Unfolding the Human Hippocampus With High Resolution Structural and Functional MRI

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The hippocampus is a region of the brain that is crucial to memory function. Functional neuroimaging allows for the noninvasive investigation of the neurophysiology of human memory by observing changes in blood flow in the brain. We have developed a technique that employs high-resolution functional magnetic resonance imaging (fMRI) in combination with cortical unfolding to provide activation maps of the hippocampal region that surpass in anatomic and functional detail other methods of in vivo human brain mapping of the medial temporal lobe. We explain the principles behind this method and illustrate its application to a novelty-encoding paradigm. Anat Rec (New Anat) 265: 111–120, 2001. © 2001 Wiley-Liss, Inc.

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One of the most influential findings in the history of neuroscience involves a patient known as HM, who suffered from intractable epilepsy. To treat his epilepsy, the neurosurgeon Scoville performed a bilateral resection of HM’s medial temporal lobes (Scoville et al., 1957). At the heart of the medial temporal lobe is the hippocampus, a site of neuroanatomic convergence in the brain. At the time, little was known about its importance, and the procedure reduced the severity of HM’s seizures. However, HM was left with significantly impaired memory function. Specifically, he had severe anterograde amnesia (an inability to learn new information) and mild retrograde amnesia (an inability to recall past events). From the data of HM and patients like him, it has become increasingly clear that the medial temporal lobe is a structure vital for memory formation and retrieval (Squire, 1992).

This finding has sparked an entire field of research, spanning the gamut from molecular to systems neuroscience, that explores the role the hippocampus plays in memory. At the molecular level, scientists are studying the behavior of ion-channels that facilitate potentiation of neuronal responses, a phenomenon that is thought to be the molecular signature of memory formation (Milner et al., 1998; Bennett, 2000). At the level of individual cells, researchers record from rats and monkeys to identify cellular and network correlates of object and place memory (O’Keefe and Nadel, 1978; Rolls and O’Mara, 1995; Buzsáki, 1996; Rolls, 1999). Finally, at the level of cellular populations, neuroimaging researchers measure the metabolic consequences of changes in neuronal firing in the awake human during memory tasks. The question is the same at all levels: how does the hippocampus engage in the storage and retrieval of information?

FUNCTIONAL BRAIN IMAGING: HOW IT WORKS

Functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) both rely on an epiphenomenon of neural activity: when many neurons in a region of the brain increase their firing rate, local blood flow increases to that region of the brain (Roland, 1993). Both PET and fMRI attempt to measure this change in blood flow. With PET, radioactive H2O15 is injected intravenously in subjects, and by tomographic reconstruction of radioactive decay events, a map of blood flow in the brain emerges. With arterial blood sampling, one may obtain quantitative maps with units of blood flow in milliliters per minute per gram. Statistical comparisons between maps of blood flow during resting states and maps of blood flow during memory

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tasks can reveal changes in blood flow accompanying mnemonic processes (Cherry and Phelps, 1996).

The power of fMRI is that it accomplishes similar goals noninvasively. In fMRI, continuous MRI scanning occurs while a subject is engaged in a task, with the acquisition of a complete volume of the brain every few seconds over a period of minutes (this stands in contrast to structural MRI, in which only one volume is acquired). As reviewed by Cohen (1996) and by Mori and Barker (1999), MR images are produced by placing a subject in a strong, uniform magnetic field and applying radio frequency (RF) pulses of electromagnetic energy to synchronously alter the precession of protons in H2O. After an RF energy pulse, the protons are precessing in phase, and this precession can be measured with a receiver coil. The precession dephases with time resulting in signal decay. In areas of the brain where the magnetic field is less uniform, the dephasing occurs faster; consequently, signal decay is greater, resulting in darker pixels. Images whose contrast is based on measuring such differences in dephasing are termed T2-weighted images.

When blood flow increases to a part of the brain in response to an increase in neural firing, there is an increase in incoming oxyhemoglobin. In fact, the increase in oxyhemoglobin delivery is greater than the increase in oxygen consumption (Fox and Raichle, 1986). The net effect is an increase in venous oxyhemoglobin compared with deoxyhemoglobin several seconds after the neural event. Deoxyhemoglobin causes more perturbation, that is, more deviation from uniformity in the magnetic field in comparison to oxyhemoglobin. Thus, more deoxyhemoglobin results in more signal decay and lower signal intensity; conversely, more oxyhemoglobin results in less signal decay and higher signal intensity (i.e., brighter pixels). With the greater relative venous oxyhemoglobin concentration that accompanies activation, less signal decay occurs in those activated pixels, and those pixels are brighter in an image (Cohen and Bookheimer, 1994). This type of functional contrast is termed blood oxygenation-level dependent (BOLD) MRI. Hence, images are taken during a resting condition and during a memory condition, and statistics are performed to identify which pixels have an increase in intensity during the memory condition, that is, an increase in BOLD contrast, resulting from a change in blood flow.

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ANATOMY OF THE HIPPOCAMPAL REGION

The hippocampal region is the centerpiece of the medial temporal lobe, and it is a compact structure with several components (Fig. 1). It can be roughly divided as having two constituents: nearby cortical areas, and the hippocampus proper. The nearby cortical areas project to the hippocampus proper, and the hippocampus proper has an internal system of projections that sends output back to the nearby cortical areas. The entorhinal cortex (ERC), which lies in the anterior portion of the parahippocampal gyrus and borders the anterior hippocampus, is the main gateway of input to the hippocampus proper (Fig. 2) (Suzuki and Amaral, 1994b). It receives most of its projections from two adja-
component in the hippocampal re-
match the sample stimulus). Of all the
ond stimulus is not identical to the
digm, the monkey sees a stimulus and
ception to fields CA 3 (Cornu Ammonis 3), CA 1, the subiculum, and
back to the entorhinal cortex, where projections return to the parahipp-
pocampal and perirhinal cortices. A
from CA 3 via the fornix, a white matter tract that at-
taches to the inferior border of the
septum pellucidum and projects to the mamillary nuclei within the hypothalamus as well as the septal nu-
cleis.

The essential circuit diagram of the
hippocampus proper is a loop (Fig. 2).

Recent higher field MR technologies
increase signal-to-noise and make scanning at high resolution possible.

CORTICAL UNFOLDING TECHNIQUES

The human cerebral cortex is highly folded, presumably to increase the amount of cortical surface area in the limited volume of the human skull. This gyrification has posed multiple difficulties for localizing and displaying blood flow changes in the brain. First, individuals vary in their pattern of gyri and sulci. Hence, typical techniques of volumetric aligning and processing data across subjects often blur together gyri and sulci because they do not take into account individual differences. Second, visualization of activation is difficult because signal changes may occur either on the gyrus or inside the sulcus.

Cortical unfolding techniques can successfully address these two difficulties: they account for across-subject variability by individual segmen-
tation, and they flatten the data to produce simple two-dimensional maps (Van Essen et al., 1998). To perform cortical unfolding, high resolution structural and functional images are taken of individual subjects. Structural images have only one time point and have high spatial resolution, while functional images have multiple time points and have high temporal resolution that facilitates the measurement of BOLD contrast. The structural images are segmented; that is, pixels in the image are labeled as gray matter, white matter, and CSF (Teo et al., 1998; Van Essen et al., 1998). The gray matter surface is then extracted and stretched until it is a two-dimensional flat surface. Statistics are computed on the functional images to generate activation maps (Cohen, 1997), and these activation maps are then superimposed on the flat maps. This technique was first used with fMRI in early visual areas to provide maps of retinotopy, the mapping of the visual field from the retina to visual cortex (Engel et al., 1994; Sereno et al., 1995). Primary visual cortex lies in the occipital lobe along the calcarine sulcus, a particularly winding sulcus, and visualizing retinotopy is difficult in three dimensions. Segmentation and unfolding converts the complex sulcal pattern to a simpler flat representation where areas V1, V2, and V3 can be easily visualized.

**UNFOLDING THE HIPPOCAMPUS**

The hippocampus is a compact structure, and the region contains potentially heterogeneous areas as alluded to previously. Unfolding techniques are ideally suited for reducing the anatomic complexity of the region to a more tractable two-dimensional problem. By applying unfolding techniques to the hippocampal region, we can localize activity with greater precision than has ever been possible to the different subregions of the medial temporal lobe, namely, the CA fields, subiculum, and parahippocampal areas (Zeineh et al., 2000b). Furthermore, we have created a hippocampal reference template based on the subjects in our studies that facilitates the registration of subjects in the same space (Thompson et al., 2000; Zeineh et al., 2000a). This allows us to perform powerful group statistics while maintaining the high resolution necessary for localizing activation to the different anatomic substructures (Fischl et al., 1999b; Zeineh et al., 2000a).

We describe the unfolding procedure in two parts: structural unfolding and functional mapping. To accomplish the structural unfolding, high spatial resolution structural scanning takes place, followed by segmentation, identification of boundaries between the medial temporal subregions, and computational flattening. For the functional mapping, we acquire coplanar functional scans and perform statistical processing to generate activation maps. Overlaying the activation maps on the flattened hippocampi from the structural unfolding delivers the high-resolution functional activation maps.

**Structural Unfolding of the Hippocampus**

The goal of the structural unfolding procedure is to create a gray matter volume (a three-dimensional set of pixels that are classified as gray matter) and stretch and unfold this volume until it is flat. We acquire high-resolution structural images (0.4 × 0.4 × 3 mm slices) with $T_2$ contrast so we can discern the anatomic detail of the region (Fig. 3B). In a $T_2$ weighted image, white matter is dark, and gray matter is brighter (medium $T_2$), and CSF is brightest (high $T_2$). As illustrated by the slice prescription in Figure 3A, we acquire these images perpendicular to the long axis of the hippocampus to minimize slice-to-slice variation in anatomy.

Using this image set, we proceed to segment and create a gray matter volume, that is, an organized array of pixels covering all of the medial temporal structures of interest that we will subsequently unfold. In our unfolding technique, we manually label pixels as white matter and CSF. A region expansion algorithm grows out layers of gray matter, with the first layer starting at the white matter surface, the second layer starting on top of the first, and the last layer ending adjacent to the CSF boundary (Fig. 3B, bottom) (Teo et al., 1998). The goal in segmenting white matter is to provide a continuous strip of pixels upon which the gray matter in the hippocampus and adjacent cortex rests. Toward this end, as Figure 3B illustrates, we classify pixels as white matter in the parahippocampal and fusiform gyri and extend the white matter all the way around to the top of the hippocampus. The goal in segmenting CSF is to limit the extent to which gray matter is grown (i.e., so that it does not cross a sulcal boundary or extend into the CSF). We classified the collateral sulcus as CSF, facilitating the separation of adjacent cortex on different banks of the sulcus (inferior no. 5), as well as the hippocampal fissure (superior no. 5), facilitating the separation of fields CA2, 3, and the dentate gyrus from field CA 1 and the subiculum. Classifying the boundary of the segmentation as CSF allows for a limited volume of gray matter to be grown that encompasses only hippocampal and adjacent parahippocampal gray matter.

At the end of the segmentation, we have a three-dimensional topological volume of gray matter pixels. By topological, we mean that we keep track of the spatial relationships between each gray matter pixel and its neighboring gray matter pixels. The first layer of gray matter pixels forms a thin manifold, and subsequent layers on top of this manifold form a volume. The unfolding procedure works by taking the first layer manifold, and stretching it so that it is flat while maintaining topology and minimizing distance errors (Wandell et al., 1996; Engel et al., 1997; Zeineh et al., 2000b). Distance errors are the difference in distance between two pixels along the manifold before unfolding and along the flat map after unfolding; such errors are inevitable in the distortion that results from flattening. If you recall flat maps of the earth, the Northern and Southern poles are heavily distorted to maintain continuity. This distortion can be eliminated if cuts are made on latitude lines. Similarly, when the whole brain is flattened, researchers often make cuts to reduce overall distortion (Drury et al., 1996; Van Essen and Drury, 1997; Fischl et al., 1999a). Fortunately, we unfold only the medial temporal lobe, and minimal distortion is incurred in the unfolding.
process on a relatively small stretch of cortex, so we do not cut any part of our segmentation (Zeineh et al., 2000b). After the first layer is unfolded, subsequent layers are compressed onto this layer and the cortical volume has been flattened. Thus, we have a transform that takes pixels from 3D volumetric space to 2D flat space.

Once we have flat structural images, we create a map of the different architectonic subregions (Fig. 3C). Using a rule-based system in concert with histological and MRI atlases, we demarcate the boundaries between the following regions: fusiform gyrus (FG), parahippocampal cortex (PHC), perirhinal cortex (PRC), entorhinal cortex (ERC), subiculum (Sub), CA 1, and CA fields 2, 3, and dentate gyrus (CA23DG).
The last category combines several histological fields that even at our high resolution are inseparable. We define these boundaries on the structural 3-D images, and using the transform from 3-D to 2-D space, project these boundaries to the flat maps, thus defining the structure of the flat maps (Fig. 3D). Curves are fit to the boundaries for easier viewing of activation maps.

**Functional Activation Maps in Flat Space**

For the functional imaging, we take echo-planar MR images while the subject is performing a memory task. Echo-planar imaging (EPI) is a method of MR imaging that optimizes temporal resolution by acquiring an entire two dimensional slice after a single RF pulse (Cohen, 1996), and it enables us to acquire volumes of the hippocampus with a resolution of $1.6 \times 1.6 \times 3$ mm every 3.5 sec. The resulting set of images has three spatial dimensions and one temporal dimension, and we term this data set a time series of volumes. The spatial resolution is adequate for registration with the high-resolution structural data, and the temporal resolution enables us to observe BOLD hemodynamic signal changes, which peak about 5–7 sec after a change in neuronal firing (Cohen, 1997).

We applied the flat technique to a novelty-encoding paradigm, a memory activation task that compares the encoding of novel stimuli the subject has never seen before with the same stimulus repeated several times. Because an intact medial temporal lobe is required to create new memories, one would expect medial temporal activity when subjects are instructed to encode novel stimuli. In monkeys, researchers have found temporal lobe neurons that respond greater to novel...

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**Figure 4.** Blood flow response: dark lines are the boxcar waveform, and shaded lines are the boxcar waveform smoothed and delayed to model typical hemodynamic response latencies. Reproduced from Zeineh et al., 2000b with permission of the publisher.

**Figure 5.** Single-subject correlation map superimposed on anatomy in (A) coronal space, anterior and posterior slices; and (B) flat space, right and left hippocampi. Blue pixel outlines indicate areas of low sensitivity. Reproduced from Zeineh et al., 2000b, with permission of the publisher.
stimuli in comparison to repeated stimuli (Riches et al., 1991). The novelty-encoding task tests the idea that the medial temporal lobes encode novel stimuli by contrasting epochs of novel stimuli with epochs of repeated stimuli. The paradigm has been known to elicit activation in the “hippocampal region,” but has been lacking a more precise localization to either the hippocampus proper or the adjacent parahippocampal regions (Tulving et al., 1994; Stern et al., 1996; Gabrieli et al., 1997). This paradigm thus served as a perfect target for our method. Each stimulus was a complex color image of indoor or outdoor scenery, and subjects were viewing the images with magnet-compatible goggles. There were 10 alternating blocks of novel and repeated stimuli, and blocks were 40 sec long with an image was presented every 2.5 sec (Fig. 4). During the novel blocks, subjects viewed color pictures of complex scenery they had not seen previously, and during the repeat blocks, subjects viewed the same two pictures repeatedly. To ensure subjects paid attention to both the novel and repeat blocks, we instructed subjects to press different mouse buttons for indoor and outdoor scenes.

The hypothesis is that, during novel blocks, subjects will engage in more encoding than during repeat blocks, and involved medial temporal areas will be more metabolically active and have an increase in BOLD signal. In other words, pixels that contain veins that drain active regions will have a “boxcar” temporal pattern of signal intensity with increases during the novel blocks and baseline during the repeat blocks (Fig. 4). Before looking for such pixels, we perform motion-correction on the data by aligning all volumes with the first volume in the time series (Woods et al., 1998). This corrects for signal intensity changes associated with movement of the head. After coregistering the functional EPI data with the high-resolution structural data, we project each three dimensional volume onto flat space using the unfolding transformation to arrive at a two dimensional time series of images (Zeineh et al., 2000b). After these processing steps, correlation maps are computed to identify pixels that have a boxcar time series (Cohen, 1997). For each pixel, we calculate the Pearson correlation between that pixel’s time series and a smoothed boxcar function (smoothed and delayed to model a typical hemodynamic response, see Fig. 4), and we threshold the correlation map and superimpose it on the anatomical map. A single subject’s correlation map is illustrated on Figure 5, both in the coronal plane and in flat space, with red indicating positively activated pixels, or pixels positively correlated above a 0.25 threshold with the smoothed boxcar waveform, and blue indicating negatively activated pixels, or pixels with a negative correlation with the smoothed boxcar waveform. Although activation can be discerned posteriorly in the coronal images, it is much easier to view the location and extent of activation on the flat maps. On both sides, activation lined the collateral sulcus in the fusiform gyrus and parahippocampal cortex, and on the left hemisphere, activation included the subiculum and fields CA23DG. This offered concrete evidence that both the hippocampus and adjacent cortical areas are involved in novelty-encoding processes.

The procedure was applied to seven other subjects with results similar to that of Figure 5. To better characterize the signal changes, the time course for each region was computed (Fig. 6). That is, in parahippocampal cortex as an example, the time series was averaged across all pixels in the parahippocampal cortex over all subjects in both hemispheres (Zeineh et al., 2000b). The resulting time series confirmed that both hippocampal and parahippocampal structures were active in the paradigm. Furthermore, hippocampal structures (Sub and CA23DG) exhibited a considerable delay in the development of a strong contrast between novel and repeat stimuli, and activity was significantly greater at the end compared with the beginning of the paradigm. We propose that encoding processes were occurring for the repeat stimuli during the first few blocks, and only late in the paradigm did encoding truly stop and the contrast became apparent. This can be differentiated from the parahippocampal and fusiform response, which is more consistent with direct visual stimulation. Using this technique, we have been able to not only pinpoint the localization of activation within the medial temporal lobe but also discern differences in temporal characteristics.

WARPING DATA IN FLAT SPACE

In addition to offering a superior visualization of activation patterns, transforming subjects to flat space facilitates the comparison of data across subjects. In flat space, the relationship between the pattern of activation and the anatomy is clear, and the anatomic consistency across subjects is adequate to facilitate inspection by eye. Furthermore, by using computational warping techniques, we can align data across subjects into the same space (see also Toga and Thompson, 2001, for discussion of warping). Typically, researchers align subjects in 3D volumetric space and perform group statistics to increase their power. The alignment is imper-
effect, however, because of anatomic variability across subjects, and 3D smoothing is applied to minimize the impact; the net effect is a loss in resolution and localization confidence. Through segmentation of individual subjects, we account for anatomical differences, and by unfolding, we display individual subjects in 2-D space. By warping data to a flat template, we can compute powerful group statistics while maintaining the high resolution necessary to distinguish medial temporal subregions (Fischl et al., 1999b; Zeineh et al., 2000a).

The first step is to create an average hippocampal template based on the anatomic boundaries of all of our subjects (n = 8). We take each of the curves fitted to these boundaries in flat space (Fig. 7A top), resample them so that each anatomical curve contains an equal number of uniformly spaced points, and average the resampled boundaries over all of the subjects (Fig. 7A bottom) (Thompson et al., 2000). This set of averaged curves thus forms the flat hippocampal template, and it is representative of the anatomy of our subject population. A transform is computed that warps each subject’s anatomical image to the template. This transform exactly overlays each of the nine boundaries and continuously warps the entire image while minimizing changes in area as measured by a warping energy function (Thompson and Toya, 1998). Application of this transform to each time series image (Fig. 7B) results in a full time series of data in a common space. One can then employ the general linear model on each pixel in flat space and perform either fixed-effects or random-effects analyses (Worsley and Friston, 1995; Holmes and Friston, 1998).

We chose to employ a random-effects analysis to generate maps of activation representative of the population: for each pixel and in each subject, the slope of the regression was calculated with the pixel’s time series as the ordinate (Y) and the smoothed-boxcar waveform as the abscissa (X). Computing a \( t \)-statistic that tests the significance of the slope in each pixel averaged across subjects resulted in a \( t \)-statistic activation map (Holmes and Friston, 1998). As random-effects analyses are very conservative, an uncorrected threshold of 0.05 was suitable for creating the overlay maps depicted in Figure 7. These maps illustrate clear activation

Figure 7. A: Creating a right hippocampal template by averaging the 9 boundaries across subjects. B: Warping a time series image to the template. C: Novelty activation maps produced by random-effects analyses of group data (n = 8).
of the parahippocampal gyrus and fusiform gyrus bilaterally, as well as subicular and CA23DG activity, consistent with the data from the group time courses.

CONCLUSIONS

Our study has shown that the contingent novelty-encoding paradigm elicits activation in both the hippocampus proper and parahippocampal cortex (Zeineh et al., 2000b). We have further dissociated the temporal responses from these two regions and measured a significant response latency in the hippocampus proper; this may concord with the cessation of encoding processes for repeated stimuli. The application of cortical unfolding techniques to fMRI facilitated this accomplishment by delivering high-power group activation maps with a resolution that maintains the distinction among the different anatomic subregion.

FUTURE DIRECTIONS

With the tools to localize activation to specific substrata within the hippocampus, we will begin to engage in focused hypotheses concerning hippocampal function in memory. These include differential specificity of the perirhinal, entorhinal and parahippocampal cortices as well as the elements of the hippocampus proper. As MRI hardware advances, software techniques such as cortical unfolding will bring us closer to the kind of data currently obtained only in animal studies. The convergence of human imaging with animal electrophysiology will propel the neuroscientific study of memory by enabling neuroscientists to generate and test models of memory that span the gamut of systems to molecular neuroscience.

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LITERATURE CITED

dence from functional magnetic resonance imaging. Proc Natl Acad Sci. USA 93:8660–8665.