Interobserver and within-subject variances of $T_2$-relaxation time and $^1$H-metabolite ratios in the normal hippocampus

Variabilités inter et intraobservateur du temps de relaxation T2 et des ratios de métabolites dans l’hippocampe normal

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KEYWORDS
Epilepsy; Hippocampal sclerosis; Magnetic resonance imaging; Volumetry; $T_2$-relaxometry; Spectroscopy

Abstract
Purpose. — To investigate the magnetic resonance (MR) reproducibility of normal hippocampal volume (HV), temporal lobe volume (TLV), transversal relaxation time ($T_2$) and $^1$H-MR spectroscopy ($^1$H-MRS) metabolite ratios.

Materials and methods. — Two sets of HV, TLV, $T_2$ and MR spectroscopic metabolite signal ratios were determined in 27 healthy volunteers. HV and TLV were measured with a $T_1$-weighted MR sequence; whereas $T_2$ measurements were performed with conventional spin-echo (CSE) and fast spin-echo (FSE) MR imaging sequences. The interobserver and within-subject variances of $T_2$ measurements were estimated.

Results. — Estimated right and left HV coefficients of variation (CV) = 0.13. FSE $T_2$ measurements showed no significant differences in the interobserver (CV = 0.02) and within-subject variances (CV = 0.02). Measurements showed no differences in the interobserver (CV = 0.02) and within-subject (CV = 0.04) variances for the CSE $T_2$ of the right and left hippocampi. Metabolite ratios between $N$-acetyl aspartate (NAA) and creatine (Cr), choline (Cho) and creatine, and NAA and choline plus creatine (Cho + Cr) for the right hippocampus were $2.29 \pm 0.19$, $1.52 \pm 0.14$ and $0.91 \pm 0.05$, respectively. Metabolite ratios for the left hippocampus were $2.18 \pm 0.10$, $1.48 \pm 0.10$ and $0.88 \pm 0.06$, respectively.
Interobserver and within-subject variances of T2-relaxation time and \(^1\)H-metabolite ratios in the normal hippocampus

30% of the contralateral HV. Furthermore, T2 relaxometry situations where the ipsilateral HV is reduced by more than relaxation time (T2) \([11]\) and decreased longitudinal relaxation (T1) \([11]\) and an increased apparent diffusion coefficients \([6]\). Townsend et al. \([26]\) have, for example, demonstrated that there is an increase in the T2 of the white matter T2 in 78% of patients with hippocampal atrophy. In addition, hippocampal atrophy can be detected by visual inspection of hippocampal volume (HV). However, Reutens et al. \([23]\) have shown that this approach is useful only in situations where the ipsilateral HV is reduced by more than 30% of the contralateral HV. Furthermore, T2 relaxometry has proven capable of detecting bilateral hippocampal abnormalities, hippocampal gliosis and epileptic activity, and also has the advantage of being a relatively accurate measurement technique.

Conclusions. — HV, TLV, T2 and \(^1\)H MRS metabolite ratio measurements showed fair reproducibility with small CVs, and no differences in the interobserver and within-subject variances, including no differences between right and left TLV, and in the right and left T2.

**Introduction**

Hippocampal sclerosis (HS) in the form of hippocampal atrophy and hippocampal gliosis is found in approximately two-thirds of patients with temporal lobe epilepsy \([16]\). Epileptic seizures can be associated with foci in all four cerebral lobes; however, surgical resection of the hippocampus and the anterior temporal lobe has cured about 90% of patients suffering from partial seizures of temporal-lobe origin \([2]\). A successful surgical outcome requires, nevertheless, a precise preoperative diagnosis.

Different imaging techniques are used for diagnosis and treatment guidance of temporal-lobe epilepsy. Magnetic resonance imaging (MRI) and \(^1\)H magnetic resonance spectroscopy (\(^1\)H MRS) are useful in the evaluation of lateral and localized epileptogenic foci, and in the determination of their spatial distribution \([3,23,27]\). Relaxometric measurements are occasionally applied as a sign of hippocampal gliosis and is accompanied by an increased transversal relaxation time (T2) \([11]\) and decreased longitudinal relaxation time (T1) \([11]\) and an increased apparent diffusion coefficient \([6]\). Townsend et al. \([26]\) have, for example, demonstrated that there is an increase in the T2 of the white matter T2 in 78% of patients with hippocampal atrophy. In addition, hippocampal atrophy can be detected by visual inspection of hippocampal volume (HV). However, Reutens et al. \([23]\) have shown that this approach is useful only in situations where the ipsilateral HV is reduced by more than 30% of the contralateral HV. Furthermore, T2 relaxometry has proven capable of detecting bilateral hippocampal abnormalities, hippocampal gliosis and epileptic activity, and also has the advantage of being a relatively accurate measurement technique.

In some cases, measurements of T2 have been able to detect hippocampal gliosis and epileptic activity, even when the HV data were apparently normal \([11]\). T2 relaxometry is able to detect bilateral hippocampal abnormalities in patients with unilateral or no hippocampal atrophy, and can correctly pinpoint the site in most patients \([1]\).

Unfortunately, radiological inspection occasionally misses minor lesions and bilateral atrophies \([3,5]\). In fact, it has been shown that measurements of HV are inadequate for revealing bilateral abnormalities with minor hippocampal atrophy \([12,23]\) and, interestingly, Sørensen et al. \([25]\) have noted that diagnostic expertise does not increase the reproducibility of HV when inspected visually.

\(^1\)H MRS is frequently employed to supplement volumetric and relaxometric measurements \([3,18]\), as the metabolite ratios of NAA to (Cho + Cr), NAA to Cr, and Cho to Cr are predictors of epilepsy and sclerosis. \(^1\)H MRS is, however, influenced by various factors that restrict the ability to reproduce acquired metabolic spectra; of particular concern is the relatively low signal-to-noise ratio (SNR) observed using clinically available MRS systems. Furthermore, factors such as magnetic-field non-homogeneity, changes in positioning and changes in pulse-sequence parameters critically reduce the possibility of reproducing \(^1\)H MRS spectra.
The aim of this study was to investigate the interobserver and within-subject variances in measurements of HV, temporal lobe volume (TLV), T₂ and metabolite ratios in healthy adult brains that may potentially serve as references in the clinical evaluation of hippocampal disorders.

Materials and methods

The study included 27 healthy volunteers, aged between 24 and 58 years (36.9 ± 9.2 years), of whom 21 were men and 6 were women. Measurements of HV, TLV and T₂ were performed on a 1.5 T Philips Gyroscan MR system (Philips Medical Systems, Best, Netherlands). One operator performed two sequential measurements of HV, TLV and T₂ of the right and left hippocampi in each subject. Following the first set of measurements, the subject was repositioned and the procedure repeated. HV and TLV were measured with manual segmentation on images acquired with a coronal TR₁-weighted fluid-attenuated inversion recovery (FLAIR) sequence using the following parameters: TE/TR/TR₁ = 9.6 ms/1500 ms/700 ms, matrix = 256 × 256, number of transients (NT) = 2, slice thickness = 4 mm, FOV = 22 × 22 cm². T₂ was measured with a coronal fast spin-echo (FSE) sequence using TE/TR/TE₁/TR₁ = shortest and 100 ms/1000 ms/2260 ms/500 ms, matrix = 128 × 128, NT = 1, FOV = 20 × 20 cm², slice thickness = 5 mm. T₂ was also measured with a conventional spin-echo (CSE) sequence using TE = 35, 70, 105, 140 ms, TR = 2400 ms, matrix = 128 × 128, FOV = 22 × 16 cm², slice thickness = 5 mm. HV, TLV and T₂ were measured twice; each time, the volunteer was removed and repositioned.

¹H MRS was conducted on a 1.5 T GE Signa EchoSpeed system (General Electric, Milwaukee, WI), as our Philips system has no spectroscopic module. Experiments were performed using both a single-volume point-resolved spectroscopy (PRESS) sequence with TR/TE = 2000 ms/270 ms, and a stimulated-echo acquisition-mode (STEAM) sequence with TR/TE = 2000 ms/25 ms both employed with water suppression. Other parameters were FOV = 24 × 24 cm², slice thickness = 20 mm, and NT = 256. Both right and left hippocampi were measured with PRESS, whereas measurements with STEAM were applied to the left hippocampus only (Fig. 1). The area and height of the metabolic peaks were processed using the SAGE technique (Horison EchoSpeed; GE Medical Systems, Milwaukee, WI). Only spectra that satisfied the following criteria were included in the analysis: 1) the lowest point between the Cho peak and the Cr peak was less than the half width of the smaller of the two; and 2) SNR > 8 for the Cr peak. The ratios of NAA to (Cr + Cho), NAA to Cr, and Cho to Cr were calculated, and the volume of interest was positioned in the center of the hippocampus (2 × 2 × 2 cm³) (Fig. 2).

Each subject gave his/her informed consent. The study was approved by the local institutional review board.
Statistics

Means and standard deviations (S.D.) were calculated for all parameters. HV and TLV were measured by two experienced observers, and the HV and TLV interobserver and within-subject variances calculated. Repeated measurements of HV, TLV and $T_2$ were analyzed by the same observer to evaluate the within-subject variance. Statistical comparison was performed using a $t$-test (equality of mean), Fisher’s $F$-test (equality of variance) and a $\chi^2$-test (normality). A significance level of 95% was used.

Results

Two sets of volumetric measurements of HV and TLV were successfully performed in 25 participants. The right and left HVs for each volunteer were $4.3 \pm 0.6$ cm$^2$ (within-subject variance of $0.5$ cm$^2$, interobserver variance of $0.2$ cm$^2$) and HV of $4.1 \pm 0.5$ cm$^2$ (within-subject variance of $0.5$ cm$^2$, interobserver variance of $0.2$ cm$^2$), respectively (Fig. 3). TLVs were $73.4 \pm 8.3$ cm$^2$ and $71.2 \pm 7.4$ cm$^2$ for the right and left hemispheres, respectively. Statistical evaluation revealed a significant difference between right and left HVs ($P < 0.0001$) and between right and left TLVs ($P < 0.01$), with coefficients of variance (CV) for the right and left HVs of 0.13. The reproduced measurements of right and left HVs, and right and left TLVs, demonstrated normality based on a $\chi^2$-test, equal mean values according to a $t$-test and a comparable variance according to Fisher’s $F$-test.

Table 1 shows the results of $T_2$ measurements, based on the FSE sequence, demonstrating no significant difference in the interobserver and within-subject variances. However, the interobserver variance showed a small, but statistically significant, difference in $T_2$ measurements on the left side. No significant difference between $T_2$ of the right and left hippocampi was observed. The mean $T_2$ of the right hippocampus using FSE was $104.8 \pm 4.5$ ms (CV = 0.04, within-subject variance of 2.3 ms), and $103.8 \pm 4.5$ ms (CV = 0.04, within-subject variance of 2.4 ms) for the left hippocampus.

Table 2 shows $T_2$ measured by the CSE sequence demonstrating no significant difference in the interobserver and within-subject variances, except for a significant difference in the first within-subject variance measurement of the left $T_2$ ($P = 0.047$). Similarly, CSE measurements of $T_2$ demonstrated no significant difference between the right and left hippocampi. The mean $T_2$ for the right hippocampus was $98.8 \pm 5.3$ ms (interobserver variance of 2.3 ms) and $98.4 \pm 5.9$ ms for the left hippocampus (interobserver variance of 3.3 ms). For the right hippocampus, CSE $T_2$ produced a CV of 0.04 and, for the left hippocampus, CSE $T_2$ produced a CV of 0.02, indicating fair repeatability of $T_2$.

$^1$H MRS metabolite ratios were successfully measured in 19 left and 21 right hippocampi (70% and 77%, respectively). The $^1$H MRS metabolite ratios measured with the PRESS sequence calculated by peak height estimation showed no difference between the right and left hippocampi (Table 3). Metabolite ratios of the right hippocampus were $0.91 \pm 0.05$, $2.29 \pm 0.19$ and $1.52 \pm 0.14$ for NAA to (Cr

![Figure 3](image-url) Right and left HVs. The mean is indicated by the horizontal line, and broken lines represent mean ± 2 S.D. In the right hippocampus, the interobserver variance was 0.19 cm$^2$ and within-subject variance was 0.52 cm$^2$. In the left hippocampus, the interobserver variance was 0.17 cm$^2$, and within-subject variance was 0.48 cm$^2$.
+ Cho), NAA to Cr, and Cho to Cr, respectively, with CV percentages of 5.3%, 8.5% and 9.4%. Metabolite ratios of the left hippocampus were 0.88 ± 0.06, 2.18 ± 0.17 and 1.48 ± 0.10, respectively, with CV percentages of 6.8%, 8.0% and 6.8%. These results agree with others published previously [4,9,10,17,24]. Metabolite ratios of the left hippocampus measured with the STEAM sequence were 0.73, 1.41, 0.93 with CV of 8.0%, 8.5% and 9.5%, respectively, indicating a relatively higher uncertainty compared with the PRESS sequence. There was, however, no difference between ratios calculated by peak areas and peak heights estimated by the PRESS and the STEAM sequences.

Discussion

The purpose of this study was to investigate values of HV, TLV, T2 and metabolite ratios in healthy persons, and also to evaluate the interobserver and within-subject variances of these parameters.

In our hospital, HV and TLV are usually measured by the same operator to ensure repeatability. Measurements of the intraobserver variance of HV and TLV were, therefore, not performed in this study. The statistically significant differences measured between right and left HVs, and right and left TLVs, were in agreement with previous findings by Fernandez et al. [7] T2 measurements were performed both with a CSE and a FSE to investigate the difference in methodology. This study surprisingly demonstrated a marked difference in the T2 measurements performed by observers 1 and 2, and a small difference between subjects (P < 0.05) in the first measurement of left T2, which we could not explain. However, the observed T2 values were comparable with those reported in healthy brains [11,27]. In agreement with these results, we used the HV difference (right–left) of -0.5 cm² as a lower cut-off level and +0.8 cm² as an upper cut-off level, and differences in HV outside this interval were then identified as abnormal.

The wide variation in normal HVs suggests that diagnosis of hippocampal atrophy and bilateral abnormalities cannot be based entirely on visual assessments of HV. However, diagnostic evaluation of temporal-lobe epilepsy is significantly improved when volumetry and measurement of T2 are combined [27]. It has likewise been reported that combined measurements of HV and metabolite ratios improve the diagnostic lateralization of HS [14]. Experimental studies in animals and humans suggest that hippocampal neurons are particularly susceptible to aging, suggesting that the volumetric findings in our study should be related to age. Such analyses have not been done in this study, as

Table 2 Measurements of normal T2 with a CSE

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Right (N = 26)</th>
<th>Left (N = 26)</th>
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<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>CSE T2 (ms)</td>
<td>98.1 ± 3.7</td>
<td>98.3 ± 2.9</td>
</tr>
<tr>
<td>CV</td>
<td>0.04</td>
<td>0.03</td>
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<tr>
<td>P</td>
<td>0.894</td>
<td>0.188</td>
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CV: coefficient of variation; P: probability coefficient.

Table 3 Mean values of area and height of metabolite ratios in the right and left hippocampi. Differences between right and left side were not significant (P > 0.05)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Right (N = 19)</th>
<th>Left (N = 21)</th>
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<tbody>
<tr>
<td></td>
<td>Mean CV%</td>
<td>Mean CV%</td>
</tr>
<tr>
<td>NAA/(Cr + Cho)</td>
<td>0.94 6.5</td>
<td>0.89 9.4</td>
</tr>
<tr>
<td>NAA/CT</td>
<td>2.39 10.0</td>
<td>2.27 12.9</td>
</tr>
<tr>
<td>Cho/CT</td>
<td>1.56 12.1</td>
<td>1.55 13.9</td>
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Press Right (N = 19) Left (N = 21)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean CV%</th>
<th>Mean CV%</th>
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<tbody>
<tr>
<td>NAA/(Cr + Cho)</td>
<td>0.91 5.3</td>
<td>0.88 6.8</td>
</tr>
<tr>
<td>NAA/CT</td>
<td>2.29 8.5</td>
<td>2.18 8.0</td>
</tr>
<tr>
<td>Cho/CT</td>
<td>1.52 9.4</td>
<td>1.48 6.8</td>
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Press Left (N = 20) Left (N = 18)

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<tr>
<th>Parameter</th>
<th>Mean CV%</th>
<th>Mean CV%</th>
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<tbody>
<tr>
<td>NAA/(Cr + Cho)</td>
<td>0.63 5.7</td>
<td>0.73 8.0</td>
</tr>
<tr>
<td>NAA/CT</td>
<td>1.23 7.6</td>
<td>1.41 8.5</td>
</tr>
<tr>
<td>Cho/CT</td>
<td>0.95 8.9</td>
<td>0.93 9.5</td>
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STEAM

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<tr>
<th>Parameter</th>
<th>Mean CV%</th>
<th>Mean CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA/(Cr + Cho)</td>
<td>0.63 5.7</td>
<td>0.73 8.0</td>
</tr>
<tr>
<td>NAA/CT</td>
<td>1.23 7.6</td>
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<td>Cho/CT</td>
<td>0.95 8.9</td>
<td>0.93 9.5</td>
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reports of age-related hippocampal changes in MRI volumetry have been inconsistent [19,22]. Age-related HV loss has been reported by some authors [13,21], but not others [15].

1H MRS estimation of peak heights showed that the CVs for the left hippocampus measured with the STEAM sequence decreased in precision compared with the CVs measured with the PRESS sequence, which presumably could be explained by a lower SNR in spectra obtained with STEAM compared with those obtained with PRESS. It has been demonstrated that epilepsy patients present with significant reductions in the NAA signal and in the NAA-to-(Cr + Cho) ratio, together with an increase in the Cr and Cho signals [8]. Reduction of NAA was interpreted as neuronal loss or damage and increase of Cr and Cho as reactive astrocytosis. It was, therefore, suggested that measurements of the NAA-to-(Cr + Cho) ratio may serve as an important parameter in the evaluation of neuronal loss in the hippocampus.

Several considerations are involved in performing a single-voxel 1H MRS study. The employed TR makes a trade-off between SNR and acquisition time (for a fixed number of averages), whereas a long TE minimizes unwanted lipid and water resonances at the expense of SNR as well as the ability to detect long T2 components (NAA, Cr, Cho). In addition, the choice of pulse sequence can be critical, as PRESS usually produces a twofold better SNR (for the same TE) than STEAM, whereas STEAM is known to generate both a better volume of interest selection and increased water suppression. In the current study, both techniques were investigated, but with a relatively lower TE for the STEAM sequence according to the general use in clinical settings.

Another important criterion is related to the placement of the volume of interest. Although neuronal anatomy dictates the selected volume, several factors need to be considered. First, the quality of the collected spectra depends critically on magnetic-field homogeneity and, therefore, regions near air-filled sinuses, vessels or bone, or other sources that can change the local magnetic susceptibility can compromise the quality of the acquired data. This goes along with the second criterion, that it should be an experienced person handling the 1H MRS measurement.

Various methods have been used to measure peak areas, including the sophisticated LCModel minimization method introduced by Provencher [20], which models the spectra upon each of the expected components in the brain as a basis set for minimization.

This study generally showed that HV and T2 measurements were at least as precise as MRS measurements having CVs in the range of 5-14%. Based on these results, we recommend performing T2 measurements in combination with hippocampal volumetry. However, in patients with bilateral abnormalities, normal HV and T2, or who are about to undergo surgical procedures, we additionally recommend that 1H MRS be added to the MR protocol.

In conclusion, the relatively small CVs in measurements of HV, TLV, T2 and metabolite ratios, with T2 measurements showing no differences between variances within subjects and between observers, suggest that measurements of HV, TLV, T2 and metabolite ratios are repeatable. Volumetry is used in combination with relaxometry in clinical practice, and 1H MRS measurements are likely to be useful in situations with bilateral abnormality or with normal-appearing HV and T2, especially as quantitative 1H MRS can reveal important hippocampal abnormalities. It must, however, be noted that, although the observed data reported in this study may serve as a reference for healthy adults (taking the possible age-dependency into consideration), these results may not necessarily comply with measurements performed at other magnetic field strengths or in individuals with pathological disorders.

References

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