Localized Single-Voxel Spin-Echo Proton MR Spectroscopy of Normal Hippocampal Area at 1.5 T: Optimal Voxel Volume and Reproducibility of Metabolite Ratios

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Purpose: To determine the optimal voxel volume covering the hippocampal area in single-voxel proton magnetic resonance (MR) spectroscopy and to evaluate the reproducibility of metabolite ratios of the spectra.

Materials and Methods: Localized single-voxel proton MR spectroscopy was applied to the right hippocampal area of five healthy volunteers at 1.5 T (Siemens Vision), using a standard head coil, and we employed the spin-echo or point resolved spectroscopy sequence. Voxel volume was changed from 1 ml to 5 ml but other operator-dependent measurement parameters were fixed, as follows: repetition time/echo time = 1500/135 msec, number of scans = 300. Using the same voxel volume, five consecutive measurements were obtained in each subject. Signal to noise ratio (SNR) of N-acetylaspartate (NAA), NAA/Choline containing compounds (Cho) and NAA/Cr (creatine and phosphocreatine) + Cho ratios were calculated for all 25 spectra.

Results: The SNR of NAA peaks increased significantly as voxel volume was increased to 3 ml (p < 0.01, Kruskal-Wallis test and Wilcoxon rank sum test). There were, however, no significant differences in values among the spectra obtained with voxel volumes of 3, 4 and 5 ml (p > 0.1); in those obtained with voxel volume of 2—4 ml, the standard deviations of NAA/Cho (10.6—13.2% of mean values) were similar to those of NAA/Cr + Cho (8.5—13.2% of mean values).

Conclusion: For spin-echo proton MR spectroscopy of the hippocampal area, the optimal voxel volume may be more than 3 ml in a setting of TR/TE = 1500/135 msec and number of scans = 300. In this situation, standard deviations of metabolite ratios may reach about 8—13% of mean values.

Index Words: Brain, MR
Brain, metabolism

The hippocampus is an important limbic structure and is located within the temporal horn of the lateral ventricle. In patients with temporal lobe epilepsy (TLE), sclerosis of the hippocampus is a major pathologic substrate which on microscopic examination shows neuronal loss and gliosis (1). During the last decade, morphologic changes in this small structure have been thoroughly evaluated by qualitative and quantitative magnetic resonance imaging (MRI) techniques (2—5), and MR imaging of the hippocampus is currently an essential part of the preoperative evaluation protocol in TLE patients. Some TLE patients, however, show no definite morphologic changes of the hippocampus on MRI.

Proton MR Spectroscopy (MRS) has recently been applied to the hippocampal areas of TLE patients (7—10); earlier reports had shown promising potential of this technique, especially in patients in whom no definite hippocampal abnormalities were seen on MRI (10). So far, various kinds of pulse sequences, voxel sizes, echo times (TE) and repetition times (TR) have been tested and on the basis of these, spectroscopic data of normal hippocampus have been produced.
In general, because of the advantages of signal-to-noise ratio, the spin-echo sequence or point resolved spectroscopy (PRESS) is preferred to the stimulated echo acquisition mode (STEAM). As the hippocampal area is a region with large differences in susceptibility, the single-voxel technique may be an easier and safer than multivoxel or spectroscopic imaging. To take advantage of flat baselines, TE as long as 135–270 milliseconds is usually chosen. For operators, the remaining practical considerations would be the choice of optimal measurement parameters such as voxel volume, TR and number of scans. These operator-dependent parameters affect anatomical resolution, measurement time and signal-to-noise ratio of the acquired spectrum. It is therefore necessary to optimize measurement parameters, especially voxel volume, and evaluate reproducibility of the spectra obtained using such parameters, but reports of this are rare.

In this study, we have tried to optimize the voxel volume covering the hippocampal area in the setting of TR/TE = 1500/135 msec and number of scans = 300, and have evaluated the reproducibility of metabolic ratios of the spectra obtained from the 1.5 Tesla MR system.

**Subjects and Methods**

Five healthy adults aged 25–33, without a history of central nervous system disorder, participated in this study; the male-to-female ratio was 3:2. All MR imaging and spectroscopic study utilized the same 1.5 Tesla MR machine, using the manufacturer’s standard circular polarized head coil (Magnetom Vision, Siemens Medical Systems, Erlangen, Germany). At the time of this study, commercially available spectroscopy programs did not allow localizer images of oblique planes; to obtain axial localizer images parallel to the long axis of the hippocampus, we therefore changed the angle of the head by about 30°, placing soft cushions under the neck. After head positioning, three plane localizer images covering the right hippocampal head and body areas were obtained, using a turbo spin echo sequence (TR/TE = 3500/128 milliseconds, echo train length = 23; field of view = 230mm; matrix size = 138 × 256; slice thickness/gap = 5/0–3 mm; number of slices = 10; number of acquisitions = 2; acquisition time = 23 seconds).

We employed the single voxel spin-echo sequence provided in the routine Siemens MRS package, and its parameters were as follows: TR/TE = 1500/135 milliseconds, number of scans = 300; acquisition time = 7 minutes 30 seconds. The data file size was 1,024 complex data points. Each of five volunteers was examined using the same sequence parameters but with a different voxel volume (1ml, 10 × 10 × 10 mm in volunteer 1; 2ml, 10 × 12 × 16.5 mm in volunteer 2; 3ml, 10 × 15 × 20mm in volunteer 3; 4ml, 13.3 × 15 × 20 mm in volunteer 4; 5ml, 13.3 × 19 × 20mm in volunteer 5). The smallest voxel (1ml) was composed almost entirely of hippocampal tissue. As voxel volume increased, larger voxels contained more perihippocampal tissues such as subiculum, parahippocampal gyrus and perihippocampal deep white matter than smaller ones. A voxel was therefore positioned carefully, using three plane localizer images to cover the head and anterior body portion of the right hippocampus as much as possible (Fig. 1). After automatic global shimming and setting of total receiver gain to 80 decibels, manual localized shimming was performed on the voxel and usual full widths at half maximum (FWHM) of the water peaks were 8–10 hertz. To suppress the dominating water peak, the amplitude of a frequency-selective Gaussian type radio-frequency pulse was adjusted manually, resulting in the usual values of analog-to-digital converter (ADC) voltage below 0.5. To check the reproducibility of metabolite ratios, five consecutive measurements were taken during each spectroscopic examination session.

Once data acquisition was complete, each spectrum was post-processed according to the protocol described in the manufacturer’s spectroscopy analysis program. Typical protocol consisted of Gaussian

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**Fig. 1.** Location of a voxel with 2ml volume (10 × 12 × 16.5 mm).
filtering of the time domain signal (filter center width: 0, 250 milliseconds), subtraction of the residual water signal after water reference processing of the frequency shift, Fourier transformation into frequency domain, constant and linear phase correction, and baseline correction by a polynomial function. Eddy current compensation by the use of water signal was not applied.

Using the same parameters and same voxel volume, we examined each volunteer five times, obtaining a total of 25 spectra. Major peaks at 2.0 ppm, 3.0 ppm, and 3.6 ppm were assigned to N-acetylaspartate (NAA), creatine/phosphocreatine (Cr), and choline-containing compounds (Cho), respectively. The values of integral amplitude and FWHM of NAA, Cho, and Cr peaks were calculated using the manufacturer’s peak information analysis program. The metabolite ratios of normal hippocampus were expressed as NAA/Cho and NAA/Cr + Cho; the signal to noise ratio (SNR) of NAA peak in a spectrum was calculated as follows (14):

\[
\text{SNR of NAA} = \frac{\text{signal amplitude of NAA peak}}{\sqrt{\text{RMS noise}}}
\]

\[
\text{RMS noise} = \frac{\text{peak to peak noise}}{2.5}
\]

In order to avoid-as far as possible-distortion of the spectral baseline, peak-to-peak noise was measured in the low frequency region (8 – 10 ppm).

Mean values of the data obtained from various voxel volumes were analysed using the nonparametric Kruskal-Wallis test and Wilcoxon rank sum test (SPSS 6.0 for Windows). P-values less than 0.05 were considered as significant.

Results

On visual inspection of three-plane localizer T2-weighted images, signal intensity and size of the

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Fig. 2. The representative spectra obtained from five volunteers.
A. Two spectra with a voxel volume of 1 ml.
B. Two spectra with a voxel volume of 2 ml.
C. Two spectra with a voxel volume of 3 ml.
D. Two spectra with a voxel volume of 4 ml.
E. Two spectra with a voxel volume of 5 ml. The peak amplitude and width of major peaks are varied unpredictably during consecutive measurements with the same acquisition parameters. The same scales are used in all spectra for comparison.
examined hippocampus were seen to be normal. Representative spectra obtained from the five volunteers are shown in Fig. 2. The means and standard deviations of integral value and SNR of NAA, NAA/Cho and NAA/Cr+Cho are shown in Table 1. These numeric data are displayed graphically in Fig. 3. The integral values of NAA, Cr and Cho peaks are similarly displayed in Fig. 4.

### Table 1. Mean (Standard Deviation of Four Variables of Spectroscopic Measurement in Various Voxel Volumes.

<table>
<thead>
<tr>
<th>Sex/Age</th>
<th>Voxel Volume (ml)</th>
<th>No. of Measurement</th>
<th>Integral of NAA</th>
<th>SNR of NAA</th>
<th>NAA/Cho</th>
<th>NAA/Cr+Cho</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. F/25</td>
<td>1</td>
<td>5</td>
<td>5.20 ± 1.3(25.6)</td>
<td>3.46 ± 0.25(7.2)</td>
<td>1.55 ± 1.20(77.4)</td>
<td>0.63 ± 0.30(47.6)</td>
</tr>
<tr>
<td>2. M/29</td>
<td>2</td>
<td>5</td>
<td>12.03 ± 1.33(11)</td>
<td>5.16 ± 0.76(14.7)</td>
<td>1.11 ± 0.13(11.7)</td>
<td>0.64 ± 0.08(12.5)</td>
</tr>
<tr>
<td>3. M/33</td>
<td>3</td>
<td>5</td>
<td>18.90 ± 1.89(10)</td>
<td>8.10 ± 1.01(12.5)</td>
<td>1.23 ± 0.13(10.6)</td>
<td>0.59 ± 0.05(8.5)</td>
</tr>
<tr>
<td>4. F/25</td>
<td>4</td>
<td>5</td>
<td>17.76 ± 1.49(8.3)</td>
<td>8.98 ± 0.92(10.2)</td>
<td>1.14 ± 0.15(13.2)</td>
<td>0.53 ± 0.07(13.2)</td>
</tr>
<tr>
<td>5. M/25</td>
<td>5</td>
<td>5</td>
<td>19.50 ± 2.67(13.7)</td>
<td>9.46 ± 1.39(14.7)</td>
<td>1.15 ± 0.14(12.2)</td>
<td>0.56 ± 0.10(17.9)</td>
</tr>
</tbody>
</table>


The number within the parenthesis is the standard deviation calculated as percentile of a mean value.

**Fig. 3.** Scattergram display of integral values and SNR of NAA, NAA/Cho, NAA/Cr+Cho.

A, B. As the voxel volume increases, integral value and SNR of NAA increases linearly to 3ml of the voxel volume.

C, D. The variation of NAA/Cho ratio is smaller than that of NAA/Cr+Cho ratio. The same abbreviations are used as in the text.
As shown in Fig. 3, the integral values and SNR of NAA peaks increased significantly as the voxel volume increased to 3ml (p < 0.005, Kruskal-Wallis test). There were, however, no significant differences in value among voxel volumes of 3, 4 and 5ml (p > 0.1, Wilcoxon rank sum test). As far as NAA/Cho and NAA/Cr+Cho ratios are concerned, there were no significant differences in metabolite ratios among the spectra obtained with voxel volumes of 2–5ml (p = 0.67, Kruskal-Wallis test).

As shown in Fig. 4, integral values of NAA, Cr+PCr and Cho changed unpredictably during the five consecutive measurements; this was due to the random variations of full width at half maximum (FWHM) and the amplitude of metabolite peaks. Calculated NAA/Cho and NAA/Cr+Cho ratios also changed, and this was according to variation in the integral values of NAA, Cr and Cho metabolites. In the spectra obtained with voxel volume of 2–4ml, the standard deviations of NAA/Cho (10.6–13.2% of mean values) were similar to those of NAA/Cr+Cho (8.5–13.2% of mean values). In spite of the low SNR of NAA, the inter-measurement variation of NAA/Cr+Cho, shown by data obtained with a voxel volume of 2ml, was similar.

![Integral Values of NAA, Cr, and Cho Peaks](image1)

**Fig. 4.** Variation of integral values of NAA, Cr, and Cho peaks. A–E. Integral values of major metabolites fluctuate unpredictably during the five consecutive measurements in spite of the same measurement parameters. The same abbreviations are used as in the text. The same scales are used for comparison.
to the variation seen when voxel volume was 3–5 ml.

**Discussion**

Our observations suggest that with measurement parameters such as TR/TE = 1500/135 msec and number of scans = 300, optimal voxel volume should be more than 3 ml and the standard deviation of metabolite ratios may be about 8–13% of mean values.

Spectra obtained from the hippocampal area showed much lower SNRs (maximum SNR < 10) than those obtained from other brain regions such as the frontal, parietal or occipital areas. It is possible that as the hippocampal area is near the skull base and sphenoid sinus, protons of metabolites suffer more intra-voxel phase dispersion due to magnetic field inhomogeneity than those of other brain regions. In this study, increased voxel volume did not result in linearly increased SNR, which almost reached a plateau when voxel volume was greater than 3 ml (Fig. 3). This pattern of SNR behavior of NAA could, of course, be attributed to an error in the SNR calculation or to anatomic variation among the participants in this study; the formula for calculating the SNR of NAA is shown in the Methods section. The amplitude of an NAA peak was calculated using the manufacturer’s peak information analysis program, but RMS spectrum noise was calculated manually by measuring peak-to-peak noise in the frequency region of 8–10 ppm. The spectra baselines were usually flat in this region, but not always. Distorted baselines may lead to an increase in peak-to-peak noise and a decrease in calculated SNR of the spectra; individual variation of anatomic structures and the biologic composition of the region of interest may also lead to variations in SNR, despite the same spectroscopic measurement parameters. The other possibility, however, is that SNR of NAA decreased due to increased intra-voxel phase dispersion of large voxel volumes. In this study, the number of subjects was too small to clarify the signal behavior of NAA according to changes in the volume of voxels, and further investigation of this point should be pursued in another study.

Ideally, it is desirable to obtain metabolic information from small volumes of interest (1–2 ml) covering only the hippocampal tissue. Spectra obtained, however, may require roughly a three or four-fold increase of acquisition time to ensure an SNR equivalent to obtained from 3–4 ml of voxel, and as scan time alone extends to about 30 minutes per measurement, this approach would be impractical in the clinical situation. In most such situations, not only is prolonged examination time not permitted, but it is also undesirable because of the possibility that a patient will move. In the clinical situation, it is desirable to acquire a spectrum within 10 minutes and to complete the examination of both hippocampi within 30 minutes.

<p>| Table 2. Reported Metabolite Ratios of Normal Hippocampal or Mesial Temporal Area |</p>
<table>
<thead>
<tr>
<th>Author</th>
<th>Sequence</th>
<th>TR/TE [msec]</th>
<th>No. of Scan</th>
<th>No. of Voxel Size [ml]</th>
<th>No. of Subjects</th>
<th>Integral of NAA/Cho</th>
<th>Integral of NAA/Cr+Cho</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connelly [9]</td>
<td>SE-SVS</td>
<td>1600/135</td>
<td>256–512</td>
<td>8</td>
<td>13</td>
<td>NA</td>
<td>0.92 ± 0.16 (17)</td>
<td>no asymmetry between right and left</td>
</tr>
<tr>
<td>Ng [10]</td>
<td>SE-CSI</td>
<td>1500/135</td>
<td>16 ×16*</td>
<td>1.4–2.7</td>
<td>12</td>
<td>1.56 ± 0.21 (13)</td>
<td>NA</td>
<td>between right and left partial volume effect</td>
</tr>
<tr>
<td>Strauss [11]</td>
<td>SE-SVS</td>
<td>2000/136</td>
<td>60–256</td>
<td>0.9</td>
<td>8</td>
<td>0.97–1.57</td>
<td>NA</td>
<td>phase-array coil used</td>
</tr>
<tr>
<td>Bernard [12]</td>
<td>STEAM-SVS</td>
<td>1500/272</td>
<td>400</td>
<td>8</td>
<td>28</td>
<td>Right-hander</td>
<td>NA</td>
<td>asymmetry in right-handers</td>
</tr>
<tr>
<td>Ende [13]</td>
<td>SE-CSI</td>
<td>1800/135</td>
<td>24 ×24*</td>
<td>4</td>
<td>16</td>
<td>1.63 ± 0.18 (11)</td>
<td>0.81 ± 0.06 (7)</td>
<td>no asymmetry between right and left</td>
</tr>
</tbody>
</table>

TR/TE; repetition time/echo time, No.; number, NAA; N-acetylaspartate, Cho; choline containing compounds, Cr; creatine + phosphocreatine, SE; spin echo, STEAM; stimulated echo acquisition mode, SVS; single voxel spectroscopy, CSI; chemical shift imaging, * number of phase encoding steps in 2 dimensional spectroscopic imaging, the number within the parenthesis is standard deviation calculated as percentile of a mean value, the number within the bracket is the reference number cited in the text.
spectra obtained with voxel volumes of 2–5 ml (10.6–13.2% of mean values). This relatively high variability of NAA/Cho may be attributed to systemic causes, problems of post-processing, or low SNR of spectra obtained from the hippocampal area; systemic causes, however, cannot be removed, and as we employed the manufacturer's post-processing protocol, with only minor modifications of baseline or phase correction, it seems that post-processing itself was not a significant factor of metabolite ratio variation. We believe that the low SNR of spectra obtained from the hippocampal region is the major cause of the reproducibility problem. To improve the SNR of the spectra, careful shimming is essential, but in the hippocampal area, it is usually difficult to obtain good shim values, due to relatively strong magnetic field inhomogeneity. The problem of SNR can be improved partly by using a specially designed phased-array coil for the temporal lobes instead of conventional volume head coils, but this is not available commercially (15).

SNR of a spectrum can, in addition, be improved by increasing voxel volume at the expense of spatial resolution, or by increasing the number of scans at the expense of examination time. The former approach should consider the possibility of intra-voxel phase dispersion as mentioned above; to achieve SNR improvement, the latter approach would—in spite of the prolonged examination time and risk of a patient moving—be a safer method than the former.

A wide range of normal NAA/Cho ratios has been reported in literature describing spectroscopy of the temporal lobe (9–13); published data are shown in Table 2. It may be presumed that these variations are secondary to the choice of pulse sequence, TR/TE, coil type, voxel volume and location of the voxel. In many reports, we could not find detailed descriptions of the SNR of spectra, or the reproducibility of the selected measurement parameters, though to determine optimal measurement parameters for producing reliable metabolic information regarding hippocampal areas, such descriptions are essential. Data variation due to spectroscopic measurement itself may partly contribute to variations in the spectroscopic data of normal hippocampal area, in addition to individual variations in anatomy or biochemical composition.

In summary, we observed that when performing spin-echo spectroscopy for the hippocampal area, optimal voxel volume may well be more than 3 ml in the setting of TR/TE = 1500/135 milliseconds and number of scans = 300. In this setting of measurement parameters, standard deviations of NAA/Cho and NAA/Cr + Cho may reach about 8–13% of mean values. MRS of the hippocampal area may not easy, since it requires accurate localization of the voxel and adequate SNR from a small volume of interest and must also overcome the susceptibility effect in the anteromedial temporal lobe. Careful consideration and evaluation of suitable measurement parameters may be necessary before applying this technique to clinical patients.

References

목 적 : 해마부위의 단일 화적소 양성자 자기공명분광법을 시행함에 있어서 적정 체적 용적 및 대사물질 비율의 재연성을 알아보기 위함.

대상 및 방법 : 다섯명의 건강한 정상 성인의 우측 해마부위에서 단일 화적소 스피니에코 양성자 자기공명분광법을 시행하였다. 기기는 1.5 Tesla 자기공명영상장치(Siemens Vision)와 표준 두부 코일을 이용하였고 스피니에코 기법을 적용하였다. 각 대상 지원자에서 측정 변수 중 반복시간/에코시간, 스캔 횟수를 각각 1500/135 msec, 300회로 고정한 상태에서 화적소 용적을 1ml에서 5ml 중 하나로하며 5회 연속 촬영하였다. 이렇게 얻은 25개의 스펙트럼에서 N-acetylaspartate(NAA)의 신호대 잡음비, NAA/Choline containing compounds(Cho), NAA/ Creatine(Cr) + Cho 비를 계산하였다.

결 과 : NAA의 신호대 잡음비는 화적소 용적이 1ml에서 3ml로 증가함에 따라 선형적으로 증가하였으나 3, 4, 5ml 간에는 큰 차이가 없었다. 화적소 용적이 2-4ml 범위인 경우 NAA/Cho 및 NAA/Cr+Cho 값의 평균치 및 표준편차 간에 큰 차이가 없었으며 표준 편차는 평균치의 8-13%의 범위였다.

결 론 : 측정 변수 중 반복시간/에코시간, 스캔 횟수 각각 1500/135msec, 300회인 상태에서 적당한 화적소 용적은 최소한 3ml 이상이 필요함 것으로 보이며 이 조건하에서 대사물질 비율의 표준 편차는 평균치의 8-13% 로 비교적 높은 값으로 보였다.