Published in final edited form as:

Neuroimage. 2001 November ; 14(5): 1122–1127. doi:10.1006/nimg.2001.0926.

Functional Inferences Vary with the Method of Analysis in fMRI

Mary M. Machulda[†], Heidi A. Ward[‡], Ruth Cha[§], Peter O'Brien[§], and Clifford R. Jack Jr.[‡]

[‡] Department of Radiology, Magnetic Resonance Research, Mayo Clinic, Rochester, MN

[†] Department of Psychiatry and Psychology, Mayo Clinic, Rochester, MN

[§] Department of Biostatistics, Mayo Clinic, Rochester, MN

Abstract

Neuroanatomic substrates of specific cognitive functions have been inferred from anatomic distributions of activated pixels during fMRI studies. With declarative memory tasks, interest has focused on the extent to which various medial temporal lobe anatomic structures are activated while subjects encode new information. The aim of this project was to examine how commonly used variations in fMRI data processing methods affect the distribution of activation in anatomically defined medial temporal lobe regions of interest (ROIs) during a complex scene-encoding task. ROIs were drawn on an MRI anatomic template formed from 3d-SPGR scans of 8 subjects combined in Talairach space. Separate ROIs were drawn for the posterior and anterior hippocampal formation, parahippocampal gyrus, and entorhinal cortex. Twelve different activation maps were created for each subject by using four correlation coefficients and three cluster volumes. Friedman's two-way ANOVA by ranks was used to test the hypothesis that the distribution of activated pixels among defined anatomic ROIs varied as a function of the data processing method.

By simply varying the combination of correlation-coefficient and cluster volume, significantly different distributions of activation within named medial temporal lobe structures were obtained from the same fMRI datasets ($p < 0.015$; $p < 0.001$). The number of subjects studied ($n=8$) is in a range commonly found in the literature yet this clearly resulted in spurious associations between processing parameter variations and activation distribution. Using data processing methods that are independent of the arbitrary selection of cutoff values for thresholding activation maps may reduce the likelihood of obtaining spurious results.

Introduction

Fundamental mechanisms of brain function are regularly inferred on the basis of the neuroanatomic distribution of activated pixels in fMRI studies. However, as of yet, there are no consistent methods of fMRI data analysis that are universally accepted to indicate "true" activation. It is well known that medial temporal lobe structures are crucial for memory retrieval and the encoding of new information. Within the past few years, several independent groups of investigators have reported fMRI activation of the medial temporal lobe using a similar complex scene encoding task (Detre *et al.*, 1998; Gabrieli *et al.*, 1997; Rombouts *et al.*, 1997; & Stern *et al.*, 1996).

With declarative memory paradigms that activate the medial temporal lobe limbic structures, interest has focused on the significance of anterior vs. posterior, right vs. left-sided, and hippocampal vs. parahippocampal activation patterns (Dolan and Fletcher, 1999; Fernandez

To whom correspondence should be addressed: Clifford R. Jack, M.D., Department of Radiology, Mayo Clinic, 200 1st Street SW, Rochester, MN 55905, Telephone: 507-284-8548, Fax: 507-284-2405, jack.clifford@mayo.edu.

et al., 1998; Grady *et al.*, 1995; Haxby *et al.*, 1996; Kelley *et al.*, 1998; Rombouts *et al.*, 1997; Stern *et al.*, 1996; Wagner *et al.*, 1998). Task specific differences in the anatomic distribution of fMRI activation, when averaged across subjects, have been cited as proof of functional specialization of different brain areas. One would assume that such fundamental features of cognitive function would not be affected simply by slight variations in the standard methods of fMRI data processing.

The aim of this project was to examine the effect of fMRI data processing methods on the distribution of activation in anatomically defined medial temporal lobe regions of interest (ROIs) during a complex scene-encoding task. We systematically varied standard data processing parameters in order to test the hypothesis that straightforward variation in these parameters significantly influences the distribution of activated pixels in anatomically defined regions of interest.

Methods

Subjects and Activation Task

Eight healthy, subjects [6 males, 2 females; 7 right-handed, 1 left-handed; mean age 31.6 (± 8.6) years; mean level of education 17.8 (± 1.7) years] were scanned while performing an encoding task. Each fMRI run consisted of 5 cycles of the encoding task (18 seconds) alternated with 5 cycles of visual fixation (18 seconds). Photographs were presented every three seconds during the encoding task. In order to verify that subjects were attending to the stimuli, they were required to distinguish indoor from outdoor scenes by squeezing a pneumatic bulb each time an indoor scene was shown.

Image Acquisition

Whole brain gradient-echo, echoplanar fMRI (TR 3s, TE 45 ms, FOV 24 cm, matrix 64×64) was performed using a 1.5T GE scanner. Twenty-seven contiguous, sagittal 5 mm slices were acquired in order to provide coverage of the entire brain. Each subject also underwent a 3D SPGR anatomic reference scan (TR 26 ms, TE 9 ms, FOV 24 cm, matrix $256 \times 192 \times 128$, 1.5 mm thick sagittal images) during the same scanning session as the functional runs.

Data Analysis

The fMRI data sets were processed in AFNI, using a cross-correlation analysis with a sinusoidal stimulus waveform function (Cox, 1996). Twelve different activation maps were created for each individual subject by using combinations of four correlation coefficients ($r = .39$, $p < .01$; $r = .42$, $p < .005$; $r = .46$, $p < .001$; $r = .50$, $p < .0001$) and three cluster volumes [70 mm^3 (1 voxel, i.e., no clustering), 140 mm^3 (2 voxels), 700 mm^3 (10 voxels)]. The cross-correlation coefficient [r] represented the association between the time-course of the signal intensity and the stimulus waveform function. The probability of a false positive activation [p] was estimated by a data reshuffling analysis corresponding to each particular r -value (Bullmore *et al.*, 1996). The cluster volumes, comprised of 2 and 10 voxels, were defined in a manner that accepted all neighboring voxels sharing a side, edge, or corner. No spatial smoothing was used.

Regions of interest were manually outlined on a coronal MRI anatomic template formed from the 3d SPGR scans of all 8 subjects which had been combined in Talairach space. The medial temporal lobe was divided into four anatomic compartments. These regions of interest were the posterior hippocampal formation, anterior hippocampal formation, parahippocampal gyrus, and entorhinal cortex. Figure 1 shows two views of the ROIs.

The anterior portion of the parahippocampal gyrus and associated white matter was labeled entorhinal cortex while the posterior parahippocampal gyrus was simply labeled

parahippocampal gyrus (Insausti *et al.*, 1998). The anterior boundary of the medial temporal lobe ROIs was defined as the slice on which the anterior commissure was visualized. The midpoint, used to divide the medial temporal lobe into anterior vs. posterior segments, was determined by the slice on which the intra-limbic gyrus was first visualized when progressing from posterior to anterior. The slice on which the hippocampal fornices were seen in full profile (Jack *et al* 1997) defined the posterior boundary of the medial temporal lobes. Right and left-sided ROIs were drawn for each named anatomic structure resulting in a total of eight anatomic ROIs.

Analysis of the twelve fMRI activation maps was performed after transformation into Talairach space. As the fMRI activation maps were transformed to Talairach space, they were reformatted to a 1 mm³ grid using nearest-neighbor interpolation.

For a given set of correlation coefficient and cluster volume values, pixel counts were computed for the named ROIs for each subject. In order to analyze the anatomic distribution of fMRI activation as a function of data processing parameters, three difference variables were created (D_1 , D_2 , and D_3). These variables described the distribution of pixel counts among different anatomic compartments, measured as a difference score. D_1 compared right versus left side activation, and was defined as the sum of activated pixels in the right-sided ROIs minus the sum of activated pixels in left-sided ROIs. D_2 compared anterior versus posterior activation, and was defined as the sum of activated pixels in the anterior hippocampal and entorhinal cortex ROIs minus the sum of activated pixels in the posterior hippocampal and parahippocampal ROIs. D_3 compared superior vs. inferior activation and was defined as the sum of activated pixels in the hippocampal ROIs minus the sum of activated pixels in parahippocampal and entorhinal cortex. The combinations of the four correlation coefficients and three cluster volumes defined 12 experimental conditions. Volumes (in cm³) of the anatomic ROIs analyzed were right 16.3, left 15.4, superior 15.6, inferior 16.1, anterior 13.8, and posterior 17.9.

Because much of the data were highly skewed, non-parametric statistical tests were used. We first considered each experimental condition separately. To test for a difference between right and left sides, performed signed rank tests on the D_1 values. Similarly, signed rank tests were performed on D_2 and on D_3 . In each case, the 12 signed rank tests provide a qualitative assessment of how inferences may vary depending on which set of FMRI data processing parameters were used.

For each difference variable, we used Friedman's two-way ANOVA by ranks to directly test the hypothesis that the anatomic distribution of activation pixels varied significantly as a function of FMRI data processing method.

In a secondary analysis we assessed the possibility that results obtained from the analysis above were influenced by differences in the signal to noise (SNR) among the different regions of the brain that were analyzed. The median SNR was computed for each of the 6 defined anatomic regions, for each of the 8 subjects. SNR was computed as the mean signal intensity across all EPI images in the fMRI time series divide by the standard deviation. Signed rank tests were used to test for differences in SNR between the anterior vs posterior, superior vs inferior, and right vs left ROIs.

We also wished to test the hypothesis that regional differences in SNR were associated with regional differences in the level of functional activation. The median number of activated pixels in each ROI across all 12 variations in data processing was computed for each of the 8 subjects. Spearman correlation was used to test the hypothesis that regional functional activation was related to regional variation in SNR.

Results

The median differences in the number of activated pixels between ROI pairs (anterior vs posterior, superior vs inferior, and right vs left) and the corresponding tests of significance, as a function of data processing method are listed in Table 1. The differences observed and the corresponding statistical significance varied markedly depending on the selection of data processing parameters. The difference among the 12 data processing methods was statistically significant for anterior vs. posterior distribution ($p < 0.001$), and for superior vs. inferior distribution ($p = 0.015$), but not for right vs. left-sided activation. The following figures illustrate how the activation distribution varies as a function of processing method for this experiment using the combined activation maps of all eight subjects. Figure 2 illustrates the different activation patterns in the posterior hippocampus that result from varying the correlation coefficient. The cluster volume was held constant at 140 mm^3 . Figure 3 illustrates the different activation patterns in the posterior hippocampus that result from varying the cluster volume using a constant correlation coefficient ($r = .42$; $p < .005$). As the correlation coefficients and cluster volumes increase, the number of activated voxels that meet the threshold criteria gradually diminishes, but the anatomic distribution changes as well.

The results shown in Table 1 motivated us to assess the possibility that image SNR varied with anatomic location and whether regional variation in functional activation was dependent on image SNR. Analysis of regional SNR variation is found in Table 2. On average, SNR was greater in the posterior than the anterior ROIs, greater in the left than the right ROIs, but not significantly different between the superior and inferior ROIs. No significant association was found between regional differences in SNR and regional differences in functional activation.

Discussion

The distribution of fMRI activated pixels among different named medial temporal structures have been used to deduce fundamental mechanisms for information processing during various memory tasks (Schacter & Wagner, 1999). For example, right vs. left-sided fMRI activation has been invoked as proof of the neuroanatomic substrate of verbal vs. non-verbal memory function (Dolan & Fletcher, 1997; Grady *et al.*, 1995; Haxby *et al.*, 1996; Kelley *et al.*, 1998). Anterior vs. posterior medial temporal lobe fMRI activation has been labeled as proof of the neuroanatomic substrate of retrieval vs. encoding (Gabrieli *et al.*, 1997). The role of the hippocampus vs. entorhinal cortex in processing information for memory storage has been inferred from superior compartment (hippocampus) vs. inferior compartment (parahippocampal gyrus and entorhinal cortex) fMRI activation (Rombouts *et al.*, 1997; Stern *et al.*, 1996;). However, we found that significant differences in the distribution of activated pixels among named anatomic medial temporal lobe structures can simply be the result of the method of processing *the same* fMRI data set. The number of normal subjects we studied ($n = 8$) was fairly typical of many published studies in this area.

In a second analysis we assessed whether variation in functional activation among ROIs could simply be due to basic regional differences in SNR in the raw EPI image data. Magnetic susceptibility induced signal loss in brain areas adjacent to air-bone interfaces at the skull base is accentuated by the long read out times of the EPI data acquisition commonly used in fMRI experiments. Based on differences in proximity to the petrous bone and sphenoid sinus, we expected that susceptibility induced signal loss would be greater (and SNR lower) in the anterior than the posterior ROIs, and greater in the inferior than the superior ROIs. We expected no difference in SNR in right vs left ROIs. The expected finding was observed in the anterior vs posterior ROI comparison – i.e. the ROI closer to the sphenoid sinus typically had lower SNR. As anticipated the superior ROIs, which were further away from the skull base, had greater SNR than the inferior ROIs, however this association did not reach significance. An

unexpected and difficult to explain finding was greater SNR on the left than right. Most importantly though, the test for associations between SNR and functional activation magnitude was not significant. We can therefore safely conclude that differences in functional activation among the ROIs evaluated are not simply due to artifactual signal loss in the raw EPI images of the fMRI time series data.

We employed standard, widely used methods for acquisition and post-processing of the functional data. We also used accepted neuroanatomic criteria for determination of regions of interest within the medial temporal lobe. Alternate fMRI acquisition parameters could have been used, however, it is not clear that would have materially affected the results obtained. For example, the number of image volumes in our paradigm and the duration of the paradigm were less than in comparable studies. Theoretically, longer paradigms with more images in the time series should translate directly into greater fMRI signal stability. It is almost certain, however, that this is not true for in vivo studies. It has been our experience, as well as that of many other groups, that the major source of data variability in fMRI is bulk head motion which occurs over the course of the time series acquisition. The probability of head motion is inversely related to the length of individual fMRI runs as well as the overall duration of the exam. The optimal duration of any particular fMRI paradigm has not been established.

The plane in which our EPI time series data was acquired was not perpendicular to the longitudinal axis of the hippocampal body. Thus, partial volume effects might have lowered SNR. We gave careful consideration to the plane of acquisition at the outset. The sagittal plane was chosen for our acquisition because others and we have empirically observed that the most frequent and most significant direction of bulk head motion in fMRI is sagittal rotation, that is rotation about the right-left axis. If the image data is acquired in the sagittal plane, then retrospective volume to volume registration can partially correct this type of motion. Had we acquired the data in a different plane, then through-plane registration would have been required, which is far more problematic. Also, the narrowest dimension of the head is in the sagittal direction. A volume acquisition can be accomplished more quickly in the sagittal than the coronal direction, which translates into greater temporal resolution per volume.

We might have improved specificity of medial temporal lobe activation with a different control task to remove sensitivity to visual activation (Binder *et al.*, 1999). However, given the fact that the data was derived entirely from medial temporal lobe regions of interest, it seems unlikely that a different task resulting in greater anatomic specificity would have changed the result. A different control task might have eliminated activation in other areas not directly engaged in encoding of memory (for example, areas in visual association cortex involved in processing of the complex visual scenes). However, areas outside the medial temporal lobe ROIs were not analyzed in our data, and therefore should not influence our results comparing data processing methods.

Differences in the size of ROIs can influence quantitative comparisons of fMRI activation between ROIs. Threshold – pixel counting methods are more vulnerable to this phenomenon than analysis methods that are done over a range of threshold values (Constable *et al* 2000). However, the superior-inferior and the right-left ROI pairs in our analysis were essentially equal in size. Therefore, our results can not simply be explained by differences in size of the ROI pairs analyzed.

Methods of data analysis that are not dependent on the selection of a single parameter for fMRI data processing have been suggested as a preferred alternative to the more common practice of arbitrarily selecting cutoff values for thresholding activation maps. Receiver operating characteristic curves (Constable *et al.*, 1995) using the sum of t-values (Constable *et al* 2000), using z-scores or other methods that overweight pixels with high correlation coefficients and

underweight those with low correlation coefficients have been suggested as ways to improve reliability. Our data reinforce this message and our primary conclusion is that inferring fundamental principles of brain function from anatomic patterns of fMRI activation in a limited number of subjects using pixel counts of thresholded activation maps should be approached judiciously.

Acknowledgments

The authors wish to thank Bret Borowski, Chris LaPlante, Phil Rossman, and Tom Hulshizer for technical assistance.

Grant support: NIH, CA73691 and AG19142

References

- Binder J, Frost J, Hammeke T, Bellgowan P, Rao S, Cox R. Conceptual processing during the conscious resting state: a functional MRI study. *Journal of Cognitive Neuroscience* 1999;11:80–93. [PubMed: 9950716]
- Bullmore E, Brammer M, Williams SCR, Rabe-Hasketh S, Janot N, David A, Mellers J, Howard R, Sham P. Statistical methods of estimation and inference for functional MR image analysis. *Magn Reson Med* 1996;35:261–277. [PubMed: 8622592]
- Constable R, Skudlarski P, Gore J. An ROC approach for evaluating functional brain MR imaging and post processing protocols. *Magn Reson Med* 1995;34:57–64. [PubMed: 7674899]
- Cox RW. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research* 1996;29:162–173. [PubMed: 8812068]
- Detre JA, Maccotta L, King D, Alsop DC, Glosser G, D’Espito M, Zarahn E, Aguirre GK, French JA. Functional MRI lateralization of memory in temporal lobe epilepsy. *Neurology* 1998;50:926–932. [PubMed: 9566374]
- Dolan RJ, Fletcher PF. Encoding and retrieval in human medial temporal lobes: an empirical investigation using functional magnetic resonance imaging (fMRI). *Hippocampus* 1999;9:25–34. [PubMed: 10088897]
- Fernandez G, Weyerts H, Schrader-Bolsche M, Tendolkar I, Smid HGOM, Tempelmann C, Hinrichs H, Scheich H, Elger CE, Mangun GR, Heinze H-J. Successful verbal encoding into episodic memory engages the posterior hippocampus: a parametrically analyzed functional magnetic resonance imaging study. *The Journal of Neuroscience* 1998;18:1841–1847. [PubMed: 9465008]
- Gabrieli JDE, Brewer JB, Desmond JE, Glover GH. Separate neural bases of two fundamental memory processes in the human medial temporal lobe. *Science* 1997;276:265–266.
- Grady CL, McIntosh AR, Horwitz B, Maisog JM, Ungerleider LG, Mentis MJ, Pietrini P, Schapiro MB, Haxby JV. Age-related reductions in human recognition memory due to impaired encoding. *Science* 1995;269:218–221. [PubMed: 7618082]
- Haxby JV, Ungerleider LG, Horwitz B, Maisog JM, Rapoport SI, Grady CL. Face encoding and recognition in the human brain. *Proc Natl Acad Sci USA* 1996;93:922–927. [PubMed: 8570661]
- Insausti R, Juottonen K, Soininen H, Insausti A, Partanen K, Vainio P, Laakso M, Pitkanen A. MR volumetric analysis of the human entorhinal, perirhinal, and temporopolar cortices. *AJNR* 1998;19:659–671. [PubMed: 9576651]
- Jack CR, Petersen RC, Xu YC, Waring SC, O’Brien PC, Tangalos EG, Smith GE, Ivnik RJ, Kokmen E. Medial temporal atrophy on MRI in normal aging and very mild Alzheimer’s disease. *Neurology* 1997;49:786–794. [PubMed: 9305341]
- Kelley WM, Miezin FM, McDermott KB, Buckner RL, Raichle ME, Cohen NJ, Ollinger JM, Akbudak E, Conturo TE, Snyder AZ, Petersen SE. Hemispheric specialization in human dorsal frontal cortex and medial temporal lobe for verbal and nonverbal memory encoding. *Neuron* 1998;20:927–936. [PubMed: 9620697]
- Lepage M, Habib R, Tulving E. Hippocampal PET activations of memory encoding and retrieval: the HIPER model. *Hippocampus* 1998;8:313–322. [PubMed: 9744418]

- Rombouts S, Scheltens P, Machielsen W, Barkhof F, Hoogenraad F, Veltman D, Valk J, Witter MP. Parametric fMRI analysis of visual encoding in the human medial temporal lobe. *Hippocampus* 1999;9:637–643. [PubMed: 10641756]
- Schacter DL, Wagner AD. Medial temporal lobe activations in fMRI and PET studies of episodic encoding and retrieval. *Hippocampus* 1999;9:7–24. [PubMed: 10088896]
- Stern CE, Corkin S, Gonzalez G, Guimares AR, Baker JR, Hennings PJ, Carr CA, Suigiura RM, Vedantham V, Rosen BR. The hippocampal formation participates in novel picture encoding: evidence from functional magnetic resonance imaging. *Proc Natl Acad Sci USA* 1996;93:8660–8665. [PubMed: 8710927]
- Wagner AD, Schacter DL, Rotte M, Koustaal W, Maril A, Dale AM, Rosen BR, Buckner RL. Building memories: remembering and forgetting of verbal experiences as predicted by brain activity. *Science* 1998;281:1188–1191. [PubMed: 9712582]
- Zeineh MM, Engel SA, Bookheimer SY. Application of cortical unfolding techniques to functional MRI of the human hippocampal region. *NeuroImage* 2000;11:668–683. [PubMed: 10860795]

Figure 1. Two views of the ROIs

Figure 2. Effect of varying correlation coefficient threshold

Three activation maps derived from the same data set created by varying the correlation coefficient used for thresholding. The cluster volume was held constant at 140 mm^3 .

Figure 3. Effect of varying cluster volume

Three activation maps derived from the same data set created by varying the cluster volume. The correlation coefficient threshold was held constant at $r = 0.42$.

Table 1
Distribution of FMRI Activation as a Function of Data Processing Method

r value; cluster volume	anterior vs. posterior (D₂) (A-P)	superior vs. inferior (D₃) (S - I)	right vs. left (D₁) (R-L)
r = .39; 70 mm ³	-781; p ≤ .01	-342; p ≤ .02	77; p > .10
r = .42; 70 mm ³	-491; p ≤ .01	-201; p ≤ .01	-21; p > .10
r = .46; 70 mm ³	-353; p ≤ .01	-121; p ≤ .05	32; p > .10
r = .50; 70 mm ³	-350; p ≤ .02	-138; p ≤ .10	12; p > .10
r = .39; 140 mm ³	-619; p ≤ .05	-265; p ≤ .10	16; p > .10
r = .42; 140 mm ³	-304; p ≤ .05	-111; p ≤ .05	30; p > .10
r = .46; 140 mm ³	-131; p ≤ .10	-53; p ≤ .05	0; p > .10
r = .50; 140 mm ³	-116; p ≤ .10	-42; p ≤ .10	0; p > .10
r = .39; 700 mm ³	-511; p ≤ .05	-237; p ≤ .10	14; p > .10
r = .42; 700 mm ³	-148; p ≤ .05	-144; p ≤ .05	0; p > .10
r = .46; 700 mm ³	-115; p ≤ .10	-97; p > .10	0; p > .10
r = .50; 700 mm ³	0; p > .10	0; p > .10	0; p > .10

For each of the various combinations of correlation coefficients and cluster volumes that were used as independent variables, the median difference score (D₁, D₂, or D₃) is shown along with significance values for the signed-rank tests.

Table 2

Signal to Noise Measurements by ROI

ROI comparison	Difference ¹	p-value ²
Right vs. Left	– 39	p < 0.01
Anterior vs. Posterior	–31	p < 0.01
Superior vs. Inferior	6	ns

¹ Median difference in SNR between the 2 ROIs tested.

² Test of the hypothesis that the SNR was significantly different between ROIs indicated.