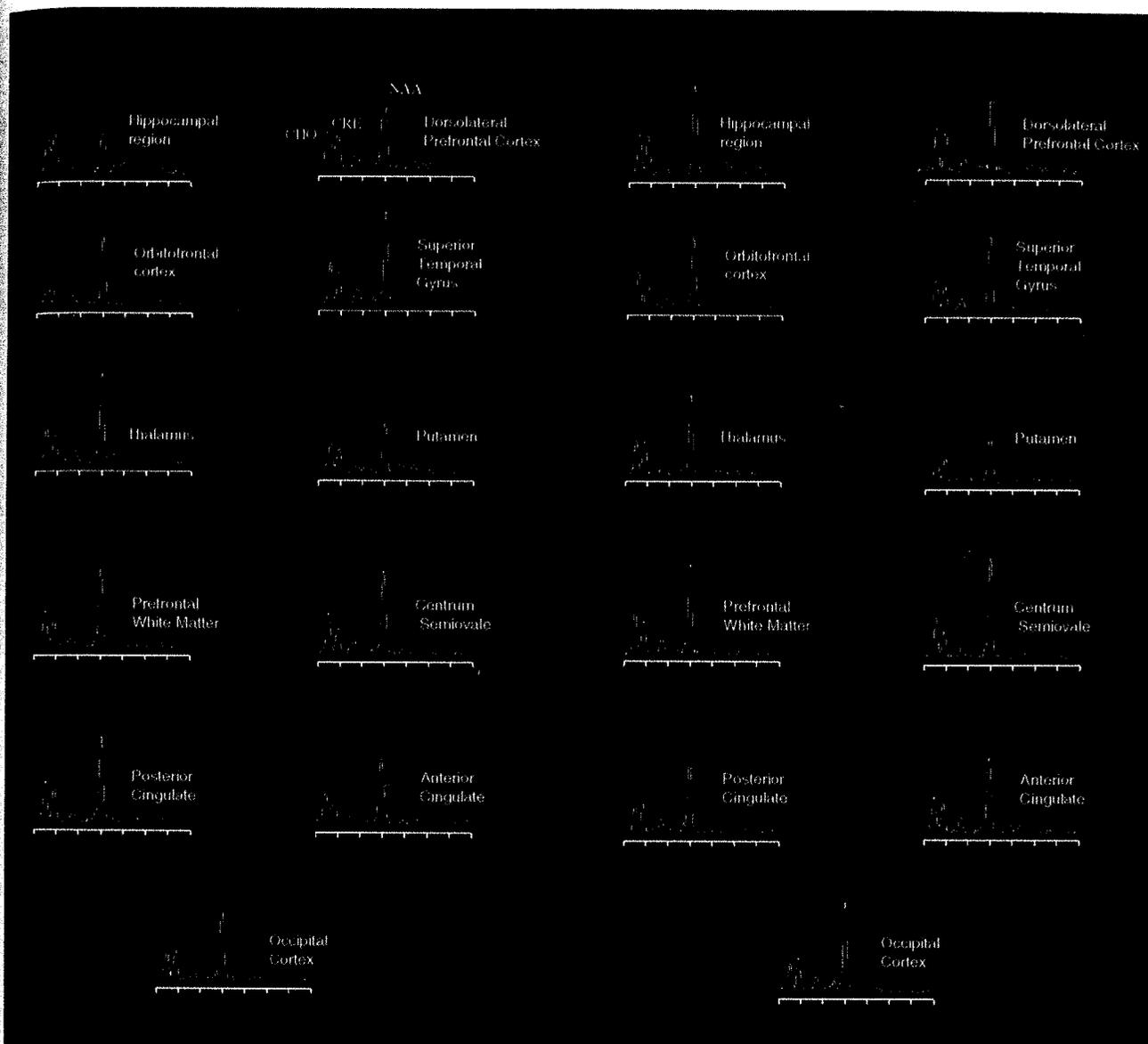
Morphometry

Sagittal volume scans were re-sliced into a coronal stack (1.87 mm thick) by using public-domain software (NIH Image 1.57) on a Macintosh computer. The same software package was then used to manually outline the hippocampal region on the coronal images with the mammillary bodies as an anatomical reference point. The hippocampal region area was outlined bilaterally in the three slices anterior to the mammillary bodies and in the 10 slices posterior to them for a total of 14 slices. The values obtained were then summed and multiplied by the thickness of the slice (1.87 mm) to produce estimates of the hippocampal region volume in cubic centimeters. The hippocampal region measured in this manner encompassed a major portion of the amygdala and the hippocampus. Since the resolution of spectroscopic images does not resolve between amygdala and hippocampus, we did not differentiate between these structures. The measurements were made blind to diagnosis. To evaluate the reliability of the volumetric measurements, a different rater (S.N.), also blind to diagnosis, measured the hippocampal region areas in the same manner in 10 randomly selected subjects. The ICC for the left and right hippocampal region volumes was 0.75.

Clinical Measures

All patients had previously undergone neuropsychological testing, which included the Wisconsin Card Sorting Test, Continuous Performance Test, WAIS, and California Verbal Learning Test. On the same day of the ¹H-MRSI procedure, positive and negative symptoms were rated by a single physician (S.N.) using the Brief Psychiatric Rating Scale (BPRS) and the Scale for the Assessment of Negative Symptoms (SANS).

FIGURE 3. Representative Spectra From a Patient With Schizophrenia and From a Comparison Subject in 11 Anatomical Regions^a



^aThe left two columns of spectra are from the patient and the two right columns are from the comparison subject.

Statistical Analysis

Differences between patients and comparison subjects were tested separately for each metabolite ratio and for each region of interest by a two-way repeated measures analysis of variance (ANOVA), with hemisphere (left or right) as the within-group factor and diagnosis (patients or comparison subjects) as the between-group factor. Post hoc analysis was performed by using the Tukey honest significance difference test. Bonferroni correction for the number of regions ($N=11$) was applied. The relationship between hippocampal region metabolite ratios and the hippocampal region volume was assessed by using the Spearman correlation test. Spearman correlational analysis was also used to test for correlations between metabolite ratios and clinical measures, including age, age at onset of illness, length of illness, duration of exposure to neuroleptics, dose of neuroleptics at the time of the study, BPRS and SANS scores, number of hospitalizations, and number of years of education.

RESULTS

¹H-MRSI Analysis

Figures 1 and 2 show metabolite maps of NAA, CHO, and CRE signal intensities and coaxial MRIs with the regions of interest corresponding to the hippocampal region and the dorsolateral prefrontal cortex shown in a patient and in a comparison subject. Figure 3 shows representative spectra of a patient and a comparison subject from all regions of interest. Figures 4–6 show mean regional variations of the three metabolite ratios (NAA/CRE, NAA/CHO, and CHO/CRE).

In the hippocampal region, patients with schizophre-

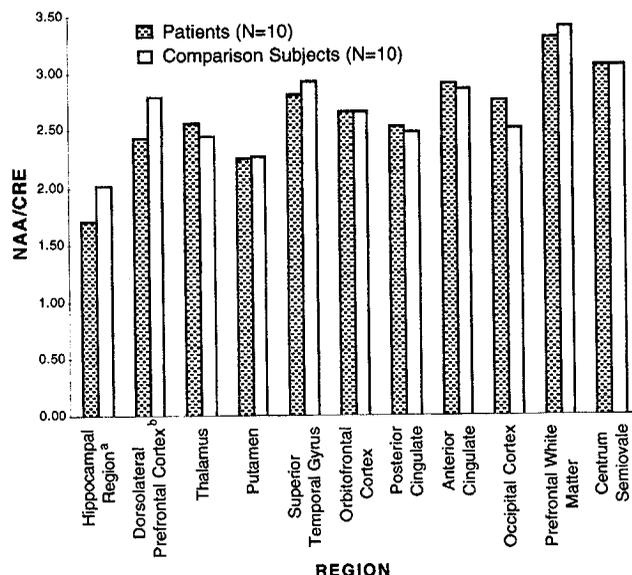
3 voxels, $SD=2.7$, $n=12.6$ voxels, $SD=$
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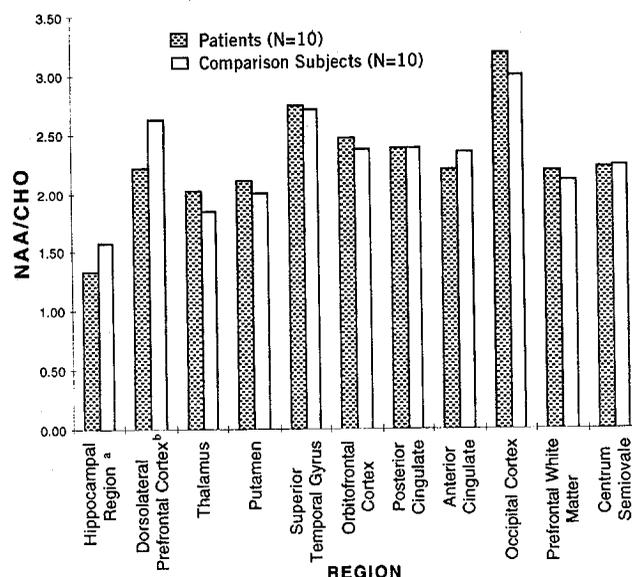
FIGURE 4. Mean Regional Variations in NAA/CRE in Patients With Schizophrenia and Comparison Subjects



^aSignificant difference between patients and comparison subjects ($F=11.5$, $df=1, 18$, $p<0.003$; $p<0.03$ after Bonferroni correction).

^bSignificant difference between patients and comparison subjects ($F=11.7$, $df=1, 18$, $p<0.002$; $p<0.03$ after Bonferroni correction).

FIGURE 5. Mean Regional Variations in NAA/CHO in Patients With Schizophrenia and Comparison Subjects

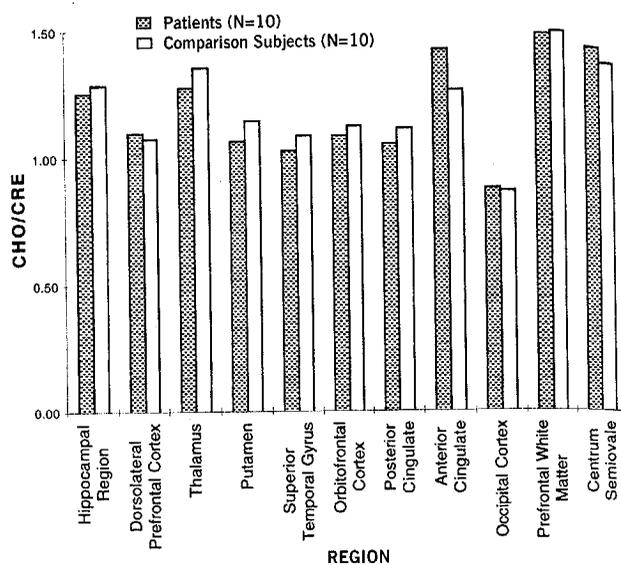


^aSignificant difference between patients and comparison subjects ($F=9.1$, $df=1, 18$, $p<0.007$; $p<0.07$ after Bonferroni correction).

^bSignificant difference between patients and comparison subjects ($F=6.6$, $df=1, 18$, $p<0.001$; $p<0.11$ after Bonferroni correction).

nia had lower NAA/CRE (mean=1.71, $SD=0.23$) (figure 7) and NAA/CHO (mean=1.33, $SD=0.21$) than comparison subjects (NAA/CRE mean=2.02, $SD=0.26$, and

FIGURE 6. Regional Variations in CHO/CRE in Patients With Schizophrenia and Comparison Subjects



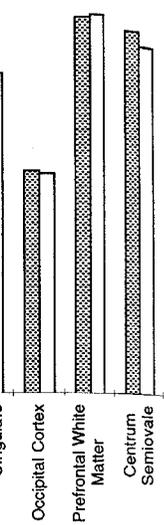
NAA/CHO mean=1.57, $SD=0.22$). ANOVA revealed a significant effect of diagnosis only for NAA/CRE (figure 4) and NAA/CHO (figure 5). Post hoc analysis showed that patients with schizophrenia had reduced NAA/CRE ($p<0.003$) and NAA/CHO ($p<0.007$). The difference between patients and comparison subjects in NAA/CRE remained significant even after Bonferroni correction ($p<0.03$); the difference in NAA/CHO did not quite achieve significance ($p<0.07$). No main effects or interactions were found for CHO/CRE.

In the dorsolateral prefrontal cortex, patients with schizophrenia had lower NAA/CRE (mean=2.44, $SD=0.27$) and NAA/CHO (mean=2.23, $SD=0.30$) than comparison subjects (NAA/CRE mean=2.80, $SD=0.28$, and NAA/CHO mean=2.63, $SD=0.45$). ANOVA revealed again a significant effect of diagnosis only for NAA/CRE (figure 4) and NAA/CHO (figure 5). Post hoc analysis showed that patients had significantly decreased NAA/CRE ($p<0.003$) and NAA/CHO ($p<0.01$). Again, the difference between patients and comparison subjects in NAA/CRE remained significant even after Bonferroni correction ($p<0.03$), whereas the difference in NAA/CHO did not ($p<0.11$). No main effects or interactions were found for CHO/CRE.

No significant effect due to diagnosis was found in any other region of interest for all metabolite ratios. ANOVA revealed sporadic effects of hemisphere and no interaction of diagnosis by hemisphere other than in the thalamus for NAA/CRE. None of the sporadic results survived Bonferroni correction, even at the trend level.

As a post hoc analysis to evaluate whether the metabolite ratio changes seen in the patients were a result of changes in the numerator or denominator terms, the mean integrated areas of NAA, CHO, and CRE resonances were normalized to the corresponding mean integrated areas

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in the centrum semiovale (i.e., NAA in the hippocampal region/NAA in the centrum semiovale, CHO in the hippocampal region/CHO in the centrum semiovale, CRE in the hippocampal region/CRE in the centrum semiovale, etc.). We used the centrum semiovale as a reference because a previous study (32) found that its integrated metabolite signal intensities showed the lowest coefficients of variation among several other regions of interest. Again, a statistically significant reduction of normalized NAA was found bilaterally in the hippocampal region (two-way repeated measures ANOVA $F=4.7$, $df=1, 18$, $p < 0.04$) and in the dorsolateral prefrontal cortex ($F=11.1$, $df=1, 18$, $p < 0.003$). No significant differences were found for centrum-semiovale-normalized CHO in the hippocampal region ($F=0.07$, $df=1, 18$, $p > 0.7$) and the dorsolateral prefrontal cortex ($F=0.07$, $df=1, 18$, $p > 0.8$) and for centrum-semiovale-normalized CRE in the hippocampal region ($F=0.6$, $df=1, 18$, $p > 0.4$) and the dorsolateral prefrontal cortex ($F=0.01$, $df=1, 18$, $p > 0.8$). These analyses indicate that the NAA ratio differences between patients and normal comparison subjects were the result of differences in the numerator, i.e., in NAA.

No detectable lactate signal was found in any of the subjects studied. This result is consistent with the normal intracerebral lactate concentration (about 0.5 mmol/g) being close to or below the detection limit of our method.

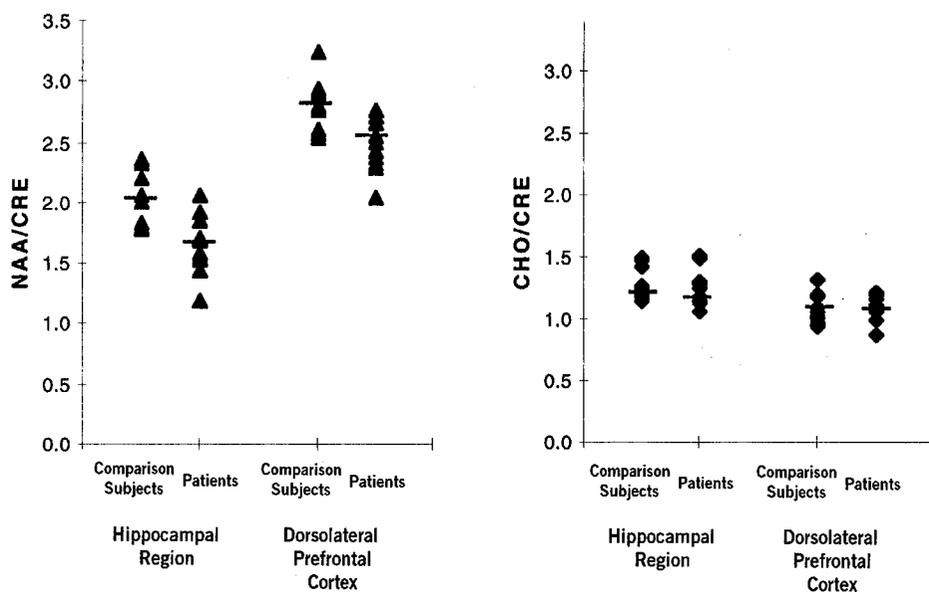
Hippocampal Volume

In this relatively small group of patients, the hippocampal region volume of the patients (left mean=2.05, $SD=0.35$, right mean=2.05, $SD=0.23$) did not differ significantly from that of the comparison subjects (left mean=2.19, $SD=0.22$, right mean=2.19, $SD=0.29$).

Correlations

A few sporadic and inconsistent correlations emerged in patients with schizophrenia between NAA/CRE in some regions of interest and the neuropsychological data. None of them was significant when Bonferroni-corrected for multiple regional comparisons. No significant correlations were found in the patients between metabolite ratios in the hippocampal region or in the dorsolateral prefrontal cortex and any of the following: hippocampal region volume, BPRS score, SANS score,

FIGURE 7. Scatterplots of NAA/CRE and CHO/CRE in the Hippocampal Region and the Dorsolateral Prefrontal Cortex of Patients With Schizophrenia and Comparison Subjects



age, age at onset, length of illness, length of exposure to neuroleptics, dose of neuroleptics at the time of the study, education, and number of hospitalizations. Furthermore, metabolite ratios did not correlate with number of voxels within any region of interest. No statistically significant correlation was found between metabolite ratios and the hippocampal region volume, education, or age in normal comparison subjects.

DISCUSSION

The main findings of our multislice 1H -MRSI study of schizophrenia are 1) neurochemical pathology, as evidenced by reductions of NAA/CRE and NAA/NAA in the centrum semiovale, was confined to the hippocampal region and the dorsolateral prefrontal cortex bilaterally and occurred in none of the other cortical and subcortical regions surveyed; 2) the NAA ratio values in the hippocampal region did not correlate with the volume of the hippocampal region; 3) there were no differences in centrum semiovale CHO/CRE, CHO/CHO, and CRE/CRE in the hippocampal region, in the dorsolateral prefrontal cortex, or in any other region surveyed.

Comparison With Other Studies

Our findings are partially consistent with previous postmortem neurochemical and in vitro spectroscopy studies of brain tissue of patients with schizophrenia. Tsai et al. (33), in a controlled postmortem study, found an increase of *N*-acetyl-aspartyl glutamate (NAAG) and a reduction of *N*-acetyl- α -linked-acidic-dipeptidase (the enzyme responsible for the cleavage of NAAG into

NAA and glutamate) in the hippocampus and prefrontal cortex and a reduction of NAA in the parahippocampus. Komoroski et al. (34) reported in abstract form the results of an *in vitro* ^1H -MRS study of brain extracts of patients with schizophrenia in which they found indirect evidence of a reduction of NAA in the thalamus but no change in the frontal pole, amygdala, temporal lobe, and cerebellar vermis. A comparison between our results and these two earlier studies is difficult because the degree to which *in vivo* spectroscopy and postmortem and *in vitro* histochemistry are comparable is uncertain.

Our findings of bilaterally reduced NAA ratios in the hippocampal region and the dorsolateral prefrontal cortex are also in partial agreement with previously published results of *in vivo* single-voxel ^1H -MRS studies of schizophrenia. Buckley et al. (22) assayed left frontal and temporal lobes with an 11-cm^3 voxel and reported no overall group differences between patients and comparison subjects. However, in males as a separate group, a significant reduction of NAA and a significant increase of CHO was found in the left frontal lobe. Our data cannot address the hypothesis that male patients show more anatomical and functional abnormalities than female patients because our group consisted primarily of male patients.

Nasrallah et al. (20) surveyed hippocampal/amygdala regions bilaterally (12-cm^3 voxel) and found a reduction of NAA only on the right. Yurgelun-Todd et al. (18) (12-cm^3 voxel) found a 20% reduction of NAA/CRE bilaterally in the temporal lobe, the only region assayed. Renshaw et al. (19) (8-cm^3 voxel) also found a bilateral reduction of NAA/CRE in the temporal lobes (the only region assayed) of patients with first-episode psychosis. Fukuzako et al. (24) studied the left medial temporal (8-cm^3 voxel) and frontal (27-cm^3 voxel) lobes and found reduced NAA/CRE and NAA/CHO and increased CHO/CRE in the temporal lobe but no changes in the frontal lobe.

Our data are also partially consistent with some single-voxel MRS studies that have been reported only in abstract form, but a comparison between our results and these, communicated in such limited reports, is problematic. Stanley et al. (23) reported no differences of NAA in the dorsolateral prefrontal cortex of patients assayed with an 8-cm^3 voxel. Komoroski et al. (25) (8-cm^3 voxel) found that a reduction in NAA/CRE approached significance in the temporal lobe but not in the frontal lobe, thalamus, or caudate of the left hemisphere.

Inconsistencies between our results and earlier studies could arise from several sources, including differences in patients and methods. The voxel sizes used in previous single-volume ^1H -MRS studies do not permit accurate anatomical localization and differentiation of white and gray matter. NAA, CHO, and CRE vary considerably across anatomical regions (32, 35–37) and between gray and white matter. For these reasons and because of other factors, such as subject positioning, movement correction, volume selection, and variations

in anatomy, the use of small voxels is advantageous. It should be noted, however, that our voxel dimensions are still subject to considerable partial volume effects and do not completely separate the cortical gray ribbon from the surrounding white matter.

Almost all of the studies in the literature, including our own, used ratios of NAA to other signals as a form of normalization. Although this approach controls for variance associated with assay irregularities across regions and subjects (e.g., magnetic field inhomogeneities), it is potentially less sensitive to changes that affect multiple metabolite signals. Maier et al. (21), in an effort to avoid this limitation, measured absolute metabolite levels bilaterally in the hippocampus. In a single-voxel ($4\text{--}9\text{-cm}^3$) study, they reported significantly reduced NAA, CHO, and CRE concentrations only in the left hemisphere of patients as well as a reversed asymmetry for NAA and CRE. These authors suggested that a general reduction in brain cellularity may account for a reduction in all three metabolites. Absolute metabolite concentrations represent a potential advantage over ratios in terms of their sensitivity, especially if all metabolites are changed, but they are more subject to errors arising from changes in tissue volume.

As a check on our ratio results, we also normalized each metabolite to its counterpart in the centrum semiovale, a region likely to be relatively spared by the pathology associated with schizophrenia. The results of our two different ratio measurement approaches suggest that in the hippocampal region and the dorsolateral prefrontal cortex of our patients there was a reduction of NAA and not of CHO and CRE. This discrepancy between our data and those of Maier et al. (21) cannot be resolved at this time and must await additional studies.

We could not find a relationship between the NAA ratios and current or previous neuroleptic administration. This is consistent with the results of Renshaw et al. (19) in first-episode patients and those of Buckley et al. (22), who found no prominent differences in ^1H -MRS among four neuroleptic-naive and 24 neuroleptic-treated patients. It should also be noted that NAA levels were found not to change in at least one study of rats treated chronically with neuroleptics (38). Finally, in a preliminary analysis of ^1H -MRSI data from a small study group of drug-naive patients with schizophrenia, we have found virtually the same magnitude and regional pattern of differences between patients and comparison subjects reported here (39).

Several artifacts might be considered as potential explanations for our MRSI findings. We used a relatively long TR (TR/T_1 of NAA >1) and a long TE (TE/T_2 of NAA <1) for the acquisition of the data. T_1 and T_2 relaxation time differences of ^1H -MRS metabolites could potentially cause differences in signal intensities. Therefore, we cannot exclude T_1 or T_2 effects on the calculated ratios. Yet, to our knowledge, there is no evidence that ^1H -MRS metabolites have abnormal relaxation times in patients with schizophrenia. Water proton relaxation times have been reported as changed in schizophrenia in different regions (40), but this is

not likely to affect the relaxation times of NAA, CHO, and CRE. Moreover, even if a change in relaxation times could account for reductions in NAA/CRE and NAA/CHO, this effect would be unlikely to explain similar reductions in NAA normalized to the centrum semiovale.

Another potential methodological confound that may be more problematic in patients with schizophrenia is motion. This artifact could conceivably attenuate spectroscopic signals, and the effects might vary somewhat from one region to another. We visually inspected the T_1 scans before and after the ^1H -MRSI acquisition for evidence of motion and excluded those studies of subjects (two patients, one comparison subject) who had obviously moved. We further examined the spectroscopic data for motion by looking for lipid signals outside the skull and scalp region. Since lipid suppression was performed, any motion would cause lipid signals to bleed beyond these extracerebral regions. The lipid intensity in spectra beyond the skull and scalp region was between 5% and 12% of lipid signals in the skull (i.e., the noise level) in patients and between 4% and 14% in normal subjects. Therefore, we doubt that subject motion during the scan resulted in significant artifacts, although we cannot exclude more subtle effects.

Finally, our failure to find a correlation between metabolite ratios and the volume of the hippocampal region might reflect partial volume effects inherent in current spectroscopy techniques. The hippocampal region sampled with the spectroscopic region of interest analysis was approximately 14 cm^3 (10 voxels \times 1.4 ml of voxel resolution). The volume of our anatomical hippocampal region, as measured on coronal MRIs, was on the order of 2 cm^3 . Thus, our MRSI hippocampal region might have misrepresented the true hippocampal signal. In a recent localized ^1H -MRS study centered on the rostral hippocampus, Strauss et al. (41) have shown a trend for an increase in NAA/CRE as voxel size increased. Alternatively, the lack of a correlation between volume and metabolites in our data might support other evidence that NAA is more than a measure of neuronal density and is, perhaps, related to neuronal function. Further studies are needed to clarify these issues.

Theoretical Implications

Our results have implications in terms of regional neuroanatomy and neurochemistry. The finding of a focal change confined to the hippocampal region and the dorsolateral prefrontal cortex is consistent with a growing body of research implicating prefrontal-temporolimbic neural systems and their function as important components of the neural circuitry affected in schizophrenia (42-44). The neurochemical implications of the selective NAA reductions are less clear. The role of NAA in neurons has yet to be fully elucidated (45). NAA has been regarded as a marker of either neuronal density, neuronal viability, or neuronal dys-

function as observed in both clinical (46, 47) and experimental (48, 49) investigations.

We did not find a correlation between NAA/CRE and NAA/CHO and the length of illness. This result in our 10 chronically ill patients, combined with the findings of Renshaw et al. (19) in the temporal lobes of patients with first-episode psychosis, suggests that the loss of NAA signal is not a reflection of an evolving neurodegenerative process. In this regard, it is interesting that we did not find changes in the CHO signal, which arises mainly from anabolites and catabolites of membrane phospholipids. The CHO signal, however, can also be influenced by dietary intake of choline (50), making it less reliable when diet is not controlled. Several studies of patients with temporal lobe epilepsy have shown decreased NAA and increased CHO in the mesial temporal lobe and in the diseased hippocampus (51-54). It has also been demonstrated with ^1H -MRS of extracts of cultured neural cells that astrocyte and oligodendrocyte cells express higher concentrations of CHO than cerebellar granule neurons (15). Bearing in mind the limitations associated with the interpretation of CHO signal when diet is not controlled, these studies suggest that astrogliosis in the context of neuronal pathology tends to be associated with enhanced CHO signal.

The reduction of NAA in our 10 patients is suggestive of neuronal pathology, and the lack of an increase in CHO signal might suggest that no active gliosis is taking place. This latter suggestion is consistent with the results of quantitative neuropathological studies in schizophrenia that tend to describe neuronal loss or dysplasia/hypoplasia but tend not to describe gliosis, and with the hypothesis that schizophrenia is not of neurodegenerative origin (42, 55).

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