Lower Concentration of Hippocampal N-Acetylaspartate in Familial Bipolar I Disorder

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Objective: Previous studies attempting to identify neuropathological alterations in the hippocampus in bipolar disorder have been inconclusive. The objective of this study was to determine if the concentration of N-acetylaspartate, a neuronal and axonal marker, was lower in subjects with familial bipolar I disorder than in healthy comparison subjects, suggesting possible neuronal loss, neuronal dysfunction, or neuropil reduction in bipolar I disorder.

Method: N-acetylaspartate, choline, and creatine in the right and left hippocampus were measured in 15 euthymic male patients with familial bipolar I disorder and 20 healthy male comparison subjects by using proton magnetic resonance spectroscopy (1H-MRS).

Results: Relative to the comparison group, the patients with bipolar I disorder demonstrated significantly lower concentrations of N-acetylaspartate and creatine but normal choline concentration in both the right and left hippocampus. There were no group or lateralized differences in the percentages of different tissue types within the MRS voxels, suggesting that the hippocampal N-acetylaspartate and creatine alterations were not an artifact of variations in tissue types represented in the voxels. There was also a significant negative correlation between N-acetylaspartate concentration in the right hippocampus and illness duration, after adjustment for the effects of age.

Conclusions: This preliminary study provides support for the existence of neuronal loss, neuronal metabolic dysfunction, or interneuronal neuropil reduction in the hippocampal region in male patients with familial bipolar I disorder. The finding of normal hippocampal choline levels in these patients does not provide support for ongoing myelin breakdown or glial cell proliferation in this brain region in familial bipolar I disorder. The significant association between illness duration and N-acetylaspartate concentration in the right hippocampus supports the idea that neuronal pathology may increase with disease progression and that this effect may be lateralized, involving the right but not the left hippocampus.

Evidence has been far from conclusive as to whether there are abnormalities of hippocampal structure and function in bipolar disorder. Whereas a smaller total number and smaller density and size of nonpyramidal hippocampal neurons have been reported by Benes and colleagues (1), six of the seven published volumetric magnetic resonance imaging (MRI) studies of bipolar disorder that we are aware of reported no differences in hippocampal volumes of bipolar disorder patients relative to comparison subjects (2–7). Only one study noted a smaller right hippocampus in the bipolar disorder group (8). However, three high-resolution MRI studies of recurrent major depression demonstrated significantly smaller hippocampal volumes (9–11). Furthermore, in one study, smaller hippocampal volumes were correlated with the longer duration of major depression, suggesting that recurrent episodes of depression may result in hippocampal neuronal loss (9). One question that arises from these studies of mood disorders is whether hippocampal pathology in bipolar disorder is more subtle than that observed in major depression, perhaps too subtle to be reliably detected by MRI volumetric techniques. Furthermore, MRI volumetric techniques are currently not able to differentiate between smaller gray matter tissue volumes resulting from neuronal loss or atrophy and smaller volumes resulting from reductions in glial cell number or density.

N-Acetylaspartate is the second most abundant amino acid in the central nervous system and is reliably measured by proton magnetic resonance spectroscopy (1H-MRS). N-Acetylaspartate is formed in mitochondria from acetyl coenzyme A and aspartate by the membrane-bound enzyme L-aspartate N-acetyltransferase, an enzyme selectively found in brain (12). In studies using immunohistochemical techniques, N-acetylaspartate has been shown to be predominantly localized to neurons, axons, and dendrites within the CNS (13). Animal models of neuronal injury have been shown to give good correlations between N-acetylaspartate levels as measured by 1H-MRS and neuronal counts as measured by histological techniques (14–16). Low N-acetylaspartate is thought to represent loss of
neurons and/or axons, reduction of interneuronal neuropil, and neuronal or axonal metabolic dysfunction or damage (17–20). Our previous study of patients with schizophrenia (21) found low N-acetylaspartate bilaterally in the absence of smaller hippocampal volume as measured by MRI, supporting the idea that N-acetylaspartate may be a more sensitive marker of neuronal damage or loss than quantitative MRI measurements of tissue loss.

The choline signal measured by $^1$H-MRS is derived predominantly from constituents of membrane phospholipid metabolism (22, 23), such as glycerophosphocholine and phosphocholine, which account for more than 50% of the resonance, and glycerophosphoethanolamine and phosphoethanolamine, which account for between 10% (in adults) and 25% (in the newborn) of the resonance. The choline signal is known to be significantly higher in conditions where there is ongoing myelin breakdown and/or glial cell proliferation, such as multiple sclerosis, adrenoleukodystrophy, brain tumors, and reactive gliosis (24, 25). While two earlier studies (26, 27) reported that changes in dietary choline could affect the choline signal, more recent studies have demonstrated that neither short-term nor long-term administration of oral choline appears to alter the $^1$H-MRS choline resonance or the proton-decoupled phosphorous MRS glycerophosphorylcholine and phosphorylcholine resonances in cortical gray matter, cortical white matter, basal ganglia, thalamus, and cerebellum (28, 29).

To our knowledge, no published studies to date have used $^1$H-MRS to investigate hippocampal neuronal loss or dysfunction in bipolar disorder. Renshaw and colleagues (30) reported lower N-acetylaspartate measures bilaterally in the temporal lobe in 13 patients with first-episode psychosis, including six bipolar disorder patients; however, their findings were not specific to the hippocampus. Therefore, we used $^1$H-MRS to test the hypothesis that hippocampal N-acetylaspartate was lower in patients with bipolar I disorder, suggesting neuronal loss and/or dysfunction. A second objective was to determine if the choline level in this region was higher, suggesting possible myelin breakdown or significant glial cell proliferation. A third objective was to determine if lower measures of N-acetylaspartate in the hippocampus were associated with illness duration. To avoid the potential confounding effects of gender and clinical-state-dependent changes on N-acetylaspartate measures, and because emerging evidence has suggested that abnormalities in brain structure and function may be more prominent in familial mood disorders (31, 32), this study examined only euthymic male patients with familial bipolar I disorder.

Method

Subjects

Fifteen male patients who met the DSM-IV criteria for bipolar I disorder (mean age=39.3 years, SD=10.3) and 20 male comparison subjects (mean age=36.0 years, SD=10.7) gave written informed consent for participation in the study after the procedures had been fully explained. The procedures were approved by the University of California, San Francisco, Committee on Human Research. Subjects were recruited from the San Francisco Veterans Affairs Medical Center, local outpatient mental health clinics, and the local community by means of a newspaper advertisement. All subjects with bipolar I disorder were stable outpatients at the time of the study. The demographic and clinical characteristics of the patients are summarized in Table 1. The diagnosis of bipolar I disorder was confirmed by using the Structured Clinical Interview for DSM-IV (SCID) (33). The mean time since onset of illness was 18.7 years (SD=9.5). The mean number of years of education was 14.3 (SD=2.5) for the patients and 15.4 (SD=2.7) for the comparison subjects. To avoid the potential confounding effect of gender, only male subjects were included in the study. All of the patients had a family history of major mood disorder in first-degree relatives (parent, sibling, or offspring). The comparison subjects were also assessed by using the SCID to rule out any major axis I diagnoses. All subjects were right-handed, as assessed by the Annett Handedness Questionnaire (34). All but three of the patients were taking medication at the time of the study. The three patients who were not taking medication at the time of the study had elected to temporarily discontinue their medications several months before the study during a period of sustained euthymia. The patients had all been euthymic for at least 2 months before the study, as documented by clinical interview, history, and scores on the Young Mania Rating Scale (35) and Hamilton Depression Rating Scale (36) on the day of the $^1$H-MRS study. All patients achieved a score of 6 or less on each of these separate rating scales. None of the patients had a history of head injury, organic mental disorder, neurological disorder, cerebrovascular disease, schizophrenia, or anxiety disorder, and none had a history of clinically significant alcohol or substance abuse in the 12 months before the study. None of the comparison subjects had a history of significant medical illness, head injury, neurological disorder, psychiatric disorder, or alcohol or substance abuse, and none had a family history of psychiatric disorder. The patient and comparison groups had no significant differences in age ($t=0.91, df=33, p=0.37$), years of education ($t=1.19, df=33, p=0.24$), or years of parental education ($t=0.80, df=33, p=0.43$).

MRI/$^1$H-MRS Examinations

All MRI/$^1$H-MRS studies were performed at the Magnetic Resonance Unit of the San Francisco Veterans Affairs Medical Center on a Siemens 1.5-T Magnetom VISION magnetic resonance imaging/spectroscopy (MRI/MRS) system (Siemens, New York) with a standard circularly polarized head coil. Each subject was assigned a numeric code to allow measurement and data processing by researchers who were blind to the subject’s group. MRI sequences included 1) $T_1$-weighted scout views in the sagittal, coronal, and axial planes for positioning MRI and $^1$H-MRS slices, 2) axial $T_2$-weighted images from double spin echo (TR=3000 msec, TE1=20 msec, TE2=80 msec, resolution=1×1.4 mm$^2$, slice thickness=3 mm), oriented –10° off the planum sphenoidale, and 3) coronal $T_1$-weighted images from three-dimensional magnetization-prepared rapid acquisition gradient echo (MP-RAGE) (TR=10 msec, TI=250 msec, TE=4 msec, resolution 1×1 mm$^2$ resolution, 1.4 mm slice thickness), orthogonal to the long axis of the hippocampus. The MRI scans from both the comparison subjects and the patients were evaluated by a board-certified neuroradiologist (R.E.) to determine if any abnormalities were present. $^1$H-MRS data were acquired by using a spin echo $^1$H-MRS sequence at TR=1800 msec and TE=135 msec with preselection of a region of interest for point-resolved spectroscopy. The point-resolved spectroscopy volume for the hippocampal region was angulated parallel to the long axis of the hippocampi as seen from the sagittal scout images and positioned on the axial plane to include both hippocampi in their entire length and adjacent sections of the midbrain and
TABLE 1. Demographic and Clinical Characteristics of Euthymic Male Outpatients With Bipolar I Disorder in a Proton Magnetic Resonance Spectroscopy Study of Hippocampal N-Acetylaspartate Concentration

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Race</th>
<th>Education (years)</th>
<th>Parental Education (years)</th>
<th>Illness Duration (years)</th>
<th>Young Mania Rating Scale Score</th>
<th>Hamilton Depression Rating Scale Score</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>Caucasian</td>
<td>18</td>
<td>16</td>
<td>10</td>
<td>3</td>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>Caucasian</td>
<td>12</td>
<td>14</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>Divalproex, 1500 mg/day; sertraline, 50 mg/day</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>Caucasian</td>
<td>14</td>
<td>12</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>Divalproex, 1500 mg/day</td>
</tr>
<tr>
<td>4</td>
<td>54</td>
<td>Caucasian</td>
<td>12</td>
<td>16</td>
<td>30</td>
<td>2</td>
<td>5</td>
<td>Divalproex, 2000 mg/day; olanzapine, 10 mg/day</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>Caucasian</td>
<td>18</td>
<td>16</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>Lithium, 900 mg/day</td>
</tr>
<tr>
<td>6</td>
<td>56</td>
<td>Caucasian</td>
<td>16</td>
<td>13</td>
<td>22</td>
<td>3</td>
<td>0</td>
<td>Lithium, 1350 mg/day; divalproex, 2000 mg/day</td>
</tr>
<tr>
<td>7</td>
<td>39</td>
<td>Caucasian</td>
<td>13</td>
<td>10</td>
<td>29</td>
<td>0</td>
<td>1</td>
<td>Lithium, 900 mg/day</td>
</tr>
<tr>
<td>8</td>
<td>40</td>
<td>Caucasian</td>
<td>18</td>
<td>14</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>Divalproex, 1000 mg/day; bupropion, 225 mg/day</td>
</tr>
<tr>
<td>9</td>
<td>45</td>
<td>Caucasian</td>
<td>12</td>
<td>9</td>
<td>30</td>
<td>4</td>
<td>0</td>
<td>Lithium, 1200 mg/day; paroxetine, 40 mg/day</td>
</tr>
<tr>
<td>10</td>
<td>41</td>
<td>Caucasian</td>
<td>12</td>
<td>14</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>Divalproex, 1500 mg/day</td>
</tr>
<tr>
<td>11</td>
<td>33</td>
<td>Caucasian</td>
<td>16</td>
<td>16</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>12</td>
<td>36</td>
<td>Caucasian</td>
<td>13</td>
<td>15</td>
<td>14</td>
<td>0</td>
<td>5</td>
<td>Divalproex, 750 mg/day; bupropion, 300 mg/day</td>
</tr>
<tr>
<td>13</td>
<td>23</td>
<td>Caucasian</td>
<td>11</td>
<td>17</td>
<td>11</td>
<td>5</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>14</td>
<td>49</td>
<td>Caucasian</td>
<td>14</td>
<td>15</td>
<td>15</td>
<td>3</td>
<td>4</td>
<td>Olanzapine, 10 mg/day; trazodone, 300 mg/day</td>
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<tr>
<td>15</td>
<td>30</td>
<td>Caucasian</td>
<td>16</td>
<td>18</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>Olanzapine, 10 mg/day</td>
</tr>
</tbody>
</table>

a Average years of education of parents.

The MRI tissue segmentation and volumetric methods have been previously described (38) and have been validated (39) by using an MRI simulator and digital brain phantoms developed by investigators at the Montreal Neurological Institute (40, 41), with percentage volume differences of less than 5% and spatial distribution overlaps greater than 0.94 (1.00 is perfect). The double first-pass autosegmentation, which separated the brain into gray matter, white matter, and CSF. In-house software was used to reliably differentiate gray matter and white matter within the hippocampus itself. Reliability studies for 10 healthy subjects on two separate occasions revealed 1) interoperator correlations of 0.99 for ventricular CSF; 0.98 for sulcal CSF; 0.95 for cortical gray matter, 0.92 for white matter, 0.94 for the percentage of white matter signal hyperintensity, 0.94 for the right hippocampal tissue volume mask, and 0.95 for the left hippocampal tissue volume mask, and 2) intraoperator correlations of 0.99 for ventricular CSF; 0.99 for sulcal CSF; 0.97 for cortical gray matter, 0.93 for white matter, 0.97 for percentage of white matter signal hyperintensity, 0.98 for the right hippocampal tissue volume mask, and 0.98 for the left hippocampal tissue volume mask.

MRI Tissue Segmentation

The MRI tissue segmentation and volumetric methods have been previously described (38) and have been validated (39) by using an MRI simulator and digital brain phantoms developed by investigators at the Montreal Neurological Institute (40, 41), with percentage volume differences of less than 5% and spatial distribution overlaps greater than 0.94 (1.00 is perfect). The double spin echo study was in the axial plane with 3-mm interleaved slices. The three-dimensional T1 study used the Siemens MP-RAGE sequence, which gathers 1.4-mm thick coronal slices with an in-plane pixel size of 0.97×0.97 mm. Analysis began with the first-pass autosegmentation, which separated the brain into gray matter, white matter, and CSF. In-house software was used to 1) remove the skull and meninges from the images, 2) coregister each of the interleaves of the spin echo images to T1 images reformatted to the axial plane, 3) correct radio frequency inhomogeneity in three dimensions, 4) define seeds (based on peaks in the three-dimensional histogram of T1 pixel intensities) for the K-means cluster analysis, and 5) transfer the data to statistical software that performs the actual cluster analysis. The initial process was followed by computer-assisted editing of the data to separate cortical from subcortical gray matter, to separate ventricular CSF from sulcal CSF, and to reclassify white matter incorrectly classified in the first pass into a category of white matter with an abnormal MRI signal or of white matter signal hyperintensity. This was followed by manual delineation of the boundaries of the cortical regions, subcortical structures, the cerebellum, and the hippocampus. For the hippocampus, manual tracing of the boundaries on each coronal MP-RAGE slice where the hippocampus was visible was performed to create right and left hippocampal tissue volume masks. The tracing was verified by a neuroradiologist (R.E.). Creation of hippocampal tissue volume masks was necessary because the tissue segmentation method was not able to.

MRI/MRSI Coanalysis

After acquisition, the 1H-MRS data were zero filled to a rectangular matrix of 32×32×1024 points, Fourier transformed, phase corrected, and baseline corrected by using software developed in house (42). Four-Hz Gaussian line broadening was used in the spectral direction, and mild Gaussian apodization was applied along the spatial directions to reduce Gibbs ringing effects, resulting in an effective volume of approximately 1.6 ml for the MRS voxels. By using spectroscopic imaging display software (42), a reference image of the total 1H signal was generated together with higher-resolution, spatially coregistered MR axial double spin echo images. A single operator who was blind to the subjects’ diagnostic group adjusted the coregistered MR axial image intensity and contrast to optimize visualization of the hippocampus and then selected three voxels each in the right and left hippocampus for each subject. Figure 1 (upper panel) illustrates three representative right hippocampal voxels. 1H spectra from these voxels were fit by an automated curve fitting software program developed in our laboratory (43). A typical spectrum from a single hippocampal MRS voxel is shown in Figure 1 (lower panel). Absolute integrals were corrected for differential head coil loading and receiver gain for each subject to obtain concentration estimates of N-acetylaspartate, choline, and creatine in institutional units. To help control for any motion artifacts, line widths for N-acetylaspartate, choline, and creatine were measured and found not to be significantly different between groups. N-Acetylaspartate, choline, and creatine concentration estimates were obtained.
rather than metabolite peak ratios such as the ratio of N-acetylaspartate to creatine or of N-acetylaspartate to choline, because N-acetylaspartate metabolite ratios must account for alterations in the creatine reference peak that invalidate their usefulness as an internal reference for the other metabolite peaks of the proton spectrum (24).

To obtain tissue-volume-corrected N-acetylaspartate, choline, and creatine concentration estimates and to verify that any alterations in these metabolites were not an artifact of variations in the tissue composition of the MRS voxels, the tissue enclosed in each voxel was analyzed for amounts of hippocampus, surrounding white matter outside the hippocampus (extrahippocampal white matter), surrounding gray matter outside the hippocampus (extrahippocampal gray matter), and CSF. By using in-house software, the tissue composition of each MRS voxel was determined from MRI segmentation data, taking into account the point spread function, chemical shift offset due to use of slice-selective gradients in the presence of radio frequency pulses, the slice profile, and post-acquisition spatial filtering functions used to reduce voxel bleeding. The tissue content, p, in each voxel was estimated with the following equation: 

\[
p = \frac{\text{hippocampus}}{\text{hippocampus} + \text{extrahippocampal gray matter}}
\]

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\[
p = \frac{\text{hippocampus}}{\text{hippocampus} + \text{extrahippocampal gray matter}}
\]
right and left hippocampi. There were no significant group or lateralized differences for choline, MRS voxel % hippocampus, MRS voxel % extrahippocampal white matter, or MRS voxel % extrahippocampal gray matter.

ANCOVA analysis revealed that neither variations of the MRS voxel % hippocampus, MRS voxel % extrahippocampal white matter, or MRS voxel % extrahippocampal gray matter contributed significantly to the group differences in N-acetylaspartate and creatine. These results suggest that the group differences in N-acetylaspartate and creatine are not simply an artifact of variations in the tissue composition of the selected voxels.

In the bipolar I disorder group, there was a significant negative correlation between right hippocampal N-acetylaspartate concentration and years of illness (r=-0.66, N=15, p=0.003) after adjusting for age (Figure 2). There was no significant correlation between left hippocampal N-acetylaspartate concentration and years of illness. With regard to mood stabilizing medications, there were no significant correlations between 1) N-acetylaspartate concentration and divalproex dose for either the right or left hippocampus or 2) N-acetylaspartate concentration and lithium dose for either the right or the left hippocampus.

Discussion

To our knowledge, this is the first published report of significantly lower N-acetylaspartate in both the right and left hippocampus in euthymic male patients with familial bipolar I disorder, relative to healthy comparison subjects. Previous 1H-MRS studies of other brain regions in bipolar disorder have reported lower N-acetylaspartate bilaterally in the dorsolateral prefrontal region (46), as well as normal N-acetylaspartate measures in the lenticular nuclei (47) and frontal lobes (48). Given that N-acetylaspartate is found only in neurons and axons but not mature glial cells, lower hippocampal N-acetylaspartate measures suggest loss of neurons and/or axons, reduction of interneuronal neuropil, neuronal or axonal metabolic dysfunction, or some combination of these processes. The finding of normal choline levels in the bipolar I disorder patients suggests that there is no biochemical evidence of myelin breakdown or glial cell proliferation in the hippocampus. A finding of lower hippocampal N-acetylaspartate is consistent with the smaller total number, cell density, and cell size of nonpyramidal hippocampal neurons observed in the postmortem study by Benes and colleagues (1).

Lower hippocampal N-acetylaspartate may be the result of stress-induced cellular changes mediated by higher levels of glucocorticoids. The two major structural changes in the hippocampus that occur as a result of stress are atrophy of CA3 pyramidal neurons (49) and reduced adult neurogenesis of granule cells in the dentate gyrus (50, 51). A number of studies over the years have demonstrated how stress and adrenal glucocorticoids directly cause hippocampal neuronal atrophy as well as reduce cellular resilience, thereby making neurons more vulnerable to ischemia, hypoglycemia, and excitatory amino acid toxicity (49, 52, 53). Since hypercortisolism is a frequent finding during episodes of major depression, elevations in gluco-

### Table 2. Right and Left Hippocampal N-Acetylaspartate, Choline, and Creatine Concentrations and MRS Voxel Tissue Composition in Euthymic Male Outpatients with Bipolar I Disorder and Healthy Comparison Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients With Bipolar I Disorder (N=15)</th>
<th>Comparison Subjects (N=20)</th>
<th>Repeated-Measures Analysis of Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>N-Acetylaspartate</td>
<td>2.75</td>
<td>0.33</td>
<td>2.87</td>
</tr>
<tr>
<td>Choline</td>
<td>7.67</td>
<td>1.17</td>
<td>7.94</td>
</tr>
<tr>
<td>Creatine</td>
<td>2.77</td>
<td>0.32</td>
<td>2.87</td>
</tr>
<tr>
<td>Hippocampal tissue in MRS voxels (mean %)</td>
<td>28.99</td>
<td>2.75</td>
<td>28.95</td>
</tr>
<tr>
<td>Extrahippocampal white matter tissue in MRS voxels (mean %)</td>
<td>45.48</td>
<td>6.96</td>
<td>46.26</td>
</tr>
<tr>
<td>Extrahippocampal gray matter tissue in MRS voxels (mean %)</td>
<td>24.39</td>
<td>5.97</td>
<td>24.61</td>
</tr>
</tbody>
</table>

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**Notes:**

- Acetylaspartate, Choline, and Creatine Concentrations and MRS Voxel Tissue Composition in Euthymic Male Outpatients with Bipolar I Disorder and Healthy Comparison Subjects.

- Measured in institutional units (metabolite intensity per tissue volume). Metabolite intensity is the area under the metabolite resonance curve, including corrections for instrumental variables (such as receiver gain and differential head coil loading).

- Tissue enclosed in MRS voxels on each side of the hippocampus.
corticoid levels may induce similar hippocampal neuronal alterations as a result of repeated episodes of depression; however, the evidence for this relationship at present remains indirect (53). Therefore, it is reasonable to hypothesize that glucocorticoid-induced cell damage or loss, thought to be responsible for the smaller hippocampal volumes observed by means of MRI in major depression, may also be occurring in bipolar I disorder, resulting in compromised neuronal function or loss and lower hippocampal N-acetylaspartate measures. However, it should be noted that none of the bipolar I disorder patients in this study were assessed for elevations in glucocorticoids.

Alterations in neurotrophic or neuroprotective factors may also underlie lower hippocampal N-acetylaspartate measures. Stress appears to decrease expression of brain-derived neurotrophic factor (BDNF) (54), which is critical for both the survival and function of neurons in the developing and adult brain (55). Thus, stress-induced reductions in BDNF could lead to damage and loss of hippocampal neurons. Another major neuroprotective and neurotrophic factor, B-cell lymphoma/leukemia-2 gene proteins (Bcl-2), inhibits programmed cell death (56, 57); yet, when overexpressed, Bcl-2 promotes regeneration of axons and regulates neurite sprouting (58). It is interesting to note that lithium and valproate appear to robustly increase Bcl-2 levels in both in vitro and in vivo experimental models (20). Both BDNF and Bcl-2 expression are regulated by the transcription factor cyclic adenosine monophosphate response element-binding protein (CREB), and recent studies have shown lower levels of CREB in the temporal cortex in depressed patients (59) and up-regulated CREB and hippocampal expression of BDNF in response to chronic antidepressant treatment (60–62). Findings such as these support the idea that major mood disorders could result from a failure or compromise of mechanisms that regulate neuronal plasticity. It is therefore conceivable that reductions in BDNF and/or Bcl-2 are occurring in bipolar I disorder, adversely affecting hippocampal neuronal plasticity and survival and resulting in a lower hippocampal N-acetylaspartate levels.

Alternatively, a lower hippocampal N-acetylaspartate may be a consequence of alterations in reelin, a secretory glycoprotein responsible for normal lamination of the brain. In the adult mammalian brain, reelin is localized to layer I cortical Cajal-Retzius cells, cortical and hippocampal interneurons, and cerebellar granule cells (63, 64). Reelin contributes to neuronal plasticity in the brains of adults by acting on integrin receptors expressed in spine postsynaptic densities. An important consequence of reelin signaling is the activation of the focal adhesion tyrosine kinase system, which is a component of a postsynaptic mechanism responsible for an increase in the number of synapses and alterations in postsynaptic structure in axons, dendrites, and the intermediate filament cytoskeleton of astrocytes (63, 65). The heterozygous reeler mouse, which expresses only 50% of the reelin protein levels seen in wild-type mice, exhibits reduced cortical neuropil expression, neuronal packing density, and dendritic spine density (66). Recent studies of patients with bipolar disorder have reported 1) lower blood levels of reelin and its isoforms (63, 2) lower reelin mRNA in prefrontal cortex and cerebellum (64, 67), and 3) lower reelin protein in the CA4 areas of the hippocampus (68). Therefore, lower levels of hippocampal reelin in bipolar disorder could result in lower hippocampal N-acetylaspartate, reflecting shrinkage of the interneuronal neuropil.

The second finding of this study was a lower concentration of hippocampal creatine bilaterally in the bipolar I disorder patients than in the comparison subjects. The creatine signal measures both creatine and phosphocreatine together, which are in rapid chemical enzymatic exchange in the human brain. In addition, the level of creatine in the brain can be significantly altered by osmotic forces as well as by extracerebral events due to the complex biosynthetic pathway through liver and kidney enzymes (24). Because of this complexity, it is difficult to attribute differences in creatine simply to local derangements of cellular energy metabolism. Nevertheless, since phosphocreatine is linked to ATP through the creatine-kinase equilibrium, alterations in creatine could be related to changes in hippocampal metabolism.

The third finding of this study was that lower hippocampal N-acetylaspartate and creatine concentrations cannot be attributed to differences in voxel tissue heterogeneity. This analysis was made possible by using information from coregistered MRI tissue segmentation data. This result is important because it documents that the observed alterations in hippocampal N-acetylaspartate and crea-
tine are not merely a reflection of differences in the tissue composition of the MRS voxels. Furthermore, it emphasizes the value of using MRI-derived tissue segmentation information to improve the analysis of spectral metabolite data from $^1$H-MRS investigations.

The fourth finding of this study was the presence of a significant negative correlation between right hippocampal N-acetylaspartate concentration and illness duration. This finding supports the idea that neuronal atrophy, loss, or dysfunction may increase in this region with disease progression. To our knowledge, this is the first in vivo $^1$H-MRS study of bipolar disorder to report such a correlation. Furthermore, none of the $^1$H-MRS studies of schizophrenia have reported a significant negative correlation between illness duration and hippocampal or medial temporal lobe N-acetylaspartate measures (21, 69–77). This finding is consistent with previous findings of 1) a significant negative correlation between right temporal lobe volume and duration of illness in male patients with bipolar disorder (78), 2) a negative correlation between age at onset of bipolar I disorder and N-acetylaspartate measures in the right basal ganglia (47), and 3) negative correlations between illness duration and both right and left prefrontal N-acetylaspartate measures (46). Taken together, these findings suggest that illness progression may result in further neuropathological changes in multiple brain regions in bipolar disorder and may perhaps involve brain regions in the right hemisphere more than the left.

With regard to the limitations of the present study, the long-term effects of medications on the hippocampus, which are not known, may have influenced the results. Although no significant associations between N-acetylaspartate concentrations and either valproic acid or lithium doses were found, it is conceivable that long-term exposure to neuroleptics, mood stabilizers, or antidepressants may be associated with alterations in both the structure and neuronal integrity of the hippocampus. Preliminary studies have suggested that both atypical and typical neuroleptics may increase previously low N-acetylaspartate levels toward more normative values in the anterior cingulate and dorsolateral prefrontal regions in schizophrenia (79, 80). Studies of healthy volunteers and bipolar disorder patients demonstrated that 4 weeks of lithium administration significantly increased N-acetylaspartate in the frontal, temporal, parietal, and occipital lobes (81), as well as significantly increased total gray matter content in the bipolar disorder patients (81, 82). There is also evidence that valproate activates pathways that regulate BDNF, robustly increases trophic factors such as Bcl-2 and growth cone proteins, and promotes neurite outgrowth in as well as prolongs survival of human neuroblastoma cells (20). Although antidepressant effects on N-acetylaspartate measures have not been investigated, chronic administration of several different classes of antidepressants has been shown to increase neurogenesis in the adult rodent hippocampus (83–86), and this effect may be related to antidepressant-induced up-regulation of hippocampal CREB and BDNF (60–62). If chronic administration of antidepressants, lithium, and valproate can all potentially increase N-acetylaspartate by promoting neurogenesis, this would mean that hippocampal N-acetylaspartate measures in unmedicated bipolar I disorder patients might be even lower than those reported in the present study of mostly medicated patients.

Finally, the number of subjects was small, and alterations in the T1 or T2 of proton metabolites in the hippocampal region of the patients might have contributed to the observed differences. In other words, as a consequence of relaxation time differences, the N-acetylaspartate measures observed in this study may have to some degree reflected differences in metabolite variability as well as metabolite concentrations; however, there is no evidence that N-acetylaspartate in bipolar disorder patients has abnormal relaxation times. Nevertheless, future studies will probably need to determine if there are significant alterations in N-acetylaspartate relaxation times in the hippocampus of patients with bipolar I disorder.

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