A multicenter reproducibility study of single-voxel $^1$H-MRS of the medial temporal lobe

Abstract Proton magnetic resonance spectroscopy ($^1$H-MRS) has provided evidence for a reduction of N-acetylaspartate (NAA) in the medial temporal lobe (MTL) in cerebral disorders such as Alzheimer’s Disease. Within the $^1$H-MRS study of the German Research Network on Dementia, we determined the multicenter reproducibility of single-voxel $^1$H-MRS of the MTL. At five sites with 1.5T MR systems, single-voxel $^1$H spectra from the MTL of an identical healthy subject were measured. The same subject was also examined at one of the sites five times to assess intracenter stability. The protocol included water-suppressed spectra with TE 272 ms and TE 30 ms and unsuppressed spectra for absolute quantification of metabolite concentrations. The intracenter reproducibility of absolute NAA concentration, expressed as coefficient of variation (CV), was 1.8%. CV for the concentrations of creatine (Cr), choline (Cho), and myoinositol (MI) and for the ratios NAA/Cr, NAA/Cho, and MI/NAA varied by 11–16%. Intercenter CV was 3.9% for NAA and were below 10% for all other metabolites and metabolic ratios. Our study demonstrates that quantitative assessment of NAA with single-voxel MRS can be performed with high intercenter reproducibility. This is the basis for applying $^1$H-MRS in large-scale early recognition and treatment studies in MTL affecting disorders. 

Keywords Reproducibility study · Dementia · Medial temporal lobe · Magnetic resonance spectroscopy · Multicenter

Introduction

Proton magnetic resonance spectroscopy ($^1$H-MRS) is a well-established, noninvasive technique for the detection of cerebral metabolites and for the quantification of their in-vivo concentrations in brain tissue. Extensive literature supports its usefulness for the assessment of brain tumors, epilepsy, and other neurological disorders [1, 2]. In the case of dementia, the medial temporal lobe (MTL) represents the focus of earliest and most pronounced tissue degeneration and metabolic alterations and therefore turns out to be a target of particular interest. A few groups have already reported a reduction of the neuronal marker N-acetyl aspartate (NAA) in the MTL of Alzheimer’s disease (AD)
patients cross-sectionally and longitudinally [3–10]. A novel study has found a reduction of NAA also in the MTL of patients with mild cognitive impairment (MCI) as a transitional stage between health and dementia [11]. NAA might be of particular value in dementia because its decrease is not correlated with the atrophy detected with structural MRI [4, 9], and it might be more sensitive to early neuronal damage than gross volume reduction. In addition to its potential role in the early recognition of neurodegeneration, NAA mirrors short-term treatment effects of antidementia drugs [12], which might establish its role in the prediction of treatment response.

To assess the potential contribution of NAA changes in the MTL to the early recognition of dementia and in treatment monitoring, large multicenter trials would be of benefit. There is, however, limited experience with 1H-MRS multicenter protocols in such patients. Two groups have reported multicenter trials in neurooncologic patients and demonstrated the general feasibility of this approach [13, 14]. Since brain tumors lead to drastic changes in the metabolite signal, such a disorder might be of limited value to test the intercenter reproducibility of the method. Two multicenter 1H-MRS trials of HIV encephalopathy have demonstrated the general feasibility of multicenter 1H-MRS trials. No studies have addressed the intercenter reproducibility of: (1) Measurements of the same individual subject (2) Absolute metabolite concentrations (vs. metabolic ratios) and (3) 1H-MRS of the medial temporal lobe, as a region of high interest for neuropsychiatric disorders but subject to spectral artifacts and to line broadening by strong susceptibility gradients

In preparation of the early recognition and treatment study of the German Research Network on Dementia, which includes a single-voxel 1H-MRS study of the MTL in cognitively impaired subjects, we performed a multicenter reproducibility trial to assess these questions. In these pilot examinations, we wanted to ascertain whether signal ratios and absolute concentrations of NAA and other metabolites could be determined at the participating sites with a sufficiently low variability comparable with a single-center test-retest study. If so, the protocol would yield sufficiently accurate results to be employed in large multicenter trials to thoroughly assess the potential role of 1H-MRS of the MTL in early recognition and treatment monitoring in dementia.

Materials and methods

Sites

The 1H-MRS raw data from all five participating sites were collected and centrally postprocessed at one of the sites serving as reference and organization center for the MRS study. All five MR scanners were operating at a magnetic field of 1.5 T and used a radiofrequency transmit/receive head coil suited for cerebral MRI and MRS. Table 1 lists the technical equipment of each site.

Subject measurements

Single-voxel 1H-MRS was performed at all sites in the same 32-year old male healthy subject. The intercenter measurements were completed within a period of 1 month. To assess the intracenter variation, five independent 1H-MRS examinations were performed at the MRS reference site. These were obtained as part of the regular control of scanner quality involving phantom and in vivo scans and covered a total time frame of almost 1 year.

The spectroscopic volume of interest (VOI) of 35×25×20 mm was positioned in the left medial temporal lobe in temporal angulation and was centered on the hippocampus (Fig. 1). The angulation of the VOI was achieved either directly by appropriate switching of the magnetic field gradients or by reclining the subject’s head in an adequate position. Anatomical MR scans were obtained for image-guided positioning of the voxel, and repositioning in the repeated MRS examinations was accurately referenced to image documentation of the voxel cross-section in all three orientations (Fig. 1) and was always performed by the same member of the study group (FT). Water-suppressed 1H-MRS spectra with volume selection by PRESS [23] were acquired with TR 2000 ms and 128 signal averages. Sampling of 512 data points with 1 kHz bandwidth corresponded to a nominal frequency resolution of 2 Hz/point. Metabolite signal ratios of NAA, of choline containing compounds (Cho), and of total creatine (Cr) were determined from spectra with a TE of 272 ms (Fig. 2a) while the signal ratios of myoinositol (MI) to NAA were obtained from spectra with TE 30 ms ad-
ditionally presenting a variety of short T2 or spin-coupled components (Fig. 2b). In semiautomatic postprocessing, Lorentz-Gauss filtering was applied to improve the signal-to-noise ratio (SNR) and to reduce spectral overlap, particularly in the water-suppressed TE 30 ms spectra, by converting the lines into a more Gaussian shape. Filter widths were adapted to spectrum parameters (short/long TE) but were held identical at all sites and in all repetitions of the same type of spectrum. Then, operator-independent quantification was performed using the AMARES algorithm [24] of the MRUI software package [25] for time-domain fitting of 3 (for TE 272 ms) or 18 (for TE 30 ms) Gaussian components, respectively. At TE 30 ms, compared with even shorter TE settings, spectral contributions from broad underlying macromolecular resonances are already strongly reduced due to their fast T2 decay, as well as other baseline distortions caused, e.g., by eddy currents. Moreover, the time-domain quantification supplied by AMARES is less sensitive to phasing and baseline errors than frequency-domain techniques [26]. The JAVA version [27] of the MRUI package was capable of handling the different export data formats from all participating sites and was therefore used for the automated off-line data processing at the reference center.

Absolute concentrations of NAA were determined from unsuppressed spectra of the VOI acquired with TR/TE 3000/272 ms and 32 averages (Fig. 2c) using the tissue water signal as internal reference [28]. To determine NAA/H2O concentration ratios by extrapolation to TE 0 and to correct for the CSF contents within the voxel, T2 relaxation times and relative fractions of tissue water and CSF were obtained by a biexponential, four-parameter fit to a series of seven unsuppressed spin-echo spectra with TE 30/70/136/272/400/700/1000 ms, and four signal averages each (Fig. 2d). Due to software restrictions, longest available TE was 300 ms at two of the sites, and a slightly reduced set of acquisitions had to be taken there. Errors in the extrapolation to TR ∞ due to variations in the assumed T1 values of mixed gray/white matter (800 ms) and of CSF (3,000 ms) were minimized, applying a long TR of 6,000 ms. Total acquisition time for the complete set of water-suppressed and unsuppressed spectra was 15 min, thus being well suited also for the scheduled multicenter trial on cognitively impaired patients. The T2 relaxation times of the me-

### Table 1 Magnetic resonance (MR) system equipment used at the participating sites

<table>
<thead>
<tr>
<th>Site</th>
<th>MR scanner</th>
<th>Max. gradient strength [mT/m]</th>
<th>Type of radiofrequency head coil</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.5 T Philips Gyrosan Intera</td>
<td>30</td>
<td>Quadrature transmit/receive</td>
</tr>
<tr>
<td>B</td>
<td>1.5 T Philips Gyrosan Intera</td>
<td>30</td>
<td>Quadrature transmit/receive</td>
</tr>
<tr>
<td>C</td>
<td>1.5 T Siemens Magnetom Vision+</td>
<td>25</td>
<td>Quadrature transmit/receive</td>
</tr>
<tr>
<td>D</td>
<td>1.5 T Siemens Magnetom Sonata</td>
<td>40</td>
<td>Quadrature transmit/receive</td>
</tr>
<tr>
<td>E</td>
<td>1.5 T Siemens Magnetom Vision</td>
<td>30</td>
<td>Quadrature transmit/receive</td>
</tr>
</tbody>
</table>

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**Fig. 1** Position of the PRESS-localized 1H-magnetic resonance spectroscopy (MRS) voxel (35×25×20 mm) in the left medial temporal lobe of the 32-year old healthy subject displayed on temporally angulated transverse T1-weighted gradient echo sequence and on coronal and sagittal turbo gradient echo images.
tabolites were measured by a spin-echo series of five water-suppressed spectra with TR 3,000 ms, TE 50/136/272/400/550 ms and 32 averages each. As this additional measurement was rather time consuming (8 min); it was only performed once (at the reference site). The obtained metabolite T2 results were applied in the extrapolation of the TE 272 ms data to TE 0 as fixed parameter values at all sites. Although some T1 saturation of the metabolite signals at the selected TR of 3,000 ms for these unsuppressed spectra has also to be taken into account, even an error of 15% in the assumed values of the metabolite T1 relaxation times (1,450 ms for NAA and Cho, and 1,250 ms for Cr) would influence the results for the absolute concentrations by less than 5%.

The fractions of brain tissue and CSF were expressed in volume percent of target voxel, and NAA content was calculated as molar concentration per liter of brain tissue using a water contents of 72% in mixed gray/white matter [28] corresponding to 40 mol/l. Finally, absolute concentrations of Cho and Cr were determined from the signal ratios to

![Fig. 2 Examples of 1H-magnetic resonance spectroscopy (MRS) spectra acquired from the medial temporal lobe (MTL) voxel displayed in Fig. 1.](image)

a TR/TE=2000/272 ms, water-suppressed, 128 signal averages. b TR/TE=2000/30 ms, water-suppressed, 128 signal averages. c unsuppressed spectrum with TR/TE=3000/272 ms, 32 signal averages. Amplitudes on different scale than in Fig. 2a: d multistack display of H2O spectra with TE=30/70/136/272/400/700/1000 ms, TR=6000 ms, four signal averages each
NAA obtained in the water-suppressed spectra at TE 272 ms and extrapolated to TE 0. This procedure was preferred to calculating the concentrations from the signal ratio to water in the unsuppressed spectra at TE 272 ms, as the lines of Cho and Cr are much more subject to distortions by the slope of the intense water component in these spectra than the NAA resonance (Fig. 2c). Absolute concentration of MI was calculated using the signal ratio to Cho taken from the water-suppressed spectra at TE 30 ms. Again, saturation effects cannot completely be neglected at a TR of 2,000 ms for the suppressed acquisitions; however, only T1 alterations being different for the considered metabolites could affect the determined concentrations.

Statistics

Coefficients of variance (CV, expressed as standard deviation in percent of the mean values) of the concentrations of NAA, Cho, Cr, MI and the metabolic ratios NAA/Cr and MI/NAA were calculated for the intracenter reproducibility of the data sets acquired at the MRS reference site and for the intercenter reproducibility of the data sets from the other sites, including the means of the intracenter study. Comparison between intra- and intercenter results was performed by the nonparametric Mann-Whitney U test for unpaired samples using the SPSS software package (SPSS Inc., Chicago, IL, USA).

Results

Measurement of the metabolite T2 relaxation times performed at the MRS reference site yielded the following values for the MTL region (± errors of monoeXponential regression): T2 (NAA)=329±32 ms, T2 (Cho)=324±40 ms, T2 (Cr)=202±29 ms. These were applied in the extrapolation to TE 0 from the TE 272 ms and TE 30 ms data of all centers. Mean line width (± standard deviation) for the NAA-CH3 resonance (fitted with Gaussian line shape) in the watersuppressed TE 30 ms spectra was 3.5±0.6 Hz (intracenter, reference site) and 3.7±0.5 Hz (intercenter), respectively. Mean SNR (defined as ratio of peak amplitude to noise standard deviation) of this line was 30.7 (reference site, range 21–42) and 27.0 (intercenter, range 14–40), respectively, in the water-suppressed spectra with TE 272 ms. In the unsuppressed spectra, TR/TE 3,000/272 ms acquired with 32 averages only, the respective SNR of NAA was 16.4 (intracenter, range 11–27) and 17.7 (intercenter, range 12–23).

The results for the water T2 relaxation times, the percentage of brain tissue (BT) within the VOI, the absolute concentrations of the metabolites (expressed as mmol/l brain tissue), and the signal ratios NAA/Cho and NAA/Cr at TE 272 ms and MI/NAA at TE 30 ms are given in Tables 2 and 3. Table 2 lists the individual values, means, and standard deviations (SD) of the repeated measurements of the subject at the reference site, and Table 3 lists the respective data from the multicenter study. The Mann-Whitney U test for unpaired samples revealed no significant differences ($p>0.05$ for all parameters) between the results of the intracenter measurements at the reference site and those of the multicenter examinations.

The metabolites of main interest with respect to assessment and treatment monitoring of dementia are NAA and MI. The concentration of NAA showed a coefficient of variance of only 1.8% within the reference center and a CV of 3.9% between centers. In comparison, intracenter values of the metabolic ratios NAA/Cho and NAA/Cr, measured at TE 272 ms, varied by about 10% while the variation between centers was 6% and 10%, respectively. The ratio MI/NAA, obtained from the TE 30 ms spectra, showed a CV of 16% within one center and only 1.7% between centers.

The absolute concentrations of Cho, Cr, and MI, which were derived as “second pass” parameters from the results of the NAA quantification and from the measured metab-

### Table 2 Intracenter reproducibility (reference site) for 1H-magnetic resonance spectroscopy (MRS) of the selected medial temporal lobe (MTL) voxel

<table>
<thead>
<tr>
<th>Examination</th>
<th>T2 water [ms] (WM+GM)</th>
<th>% BT (WM+GM)</th>
<th>NAA [mmol/l brain]</th>
<th>Cho [mmol/l brain]</th>
<th>Cr [mmol/l brain]</th>
<th>MI [mmol/l brain]</th>
<th>NAA/Cho (TE 272 ms)</th>
<th>NAA/Cr (TE 272 ms)</th>
<th>MI/NAA (TE 30 ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>81.39</td>
<td>92.57</td>
<td>12.05</td>
<td>3.22</td>
<td>9.73</td>
<td>7.00</td>
<td>1.26</td>
<td>1.94</td>
<td>0.67</td>
</tr>
<tr>
<td>2</td>
<td>80.85</td>
<td>94.32</td>
<td>11.60</td>
<td>2.41</td>
<td>7.86</td>
<td>6.52</td>
<td>1.63</td>
<td>2.35</td>
<td>0.79</td>
</tr>
<tr>
<td>3</td>
<td>80.09</td>
<td>92.84</td>
<td>12.00</td>
<td>2.62</td>
<td>8.39</td>
<td>5.38</td>
<td>1.55</td>
<td>2.24</td>
<td>0.52</td>
</tr>
<tr>
<td>4</td>
<td>84.79</td>
<td>94.81</td>
<td>11.67</td>
<td>2.39</td>
<td>9.12</td>
<td>5.66</td>
<td>1.65</td>
<td>2.00</td>
<td>0.77</td>
</tr>
<tr>
<td>5</td>
<td>78.63</td>
<td>93.93</td>
<td>11.66</td>
<td>2.63</td>
<td>7.45</td>
<td>5.69</td>
<td>1.50</td>
<td>2.45</td>
<td>0.62</td>
</tr>
<tr>
<td>Mean</td>
<td>81.15</td>
<td>93.69</td>
<td>11.80</td>
<td>2.65</td>
<td>8.51</td>
<td>6.05</td>
<td>1.52</td>
<td>2.19</td>
<td>0.67</td>
</tr>
<tr>
<td>SD</td>
<td>2.28</td>
<td>0.96</td>
<td>0.21</td>
<td>0.34</td>
<td>0.92</td>
<td>0.68</td>
<td>0.16</td>
<td>0.22</td>
<td>0.11</td>
</tr>
<tr>
<td>SD (% of mean)</td>
<td>2.8</td>
<td>1.0</td>
<td>1.8</td>
<td>12.7</td>
<td>10.8</td>
<td>11.2</td>
<td>10.3</td>
<td>10.0</td>
<td>16.3</td>
</tr>
</tbody>
</table>

BT brain tissue, NAA N-acetyl-aspartate, Cho choline, Cr creatine, MI myoinositol, SD standard deviation
Table 3  Intercenter reproducibility for $^1$H-magnetic resonance spectroscopy (MRS) of the selected medial temporal lobe (MTL) voxel

<table>
<thead>
<tr>
<th>Site</th>
<th>T2 water [ms] (WM+GM)</th>
<th>% BT (WM+GM)</th>
<th>NAA [mmol/l brain]</th>
<th>Cho [mmol/l brain]</th>
<th>Cr [mmol/l brain]</th>
<th>MI [mmol/l brain]</th>
<th>NAA/Cho (TE 272 ms)</th>
<th>NAA/Cr (TE 272 ms)</th>
<th>MI/NAA (TE 30 ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>81.15</td>
<td>93.69</td>
<td>11.80</td>
<td>2.65</td>
<td>8.51</td>
<td>6.05</td>
<td>1.52</td>
<td>2.19</td>
<td>0.67</td>
</tr>
<tr>
<td>B</td>
<td>79.73</td>
<td>96.16</td>
<td>12.00</td>
<td>2.39</td>
<td>8.01</td>
<td>5.96</td>
<td>1.69</td>
<td>2.34</td>
<td>0.68</td>
</tr>
<tr>
<td>C</td>
<td>74.37</td>
<td>84.83</td>
<td>12.24</td>
<td>2.63</td>
<td>9.60</td>
<td>6.02</td>
<td>1.55</td>
<td>1.83</td>
<td>0.69</td>
</tr>
<tr>
<td>D</td>
<td>78.12</td>
<td>93.49</td>
<td>12.98</td>
<td>2.34</td>
<td>8.96</td>
<td>5.92</td>
<td>1.71</td>
<td>2.27</td>
<td>0.67</td>
</tr>
<tr>
<td>E</td>
<td>76.41</td>
<td>81.50</td>
<td>12.66</td>
<td>2.76</td>
<td>8.46</td>
<td>6.91</td>
<td>1.55</td>
<td>2.35</td>
<td>0.69</td>
</tr>
<tr>
<td>Mean</td>
<td>77.96</td>
<td>89.93</td>
<td>12.33</td>
<td>2.56</td>
<td>8.71</td>
<td>6.17</td>
<td>1.60</td>
<td>2.20</td>
<td>0.68</td>
</tr>
</tbody>
</table>

SD (% of mean)  

| SD (%) | 3.4 | 7.1 | 3.9 | 7.1 | 6.9 | 6.7 | 5.7 | 9.8 | 1.7 |

BT brain tissue, NAA N-acetyl-aspartate, Cho choline, Cr creatine, MI myoinositol, SD standard deviation

Discussion

To our knowledge, the present study is the first assessment of the multicenter reproducibility of quantitative $^1$H-MRS where the MTL in one single subject was investigated with different technical equipment at the participating sites. This allows intra- and intercenter comparisons without an additional bias from interindividual physiological variations in metabolite concentrations and relaxation times.

The most important finding is a high reproducibility for the measurements of the NAA concentration in the MTL across centers and in repeated measurements within the reference center. This clearly supports the general feasibility of absolute quantification by single-voxel $^1$H-MRS of the MTL in clinical multicenter trials. Our intra- and intercenter coefficients of variation are in the range 2–4%.

These data for NAA are in agreement with those found in single-center intra- and intersubject MRS reproducibility studies [29, 30] and in the dual-center intersubject MRSTI study of Chard et al. [18]. Moreover, the mentioned single-voxel $^1$H-MRS reproducibility studies both acquired spectra from VOI located in (occipito)parietal white matter. These are much less influenced by susceptibility broadening and by spectral distortions due to movement and insufficient water-suppression than spectra from voxels placed within the MTL.

Importantly, in our study, the widely employed metabolic ratios NAA/Cr and NAA/Cho revealed CV of 6–10% within and across sites, which are considerably larger than those obtained for the absolute concentration of NAA.

Similar observations were also reported in the publication of Schirmer and Auer [29] and were analyzed in more detail in a single-center MRSI reproducibility study [31]. Therefore, correlated effects (e.g., $B_0$ or $B_1$ instabilities, changes in partial CSF volume due to inaccurate VOI re-positioning), which influence all metabolite signals to the same degree (and thus are potentially corrected by forming signal ratios), appear to contribute only marginally to the observed variability.

The metabolites Cho and Cr showed a variation of 7% between centers in the results for their absolute concentrations; however, they revealed higher CV in repeated single-center measurements. While larger variations for the absolute concentrations of Cho and Cr than for the NAA quantification might result from the lower SNR compared with NAA and also from error propagation as they additionally rely on the measured ratios NAA/Cho and NAA/Cr, the reason for the higher intracenter CV is not yet clear. It can only be speculated that discrete metabolic inner-subject changes over the rather long temporal spread of the intracenter examinations might be responsible for this effect. A recent single-center MRS study [32] performed at 3 T in a test-retest design has reported even larger differences attributed to physiologic variability of 10–15% for absolute NAA, Cho, and Cr concentrations, and up to 28% for MI concentration in the MTL.

The potential role of myoinositol in the MR spectroscopic diagnosis of dementia is hardly predictable at present. Although an absolute or relative increase of MI concentration was observed in some patient studies, these findings could not be reproduced by all MRS trials on dementia [10, 12, 33–35]. This is probably due to uncertainties in the quantification of MI caused by the stronger line overlap in the short TE spectra, which is also reflected in the comparably higher CV found for MI in the other MRS reproducibility studies. Although the difficulties in component separation are even more pronounced for MTL voxels, in our study, the absolute concentration of MI could be reproduced in repeated single-center and in multicenter measurements within the same accuracy as the results obtained for Cho and Cr.

A major advantage of the quantification technique applied in our reproducibility study appears to be the direct determination of the CSF contents within the MRS voxel. This allows for correction for partial volume and for trans-
lation of measured metabolite concentrations within the VOI into absolute concentrations in brain tissue. Considering the rather large voxel size of 17.5 cm³, which was selected to provide MTL spectra with high SNR in sufficiently short acquisition time also in the patient population, such a correction for brain atrophy will be of particular importance in the designed multicenter trial. Also, repositioning errors or voxel misregistration leading to varying contents of brain tissue within the VOI in repeated measurements have only minor influence on the results of metabolite quantification. A larger intercenter variation of 7% for the BT fraction in the selected voxel was indeed observed (presumably due to the necessity of reclining the subject’s head to achieve temporal angulation of the VOI at some of the participating sites) compared with the low CV of 1% for the intracenter repetitions with gradient-controlled angulation of the VOI.

In general, intercenter variability was less than 10% for all of the investigated metabolite concentrations and ratios, and proved even better than long-term intracenter reproducibility except for absolute NAA contents. However, the data sample is still quite small, and repeated MRS examinations of the same subject at all participating sites would be of benefit to address intracenter variability more extensively and to identify possible center-related bias effects. These items will be investigated in further control examinations accompanying the initial stage of the projected clinical multicenter trial on cognitively impaired patients. Of course, systematic over- or underestimation of the “true” metabolite concentrations potentially inherent to the applied MRS quantification technique and common to all centers cannot be excluded, as no “gold standard” method for a noninvasive, in vivo determination of such concentrations is available for comparison.

References