Proton Magnetic Resonance Spectroscopic Imaging of Cortical Gray and White Matter in Schizophrenia

Kelvin O. Lim, MD; Elfar Adalsteinsson, PhD; Daniel Spielman, PhD; Edith V. Sullivan, PhD; Margaret J. Rosenbloom, MA; Adolf Pfefferbaum, MD

Objective: To apply in vivo proton magnetic resonance spectroscopy imaging estimates of N-acetylaspartate (NAA), a neuronal marker, to clarify the relative contribution of neuronal and glial changes to the widespread volume deficit of cortical gray matter seen in patients with schizophrenia with magnetic resonance images.

Methods: Ten male veterans meeting criteria of the DSM-IV, for schizophrenia and 9 healthy age-matched men for comparison were scanned using spectroscopic, anatomical, and field-map sequences. Instrument and collection variables were standardized to allow an estimation of comparable values for NAA, choline, and creatine for all subjects. Metabolite values from each voxel on 3 upper cortical slices were regressed against the gray tissue proportion of that voxel to derive estimates of gray and white matter NAA, creatine, and choline concentrations.

Results: The volume of cortical gray matter was reduced in patients with schizophrenia, but NAA signal intensity from a comparable region was normal. In contrast, the volume of cortical white matter was normal in patients with schizophrenia, but NAA signal intensity from a comparable region was reduced.

Conclusions: The lack of reduction in gray matter NAA signal intensity suggests that the cortical gray matter deficit in these patients involved both neuronal and glial compartments rather than a neurodegenerative process in which there is a decrease in the neuronal relative to the glial compartment. Reduced white matter NAA signal intensity without a white matter volume deficit may reflect abnormal axonal connections.

Arch Gen Psychiatry. 1998;55:346-352
SUBJECTS AND METHODS

SUBJECTS

Demographic and clinical variables of both groups are summarized in Table 1. Patients who met criteria of the DSM-IV for schizophrenia gave written informed consent to participate in this study. Patients who met criteria of the DSM-IV for alcohol or substance abuse in the past 3 months or who had ever met the criteria for posttraumatic stress disorder and alcohol or substance dependence were excluded. Five patients had met criteria for alcohol or substance abuse but not in the past 3 years. Other exclusion factors were significant medical illness or head injury resulting in a loss of consciousness exceeding 30 minutes. The DSM-IV diagnoses were determined by consensus between a psychiatrist or clinical psychologist who conducted a clinical interview and a trained research assistant who administered the Structured Clinical Interview for Diagnosis. One patient was excluded before analysis after his MRI scan revealed a basal ganglia lacunar infarct. All patients were receiving antipsychotic medications. Their clinical condition was evaluated using an average of the 18-item Brief Psychiatric Rating Scale scores obtained by 2 raters with established reliability. Premorbid intelligence was assessed using the National Adult Reading Test and parental socioeconomic status was determined using the Hollingshead 2-Factor Scale.

Healthy Control Subjects

Nine control subjects, 6 of whom were veterans, were recalled specifically for this study from a roster of healthy men who had served in the past as control subjects for other neuropsychiatric studies. All gave written informed consent to participate in the study.

CORTICAL GRAY MATTER VOLUME ASSESSMENT

Cortical gray and white matter volumes were measured from the upper 4 slices of a spin-echo axial image (cardiac-gated repetition time [TR], >2400 milliseconds; echo times [TEs], 20 and 80 milliseconds; field of view, 24 cm; 256×256 matrix; 3-mm-thick slices, with a 2.5-mm skip) in an oblique plane passing through the anterior and posterior commissures (Figure 2). For each data set, the most inferior slice above the level of the orbits, where the anterior horns of the lateral ventricles could be seen bilaterally, was identified as an index slice (slice 7 in Figure 2). Seven consecutive slices, beginning at the index slice and proceeding superiority, were segmented into cerebrospinal fluid (CSF) and gray and white matter compartments, using a semiautomated image analysis technique. Gray and white matter pixels were summed over the upper 3 slices to measure raw gray and white matter volume of the region from which spectroscopic data were collected. To account for differences associated with normal variation in head size or age, a 2-step regression analysis, based on data obtained previously from 73 normal control subjects, was applied. Six controls had served earlier in the normative sample, but a new axial scan was obtained for this study. This analysis yields z scores that have a mean of 0 and an SD of 1 in the full normative sample and that estimate the extent to which a person’s gray and white matter volumes deviate from head-size and age norms. Raw and z score measurements of white and gray matter volumes are summarized in Table 1.

SPECTROSCOPIC DATA ACQUISITION AND ANALYSIS

Data Acquisition

Proton MRS imaging scans were acquired using a quadrature head coil on a 1.5-T MRI scanner (Signa, General Electric, Milwaukee, Wis; 5.4 system software) with standard gradient hardware (1 G/cm maximum gradient amplitude, and 600 milliseconds minimum rise time). Foam padding wedged around the subject’s head reduced involuntary head movements. Sedation with up to 2 mg of lorazepam or equivalent was used for 5 patients.

Spatial coordinates of the base of the frontal lobe were identified on the midsagittal slice of a sagittal gradient echo scout series (TR, 30 milliseconds; TE, 6 milliseconds; flip angle, 30°; 5-mm skip; 2.5 mm; number of averages, 1; time, 30 seconds). These locations were then used to compute slice positions with 0.5-mm accuracy for all 3 scans in this protocol (anatomical, field map, MRS imaging). Anatomical images were acquired with an axial fast-spin-echo protocol (TR, 3000 milliseconds; TE, 20/80 milliseconds; echo train length of 8; 3-mm skip, 0.2 mm; 256×256 matrix, field of view, 24 cm; number of averages, 1; time, 3 minutes 18 seconds). Sixteen slices were collected, the most inferior slice being 20 mm above the base of the frontal lobe.

A given volume of gray matter may contain varying numbers and sizes of neurons packed at varying densities. Neurons are not replaced if destroyed, do not undergo division, and are usually incapable of regeneration after the first few weeks of birth. Glia consist of different cell types that can also vary in number, size, and density for a given volume of gray matter, but unlike neurons, can proliferate, often as a reaction to neuronal degeneration. Thus an MRI-visible reduction in gross volume of gray matter may be associated with fewer and/or smaller neurons and/or fewer and/or smaller glia (Figure 1). We propose to use spectroscopic measurements of NAA signal intensity as a tool to investigate the pathogenesis of the gross volume deficit in cortical gray matter in schizophrenia. To do so, we assume that NAA is absent in the glial compartment and that all neurons, regardless of size, have an equal NAA concentration in their cytoplasm. Thus, large neurons will have more NAA than smaller neurons, but the NAA concentration (amount of NAA per neuronal volume) will be the same in all neurons.

In this report, we apply tissue segmentation, image alignment, and statistical regression approaches to derive estimates of gray and white matter NAA concentration. We use standardized metabolite values and apply the assumptions delineated above to examine the pathogenesis of observed reduced gross volume of cortical gray matter in schizophrenia.
These slices were later segmented into CSF and gray and white matter to provide detailed information about the composition of each voxel selected for spectroscopic analysis. Automated shimming, based on 16 slices (each 0.3 mm thick) extending ±4 cm around a center position, 47.5 mm above the base of the frontal lobe, first used a map of the main magnetic field (B₀ field map; measured from the high signal-to-noise ratio water image) and a least-squares fitting procedure. ¹²,¹³ Gradient coils provided the linear terms, and a computer-controlled resistive shim supplied higher-order terms (xy, x²y², zx, zy, z², and z³), which increased the number of usable spectroscopic voxels by 30% over the linear terms. ⁴³ After the desired shim was achieved, a final 3-dimensional field map was collected at a resolution of 64 × 64 × 16 (TR, 40 milliseconds; TE, 10 milliseconds; flip angle, 20°; effective slice thickness, 6.4 mm; field of view, 24 cm; matrix, 64 × 64), covering a region ±3.1 cm around the center position, to measure residual field inhomogeneity.

Spectroscopic data were collected using a 3-dimensional protocol ⁴⁴ preceded by an inversion pulse for lipid suppression ⁴⁵ and spectral-spatial pulses ⁴⁶ for water suppression and spatial selection. Collection variables were as follows: TR, 2 seconds; inversion time, 170 milliseconds; TE, 144 milliseconds; field of view, 24 cm; circular 18 × 18 pixel sampling matrix in k-space (kx, ky) plane, effective slice thickness, 6.4 mm; and effective voxel size, 1.1 cm³. Corrections for receiver gain and coil loading were made when images were reconstructed ⁴⁵ to allow comparability of metabolite data (Figure 3) between subjects.

Data Analysis

All analyses were performed by analysts blind to subjects’ identity, age, and diagnosis. Anatomical images were stripped of scalp and skull voxels and segmented into CSF and gray and white matter compartments using a fully automated image analysis technique. This technique first segments CSF from tissue using a minimum-error-thresholding algorithm ⁴⁷ and then applies a nonparametric histogram technique ⁴⁸ to differentiate gray matter from white matter. ⁴⁹ This technique has been validated by a comparison of automated segmentation against operator-driven thresholding techniques ⁵⁰ and correlations obtained of 0.96 for tissue and fluid and 0.73 for gray and white matter. Two anatomical slices were combined to match the 6.4-mm-thick slices drawn from the 3-dimensional field-map and spectroscopic data sets. Metabolite images were generated for NAA, choline, and creatine by fitting a gaussian line shape to the magnitude spectrum from each voxel, where the line width of the gaussian model was 8 Hz (measured as a full width at half of maximum). The residual main field inhomogeneity was estimated for each voxel by using NAA, choline, and creatine as internal reference signals to determine bulk shifts in the main field. Estimated metabolite concentrations were then displayed in a 32 × 32-pixel image format for each slice (Figure 4).

The next step involved merging information from the anatomical, spectroscopic, and field-map images. Residual scalp signal was manually removed from the spectroscopic images (Figure 4, B), and the gray scale of the late echo image from the anatomical acquisition was reversed (Figure 4, A) to make fluid-tissue contrast (dark CSF and light tissue) similar to that in field-map and spectroscopic images. Rigid body transformations using 3 translations and 3 rotations ⁵ⁱ defined the reslicing of the 3-dimensional spectroscopic data (NAA, choline, and creatine) to the 2-dimensional anatomical slice positions. The field-map data were registered with anatomical data in a similar manner using spin-density images (Figure 4, C) collected as part of the field-map acquisition (to provide the anatomical resolution absent from the actual field map) and interpolated to 32 × 32 pixels. After the metabolite (Figure 4, D) and field-map data had been resliced to correspond to the structural anatomical images, voxels with good homogeneity (B₀ shifts within the range of ±5 Hz) and without lipid artifact from subcutaneous scalp fat (>60% tissue) were selected for analysis from 3 slices (1-3 in Figure 4) covering cortical gray and white matter but excluding ventricular CSF and subcortical structures. A metabolite signal intensity-tissue volume ratio (an indicator of metabolite concentration) for each voxel was then regressed against the gray matter-total tissue (but not CSF) ratio for each voxel using a weighted least-squares procedure to yield unbiased estimates of slope. Intercept values at 1 and 0 represent metabolite signal intensity for gray and white matter, respectively. Standard errors for gray and white matter estimates, which included both additive noise and partial voluming effects ⁵² were also calculated. This regression analysis was performed separately for NAA, creatine, choline, NAA:creatine, and NAA:choline. Repeated-measures analyses of variance were performed for the factors of group (patients with schizophrenia and controls) and tissue (white and gray matter) for each metabolite signal-intensity measures, using a standard software program (StatView, Abacus Concepts, Inc, Berkeley, Calif). Where significant group or tissue differences were observed, follow-up 2-tailed Student t tests and nonparametric statistics were applied, with the α set at P < .05.

RESULTS

The groups did not differ significantly in age. As expected, the control subjects had more years of education than patients, but the groups did not differ significantly in premorbid intelligence as measured by the National Adult Reading Test or on parental socioeconomic status. The absolute bid intelligence as measured by the National Adult Read-

ARCH GEN PSYCHIATRY/VOL 55, APR 1998

348

Downloaded From: http://archpsyc.jamanetwork.com/ on 11/07/2016 ©1998 American Medical Association. All rights reserved.
expressed as a ratio of either creatine or choline was also higher in gray matter than in white matter in both groups. For the NAA:creatine ratio, the group-tissue interaction was significant, indicating that NAA:creatine was disproportionately lower in 1 tissue type in patients with schizophrenia relative to controls. Follow-up t tests revealed that the NAA:creatine was lower in patients with schizophrenia than in controls in white matter ($t_{(1,17)} = 3.03; P = .008$), but not in gray matter ($t_{(1,17)} = 0.71; P = .49$); nonparametric testing yielded the same results (Table 2).

**COMMENT**

We assessed the gross volumes of gray and white matter from the upper portions of the prefrontal, temporal, parietal, and occipital lobes, as well as the centrum semiovale, and estimated NAA signal intensity for these white and gray matter volumes. Although the gross gray matter volume of this region was 18% less in patients with schizophrenia than in control subjects, no significant difference in gray matter NAA signal intensity was detected. In contrast, white matter volume was not less in patients with schizophrenia than in controls, but white matter NAA signal intensity was 7% less in those with schizophrenia.

Our methods included several innovations not previously applied to studies of patients with schizophrenia. First, we normalized coil loading and receiver gain settings to a standard value in postacquisition processing to provide both absolute metabolite measurements and ratio measures (NAA:creatine and NAA:choline). Ratio measures are included here to enable a comparison between our results and those of other laboratories. Second, we used regression analysis to estimate gray and white matter NAA, creatine, and choline.

These techniques yielded significantly higher signal intensity in gray matter than white matter for NAA and creatine, but not for choline across all subjects, consistent with in vitro studies using high-performance liquid chromatography and some in vivo MRS studies. Differences from other published results may arise from differences in acquisition methods and variables. Higher signal intensity for NAA in gray matter than white matter presumably reflects the greater proportion of gray matter than of white matter taken up by the neuronal compartment.

**Table 1. Demographics, Clinical Characteristics, and Brain Measures of Patients With Schizophrenia and Control Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Patients With Schizophrenia (n = 10)</th>
<th>Control Subjects (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>Mean (SD) 43.6 (5.9)</td>
<td>41.6 (7.2)</td>
</tr>
<tr>
<td></td>
<td>Range 34-54</td>
<td>28-52</td>
</tr>
<tr>
<td><strong>Education, y</strong></td>
<td>Mean (SD) 13.2 (1.6)†</td>
<td>16.11 (0.5)</td>
</tr>
<tr>
<td></td>
<td>Range 12-17</td>
<td>13-20</td>
</tr>
<tr>
<td><strong>Parental SES</strong></td>
<td>Mean (SD) 3.0 (0.7)</td>
<td>3.1 (1.6)</td>
</tr>
<tr>
<td></td>
<td>Range 2-4</td>
<td>1-5</td>
</tr>
<tr>
<td><strong>Brief Psychiatric Rating Scale</strong></td>
<td>Mean (SD) 35.2 (7.4)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Range 24-44</td>
<td>...</td>
</tr>
<tr>
<td><strong>Onset age of schizophrenia, y</strong></td>
<td>Mean (SD) 24.9 (4.1)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Range 18-30</td>
<td>...</td>
</tr>
<tr>
<td><strong>Brain size, cm³</strong></td>
<td>Mean (SD) 112.4 (7.3)</td>
<td>112.1 (7.6)</td>
</tr>
<tr>
<td></td>
<td>Range 103-124</td>
<td>99-120</td>
</tr>
<tr>
<td><strong>Cortical gray matter, cm³</strong></td>
<td>Mean (SD) 70.26 (8.0)†</td>
<td>85.91 (9.37)</td>
</tr>
<tr>
<td></td>
<td>Range 59.16-87.73</td>
<td>73.46-96.62</td>
</tr>
<tr>
<td><strong>Cortical gray matter, z score</strong></td>
<td>Mean (SD) −1.25 (0.99)‡</td>
<td>0.83 (0.98)</td>
</tr>
<tr>
<td></td>
<td>Range −2.11 to 1.13</td>
<td>−0.26 to 2.12</td>
</tr>
<tr>
<td><strong>Cortical white matter, cm³</strong></td>
<td>Mean (SD) 70.35 (3.8)</td>
<td>72.64 (14.83)</td>
</tr>
<tr>
<td></td>
<td>Range 64.8-77.11</td>
<td>45.16-97.62</td>
</tr>
<tr>
<td><strong>Cortical white matter, z score</strong></td>
<td>Mean (SD) −0.33 (0.81)</td>
<td>0.02 (0.82)</td>
</tr>
<tr>
<td></td>
<td>Range −1.48 to 0.86</td>
<td>−0.9 to 1.66</td>
</tr>
</tbody>
</table>

*The 2-tailed $t$ ($1,17$) test was used to determine significance. SES indicates socioeconomic status; NART-IQ, National Adult Reading Test intelligence quotient; and ellipses, not applicable.

†$P<.05$.

‡$P<.001$. 

Figure 1. Each box represents the total magnetic resonance imaging–derived volume of cortical gray matter from different hypothetical subjects with different proportions of neurons (N) and glia (G). Box C represents a hypothetically normal brain, and boxes I, II, and III represent 3 cases with reduced cortical gray matter volume. This figure illustrates that grossly reduced gray matter volume may involve an equivalent diminution of both neuronal and glial compartments (I) or preferential diminution of 1 compartment over the other (II and III). Neuronal gray matter proportion, and hence, N-acetylaspartate (NAA) concentration, in II would be equal to that in controls (C). Neuronal gray matter proportion, and hence, NAA concentration, in II would be less than that in controls (C) and in III would be greater than in controls.

Figure 2. Eight axial slices from a spin-echo sequence segmented into gray matter (dark gray), white matter (light gray), and cerebrospinal fluid (black). The superior slices (1-3) from this sequence correspond to the 3 superior 6.4-mm slices (1-3) from the spectroscopic sequence (Figure 4).
According to the model for gray matter composition outlined above, our finding of no difference in gray matter NAA signal intensity between patients with schizophrenia and control subjects, despite a gray matter volume deficit, is consistent with a process affecting neuronal and glial compartments equally (model I in Figure 1). Not supported by this finding are scenarios illustrated as models II and III in Figure 1 that could occur with either neurodegeneration (model II) or a failure in dendritic pruning, leading to an overrepresentation of the neuronal compartment (model III). Although we found no evidence to support a neurodegeneration model in schizophrenia, our technique is sensitive enough to detect an age-related reduction in the ratio of gray to white matter for NAA.

Although the cortical gray matter deficit is widespread in patients with schizophrenia, it is worse in the prefrontal and temporal lobes than in other regions (such as the occipital lobes). Frontal lobe deficits in NAA, with normal values elsewhere, have been reported by other investigators. It remains to be established whether a similar pattern can be found using the techniques described in this article. The cortical sample examined for gray matter volume and NAA signal intensity in this study was defined to exclude lateral ventricular fluid, of which there was an insignificantly greater amount in patients with schizophrenia (26 cm³) than in controls (19 cm³). Despite this, the 3 slices used for analysis were at the same position relative to the index slice in all subjects, and thus, comparable regions of cortex were analyzed in both groups.

Our in vivo MRI and MRS imaging data do not provide adequate resolution to directly address the relative contribution of changes in neuronal and glial compartments to gross changes in cortical gray matter volume. In contrast, in vitro techniques enable detailed analyses of the number, size, and density of neurons and glia separately. One study reported equivalent numbers of neurons in schizophrenic patients and control subjects, despite reduced cortical volumes, suggesting increased neuronal density in the cortex. Selemon et al found that neuronal density was higher in both prefrontal and occipital regions in schizophrenic than in control subjects. Glial density was also increased in both regions, an effect that did not reach statistical significance, perhaps because fewer cases were available for this analysis. There was also a reduction of neuronal but not glial size in the pre-
The NAA deficit in schizophrenia is located exclusively in white rather than gray matter.

**CONCLUSION**

This study demonstrates the value of differentiating between brain white and gray matter origins for NAA signal intensity, provides new insights into mechanisms underling the gross deficit in cortical gray matter observed in schizophrenia, and may provide a new approach for assessing the role of impaired cortical and subcortical connectivity in the pathogenesis of schizophrenia.

Accepted for publication July 30, 1997.

This work was supported by the Department of Veterans Affairs, Palo Alto, Calif; the National Institutes of Health, Bethesda, Md (MH 30854, AG11427, RR09784, CA48269); and the Norris Foundation, Stanford, Calif.

Earlier versions of this report have been presented at the annual meetings of the Society for Biological Psychiatry, Chicago, Ill, May 3, 1996; the American College of Neuropsychopharmacology, San Juan, Puerto Rico, December 9-13, 1996; and the International Congress of Schizophrenia Research, Colorado Springs, Colo, April 12-16, 1997.

The following people provided invaluable roles in this project: Sarah Rawson, MA, and the staff of the Mental Health Clinical Research Center, Palo Alto, Calif, for the recruitment and clinical description of schizophrenic patients, Amy Lutz, BA, for scheduling the control subjects, and James Kucik, BA, and Monika Tucker, BA, for assisting in spectroscopic image acquisition.

Reprints: Kelvin O. Lim, MD, Division of Medical Physics, Nathan S. Kline Institute for Psychiatric Research, 140 Old Orangeburg Rd, Orangeburg, NY 10962 (e-mail: lim@nki.rfmh.org).

©1998 American Medical Association. All rights reserved.
REFERENCES


