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Age-related metabolite changes and volume loss in the hippocampus by magnetic resonance spectroscopy and imaging[★]

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Abstract

Magnetic resonance imaging (MRI) studies have produced controversial results concerning the correlation of hippocampal volume loss with increasing age. The goals in this study were: 1) to test whether levels of N-acetyl aspartate (NAA, a neuron marker) change in the hippocampus during normal aging and 2) to determine the relationship between hippocampal NAA and volume changes. Proton magnetic resonance spectroscopic imaging (¹H MRSI) and MRI were used to measure hippocampal metabolites and volumes in 24 healthy adults from 36 to 85 years of age. NAA/Cho decreased by 24% ($r = 0.53$, $p = 0.01$) and NAA/Cr by 26% ($r = 0.61$, $p < 0.005$) over the age range studied, whereas Cho/Cr remained stable, implying diminished NAA levels. Hippocampal volume shrank by 20% ($r = 0.64$, $p < 0.05$). In summary, aging effects must be considered in ¹H MRSI brain studies. Furthermore, because NAA is considered a marker of neurons, these results provide stronger support for neuron loss in the aging hippocampus than volume measurements by MRI alone.

Keywords

Magnetic resonance spectroscopy; Magnetic resonance imaging; Aging; Hippocampus; Atrophy; Neuron loss; N-acetyl aspartate

1. Introduction

Considerable evidence suggests that normal aging is associated with gradual impairment of memory functioning [16]. The medial temporal lobe, especially the hippocampus, plays a central role in declarative memory processing [31]. Some in-vivo studies using magnetic

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resonance imaging (MRI) reported atrophy of the hippocampus [13,14] with advancing age. Other MRI studies, however, did not identify age-related hippocampal volume losses [17,18, 32]. Although volume loss has been interpreted to reflect neuron loss, it is a non-specific indicator of neuronal integrity as volume loss may also reflect shrinkage of neuron cell bodies and non-neural changes, such as loss of glial components.

Proton magnetic resonance spectroscopy (^1H MRS) and ^1H MR spectroscopic imaging (MRSI) detect cerebral metabolites in-vivo, most commonly N-acetyl aspartate (NAA), and choline (Cho) and creatine (Cr) containing compounds. NAA is described as a neuron marker, because it is found at high concentration almost exclusively in neurons [24], but is virtually undetectable in various other cell types, including glial cells [35]. Using ^1H MRSI, we found diminished NAA levels in the hippocampal region in several conditions, including epilepsy [8,36], post-traumatic stress disorder [28], and Alzheimer's disease [29]. Furthermore, measurements of NAA with ^1H MRSI seemed in some instances to be more sensitive to subtle changes of neuronal losses than volume measurement with MRI [8]. Therefore, ^1H MRSI may be useful in assessing the integrity of neurons in the hippocampus during aging.

The Cho resonance measured by ^1H MRSI originates predominantly from choline itself and from glycerophosphocoline and phosphocholine, which are constituents of membrane phospholipids. Cho is markedly increased in conditions with myelin breakdown [1], which is thought to contribute to some cognitive deficits in aging [25]. Decreased NAA along with increased Cho may therefore be useful in differentiating between axonal damages that involve myelin breakdown and those that do not. Finally, the Cr resonance represents the sum of creatine and phosphocreatine, which are both present in neural and glial cells, making Cr alterations somewhat difficult to interpret.

Age-related changes of these metabolites have been reported earlier by several ^1H MRS studies [4,5,9,19]. However, most of these ^1H MRS studies were performed in brain regions that are not particularly known for an involvement in declarative memory functioning. This laboratory has been performing ^1H MRSI studies of the hippocampus on patients with temporal lobe epilepsy [8] and Alzheimer's disease [29]. The data from the age-matched controls of these studies provided an opportunity to examine the effects of age on hippocampal NAA. Therefore, the first goal of this study was to determine whether age-related changes of NAA were present in the hippocampus of healthy subjects. The second goal was to determine the relationship between hippocampal NAA and volume changes.

2. Methods

2.1. Recruitment and examination of subjects

The study included 24 healthy subjects (age range 36 to 85, 13 women, 11 men), who had been previously recruited to participate as controls in two separate studies. Sixteen subjects between 61 and 85 years old were recruited from the Northern California Alzheimer Center and University of California at Davis and the Mount Zion Center of Aging in San Francisco as part of ongoing studies on Alzheimer's disease and subcortical vascular dementia in this laboratory. These subjects were examined by a neurologist and had the standard battery of neuropsychologic tests at the Centers to judge that they were cognitively normal for their age and had no signs of mild cognitive impairment or early dementia. The eight young subjects were between 36 and 56 years old and were originally enrolled in MRI/MRSI studies of epilepsy in our laboratory. They had been recruited by posting flyers in the community. The MRI scans of all subjects were read by a neuroradiologist (D.N.). No subject had evidence of stroke, cortical or subcortical infarctions, or other major abnormalities on MRI scans. The Committee on Human Research at UC San Francisco approved the study protocol, and written informed consent was obtained from all participants of the study.

2.2. MRI/MRSI Examinations

All studies were performed on the same 1.5 Tesla Magnetom VISION™ system (Siemens Inc., Iselin NJ), equipped with a standard quadrature head coil. A vacuum-molded head holder (Vac-Pac, Olympic Medical, Seattle WA) was employed to restrict head motion. Axial and sagittal T₁ weighted localizer MRI scans were acquired to guide the positioning of subsequent MRI and ¹H MRSI scans. A volumetric (3D) magnetization prepared rapid gradient echo (MP-RAGE) sequence with TR/TI/TE = 10/250/4 ms, 15° flip angle, 1.0 × 1.0 mm² resolution, and 1.4 mm thick partitions was employed to obtain T1-weighted coronal images oblique to the long axis of the hippocampus. These images were used to measure hippocampal and the total intracranial volumes. In addition to MP-RAGE, subjects from the Alzheimer's study were scanned with a double spin-echo (DSE) sequence which yields proton density and T2-weighted images. The information from DSE is needed to perform image segmentation into brain tissue and cerebrospinal fluid (CSF) and, furthermore, to correct the metabolite signal from ¹H MRSI for partial volume effects with varying amounts of tissue and CSF. The metabolite changes were expressed in terms of ratio values instead of absolute concentrations to minimize partial volume differences between the ¹H MRSI data of the subjects. Although this laboratory reported earlier absolute metabolite concentrations with volume corrections from tissue segmented MRI data [29], this could not be accomplished in this study because subjects recruited for the epilepsy study had no DSE MRI scans which are required for reliable MRI tissue segmentation. All other parameters of the Alzheimer's and epilepsy studies were identical and data acquisitions of the two studies were accomplished during the same time period.

¹H MRSI data sets were acquired using a spin-echo sequence (TR/TE = 1800/135 ms) with preselection of a region of interest (PRESS volume). The PRESS volume was angulated parallel to the long axis of the hippocampi as seen from the sagittal scout images and positioned on the axial plane to cover both hippocampi in their entire length and adjacent sections of the midbrain and the temporal lobes. The MRSI field of view was 210 × 210 mm and was sampled using a circular k-space scheme equivalent to a maximum of 24 × 24 phase encoding steps [22], resulting in a nominal voxel resolution of 1.1 mL. The spectral sweep width was 1000 Hz. Fig. 1 shows an axial T1-weighted MR image from a control subject at the position of the hippocampus and the corresponding NAA image, restricted to the sensitive area of the PRESS volume. Also shown in Fig. 1 is a representative ¹H MR spectrum selected from the hippocampal body (location and approximate size of the MRSI voxel is indicated by a circle in the corresponding MRI), exhibiting the three prominent resonances from NAA, Cho, and Cr.

Volume measurements of the hippocampus were performed using software developed in house (G.F.). Quantitative estimates of the right and left hippocampal volumes were obtained from coronal T₁-weighted MP-RAGE images, reformatted orthogonal to the long axis of the hippocampus. Boundaries of the hippocampus were drawn following the guidelines of Watson et al. [37]. The areas of all regions of interest were then calculated and the total volume derived by multiplying that value by the slice thickness. Volume measurements were performed by one rater (D.A.), who had been extensively trained by an experienced operator (R.K). Calculated inter- and intrarater reliabilities were better than 0.92 [29]. Each subject's data sets were encrypted using a random number assignment procedure to ensure that the operators were blinded to subject clinical data. Hippocampal volumes were normalized for differences in total intracranial volume among subjects by an analysis of covariance approach [12], according to

$$V(a) = V(o) - b * (TIV^i - \bar{TIV}).$$

$V(a)$ is the adjusted volume, $V(o)$ is the observed volume, b is the regression slope of the hippocampal volume regressed against total intracranial volume (TIV), TIV^i is the intracranial volume of the i th subject, and TIV is the mean intracranial volume. Intracranial volumes were calculated from image segmentation by summing over all segmented pixels, which represented either brain tissue or cerebrospinal fluid. The analysis of covariance approach was used because this adjustment accounts for sex and age effects. Furthermore, this approach maintains the dimension of the adjusted volumes in cubic millimeters, whereas other adjustments that use volume/TIV ratios produce dimensionless values.

2.3. MRSI analysis

After acquisition, the ^1H MRSI data were zero-filled to a rectangular matrix of $32 \times 32 \times 1024$ points, Fourier transformed, and phase- and baseline corrected using software developed in house [21]. Gaussian broadening with 4 Hz linewidth was used in the spectral direction and mild Gaussian apodization was applied along the spatial directions, yielding MRSI voxels with approximately 1.6 mL of volume. Voxels were selected from head, body, and tail of the right and left hippocampus, and the resonances from NAA, Cr, and Cho were curve fitted using NMR1TM software (New Research Methods Inc, Syracuse NY) and expressed in terms of ratio values (NAA/Cr, NAA/Cho, and Cho/Cr). Spectra from the hippocampal body are best suited to measure metabolic changes in the hippocampus and were therefore used exclusively for analysis in this study. Previous ^1H MRSI studies of hippocampus from this laboratory [29] in combination with MRI tissue segmentation showed that MRSI voxels positioned in the hippocampal body contained more hippocampal tissue than voxels from the tail, which often extend into ventricular spaces and may include sections of the adjacent temporal lobe.

2.4. Statistical analysis

Multivariate analysis of variance was used to assess the effects of age and gender on the metabolite ratios and volume measures. Pearson product-moment correlation was used to test for the correlation between age and MRSI/MRI measures. Regression analysis was used to determine the rates of the metabolite changes and volume loss with age. Values are presented as mean $\pm$ standard deviation, unless otherwise noted. The level of significance was $p < 0.05$.

3. Results

NAA/Cho decreased significantly with advancing age ($F(1,23) = 5.08$; $p = 0.02$) and without a significant effect by gender ($F(1,23) = 2.05$; $p = 0.15$). A similar decrease with age was observed for NAA/Cr ($F(1,23) = 6.74$, $p = 0.006$) and no significant gender effect ($F(1,23) = 0.41$; $p = 0.7$). Although NAA/Cho was about 4% higher in the right than in the left hippocampus and NAA/Cr was 3% higher in the left than in the right hippocampus, these differences were not statistically significant ($p > 0.2$, paired t -test). Cho/Cr did not change significantly with age or gender. The hippocampal volumes were significantly decreased with advancing age ($F(1,23) = 7.89$; $p = 0.003$) and without a significant effect by gender ($F(1,23) = 1.01$; $p = 0.4$). The right hippocampus was on average 4% larger than the left across the age groups, but this difference was not significant ($p = 0.11$, paired t -test). Tests for associations between the metabolite and volume changes showed that NAA/Cho and volume reductions were significantly correlated ($r = 0.58$, $p = 0.01$) in the left hippocampus. No other significant metabolite and volume relations were found. Because no significant hemisphere effect was found, the measurements from the right and left hippocampus were averaged in the remainder of the analyses. When a similar statistical analysis was performed separately on the two subgroups of subjects recruited for epilepsy (those ≤ 56 years) and Alzheimer's studies (those > 56 years), there were no significant age effects within each group.

The scatterplots of Fig. 2 depict the relationship of the metabolite ratio and volume changes with age, separately for men and women. Both, NAA/Cho ($r = -0.53$, $p = 0.01$) and NAA/Cr ($r = -0.61$, $p < 0.005$) were significantly correlated with age, so was hippocampal volume ($r = -0.64$, $p < 0.005$). Over the age range studied, NAA/Cho declined by 24% from a value of 1.66 ± 0.10 (standard error) at the age of 36 to 1.25 ± 0.24 at the age of 85 based on the regression. Similarly, NAA/Cr declined by 26% from a value of 1.98 ± 0.11 at the age of 36 to 1.46 ± 0.25 at the age of 85. Hippocampal volume diminished by 20% from $3,584 \pm 136 \text{ mm}^3$ at age 36 to $2,870 \pm 220 \text{ mm}^3$ at age of 85, corresponding to a volume loss of about $14.6 \text{ mm}^3/\text{year}$. To determine if the relative changes of NAA/Cho and NAA/Cr over the entire age range were significantly larger than the relative volume change, we calculated the contribution of the product terms (NAA/Cho $\times$ volume and NAA/Cr $\times$ volume) to the regressions. We found no significant contribution, indicating that hippocampal NAA/Cho, NAA/Cr, and volume changed at similar relative rates with advancing age. Because metabolite ratios are not affected by tissue volume, the similar rate of NAA ratio change and volume change is consistent with the view that volume reductions are due to neuron loss. When correlation tests were performed separately on the two subgroups of recruited subjects, no significant correlations with age were found within each group.

4. Discussion

The major findings of this study were: 1) Hippocampal NAA/Cho and NAA/Cr decreases with advancing age, whereas Cho/Cr remains relatively stable. This implies that NAA ratio reductions are primarily due to decreases of NAA. 2) Hippocampal volume decreases with age, confirming previous reports [13,14]. 3) Hippocampal NAA ratios and volume change at similar relative rates with advancing age. This is consistent with the view that hippocampal volume loss is due to neuronal loss.

The finding that NAA/Cho and NAA/Cr but not Cho/Cr were reduced with older age suggests that these changes are primarily due to diminished levels of NAA and not due to elevated levels of choline and/or creatine containing compounds. Possible explanations for the reduced NAA levels are diminished neuronal density and/or loss of neurons in the aged hippocampus. Age-related losses of hippocampal neurons have been demonstrated by several autopsy studies [23,26,38]. West [38] reported neuron losses of 31% to 52% across an age range of 13 to 85 years in some substructures of the hippocampus using highly accurate stereological methods. An alternative explanation for reduced NAA levels, which must be considered, is a diminished concentration of NAA in neurons. Several MRS studies reported reversible NAA levels in the presence of acute ischemia [3,7,11,15], indicating that NAA concentrations are a product of oxidative metabolism [2]. Therefore, reduced NAA levels may also reflect impairment of neuronal metabolism. Impaired oxidative metabolism in the normal elderly brain seems a less likely explanation.

A single voxel ^1H MRS study by Fukozako et al. [9] also reported decreased NAA/Cr in the medial temporal lobe (which included the hippocampus) of older subjects. Other ^1H MRS studies found stable NAA levels with increasing age in other regions of the brain, including fronto-parietal brain gray and white matter regions [30] and regions of the frontal cortex [4]. However, Lim et al. [19] found lower NAA levels in cortical gray matter of older subjects when compared with younger subjects. This suggests that there may be regional differences of age related NAA changes.

The finding that hippocampal volume decreases with advancing age is consistent with earlier MRI reports [13,14]. Other MRI studies [17,18,32], however, found no correlation between hippocampal volume and age on a large number of subjects. The heterogeneity of the different study populations, including the polymorphism of the apolipoprotein E (ApoE) gene, which

is associated with Alzheimer's disease could be responsible for the lack of consistent findings among the MRI studies. Recent studies [27,34] suggest that normal subjects carrying the e4 allele of ApoE (considered a risk for developing Alzheimer's disease) have markedly smaller hippocampi than individuals without the e4 allele. There was no control for the ApoE status in this study nor in the MRI studies that found no age-related volume losses. Another explanation for the inconsistent findings is that other cellular mechanisms that were not related with aging may have contributed to volume loss. Volume measurements are not a specific indicator of neuron loss but may also reflect shrinkage of cell bodies and loss of glial components. Similarly, because age-related neuron loss can also be accompanied by reactive gliosis [6], volume reductions may be attenuated to the extent that neurons are replaced by glial cells. Because no autopsy data are available from the subjects of this study, we cannot conclusively determine to what extent other tissue components than neurons may have contributed to the hippocampal volume changes.

The third finding of this study was that the relative decline of hippocampal NAA ratios with age was similar to the relative decline of hippocampal volume. Because the NAA ratio changes were most likely due to decreases of NAA, this finding is consistent with the view that volume reductions in the hippocampus are due to neuron loss. Because diminished NAA levels are considered to reflect reduced density or numbers of neurons but not changes in non-neural processes, these results provide stronger support for neuron loss in the aging hippocampus than volume measurements alone.

The major limitation of this study is that the young subjects (36 to 56 years) were initially not recruited for this aging study and had screening procedures that were different from those applied to older subjects. Because we could not detect significant age effects within each of the subgroups, it is possible that differences other than age between the younger and older subjects might account for the apparent age effects. These differences might include alcohol use, ApoE status, and other factors that are thought to affect hippocampal size and neuron density [33,34]. Ultimately, age effects on hippocampal NAA and volume need to be studied in a prospective longitudinal study over several years. We expect, however, that a MRSI/MRI study on a larger cross-sectional sample of carefully screened subjects with control for all factors that are associated with hippocampal size, would provide enough information to establish more firmly age-related metabolite changes in the hippocampus, and will hopefully confirm our findings.

There are several other limitations to this study: 1) The data were presented as metabolite ratios instead of absolute metabolite changes, complicating the interpretation of the results. This laboratory [20] and others [10,19] have reported methods to quantify metabolite MRSI data, including corrections for partial volume effects using the information from tissue segmented MRI data. Unfortunately, the DSE MRI sequence, which is necessary for tissue and CSF segmentation and partial volume correction was not performed in this study on all subjects. Therefore, quantitative MRSI data yielding metabolite concentrations could not be obtained on every subject. Similar to NAA ratios, volume corrected NAA is independent of changes in tissue volume. In an earlier study on Alzheimer's disease [29] we have found that volume corrected NAA changes can be larger in magnitude than changes of NAA ratios. Therefore, it is possible that volume corrected NAA of the hippocampus might be a more sensitive measure of aging effects than NAA ratio measurements. 2) The major technical limitation of this study is the coarse spatial resolution of ^1H MRSI that may have resulted in volume averaging of non-hippocampal structures, such as the entorhinal cortex. Therefore, the decline of NAA ratios may also reflect neuron loss outside the hippocampus. Some improvement in the spatial resolution of ^1H MRSI can be accomplished by increasing the signal to noise ratio of ^1H MRSI experiments, i.e., by acquiring the MR signal at much shorter spin-echo times. Such experiments are currently underway in our laboratory. Furthermore, T1 and T2 relaxation rates

of the metabolites were not measured because of the prohibitively long duration of data acquisition. Therefore, it is possible that some of the NAA/Cho and NAA/Cr changes may be due to alterations of the metabolite relaxation rates and not due to reduced NAA concentrations. Finally, this study comprised only a small number of subjects and in particular included only 2 women under the age of 60. Other MRI studies on aging [17,32] reported data from much larger samples, which may have presented more adequately the general population than this study.

Notwithstanding these limitations, the current findings demonstrate significant reductions of NAA metabolite ratios in hippocampus with increasing age. Therefore, age effects must be considered in ¹H MRSI studies of human brain disease and strong attempts should be made to provide careful age matching between study groups. Furthermore, because the reductions of NAA ratios are considered to reflect reduced density or numbers of neurons but not changes in non-neural processes, the results from this study provide stronger support for neuron loss in the aging hippocampus than volume measurements by MRI alone.

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Fig. 1. Axial MR image from a control subject at the position of the hippocampus, and the corresponding NAA image from this region, restricted to the sensitive area of the PRESS volume. Contours of the MRI are superimposed in the NAA image for better anatomical reference. Also shown is a representative ^1H MR spectrum selected from the hippocampal body (location indicated by a circle in the MRI).

Fig. 2.
Regression plots of hippocampal NAA/Cho (a), NAA/Cr (b), Cho/Cr (c), and volume (d) as a function of age. Men (•), women (○).