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Localized Proton MRS of the Human Hippocampus: Metabolite Concentrations and Relaxation Times

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Absolute concentrations and proton relaxation times of major metabolites in the human hippocampus were determined with use of fully relaxed, short-echo time STEAM localization sequences at 2.0 T (20 normal adults). Mean metabolite concentrations were 7.6 ± 0.9 mM for total *N*-acetylaspartate (tNAA), 6.9 ± 0.8 mM for total creatine (tCr), 2.1 ± 0.3 mM for choline-containing compounds (Cho), and 6.2 ± 0.9 mM for *myo*-inositol (Ins). The observation of relatively low tNAA and high Cho and Ins levels compared with cortical gray and white matter corresponds to a lower neuronal density and higher glial density than in the neocortex, in agreement with histologic findings. The data do not support a lateralization of metabolites. T1 and T2 relaxation times were in the range of 1400–1730 and 140–330 msec, respectively, similar to those in other brain regions. Magn Reson Med 41:204–207, 1999. © **1999 Wiley-Liss, Inc.**

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THE HIPPOCAMPUS and parahippocampal gyrus are important anatomical structures involved in various disease processes such as Alzheimer's disease, schizophrenia, limbic or herpetic encephalitis, and temporal lobe epilepsy (TLE). In particular, the pathogenesis of TLE reveals a characteristic topographic pattern of neuronal loss and gliosis (1) that has been addressed by in vivo proton magnetic resonance spectroscopy (MRS) of metabolites such as total *N*-acetylaspartate (tNAA), total creatine (tCr), and choline-containing compounds (Cho) in hippocampal areas of TLE patients (2–8). So far, however, most studies were performed at long echo times, and, except for two reports using the possibly altered water signal as an internal reference (4,7), the spectroscopic information was evaluated only as relative ratios of metabolite resonance signals, i.e. NAA/Cr, NAA/Cho, or NAA/(Cr + Cho).

Because the availability of absolute metabolite concentrations allows an assessment of individual metabolites in direct relation to those of age-matched controls, i.e., independent of relative changes and hemispheric comparisons, the primary aim of the present study was to extend previous work on quantitative proton MRS of other brain regions (9) to hippocampal areas. Specific problems arise from the small volume of the hippocampus and the presence of strong susceptibility differences between the anterior temporal lobe and surrounding air cavities within the sphenoid sinus and petrous bone. We therefore first identified the experimental conditions under which reliable short-echo-time proton MR spectra of reasonable quality may be obtained, and in a second step, used such conditions to determine absolute metabolite concentrations as well as T1 and T2 relaxation times of respective proton resonances.

MATERIALS AND METHODS

A total of eight subjects participated in a pilot study to determine the optimum location and size of a volume of interest (VOI) in the hippocampus. The main study consisted of bilateral investigations of hippocampal areas in 25 healthy volunteers (11 female/14 male; age range 22–43 years, mean 30 ± 6 years). In all cases informed written consent was obtained before the examinations. The subjects' handedness was determined by asking for the preferred hand for writing, drawing, and throwing; the preferred foot for kicking; and the preferred eye for telescope viewing following the questionnaire in Bernard et al (10).

Proton MRS was conducted at 2.0 T (Siemens Magnetom Vision, Erlangen, Germany) using the standard headcoil. The volunteers were positioned with the neck slightly hyperextended to align the long axis of the hippocampus proper with the transverse direction. Fully relaxed, short-echo-time proton MR spectra were recorded with use of a STEAM sequence (TR/TE/TM 6000/20/10 msec, 64 accumulations) and VOIs ranging from 2.2 to 8.0 mL. The time-domain data were corrected for residual phase distortions and calibrated in proportion to the actual coil loading (9).

Automated quantitation of absolute metabolite concentrations was accomplished by LCModel (11). Concentrations are expressed as mM, i.e., mmol/liter VOI, and are not corrected for residual T2 relaxation effects and CSF contributions, which were estimated to approximately 5–10%. T1 saturation was ignored because of a sufficiently long repetition time. Because of limited spectral resolution in hippocampal areas, *N*-acetyl compounds are given as total NAA (tNAA), i.e., the sum of *N*-acetylaspartylglutamate (NAAG) and NAA. Accordingly, total Cr (tCr) represents both creatine (Cr) and phosphocreatine (PCr). The level of choline-containing compounds (Cho) predominantly reflects contributions from glycerophosphorylcholine (GPC) and phosphorylcholine (PCh). Complementary to the role of tNAA as a marker of viable neurons (12), *myo*-inositol (Ins) distinctly indicates glial cells (13). Interindividual hemispheric asymmetry was assessed by comparing mean concentrations from right and left hippocampal areas averaged across subjects (two-sided unpaired *t*-test assuming unequal variances).

Estimates of T1 and T2 relaxation times of major metabolite resonances were obtained from a subgroup of eight subjects using three differently T1- or T2-weighted spectra. T1 weighting was achieved by varying the TR from 1500 to

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3000 and 6000 msec (TE/TM 20/10 msec), whereas T2 weighting was based on echo times TE of 20, 135, and 270 msec (TR/TM 6000/10 msec). Assuming monoexponential relaxation, T1 and T2 values were calculated from the LCModel analysis of only the singlet resonances of tNAA, tCr, Cho, and Ins using either a nonlinear Gauss-Newton method (for T1) or a linear least-squares fitting procedure (for T2).

RESULTS

Taking both anatomical and technical issues into account, the size and shape of the hippocampal VOI was optimized as a rectangular box of dimensions $13 \times 18 \times 23$ mm^3 (5.4 mL), as shown in Fig. 1. Special care was taken to avoid susceptibility effects, to cover most of the hippocampus, and to minimize partial volume effects with other tissues. The resulting VOI consisted mainly of the head and body portions of the hippocampus, parahippocampal gyrus, and small amounts of perihippocampal white matter and cerebrospinal fluid (CSF). The anterior medial temporal lobe containing the uncus, amygdala, and hippocampal heads was neglected because of its considerable sensitivity to susceptibility differences, which resulted in low spectral quality and poor reproducibility. Despite these precautions, Fig. 2 demonstrates that residual uncompensated susceptibility effects yield hippocampal spectra with broader resonance linewidths (5.1–6.8 Hz for tCr at 2.0 T) than obtained in cortical gray and white matter (3.3–4.1 Hz); see Pouwels and Frahm (9).

Table 1 summarizes the hippocampal concentrations of tNAA, tCr, Cho, and Ins averaged across 20 subjects and both hemispheres (mean value ± SD, $n = 40$). Further concentrations may be obtained from an LCModel analysis of the sum of all 40 spectra exhibiting a largely improved signal-to-noise ratio for the determination of less concentrated metabolites. This strategy yielded 7.1 mM for glutamate, 4.7 mM for glutamine, and 0.6 mM for lactate. The data from 5 of the 25 subjects were disregarded because LCModel estimated an uncertainty of the evaluated concentrations of more than 10% SD for NAA, Cr, and Cho or more than 15% SD for Ins in either hemisphere. In these cases the underlying poor spectral resolution was not caused by any external factors such as metallic dental prothesis, but must be ascribed to intrinsic structural inhomogeneities in and around the hippocampal areas. Both the short-term reproducibility (i.e., within one examination) or long-term reproducibility of data acquisition and evaluation (i.e., between several examinations) yielded 3–8% SD for tNAA, tCr, and Cho and 11% SD for Ins.

The data in Table 1 were pooled for both hemispheres because a statistical analysis of the interindividual asymmetry did not support any lateralization of metabolite levels ($P > 0.05$, $n = 20$). More specifically, there was no statistically significant interindividual asymmetry in a subgroup of subjects with a right-sided preference in at least four of

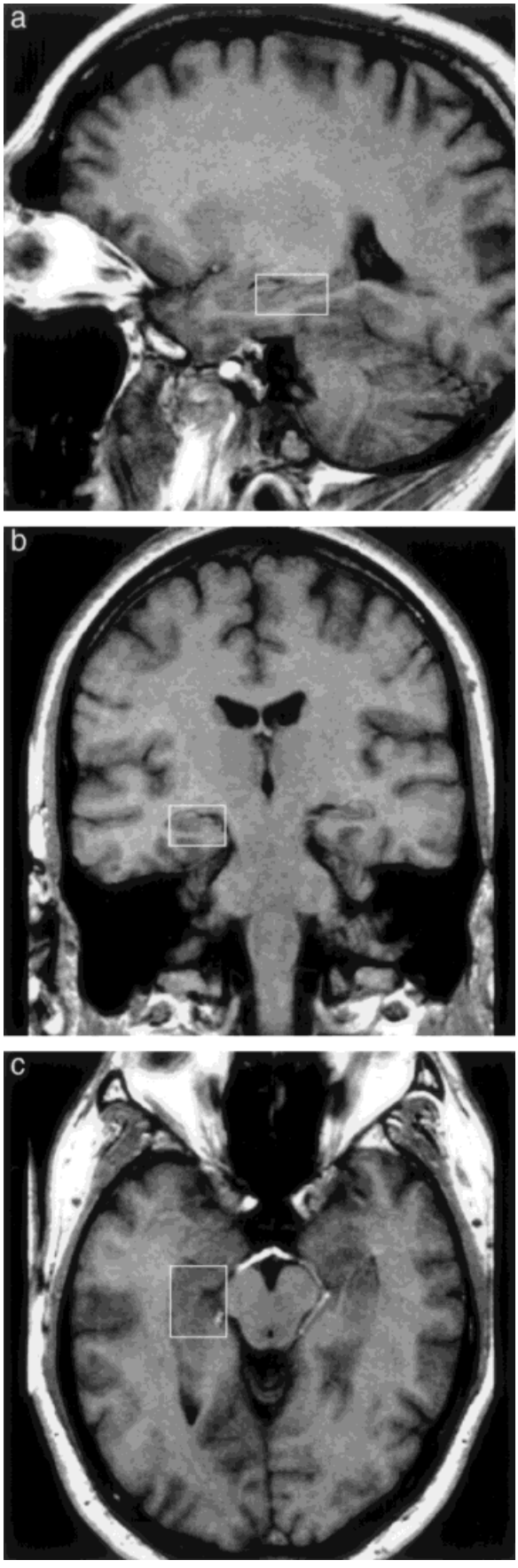

FIG. 1. **a–c:** T1-weighted MR images showing a typical VOI ($13 \times 18 \times 23$ mm^3 = 5.4 mL) selected for proton MRS of the human hippocampus (RF spoiled 3D FLASH, TR/TE 15/6 msec, flip angle 20°).

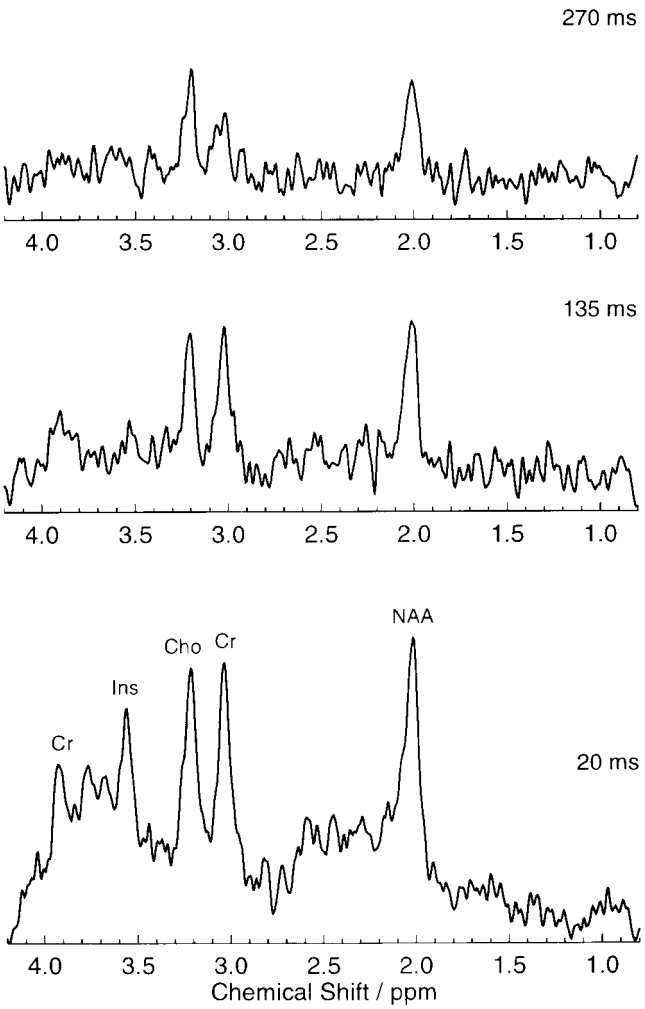

FIG. 2. Localized proton MR spectra of the hippocampus of a young healthy adult for three different echo times TE of 20, 135, and 270 msec (STEAM, TR/TM 6000/10 msec, 64 accumulations, VOI 5.4 mL). For display purposes only, spectral processing involved zero-filling to 4 K complex data points (2048 msec), Gaussian filtering (half-width 317 msec), and manual phase correction.

five items of the handedness questionnaire ($P > 0.05$, $n = 16$, right dominancy).

The T1 and T2 relaxation times of proton resonances of hippocampal metabolites given in Table 1 are in general agreement with previous findings in other brain regions, e.g., compare Kreis (14). Despite the large experimental uncertainties, the data suggest the absence of major regional differences. To our knowledge, metabolite relaxation times of hippocampal areas have not been reported so far.

Table 1
Concentrations and Proton T1 and T2 Relaxation Times of Major Metabolites in the Human Hippocampus

Metabolite	Concentration mM ± SD[a] ($n = 40$)[b]	T1 msec ± SD ($n = 12$)[c]	T2 msec ± SD ($n = 12$)[c]
tNAA	7.6 ± 0.9	1408 ± 253	283 ± 62
tCr	6.9 ± 0.8	1554 ± 317	191 ± 43
Cho	2.1 ± 0.3	1438 ± 365	334 ± 106
Ins	6.2 ± 0.9	1726 ± 512	138 ± 14

[a]mM refers to mmol/liter VOI.
[b]Bilateral spectra from 20 subjects.
[c]Bilateral experiments from eight subjects.

DISCUSSION

Although the optimization of a single VOI for proton MRS of hippocampal areas includes minor contributions from perihippocampal white matter and CSF, the chosen approach establishes a practical protocol for future studies of patients with TLE that (a) minimizes examination times, (b) allows for simple, fast, and fully automated data evaluation, and (c) provides a maximum of clinically useful information.

In comparison to a methodologically identical study (9), the hippocampal tNAA level of 7.6 ± 0.9 mM is significantly lower than those in frontal, parietal, and occipital white matter (9.6–10.6 mM), gray matter (8.4–10.6 mM), cerebellum (8.7–11.0 mM), and thalamus (10.5 mM). Putatively larger contributions from CSF are unlikely to account for this observation because similar findings apply to the previous work. Relatively low tNAA in the medial temporal lobe compared with thalamus or frontal white matter has also been reported in another single-voxel MRS study (4) and explains the decrease of the tNAA/Cr intensity ratio with decreasing voxel size (6). In contrast, much higher absolute tNAA levels of 11–12 mM (4,7) may be explained by differences in quantitation using water as an internal reference. This is further supported by the fact that these studies find much higher values for all other metabolites as well, which limits their usefulness for a detailed comparison with present results.

Taking tNAA as a neuronal marker and its concentration as an indicator of neuronal density (12,15), the lower tNAA content than in neocortex is in good agreement with reduced neuronal densities reported for the hippocampus and parahippocampal gyrus from embryologic and histologic evidence (16). Unlike the six-layered neocortex of the cerebrum, the hippocampus is phylogenetically the oldest cortex (''archicortex''), consisting of only a thick superficial molecular layer, a pyramidal neuron layer, and a cell-sparse polymorphic layer. Already at the end of 9 gestational weeks, a clear morphologic distinction can be made between the neocortex and archicortex. The dorsomedial portion of the fetal cerebral wall, which constitutes the future archicortex, reveals a thinner ventricular layer (neuroblast zone) and a thicker cell-sparse layer than the adjacent prospective neocortex. The hippocampal pyramidal or polymorphic cell layers correspond to the deeper layers 5 and 6 of the neocortex, whereas no cell populations are found that correspond to the superficial layers 2, 3, and 4 of the neocortex.

The hippocampal Cho concentration of 2.1 ± 0.3 mM is significantly higher than in cerebral gray matter (0.9–1.4 mM) or white matter (1.6–1.8 mM) but similar to that of cerebellum (2.2 mM) (9). Similar findings apply to the Ins concentration of 6.2 ± 0.9 mM, which is higher than in cerebral gray matter (4.1–4.7 mM) and even cerebellum (5.6–5.8 mM) (9). In normal adults, regional differences of Cho and Ins concentrations mainly depend on the cellular composition of the brain tissue. Detailed studies of cell type-specific cultures revealed that Cho concentrations in astrocytes or oligodendrocytes were two to threefold higher

than in neurons (17) and demonstrated that Ins may be considered a glial (astrocytic) marker that is not present in neurons (13). Thus, a most likely explanation for the high Cho and Ins concentrations in hippocampal areas is an increased glial cell density relative to the cerebrum. This may especially hold for the thick molecular and polymorphic layers with very low neuronal densities and is further supported by the general finding that the glial density is roughly inversely proportional to the neuronal density in cerebral cortex (18).

Because tCr is contained in both hippocampal neurons and glial cells, the finding of a hippocampal tCr concentration of 6.9 ± 0.8 mM similar to that of cerebral gray matter (6.4–7.0 mM) (9) suggests a similar overall cell density as in the neocortex but with more glial cells and less neurons. One may speculate that the need for large numbers of astrocytes may be caused by the high energy demand of hippocampal pyramidal neurons, which have a higher synaptic density per neuron than other neocortical neurons (19). It is also supported by high amounts of cytosolic or mitochondrial creatine kinase isoenzymes found in granular and pyramidal cell layers of the hippocampus during postnatal development of rats as well as by high levels of expression of the creatine transporter in the pyramidal cell layer of adult rat brains (20,21). In humans, there is even evidence of muscle-type creatine kinase in pyramidal cells of the hippocampus proper (22).

CONCLUSIONS

Single-voxel short-echo-time proton MRS examinations of hippocampal areas were successful in about 80% of normal adults. Whereas T1 and T2 relaxation times of major brain metabolites are rather similar to those found in other brain regions, the hippocampus reveals a unique pattern of metabolite concentrations that is characterized by relatively low levels of tNAA and high levels of Cho and Ins. These findings are in line with the hippocampal cytoarchitecture and mixture of cell populations consisting of a lower neuronal density and higher glial density than in neocortical tissues. Pertinent insights will help to develop further quantitative proton MRS of patients with TLE toward its ultimate role as a tool for noninvasive histopathology.

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