N-Acetylaspartylglutamate (NAAG) and N-Acetylaspartate (NAA) in Patients With Schizophrenia

Frank Jessen*,1,2, Natascha Fingerhut1, Alois M. Sprinkart3, Kai-Uwe Kühn4, Nadine Petrovsky1, Wolfgang Maier1,2, Hans-H Schild3, Wolfgang Block3, Michael Wagner1, and Frank Träber3

1Department of Psychiatry and Psychotherapy, University of Bonn, Germany; 2German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany; 3Department of Radiology, University of Bonn, Germany; 4Department of Psychiatry and Psychotherapy, University of Saarland, Homburg, Germany

*To whom correspondence should be addressed; tel: +49-228-287-11109, fax: +49-228-298-16097, e-mail: frank.jessen@ukb.uni-bonn.de

Introduction

The "glutamatergic hypothesis" of schizophrenia originates from the induction of schizophrenia-like symptoms in animals and volunteers by glutamate release stimulating compounds such as the N-methyl-D-aspartate receptor (NMDAR) antagonists phencyclidine (PCP) and ketamine.1 The presynaptic metabotropic glutamate receptor 3 (mGluR3) inhibits glutamate release. The mGluR2/3 agonist LY404039 reverses PCP induced behavior in animals and has shown clinical effects by reducing psychotic symptoms in patients with schizophrenia.2

Today only postmortem investigations are available, which provide evidence for both, increased and reduced NAAG concentrations in patients with schizophrenia.3,4 In this study, we present the first in vivo evidence for altered NAAG concentration in patients with schizophrenia.

Key words: schizophrenia/N-acetylaspartate/N-acetylaspartylglutamate/magnetic resonance spectroscopy/anterior cingulate gyrus/frontal lobe

Background: Imbalance of glutamatergic neurotransmission has been proposed as a key mechanism underlying symptoms of schizophrenia. The neuropeptide N-acetylaspartylglutamate (NAAG) modulates glutamate release. NAAG provides a component of the proton magnetic resonance spectrum (1H-MRS) in humans. The signal of NAAG, however, largely overlaps with its precursor and degrading product N-acetylaspartate (NAA) that by itself does not act in glutamatergic neurotransmission. Methods: We quantified NAAG and NAA separately from the 1H-MRS signal in 20 patients with schizophrenia and 20 healthy comparison subjects on a 3.0 Tesla MR scanner. The 1H-MRS voxels were positioned in the anterior cingulate cortex (ACC) and in the left frontal lobe. Psychopathological symptoms and cognitive performance were assessed. Results: In the ACC, the ratio NAAG/NAA was increased (P = .041) and NAAG was increased at a trend level (P = .066) in patients, while NAA was reduced (P = .030). NAA correlated with attention performance in patients (r = .64, P = .005) in the ACC. There was no group difference of NAAG, NAA, or NAAG/NAA in the frontal lobe but an inverse correlation of NAAG with negatives symptoms (Positive and Negative Symptoms Scale [PANSS] negative, r = -.58, P = .018) and with the total symptom score (PANSS total, r = -.50, P = .049). In addition, there was a positive correlation of frontal lobe NAAG (r = .53, P = .035) and NAAG/NAA (r = .54, P = .030) with episodic memory in patients. Conclusions: In this study, we present the first in vivo evidence for altered NAAG concentration in patients with schizophrenia.
There are numerous reports on the combined [NAA+NAAG] peak in schizophrenia. Common observations are signal reductions in the frontal lobe and in the anterior cingulate cortex (ACC). The reduction of the combined [NAA+NAAG] signal is conventionally interpreted as an indicator of reduced NAA concentration only because NAA is highly concentrated in the brain. NAA levels are considered to reflect mitochondrial function of neurons because the synthesis of NAA is exclusively located in neurons and NAA concentration correlates with adenosine diphosphate levels. The metabolic role of NAA in the nervous system is not fully understood. Involvement in myelination, osmoregulation, and axon-glial signaling has been proposed. NAA does not act in neurotransmission. Thus, the separation of the NAAG from the NAA signal is required to study NAAG as a component of glutamatergic neurotransmission.

To date, 2 1H-MRS studies with separate measures of NAAG and NAA in healthy volunteer have been reported. These showed that the combined [NAA+NAAG] peak is composed of roughly 90% NAA and 10% NAAG. Considerably, higher NAAG fractions were observed in parietal and occipital white matter.

In the present 1H-MRS study, we separately quantified NAAG and NAA and determined the NAAG/NAA ratio in patients with schizophrenia and compared these measures with those of healthy individuals. We also assessed the correlation of NAAG, NAA, and NAAG/NAA with cognitive measures and psychopathological symptoms. We chose the left frontal lobe and the ACC as the target regions as both are considered to be functionally altered in schizophrenia.

Methods
Subjects
We recruited 20 patients with schizophrenia according the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) from the inpatient unit of the Department of Psychiatry and Psychotherapy, University of Bonn. All were on stable atypical antipsychotic medication (11 on monotherapy and 9 on combination therapy) and in partial or full remission with regard to positive symptoms. The mean time since first episode was 7.2 years (SD: 8.5). Current symptoms were assessed with the Positive and Negative Symptoms Scale (PANSS). Demographic data and PANSS scores are listed in table 1.

Twenty comparison subjects were recruited from the general population by advertisement. None of these control subjects suffered from any psychiatric or neurological disorder at present or in the past or was taking any medication. The demographic data are listed in table 1.

All participants gave written informed consent prior to the study. The protocol was approved by the local ethical committee.

Neuropsychological Assessment
A neuropsychological test battery was applied in all participants to correlate cognitive performance with 1H-MRS data. The battery included the Auditory Verbal Learning and Memory Test (AVLT), the digit span test and block span test of the revised Wechsler Memory Scale, the letter-number-sequencing test, and the digit symbol substitution test (DSST), and the trail making test (TMT) A and B.

We created composite scores for 3 cognitive domains by averaging z-transformed raw scores. These composite scores were (1) a verbal memory score derived from AVLT trials 1 to 5, 6, and 7 (Cronbach Alpha: .95), (2) a working memory score derived from the digit span test, the block span tests, and the letter-number-sequencing test (Cronbach Alpha: .94), and (3) an attention score composed of TMT A, TMT B, and DSST (Cronbach Alpha: .92). All neuropsychological raw data and the composite scores are listed in table 1.

Proton MR-Spectroscopy (1H-MRS)
The single voxel 1H-MRS study was performed on a 3.0 Tesla MR scanner (Achieva 3.0, Philips Healthcare) using a 12-element birdcage quadrature head coil (diameter 28 cm and length 23 cm) for signal transmission and reception. We positioned one voxel within the ACC (6-ml voxel size) and one voxel within the left frontal lobe (8-ml voxel size) (figure 1). The ACC voxel, placed just superior to the genu of the corpus callosum and parallel to its surface, was constrained to the left hemisphere to reduce susceptibility broadening of the spectral lines caused by inclusion of the hemispheric fassure. As a consequence, the lateral extension was reduced to only 12 mm to avoid spatial overlap with the other voxel. The second voxel was positioned in the medial lateral part of the left frontal lobe. Patient movement was restrained by foam pads lateral to the head and by fixing the forehead with Velcro straps, and the magnetic resonance imaging (MRI) scans used for voxel planning were repeated after completion of the MRS acquisitions to detect possible shifts of the head position. Data of sufficient quality from the frontal lobe voxel were only obtained in 18 patients, while in the other 2 cases, the spectral data from this region had to be discarded due to patient movement.

Water-suppressed 1H-MR spectra with a repetition time (TR) of 2000 ms and echo times (TE) of 30 ms and 140 ms were obtained from these voxels using image-guided PRESS localization. NAAG and NAA measures were taken from the spectra at TE 140 ms because at TE 30 ms, the overlapping signal of the \( ^3 \text{CH}_2 \) protons of glutamate (Glu) and glutamine (Gln) in the
NAAG and NAA in Schizophrenia

Table 1. Demographic Data and Neuropsychological Measures (Mean and SD)

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 20)</th>
<th>Controls (n = 20)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (m/f)</td>
<td>14/6</td>
<td>11/9</td>
<td>P = .514</td>
</tr>
<tr>
<td>Age (years)</td>
<td>34.5 (10.2)</td>
<td>30.7 (9.1)</td>
<td>P = .217</td>
</tr>
<tr>
<td>PANSS positive</td>
<td>10.8 (3.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PANSS negative</td>
<td>13.9 (3.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PANSS general</td>
<td>26.4 (6.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PANSS total</td>
<td>51.0 (11.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLMT, immediate recall</td>
<td>50.1 (11.5)</td>
<td>61.9 (7.6)</td>
<td>P = .001</td>
</tr>
<tr>
<td>VLMT, delayed recall</td>
<td>9.3 (3.2)</td>
<td>13.9 (1.1)</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>VLMT, recognition</td>
<td>11.8 (3.7)</td>
<td>14.1 (1.8)</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>Digit span</td>
<td>13.1 (4.3)</td>
<td>19.1 (2.9)</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>Block span</td>
<td>13.5 (3.4)</td>
<td>17.9 (2.8)</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>Letter-number-sequencing</td>
<td>13.6 (4.2)</td>
<td>18.5 (3.2)</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>DSST</td>
<td>46.1 (13.8)</td>
<td>69.0 (7.1)</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>TMT A (s)</td>
<td>36.5 (19.6)</td>
<td>25.2 (10.4)</td>
<td>P = .032</td>
</tr>
<tr>
<td>TMT B (s)</td>
<td>76.9 (42.6)</td>
<td>50.9 (17.6)</td>
<td>P = .018</td>
</tr>
<tr>
<td>Composite episodic memory score</td>
<td>-0.66 (0.96)</td>
<td>0.56 (0.48)</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>Composite working memory score</td>
<td>-0.67 (0.86)</td>
<td>0.47 (0.61)</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>Composite attention score</td>
<td>-0.54 (1.04)</td>
<td>0.46 (0.49)</td>
<td>P &lt; .001</td>
</tr>
</tbody>
</table>

Note: PANSS, Positive and Negative Symptoms Scale; VLMT, Auditory Verbal Learning and Memory Test; DSST, Digit symbol substitution test; TMT, Trail making test.

range 2.00–2.07 ppm contributes about 10% to the peak as determined by density-matrix calculations with the nuclear magnetic resonance (NMR)-spectra calculation using operators (SCOPE) (NMR-SCOPE) module of the magnetic resonance user interface (MRUI) software package. At the longer TE, this signal contribution was calculated to be less than 3% at the NAAG position due to dephasing of the coupled spin system of glutamate/glutamine, and it is further slightly reduced by the shorter T2 relaxation time of these components. These calculations were confirmed by phantom experiments using model solutions containing NAA, Glu, and Gln in concentrations approximately twice the cerebral values but with concentration ratios 1:0.75:0.3 closely matching the in vivo ratios for human brain. While at TE 30 ms, the Glx component at 2.08 ppm indeed has a 16%

![Image](https://example.com/image1.png)

![Image](https://example.com/image2.png)

Fig. 1. Position of the 1H-MRS voxels in the (a) anterior cingulate cortex and the (b) left frontal lobe.
peak area relative to NAA with considerable residue at the
NAAG frequency of 2.04 ppm and at TE 80 ms, the residual Glx signal at 2.05 ppm has already decreased
to 3% of NAA intensity and is no longer detectable above
noise at the TE of 140 ms (figure 2a).
In vivo spectra were sampled with 1024 data points at
2-kHz bandwidth, yielding a noninterpolated frequency
resolution of 2 Hz/pt and were averaged over 128 signal
acquisitions. Signal averaging was performed online but
possible line broadening and phase shifts caused by patient
movement during the MRS scan were suppressed by mea-
suring the Larmor frequency in each TR interval for drift
correction. Absolute metabolite concentrations in mmol/l
brain tissue were determined by referencing the
[NAA+NAAG] signal to the internal tissue water signal
from unsuppressed 1H-MRS, immediately accomplished
with 32 signal averages after acquiring the TE 140-ms spec-
trum. These acquisitions of both [NAA+NAAG] and wa-
ter signal were performed with TR 4000 ms and an
intermediate TE of 80 ms to minimize errors due to T1
saturation, T2 relaxation, and underlying glutamate/gluta-
mine 3CH2 components in the metabolite spectra. The
metabolite relaxation times for frontal lobe and ACC at 3.0 T
required in the extrapolation to TE 0 were taken from ear-
lier data, as T2 values for [NAA+NAAG] we used 279
ms (frontal lobe) and 254 ms (ACC), respectively. Further,
it was assumed that the relaxation times of the acetyl moi-
ety in NAA and NAAG are not significantly different. The
absolute concentrations of [NAA+NAAG] were then
corrected for partial cerebro spinal fluid (CSF) volume,
which was obtained from biexponential 4-parameter fit-
ting of the T2 values and the relative fractions of brain tis-
sue water and CSF to an additional series of unsuppressed
spectra, with TR 6000 ms and TE increasing in 7 steps
from 35 to 800 ms. The concentrations of the other metab-
olites choline compounds (Cho), total creatine ((P)Cr),
and myo-inositol (MI) were derived by referencing
against [NAA+NAAG] in the water-suppressed spectra
obtained at TE 140 ms (Cho, (P)Cr) and at TE 30 ms
(MI), respectively. For MI, an effective T2 of 75 ms
was used in the extrapolation from TE 30 ms to 0 TE tak-
ing into account the modeled signal decay due to dephas-
ing of the J-coupled C1/C3 and C4/C6 protons as well as
T2 relaxation.
The metabolic ratios Glx/(P)Cr and Glu/(P)Cr were deter-
mined from the short-TE spectra at 30 ms without relax-
ation correction, with Glx defined as the summation
over the overlapping glutamate/glutamine/GABA-CH2
components in the 2.1- to 2.4-ppm frequency range
and Glu taken from the 4CH2 resonance of glutamine
at 2.47 ppm. Table 2 lists the respective 1H-MRS sequen-
ces at with which the metabolite concentrations and
ratios were determined.
Spectra were preprocessed by apodization with matched Lorentz-Gauss filtering (5-Hz Gaussian broad-
ening followed by -6-Hz Lorentzian narrowing) and
time-domain quantified using the advanced method for
accurate, robust and efficient spectral fitting (AMARES)
NAAG and NAA in Schizophrenia

Table 2. 1H-MRS Sequences Used for Quantification of Metabolite Concentrations

<table>
<thead>
<tr>
<th>TR (ms)</th>
<th>TE (ms)</th>
<th>2000 30 ws</th>
<th>2000 140 ws</th>
<th>4000 80 us</th>
<th>6000 35/60/100/140/280/500/800 us</th>
</tr>
</thead>
<tbody>
<tr>
<td>[NAA+NAAG]</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[NAAG]</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[NAA]</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAAG/NAA</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Cho]</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>([P]Cr</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[MI]</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glx/(P)Cr</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln/(P)Cr</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2 (H2O) and % CSF in MRS voxel</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: NAA, N-acetylaspartate; NAAG, N-acetylaspartylglutamate; Cho, Choline compounds; (P)Cr, Total creatine; MI, Myo-inositol; Glx, Glutamate/glutamine/GABA-CH2; Gln, Glutamine (ws = H2O-suppressed, us = unsuppressed).

algorithm of the MRUI software package. Mean full with half maximum (FWHM) linewidth (after apodization) of the NAA methyl resonance in the long-TE spectra was 4.4 Hz for the frontal lobe voxel and 4.2 Hz in the ACC, thus allowing independent fitting of the NAAG component separated from NAA by 4.6 Hz at 3.0 T (figure 3). This frequency spacing was introduced as fixed value in the AMARES fit, and the linewidths of both components were kept equal. To improve the reliability of the results, only spectra with resulting NAA linewidths less than 6 Hz and the small NAAG component fitted by AMARES with an accuracy (Cramér-Rao lower bounds [CRLB]) better than 50% were further included in the evaluation of NAAG/NAA ratios and NAAG and NAA concentrations. Following both of these criteria, the NAAG data from both voxels in one patient and from the frontal lobe voxel of one control had to be omitted. NAAG could be determined in both target regions with mean CRLB of only 13%. Because the CRLB values for NAA were less than 2.5% in all cases (mean value 1.4%), also the accuracy of the ratios NAAG/NAA determined by error propagation is almost entirely defined by the CRLB of NAA.

The reliability of the separate determination of NAAG and NAA concentrations by the applied quantification method was also tested in phantom experiments using aqueous solutions with 3 mmol/NAAG and 25 mmol/l NAA. While phantom T1 (1280 ms for NAA) resembled the in vivo values, phantom T2 was much longer (>750 ms for both NAAG and NAA) and measured NAAG/NAA ratios reproduced the nominal concentration ratio 0.12 within 7% at all TE 30/80/140 ms. CRLB of NAAG and NAA were 3.2% and 0.5%, respectively, at TE 140 ms and did not increase after additional 5-Hz Gaussian broadening to match in vivo linewidths (figure 2b).

Statistics

We compared all metabolic concentrations and ratios between patients and comparison subjects with a MANOVA including all metabolic concentrations and ratios.

Fig. 3. Examples of 1H-MRS spectra (TE = 140 ms) from (a) the anterior cingulate cortex (ACC) of a patient with (from top to bottom) the raw spectrum, the fitted components, and the residues. The frequency positions of the methyl resonances of NAAG (2.04 ppm) and NAA (2.01 ppm) are marked with dashed and dotted lines, respectively. The linewidth (FWHM) of NAAG and NAA was 3.8 Hz and the ratio NAAG/NAA determined by the AMARES fit was 0.15. Signal-to-noise ratio was 5.3 for NAAG and 32.1 for NAA in the raw spectrum, and fitting accuracy (CRLB) of NAAG and NAA were 8% and 1.3%, respectively. (b) The ACC of a healthy control. NAAG and NAA linewidth was 3.4 Hz in this case, and the NAAG/NAA ratio was 0.08. (c) The left frontal lobe of a patient, with NAAG and NAA linewidth of 4.7 Hz and a NAAG/NAA ratio of 0.14.
concentration of either NAAG or NAA or with the ratio NAAG/NAA. NAAG is increased in patients at a trend level. NAAG is reduced in patients and the ratio NAAG/NAA is increased. NAAG is increased in patients at a trend level.

Anterior cingulate gyrus

In addition, we tested the correlations of the concentrations of NAAG and NAA and of the metabolic ratio NAAG/NAA with the PANSS subscores in patient group with the Pearson correlation coefficient. The correlations of the concentrations of NAAG and NAA and of the metabolic ratio NAAG/NAA with the 3 cognitive composite scores were tested for both groups separately by Pearson correlation. All analyses were performed for each 1H-MRS voxel separately.

Results

Means and SDs of the absolute concentrations of NAA, NAAG, Cho, (P)Cr, and MI and of the ratios NAAG/NAA, Glx/(P)Cr, and Gln/(P)Cr from the patients and the comparison subjects are listed in table 3.

Anterior Cingulate Cortex

The MANOVA over all metabolic measures revealed a significant group effect ($P = .044$). A significant increase in patients was observed for the ratio NAAG/NAA ($P = .041$) and the NAAG concentration at a statistical trend level ($P = .066$). NAA in the patient was reduced ($P = .030$). The other metabolic concentrations or metabolic ratios did not differ between both groups. None of the PANSS subscores correlated significantly with the concentration of either NAAG or NAA or with the metabolic ratio NAAG/NAA. The attention composite score correlated with the concentration of NAA ($r = .64$, $P = .005$) in patients. In the comparison subjects, there was no significant correlation of the concentrations of NAAG and NAA or the ratio NAAG/NAA with cognitive measures.

Frontal Lobe

The group effect of the MANOVA was not significant, and there were no differences in any metabolic concentration or metabolic ratio between both groups within the frontal lobe voxel. In patients, the concentration of NAAG correlated inversely with the PANSS negative scale ($r = -.58$, $P = .018$) (figure 4) and with the PANSS total score ($r = -.50$, $P = .049$). In patients, there was a positive correlation of the episodic memory composite score with the concentration of NAAG ($r = .53$, $P = .035$) and with the ratio NAAG/NAA ($r = .54$, $P = .030$). In the comparison subjects, there was no significant correlation of
the concentrations of NAAG and NAA or the ratio NAAG/NAA with any cognitive measure.

Discussion
The determination of NAAG concentration is crucial for gaining further insight into the glutamatergic dysfunction in schizophrenia and for assessing modes of action of novel antipsychotic drugs candidates. In this first 1H-MRS report on NAAG in patients with schizophrenia, we found an increased NAAG/NAA ratio and a trend toward an increased concentration of NAAG in the ACC. In the left frontal lobe, we observed an inverse correlation of NAAG concentration with negative symptoms and a positive correlation of NAAG with episodic memory in patients. Our data contribute to earlier postmortem studies that delivered partly controversial results. Tsai et al found increased NAAG in the hippocampus of patients with schizophrenia but not in the cingulate cortex or the frontal lobe. Nudmanud et al reported reduced NAAG levels in the superior temporal gyrus in patients and no difference in the frontal lobe compared with a control sample. In recent postmortem gene expression studies, mGluR3 was lower in the prefrontal cortex in patients, while the concentration of the NAAG degrading enzyme GCP II has been found to be both higher and lower, suggesting altered regulation of NAAG levels.

Based on the ameliorating effect of inhibitors of the NAAG degrading enzyme GCP II on schizophrenia-like symptoms in animals and the antipsychotic efficacy of the mGluR2/3 agonist LY404039 in humans, reduced NAAG concentration has been proposed as a pathological condition associated with symptoms of schizophrenia in vivo. Recent experiments in neuroblastoma cells revealed that antipsychotic treatment may increase NAAG concentration, suggesting reversal of low NAAG in patients by antipsychotic treatment. This may explain increased NAAG levels that have been observed in postmortem brain tissue of patients with schizophrenia. The effects of antipsychotics in patients may also explain the increased NAAG levels within the ACC observed in the present study because all were on stable antipsychotic treatment and in partial or full remission with regard to positive symptoms. High NAAG concentration in the frontal lobe correlated with less negative symptoms and with better memory performance within the group of patients, suggesting beneficial effects of high NAAG levels. This finding supports the concept of increasing NAAG as a potential treatment approach that is derived from animal and human studies.

In a recent publication, the role of NAAG as an agonist at the mGluR3 has been questioned. While the authors did not observe a direct effect of NAAG on the mGluR3, they did report modulatory effects of NAAG on the mGluR3 response to glutamatergic stimulation. This highlights the need for refined characterization of the action of NAAG in the glutamatergic system. The relevance of NAAG for schizophrenia, which is supported by different lines of evidence, however, is not generally questioned by the uncertainty on the interplay of NAAG, mGluR3, and glutamate.

NAAG was reduced in the ACC in patients with schizophrenia in the present study. This is in agreement with many but not all 1H-MRS studies that report a reduction of the combined [NAA+NAAG] peak. In addition, we found a correlation of NAAG in the ACC with attentional performance in the patient group. As NAAG indicates the status of neuronal mitochondria, the correlation of NAAG with performance in attention tasks in patients is in agreement with reports on the crucial role of the ACC in attention modulation in schizophrenia.

The increase of NAAG and the decrease of NAA as well as the significant difference of the NAAG/NAA ratio between patients and comparison subjects in the ACC in our study must raise caution, when interpreting the 1H-MRS studies on the commonly used combined [NAA+NAAG] peak. Change of NAAG as a component of glutamatergic neurotransmission and of NAA as a compound that does not act in neurotransmission may mask each other and may induce wrong conclusion about the individual concentration of both in schizophrenia. This potential confound also extends to 1H-MRS reports on other diseases.

We cannot confirm the reports on reduced NAA or [NAA+NAAG] in the frontal lobe. In a meta-analysis of 27 studies on frontal gray matter, [NAA+NAAG] was reported to be lower in patients with schizophrenia even though several individual studies did not find a significant difference between patients and comparison subjects. The differences in several parameters of individual studies beyond sample size such as field strength, voxel placement, and patients’ characteristics may contribute to the in part discrepant findings. Also, potential disease-related alterations in metabolite relaxation times may mimic concentration changes and could account for the heterogeneity of the study results. This is also a limitation of our study because with respect to tolerable examination time, it was not possible to measure metabolite T2 times individually and to acquire only fully T1-relaxed spectra. However, by the choice of an intermediate TE of 80 ms and a long TR of 4000 ms for the concentration referencing of [NAA+NAAG] against internal tissue water, we tried to minimize the influence of metabolite relaxation times on absolute concentration values.

We did not detect differences in metabolic ratios that include glutamate or glutamine. Some studies have reported reduced glutamate in different brain areas in schizophrenia, while others found increased concentration or no change. With regard to glutamine,
a reduction in the ACC of chronic patients has been reported in one study. Differences in patient characteristics may contribute to the lack of glutamine difference in our data.

Separate measurement of cerebral NAAG and NAA concentrations by 1H-MRS in vivo has thus far only been reported in 2 studies on healthy volunteers. This is due to the strong overlap of the methyl resonances of both metabolites, which allows discrete quantification only at high magnetic fields, while the majority of 1H-MRS studies on patients with schizophrenia and other disorders has been performed at field strengths less than 3T. Moreover, according to the first study on separate measures of NAAG and NAA, in some brain structures such as the frontal lobe, NAAG is lower than in other cerebral regions, which makes separate quantification even more demanding. While in the more recent study the investigators discriminated NAAG from NAA by spectral editing in separate acquisitions, where quantification accuracy strongly depends on the degree of coediting the respective other component, our time-domain-fitting procedure is similar to that of the first report on separate measures of NAAG and NAA who used LC model fitting of NAAG and NAA signals at 2T. The NAAG fractions of 0.08 and 0.18 obtained in that study for frontal gray and white matter, respectively, are in agreement with our NAAG/NAA ratio of 0.10 in the healthy control group in our data.

Overall, it needs to be stressed that in vivo measurement of NAAG and NAA separately is highly challenging, and replication of our data in patients with schizophrenia is needed.

Our study is limited by the inclusion of patients on stable medication only as opposed to untreated subjects. Ultimately, 1H-MRS studies in patients before and after the initiation of antipsychotic treatment will disclose whether a reduction of NAAG is a phenomenon of schizophrenia per se and whether an increase of NAAG is related to improvement of symptoms by antipsychotic treatment.

We conclude that the concentrations of NAAG and NAA act differently in schizophrenia, which impacts on the interpretation of the large body of literature on the [NAA+NAAG] peak in this disorder and in other diseases. The opportunity to measure NAAG in patients creates a new and promising approach for intensified research on the glutamatergic systems and on effects of novel antipsychotic drugs candidates.

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References

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