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Heterogeneity of functional activation during memory encoding across hippocampal subfields in temporal lobe epilepsy

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ABSTRACT

Pathology studies have shown that the anatomical subregions of the hippocampal formation are differentially affected in various neurological disorders, including temporal lobe epilepsy (TLE). Analysis of structure and function within these subregions using magnetic resonance imaging (MRI) has the potential to generate insights on disease associations as well as normative brain function. In this study, an atlas-based normalization method (Yushkevich, P.A., Avants, B.B., Pluta, J., Das, S., Minkoff, D., Mechanic-Hamilton, D., Glynn, S., Pickup, S., Liu, W., Gee, J.C., Grossman, M., Detre, J.A., 2009. A high-resolution computational atlas of the human hippocampus from postmortem magnetic resonance imaging at 9.4 T. NeuroImage 44 (2), 385–398) was used to label hippocampal subregions, making it possible to examine subfield-level functional activation during an episodic memory task in two different cohorts of healthy controls and subjects diagnosed with intractable unilateral TLE. We report, for the first time, functional activation patterns within hippocampal subfields in TLE. We detected group differences in subfield activation between patients and controls as well as inter-hemispheric activation asymmetry within subfields in patients, with dentate gyrus (DG) and the anterior hippocampus region showing the greatest effects. DG was also found to be more active than CA1 in controls, but not in patients' epileptogenic side. These preliminary results will encourage further research on the utility of subfield-based biomarkers in TLE.

Introduction

Temporal lobe epilepsy (TLE) is a common neurological disorder in which seizures arise from the hippocampus. Approximately 30% of patients with TLE are refractory to medical therapy and are candidates for resective surgery as the only remaining treatment option (Engel, 1996). Although temporal lobectomy has been shown to provide highly significant benefits from the perspective of seizure reduction or elimination, it can be complicated by memory deficits, since the hippocampus normally plays a critical role in memory consolidation (Squire, 1992) and retrieval (Nadel and Moscovitch, 1997). Accordingly, pre-surgical evaluation for temporal lobectomy includes both seizure lateralization and attempts to assess the functional integrity of the hippocampus that will be resected.

Structural and functional abnormalities in the epileptogenic hippocampi in patients have been documented in TLE using MRI for more than a decade (Bellgowan et al., 1998; Bookheimer, 1996; Lencz et al., 1992). Functional activation in the hippocampus has been shown to predict post-surgical seizure outcome (Killgore et al., 1999) as well as cognitive outcome (Rabin et al., 2004). Inter-hemispheric activation asymmetry in the hippocampus has also been used to lateralize memory function (Deblaere et al., 2005; Golby et al., 2002; Jokeit et al., 2001) during pre-surgical evaluation.

The hippocampus consists of anatomically distinct subregions, known as hippocampal subfields that contain different neuronal cell types, and are connected with each other and with surrounding subcortical and cortical structures in the medial temporal lobe (MTL) in different ways. Accordingly, the hippocampal region is affected by various neurological disorders in a spatially non-uniform, complex fashion (Huesgen et al., 1993; Saravia et al., 2006; Sass et al., 1991). In a recent pioneering MRI-based volumetry study in a cohort of TLE patients, atrophy was found in dentate gyrus (DG) and CA3, and sometimes in CA1 and CA2 subfields (Mueller et al., 2009). This was the first attempt to segment and measure volumes of hippocampal subfields in TLE. The same group has also reported correlation of memory impairment with volume loss in subfields (Mueller et al., 2011). Recent histopathological studies have found plastic changes and abnormal sprouting of mossy fibers—which connect DG with CA3—due to epileptogenic activity or neuron death (Andrade-Valença et al., 2008; McAuliffe et al., 2011). Therefore, focal measurements based on individual subfields may provide valuable insight about the disease process in TLE.

Most prior functional imaging studies in TLE have considered the hippocampus as a single region of interest (ROI). A few studies have segregated group effects into anterior and posterior regions (Bettus et al.,

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2009; Das et al., 2009; Figueiredo et al., 2008), but to our knowledge, none have examined functional activation patterns across hippocampal subfields. Subfield-based structural morphometry, however, has been shown to provide superior information than whole hippocampus based measurements (Mueller et al., 2009), correlated with performance in memory tasks (Mueller et al., 2011), and used to study other neurological disorders such as Alzheimer's disease (Mueller et al., 2008) and post-traumatic stress disorder (Wang et al., 2010). Further, in non-clinical populations, hippocampal subfields have been shown to exhibit dissociation of cognitive function (Bakker et al., 2008; Duncan et al., 2011; Suthana et al., 2010; Zeineh et al., 2003). Based on these findings, we hypothesize that analysis of functional activation within hippocampal subfields will augment existing knowledge on TLE pathology as well as normal memory function mediated by the hippocampus and can potentially be more sensitive to disease effects than whole hippocampus-based measurements.

There are a number of existing methods for analyzing functional activation in hippocampal subfields (Stark and Okado, 2003; Zeineh et al., 2003). These methods vary in the type of structural images used to label subfields or the labeling technique used, or both. A method used in Zeineh et al. (2003), and recently enhanced in Ekstrom et al. (2009), requires an initial manual segmentation of hippocampal gray matter, white matter and cerebrospinal fluid (CSF), in images with high in-plane resolution of 0.4×0.4 mm in slices oriented obliquely along the long axis of the hippocampus. It then uses a computational flattening technique that allows the gray matter sheet in MTL to be transformed into a flat space, where activations within subfields can be computed, and group-wise statistical analysis can be performed. The ROI-AL technique (Bakker et al., 2008; Miller et al., 2005; Stark and Okado, 2003) uses more common T1-weighted structural images with $\approx$1 mm isotropic resolution, as we use in the present study, and uses image registration to an atlas containing subfield labels to segment ROIs in individual subjects. The atlas is constructed by manually segmenting subfield ROIs in *in vivo* images with 0.75 mm isotropic resolution from several subjects, and averaging the ROI labels in a common space after spatial normalization driven by the label images (Kirwan et al., 2007). In contrast, we use shape-based normalization to a high-resolution atlas (Yushkevich et al., 2009) that was constructed from *ex vivo* MRI scans of resolution $0.2\times0.2\times0.2$ mm or $0.2\times0.3\times0.2$ mm to label subfields in individual hippocampi. The benefit of this approach is that the subfields can be distinguished in the postmortem images reliably, with the tradeoff that no intensity information is used from the *in vivo* image, where subfields are difficult to distinguish (see Section Labeling of subfields using shape-based normalization for details). In our previous work, we have used shape-based normalization to establish voxel-by-voxel correspondence within the hippocampus (Das et al., 2009; Yushkevich et al., 2007) to perform group statistical analysis of activation maps, but this work did not include subfield ROI. In this study, we used subfield labels to determine functional activation within subfields in both healthy controls and patients with TLE. We then compared activations in different subfields across subject groups. We also studied inter-hemispheric activation asymmetry, a measure that is often used to lateralize pre-surgical cognitive function in TLE (Deblaere et al., 2005; Golby et al., 2002; Jokeit et al., 2001). We demonstrate both subfield-specific group differences in functional activation, and hemispheric differences in subfield activation within the same subject.

Materials and methods

Image acquisition

This paper analyzes data from two independent TLE studies. The first study, denoted TLE-HR, was designed with detailed hippocampal morphometry in mind and collected high-resolution fMRI data. An older study, denoted TLE-SR (for standard resolution), collected more routine 3 mm isotropic fMRI data. MRI images were obtained from a 3T Siemens Trio scanner using a product T/R head coil and body coil transmitter. For both datasets, the imaging protocol consisted of a localizer scan, followed by an anatomical scan, and a functional MRI (fMRI) scan while the subjects performed a complex scene encoding task in a blocked design experiment. The T1-weighted anatomical scans used the MP-RAGE sequence with the following parameters: TR = 1620 ms, TE = 3.87 ms, TI = 950 ms, flip angle = 150, 160 sagittal slices, matrix size = 256×192 and voxel size = $0.9375\times0.9375\times1\ mm^3$. The BOLD fMRI scans used a gradient echo echoplanar (EPI) sequence with TR = 3000 ms, TE = 30 ms. The TLE-HR dataset used a resolution of $1.95\times1.95\times2\ mm^3$ (flip angle = 90, 30 oblique slices, matrix size = 128×128), did not cover the whole brain, but included the entire temporal lobe. The TLE-SR dataset used a $3\times3\times3\ mm^3$ (flip angle = 90, 40 axial slices, matrix size = 64×64) resolution and imaged the whole brain. Note that the former dataset—having higher spatial resolution that yielded voxels that are more than 3 times smaller than the latter—was more suitable for studies of BOLD activation in small hippocampal subfields. A resolution of 2 mm or lower is typically considered to be high-enough resolution (Carr et al., 2010) for such studies. As such, the TLE-HR dataset should be considered our primary dataset for the current study. Nonetheless, the motivation for presenting independent analyses on both datasets are twofold: 1) On the one hand, if broadly similar effects can be demonstrated in two disjoint cohorts, it serves as a validation of the results, 2) On the other hand, the way the results differ in the two datasets can also help interpret them, and inform us about the possible effects of spatial resolution. Also note that activation within some larger subfield ROIs may be distinguishable in the lower resolution TLE-SR dataset.

Experimental paradigm

In both datasets, alternating blocks consisted of a task condition when the subjects were instructed to remember visual scenes of people, landscape and human-created environments, and a control condition when they viewed randomly scrambled scenes. In the TLE-HR cohort, subjects also indicated whether the scene was meaningful to them in some way or not during the task condition, and performed a visual search task during the control condition where they had to locate an embedded “X" or “T" in the scrambled scene. Subjects indicated their binary choice for both conditions by a button press. Further details of the task can be found in (Rabin et al., 2004).

Subjects

The TLE-HR dataset consisted of 15 patients with TLE and 19 healthy volunteers with no history of neurological illness. 8 patients had seizures originating in the left hemisphere and 5 on the right hemisphere. 2 had bilateral seizure foci. The TLE-SR dataset consisted of 18 patients with TLE and 19 healthy volunteers with no history of neurological illness. 9 patients had seizures originating in the left hemisphere and 7 on the right hemisphere. 2 had bilateral seizure foci. Patients with bilateral seizures were not included in the analysis. Structural and functional imaging data were obtained for the healthy volunteers in the same way as for the patients.

Image processing

Whole hippocampus segmentation

Each subject's hippocampi were segmented using a semi-automated protocol (Pluta et al., 2009), in which a few user-defined landmarks are used to drive diffeomorphic normalization of the subject's MRI to a disease-specific template with manual whole-hippocampus segmentations. This produced a whole hippocampus label in the subject space that was edited by an expert. The initial landmark placement and final editing of the mask required 15 min on average for a trained rater. This procedure had an inter-rater reliability of 89.5% as measured by DICE overlap (Dice, 1945).