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High-field magnetic resonance imaging of the human temporal lobe☆

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ABSTRACT

Background: Emerging high-field diffusion weighted MR imaging protocols, along with tractography, can elucidate microstructural changes associated with brain disease at the sub-millimeter image resolution. Epilepsy and other neurological disorders are accompanied by structural changes in the hippocampal formation and associated regions; however, these changes can be subtle and on a much smaller scale than the spatial resolution commonly obtained by current clinical magnetic resonance (MR) protocols *in vivo*.
Methods: We explored the possibility of studying the organization of fresh tissue with a 17.6 Tesla magnet using diffusion MR imaging and tractography. The mesoscale organization of the temporal lobe was estimated using a fresh unfixed specimen obtained from a subject who underwent anterior temporal lobectomy for medically refractory temporal lobe epilepsy (TLE). Following *ex vivo* imaging, the tissue was fixed, serial-sectioned, and stained for correlation with imaging.
Findings: We resolved tissue microstructural organizational features in the temporal lobe from diffusion MR imaging and tractography in fresh tissue.
Conclusions: Fresh *ex vivo* MR imaging, along with tractography, revealed complex intra-temporal structural variation corresponding to neuronal cell body layers, dendritic fields, and axonal projection systems evident histologically. This is the first study to describe in detail the human temporal lobe structural organization using high-field MR imaging and tractography. By preserving the 3-dimensional structures of the hippocampus and surrounding structures, specific changes in anatomy may inform us about the changes that occur in TLE in relation to the disease process and structural underpinnings in epilepsy-related memory dysfunction.

1. Introduction

Sub-millimeter resolution magnetic resonance (MR) imaging has been proposed as a tool for the study of volumetric changes in the subfields of hippocampal formations in neuropsychiatric and degenerative diseases (Wisse et al., 2012; Yushkevich et al., 2010). Epilepsy is one of the most common neurological disorders in humans, afflicting more than 65 million people worldwide (England et al., 2012). Over 30% of patients have seizures refractory to commonly used anti-convulsant drugs (Bauer and Burr, 2001). The most common form of epilepsy is temporal lobe epilepsy (TLE) and mesial temporal lobe

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sclerosis is the most common pathological abnormality in TLE (Bronen et al., 1997). The histopathological hallmarks of hippocampal sclerosis include loss of pyramidal neurons, granule cell dispersion, and reactive gliosis (Kienzler et al., 2009; Sutula et al., 1989). Recent advances in neuroimaging have demonstrated macroscale white matter abnormalities in TLE (Concha et al., 2005, 2009; Gross, 2011; Liu et al., 2012, 2014). Changes in the integrity of the hippocampus and white matter surrounding it have been postulated to influence overall temporal lobe network connectivity, hippocampal efficiency (Cadotte et al., 2009), and memory function (Eichenbaum et al., 2007, 2012; Liao et al., 2011a,b). Sub-millimeter highly resolved MR imaging may contribute to the study of these changes in brain structure and re-organization in brain tissue in disease.

The cytoarchitectonic features of nervous tissue and cellular organization influences how water diffuses in the brain (Beaulieu, 2002). For this reason, diffusion tensor imaging is becoming the standard magnetic resonance (MR) method for studying white matter *in vivo* and non-invasively (Hagmann et al., 2006). Diffusion MR imaging has shown promise as a diagnostic tool because it describes microstructural changes much earlier than structural scans, such as fluid-attenuated inversion recovery (FLAIR) (Gerdes et al., 2014) and subtle structural changes due to apoptosis and inflammation (Johnson et al., 2014). *In vivo* diffusion MR imaging protocols at 3 Tesla (T) magnets are limited by the image resolution achievable at this field strength, which restricts the accurate estimation of white matter nervous tissue structure to large fiber tracts (Cammoun et al., 2012; Hagmann et al., 2008). Volume averaging with large voxels (>1 mm^3) precludes resolution of finer internal anatomical organization. Hence, current *in vivo* diffusion MR imaging approaches are unable to discriminate the defining structural pathological changes of TLE, such as mossy fiber sprouting, loss of CA1 and dentate gyrus neurons in mesial temporal sclerosis and fimbria, and perforant pathway injury (Kienzler et al., 2009; Parekh et al., 2010). High-resolution structural information of the human brain therefore would be a useful approach to study these changes and to test anatomical predictions derived from animal pathway tracing studies (Amaral et al., 2014; Oh et al., 2014; Silasi and Murphy, 2014). However, diffusion MR imaging at sub-millimeter isotropic image resolution is rarely feasible in current clinical MR scanners.

Developing improved high-resolution MR methods can be facilitated by the use of *ex vivo* tissue from individuals undergoing resective surgery for intractable epilepsy. The impact of high-resolution diffusion MR imaging over volumetric studies (Wisse et al., 2012) lies in its ability to provide sufficient contrast in order to identify sub-millimeter fields within tissues (Shepherd et al., 2007). Fresh tissue specimens with undisturbed internal cytoarchitecture can be subjected to protracted high-resolution imaging approaches currently unattainable *in vivo*. Long *ex vivo* acquisition times can yield high spatial resolution images with sufficient information to study small structures that may provide important information about the etiology, evolution, and progression of epilepsy. Both hippocampal subfield and temporal lobe structural analyses may produce more sensitive markers for epilepsy and neuropsychiatric disorders than whole-hippocampal volumetric analysis, especially early in disease. Therefore, such information is important for studies of brain pathology and in making decisions in clinical practice.

The brain is typically described on three scales: microscale, mesoscale, and macroscale (Silasi and Murphy, 2014; Sporns et al., 2005). Microscale refers to cellular features, mesoscale refers to neuronal group connections, and macroscale refers to the features of large anatomical brain regions. Particular interest is drawn to the mesoscale, with which great advances have been made using anterograde tracers (Amaral et al., 2014; Oh et al., 2014; Silasi and Murphy, 2014). MR imaging can be a great tool to assess mesoscale structure in the human brain by using tissue resected from patients undergoing treatment for epilepsy (Silasi and Murphy, 2014). In this study, a high-angular resolution diffusion imaging (HARDI) sequence was employed with micrometer scale spatial-resolution to estimate the mesoscale organization of the temporal lobe in an individual with pharmacoresistant TLE. This report describes 1) high-field (17.6) MR imaging as a tool for the study of mesoscale organization of freshly resected tissue resected from an epileptic patient, 2) qualitative validation of MR imaging with conventional histology, 3) interpretations of diffusion MR imaging and tractography, and 4) utility of streamline tractography for the study of mesoscale brain structure.

2. Methods

2.1. Participant information

The University of Florida Institutional Review Board approved this study. The patient is a 10-year-old right-handed boy who came to University of Florida Health Center hospital, a high complexity epilepsy center of excellence, in July 2010 because of a new onset status epilepticus. His seizures began with left-sided manual and oral automatisms, which then progressed to left arm and leg tonic/clonic seizures and left-sided head and eye deviation. He was started on oxycarbamazepine, which he remained on for 9 months. Over the ensuing months, his seizures recurred at every 2-week interval with each seizure lasting between 45 s and 1 min. His antiepileptic medication treatment was changed to lamotrigine, and clonazepam was later added. Subsequently, his seizure occurrence decreased to every 4–6 weeks. Topiramate and levetiracetam were later added to no avail. Surface electroencephalography showed right temporal lobe epileptiform discharges with right focal temporal region theta slowing. The patient was deemed medically intractable or drug resistant and therefore evaluated for resective epilepsy surgery. Phase one right electroencephalography showed posterior temporal and right parietal sharp epileptiform discharges. A phase two right temporal lobe subdural grid assessments showed right temporal lobe onset. Positron emission tomography was normal. Formal neuropsychometric and Wada procedure demonstrated moderate deficits for declarative memory systems for verbal and nonverbal material. Magnetoencephalography and single photon spectroscopy were not performed. The patient underwent a right anterior temporal lobectomy on February 2012. He was a previously healthy boy without any remarkable medical history. His neurological development was normal. There were no specific epilepsy risk factors including a history of prematurity, head trauma, encephalitis/meningitis, febrile seizures, or family history of epilepsy.

2.2. MR data acquisition

The patient was consented and scanned with a diffusion-weighted sequence in a Siemens 3 T magnet. TR/TE = 15,200/81 ms, 2 images without diffusion weighting, 6 images with b = 100 s/mm^2, and 64 with b = 1000 s/mm^2 and an image resolution of 2 mm isotropic. The diffusion gradients were distributed following an electrostatic repulsion model (Jones et al., 1999). Brain MR imaging demonstrated minimal signal abnormality near the tail the right hippocampus and within the right parahippocampal gyrus. The left hippocampus was grossly normal.

Immediately after the right anterior temporal lobe resection and before tissue fixation, the tissue block was imaged *ex vivo* and fresh at the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility of the McKnight Brain Institute at the University of Florida. The tissue was scanned with a diffusion weighted spin echo sequence in a 17.6 T vertical bore magnet (Bruker Corp., Billerica, MA) for 5 h. The tissue was placed in a 20 mm NMR tube (model 20PP, Wilmad-LabGlass, Vineland, NJ) in Flourinert (3 M, St. Paul, MN). The scan parameters were TR/TE = 4000/28 ms, matrix size of 86 × 86 with 75 slices with 220 μm thickness, yielding a final isotropic image resolution of 220 μm. There were a total of 52 diffusion gradients distributed over a sphere following a model