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Development of a histologically validated segmentation protocol for the hippocampal body

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ABSTRACT

Background: Recent findings have demonstrated that hippocampal subfields can be selectively affected in different disease states, which has led to efforts to segment the human hippocampus with in vivo magnetic resonance imaging (MRI). However, no studies have examined the histological accuracy of subfield segmentation protocols. The presence of MRI-visible anatomical landmarks with known correspondence to histology represents a fundamental prerequisite for in vivo hippocampal subfield segmentation. In the present study, we aimed to: 1) develop a novel method for hippocampal body segmentation, based on two MRI-visible anatomical landmarks (stratum lacunosum moleculare [SLM] & dentate gyrus [DG]), and assess its accuracy in comparison to the gold standard direct histological measurements; 2) quantify the accuracy of two published segmentation strategies in comparison to the histological gold standard; and 3) apply the novel method to ex vivo MRI and correlate the results with histology.

Methods: Ultra-high resolution ex vivo MRI was performed on six whole cadaveric hippocampal specimens, which were then divided into 22 blocks and histologically processed. The hippocampal bodies were segmented into subfields based on histological criteria and subfield boundaries and areas were directly measured. A novel method was developed using mean percentage of the total SLM distance to define subfield boundaries. Boundary distances and subfield areas on histology were then determined using the novel method and compared to the gold standard histological measurements. The novel method was then used to determine ex vivo MRI measures of subfield boundaries and areas, which were compared to histological measurements.

Results: For direct histological measurements, the mean percentages of total SLM distance were: Subiculum/CA1 = 9.7%, CA1/CA2 = 78.4%, CA2/CA3 = 97.5%. When applied to histology, the novel method provided accurate measures for CA1/CA2 (ICC = 0.93) and CA2/CA3 (ICC = 0.97) boundaries, but not for the Subiculum/CA1 (ICC = −0.04) boundary. Accuracy was poorer using previous techniques for CA1/CA2 (maximum ICC = 0.85) and CA2/CA3 (maximum ICC = 0.88), with the previously reported techniques also performing poorly in defining the Subiculum/CA1 boundary (maximum ICC = 0.00). Ex vivo MRI measurements using the novel method were linearly related to direct measurements of SLM length (r^2 = 0.58), CA1/CA2 boundary (r^2 = 0.39) and CA2/CA3 boundary (r^2 = 0.47), but not for Subiculum/CA1 boundary (r^2 = 0.01). Subfield areas measured with the novel method on histology and ex vivo MRI were linearly related to gold standard histological measures for CA1, CA2, and CA3/CA4/DG.

Conclusions: In this initial proof of concept study, we used ex vivo MRI and histology of cadaveric hippocampi to develop a novel segmentation protocol for the hippocampal body. The novel method utilized two anatomical landmarks, SLM & DG, and provided accurate measurements of CA1, CA2, and CA3/CA4/DG subfields in comparison to the gold standard histological measurements. The relationships demonstrated between histology and ex vivo MRI supports the potential feasibility of applying this method to in vivo MRI studies.

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Introduction

The hippocampus plays a crucial role in human memory (Scoville and Milner, 1957) and is prominently affected in both Epilepsy (Bouchet and Cazauvieihlh, 1825) and Alzheimer's disease (Braak and Braak, 1991). Hippocampal subfields, which are discrete histological subregions of the hippocampus, are thought to subserve distinct aspects of memory function. The dentate gyrus (DG) has been implicated in discrimination of novel stimuli (pattern separation) during memory encoding (Neunuebel and Knierim, 2014). Conversely, the CA3 subregion is likely responsible for recall of a memory based on partial cues (pattern completion) during learning (Neunuebel and Knierim, 2014). Finally, CA1 appears to be critical for autobiographical and episodic memory retrieval (Bartsch et al., 2011). In addition to their distinct cognitive functions, hippocampal subfields are differentially involved in several neurological conditions.

Alzheimer's disease (AD) is characterized by the progressive development of neurofibrillary pathology in specific hippocampal subfields. The earliest stages of AD are accompanied by pathology restricted to the transentorhinal and entorhinal cortices of the hippocampal formation (Braak et al., 2006). When the disease reaches the hippocampus proper, pathology remains strikingly subfield-specific with preferential involvement of Subiculum and CA1 (West et al., 1994). Progression of pathological changes from the entorhinal cortex to the hippocampus is strongly correlated with the development of dementia (Thal et al., 2000). Measurement of pathology in hippocampal subfields could, therefore, represent a preclinical biomarker for early diagnosis of AD (Jack et al., 2010).

Hippocampal subfield pathology also has potential direct clinical relevance to the treatment of epilepsy. Hippocampal sclerosis (HS) is the most common pathological finding in drug-resistant epilepsy (Thom et al., 2008). While pathology can be seen throughout all hippocampal subfields in HS (Steve et al., 2014), hippocampal subfields are differentially affected in classical HS, with more severe involvement of the CA1 region and relative sparing of the CA2 subfield (Sommer, 1880; Spielmeyer, 1927). While surgery can potentially cure patients who fail medical treatment, recent studies report long-term seizure freedom rates of only 50% (de Tisi et al., 2011). However, detection of neuronal loss in CA1 and CA4 subfields with postoperative pathology is consistently associated with a high likelihood of seizure freedom postoperatively (Blumcke et al., 2007, 2013; Thom et al., 2010). Detection of subfield-specific pathology in vivo could therefore potentially allow more accurate preoperative prediction of seizure-free outcomes in patients with epilepsy.

Measurement of hippocampal subfields in vivo has recently become feasible due to advances in magnetic resonance imaging (MRI) spatial resolution. Alterations in hippocampal shape have been used by some investigators to measure subfield-specific atrophy (La Joie et al., 2010; Wang et al., 2003), while cortical thickness measurements have been utilized in some protocols to determine subfield locations (Kerchner et al., 2012). The majority of published techniques have used a combination of anatomical landmarks and geometric rules to define subfield boundaries (Malykhin et al., 2010; Mueller et al., 2007; Winterburn et al., 2013; Wisse et al., 2012; Yushkevich et al., 2010). In total, at least twenty-one distinct methods have been used by different investigators to perform hippocampal subfield segmentation (Yushkevich et al., 2015b).

While previous segmentation protocols have been based on histological references (Duvernoy, 2005), they have not been systematically validated in comparison to histology. Although subfield volumes have been the primary focus of hippocampal segmentation research to date (Yushkevich et al., 2015b), accurate volumetric information is critically dependent upon correct localization of subfield boundaries (van Strien et al., 2012). Hippocampal subfields are defined by transitions in cytoarchitecture detected with histological analysis (Braak, 1980; Duvernoy, 2005; Lorente de No, 1934). However, the accuracy of MRI-based subfield boundary delineation in comparison with histology remains unknown (Eugenio Iglesias et al., 2015; Yushkevich et al., 2009). *Ex vivo* MRI has recently been used to allow histologically accurate measurement of the entorhinal cortex (Fischl et al., 2009). In addition, coregistration of hippocampal histology with *ex vivo* MRI has been described (Adler et al., 2014; Coras et al., 2014; Goubran et al., 2013), but such techniques have not yet been used to develop a histologically valid segmentation protocol for application to in vivo MRI.

In the present study, we aimed to: 1) develop a novel histology-based method for hippocampal body segmentation, using MRI-visible anatomical landmarks, and assess its accuracy in comparison to the gold standard direct histological measurements; 2) quantify the accuracy of two published segmentation strategies in comparison to the histological gold standard; and 3) apply the novel method to ex vivo MRI and correlate the results with histology.

Materials and methods

Six temporal lobes (3 Left / 3 Right) were removed post-mortem from six subjects (age 61–96) with no known history of neuropsychiatric disorders. Brain specimens were fixed in formaldehyde for an average of six months. Surgical cuts were placed in the temporal neocortex to aid coregistration of *ex vivo* MRI and subsequent histology (see section 2.5). Specimens were trimmed to fit into a 50 mL centrifuge tube and submerged in an MRI-compatible liquid fluorocarbon (Fluorinert, 3 M). Imaging was performed on a 4.7 T MRI system (Varian, Palo Alto, CA) using a custom-built mouse coil. A T2-weighted (inverted contrast) fast-spin echo technique was used to acquire 40 contiguous 0.5-mm-thick slices, perpendicular to the long axis, across 2 cm of the hippocampal body [echo time (TE) = 39 ms, repetition time (TR) = 10,000 ms, FOV 40×40 mm, in-plane matrix 200×200] yielding a native resolution of 0.2×0.2×0.5 mm^3 in nine minutes of total scan time.

Following MRI, each hippocampus (n=6) was dissected from the surrounding temporal lobe and cut, perpendicular to the long axis of the hippocampal body, into four equal 5 mm blocks (n=4×6=24). Previous studies have demonstrated functional differences along the hippocampal long axis (Poppenk et al., 2013), while some hippocampal pathologies can also demonstrate variability along the anteroposterior extent of this structure (Thom et al., 2012). Therefore, the location of the blocks along the anterior-posterior axis of the hippocampus was noted and included in order to allow block-wise analyses along the hippocampal long axis. The blocks were embedded with paraffin and a *single* 5 μm histological section was taken from each 5 mm block. Two of the blocks contained only tissue from the hippocampal head and were therefore not analyzed. This yielded a final n=22 histological sections for our study.

Gold standard histology measurements

Histological sections (n=22) were stained with cresyl violet / luxol fast blue (Fig. 1i) as described elsewhere (Blumcke et al., 2015). The length of the stratum lacunosum moleculare (SLM) from the superficial hippocampal sulcus to the blades of the DG was measured using the curvilinear measurement tool in ImageJ (Schneider et al., 2012) for each of the histological sections (n=22). Hippocampal subfields were delineated according to histological criteria (Braak, 1980; Duvernoy, 2005; Lorente de No, 1934) by an experienced neuropathologist (RC), who has substantial experience measuring hippocampal subfields in surgical specimens (Fig. 1v).

The distances of three hippocampal subfield boundaries (Subiculum/CA1, CA1/CA2, and CA2/CA3) along a line from the superficial hippocampal sulcus to the blades of the DG were directly measured (Fig. 1 vi). The distances to these subfield boundaries were used: i) as the basis for the novel method (section 2.2) and ii) as the