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Medial Temporal Cortices in Ex Vivo Magnetic Resonance Imaging

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Abstract

This review focuses on the ex vivo magnetic resonance imaging (MRI) modeling of medial temporal cortices and associated structures, the entorhinal verrucae and the perforant pathway. Typical in vivo MRI has limited resolution due to constraints on scan times and does not show laminae in the medial temporal lobe. Recent studies using ex vivo MRI have demonstrated laminae in the entorhinal, perirhinal, and hippocampal cortices. These studies have enabled probabilistic brain mapping that is based on the ex vivo MRI contrast, validated to histology, and subsequently mapped onto an in vivo spherically warped surface model. Probabilistic maps are applicable to other in vivo studies.

INDEXING TERMS

cortical localization; entorhinal cortex; verrucae; perirhinal cortex; perforant pathway

The medial temporal lobe houses structures that are critical to normal memory function: the entorhinal cortex and the hippocampus. The circuit that memory formation relies on has been well established. Neural information from multiple modalities converge in the entorhinal cortex and then project to the hippocampus via the perforant pathway (Van Hoesen et al., 1972; Van Hoesen and Pandya, 1975a,b). When this circuit is damaged surgically (Scoville and Milner, 1957) or damaged due to neurodegenerative pathology such as Alzheimer's disease (Hyman et al., 1984), memory function fails. The two pathological hallmarks in Alzheimer's disease, neurofibrillary tangles and amyloid plaques, manifest differently spatially and temporally in the human brain (Braak and Braak, 1991). The

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CONFLICT OF INTEREST

BF has a financial interest in CorticoMetrics, a company whose medical pursuits focus on brain imaging and measurement technologies. BF's interests were reviewed and are managed by Massachusetts General Hospital and Partners HealthCare in accordance with their conflict of interest policies. The other authors have nothing to disclose.

ROLE OF AUTHORS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: JCA, AVDK, BF. Acquisition of data: JCA, AVDK. Analysis and interpretation of data: JCA, BF. Drafting of the article: JCA. Critical revision of the article for important intellectual content: JCA, AVDK, BF. Statistical analysis: JCA. Obtained funding: JCA, BF. Technical support: AVDK. Study supervision: JCA, BF.

entorhinal and perirhinal cortices exhibit the first neurofibrillary tangles in Alzheimer's disease in their superficial layers. The anterior parahippocampal gyrus includes entorhinal and perirhinal cortices while the posterior parahippocampal gyrus contains posterior parahippocampal cortex (Van Hoesen, 1982). Amyloid plaques appear prior to neurofibrillary tangles primarily in isocortical areas distal to the temporal lobe; however, once neurofibrillary tangles accumulate in the anterior parahippocampal gyrus (entorhinal and perirhinal cortices) and the hippocampus, cognitive impairment is observed (Bennett et al., 2005; Nelson et al., 2009; Savva et al., 2009). Neurofibrillary tangles and neuronal death correlate strongly with cognitive impairment and the density of neurofibrillary tangles and decreased neuronal numbers endure as reliable correlates that predict dementia (Arriagada et al., 1992; Gomez-Isla et al., 1997; Hof et al., 2003; Giannakopoulos et al., 2003). Brain imaging has been invaluable in understanding anatomical and functional properties of these vulnerable cortices in healthy individuals and as well patterns of change with disease (Insausti et al., 1998a,b; De Toledo-Morrell et al., 2000; deToledo-Morrell et al., 2004; Desikan et al., 2008, 2009a,b, 2010; Dickerson et al., 2009, 2011). Even so, brain mapping with specificity and improved accuracy is sought after to improve imaging methods for healthy and disease states.

Over a century ago, Brodmann parcellated the cerebral cortex into cytoarchitectural areas based on structural properties, neuronal size, neuronal packing density, and laminar organization (Brodmann, 1909, 1994). Almost a century after that, the development of magnetic resonance imaging (MRI) (Lauterbur, 1973; Mansfield and Grannell, 1973) facilitated the noninvasive imaging of brain tissue in the living person (Doyle et al., 1981). Structural and functional MRI scans have become staples in assessing brain integrity and function. Functional MRI studies have opened the ability to pose questions about performing tasks in the human brain (Belliveau et al., 1990; Ogawa et al., 1990) and software automates anatomical segmentation for structural MRI (Ashburner and Friston, 1999; Fischl et al., 1999a,b, 2002, 2004b; Smith et al., 2004; Jenkinson et al., 2012). Combining structural and functional brain maps in the same space has enriched neuroanatomical mapping (Amunts and Zilles, 2001; Eickhoff et al., 2006a). Still, more detailed brain mapping is needed especially in clinically vulnerable areas such as the medial temporal cortices. Furthermore, investigating cellular based pathologies in MRI is not possible with in vivo imaging. In vivo imaging, whether it is structural or functional MRI, permits limited resolution due to constraints on scan time, limited signal-to-noise ratio (SNR), and direct validation in the brain tissue. To tackle these issues, a relatively new model in neuroimaging has emerged that used a tripartite approach (Fischl et al., 2009; Geyer et al., 2011). The tripartite approach utilizes ex vivo imaging, histology, and in vivo probabilistic brain mapping. This review will describe the components of this neuroimaging method and details some of the findings related to the medial temporal lobe. The review commemorates the life and work of Dr. Gary Van Hoesen, who contributed greatly throughout his career to study the anatomy and connectivity of the hippocampus and parahippocampal gyrus and how these relate to the pathology of Alzheimer's disease. The review focuses primarily on the following structures: the entorhinal cortex, the entorhinal verrucae, the perirhinal cortex, the hippocampal formation, and the perforant pathway and how each has been visualized and modeled with ex vivo MRI.

EX VIVO IMAGING

Utilizing this tripartite approach, ex vivo imaging provides an opportunity to make progress where in vivo imaging has limitations of scan time, resolution, and lacks validation. Ex vivo imaging implies scanning postmortem tissue and allows for long scan sessions at high field strengths that yield ultra-high resolution ($100\ \mu\text{m}$)³ or less and higher SNR images. Equally as important, ex vivo imaging facilitates validation of histological properties in high-resolution MRI. Notably, this model has no delay between scan and histology where the aging processes can intervene and change properties, which can be a confounding variable in in vivo imaging. Ex vivo imaging also allows correlation of pathological lesions with MRI intensities (albeit ex vivo) (Blezer et al., 2007; Kangarlu et al., 2007; Baltes et al., 2011; Cowin et al., 2011; Garbelli et al., 2011; Nabuurs et al., 2011; Riddle et al., 2011). Although the ex vivo model utilizes cross-sectional data, it establishes MRI parameters for that particular lesion and helps develop engineering hardware and computational tools that can one day be applicable to in vivo studies. Ex vivo MRI produces isotropic 3D volumes and permits visualization in several viewing planes, unlike histological data that is typically sectioned in the coronal plane. The ultimate advantage of ex vivo imaging is that it validates the MRI and helps determine what the MRI contrast equals in the histological stained section.

MEDIAL TEMPORAL LOBE PARCELLATION WITH EX VIVO MRI

This section details the ex vivo MRI contrast in the medial temporal lobe (Augustinack et al., 2005, 2013; Fischl et al., 2009) and is the basis for the probabilistic maps described in later sections. Entorhinal, perirhinal, and hippocampal cortices will be described. The first figure shows the hippocampus and adjacent parahippocampal cortices in a sagittal slice (Fig. 1A). This plane of cut catches several cortices in the medial temporal lobe at various anterior–posterior levels. The entorhinal cortex (Brodmann's area 28) has a distinctive architecture and corresponds to the anterior parahippocampal gyrus. Surface bumps, known as the verrucae, cover the entorhinal cortex. The entorhinal layer II islands lie directly below the verrucae and are observed in Nisslstained sections and ex vivo MRI. The entorhinal layer II islands, which represent the neuron-dense clusters in layer II, show up as bright intensities in fast-low-angle-shot (FLASH) images in MRI (Augustinack et al., 2005). The ex vivo FLASH images comprise several types of contrast but T2* dominates (Fischl et al., 2004a). Entorhinal cortex layer III is wide and not as bright as layer II. Lamina dissecans appears as a dark band that segments the supragranular layers from the infragranular layers. Entorhinal cortex layer IV appears bright and homogenous. The entorhinal cortex displays its typical architecture with cell clusters in a sagittal slice (Fig. 1A) as well as coronal slices at the level of the anterior hippocampal head (Fig. 1B) and posterior hippocampal head (Fig. 1C). These MRI lamina have been histologically validated in previous studies (Augustinack et al., 2005, 2013; Fischl et al., 2009) and corresponding Nissl-stained sections are illustrated at the level of the hippocampal head (Fig. 1D) and the hippocampal uncus/body (Fig. 1E).

Perirhinal cortex (Brodmann's area 35) also shows bright intensities in its superficial layers (layer II–III columns in area 35a). Between perirhinal area 35a and 35b, the unique lamina (Illu of Ding et al., 2009) forms an oblique layer that begins in area 35a and ends in area 35b

(Ding and Van Hoesen, 2010; Augustinack et al., 2013). Braak and Braak (1985) were the first to describe this same oblique layer in transentorhinal cortex (Brodmann's perirhinal area 35a). Specifically, the large neurons that occupy the superficial portion of entorhinal cortex curve downward to eventually reside in the deeper lamina in isocortex. The superficial layers of area 35b in perirhinal cortex shows a bright homogeneous intensity in ex vivo MRI but not organized in columns or islands as do the periallocortical areas (areas 28 and 35a) of the medial temporal lobe. Accordingly, the supragranular layers have a homogeneous bright intensity but the infragranular layers have a dark intensity for that same area (35b). Perirhinal area 35b exhibits a wide dark band in ex vivo MRI and it corresponds to the lateral part of the oblique wedge in ex vivo MRI (unique IIIu) (Augustinack et al., 2013). The illustrated sagittal plane shows three portions of perirhinal area 35, dorsally at the temporal incisura (between straight white arrows), lateral to the rhinal sulcus (between dotted white arrows), and mostly medial to the collateral sulcus (between curved white arrows) (Fig. 1A). This wide dark band in the middle portion of the lamina is typical of periallocortex/proisocortex in ex vivo MRI (Fig. 1A) and in histology (Fig. 1D,E) (Ding et al., 2009; Ding and Van Hoesen, 2010; Augustinack et al., 2013). Brodmann's area 35 spills over the collateral sulcus (laterally) in this case (Fig. 1B,D). When the wide dark band ends, it signifies the boundary between proisocortex (perirhinal 35b) and temporal isocortical area 36. In ex vivo MRI, temporal isocortical area 36 displays a thin dark line that corresponds to layer IV (Fig. 1A, white carets). Layer IV shows contrast in ex vivo MRI likely due to the intracortical myelin in that layer (Eickhoff et al., 2005; Augustinack et al., 2013). Our corresponding histological analyses have confirmed that it is layer IV in our previous study. We have argued that area 36 is temporal isocortex due to the presence of a granular layer IV from our own observations in the human (Van Hoesen et al., 2000; Augustinack et al., 2013) and as illustrated and labeled by Amaral et al. (1987) in the monkey brain. In their report, Amaral et al. (1987) show a clear layer IV in area 36 (rostral and caudal) in the monkey brain. Brodmann noted that, "area 36 — the entorhinal area — lies, as its name implies directly lateral to the rhinal sulcus and represents the first area of neopallium adjacent to the archipallium." Moreover, area 36 has six distinct laminae and distinct granularity, both of which defines the isocortical tissue type (Brodmann, 1909; Filimonov, 1949; Sanides, 1969; Stephan, 1975; Mesulam and Mufson, 1982; Pandya and Yeterian, 1985; Gloor, 1997; Van Hoesen et al., 2000). In the human brain, we have observed that it is perirhinal area 35b that displays a poor layer IV (i.e., incipient) but that area 36 has a fairly well-developed layer IV. It is important to note that, in the human brain, perirhinal area 35 represents a bipartite cortex that is periallocortex (35a) and proisocortex (35b), while area 36 is isocortex (Van Hoesen et al., 2000; Augustinack et al., 2013). The modularity and distinctive structures of entorhinal and perirhinal cortices allow for straightforward parcellation in ex vivo MRI. In fact, we routinely observed architectonic field boundaries in ex vivo MRI in this region before histological analyses were carried out.

The subicular cortices are located inferior to the hippocampus (Fig. 1). In the illustrated slice, the presubiculum extends the entire length of the hippocampal head and body between the asterisks (Fig. 1A). The presubiculum routinely displays presubicular clouds (grouped neurons in the presubiculum that the perforant pathway projects through (Van Hoesen and Pandya, 1975b) that appear bright in ex vivo MRI, while the parasubiculum reveals a

homogeneous layer superficially (Fig. 1C). Several lamina in the hippocampus are also discernible: the alveus, the molecular layer of the hippocampus (stratum lacunosum of Lorente de N6, 1934), and the pyramidal cell layer. The alveus and molecular layer appear consistently dark in ex vivo MRI, while the pyramidal layer shows a bright appearance. With enough averages and a brain with good contrast, the mossy fiber layer (stratum lucidum of Lorente de N6, 1934) is observed as a dark band inferior to the lighter pyramidal layer (Fig. 1C). The granule layer of the dentate gyrus conveys a bright intensity in ex vivo MRI. Typically, the granule cell layer is as bright as the entorhinal islands. Our MRI findings in medial temporal lobe suggest that bright intensities in ex vivo MRI represent densely packed neuronal layers (i.e., entorhinal islands, perirhinal columns, and granule cells of dentate gyrus) and that dark intensities represent myelin-rich lamina or neuronal-sparse areas (i.e., the inter-islands in area 28) in T2*-weighted FLASH images.

PROBABILISTIC MAPPING

The ability to visualize populations of neurons and density of myelin with high-resolution MRI has had an extensive impact and reshaped the field of brain mapping (Clark et al., 1992; Barbier et al., 2002; Fatterpekar et al., 2002; Walters et al., 2003; Eickhoff et al., 2005; Augustinack et al., 2005; Bridge and Clare, 2006; Duyn et al., 2007; Post, 2008). The capability to correlate MRI with histology has provided validated maps based on cytoarchitecture, myeloarchitecture, multireceptor architecture, and pathoarchitecture and adding depth to neuroanatomical imaging (Rademacher et al., 2001; Amunts and Zilles, 2001; Fatterpekar et al., 2002; Amunts et al., 2005, 2007; Eickhoff et al., 2005, 2006b; Scheperjans et al., 2008a,b; Zilles and Amunts, 2009; Howe et al., 2010; Augustinack et al., 2012b, 2013). Based on the ex vivo MRI contrast described in the previous sections, the boundaries of entorhinal and perirhinal cortices (Brodmann's area 28 and 35, respectively) were determined on the high-resolution images (Fischl et al., 2009; Augustinack et al., 2013). High-resolution ex vivo data were manually labeled using anatomically defined protocols to create labels of entorhinal and perirhinal cortices. These structures were labeled across several cases at $(120\text{ }\mu\text{m})^3$ and each label was registered onto its respective hemisphere volume $(1\text{ mm})^3$ using Register (Register; MNI toolkit, Montreal, Canada, <http://www.bic.mni.mcgill.ca>). Subsequently, the labels of many cases were transformed onto an average surface template that predicts the cortical localization. This creates the histologically validated entorhinal (Fig. 2A) and perirhinal (Fig. 2B) probability maps based on spherical warping. The entorhinal label extends from the primary olfactory cortex to midway on the parahippocampal gyrus (Fig. 2A). Throughout its course, entorhinal cortex remains on the crown of the parahippocampal gyrus. The perirhinal label extends from the anterior temporal incisura area to midway on the parahippocampal gyrus (Figs. 1A, 2B). The depth of the collateral sulcus conceals the middle portion of perirhinal label in the partially inflated brain. The sulci in the medial temporal lobe complicate the topography of perirhinal cortex. Perirhinal cortex (Brodmann's area 35) involves two different sulci, the collateral and the rhinal sulcus and extends slightly into the temporal incisura. For the majority of its territory, perirhinal cortex resides on the lateral side of the rhinal sulcus but at middle levels, it resides on the medial side of the collateral sulcus. At the posterior levels, perirhinal label appears on the crown of parahippocampal gyrus just briefly, before it ends (Fig. 2C,D). With

the accomplishment of being able to visualize the lamina of the medial temporal cortices, it has become possible to parcellate the entorhinal and perirhinal cortices in ex vivo MRI, to establish areal boundaries, and to create histologically validated labels for application to future in vivo studies. The labels generated can be applied to structural and functional MRI in vivo brain mapping. For example, application to larger cohort studies in aging and disease studies shows differences in cortical thickness among diagnostic groups (Fischl et al., 2009; Augustinack et al., 2013). In sum, ex vivo imaging provides the ability to improve brain mapping by linking the ground truth histology with MRI based surface models that apply to in vivo imaging models.

SULCAL COMPLEXITY IN THE MEDIAL TEMPORAL LOBE

The rhinal sulcus varies considerably in the human brain (Fig. 3) (Ono, 1990; Hanke, 1997; Insausti et al., 1998b; Van Hoesen et al., 2000). The rhinal sulcus ranges from a significant one (Fig. 3A) to a more subtle one (Fig. 3B,C) to a shallow groove (Fig. 3D). Zuckerkandel (1887) noted that 86% of brains in his collection did not have a rhinal sulcus. The Victorian comparative anatomist Richard Owen coined the term “ecto-rhinal” or “rhinal sulcus” to denote the border between olfactory cortex (the olfactory peduncle) and frontal cortex (lateral to the peduncle) in the human brain (Owen, 1868). Thus, the term “rhinal sulcus” was fixed to the border between rhinencephalon and other cortex. Owen used the terms ecto-rhinal and rhinal interchangeably and later William Turner (1890) shortened the term ecto-rhinal sulcus to rhinal sulcus. Several turn of the century neuroanatomists neglected to label the rhinal and collateral sulci in primates (Broca, 1878; Turner, 1890; Retzius, 1896; Brodmann, 1909; Ariens-Kappers et al., 1967), while others mislabeled the sulci (Smith, 1903; Connolly, 1950; Krieg, 1973; Netter, 1989). Both trends were likely due to the sulci variability and nomenclature uncertainty. Monkeys (Fig. 4A) and great apes have a long rhinal sulcus that extends from the anterior medial part of the temporal lobe to the posterior levels of the parahippocampal gyrus. The sulcal topography in monkeys differs from great apes in that monkeys do not have a collateral sulcus, whereas apes and humans do (Figs. 3, 4A,B). The complication that a cortical area (perirhinal cortex, area 35) weaves through several sulci and that many human brains do not have a rhinal sulcus underscores an important point that cytoarchitecture-based analyses produce more accurate architectonic mapping than sulcal-based ones (Amunts et al., 2005; Augustinack et al., 2013). Probabilistic mapping validated with histological architecture provides a dependable method to map regardless of sulcus presence, absence, or particular depth.

SURFACE GEOMETRY OF THE ENTORHINAL CORTEX

High-resolution imaging with ex vivo samples has advanced the modeling of small structures. In the human brain, the entorhinal cortex displays small bumps on its surface (Retzius, 1896; Klingler, 1948). The surface bumps are termed entorhinal verrucae (Fig. 4B,C) and clusters of layer II entorhinal islands lie directly beneath them. Thus, the verrucae correspond with the entorhinal islands, one verruca for each entorhinal island. Notably, the monkey entorhinal cortex does not show verrucae (Fig. 4A) and this suggests that the verrucae are a human entorhinal feature. In the monkey entorhinal cortex, layer II organizes into islands but to a lesser degree than the human entorhinal cortex. The entorhinal layer II

in the monkey brain tends to elongate and is less circular than observed in the human entorhinal cortex. In humans, the majority of the entorhinal cortex displays clusters of entorhinal islands. It is unknown whether the absence of verrucae in monkeys is due to neuronal size or island shape (i.e., elongated island) but further comparative studies about the verrucae would establish definitive evidence. In the human brain, it is thought that the large neurons that make up layer II cause the bulging onto the surface but synapses may also play a role. In Alzheimer's disease, the entorhinal verrucae disappear (Van Hoesen and Solodkin, 1993; Solodkin and Van Hoesen, 1996; Van Hoesen et al., 2000; Augustinack et al., 2012b). Simic et al. (2005) documented that entorhinal verrucae decrease in surface area during aging, yet this brain collection revealed a laterality relationship (more verrucae on the left) and an increase in number of verrucae with age. Volumetric measures in the medial temporal lobe in patients have shown that atrophy in the right entorhinal cortex predicts the conversion from healthy to mild cognitive impairment (De Toledo-Morrell et al., 2000). Although these two studies were assessed in different conditions (in vivo versus ex vivo), it may be postulated that verrucae represent a structural marker of cognitive resilience. Further studies are necessary to pinpoint the factors contributing to cognitive resilience.

ASSAYING ENTORHINAL VERRUCAE QUANTITATIVELY AND QUALITATIVELY

High-resolution ex vivo imaging gives us a closer look at the entorhinal surface and provides a model for quantitative measurement of individual verrucae. From ex vivo MRI volumes, a 3D isosurface is generated with Freeview (FreeSurfer, <http://surfer.nmr.mgh.harvard.edu>). The gross morphometry (or photographic image) (Fig. 4B) validates the verrucae isosurface reconstruction (Fig. 4C) and the isosurface represents a detailed 3D model of the surface. The isosurface allows for measurement of individual verruca (Fig. 4C). Our verrucae metric algorithm uses an optimal least squares fitting plane at the base of the isosurface and then measures verruca height, width, surface area, and calculates verruca volume (Fig. 5A) (Augustinack et al., 2012b). Verrucae qualitative ratings correlate with verrucae height and volume (Augustinack et al., 2012b). The dimensions of a large verruca range from 0.20–0.25 mm in height and almost 2 mm for width. Medium-sized verrucae extend to ~0.15–0.19 mm. Observed differences in these verrucae measurements reflect a pathologic change in layer II and in diagnosis (Augustinack et al., 2012b). Differences in verrucae size, especially verrucae height, indicate mild Alzheimer's cases from control brains. Furthermore, verruca size negatively correlates with disease severity based on Braak and Braak staging (Braak and Braak, 1991; Augustinack et al., 2012b). A surface measurement below 0.10 mm denotes flat cortex. This flat cortex could be an Alzheimer's case or other types of cortex that do not exhibit verrucae (motor, prefrontal, occipital, parietal, or cingulate cortices) (Augustinack et al., 2012b). Finally, curvature measures (mean and Gaussian) in FreeSurfer correlate with verrucae height and volume as well (Augustinack et al., 2012a).

While existing in vivo technology is not able to resolve verrucae, investigating the entorhinal verrucae ex vivo allows us to better define this unique structure and examine individual differences in human populations. Since technology continually progresses, it is tempting to predict the required resolution to detect verrucae in vivo. Based on downsampling results

from control ex vivo cases, it is estimated that 300 μm isotropic resolution is needed to resolve entorhinal verrucae. When 100 μm MRI data was downsampled to 300 μm , the verrucae were still visible, but not at 500 μm . Given that this is close to current high-resolution in vivo imaging standards, imaging verrucae in vivo may soon be feasible with further technical developments.

PERFORANT PATHWAY

Diffusion imaging allows the estimation of brain fiber orientation by measuring water diffusion. In diffusion imaging, fiber pathways are inferred based on the amount of anisotropy in the measured diffusion (along a fiber in a certain orientation) (Basser, 1994; Basser et al., 1994). Because white matter is organized in axonal bundles, the diffusional anisotropy of water is higher than gray matter, which has neurons mixed with fibers. This variation in diffusional anisotropy is a useful contrast for measuring anatomical properties. Alveus, temporal stem, angular bundle, and perforant pathway all have high fractional anisotropy (bright white signal) (Fig. 6A). Diffusion voxels contain directionality and that allows visualization of fiber pathways with fiber tracking software (<http://www.trackvis.org/>). Ex vivo tractography streamlines illustrate the perforant pathway (Fig. 6B). The vertical green fibers represent the perforant pathway in this deterministic paradigm but have also been demonstrated with probabilistic tractography and fractional anisotropy (Shepherd et al., 2007; Augustinack et al., 2010). This tractography volume has been edited in order to show and highlight the perforant pathway without other fibers obstructing the view. The tractography image is a 3D volume so fibers on the right side of the image actually reside in an anterior slice (Fig. 6B) and as a result, the image (Fig. 6B) appears larger than (Fig. 6A,C). The perforant fibers appear short, appropriately so, because these fibers terminate at the outer two-thirds of the molecular layer of the dentate gyrus and the entire molecular layer of the hippocampus (stratum lacunosum), which is a short distance from the sulcus (Fig. 6B,C). The perforant pathway is the only known pathway that crosses a sulcus to reach its destination synapse. A few imaging studies have also examined the perforant pathway in vivo (Yassa et al., 2010; Zeineh et al., 2012). These studies require acquisitions with isotropic voxels to assess the small features of the perforant pathway.

TECHNICAL CONSIDERATIONS

Ex vivo MRI acquisition and surface reconstruction

Probability maps require scanning at two resolutions, a lower resolution ($1 \times 1 \times 1 \text{ mm}$) at lower field (1.5T or 3T) for surface reconstruction, and a higher resolution ($100 \times 100 \times 100 \mu\text{m}$) at 7.0T for direct visualization of microscopic features of the anatomy. MRI volumes of the entire hemisphere (i.e., lower resolution) are acquired with a routine in vivo morphometry scan and generate surface models based on spherical warping for each case (FreeSurfer, <http://surfer.nmr.mgh.harvard.edu>) (Fischl et al., 1999a,b). Subsequently, smaller blocks of the medial temporal lobes (i.e., higher resolution) scanned at 7T (Siemens, Erlangen, Germany) using a four-turn solenoid coil and a 3D spoiled gradient echo sequence generate the resolution that allows for cytoarchitectural detection. A single echo, isotropic FLASH sequence is used to acquire volumes with 100 μm isotropic resolution. Scanning

with small coils at higher field strength yields a significant increase in SNR that can be used to achieve higher resolution.

Registration

Ex vivo studies require an extra step to register the two modalities together. Registration is needed for not only ex vivo / in vivo MRI correlations (Register, MNI toolkit, Montreal, Canada) but also ex vivo histology correlation (in-house software, HistoRegister) (Sand and Teller, 2008; Wachinger, 2010; Reuter et al., 2012). Probabilistic mapping depends on good registration. At times, registration can challenge the most patient and spatially competent of us due to poor contrast and oblique and difficult-to-recognize planes and physical deformations of the tissue in the scanning tube. The integrity of human tissue varies significantly (compared to animal studies) due to many and possibly unknown factors.

CONCLUSION

Over the last several decades, the term brain mapping has had different meanings. Brain mapping has progressed from purely anatomical and cytoarchitectural maps, to connectivity tracing in animal models and recently to probabilistic mapping in a common structural and functional MRI space. Cumulative advances in brain mapping coupled with technological innovation will improve our knowledge of the medial temporal lobe as well as other regions in the human brain. Given the fact that ex vivo MRI now visualizes neuronal dense lamina and cell clusters, it may provide insight into what we will observe in future in vivo MRI.

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ABBREVIATIONS

ab	Angular bundle
alv	Alveus
AM	Amygdala
AD	Alzheimer's disease
BA	Brodmann's area
CA	Cornu ammonis

CP	Choroid plexus
CS	Collateral sulcus
DG	Dentate gyrus
EC	Entorhinal cortex
FLASH	Fast low angle shot
HF	Hippocampal fissure
HP	Hippocampus
iso	Isocortex
mf	Mossy fiber
ml	Molecular layer
MRI	Magnetic resonance imaging
OTS	Occipital temporal sulcus
ParaS	Parasubiculum
PC	Perirhinal cortex
pp	Perforant pathway
PreS	Presubiculum
Pyr	Pyramidal layer
RG	Rhinal groove
RS	Rhinal sulcus
SNR	Signal to noise ratio
SUB	Subiculum
TP	Temporal pole

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Figure 1.

Ex vivo MRI of medial temporal lobe structures at $(120 \mu\text{m})^3$. **A:** Sagittal plane through the parahippocampal gyrus and hippocampus shows lamina and neuroanatomical features. The pes of the hippocampus are shown posterior to the amygdala. The hippocampus and dentate gyrus are intertwined at posterior hippocampal head. Molecular layer, pyramidal layer, and alveus are evident in **(B,C)**. The presubiculum displays light and dark intensities and extends between the asterisks. Entorhinal layer II demarcated with light and dark intensities while layers III and IV are more homogeneous. Perirhinal cortex surrounds entorhinal cortex anteriorly (between dotted arrows) and posteriorly (between curved arrows). Note, perirhinal

cortex is also observed dorsally (between straight arrows). Temporal isocortical area 36, denoted with a dark intensity in layer IV (white caret, ^) is lateral to perirhinal area 35. B,C: Coronal planes of cut through hippocampal head and adjacent cortex. On medial bank of collateral sulcus, perirhinal cortex shows the oblique wedge with light intensity superficially and dark intensity in inferior lamina. Perirhinal cortex ends near the fundus in (B,D) and on the crown of parahippocampal gyrus as collateral sulcus ends in (C,E). **D,E:** show the equivalent slices stained for Nissl substance. In this case, the collateral sulcus ends immediately before the level of (C) for MRI and (E) for Nissl section and remains as a very subtle indentation. Since the collateral sulcus has ended in (C), the sulcus that is lateral to the collateral sulcus remnant is the occipitotemporal sulcus. Double asterisk (**) = boundary between area 28 and area 35. The black caret (^) represents the boundary between area 35 and area 36. Marked x's in hippocampal head (D) denote that CA1 is transitioning to CA2 in this section but not fully realized. alv, alveus; Am, amygdala; BA, Brodmann's area; CA, cornu ammonis; CP, choroid plexus; EC, entorhinal cortex; DG, dentate gyrus; HP, hippocampus; iso, isocortex; mf, mossy fiber layer; ml, molecular layer; OTS, occipitotemporal sulcus; PC, perirhinal cortex; pyr, pyramidal layer; TP, temporal pole; ParaS, parasubiculum; PreS, presubiculum; x, transition between CA1 and CA2. Scale bars = 1 cm in A; 0.5 cm in B–E.

Figure 2.

Average probability maps for entorhinal (**A**) and perirhinal (**B**) cortices in left hemisphere ($n = 9$; $n = 8$, respectively) and right hemispheres ($n = 7$, $n = 8$, respectively). The red represents the region of highest probability and 100% overlap of cases. In all panels, the spherical models were partially inflated and labels are displayed on the pial surface. Note the entorhinal label (Brodmann's area 28) on the crown of the parahippocampal gyrus and the perirhinal label (Brodmann's area 35) primarily in the sulcal depths and partially on the crown of the gyrus. Each label tapers posteriorly until it ends.

Figure 3. Variability of the rhinal and collateral sulci in the human brain. Rhinal sulcus is highly variable with definite sulcus in (A), shorter and more subtle in (B,C), and a groove in (D). CS, collateral sulcus; OTS, occipitotemporal sulcus; RG, rhinal groove; RS, rhinal sulcus. The collateral and occipitotemporal sulci are variable as well but they maintain a more traditional depth in these cases. Scale bar = 1 cm.

Figure 4. Entorhinal cortex in macaque monkey gross photograph (A), human gross photograph (B) and human isosurface reconstructed from MRI volume at $(120 \mu\text{m})^3$ (C). Note the verrucae in (B,C) and the lack of verrucae in (A). Black carets (^) point to individual verruca. CS, collateral sulcus; EC, entorhinal cortex; HF, hippocampal fissure; RS, rhinal sulcus.

Figure 5. Schematic drawing of entorhinal verrucae and MRI slice at $(100 \mu\text{m})^3$. The schemata details verrucae measurements obtained from our algorithm in (A). Entorhinal verrucae shown in MRI slice (zoomed to two verrucae) in (B). Scale bar = 0.5 cm.

Figure 6.

Ex vivo diffusion tensor imaging in the medial temporal lobe at $(300 \mu\text{m})^3$. Fractional anisotropy in (A), tractography streamlines in (B), and Gallyas myelin stained tissue in (C). Note the vertical oriented fibers in both (B,C). Curved arrows in (B,C) point to the perforant pathway (pp). (B) appears slightly larger than the other panels because (B) is a 3D image and some of the streamlines (i.e., green, yellow, orange tracks) actually reside anterior to the slice displayed. Thin straight arrow in (C) demarcates the hippocampal efferents projecting back to the angular bundle. Scale bar = 0.5 cm.