

ORIGINAL COMMUNICATION

High-Resolution 8 Tesla Imaging of the Formalin-Fixed Normal Human Hippocampus

DONALD W. CHAKERES,^{1*} CHASTITY D.S. WHITAKER,¹ ROGER A. DASHNER,²
DOUGLAS W. SCHARRE,³ DAVID Q. BEVERSDORF,³ ABHIK RAYCHAUDHURY,⁴
AND PETRA SCHMALBROCK¹

¹Department of Radiology, University Hospital, Ohio State University, Columbus, Ohio

²Division of Anatomy, College of Medicine & Public Health, Ohio State University, Columbus, Ohio

³Department of Neurology, College of Medicine & Public Health, Ohio State University, Columbus, Ohio

⁴Department of Pathology, University Hospital, Ohio State University, Columbus, Ohio

The purpose of this study was to evaluate the capacity of high-resolution magnetic resonance imaging (MRI) to visualize the normal anatomic features of the human hippocampus in vitro, using high field imaging equipment, parameters, and acquisition times appropriate for imaging human subjects in vivo. This research compared high field, high-resolution MRI of formalin-fixed normal human hippocampus specimens to histologic sectioning of the same hippocampus samples. Four specimens were evaluated using an 8 Tesla (T), 80 cm bore whole-body MRI scanner equipped with a 12.7 cm single strut transverse electromagnetic resonator (TEM) coil. Hahn spin echo images were acquired with a repetition time (TR) of 800 msec, echo times (TE) of 20, 50, 90, and 134 msec, and an acquisition time (TA) of 3.25 min. The image quality was superb with demonstration of most of the features of the hippocampus. High field, high-resolution MRI can be used to depict multiple layers of the formalin-fixed human hippocampus in vitro using an 8 T whole-body scanner, a TEM coil, and short acquisition times compatible with human imaging in vivo. Clin. Anat. 18:88–91, 2005. © 2005 Wiley-Liss, Inc.

Key words: brain anatomy; temporal lobe; high field MRI; TEM coil

INTRODUCTION

High field, high-resolution magnetic resonance imaging (MRI) at 7 and 8 Tesla (T) with increased signal-to-noise ratio (SNR) has generated unique imaging information in vivo not available at lower field strengths. The anatomic depiction of the microvasculature of the brain and subtle functional changes in blood flow during brain activation have been reported (Burgess et al., 1999; Vaughan et al., 2001). High field imaging has the potential to visualize the detailed anatomic structures of the hippocampus and the medial temporal lobe, which may lead to improved means of evaluating patients with disorders such as Alzheimer's disease or temporal lobe epilepsy (Duncan et al., 1996; Miller et al., 1996; Oppenheim et al., 1998; Benveniste et al., 1999; Mu et al., 1999; Killiany et al., 2002).

Realization of this potential still has many hurdles that need to be overcome including serious limitations

related to increased magnetic susceptibility artifacts and variable radio frequency (RF) head coil performance at very high fields, as well as changes in both T1 and T2 relaxation times that can lead to lower contrast (Abduljalil and Robitaille, 1999; Ibrahim et al., 2001). The RF volume coils used for high field MRI of human subjects perform much differently than routine birdcage head coils used at lower fields and are referred to as transverse electromagnetic resonator (TEM) coils. These coils have the property of producing the best image quality centrally within the head, but the region of optimal performance is not

*Correspondence to: Donald W. Chakeres, MD, Department of Radiology, The Ohio State University Hospital, 630 Means Hall, 1654 Upham Drive, Columbus, OH 43210-1228.
E-mail: chakeres-1@medctr.osu.edu

Received 23 January 2003; Revised 3 July 2003

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/ca.10232

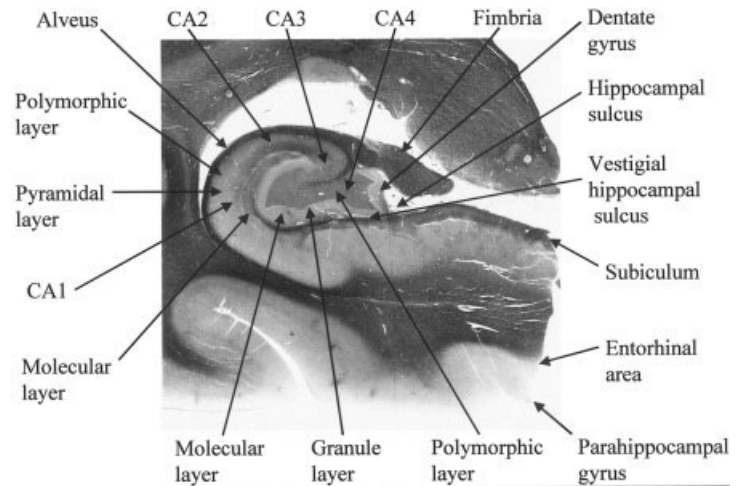


Fig. 1. Coronal histologic section of the mid-hippocampus stained with Bielschowski stain. Labeled structures include the fimbria, alveus, hippocampal polymorphic layer, pyramidal layer, CA1-4, hippocampal molecular layer, dentate gyrus, hippocampal sulcus, vestigial hippocampal sulcus, subiculum, entorhinal area, parahippocampal gyrus, dentate polymorphic layer, granule layer, and the dentate molecular layer.

easily controlled, often leading to rather poor signal in the region of the hippocampus (Ibrahim et al., 2001; Vaughan et al., 2001).

Our goal was to evaluate formalin-fixed specimens *in vitro* with an 8 T whole-body MRI scanner equipped with a single strut TEM coil. We used a pulse sequence, imaging parameters, and a short acquisition time that would be suitable for human studies *in vivo*. Most comparable studies at high field have excellent image quality, but utilize techniques that require multiple hours of acquisition time and are not compatible with routine human imaging (Beuls et al., 1993; Benveniste et al., 1999; Fatterpekar et al., 2002). We hoped to better characterize the normal anatomy visible at this field strength and develop optimal imaging techniques that may allow for the visualization of these same anatomical structures in future whole brain studies conducted on live human subjects.

MATERIALS AND METHODS

Four formalin-fixed coronal hippocampus specimens from deceased elderly subjects (ages 65, 71, 73, 73) with normal brain anatomy at autopsy were imaged. Each of the specimens measured approximately 3.8 cm in diameter and 1.9 cm in thickness and was stored in a small plastic discoid container (6.35 cm outer diameter by 5.08 cm height) filled with formalin.

The images were acquired with a Magnex-General Electric (Abingdon, UK) 8 T, 80 cm bore whole-body MRI scanner equipped with a Bruker Avance (Billerica, MA, USA) console. A single strut 12.7 cm diameter, asymmetrically shielded TEM coil was used. The specimens were imaged using the following parameters: a Hahn spin echo (SE) pulse sequence with repetition time (TR) = 800 msec, echo time (TE) = 20, 50, 90, and 134 msec, field of view (FOV) = 5.4 cm, matrix (MTX) = 256 × 256 pixels, number of

excitations (NEX) = 1, receiver bandwidth (RBW) = 50 kHz, acquisition time (TA) = 3.25 min, and a single 2-mm thick section with a final pixel size of 210 × 210 μm in-plane resolution. The MR image quality was comparable between the different specimens.

After MRI, the specimens were paraffin-embedded and cut into 5-μm histologic sections. These sections were then deparaffinized in xylene and graded alcohols, stained with H&E and Bielschowski stains, and compared directly to the imaging data.

RESULTS

The histologic sections showed the basic anatomic features of the hippocampal formation including regions such as the fimbria, dentate gyrus, cornu Ammonis, subiculum, and parahippocampal gyrus (Fig. 1). In addition, the hippocampal regions clearly demonstrated the layered anatomic features of these structures. These layers from superficial to deep include the alveus, hippocampal polymorphic layer (stratum oriens), pyramidal layer (stratum lucidum and stratum radiatum), cornu Ammonis (CA 1-4), hippocampal molecular layer (stratum lacunosum), vestigial hippocampal sulcus, dentate molecular layer, granule layer, and dentate polymorphic layer. The vestigial hippocampal sulcus is a potential space that may extend deeply into the hippocampus (Duvernoy et al., 1998). It may contain a small residual fluid space that was once continuous with the hippocampal sulcus.

The basic anatomic features of the hippocampal formation were also well visualized in the MR images (Fig. 2). The image quality was good with typical SNR values for gray matter of 118, 50, 22, and 6 for echo times of 20, 50, 90, and 134 msec, respectively (Fig. 3). The image contrast demonstrated the lowest relative signal intensity for the formalin on the shorter

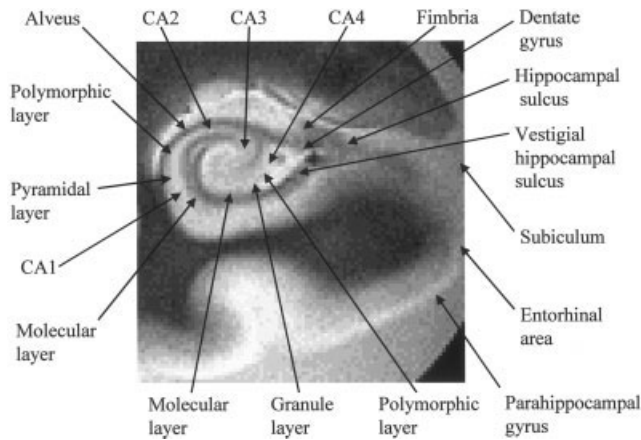


Fig. 2. MRI of the hippocampus. An 8 T coronal Hahn SE MR image at a similar level from which the histologic shown in Figure 1 was made. The image was acquired with TR = 800 msec, TE = 50 msec, and TA = 3.25 min. Many of the same detailed structures of the hippocampus are visible and labeled.

TE images. The formalin was iso-intense to the gray matter on the TE 50 msec images and higher in signal intensity on the longer TE images. Differentiation of the anatomic features was best seen on the TE 50 msec images (Figs. 2,3). Gray matter structures (parahippocampal cortex, cornu Ammonis) generally had a higher signal intensity compared to the white matter structures (fimbria, alveus, white matter of the hippocampal gyrus). The vestigial parahippocampal sulcus demonstrated a low signal intensity similar to the white matter tracts. In general, there was good contrast between the different layers of the hippocampal formation, yet a number of the thinnest layers were not distinguishable (e.g., the molecular and granule layers).

DISCUSSION

Magnetic resonance imaging of the hippocampal region has been of particular importance in the evaluation of temporal lobe epilepsy and Alzheimer's disease (Duncan et al., 1996; Miller et al., 1996; Oppenheim et al., 1998; Benveniste et al., 1999; Mu et al., 1999; Killiany et al., 2002). Because of the complicated anatomy of this region, the highest achievable spatial resolution is an important advantage particularly regarding quantitative studies of progressive brain atrophy (Benveniste et al., 1999; Mu et al., 1999; Killiany et al., 2002). Acquiring this type of high field, high-resolution imaging in a human system is difficult, but holds great potential. Most imaging of microscopic specimens (voxels $<200\ \mu\text{m}$) has been completed in small bore, high field magnets with very high gradient strengths that are not suitable for human studies in vivo (Beuls et al., 1993; Benveniste et al., 1999; Fat-

terpekar et al., 2002). In addition, the acquisition times can be extremely long (up to 24 hr). Formalin-fixed tissues are not ideal because there are significant changes in the MRI appearance due to cross-linking of the membrane molecules during fixation, which leads to relaxation time changes. These specimens were used, however, because they were the most readily available. Future research will examine fresh tissue specimens in vitro. Our long-term goal is to image the whole human brain in vivo using ultra high-resolution imaging ($200\ \mu\text{m}$ voxels).

Coronal MR imaging of the hippocampus is preferred because of its advantage of being able to depict the anatomic layers. Sagittal imaging could be used for volume measurements, but the coronal MR imaging better displays the anatomic layers. Human coronal imaging also allows for the comparison of both sides of the hippocampus and the acquisition of quantitative volumetric data. We acquired the specimen images in a similar fashion centered in the mid-hippocampal region.

Imaging at 1.5 T has generated reasonable quality images of this region and is routinely used for the evaluation of hippocampal pathology. Formalin-fixed hippocampal specimens have also been previously evaluated with MR imaging (Wiesmann et al., 1999). The hippocampal formation is highly complex and routine 1.5 T MR imaging is not capable of differentiating many of the important normal internal anatomic structures. For example, routine imaging demonstrates the temporal horn, parahippocampal gyrus,

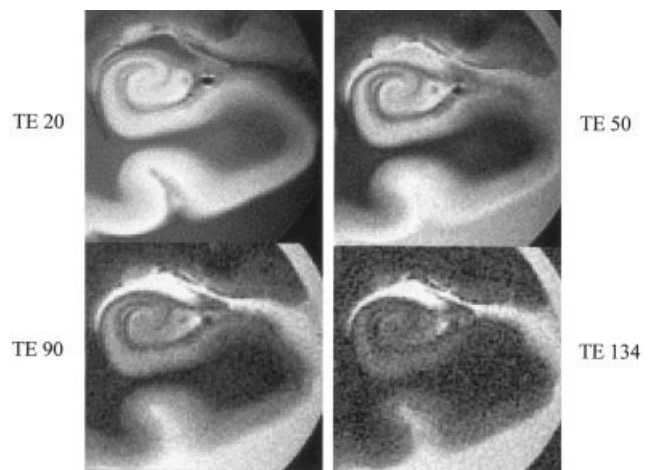


Fig. 3. Four different 8 T coronal images of the same formalin-fixed hippocampus specimen. The images are Hahn SE MR images acquired with TR = 800 msec, TE = 20, 50, 90, and 134 msec, and TA = 3.25 min. The formalin has a low signal intensity on the short TE images and a high signal intensity on the long TE images. The SNR decreased with increasing TE. The best differentiation of the various components of the hippocampal formation is seen on the TE 50 msec image. Note the magnetic susceptibility artifact related to a small bubble of air visible beneath the fimbria.

dentate gyrus, and the alveus, but only limited information related to the hippocampal layers. Identification of pathology in specific layers is not possible. This implies that extremely high-resolution imaging may be needed to detect mild forms of pathology such as those associated with early-stage Alzheimer's disease present in specific sub-regions of the hippocampal structures.

This study demonstrates that high field, high-resolution imaging utilizing a whole-body 8 T MRI system with a single strut TEM coil can visualize multiple layers of the formalin-fixed hippocampus with reasonable acquisition times (Fig. 2). The images of brain sections acquired using a large bore TEM coil can demonstrate quality similar to those obtained using small bore coils. This raises the potential that quantitative ultra high-resolution MR imaging of the medial temporal lobes in vivo may be possible in the future.

This study also demonstrates that the limiting factor in acquiring detailed high-resolution images of the hippocampus in vivo is not the SNR at high fields. High field MRI of the coronal hippocampus in human subjects in vivo is presently limited by a combination of artifacts related to magnetic susceptibility and RF magnetic field (B_1) inhomogeneities. These artifacts manifest themselves as enlargements of the low signal regions of the brain and make acquiring clear images of these areas more challenging. This "blooming" of the low signal regions is in part related to different focal magnetic susceptibilities and is much greater at 8 T than at lower field strengths (Abduljalil and Robitaille, 1999). Recent measurements of the local variability indicate that much of these signal voids has to be attributed to RF coil inhomogeneity (Mitchell et al., 2003; Whitaker et al., 2003). Although it has been demonstrated that imaging of the hippocampus and temporal lobe is feasible at 8 T, improvements in susceptibility correction methods and breakthroughs in RF engineering are needed to achieve optimal results. Overcoming some of these limitations is currently possible using a number of different techniques, but most of the widely used techniques rely on very long imaging times, which are prohibitive to the imaging of live human subjects.

ACKNOWLEDGMENTS

We would like to thank E. Herderick from the Biomedical Engineering Center at the Ohio State University for his help in digitizing the histologic sections.

REFERENCES

Abduljalil AM, Robitaille P-ML. 1999. Macroscopic susceptibility in ultra high field MRI. *J Comput Assist Tomogr* 23:832–841.

- Benveniste H, Einstein G, Kim KR, Hulette C, Johnson GA. 1999. Detection of neuritic plaques in Alzheimer's disease by magnetic resonance microscopy. *Proc Natl Acad Sci USA* 96:14079–14084.
- Beuls E, Gelan J, Vandersteen M, Adriaensens P, Vanormelingen L, Palmers Y. 1993. Microanatomy of the excised human spinal cord and the cervicomedullary junction examined with high-resolution MR imaging at 9.4 Tesla. *AJNR Am J Neuroradiol* 14:699–707.
- Burgess RE, Yu Y, Christoforidis GA, Bourekas EC, Chakeres DW, Spigos D, Kangarlu A, Abduljalil AM, Robitaille P-ML. 1999. Human leptomeningeal and cortical vascular anatomy of the cerebral cortex at 8 Tesla. *J Comput Assist Tomogr* 23:850–856.
- Duncan JS, Bartlett P, Barker GJ. 1996. Technique for measuring hippocampal T2 relaxation time. *AJNR Am J Neuroradiol* 17:1805–1810.
- Duvernoy H, Guyot J, Cabanis EA, Iba-Zizen MT, Bourguin P, Cattin F, Risold PY. 1998. The human hippocampus: functional anatomy, vascularization and serial sections with MRI. Berlin: Springer-Verlag. p 20,129.
- Fatterpekar GM, Naidich TP, Delman BN, Aguinaldo JG, Gultekin SH, Sherwood CC, Hof PR, Drayer BP, Fayad ZA. 2002. Cytoarchitecture of the human cerebral cortex: MR microscopy of excised specimens at 9.4 Tesla. *AJNR Am J Neuroradiol* 23:1313–1321.
- Ibrahim TS, Lee R, Baertlein BA, Robitaille P-ML. 2001. B_1 field homogeneity and SAR calculations for the birdcage coil. *Phys Med Biol* 46:609–619.
- Killiany RJ, Hyman BT, Gomez-Isla T, Moss MB, Kikinis R, Jolesz F, Tanzi R, Jones K, Albert MS. 2002. MRI measures of entorhinal cortex vs. hippocampus in preclinical AD. *Neurology* 58:1188–1196.
- Miller MJ, Mark LP, Ho KC, Houghton VM. 1996. MR appearance of the internal architecture of Ammon's horn. *AJNR Am J Neuroradiol* 17:23–26.
- Mitchell CA, Truong T, Ibrahim TS, Schmalbrock P. 2003. Accurate T1 measurements at 8 Tesla despite radiofrequency inhomogeneity. 11th Scientific Meeting of the ISMRM, Abstract. Toronto, Canada.
- Mu Q, Xie J, Wen Z, Weng Y, Shuyun Z. 1999. A quantitative MR study of the hippocampal formation, the amygdala, and the temporal horn of the lateral ventricle in healthy subjects 40 to 90 years of age. *AJNR Am J Neuroradiol* 20:207–211.
- Oppenheim C, Dormont D, Biondi A, Lehericy S, Hasboun D, Clemenceau S, Baulac M, Marsault C. 1998. Loss of digitations of the hippocampal head on high-resolution fast spin-echo MR: a sign of mesial temporal sclerosis. *AJNR Am J Neuroradiol* 19:457–463.
- Vaughan JT, Garwood M, Collins CM, Liu W, DelaBarre L, Adriany G, Andersen P, Merkle H, Goebel R, Smith MB, Ugurbil K. 2001. 7T vs. 4T: RF power, homogeneity, and signal-to-noise comparison in head images. *Magn Reson Med* 46:24–30.
- Whitaker CD, Truong T, Ibrahim TS, Schmalbrock P. 2003. Accuracy of 8T T2 measurements: evaluation of errors due to RF inhomogeneity. 11th Scientific Meeting of the ISMRM, Abstract. Toronto, Canada.
- Wiesmann UC, Symms MR, Mottershead JP, MacManus DG, Barker GJ, Tofts PS, Revesz T, Stevens JM, Shorvon SD. 1999. Hippocampal layers on high resolution magnetic resonance images: real or imaginary? *J Anat* 195:131–135.