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Hippocampus-Specific fMRI Group Activation Analysis Using the Continuous Medial Representation

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Abstract

We present a new shape-based approach for regional group activation analysis in fMRI studies. The method restricts anatomical normalization, spatial smoothing and random effects statistical analysis to the space inside and around a structure of interest. Normalization involves finding inter-subject correspondences between manually outlined masks, and it leverages the *continuous medial representation*, which makes it possible to extend surface-based shape correspondences to the space inside and outside of structures. Our approach is an alternative to whole-brain normalization in cases where the latter may fail due to anatomical variability or pathology. It also provides an opportunity to analyze the shape and thickness of structures concurrently with functional activation. We apply the technique to the hippocampus evaluate it using data from a visual scene encoding fMRI study, where activation in the hippocampus is expected. We produce detailed statistical maps of hippocampal activation, as well as maps comparing activation inside and outside of the hippocampus. We find that random effects statistics computed by the new approach are more significant than those produced using the Statistical Parametric Mapping framework (Friston et al., 1994) at low levels of smoothing, suggesting that greater specificity can be achieved by the new method without a severe tradeoff in sensitivity.

Keywords

Functional Neuroimaging; Hippocampus; Random Effects; Normalization; Statistical Parametric Mapping

1 Introduction

In group analysis of functional MRI, two models are commonly used. In *whole-brain analysis*, statistical maps are generated for each subject; subjects' brains are registered to an anatomical template; and group maps are computed in the space of the template. In *region-based analysis*, certain features are integrated over the volume of a region of interest (ROI) for each subject, and population-level inference is made from the integrated features. The first

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approach is well-suited for exploratory analysis, while the second approach is used to test hypotheses about specific anatomical structures. However, both approaches have shortcomings: whole-brain registration may fail to normalize structures like the hippocampus with desirable accuracy (Carmichael et al., 2005), especially in the presence of pathologies, and region-based analysis produces a single summary statistic for each structure, providing less information than a statistical map. As technological advances continue to push the limits of fMRI resolution, the need for intermediate methods arises. In this paper, we propose a technique for generating population-level statistical maps for a specific structure – the hippocampus – and we explore ways in which the specificity of these maps can be improved.

Our main contribution is the use of geometrical correspondences, rather than image registration, as the basis for intersubject normalization. Correspondences are established by fitting a deformable shape model to segmentations of the hippocampus in each subject; such segmentations are frequently available in fMRI studies, e.g., for the purpose of ROI-based analysis. Typically, geometrical correspondences are computed on the boundaries of structures (Davies et al., 2002; Styner et al., 2003b), but for fMRI analysis, correspondences over the entire volume of the structure are needed. Our work leverages the *continuous medial representation (cm-rep)* method (Yushkevich et al., 2006b), which is unique among surface-based 3D shape representations in its ability to easily extend surface-based correspondences to the interiors of structures. A secondary contribution is the use of non-uniform smoothing in order to reduce the effect of extra-hippocampal signal to measurements made in the hippocampus. The effect of both contributions is examined in the context of a functional neuroimaging study of visual memory, where hippocampal activation is expected.

This paper is organized as follows. In Sec. 2 we discuss prior work in fMRI group analysis, focusing on recently proposed alternatives to “standard” whole-brain analysis. Sec. 3 describes the shape model used to establish correspondences between subjects' hippocampi, explains how these correspondences can be leveraged in fMRI analysis, and gives details of a functional neuroimaging study used to evaluate our shape-based method and to compare it to whole-brain analysis with SPM2 (Friston et al., 1994). Sec. 4 presents the results of this comparison, showing increased sensitivity with the shape-based method at low smoothing levels, and illustrating the kinds of detailed hippocampal activation maps that our method can produce. A discussion of the results appears in Sec. 5.

2 Background

While the range of published fMRI analysis techniques is diverse, most current tools are best suited for exploratory analysis, where maps of group activation statistics are computed over the whole brain or over the entire cortical surface, and the locations of peaks and supra-threshold clusters in these maps are used to implicate anatomical structures. This whole-brain approach is exemplified by the highly prolific Statistical Parametric Mapping (SPM) method (Friston et al., 1994), as well as by the approaches based on cortical flattening, such as FreeSurfer (Dale et al., 1999; Fischl et al., 1999), BrainVoyager (Goebel, 1997) and others (Tosun et al., 2006). Intersubject normalization of brain anatomy plays a key role in these methods, as it is needed to bring the subject-level statistical maps into correspondence prior to computing group-level activation statistics. Poor alignment not only decreases sensitivity (Stark and Okado, 2003; Miller et al., 2005), but it also makes it difficult to distinguish between activation in neighboring structures (e.g., hippocampus vs. parahippocampal gyrus). Despite recent progress in image registration methodology (Thirion, 1996; Christensen et al., 1997; Davatzikos, 1997; Studholme et al., 1997; Joshi and Miller, 2000; Avants et al., 2005), the most widely used techniques tend to misalign smaller anatomical structures like the hippocampus because these structures do not contribute prominently to the objective function that drives whole-brain registration algorithms. This is especially true for parametric

registration techniques, where the possible deformations are restricted to a subspace spanned by some basis (Ashburner and Friston, 2000; Rueckert et al., 2003). For instance, in a recent survey of hippocampal alignment in elderly populations by Carmichael et al. (2005), several registration techniques were compared, and even for the best-performing fully deformable technique, overlap between aligned hippocampi ranged between 0.4 and 0.8.

Structure-oriented analysis is nearly as common in brain morphometry (Bouix et al., 2005; Styner et al., 2004; Thompson et al., 2003; Shen et al., 2002; Shenton et al., 2002; Cootes et al., 1998) as whole-brain voxel-based and deformation-based techniques (Ashburner and Friston, 2000; Chung et al., 2001; Davatzikos et al., 2001). However, in functional neuroimaging, the emphasis has been on whole-brain techniques and few structure-oriented methods have been proposed. A highly notable exception is the ROI-AL method, first introduced by Stark and Okado (2003) and later extended by Miller et al. (2005). In ROI-AL, registration is applied to a set of segmentations obtained for each subject. In the Stark and Okado method, 12-parameter affine registration is used, while Miller et al. use fully deformable diffeomorphic registration. In both methods, improved registration of the structures of interest has been shown to lead to increased statistical power of group activation maps. Our method can be seen as a variant of ROI-AL, where alignment is driven by a different set of features, and our results can be seen as confirming the findings of Miller et al. (2005) and Stark and Okado (2003). What distinguishes our method from ROI-AL is that we use specific geometrical features to align ROIs: we fit a “scaffold” to the interior of each subject’s hippocampus, and assign correspondences between points on the basis of their position relative to this scaffold. By contrast, ROI-AL deforms the ROI from each subject to a common template, basing correspondences on the hard constraints of affine registration or on the smoothness prior in diffeomorphic registration. Affine ROI registration does not account for local shape differences, and does not guarantee as high overlap between structures as deformable registration. On the other hand, diffeomorphic registration between ROIs bases correspondences in part on a local smoothness prior on deformation fields. It is not obvious whether shape-based or deformation-based correspondences model true anatomical correspondences more accurately. However, Rohlfing (2006) demonstrates that registration methods that align a set of ROIs equally well in terms of overlap can produce different deformation fields, biasing subsequent statistical analysis. In this context, explicit use of shape features to establish correspondences can be seen as an advantage of our approach. Furthermore, our approach provides a geometrical context in which to visualize and study activation. It makes it possible to relate activation to thickness and to study activation differences across structural boundaries, as we illustrate in this paper.

A different hippocampus-specific fMRI analysis approach is proposed by Zeineh et al. (2003). In this work the hippocampus is unrolled, producing a two-dimensional flat map onto which functional activation statistics are projected. The flat map is partitioned by experts into subfields, such as the dentate gyrus and cornu ammonis, using information derived from histological analysis. Zeineh et al. (2003) found that learning and retrieval in a face-name association task activated different hippocampal subfields differently. The Zeineh *et al.* study uses very high resolution 3 Tesla imaging, with structural voxels of $0.4 \times 0.4 \times 3.0\text{mm}^3$ and functional voxels of $1.6 \times 1.6 \times 5.0\text{mm}^3$. These highly non-isotropic voxels, whose long axis is along the length of the hippocampus, allow hippocampal subfields to be detected in each slice, but they make it difficult to study the hippocampus as a three-dimensional structure. In contrast, our method works with isotropic data, is less labor-intensive, and it produces both 2D and 3D statistical maps.

3 Materials and Methods

3.1 Continuous Medial Representation

There are many ways to represent shape. The *continuous medial representation (cm-rep)* describes the shape of objects in terms of symmetries. Blum (1967) showed that the symmetries of a planar object are captured by a geometrical construction called the *medial axis* or *skeleton*. The medial axis is formed by uniformly thinning the object until a curve, or a set of curves, remains, as shown in Fig. 1a. The concept extends to 3D, where the medial axis is formed by a set of surfaces called *medial manifolds* (Fig. 1b). 3D medial axes have fascinating geometry, studied recently by Damon (2005) and Giblin and Kimia (2004). The distance from the medial axis to the boundary is an easily inferred morphological feature describing thickness. This and other medial features have recently been leveraged in structural brain morphometry, including hippocampal morphometry (Bouix et al., 2005; Fletcher et al., 2004; Styner et al., 2003a; Thompson et al., 2003).

Previous techniques either derived medial axes from boundary surfaces using thinning-like algorithms (Ogniewicz and Kübler, 1995; Siddiqi et al., 1999b,a; Bouix et al., 2005), or used discrete CAD-like deformable models called *m-reps* (Pizer et al., 2003; Styner et al., 2003a). Neither approach directly supports the type of shape-based normalization of structures' interiors that we leverage in this paper: thinning approaches do not produce a parametrized description of the medial axis, while discrete deformable models do not provide a continuous parametrization of objects' interiors.¹

The *cm-rep* approach models shape by defining a *synthetic skeleton*, consisting of a medial manifold $\Omega \rightarrow \mathbb{R}^3$ and a radial (thickness) field $\Omega \rightarrow \mathbb{R}^+$, both continuous parametric functions defined on a domain $\Omega \in \mathbb{R}^2$. The boundary is derived from the synthetic skeleton using *inverse skeletonization*, i.e., the inverse of thinning. Unlike thinning, inverse skeletonization has an analytic form:

$$\mathbf{b}^\pm = \mathbf{m} + R \vec{\mathbf{U}}^\pm, \quad \text{where} \quad \vec{\mathbf{U}}^\pm = -\nabla_{\mathbf{m}} R \pm \sqrt{1 - \|\nabla_{\mathbf{m}} R\|^2} \vec{\mathbf{N}}_{\mathbf{m}}. \quad (1)$$

The boundary $\mathbf{b}$ is formed by two halves $\mathbf{b}^+$ and $\mathbf{b}^-$ that are located on opposite sides of the medial manifold. Vectors $\vec{\mathbf{U}}^+$ and $\vec{\mathbf{U}}^-$ point from the medial manifold to the boundary, have unit length, and are orthogonal to the boundary. They are defined in terms of $\nabla_{\mathbf{m}} R$, the Riemannian gradient of R on $\mathbf{m}$, and $\vec{\mathbf{N}}_{\mathbf{m}}$, the unit normal to $\mathbf{m}$. The concept of inverse skeletonization is illustrated in Fig. 1c-d.

Although inverse skeletonization is easy to compute, it is not always well-posed: under a wrong choice of $\mathbf{m}$ and R , the surfaces $\mathbf{b}^+$ and $\mathbf{b}^-$ may either intersect or be disjoint, in both cases, not forming a proper boundary. Yushkevich et al. (2006b) describe a set of sufficient conditions that $\mathbf{m}$ and R must satisfy to allow inverse skeletonization. Among them is a particularly challenging equality constraint

$$\|\nabla_{\mathbf{m}} R\| = 1 \quad \text{on} \quad \partial\Omega. \quad (2)$$

Functions $\mathbf{m}$ and R must be defined such a way that this constraint is satisfied. If $\mathbf{m}$ and R are defined as weighted sums of basis functions (Fourier harmonics, b-splines, etc.), the constraint translates into an infinite set of non-linear equations with a finite number of unknowns. So

¹See (Yushkevich et al., 2006b) for a more thorough review of medial literature.

instead, we use a set of basis functions $f_i : \Omega \rightarrow \mathbb{R}$ to define $\mathbf{m}$ and an additional *auxiliary scalar field* $\rho : \Omega \rightarrow \mathbb{R}$:

$$\mathbf{m}(\mathbf{u}) = \sum_{i=0}^N \mathbf{m}_i f_i(\mathbf{u}), \quad \rho(\mathbf{u}) = \sum_{i=0}^N \rho_i f_i(\mathbf{u}), \quad \mathbf{u} \in \Omega.$$

The main contribution of (Yushkevich et al., 2006b) was to show that the constraint (2) can be satisfied if R is derived from $\mathbf{m}$ and ρ as a solution of the following Poisson partial differential equation:

$$\Delta_{\mathbf{m}} R^2 = \rho \quad \text{subj. to} \quad \|\nabla_{\mathbf{m}} R^2\| = 2R \quad \text{on} \quad \partial\Omega, \quad (3)$$

where $\Delta_{\mathbf{m}}$ is the Laplace-Beltrami on the medial manifold. This PDE is well-behaved and can be solved efficiently in the course of deformable modeling. An example of PDE-based cm-rep model is shown in Fig. 2.

Similar to *m-reps*, *cm-rep* models are fitted to volumetric object representations using a deformable modeling algorithm that follows the Bayesian framework, with an image-driven likelihood term and prior terms that enforce inequality constraints related to inverse skeletonization, ensure correspondence between instances by minimizing distortion in the area element of $\mathbf{m}$, and incorporate a shape prior, when one is available. Since the deformable model is restricted to have a single-manifold skeleton, it matches real-world objects with some inherent level of error (Fig. 1e). In (Yushkevich et al., 2006b) we report high accuracy when fitting *cm-rep* models to hippocampi from an 87-subject schizophrenia study, with the average Dice overlap of 95.0% and the average boundary displacement error of 0.168 mm.

3.2 Hippocampal Coordinate System

Among surface-based shape representations, the *cm-rep* approach has a unique property: it allows the parametrization of the boundary to be extended to the interiors of objects. Since parametrization is a way of encoding correspondences between different instances of an anatomical structure, this unique property makes it possible to define correspondences between object interiors on the basis of boundary-based or medial-based correspondences. If the medial manifold is parametrized by $\mathbf{u} = (u_1, u_2) \in \Omega$, then every point $\mathbf{x}$ on the interior of the object can be assigned a triple of “coordinates” (u_1, u_2, ζ) , where $\zeta \in [-1, 1]$, such that $\mathbf{x}$ lies on the

line segment $\left[\mathbf{m}(\mathbf{u}), \mathbf{b}^{\bar{\zeta}} \right]$, where $\bar{\zeta}$ denotes the sign of ζ . These line segments, called *spokes*, are orthogonal to the object's boundary and they completely span the object's interior (Fig. 1f). The coordinate ξ describes the relative position of a point on its spoke, and thus, the depth of the point relative to the boundary: if $|\zeta| = 0$, the point is on the boundary; if $\zeta = 0$, the point is on the medial axis; otherwise the point is somewhere on the interior. The absolute distance from a point with coordinates $(\mathbf{u}, \zeta)$ to the boundary is $(1 - |\zeta|)R(\mathbf{u})$.

To establish a correspondence between a set of hippocampus shapes, we fit a set of *cm-rep* models to them. The fitting uses a shape prior learned in an earlier experiment (Yushkevich et al., 2006b), and a regularization prior is used to limit the distortion of area element on the surface $\mathbf{m}(\mathbf{u})$. As the result, a similar parametrization of the medial manifolds of the hippocampi is attained. Through the coordinate system, the parametrization is extended to the hippocampus interiors; points with the same $(\mathbf{u}, \zeta)$ coordinates in different hippocampi are said to be corresponding, and EPI data sampled at those points is combined for the purpose of between-subjects analysis. An example of a coordinate system fitted to the hippocampus is shown in Fig. 3.

3.3 FMRI Study

3.3.1 Subjects—The subjects in the fMRI study were healthy volunteers (ages 20-52, mean age 26, median 23) recruited from the broader University of Pennsylvania community. Overall, 30 subjects participated in the study (15 male, 15 female), but datasets from only 18 subjects (9 male, 9 female) were used for the validation experiment. Image data from the other subjects were rejected due to excessive head motion, imaging artifacts caused by a scanner upgrade, and subtle changes introduced to the study design in the early stages of the experiment. All subjects provided informed consent, following procedures approved by the institutional review board at the University of Pennsylvania.

3.3.2 Imaging Methods—All scans were acquired on a 3 Tesla Siemens Trio Scanner (Siemens, Germany) using an eight-channel head coil and body coil transmitter. The scanning protocol involved a localizer scan, followed by a high-resolution anatomical T1-weighted scan and three functional scans. The anatomical scan used the MP-RAGE sequence with the following parameters: TR = 1620 ms, TE = 3.87 ms, TI = 950 ms, flip angle = 15°, voxel size $0.977 \times 0.977 \times 1 \text{ mm}^3$. Functional scans included two BOLD fMRI scans acquired with two different voxel sizes, $2.5 \times 2.5 \times 2.5 \text{ mm}^3$ and $3 \times 3 \times 3 \text{ mm}^3$, as well as a perfusion scan that was acquired for use in a different set of experiments. Different fMRI voxel sizes were used to study the tradeoff between higher signal to noise ratio occurring at lower image resolution and decreased susceptibility artifacts and partial volume effects at higher resolution. This comparison was inspired by recent results by Szaflarski et al. (2004), who reported detecting significant activation with functional voxels as small as $2 \times 2 \times 3 \text{ mm}^3$. The order in which the two BOLD scans were acquired was alternated between subjects. The BOLD scans used the EPI sequence with FOV = $192 \times 192 \text{ mm}^2$, matrix = 64×64 , 40 axial slices, TR = 3 s, TE = 30 ms, BW = 2605 Hz/pixel, flip angle = 90°.

3.3.3 Scene Encoding Task—The experimental design from Rabin et al. (2004) was adopted for this study. Subjects were presented with complex visual scenes, which they were asked to memorize, and with baseline scenes, which they were asked to examine as they would a regular picture. Presentation followed the block design paradigm. At each EPI resolution, 172 scans were acquired. After the first 4 scans, presentation of complex visual scenes and control stimuli was alternated in blocks of 12, with this 24-scan sequence repeated 7 times ($172 = 4 + 7 \times (12 + 12)$). Each stimulus was presented for 2.6 s, with an 0.4 s interval between stimuli. Complex scenes were drawn from the Photodisc library, while the control stimuli were formed by breaking one of the photographs into small tiles and randomly scrambling the tiles.

Following each scan, subjects performed an offline recognition task, where they were presented with a subset of the scenes shown during the scan and a set of foils. The target detection rate was set at 50%. Subjects' responses were recorded on a fiberoptic response pad (FORP, Current Designs, Philadelphia, PA, USA). All subjects responded correctly at least 80% of the time.

3.4 Hippocampus Segmentation

The structure-specific approach requires the hippocampus to be segmented. In future work, we plan to fit *cm-rep* models directly to structural MRI images, but in the present study, we segmented the hippocampus manually using the ITK-SNAP tool (Yushkevich et al., 2006a). ITK-SNAP facilitates simultaneous tracing in three orthogonal image views and provides live 3D feedback, helping eliminate step edge artifacts often present in slice-by-slice segmentation. The right hippocampus was outlined using the Duvernoy (2005) atlas of the human hippocampus and previously described methods for segmenting temporal lobe regions (Chakos et al., 2005). The alveus served as the superior boundary for the hippocampus while the inferior boundary was the subiculum. Laterally, the appearance of the temporal horn of the lateral ventricles (THLV) initially defined the hippocampus, which also served as the anterior point

at which to begin segmentations of the hippocampus. Posteriorly, the hippocampus was limited by the coalescing of THLV with the lateral ventricles with the simultaneous appearance of the crux fornicis. An example segmentation is shown in Fig. 4.

3.5 Motion Correction and EPI-T₁ Co-Registration

Image alignment was performed using SPM2 software (Wellcome Department of Cognitive Neurology, <http://www.fil.ion.ucl.ac.uk/spm>). For each subject, the anatomical image and the functional time series were imported from DICOM format, along with the accompanying transformations into the patient coordinate system. Using rigid registration, the EPI time series were realigned spatially to correct for head motion and the mean realigned EPI image was computed. The mean EPI image was co-registered to the high-resolution anatomical image using rigid registration with the normalized mutual information metric (Ashburner and Friston, 1997). Registration between functional and structural data was deemed necessary because the likelihood of head motion between the fMRI scans was high, especially because the offline recognition task was inserted between consecutive scans.

3.6 Shape-Based Hippocampus Parametrization

Each hippocampus mask produced by the manual segmentation was fitted with a deformable *cm-rep* model. The fitting procedure is described in detail in (Yushkevich et al., 2006b). The fitted models establish a mapping from the interior of each hippocampus mask to a common coordinate space. A sampling grid defined in this coordinate space was mapped back into each subject's anatomical space and then further mapped into the space of each EPI image, according to the rigid transformation between each EPI image and the subject's anatomical image. This set of transformed grids was used to sample EPI data.

3.7 Smoothing of EPI Data

Smoothing is necessary because the signal at each EPI voxel is weak, and a low-pass filter can increase signal-to-noise ratio, albeit, at the cost of specificity. Precisely how much smoothing is needed is not always clear, and in our study we compare results obtained using different size kernels. Smoothing can confound the results of fMRI analysis, particularly when the structures we are interested in are small relative to the kernel size. Given strong activation in two regions neighboring a structure of interest, smoothing with an isotropic Gaussian kernel may lead us to falsely report a peak of activation in the structure itself. Thus we may even see activation in CSF and white matter regions, which is not expected, since task-related deoxyhemoglobin saturation takes place primarily in the gray matter.

In addition to smoothing with isotropic Gaussians with full width half maxima (FWHM) of 3 mm, 6mm and 9 mm, we use a non-isotropic smoothing scheme designed to minimize the contribution of extra-hippocampal signal to measurements made in the hippocampus. This smoothing scheme weights the contribution of each EPI voxel i by an estimate of the fraction of hippocampus-originating signal in that voxel. This estimate W_i is computed as the overlap between the region of space V_i occupied by the voxel i and the projection of the expert-traced hippocampus mask into the coordinate space of the EPI volume. This estimate is the simplest possible and it does not take into account the errors in T_1 -EPI co-registration and motion correction. Given the fuzzy mask image W whose voxels are the W_i 's, we compute the smoothed signal at a point x in the hippocampus as

$$F_{\sigma}(\mathbf{x}) = \frac{\int G_{\sigma}(\mathbf{x} - \mathbf{u}) W(\mathbf{u}) F(\mathbf{u}) d\mathbf{u}}{\int G_{\sigma}(\mathbf{x} - \mathbf{u}) W(\mathbf{u}) d\mathbf{u}}, \quad (4)$$

where F is the intensity of the EPI image and G_σ is a Gaussian kernel with covariance matrix $\sigma^2 I_{3 \times 3}$. This expression can be simplified as

$$F_\sigma(\mathbf{x}) = \frac{\sum_i W_i F_i \int_{V_i} G_\sigma(\mathbf{x} - \mathbf{u}) d\mathbf{u}}{\sum_i W_i \int_{V_i} G_\sigma(\mathbf{x} - \mathbf{u}) d\mathbf{u}}, \quad (5)$$

and the integral over each voxel region V_i is separable and easy to compute using the standard error function (*erf*). If the size of the bounding box containing all voxels i that overlap the hippocampus is $b_1 \times b_2 \times b_3$ voxels, then only $b_1 + b_2 + b_3 + 3$ calls to *erf* are needed to compute $F_\sigma(\mathbf{x})$.

Smoothing is effective in fMRI because the BOLD signal is spatially diffuse. However, there is no reason to believe that impulse response of the BOLD signal is spatially uniform; indeed we would expect greater task-related deoxygenation in gray matter regions than in the white matter and CSF. By masking out extra-hippocampal voxels, our smoothing scheme tries to follow such non-uniform pattern of diffusivity. It is important to note that our scheme does not increase specificity in the sense that it allows us to pinpoint a location where activation occurs any better than isotropic smoothing. However, it does increase the certainty that the activation we measure is associated with the hippocampus, and not the neighboring structures.

3.8 Statistical Analysis in Hippocampus Coordinates

Random effects (RFX) analysis was used to test for population-level positive correlation between task performance and EPI signal at a set of densely sampled points in the hippocampus coordinate frame. Following Penny et al. (2003), RFX analysis involves two sets of general linear models (GLMs). In each subject j , the time-varying signal $Y_{ij}(t)$ at the sample point $\mathbf{p}_i$ in the *cm-rep* coordinate system is modeled by the following *first-level* GLM:

$$Y_{ij}(t) = f(t) \beta_{ij} + \mu_{ij} + \epsilon_{ij}(t), \quad (6)$$

where $f(t)$ is the convolution of the box-car task reference function with a canonical model of hemodynamic response, β_{ij} and μ_{ij} are unknown parameters of the model, and ϵ_{ij} is a noise term. The parameters β_{ij} model the effect of the task, and μ_{ij} model the constant offset in the signal. Our model does not include confounds such as scanner drift and head motion because the purpose of this study is to compare two analysis methods, and we want the comparison to be made using as few parameters and assumptions as possible. The noise is modeled as non-spherical and the parameters are estimated using the *restricted maximum likelihood (ReML)* approach in SPM2 (Friston et al., 2002).

RFX analysis computes a population-level statistic at each sample point in the *cm-rep* coordinate space using the following second-level GLM:

$$B_i = \gamma_i + v_i, \quad (7)$$

where B_i is a random variable from which the ReML estimates of $\beta_{i1} \dots \beta_{iN}$ are thought to be sampled, γ_i is an unknown parameter, and v_i is a spherical noise term. Given the maximum

likelihood estimate $\hat{\gamma}_i$ and an estimate of the standard error $\hat{\sigma}_i^2$, the null hypothesis $H_0: E(B_i) = 0$ can be rejected at the level α if $p(t_{N-1}) > \alpha$, where $t_{N-1} = \hat{\gamma}_i / \hat{\sigma}_i$ follows Student's t distribution with $N - 1$ degrees of freedom.

The p -values derived from these RFX t -statistics are corrected for multiple comparisons using the *False Discovery Rate (FDR)* method (Yekutieli and Benjamini, 1999; Benjamini and Yekutieli, 2001; Genovese et al., 2002). For a given set of p -values $p_1 \dots p_k$ and level α , the

FDR method computes a threshold $q = FDR(p_1 \dots p_k, \alpha)$ that guarantees that the expected number of false positives among all tests exceeding the threshold q is α . FDR-based *adjustment* (Yekutieli and Benjamini, 1999) maps each p_j to an adjusted p -value $\bar{p}_j$, where $\bar{p}_j$ is the smallest level such that $p_j \leq FDR(p_1 \dots p_k, \bar{p}_j)$. FDR adjustment is based on the shape of the curve formed by the ‘raw’ p -values sorted in ascending order. A small ‘raw’ p -value will be mapped to a small adjusted p -value if there are many other p -values of the same magnitude, while a singleton small raw p -value will be mapped to a large adjusted p -value. FDR adjustment is less expensive to compute than correction based on permutation testing, especially for very small p -values, which require a very large number of permutations to estimate accurately.

There are various ways to visualize statistical maps computed by RFX analysis. One option is to project the statistics computed in the *cm-rep* coordinate frame onto the boundary or the medial surface of the mean hippocampus model. In figures that follow, at each point on the mean model's boundary we display the most significant statistic found along the normal vector projecting inward from the point to the medial surface (we refer to this as ‘maximum intensity projection’). To present the RFX map in anatomical context, we can project in into the space of one of the subjects.

Having access to a hippocampus shape model gives us additional tools for analyzing functional data. For instance, the *cm-rep* coordinate system can be extended beyond the hippocampus boundary, making it possible to compare activation inside the hippocampus to activation outside at each boundary location. We may also include shape features, such as thickness, as factors in RFX analysis.

3.9 Whole-Brain Analysis

As a basis for comparison, we computed RFX activation maps over the whole brain following the standard SPM2 pipeline (Friston et al., 1994). To ensure a fair comparison, all steps and parameters, except for smoothing and inter-subject normalization, were identical for whole-brain and hippocampus-specific analysis. After motion correction and EPI-anatomy co-registration, EPI data were smoothed with isotropic Gaussian kernels (FWHM = 3 mm, 6mm and 9 mm). The subject-level GLM in (6) was used to estimate parameters β_{ij} at every voxel i in each subject j . Subjects' anatomical images were normalized to the Montreal Neurological Institute (MNI) template (Evans et al., 1993) using the nonrigid registration function in SPM2, which first estimates a 12-parameter affine transformation, followed by a non-linear registration based on the discrete cosine basis (Ashburner and Friston, 1999). Next, warps generated by the normalization were used to project β -maps into the space of the template. To ensure a roughly equal number of tests in the hippocampus region between the two analysis approaches, we set the sampling in template space to be $0.5 \times 0.5 \times 0.5\text{mm}^3$. As the last step, a second-level GLM (7) was used to generate a RFX statistical map in template space.

4 Results

4.1 Hippocampus-Specific Analysis

Table 1 lists the most significant raw and adjusted p -values detected in the hippocampus with the structure-specific RFX analysis approach. There are 12 pairs of p -values, corresponding to different datasets ($2.5 \times 2.5 \times 2.5\text{mm}^3$ vs. $3 \times 3 \times 3\text{mm}^3$ voxel EPI), smoothing approaches (uniform vs. non-uniform) and the FWHM of the smoothing kernel (3mm, 6mm and 9 mm). In all cases, the most significant adjusted p -values are below 0.002. Significance increases with the size of the smoothing kernel, representing a tradeoff between sensitivity and specificity. There is no evidence to suggest a change in sensitivity due to non-uniform smoothing, even though a smaller number of EPI voxels contributes to each measurement under this scheme.

If we think of non-uniform smoothing as a way of increasing specificity, it is encouraging to find that it does not necessarily lower sensitivity.

Figs. 5 and 6 show the maximal intensity projection of the 12 RFX maps onto the boundary of the mean hippocampus shape. We observe that activation patterns are fairly consistent across the scale of Gaussian smoothing, with regions of high significance $p < 0.001$ becoming larger and more diffuse as the scale increases. The effect of non-uniform smoothing is apparent: under isotropic smoothing, smaller p -values are observed on the inferior of the tail of the hippocampus, as well as on the superior side of the structure. This difference is likely due to activation in nearby structures having an effect on hippocampal measurements. This finding is confirmed by the inside-outside analysis described later in this section. There are differences in activation patterns between 2.5mm and 3mm data. For instance, the strong peak at coordinates $(-7, -24)$ in the map “2.5mm EPI, 3mm FWHM non-uniform” in Fig. 5 does not have an equivalent in the RFX maps for 3mm EPI. Likewise, there is activation in the superior side of the tail of the hippocampus at 2.5mm (coordinates $(-14, -17)$ in Fig. 6) that is not seen in 3mm data. A possible explanation for this may be that the tail of the hippocampus is narrow, so partial volume effects at 3mm EPI are more significant than at 2.5 mm. The superior side of the hippocampal head in the region neighboring the lateral horn of the fourth ventricle (coordinates $(5, -22)$ in Fig. 6, also see Fig. 7) is not activated in both datasets. However, a direct comparison of activation maps between resolutions by means of a paired t -test does not find significant differences after FDR-based adjustment, as shown in Table 2.

4.2 Inside-Outside Comparison

In addition to looking at activation throughout the hippocampus region, the *cm-rep* coordinate system allows us to examine activation just outside of the hippocampus and to compare extra-hippocampal and intra-hippocampal activation. To sample EPI images outside of the hippocampus, we extend the ‘depth’ coordinate ξ beyond its standard range $[-1, 1]$. Extra-hippocampal samples are associated with various anatomical structures, and Fig. 7 shows a projection of the adjacent structure labels on the boundary of the ‘mean’ hippocampus. These labels were computed by fitting the *cm-rep* coordinate system to the right hippocampus ROI in the *Automated Anatomical Labeling (AAL)* atlas (Tzourio-Mazoyer et al., 2002). To compare extra- and intra-hippocampal activation, we use the following general linear model:

$$Y(\mathbf{u}, \xi, m) = \mu(\mathbf{u}, \xi, m) + \begin{cases} \beta_{\text{intra}}(\mathbf{u}, \xi) & \text{if } |\xi| < 1 \\ \beta_{\text{extra}}(\mathbf{u}, \xi) & \text{if } |\xi| > 1 \end{cases} + \epsilon,$$

where $\mathbf{u}$ and ξ are the *cm-rep* coordinates and $Y(\mathbf{u}, \xi, m)$ is a random variable describing first-level activation for subject m at $(\mathbf{u}, \xi)$. The parameters of the model are defined at each point $(\mathbf{u}, \xi)$ on the hippocampus boundary (recall that the *cm-rep* boundary consists of two halves

indexed by $\xi = \pm 1$; here ξ denotes the sign of ξ). The model includes mean within-subject

activation $\mu(\mathbf{u}, \xi, m)$ as a nuisance parameter, while the parameters of interest are $\beta_{\text{intra}}(\mathbf{u}, \xi)$ and $\beta_{\text{extra}}(\mathbf{u}, \xi)$. The t -contrast of interest is $\beta_{\text{intra}}(\mathbf{u}, \xi) - \beta_{\text{extra}}(\mathbf{u}, \xi)$. The null hypothesis associated with this contrast is that the mean activation sampled inwards from a

given point on the hippocampus boundary is equal to the mean activation sampled in the outward direction, when the differences in overall activation between subjects (μ) are taken into account.

A map of the standard z -scores associated with this contrast are plotted over the hippocampus boundary in Fig. 8. The patterns for 2.5mm and 3mm EPI data are remarkably similar. Over the region bordering the parahippocampal gyrus, we observe greater intra-hippocampal activation. Greater intra-hippocampal activation is also seen at regions bordering the CSF structures, which is to be expected. In the region adjacent to the amygdala, stronger extra-hippocampal activation is seen. Stronger extra-hippocampal activation is also seen in the regions adjacent to white matter near the thalamus. It must be emphasized that the anatomical labeling is preliminary, and a much more accurate and detailed labeling of the anatomy inside and outside of the hippocampus is required to make specific inferences about differences in activation across structures and subfields.

4.3 Comparison with Whole-Brain Analysis

In order to fairly compare RFX maps produced by whole-brain normalization to our method's RFX maps, we need to define the right hippocampus in the space of the MNI template. One option would be to use an existing hippocampus mask, e.g. from the Talairach atlas (Talairach and Tournoux, 1988) or the AAL atlas (Tzourio-Mazoyer et al., 2002). However, since the segmentation of the right hippocampus in each subject is available to us, we use the projections of these segmentations into the template space to define a probabilistic hippocampus map. For each subject, we apply the warp computed by normalization to the right hippocampus ROI and, at each voxel x in template space, we measure the fraction of warped masks covering x , giving a score between 0 and 1. The resulting “fuzzy hippocampus” map is denoted $H(x)$, and it is shown in Fig. 9. The fuzzy hippocampus map contains values between 0 and 1 because different subjects' warped hippocampus masks do not overlap exactly. In fact, the average overlap, expressed in terms of Dice similarity coefficient (Dice, 1945), is 0.53, which is consistent with other findings for low-dimensional parametric normalization methods (Carmichael et al., 2005).

Given the fuzzy hippocampus map H , we can restrict analysis to regions in template space defined as $\mathcal{R}_\eta = \{x : H(x) \geq \eta\}$, where $\eta \in (0, 1]$. These regions are decreasing in size: regions $\mathcal{R}_{0.25}$, $\mathcal{R}_{0.5}$ and $\mathcal{R}_{0.75}$ have volumes 4.6 ml, 2.6 ml and 1.1 ml, respectively. By contrast, the average volume of all subjects' hippocampus masks after projection to template space is 3.1 ml. Fig. 10 plots the most significant p -value (raw, as well as adjusted) observed in the regions $\mathcal{R}_\eta$ for different resolutions of EPI data and different smoothing parameters. The p -values increase as η increases, and do so especially quickly at low levels of smoothing. Overall, the significance is higher at higher smoothing levels.

The most significant p -values found in the hippocampus with the *cm-rep* method are also shown in Fig. 10. Notably, at 3mm FWHM smoothing, both adjusted and unadjusted p -values are lower for the *cm-rep* method for all values of η . This suggests that when smoothing levels are low, shape-based normalization has an impact on the significance of group analysis. At greater smoothing levels, where individual contrast images are more flat, the impact of normalization algorithm on sensitivity is less pronounced. Thus the apparent advantage of the *cm-rep* method is the ability to improve specificity by reducing smoothing levels without paying as high of a price in sensitivity as with the whole-brain method.

In addition to looking at the highest peaks in RFX maps, we plot the cumulative distribution functions (CDF) of these maps in Fig. 11. At 3mm FWHM smoothing, the curves for the *cm-rep* method are shifted to the right with respect to the curves for the whole-brain method,

reflecting a more sensitive statistical map. At higher smoothing levels, the curve for the *cm-rep* method with uniform smoothing tends to fall to the right of the whole-brain curves, except at 6mm FWHM in 3mm EPI data, where the curves are very close to each other. The curve for the mask-based smoothing method tends to fall behind the uniform smoothing curve at higher smoothing levels, and especially in 3mm EPI data, although the two curves tend to catch up with each other for high values of t .

The overall conclusion from the comparison of the two analysis methods is that at low levels of smoothing, structure-based normalization leads to more sensitive random effects maps, both in terms of maximal t -values and in terms of overall distribution of t -values. At higher smoothing levels, some level of improvement in sensitivity can be reported for the *cm-rep* method with uniform smoothing, but not for mask-based non-uniform smoothing. This suggests lesser impact of normalization at higher smoothing levels, as well as impact of extra-hippocampal activation on the sensitivity of intra-hippocampal maps.

5 Discussion

Our study was intended to show that hippocampus-specific group activation analysis can yield greater statistical power than whole-brain analysis in an experimental setting where hippocampal activation is likely to occur. This hypothesis was confirmed at low levels of spatial smoothing, as the activation maps yielded by the hippocampus-specific method contained higher peaks than the peaks located near the right hippocampus in whole-brain group activation maps. We suspect that much of the difference in the activation maps produced by the two methods can be attributed to the misalignment of the hippocampal gray matter by the whole-brain normalization method, as evidenced by relatively low pairwise overlap between warped hippocampus segmentations. Previous studies (Miller et al., 2005) have also found that improved hippocampal alignment leads to greater statistical power, but the effect of smoothing was not examined explicitly.

While the experiment was primarily designed to explore the benefits of the *cm-rep* approach to fMRI analysis, its findings have broader implications. An important question in the functional neuroimaging field is how much different sources of error affect the outcome of an fMRI study. Normalization, it may be argued, is not a significant source of error because the anatomical data to which it is applied has much higher resolution than the functional data and because functional images are smoothed spatially using large Gaussian kernels. The results of our experiment point to the fact that improved normalization makes it possible to decrease the amount of smoothing that is necessary to reach significance, and thus increases the specificity of analysis.

We do not presume that the *cm-rep* method is the only approach capable of reducing normalization error in fMRI group studies. Indeed, non-parametric registration techniques, especially when driven by expert-placed landmarks (e.g., (Haller et al., 1997; Miller et al., 2005)), tend to register subcortical structures more accurately than parametric registration driven only by image forces. Both non-parametric registration and *cm-rep* normalization produce correspondences that map points in the hippocampus of one subject to points in the hippocampi of other subjects. We expect that group activation statistics obtained in this way would be comparable to the ones reported in this paper. However, the *cm-rep* approach offers several unique properties that make it particularly well-suited for co-analyzing functional activation and structural atrophy. For example, the *cm-rep* method makes it easy to project volumetric group activation maps onto the surfaces of anatomical structures. It also makes it straightforward to associate voxel-wise features, such as functional activation, with shape features, such as the distance to the boundary and thickness. Thus, for instance, gray matter thickness can be used as a covariate in functional activation analysis, since functional voxels

located in regions where gray matter is thicker are less likely to suffer from partial volume effects. This may be of particular interest when comparing hippocampal activation between clinical populations with different rates of hippocampal atrophy.

Furthermore, the shape-based normalization approach does not require a well-defined one-to-one mapping between the subjects' brains, which is an advantage in cases of neuropathology or in special populations, such as neonates, where T_1 and T_2 relaxation times can vary among subjects. The shape-based method treats the expert's segmentation as the 'ground truth' definition of the hippocampus. This can be an advantage in cases where image information alone is not sufficient to match hippocampi between subjects. However, this can also be a disadvantage when segmentations are of insufficient quality.

In this paper, the *cm-rep* coordinate system was treated as a way to map activation values into a reference space. In the future, we plan to fit this shape-based coordinate system to histology and post-mortem imaging data in which the hippocampus, its neighboring structures and some of its subfields, such as the dentate gyrus and the cornu ammonis fields, have been labeled by experts. By projecting these labels back into the reference space, we would generate a probabilistic atlas of the hippocampus, which could then be used to associate peaks in hippocampal group activation maps with hippocampal subfields, similar in spirit to the work of Zeineh et al. (2003), but requiring less manual processing. We are also working to extend the *cm-rep* approach to fit deformable templates directly to anatomical image data, rather than to manual segmentations. Such an approach would leverage the method's ability of to incorporate shape and appearance priors into the Bayesian objective function that drives the fitting. The ability to automatically detect the hippocampus and map its interior into a reference frame associated with a probabilistic atlas would yield a powerful tool for hippocampal fMRI analysis.

Finally, we note that the proposed shape-based regional normalization approach is of broader interest than the application described above, since is not inherently limited to the hippocampus. We anticipate that *cm-rep* models will be able to adequately represent the parahippocampal gyrus and other structures involved in memory; indeed, discrete m-reps have been used to model a number of different brain structures besides the hippocampus (Styner et al., 2003a). Nor is our approach specific to fMRI. Regional normalization is of interest in studies that use any type of multi-modality imaging, e.g., white matter studies involving diffusion imaging, or studies of neurometabolism using FDG-PET.

6 Conclusions

We have presented an approach for using shape-based correspondences as a framework for anatomical normalization of the hippocampus in multi-subject functional neuroimaging studies. The approach makes it possible to generate random effects statistical maps over the hippocampus interior and to compare activation inside the hippocampus to extra-hippocampal activation. We compared the statistical significance of the random effects maps generated with our approach to maps computed using whole-brain normalization, in the context of a visual scene encoding experiment where hippocampal activation was expected. Our results suggest that at lower levels of smoothing, the shape-based approach is more sensitive than whole-brain normalization used in SPM2. Furthermore, our method makes it possible to define a special non-uniform smoothing scheme for EPI data, which limits the amount of extra-hippocampal signal contributing to measurements made inside of the hippocampus.

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References

- Ashburner J, Friston K. Multimodal image coregistration and partitioning—a unified framework. *Neuroimage* 1997;6(3):209–17. [PubMed: 9344825]
- Ashburner J, Friston K. Nonlinear spatial normalization using basis functions. *Human Brain Mapping* 1999;7(4):254–266. [PubMed: 10408769]
- Ashburner J, Friston KJ. Voxel-based morphometry—the methods. *Neuroimage* 2000;11(6 Pt 1):805–21. [PubMed: 10860804]
- Avants B, Schoenemann P, Gee J. Lagrangian frame diffeomorphic image registration: Morphometric comparison of human and chimpanzee cortex. *Medical Image Analysis*. 2005In Press
- Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. *The Annals of Statistics* 2001;29(4):1165–1188.
- Blum, H. Models for the Perception of Speech and Visual Form. MIT Press; 1967. A transformation for extracting new descriptors of shape.
- Bouix S, Pruessner JC, Collins DL, Siddiqi K. Hippocampal shape analysis using medial surfaces. *Neuroimage* 2005;25(4):1077–89. [PubMed: 15850726]
- Carmichael OT, Aizenstein HA, Davis SW, Becker JT, Thompson PM, Meltzer CC, Liu Y. Atlas-based hippocampus segmentation in Alzheimer's disease and mild cognitive impairment. *Neuroimage* 2005;27(4):979–90. [PubMed: 15990339]
- Chakos MH, Schobel SA, Gu H, Gerig G, Bradford D, Charles C, Lieberman JA. Duration of illness and treatment effects on hippocampal volume in male patients with schizophrenia. *Br J Psychiatry* 2005;186:26–31. [PubMed: 15630120]
- Christensen G, Joshi S, Miller M. Volumetric transformation of brain anatomy. *IEEE Transactions on Medical Imaging* 1997;16:864–877. [PubMed: 9533586]
- Chung MK, Worsley KJ, Paus T, Cherif C, Collins DL, Giedd JN, Rapoport JL, Evans AC. A unified statistical approach to deformation-based morphometry. *Neuroimage* 2001;14(3):595–606. [PubMed: 11506533]
- Cootes, T.; Edwards, G.; Taylor, C. European Conference on Computer Vision. 2. Freiburg, Germany: 1998. Active appearance models; p. 484-498.
- Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis I: segmentation and surface reconstruction. *NeuroImage* 1999;9(2):179–194. [PubMed: 9931268]
- Damon J. Determining the geometry of boundaries of objects from medial data. *International Journal of Computer Vision* 2005;63(1):45–64.
- Davatzikos C. Spatial transformation and registration of brain images using elastically deformable models. *Comp. Vis. and Image Understanding, Special Issue on Medical Imaging* 1997;66(2):207–222.
- Davatzikos C, Genc A, Xu D, Resnick SM. Voxel-based morphometry using the ravens maps: methods and validation using simulated longitudinal atrophy. *Neuroimage* 2001;14(6):1361–9. [PubMed: 11707092]
- Davies, R.; Twining, C.; Cootes, T.; Waterton, JC.; Taylor, C. 3D statistical shape models using direct optimisation of description length. 7th European Conference on Computer Vision (ECCV); 2002. p. 3-21.
- Dice LR. Measures of the amount of ecologic association between species. *Ecology* 1945;26(3):297–302.
- Duvernoy, HM. The human hippocampus, functional anatomy, vascularization and serial sections with MRI. third edition. Springer; 2005. edition
- Evans, A.; Collins, D.; Mills, S.; Brown, E.; Kelly, R.; Peters, T. 3d statistical neuroanatomical models from 305 mri volumes. IEEE Nuclear Science Symposium and Medical Imaging Conference; 1993. p. 1813-1817.
- Fischl B, Sereno MI, Dale AM. Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system. *Neuroimage* 1999;9(2):195–207. [PubMed: 9931269]
- Fletcher PT, Lu C, Pizer SM, Joshi S. Principal geodesic analysis for the study of nonlinear statistics of shape. *IEEE Trans Med Imaging* 2004;23(8):995–1005. [PubMed: 15338733]

- Friston KJ, Glaser DE, Henson RNA, Kiebel S, Phillips C, Ashburner J. Classical and Bayesian inference in neuroimaging: applications. *Neuroimage* 2002;16(2):484–512. [PubMed: 12030833]
- Friston KJ, Holmes AP, Worsley KJ, Poline J-P, Frith CD, Frackowiak RSJ. Statistical parametric maps in functional imaging: A general linear approach. *Human Brain Mapping* 1994;2(4):189–210.
- Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage* 2002;15(4):870–878. [PubMed: 11906227]
- Giblin P, Kimia BB. A formal classification of 3D medial axis points and their local geometry. *IEEE Trans Pattern Anal Mach Intell* 2004;26(2):238–51. [PubMed: 15376898]
- Goebel, R. Forschung und wissenschaftliches Rechnen. Heinz-Billing-Preis; 1997. chapter Brain Voyager: Ein Programm zur Analyse und Visualisierung von Magnetresonanztomo-graphiedaten.
- Haller J, Banerjee A, Christensen G, Gado M, Joshi S, Miller M, Sheline Y, Vannier M, Csernansky J. Three-dimensional hippocampal MR morphometry by high-dimensional transformation of a neuroanatomic atlas. *Radiology* 1997;202:504–510. [PubMed: 9015081]
- Joshi S, Miller MI. Landmark matching via large deformation diffeomorphisms. *IEEE Transactions on Image Processing* 2000;9(8):1357–1370. [PubMed: 18262973]
- Miller MI, Beg MF, Ceritoglu C, Stark C. Increasing the power of functional maps of the medial temporal lobe by using large deformation diffeomorphic metric mapping. *Proc Natl Acad Sci U S A* 2005;102(27):9685–9690. [PubMed: 15980148]
- Ogniewicz RL, Kübler O. Hierarchic Voronoi skeletons. *Pattern Recognition* 1995;28(3):343–359.
- Penny, W.; Holmes, A.; Friston, K. Random effects analysis. In: Frackowiak, R.; Friston, K.; Frith, C.; Dolan, R.; Friston, K.; Price, C.; Zeki, S.; Ashburner, J.; Penny, W., editors. *Human Brain Function*. 2nd edition. Academic Press; 2003.
- Pizer SM, Fletcher PT, Joshi S, Thall A, Chen JZ, Fridman Y, Fritsch DS, Gash AG, Glotzer JM, Jiroutek MR, Lu C, Muller KE, Tracton G, Yushkevich P, Chaney EL. Deformable m-reps for 3D medical image segmentation. *International Journal of Computer Vision* 2003;55(2):85–106.
- Rabin ML, Narayan VM, Kimberg DY, Casasanto DJ, Glosser G, Tracy JI, French JA, Sperling MR, Detre JA. Functional MRI predicts post-surgical memory following temporal lobectomy. *Brain* 2004;127(Pt 10):2286–2298. [PubMed: 15329352]
- Rohlfing, T. Transformation model and constraints cause bias in statistics on deformation fields. In: Larsen, R.; Nielsen, M.; Sparring, J., editors. *Proceedings, volume 4190 of Lecture Notes in Computer Science; Medical Image Computing and Computer-Assisted Intervention — MICCAI 2006: 9th International Conference; Copenhagen, Denmark. October 1–5, 2006; Berlin/Heidelberg: Springer-Verlag; 2006. p. 207–214.*
- Rueckert D, Frangi AF, Schnabel JA. Automatic construction of 3-D statistical deformation models of the brain using nonrigid registration. *IEEE Trans Med Imaging* 2003;22(8):1014–25. [PubMed: 12906255]
- Shen D, Moffat S, Resnick SM, Davatzikos C. Measuring size and shape of the hippocampus in MR images using a deformable shape model. *Neuroimage* 2002;15(2):422–34. [PubMed: 11798276]
- Shenton ME, Gerig G, McCarley RW, Szekely G, Kikinis R. Amygdala-hippocampal shape differences in schizophrenia: the application of 3D shape models to volumetric MR data. *Psychiatry Res* 2002;115(12):15–35. [PubMed: 12165365]
- Siddiqi K, Ahokoufandeh A, Dickinson S, Zucker S. Shock graphs and shape matching. *International Journal of Computer Vision* 1999a;1(35):13–32.
- Siddiqi, K.; Bouix, S.; Tannenbaum, A.; Zucker, SW. *Proc. Computer Vision. 2. IEEE; 1999b. The hamilton-jacobi skeleton; p. 828–834.*
- Stark CEL, Okado Y. Making memories without trying: medial temporal lobe activity associated with incidental memory formation during recognition. *J Neurosci* 2003;23(17):6748–6753. [PubMed: 12890767]
- Studholme C, Hill DL, Hawkes DJ. Automated three-dimensional registration of magnetic resonance and positron emission tomography brain images by multiresolution optimization of voxel similarity measures. *Med Phys* 1997;24(1):25–35. [PubMed: 9029539]
- Styner M, Gerig G, Lieberman J, Jones D, Weinberger D. Statistical shape analysis of neuroanatomical structures based on medial models. *Med Image Anal* 2003a;7(3):207–20. [PubMed: 12946464]

- Styner M, Lieberman JA, Pantazis D, Gerig G. Boundary and medial shape analysis of the hippocampus in schizophrenia. *Med Image Anal* 2004;8(3):197–203. [PubMed: 15450215]
- Styner, M.; Rajamani, K.; Nolte, L.; Zsemlye, G.; Székely, G.; Taylor, C.; Davies, RH. Evaluation of 3D correspondence methods for model building; International Conference on Information Processing in Medical Imaging; 2003b.
- Szaflarski J, Holland S, Schmithorst V, Dunn R, Privitera M. High resolution functional MRI at 3T in healthy and epilepsy subjects: hippocampal activation with picture encoding task. *Epilepsy and Behavior* 2004;5:244–252. [PubMed: 15123027]
- Talairach, J.; Tournoux, P. Co-planar stereotaxic atlas of the human brain. Verlag; Stuttgart: 1988.
- Thirion, J-P. Non-rigid matching using demons. CVPR '96: Proceedings of the 1996 Conference on Computer Vision and Pattern Recognition (CVPR '96); IEEE Computer Society; 1996. p. 245 ISBN 0-8186-7258-7
- Thompson P, Hayashi K, de Zubicaray G, Janke A, Rose S, Semple J, Hong M, Herman D, Gravano D, Dittmer S, D.M. D, Toga A. Improved detection and mapping of dynamic hippocampal and ventricular change in Alzheimers disease using 4D parametric mesh skeletonization. 9th Annual Meeting of the Organization for Human Brain Mapping. 2003
- Tosun D, Rettmann ME, Naiman DQ, Resnick SM, Kraut MA, Prince JL. Cortical reconstruction using implicit surface evolution: accuracy and precision analysis. *Neuroimage* 2006;29(3):838–852. [PubMed: 16269250]
- Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 2002;15(1):273–289. [PubMed: 11771995]
- Yekutieli D, Benjamini Y. Resampling-based false discovery rate controlling multiple test procedures for correlated test statistics. *J of Statistical Planning and Inference* 1999;82:171–196.
- Yushkevich PA, Piven J, Hazlett HC, Smith RG, Ho S, Gee JC, Gerig G. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage* 2006a;31(3):1116–1128. [PubMed: 16545965]
- Yushkevich PA, Zhang H, Gee J. Continuous medial representation for anatomical structures. *IEEE Trans Med Imaging* 2006b;25(2):1547–1564. [PubMed: 17167991]
- Zeineh MM, Engel SA, Thompson PM, Bookheimer SY. Dynamics of the hippocampus during encoding and retrieval of face-name pairs. *Science* 2003;299(5606):577–80. [PubMed: 12543980]

Fig. 1.

An illustration of medial geometry and the cm-rep method with 2D examples. **a.** The medial axis (skeleton) of a 2D object is a curve or a set of curves. The boundary is symmetric across the medial axis. **b.** The skeleton of a 3D object is like a scaffold formed by surface patches called medial manifolds. **c.** The cm-rep method describes objects in terms of medial geometry. First, a synthetic skeleton is defined, shown here as a curve specified by a set of control points, with each control point assigned a radius value. **d.** The interpolation of radius values along the curve leads to a continuum of circles and a definition of a solid object. The boundary of this object can be expressed analytically if the synthetic skeleton satisfies certain constraints (see text). **e.** A cm-rep model can be deformed to optimally fit a given object. The number of medial axis branches in the cm-rep model is held fixed, so the model might not fit the target object perfectly, as shown here. However, for some applications and some classes of anatomical structures, the approximation is accurate enough to be useful. **f.** The interior of a cm-rep model can be parametrized using a curvilinear coordinate system whose one axis follows the synthetic skeleton and whose other axis extends from the skeleton to the boundary and is orthogonal to the boundary. This coordinate system is used to assign correspondences between objects.

Fig. 2. Three steps of constructing a *cm-rep*. **a.** Medial manifold $\mathbf{m}(\mathbf{u})$ with a color plot of the scalar field $\rho(\mathbf{u})$. **b.** The radial scalar field $R(\mathbf{u})$ computed by solving the Poisson PDE (3) on the medial manifold. **c.** Boundary surface $\mathbf{b}^{\pm}(\mathbf{u})$ resulting from inverse skeletonization.

Fig. 3. The shape-based coordinate system induced by a *cm-rep*. **a.** A slice through the hippocampus in a T1 weighted MRI, to which a *cm-rep* template has been fitted. **b, c.** The values of the coordinates u^1 , u^2 , which span the medial manifold, plotted at each point in the hippocampus using a color map. **d.** The values of the ξ coordinate, which goes from the medial manifold to the boundary.

Fig. 4. Hippocampus segmentation result for a single subject, shown in sagittal, coronal, axial and 3D views. All four views are available to the rater during segmentation.

Fig. 5.

Random effects maps computed in the hippocampus using the structure-specific approach. The columns in this table correspond to different smoothing levels, and the rows correspond to different voxel sizes in EPI data as well as different ways of smoothing the EPI data in the hippocampus (mask-based non-uniform smoothing vs. isotropic Gaussian smoothing). The color map corresponds to the standard z-statistic.

Fig. 6.
Superior view of the 12 maps plotted in Fig. 5.

Fig. 7. Projection of the anatomical labels of adjacent anatomical structures on the hippocampus boundary. Projections are based on a single-subject anatomical atlas (Tzourio-Mazoyer et al., 2002) and are intended to give a rough idea of the structures' locations relative to the hippocampus.

Fig. 8. Statistical group-level comparison of intra-hippocampal vs. extra-hippocampal activation. Positive values in the z-map correspond to greater intra-hippocampal activation.

Fig. 9.

Top: sagittal and coronal slices through the right hippocampus in the mean anatomical image generated using whole-brain normalization to the MNI template. Bottom: probability map formed by projecting hippocampus segmentations into template space.

Fig. 10.

Comparison of minimal FDR-adjusted p -values between the structure-specific method and whole-brain SPM2 analysis. The minimal p -values for the structure-specific method with isotropic Gaussian smoothing are shown as dashed horizontal lines, and the minimal p -values for mask-based anisotropic smoothing are shown as dotted horizontal lines. Since in the whole-brain method, there is not exact definition of the hippocampus in the space of the anatomical template, we compute and plot minimal p -values over a sequence of regions associated with increasing probability of “being the hippocampus” (see text for precise definition). The x -axis in the plots above corresponds to this hippocampus probability, and the y -axis plots the p -value on a logarithmic scale.

Fig. 11.

Empirical cumulative distribution functions (CDFs) of t -statistic maps computed for the right hippocampus using hippocampus-specific analysis (HSA) and whole-brain analysis (WBA). The dashed line represents HSA with non-uniform mask-based smoothing, and the dotted line corresponds to HSA with uniform Gaussian smoothing. Solid lines represent regions $R_{1/4}$, $R_{1/2}$ and $R_{3/4}$ defined in the MNI template space, corresponding to increasing probability of “being the hippocampus.”

Minimal FDR-adjusted and raw p -values computed in the hippocampus coordinate system under different EPI resolutions and smoothing schemes. All p -values listed here are associated with positive t -statistics; in fact there were no negative t -values in the hippocampus coordinate system in either of the 12 experiments.

Table 1

FWHM:	3mm	$3.0 \times 3.0 \times 3.0 \text{ mm}^3$ EPI data			
		Non-Uniform Smoothing 6mm	9mm	3mm	Uniform Smoothing 6mm 9mm
p-raw:	$1.34 \cdot 10^{-7}$	$2.37 \cdot 10^{-8}$	$2.56 \cdot 10^{-7}$	$5.44 \cdot 10^{-8}$	$9.89 \cdot 10^{-8}$
p-adj:	$1.26 \cdot 10^{-3}$	$1.65 \cdot 10^{-4}$	$1.53 \cdot 10^{-4}$	$6.40 \cdot 10^{-4}$	$7.64 \cdot 10^{-4}$
FWHM:	3mm	$2.5 \times 2.5 \times 2.5 \text{ mm}^3$ EPI data			
		Non-Uniform Smoothing 6mm	9mm	3mm	Uniform Smoothing 6mm 9mm
p-raw:	$2.40 \cdot 10^{-8}$	$1.80 \cdot 10^{-6}$	$1.23 \cdot 10^{-5}$	$2.31 \cdot 10^{-7}$	$7.28 \cdot 10^{-6}$
p-adj:	$2.79 \cdot 10^{-5}$	$2.82 \cdot 10^{-4}$	$7.59 \cdot 10^{-4}$	$4.18 \cdot 10^{-4}$	$4.77 \cdot 10^{-4}$
					$1.34 \cdot 10^{-5}$ $3.25 \cdot 10^{-4}$

Statistical comparison of activation between $2.5 \times 2.5 \times 2.5$ mm³ and $3 \times 3 \times 3$ mm³ voxel EPI data. A paired *t*-test was performed at each point in the hippocampus coordinate system, and the minimum *p*-value over the hippocampus is reported below, with different results for different levels of spatial smoothing. Although the raw minimal *p*-values in most cases are below 0.05, there are very few such small *p*-values over the hippocampus volume, and after correction for multiple comparisons with the FDR method (Yekutieli and Benjamini, 1999), no significant differences are found.

Table 2

	3mm	Non-Uniform Smoothing		Uniform Smoothing	
		3mm	9mm	6mm	9mm
p-raw:	0.0001		0.0273	0.0328	0.0586
p-adj:	0.7392		0.5288	0.7500	0.7577