Associative Encoding of Pictures Activates the Medial Temporal Lobes

Daniela Montaldi,^{1*} Andrew R. Mayes,² Anna Barnes,³ Heather Pirie,¹
Donald M. Hadley,⁴ Jim Patterson,³ and David J. Wyper³

¹Psychology Group, Department of Applied Social Studies, University of Paisley, Paisley PA1 2BE, UK

²Department of Clinical Neurology, University of Sheffield, Sheffield S10 2JF, UK

³Department of Clinical Physics, Institute of Neurological Sciences,
Southern General Hospital, Glasgow G51 4TF, UK

⁴Department of Clinical Neuroradiology, Institute of Neurological Sciences,
Southern General Hospital, Glasgow G51 4TF, UK

Abstract: It remains unresolved whether the medial temporal lobe activations found in recent neuroimaging studies are mediated by novelty detection alone, by specific kinds of encoding or consolidation operations, or both. This study attempted to see whether associative encoding or consolidation is sufficient to cause such activation by matching for novelty across conditions. Using single-photon emission computer tomography (SPECT) (with Tc^{99m}HMPAO), we compared the activation patterns produced by the associative encoding and the perceptual matching of novel complex scenes in 10 normal subjects using both statistical parametric mapping (SPM) and a regions-of-interest (ROI) approach. During the encoding condition, significant activations were detected in the left hippocampal/parahippocampal region, the left cingulate cortex, and the right prefrontal cortex, using both statistical techniques. Additionally, activation was found in the right cingulate cortex, and a trend towards activation was found in the right hippocampal/parahippocampal region using the ROI approach. In contrast, no medial temporal activations were found during the matching condition, which produced bilateral occipito-parietal and right posterior inferior parietal (supramarginal gyrus) activations. These results not only confirm that the associative encoding and/or consolidation of complex scenes is partially mediated by medial temporal lobe structures, but also demonstrate, for the first time, that associative encoding/consolidation is sufficient to produce such an activation. The implications of the high degree of consistency revealed by the results of the SPM and ROI comparison are discussed. *Hum. Brain Mapping* 6:85–104, 1998. © 1998 Wiley-Liss, Inc.

Key words: encoding; novelty; CBF; SPECT; medial temporal lobe; prefrontal cortex; statistical parametric mapping; regions of interest

INTRODUCTION

The view that the medial temporal lobe is involved in explicit memory for episodic information (and

semantic information, at least that which is not over-learned and/or has been in storage for a long time) has long been supported by evidence that lesions to this region disrupt explicit memory for such information [reviewed in Mayes, 1988]. It has, however, proved difficult through the study of patients with medial temporal lobe lesions to determine exactly how the structures in this region mediate explicit memory for episodes and facts. In particular, it has proved hard to discover whether medial temporal lobe structures mediate the input processes of encoding and consolida-

Contract grant sponsor: Neuroscience Foundation.

*Correspondence to: Dr. Daniela Montaldi, Psychology Group, Department of Applied Social Studies, University of Paisley, High Street, Paisley PA1 2BE, Scotland, UK.

E-mail: mont-as0@paisley.ac.uk

Received for publication 30 September 1997; accepted 27 October 1997

tion, the output processes of retrieval, or both of these. The development of functional neuroimaging studies in which blood flow to the brain is compared across conditions, which are similar except for the operation of one or, at most, a few psychological processes, has made it possible to investigate the relationship between brain structure and function more methodically than was previously possible with lesion studies. It has also provided an important complement to lesion studies by offering the opportunity to investigate clinically developed hypotheses on a normal population. While the two most common neuroimaging techniques are positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), high-performance single-photon emission computer tomography (SPECT) offers an alternative method.

SPECT imaging involves not only the selection of a suitable scanner, but also the selection of a radio-pharmaceutical appropriate to the aims of the study. One such radio-pharmaceutical that has been developed for the purpose of measuring cerebral blood flow (CBF), and is particularly suitable for the requirements of cognitive activation studies, is technetium-99m hexamethylpropyleneamine oxime (Tc^{99m} d,1-HMPAO; CeretecTM, Amersham International, Amersham, UK). This has been shown to distribute in the brain in proportion to blood flow [Sharp et al., 1986]. HMPAO is administered by intravenous injection, with approximately 5% of the total dose being extracted on its first pass through the brain, where it becomes trapped in brain tissue. This trapping is thought to be due to a reduction in the lipophilicity of the molecule which occurs within the brain, thus preventing significant back diffusion through the blood-brain barrier. Since the subsequent washout rate of the trapped ligand is less than 1% per hr [Sharp et al., 1986], it is possible to image the pattern of blood flow that occurred in the brain at the time of injection, after a considerable delay. The delay between injection and scanning is limited only by the half-life of the radioisotope, which in the case of technetium^{99m} is 6 hr. This particular trapping mechanism therefore allows the administration of the radioisotope to be carried out away from the scanner, in an appropriate environment for psychological testing which is less confined and less anxiety-provoking. The scanning itself can then be carried out while the subject relaxes, or sleeps. To date, relatively few SPECT activation studies have been carried out [Robert et al., 1995; Beversdorf et al., 1995; Crosson et al., 1994]. In many cases, the potential of the studies has been limited by a lack of sensitivity and resolution resulting from the type of SPECT scanner [Robert et al., 1995] or SPECT technique [Beversdorf et al., 1995] used. While

previous studies have adopted a variety of regions-of-interest (ROI) analysis procedures, as far as the authors are aware, no SPECT activation study applying statistical parametric mapping (SPM) analysis to high-resolution imaging has yet been published.

Functional neuroimaging techniques have provided an important new method for identifying the way structures implicated in organic amnesia mediate explicit memory for episodes and facts. These techniques, relative to lesion studies, enable the investigator to separate encoding/consolidation and retrieval processes fairly easily. The use of both lesion and neuroimaging challenge studies also allows a convergent operations approach to be used in which the findings of one approach are cross-validated with those of the other. Earlier PET activation studies with episodic encoding and retrieval challenges, however, failed to find evidence that medial temporal lobe blood flow was increased either by episodic encoding or retrieval, although blood flow to frontal regions was consistently increased, and differentially so with encoding and retrieval [e.g., Kapur et al., 1994; Shallice et al., 1994; Tulving et al., 1994a]. As lesion studies strongly imply that the medial temporal lobe is involved in explicit memory for episodes and facts, the failure to find activation effects with episodic encoding or retrieval has usually been explained either in terms of this region being nearly always active so that any small increases are undetectable with PET technology, or in terms of only a very small proportion of the region being activated during episodic encoding or retrieval so that, once again, the very small blood flow increases are undetectable [Fletcher et al., 1995].

More recently, PET and fMRI studies have reported medial temporal lobe activations in association with the encoding and retrieval of both verbal and nonverbal episodic information [e.g., Squire et al., 1992; Grasby et al., 1994; Tulving et al., 1994b; Grady et al., 1995; Kapur et al., 1995; Nyberg et al., 1996; Gabrieli et al., 1997; Stern et al., 1996]. As activations have been reported during encoding as well as retrieval of episodic information, this suggests that the medial temporal lobes may play a role in both encoding/consolidating and retrieving such information. Of interest in this context is a study by Tulving et al. [1994b], which showed that studying complex colored pictures of scenes for the first time produced more medial temporal lobe activation than when they were studied for the second time. Subjects would inevitably have been retrieving episodic memories of the pictures when these were seen for the second time, suggesting that more medial temporal lobe activation was produced during encoding. Tulving et al. [1994b] proposed that

the hippocampus and possibly other medial temporal lobe structures form part of a novelty detection system, and that the detection of novelty was a critical factor in producing the activations seen in their study. As we will consider in the Discussion, Tulving et al. [1994b, 1996] seem to believe that novelty detection might be sufficient to activate an extensive neural system which includes structures in the medial temporal lobes. However, it is also likely that items, perceived as novel, are more likely to receive spontaneous and rich encoding than are items which are already highly familiar. A plausible hypothesis is that medial temporal lobe activation is proportional to the amount of encoding/consolidation engaged in. It also seems likely that the medial temporal lobe-dependent encoding will be of a particular kind, involving the formation of new associations between items [e.g., Ungerleider, 1995].

If this account is correct, then medial temporal lobe activations should be found in normal subjects when they engage in encoding of associations, even when novelty per se is controlled across conditions. Grady et al. [1995] did precisely this in a PET activation study that examined the encoding and retrieval of faces in healthy young and old subjects. The blood flow changes caused by either the encoding or retrieval of face pictures were contrasted with those produced by either a sensorimotor control task or a matching task. The matching task was of particular interest because in this, subjects saw similar pictures to those that they saw in the encoding and retrieval conditions. When encoding was contrasted with the matching condition (as well as the sensorimotor control condition), the young subjects, but not the old, showed activation in the left frontal, anterior cingulate, and right medial temporal cortices. When the retrieval condition was contrasted with both control conditions, the young and the old showed right frontal cortex activation, but only the young showed parietal cortex activation. This study, therefore, not only failed to find medial temporal lobe activation in old subjects, it failed to find it even in young subjects during the retrieval required for recognition memory. This supports the view [e.g., Ungerleider, 1995] that encoding/consolidation produces more medial temporal lobe activation than does retrieval. The comparison of the matching and encoding tasks in Grady et al. (1995) involved contrasting two conditions in which the novelty of the pictorial stimuli was equated, but presumably the encoding task engaged to a greater extent the kinds of encoding processes that activate the medial temporal lobe region. The study does little to illuminate what those processes are, because subjects in the encoding condition were merely asked to memorize the faces.

The present study had a main neuropsychological aim (which has implications for the future use of SPECT in neuroactivation), and a subsidiary methodological aim. The main aim was to see whether we could demonstrate comparable activations in the frontal cortex, medial temporal lobes, and other regions to those found by Grady et al. [1995] when faces were encoded, by using SPECT and a modification of the original procedure. Our modification used complex pictorial stimuli of the same kind as used by Tulving et al. [1994b] in their PET study of encoding activation, and by Stern et al. [1996] and Gabrieli et al. [1997] in their fMRI studies of encoding activation. None of these three studies of picture encoding controlled for novelty in the way that our study and that of Grady et al. [1995] do. As part of our main aim, we wished to determine whether the associative encoding or consolidation of complex pictures is sufficient to produce medial temporal lobe activation. Since novelty was controlled across conditions, any medial temporal lobe activation could not be a direct result of the perception of novelty, but must result from active encoding, and probably from the encoding of associations between identified objects as opposed to other kinds of encoding.

Unlike PET activation studies where as many as 6 replicated conditions are frequently used, a disadvantage of SPECT is that it is currently not possible to employ more than two conditions, although we are presently developing and evaluating a four-condition procedure [Barnes et al., 1997]. However, one important feature of SPECT which was alluded to earlier, is that due to the trapping mechanism of HMPAO, subjects perform the challenge conditions in a normal environment, which is unconstrained and relaxed, prior to being scanned. There is nevertheless skepticism about whether SPECT is able to reveal the relatively small activation effects that PET has shown are associated with cognitive processing. Our main aim, therefore, was not only to extend the studies of Grady et al. [1995] and Tulving et al. [1994b], but also to show that SPECT (using a high-quality scanner) can be effectively as sensitive as PET to these subtle cognitive changes. If this can be demonstrated, it may encourage other groups with dedicated SPECT systems to participate in this field of investigation.

Our subsidiary aim was to compare two methods of quantifying and analyzing the SPECT images produced by the encoding and matching conditions in our group of healthy adult subjects. There have been few published studies that have directly compared the results obtained using two or more different methods of analysis. This is unfortunate, as one must be able to have confidence in the location of the brain regions

TABLE I. Subjects: Demographic details and neuropsychological profile

Sex		Age		NART IQ		Word fluency (FAS)		Doors and people		
M	F	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Percentile
5	5	45.9	11.4	115.4	5.56	16.3	4.72	12.1	3.28	75th

shown to be activated or deactivated, especially in studies that reveal unexpected results. One study that did compare two methods of analysis was that of Arndt et al. [1995], which compared the use of Friston's statistical parametric mapping (SPM) procedure with that of Worsley et al. (1992) method. The two methods were found to produce broadly similar results, although some differences were found, which Arndt et al. [1995] admitted may be important in some experiments. The study compared different methods of global normalization, focusing on a predominantly statistical issue rather than on the statistical-anatomical analysis. With our data, we compared SPM [Friston et al., 1991] with an ROI approach which uses a procedure to coregister SPECT and structural MRI so as to maximize the anatomical accuracy with which regions of interest can be identified. We examined SPM because it is the most widely used procedure for analyzing neuroimaging data and compared this with an ROI approach for several reasons. First, this technique is more widely available, especially in centers with limited computing power. Second, it may have advantages over SPM when investigating subjects who have suffered serious structural brain damage and in whom distortion of brain regions may render the fitting of a brain to a standard template problematic. A further reason for considering an ROI technique is that it can be carried out on limited data sets (i.e., incomplete brain scans) more readily than SPM. This can be an important factor when dealing with patient populations or normal elderly subjects.

METHODS AND PROCEDURE

Subjects

Ten healthy right-handed subjects, mean age 46 (range, 27–59) participated in this study. All subjects were screened for any history of previous illness or neurological disorder. Normal cognitive performance was established in each subject using the National Adult Reading Test (NART) [Nelson and Willison, 1991] and the Doors and People Test [Baddeley et al., 1994] to assess intellectual and memory function, respectively (Table I). All subjects gave informed writ-

ten consent for the study, which had been approved by the Southern General Hospital ethics committee. Approval for the administration of the radioactive material was received from the Department of Health, UK.

Cognitive activation tasks

The tasks used in this study followed the design used by Grady et al. [1995]. The principal difference in this study was the use of novel complex scenes (source: *National Geographic*, pre-1990 editions) rather than faces. This difference was introduced in order to maximize associative encoding while equating novelty across conditions, so as to investigate directly the associative encoding component of the novelty-encoding network, as proposed by Tulving et al. [1994b]. Two tasks were used, a perceptual matching task and an associative encoding task. The color stimuli ($3'' \times 3''$) used in each task were presented 30–35 cm away from the subject, on cards displayed by the experimenter. The stimuli were drawn from a common pool of pictures, and while they were not counterbalanced across tasks, they were matched for content so that both sets of stimuli contained the same number of pictures drawn from a number of theme-specific categories: animal, aerial, human, and inanimate. In order to avoid any stimuli with predominantly facial information, all "human" stimuli were restricted to scenes where human faces occupied less than 20% of the picture. All practice stimuli were drawn from the original pool of stimuli and were different from those used in the activation tasks. Scan order, and therefore task order, were counterbalanced.

Perceptual matching task

The matching task followed a forced-choice matching-to-sample design in which a sample complex scene and two choices were presented simultaneously. Subjects were required to decide which of the lower two pictures in the display (left or right) was identical to the top (sample) picture. A button-pressing response (involving two buttons and both hands) was used to record subjects' choices. Each of the 60 presentations was displayed for 4 sec, with an interstimulus interval

of 1 sec; subjects were instructed to respond to each presentation, guessing when absolutely necessary. Piloting of this material ensured that the difficulty level of the task meant that the 4-sec display time was filled with perceptual matching, while allowing subjects a good chance of success on each trial. This reduced the level of associative encoding of the material while maintaining subject motivation. In order to ensure a relatively perceptual task, detailed instructions and practice sessions were provided with two specific aims in mind. First, we wanted to stress the superficial nature of the scanning strategy subjects needed to adopt in order to make a fast and accurate choice. The second aim was to emphasize both the high degree of similarity between the choices and that the nature of this similarity was in the theme of each picture and therefore, any attention paid to the theme of the pictures would not help in discriminating between them. The instructions therefore encouraged a low-level orienting task to be performed.

Memory encoding task

Subjects were presented with 60 complex scenes presented at a rate of 1 every 4 sec, with an interstimulus interval of 1 sec, and were instructed to memorize them for later recognition. Detailed instructions and practice sessions were provided prior to the task. The aims of the instructions were, first, to encourage associative encoding by asking subjects to adopt a strategy focusing on what the picture was generally about by attending to its main features, how the features related to each other within the picture (i.e., both the functional and positional relationship), and the spatial location of the features. Second, the instructions aimed to motivate subjects by informing them that the subsequent recognition test of memory contained distractors with similar features but that their positional relationships and therefore the general theme of the picture may be different. These instructions therefore encouraged the use of a high-level orienting strategy. In order to match across tasks for both the button-pressing response and the left/right selection process, subjects had to indicate whether the picture was positioned to the right or the left of the display by pressing a response button.

Assessment of task performance

Matching performance

Response accuracy on the matching task was computer-monitored, and an overall mean correct response rate was calculated.

Level of encoding

In order to establish that the level of associative encoding carried out by the subjects varied between the two tasks, several assessments of memory were used. These were carried out approximately 90 min after the activation task.

General debriefing. Following each cognitive activation task and the accompanying SPECT scan, subjects were debriefed as to their estimated performance on processing information concerning general picture comprehension, the relationship between features, and the spatial position of the features. Subjects were asked to estimate what percentage of the stimuli they processed in each of these three ways.

Recall and recognition tests. A test of free recall of any two pictures, selected by the subject, was carried out after each task. Responses were evaluated on the basis of the detail provided regarding the theme of the picture, the number of features identified and adjectives describing them, and the relationship between features. Responses were quantified on the basis of the number of correct descriptors (2 points each) attributed to each picture (1 point was deducted for each incorrect descriptor) up to a maximum of 12 descriptors across the two pictures. A cutoff score of 24 points and a cutoff time of 2 min were employed. A two-choice, forced-choice recognition test was also carried out after each task. Subjects were shown displays containing the original stimulus and one distractor and asked to identify which of the two pictures they had seen during the task performed earlier. The distractors were drawn from the original pool of stimuli and were therefore similar in content. They were not matched individually with each target, but the overall proportions drawn from each theme-specific category were the same.

Recognition debriefing. Following the recognition tests, subjects were asked to estimate their overall confidence in their responses. Subjects had to estimate on what percentage of responses they were over 90% confident, 50% confident, and less than 25% confident. They were also asked to estimate on what percentage of the responses they chose negatively (i.e., feeling that they had definitely not seen one picture, they concluded that it must have been the other).

Neuroimaging procedure

For each subject, one magnetic resonance imaging (MRI) scan and two SPECT scans (one for each cogni-

tive condition) were obtained. The MRI was carried out on a 1.5T Siemens Magnetom (Siemens PLC, UK). A magnetization-prepared rapid-acquisition gradient echo (MPRAGE) sequence was used to acquire T1-weighted 1-mm-thick slices of the whole brain. The imaging sequence used allowed the extraction of both axial and coronal slices for the purpose of anatomical localization.

Tc^{99m} HMPAO administration

Prior to each SPECT scan, a maximum dose of 500 MBq Tc^{99m}HMPAO was injected intravenously through a forearm cannula while the subject performed the cognitive task. The tasks were designed to last for 5 min, and the injection was carried out after 1.5 min of each task. This procedure was carried out in a quiet and comfortable environment, away from the scanner and in a relatively "nonclinical" setting. SPECT imaging commenced approximately 15 min after injection, with the two SPECT scans being carried out 48 hr apart.

Data acquisition

The SPECT scans were acquired using an SME 810 (Strichman Medical Equipment (NOVO Diagnostic Systems, Denmark)) Multi-detector Scanner. This neuroscanner gives better resolution, and minimizes variation in resolution across the reconstructed field of view in comparison with conventional SPECT systems based on rotating gamma cameras. For further details of this particular scanning system, see Stoddart and Stoddart [1992].

For each subject, 25 slices were acquired during the SPECT scan, thereby providing a complete brain data set for each cognitive condition. Scanning time was 120 sec per slice, with a slice thickness of 12 mm and slice spacing of 6 mm. This produced an in-plane resolution of 8 mm and longitudinal resolution of <10 mm. The scan orientation was approximately parallel to the orbito-meatal (OM) line, although this setting was not crucial, as the SME software allows 3D reorientation of the image data set. Head movement during the scanning procedure was minimized by subject cooperation and assisted by placing foam supports on either side of the subject's head, which was then held in place by a light Velcro strap. Subjects were encouraged to sleep or listen to relaxing music during the scanning session, since this or any other stimulus would have no effect on the SPECT images, as the HMPAO was already trapped in the brain tissue.

Data analysis

The MRI scans were imported from the Siemens Litebox into the SME Neuro 900 software via an Apple Macintosh (Uxbridge, UK) IIfx system. For each subject, the first SPECT scan was coregistered with the individual MRI scan, which had previously been reformatted by a neuroradiologist to a standard orientation along the anterior-posterior commissures in accordance with a stereotaxic atlas [Talairach and Tournoux, 1988]. The second SPECT scan was then coregistered to the first SPECT scan, using version 2.9 of the coregistration facility on the SME Neuro 900 software package. This software achieves optimum alignment by using an algorithm designed to detect the maximum correlation coefficient (r) between two radioisotope distributions in a reference scan (a) and a sample scan (b):

$$r = (a - \langle a \rangle)(b - \langle b \rangle) / s_a s_b$$

where s_a , s_b are the standard deviations of the distributions of a and b , respectively. The iterative technique used adopts the simplex search method [Nelder and Mead, 1965], which is terminated when the changes in translation parameters (x , y , z) are less than 0.5 mm and the changes in rotation parameters (pitch, roll, yaw) are less than 0.5°. The automated coregistration facility is run on an Apple Macintosh Quadra and an individual coregistration takes approximately 15 min to complete, using images with 128×128 pixel resolution. An evaluation of the automated coregistration procedure had previously shown that as long as complete brain data sets were used, as in this study, automated coregistration was more reliable than manual, expert coregistration [Barnes et al., 1996].

Statistical parametric mapping (SPM)

For the purpose of using SPM95 analysis [Friston et al., 1991] to explore task-related differences, all the coregistered SPECT images were transferred to a SUN SPARC Ultra work station (Sun Microsystems, Surrey, UK) and converted to ANALYZETM (CNSoftware LTD, Sussex, UK)/-compatible format. All SPECT scans were spatially normalized to the standard PET template, reformatted to voxel dimensions of $2 \times 2 \times 4$ mm³, and then smoothed with a 12-mm full width at half maximum (FWHM) Gaussian filter. The spatially normalized and smoothed SPECT CBF images were adjusted for individual differences in global blood flow, using proportional scaling to allow a direct comparison with the ROI results. However, the issue of how best to normal-

ize CBF data remains unresolved [Arndt et al., 1996]. Using the SPM95 software, the significance of CBF differences between the two tasks was tested by calculating F-ratios, which were then converted to standard z-scores. The search space for analysis was 71,878 voxels consisting of transverse sections from 34 mm below to 70 mm above the intercommissure plane. Clusters of contiguous voxels greater than 30 (approximately 480 mm²), activated at the $P < 0.01$ (uncorrected) level, with peak z-scores > 2.8 and located within hypothesized regions, were considered significant. The locations of local maxima were then expressed as coordinates in the stereotaxic space of Talairach and Tournoux [1988].

Region of interest (ROI) analysis

ROI analysis was performed using the previously coregistered data sets (both MRI and SPECT), and ROIs were individually drawn on axial (A) and coronal (C) slices (Fig. 1), using the stereotaxic atlas of Talairach and Tournoux [1988] as a reference system. For each subject, the ROI templates were drawn using an appropriate slice either axial (−6–14) or coronal (−10–6) of each individual's MRI. Quantitative CBF data were then derived for each ROI in each activation condition by measuring the activity produced when superimposing the templates from the MRI onto each of the coregistered SPECT scans. Regional CBF was normalized using proportional scaling by dividing the average counts per mm² of each ROI by the average number of counts per mm² across the relevant slices. ROI changes in HMPAO uptake were calculated by subtracting the normalized matching activity values from the normalized encoding activity values for each ROI. t-tests of this data were carried out to express comparisons between the two conditions, yielding a resultant encoding activation pattern. Conversely, subtraction of the normalized encoding activity from the normalized matching activity yielded a resultant matching activation pattern.

RESULTS

Behavioral data

Matching performance

Analysis of response choice on the matching task revealed that subjects carried out the matching procedure with a mean success rate of 81% (SD = 19.7%).

Figure 1.

Application of the proportional grid system of Talairach and Tournoux [1988] as an approximate MRI slice reference system for the localization of ROIs. Axial slice numbers are represented along the vertical axis, where slice 0 corresponds to the CA-CP line. Coronal slice numbers are represented along the horizontal axis, where slice 0 corresponds to the verticofrontal plane (VCA).

Memory performance

The results of the debriefing and memory assessments showed that, overall, the encoding task produced better memory for detailed information regarding the stimuli than did the matching task. The results of task debriefing (Table IIa) illustrated that all three estimates of information-processing distinguished between tasks. While this distinction was highly significant for information concerning both the relationship between features and the spatial location of features, the estimated picture comprehension displayed a weaker significance. The results of the recall test (Table IIa) illustrated that the subjects' ability to accurately recall the stimuli was significantly better following the encoding than the matching task, supporting the conclusion that a greater degree of associative encoding had occurred. However, the results of the forced-choice recognition test (Table IIa) did not distinguish between the two tasks, although the results obtained from the recognition debriefing measures clearly distinguished the two tasks (Table IIb). This suggests that while the objective measure of recognition did not distinguish between tasks, more subjective measures of stimulus choice confidence in the forced choice paradigm did distinguish between tasks.

Regional cerebral blood flow

Results from the SPM and ROI analysis are displayed in Table III, with the resultant encoding activa-

TABLE II. Memory performance on postactivation tasks

a. Encoding and matching debriefing									
	Picture comprehension			Identification of spatial location			Knowledge of feature relations		
	Mean %	SD	<i>P</i>	Mean %	SD	<i>P</i>	Mean %	SD	<i>P</i>
Encoding task	80.4	20.2	<0.05	81.6	16.4	<0.001	81.4	20.3	<0.0005
Matching task	65.5	33.2		39	30.2		17.5	17.5	
Tests of recall and forced-choice recognition									
	Recall			Recognition					
	Mean %	SD	<i>P</i>	Mean %	SD	<i>P</i>			
Encoding task	73.3	19.6	<0.0005	94.5	7.7	n.s.			
Matching task	25.4	14.2		93.7	3.2				
b. Recognition debriefing									
	Confidence			Negative choice					
	Mean %	SD	<i>P</i>	Mean %	SD	<i>P</i>			
Encoding task	90.3	7.5	<0.005	8.4	9.3	<0.05			
Matching task	79.7	10.4		14.4	14.2				

tions in Table IIIa and the resultant matching activations in Table IIIb. All SPM activations included in Results were either hypothesis-driven activations or were not hypothesis-driven but were confirmed using the ROI analysis. Hypothesized ROI activations were included if they were significant at an individual 0.05 *P*-level, or if a trend suggesting a hypothesis-driven activation was found.

Results using SPM analysis

Encoding activations. These results reveal several prominent activations (Table IIIa). Two large activations were identified, one in the left medial temporal region including both the hippocampus and parahippocampal gyrus, BA28/35, and one in the left cingulate gyrus in a region of the anterior cingulate cortex bordering on the anterior-posterior divide, BA23/24. The cingulate cortex activation, while displaying its peak activity on the left, spreads into the right cingulate cortex, although to a much lesser extent. The activity in the right cingulate cortex does not meet the necessary criteria for classification as an individual activation, and therefore its interpretation remains unclear. A third area of activation peaked close to the right prefrontal cortex between BA46 and BA9. A second, much weaker activation which did not quite meet the criteria for entry in Table III but is important to note, was situated in the lower portion of the right

frontal lobe, extending from the lowest point of BA45 down through BA47 to BA11, displaying 72 voxels at an individual *P* value of 0.001. Two activations were located in the lateral temporal cortex: on the right in the anterior temporal fusiform gyrus, BA21/20, and on the left in the superior temporal gyrus, BA42. Finally, a small region of activation was located across the medial frontal and cingulate cortex of the left hemisphere, BA32.

Matching activations. The largest and most significant activations were found in the left and right occipitoparietal cortex, involving predominantly BA19 but also BA7 (Table IIIb). An area of activation was found in the right middle frontal gyrus, BA9 (extending towards BA46), and one further activation was found in the inferior region of the posterior parietal cortex of the right hemisphere, the supramarginal gyrus, BA40. Finally, a spurious left-sided activation (48 voxels, $z = 3.5$) was located according to the stereotaxic coordinates, within the anterior portion of the left lateral ventricle.

Results using ROI analysis

Encoding activations. The results obtained using the ROI analysis, to a great extent, replicated the SPM findings (Table IIIa). Thus, activations were found in

TABLE IIIa. Resultant encoding activations*

z-score	Size (voxels)	X	Y	Z	BA SPM	Brain region	BA ROI	Slice	Size (mm ²)	P
3.14	233	-16	-10	-16	28/35	Left medial temporal gyrus	28/35	C-2	201	0.02
3.03		-24	-14	-24						
3.81	229	-8	-16	32	23/24	Left cingulate gyrus	23/24	A6/7	481	0.005
3.5		-6	-16	28						
3.49	126	30	24	28	46/9	Right middle frontal gyrus	46/45	A4/5	347	0.0005
2.7		22	32	16						
3.80	98	56	-4	-24	21/20	Right temporal fusiform	21/20	C0	199	n.s. (0.5)
3.1		54	-6	-28		gyrus				
3.15	48	-46	-26	16	42/39	Left superior temporal gyrus	42	A3/4	687	0.004
2.6		-50	-32	24						
3.23	48	-16	22	36	32	Left medial frontal/cingulate	32	A6	233	0.02
2.7		-16	32	28						
						Right medial temporal gyrus	28/35	C-2	188	0.08
						Right cingulate gyrus	23/24	A6/7	477	0.04

TABLE IIIb. Resultant matching activations*

z-score	Size (voxels)	X	Y	Z	BA SPM	Brain region	BA ROI	Slice	Size (mm ²)	P
3.68	139	-22	-76	36	19/7	Left cuneus	19/7	A6/7	449	0.003
2.6		-6	-80	28						
3.72	105	34	-70	36	19/7	Right cuneus	19/7	A6/7	465	0.03
2.77		24	-64	44						
2.83	75	46	24	28	9/46	Right middle frontal gyrus	9	A4/5	793	0.007
2.4		48	26	36						
3.65	46	50	-50	36	40/39	Right inferior parietal lobe	40/39	A6/7	870	0.04
3.1		56	-52	28						

* Encoding (a) and matching (b) activations revealed by using SPM and ROI. SPM activations are expressed in terms of z-scores, with their size in voxels and localized with reference to Brodmann areas (BA) and Talairach coordinates (X, Y, Z). The coordinates of the peak value for each activation are in bold; the second set of coordinates provides further information concerning the spatial extent and direction of the activation. ROI activations are expressed in terms of *P*-values, with their size expressed in terms of size, in mm², of the region analyzed and their localization with reference to Brodmann's areas (BA) and the MRI slice (see Fig. 1) on which the region was drawn.

the left medial temporal (BA28/35), cingulate (BA23/24), superior temporal (BA42), medial frontal/cingulate (BA32), and right middle frontal (BA46) cortices. An activation was also found in the right cingulate cortex (BA23/24). This is particularly interesting, as the SPM results suggested a right cingulate cortex activation but failed to distinguish it from the activation on the left. In addition, a trend suggesting an activation in the right medial temporal region (BA 28/35), the same region as that found activated on the left with both SPM and ROI, was found with this technique. The SPM activation found in the right anterior temporal fusiform gyrus was, however, not found with the ROI analysis.

Matching activations. The matching activations obtained with the ROI analysis also replicated the SPM

findings (Table IIIb). The ROI analysis produced bilateral occipito-parietal activations involving BA19 and touching on BA7, a right middle frontal activation focused on BA9, and a right inferior parietal activation crossing the BA40/39 boundary. A further activation was located in the left caudate nucleus (*P* = 0.035) following a specific analysis directed towards accurately locating the spurious SPM activation, which had been localized in the left lateral ventricle.

DISCUSSION

This SPECT study aimed to replicate and extend the findings of Grady et al. [1995] by identifying the neuroactivations produced by the encoding of novel scenes, and to test the hypothesis that medial temporal

activations will be obtained with the active encoding of new associations rather than with the spontaneous encoding that may accompany novelty. The study also aimed to compare the activation pattern produced by SPM analysis with that produced by ROI analysis.

Methodological issues

Assessment of memory processing

One of the most important aspects of the design of an activation study must be the methods of ensuring that the cognitive processes, predicted to be carried out during a task, were indeed carried out. This involves not only the careful design of the activation tasks but also the assessment of performance. With particular reference to encoding studies, it is important to establish the level of memory processing that occurred during activation by using postactivation tests, unless a retrieval activation condition is included for all stimuli. In the present study, the matching task was designed to avoid a ceiling effect by manipulating item difficulty and thereby filling the 4 sec with perceptual matching, thus reducing the amount of active encoding that might otherwise occur. The average success rate of 81% on this task suggests that these criteria may have been met. In our view (and in line with results from pilot work), in the matching condition, subjects were engaged in matching for most of or all of the 4-sec exposure so that a) their attention would have been held by each picture almost certainly for the full exposure time, and b) they would have engaged in far less associative semantic encoding than in the encoding condition. However, the true extent of the encoding that occurred can only be established through postactivation memory tests.

In this study, it is clear that the level of encoding was quite different in the two tasks, since performance on the recall task was significantly higher ($P < 0.0005$) for the encoding stimuli than for the matching stimuli. This difference was further supported by the results of the task debriefing questions, which revealed that significantly more associative encoding was reported to have been carried out during the encoding than the matching task. It is, however, important to note here that subjects' performance on the forced-choice recognition test failed to distinguish between the tasks. This failure was possibly caused by a ceiling effect, as the recognition task was unfortunately too easy. Even if the failure to find a difference was not caused by a ceiling effect, it would not mean that semantic associative memory was the same in the two conditions, because there is much evidence that item recognition is

less sensitive than free recall to encoding tasks that encourage the formation of associations [e.g., Bruce and Fagan, 1970]. The good recognition performance following the matching condition, therefore, might mean that, in this condition, subjects spontaneously engaged in enough low-level item encoding to produce good recognition. However, they did not report much associative encoding activity in the postmatching debriefing, which explains their poor recall performance. By contrast, responses to the postencoding debriefing indicated a high level of associative encoding, which in turn produced good performance on both recognition and recall. Finally, the different levels of encoding produced by the two tasks are also illustrated through the postrecognition debriefing results, which suggest that response confidence on the recognition test was significantly higher for the encoding stimuli, while negative responding was significantly higher for the matching stimuli. Therefore, although a degree of spontaneous encoding occurred during the perceptual matching task, the memory test results suggest that this was not particularly associative in nature. There is strong evidence, however, that the encoding task produced a much higher and richer level of associative encoding.

Comparing SPM and ROI analyses

The comparison of SPM and ROI analyses is important, since the accurate localization of activations is crucial to their theoretical explanation and the subsequent development of hypotheses and models. It could be argued that since SPM involves the spatial normalization of data using a standard template, the individual variability in brain size and structure is ignored in the analysis, thus threatening localization accuracy. In contrast, the ROI approach does not involve spatial normalization, and regions are identified individually on each subject's MRI. The results of this comparison display a high degree of consistency, since for both matching and encoding conditions, the majority of activations were found using both methods of analysis (Fig. 2a,b). First and most importantly, the left medial temporal lobe and cingulate cortex encoding activations were consistent across the techniques, as was the lack of any medial temporal activation in the matching condition. Second, an unexpected right middle frontal-encoding activation and two small superior temporal and medial frontal activations were also replicated using both techniques.

However, a number of inconsistencies were found which need to be addressed in some depth to allow an accurate assessment of the implications. An SPM

Figure 2.

Comparison of patterns of activation produced by SPM (a and c) and ROI (b and d) analyses during the encoding (top) and matching (bottom) tasks. ▲, SPM encoding activations; △, ROI encoding activations; ●, SPM matching activations; ○, ROI matching activations; *, “missing” activation, indicating a region of inconsistency where an activation was identified using one form of analysis only.

encoding activation was found in the right temporal fusiform region (BA21/20) which was not found using an ROI approach. There are two possible explanations for this. First, it may simply be that the specific region activated (98 voxels) was missed in the ROI analysis. Second, the activation may have been lost by adopting too large an ROI across which the CBF was averaged, thereby weakening any suggestion of an activation through partial volume effects. The remaining inconsistencies involve the reverse findings, where activations found with ROI analysis were not found using SPM. Of these, there were two encoding activations, one in the right cingulate cortex, and the other (a trend) in the right medial temporal lobe region (both of which cover the same areas as those found activated on the left). This is a particularly interesting ROI finding, as it suggests that the encoding task, used in this study, may have produced bilateral medial temporal lobe activations similar to those in Stern et al. [1996], rather than the unilateral activation suggested by SPM. However, before accepting this ROI finding, an attempt must be made to explain the inconsistency.

It becomes particularly relevant here to consider the possibility that a medial temporal lobe activation occurred during the matching task, since the behavioral data indicated that a degree of encoding, albeit spontaneous, must have taken place. The requirements of the matching task and the specific orienting instructions used, as well as the kind of debriefing responses given, make it likely that any medial temporal lobe activation that did occur was focused on the right, since the information that was processed was predominantly spatial in nature. It is also highly likely that the encoding task itself produced some right medial temporal lobe activation, since this task also involved the processing of spatial information. There is, therefore, a possibility that the encoding minus matching subtraction produced a weak right medial temporal encoding activation. However, a combination of the resultant weakness of this activation, the relatively small size of the medial temporal structures, and SPM's use of spatial warping to standardize the localization may explain SPM's failure to demonstrate this activation. Moreover, since the hippocampi and parahippocampal

TABLE IV. Summary of features contrasting SPM and ROI analytical techniques

	Statistical parametric mapping	Regions of interest
Assumptions about data	Low neuroanatomical variability	None
Recommended study population	Normal healthy subjects	Subjects with gross structural abnormalities
Resources required	Computer power	Expert knowledge Labour MRI
Study design	Exploratory Hypothesis-driven	Hypothesis-driven
Advantages	Standardized Exploratory	Permits neuroanatomical variability Facilitates analysis of regional dissociations
Limitations	Spatial warping Multiple comparisons	Labor-intensive Nonstandardized Partial volume effects

gyri are clearly definable structures on MRI, partial volume effects, which may act to weaken an ROI activation in larger structures, were unlikely to occur here.

Therefore, while the SPM and ROI analyses produce generally consistent results, it is clear that, in certain circumstances, problems may occur. An ROI activation may not be found if the activation itself is small but is situated within a large structure with few structural indicators to direct the drawing of the region. Conversely, an SPM activation may not be found if it is either weak or small and undergoes spatial warping; the combined effect would then be to “dilute” the activation. Overall, these results suggest that the SPM analysis can obtain a level of localizing accuracy broadly similar to that obtainable with the ROI analysis. In turn, this suggests that studies requiring the use of an ROI approach can be compared directly with those adopting an SPM analysis.

The choice of whether to adopt an SPM or ROI analysis technique may depend on the type of investigation being carried out (see Table IV). There are also a number of advantages and disadvantages to each technique which will be considered here. There are three main advantages that SPM has over ROI analysis. First, the ROI technique is particularly labor-intensive, since ROIs are drawn individually onto each subjects' MRI. This could be partially overcome by using an ROI template. However, this would be at the expense of the ROI technique's specific ability to consider individual neuroanatomical variability in the

analysis. A second and related disadvantage of the ROI technique is its dependence on expert knowledge to identify brain regions. Therefore, in studies where neuroanatomical variability may be a key factor, the disadvantage of the combined requirements of labor intensiveness and expert knowledge may be outweighed by the specific need to take individual variability into account in the analysis. The third advantage of SPM is that of standardization, as this technique incorporates the coordinates of the brain atlas of Talairach and Tournoux [1988] to provide a reference for the site of an activation. While it can be argued that this atlas is based on the information provided by only one brain and therefore cannot in any way allow for individual neuroanatomical variability, it does introduce a degree of standardization whereby results from different research groups can be compared directly. The ROI analysis can also provide information based on the atlas of Talairach and Tournoux [1988] to aid in the standardization of localization. However, this again is labor-intensive and requires expert knowledge.

There are two potential disadvantages of SPM. The principal disadvantage, alluded to earlier, is its use of spatial warping to achieve a level of spatial normalization that enables the mapping of the data onto the atlas of Talairach and Tournoux [1988]. The warping, which involves 12 affine parameter transformations, may reduce the accuracy of localization, and in some cases where weaker activations are being differentially displaced in each subject, the overall result of the warping may be the loss of the activation altogether. However,

it is clear from the present study that SPM produces activation results which are similar to those produced using an ROI approach and, therefore, in studies where individual neuroanatomical variability is low (as in normal healthy subjects), this disadvantage of SPM is outweighed by its advantages over the ROI approach. There is currently a second disadvantage to the SPM technique. Despite recent developments in SPM, it currently remains unclear exactly how an acceptable level of significance should be determined, taking into account whether or not an activation was hypothesized, how many comparisons were carried out, and the spatial extent of an activation. In comparison to a hypothesis-driven ROI approach, SPM is potentially much more subject to the production of false-positive errors, and while SPM studies generally report the significance criteria they adopt, practice across groups has certainly not been standardized.

The type of investigation being carried out in terms of the questions it asks and the subject groups it uses should determine which method of analysis is most appropriate (see Table IV). In studies involving normal healthy subjects where intersubject neuroanatomical variability can be assumed to be at a minimum, the SPM technique has clear advantages and should be used where the facilities (especially computing power) exist. If SPM is unavailable, an ROI approach is best used with a hypothesis-driven study, which would maximize efficiency by limiting the regions for analysis to those that are integral to the hypothesis and make use of the advantages of the ROI approach. If a study is designed to investigate neuroactivation in patients who have a gross neuroanatomical abnormality (e.g., substantial neurosurgical lesion, head injury), then the use of a hypothesis-driven ROI approach is recommended, since this technique will accommodate neuroanatomical abnormalities. The use of SPM to analyze data from groups of subjects with significant structural abnormalities would inevitably produce inaccuracies in localization, as the fitting of an abnormal brain to a standard template would make incorrect assumptions about the data. The present study has shown that SPM and ROI results in normal subjects were highly comparable, and so the use of SPM with a healthy control group and ROI for a clinical group should produce results that can be sensibly compared. Therefore, the design of comparative studies contrasting normal and brain-damaged populations might make use of both analytical techniques. Moreover, since SPM allows for a level of exploratory analysis that would be excessively labor-intensive with an ROI approach, the normal-population SPM results could be used to direct a second round of ROI analysis of the structurally

abnormal data, focusing on regions that had produced powerful but unpredicted results in the normal SPM data.

Using SPM and ROI on different sets of data (i.e., control and patient) allows us to benefit from the specific advantages of each technique. However, the two methods of analysis might also be used on the same data set. Apart from a study designed to compare SPM and ROI approaches such as the present one, an ROI analysis could also be used to clarify the localization of an SPM activation. The combined use of SPM and ROI may prove to be particularly beneficial in certain situations. For example, in the present study, an SPM matching activation whose coordinates placed it inside the left anterior lateral ventricle required explanation. This localization must be spurious, as there is no blood flow through this region. However, the set of coordinates offered for the activation did little to clarify what structures were actually being activated. A focused ROI analysis was then carried out to attempt to explain these activations. Since the coordinates for the activation projected down from $z = 20$ to $z = 16$, an ROI analysis was carried out on structures with blood flow situated immediately below the local maxima for this activation. The results of this analysis revealed an activation within the left caudate nucleus which was not found on the right. A specific ROI analysis may therefore be adopted to clarify or confirm issues of localization, as in the example described by Kapur et al. [1996], where an SPM analysis using different filter widths produced distinctly different localizations. In this case, an ROI analysis focusing on the particular regions in question might reveal important information concerning the relative activation levels of the two regions. The nature of the SPM cingulate cortex encoding activation, obtained in the present study, illustrates another example of how a combined SPM and ROI analysis might be beneficial. SPM appears to collapse activations in the left and right cingulate cortex into one activation and does not allow these two neuroanatomically distinct regions to be considered separately, therefore leaving a degree of uncertainty. However, the ROI technique analyzes the two regions separately, revealing two distinct activations. Related to this is the ability of the ROI technique to investigate the presence of single and double dissociations between blood flow changes hypothesized to occur in different regions. This direct comparison between regions whose blood flow changes may not have reached predetermined significance levels can greatly strengthen resultant neuropsychological conclusions. In this way, hypotheses relating specifically to the relative activation in two or more regions might best be tackled using a

combined ROI and SPM approach. This combined approach may be particularly appropriate when the exact location of an activation is of critical importance to a hypothesis.

Finally, the question of whether or not to accept an activation as significant in a normal subject group might also be addressed by combining SPM and ROI analyses. It is generally recognized [Friston et al., 1991] that if a hypothesis predicts an SPM activation in a given region, then an uncorrected significance can be accepted. However, if an unpredicted activation is revealed which does not maintain significance when corrected for multiple comparisons, its interpretation must be left open. Therefore, in the same way that a comparative study's SPM findings from normal subjects can direct a second round of ROIs in a brain-damaged population (as described above), an SPM-directed ROI analysis might also confirm or reject the significance of unpredicted SPM activations found in a normal population.

Neuropsychological issues

The pattern of encoding and matching activations found is generally consistent with neuropsychological theory derived from lesion studies. In this study, we accepted as activations those which were hypothesis-driven as well as those which were not, provided they were confirmed using the ROI analysis. The encoding task produced activations in the medial temporal, cingulate, temporal fusiform, and superior temporal cortices, while the matching task produced activations bilaterally in the occipito-parietal cortex and in the right inferior parietal lobe (supramarginal gyrus). The present SPECT technique only permits two conditions to be compared, so a lower-level contrast was not possible. The conclusions drawn here cannot, therefore, exclude possible interpretations involving deactivations occurring during the matching task. However, Grady et al. [1995], who included a low-level contrast, did not find evidence for such deactivations with their matching task, which was similar to ours, so this is not a serious worry. Since the task-related activations we found can be predicted on the basis of evidence available in both the lesion and neuroactivation literatures, a detailed discussion will not be provided on each. However, two specific issues are particularly relevant to the main aim of our study, and the remaining discussion will focus on these.

Frontal lobe activations and encoding

The encoding condition used in this study did not produce the left frontal activations that might have

been predicted by both the Hemispheric Encoding/Retrieved Asymmetry (HERA) [Tulving et al., 1994c] and the novelty-encoding network models. Instead, two relatively large right frontal lobe activations were revealed during encoding, together with one smaller left-sided and more medial activation. The HERA model and its related novelty-encoding network model predict that the encoding condition in the present study would produce left frontal cortex activations, reflecting both the episodic encoding component of the task and the semantic retrieval subprocess of episodic encoding. Furthermore, these models would predict little, if any, right frontal activation, as this is considered to be mediated by episodic retrieval processes. While the small left medial frontal activation may fit the HERA model, the remaining pattern of frontal lobe activations clearly fails to do so as do the results of three studies which contrasted the encoding of novel and familiar pictures [Gabrieli et al., 1997; Tulving et al., 1996; Stern et al., 1996]. Although Stern et al. [1996] found no frontal lobe activations, it is interesting that the right frontal lobe activation, reported by Gabrieli et al. [1997], partially overlapped with the region activated by encoding in our study, even though different baseline conditions were used. It is possible that this activation is associated with particular encoding operations more likely to be performed on complex pictorial stimuli than simpler verbal stimuli. One possibility would be that this region organizes spatial encoding operations. It should be pointed out, however, that HERA cannot account for the results of all studies that examine verbal encoding without post hoc modifications. For example, the study of Habib et al. [1996] of the encoding of novel and familiar words and the study of Kapur et al. [1996], which compared the reading and encoding of word pairs, both produced results that are difficult to reconcile with HERA.

The two right frontal lobe encoding activations we found reflect activity in two separate frontal regions and are likely to reflect separate components of the encoding task. The smaller of the two activations was found in the inferior frontal gyrus of area 47 bordering on area 45, while the larger and more significant activation extended across area 46 and into area 9 of the middle frontal gyrus (Fig. 3b). It is well-established that the frontal association cortex plays an executive role in the processing of information involving the monitoring, planning, and searching components of both encoding and retrieval [e.g., Mayes, 1988]. It is, therefore, probable that these two right frontal lobe activations were tapping into more than one such process. Buckner [1996] points out that the HERA model suffers from two important limitations. First, its

Figure 3.

SPM z-maps of activated regions superimposed onto SPM96b MRI template. **a:** Left cingulate cortex and left medial temporal lobe encoding activations. **b:** Right frontal cortex and left medial frontal/cingulate cortex encoding activations. The left cingulate cortex encoding activation can also be seen on the axial slice, illustrating its extension into the right cingulate cortex. **c:** Right frontal cortex, parietal lobe, and bilateral occipito-parietal cortex matching activations.

neuroanatomical definitions are too broad, and second, it focuses closely on differences between memory processes, but pays little attention to their common features. With respect to the first limitation, more attention to the precise location of activations using reference to Brodmann's areas (if not stereotaxic coordinates), rather than the basic right/left distinction, might help explain the conflicting results that neuroactivation studies are yielding. With respect to the second limitation, the particular nature and diversity of the types of processes thought to be mediated by the frontal regions call for extra care in the design and interpretation of the cognitive subtraction paradigm. Whether the operations performed by different frontal lobe regions are best described in terms of currently used memory distinctions (e.g., encoding and retrieval, and/or semantic and episodic memory), or in

terms of different executive operations and perhaps how these interact with different kinds of information, has not been resolved by neuroimaging research at this time.

It is relevant to point out that the matching condition used in the present study also produced a right frontal lobe activation which was adjacent to the larger of the two encoding activations. However, it extended more laterally into area 9 (Fig. 3c). It is possible that matching activated the right frontal lobe regions also activated by encoding, but to a lesser extent. A comparison of the executive processes involved in the matching and encoding tasks used here might conclude that while the tasks were similar in their monitoring demands, they were perhaps quite different in their searching and planning demands. The matching task, while initially involving some planning in order to

develop an appropriate strategy, becomes quite an automatic task, using the same strategy for all stimuli. The strategy would, however, involve a high level of searching. The encoding task, on the other hand, while involving some search processes, depends ultimately on planning and strategy selection, which would have to be reviewed for each stimulus because of the semantic associative nature of the encoding required. This interpretation in terms of the demands the tasks make on executive processing suggests that the right frontal lobe matching activation may reflect search processes, whereas the right frontal lobe encoding activation may reflect planning and strategy selection. Even though this interpretation is merely tentative, it is becoming apparent that an analysis of frontal lobe activations, restricted to the comparison of memory processes alone, will lead to oversimplified interpretations and misleading conclusions.

Role of the medial temporal lobe

We wished to determine whether dedicated SPECT can detect medial temporal lobe activation produced by encoding information with a view to remembering it. Our results confirmed the effectiveness of dedicated SPECT by showing a clear activation of the left medial temporal lobe in both the hippocampus and parahippocampal gyrus (Fig. 3a) when the encoding of complex pictures was compared with matching such pictures, and giving an indication that there may have been a weaker activation of the right medial temporal lobe region. We also wished to determine whether the encoding of associative information was sufficient to produce this activation. This was achieved by controlling novelty across the conditions in the manner of Grady et al. [1995] while manipulating the level and kind of associative processing across conditions through the use of specific orienting tasks. Our findings differed from those of Grady et al. [1995] as we identified predominantly left-sided activations, while their study yielded a right medial temporal activation during encoding. This is most likely to be due to differences in task design, including: a) stimulus material, which in the present study would be more readily verbalized than would faces; b) the comparison matching tasks, where the present study required more spatial processing, possibly resulting in a significant degree of single-item encoding (as explained above); and c) the nature of the encoding carried out, which in the present study was predominantly semantic. The last two possibilities receive support from a study by Martin et al. [1997], who found greater right than left activations when encoding object forms (item encoding), and greater left

than right medial temporal activation during semantic encoding of object pictures as well as words.

Since the encoding condition in the present study led to more left medial temporal activation than did the matching condition, it seems likely that this activation was caused by the encoding or early consolidation of some kind of associative information rather than by novelty detection alone. It is plausible that subjects will be more likely to think that a picture is like a familiar one or mistakenly think that it actually is familiar when they process it for meaning (as they did in the encoding condition) than when they process it at a low level (as they did in the matching condition). If this is what happens, then subjects would show more medial temporal lobe activity in the matching condition, were novelty detection alone the sole source of the activity. Our conclusion that associative encoding was sufficient to produce medial temporal lobe activation is probably conservative, although our assumption that meaningful processing makes the identification of novelty less likely needs confirmation.

Although Grady et al. [1995] found medial temporal lobe activation while controlling for novelty, it is harder to infer from their results what kind of information was encoded to produce the activation. This is because it is unclear what kind of processing subjects engaged in during the encoding condition, because no attempt was made to control this by instructing subjects to process the faces in a particular way. In our study, subjects were instructed to encode in an associative manner, and that they had done so was confirmed by debriefing questions. That encoding has led to effective storage can be confirmed by using memory testing, although the presence of effective associative encoding can only be shown by using either a recognition test in which there are foils that recombine the features encoded at study (which neither Grady et al. [1995] nor we did, even though we used straightforward picture or item recognition), or by using an appropriate recall test (which only we did). Our recognition results suggested that the encoding condition led to more confident picture recognition memory, and, more importantly, our recall results strongly suggest that this condition led to better associative memory. In contrast, Grady et al. [1995] did not report any memory test results, although they did note that another of their studies had shown that item recognition for faces was better following encoding than it was following matching. They did not show, however, that face encoding was associative, as this would have required using foils that were recombinations of studied faces.

Any encoding study which does not control for degree of novelty or familiarity across tasks leaves the explanation for why encoding causes medial temporal lobe activation even less constrained than does the study of Grady et al. [1995]. In such studies, one cannot determine whether medial temporal lobe activations have been generated by the encoding of specific kinds of information such as associations, by processes connected with the greater novelty or familiarity of the studied material, or by both of these things. Both encoding and novelty accounts have several possible variants. With the encoding account, activation could be caused by associative encoding, by the consolidation of associations that this triggers, or by both of these. With the novelty account, Tulving et al. [1994b, 1996] argued that novelty detection alone is sufficient to activate the medial temporal lobe region. Another variant of this account, considered by Tulving et al. [1996], is that priming in the familiar condition is associated with a reduced activation of the medial temporal lobe region where the encoded information is represented. Several studies have shown that activation in the regions which probably represent remembered information is typically reduced when the processing is repeated with the same information [e.g., Martin et al., 1995; Demb et al., 1995]. If the information encoded in an imaging study is associative and if associative information is partially represented in the medial temporal lobes, then it is possible that repeated or familiar associative information will produce less activation in this region. No one has attempted to distinguish between the novelty detection and priming variants of the novelty account of medial temporal lobe activations. Discrimination may be difficult, and other variants of this account may need to be considered.

Although failure to control for novelty and kind of encoding prevents the possibility of identifying whether novelty or associative encoding accounts of medial temporal lobe activation apply, such identifications have been made in picture encoding studies that did not do these things. Thus, Tulving et al. [1994b] postulated that novelty detection alone was sufficient, whereas Stern et al. [1996] postulated that associative encoding was responsible. Stern et al. [1996] also suggested that medial temporal lobe activations may be easier to produce when complex visual materials such as faces and pictures are encoded than when verbal materials are used, because the visual material is more likely to be encoded associatively. When encoding is spontaneous, it is more likely to be elaborative and associative when stimuli are novel and more complex, and this has probably been the case more often in neuroimaging studies involving encoding of

visual than of verbal stimuli. This suggestion is consistent with our view that the encoding (or early consolidation) of associations is sufficient to produce medial temporal lobe activation. Nevertheless, one would expect verbal encoding to be associative when subjects are directed to encode in this way, and it should also be associative even when it is spontaneous, provided verbal materials are complex and novel.

Therefore, it is interesting that two recent PET studies have found that encoding verbal materials led to medial temporal lobe activation. In the first study, Pardo et al. [1996] found left parahippocampal activation during the spontaneous encoding of word lists (complex and relatively novel) and concluded that the left medial temporal lobe appears specialized for the encoding of linguistic information. Unfortunately, although the encoding in this study probably was associative, because it was spontaneous and uncontrolled one cannot be sure.

In the second study, Kapur et al. [1996] compared the activation produced by the directed associative encoding of moderately related word pairs to that produced by the reading of word pairs. As in the present study, Kapur et al. [1996] encouraged associative processing in the encoding condition by requesting that subjects use the semantic link between words to develop a meaningful association that would aid memory performance later. They found several interesting encoding activations, including one in the left medial temporal lobe, which is probably related to the greater associative encoding engaged in during this condition. As they, like us, matched across conditions for novelty of the materials to be encoded, their results, like ours, imply that associative encoding (or early consolidation) is sufficient to produce medial temporal lobe activation. Kapur et al. [1996], however, raised some doubts about the validity of their medial temporal lobe activation, which appears to have been located near the border of the critical region. Their doubts, which were referred to earlier in the Discussion, were based on finding that peak activation shifted from the medial temporal region with a 10-mm FWHM filter down to the inferior fusiform gyrus with a 15-mm FWHM filter. These doubts would be unfounded if two separate activations were occurring, but that their relative size and power together with the overall resolution of the system did not allow two separate peaks to be identified. From our theoretical perspective, there should be involvement of the medial temporal region in encoding and especially in the type of encoding Kapur et al. [1996] encouraged, and the findings of the present SPECT study as well as other PET and fMRI studies lend support to this belief.

Moreover, one might also expect the encoding condition of Kapur et al. [1996] to produce a fusiform activation, reflecting the increased object processing required for associative encoding.

Provisionally at least, therefore, Kapur et al. [1996] and this study suggest that associative encoding of both verbal and visual materials is sufficient for producing medial temporal lobe, and probably hippocampal, activation. Our study is the only one to do this with complex pictures, because although three other studies showed that encoding such items causes medial temporal lobe activation, they all compared activation produced by the encoding of novel pictures with activation produced by some task that involved familiar pictures [Tulving et al., 1994b; Stern et al., 1996; Gabrieli et al., 1997]. Thus, in their PET study, Tulving et al. [1994b] compared activation produced by counting the number of novel pictures which had been recently shown (actually none in the scan window) with that produced by counting the number of novel pictures included in a set of pictures that had been shown the previous day (also none in the scan window). This study did not try to control encoding directly. Similarly, in their fMRI study, Stern et al. [1996] made a comparison between the spontaneous encoding of novel pictures and the repeated encoding of the same familiar picture. In contrast, in their fMRI study, Gabrieli et al. [1997] tried to control the encoding processes by comparing the activations produced by subjects discriminating whether a novel picture was of an outdoor or indoor scene with the activations produced by performing the same task on repeated and hence familiar pictures. In all three studies, novelty detection alone or associative priming may have been sufficient to cause the greater medial temporal lobe activation seen in the novel item condition, so they failed to show whether encoding or consolidating associations is sufficient. If one assumes that the subjects in Gabrieli et al. [1997] performed the same processing operations equally well with novel and familiar pictures, then this study shows that novelty detection alone or priming (or both) can produce a medial temporal lobe effect. This assumption of similar encoding across conditions is unlikely to apply to the other two studies, because the richness of spontaneous encoding is probably a function of perceived novelty, so that both novelty detection or priming (or both) and associative encoding may differ in the encoding condition.

Whether or not one accepts that Gabrieli et al. [1997] showed that the detection of novel pictures or priming of certain pictorial associations affects the medial temporal lobe depends on whether one believes their subjects processed the novel and familiar pictures in

the same way. The likelihood of this is very hard to determine, but presumably relates to how fully the orienting task filled the time allocated to each picture. It is interesting to note that a recent study of word encoding, which used a similar procedure to that of Gabrieli et al. [1997], failed to find that encoding novelty activated the medial temporal lobe region. In this study, Habib et al. [1996] used orthographic and semantic orienting tasks with spoken or visually presented novel or familiar words. Although novelty was associated with activation in several brain regions, it did not lead to medial temporal lobe activation. It remains to be shown whether this was because: 1) novelty has a different effect with verbal and pictorial materials, 2) the novelty effect depends on encoding associations and these were not encoded in Habib et al. [1996], or 3) novelty has no effect on the medial temporal lobe region with either words or pictures and that the appearance of this arises from experiments where novel stimuli actually are more richly encoded.

Support for the importance of novelty may be derivable from anatomical evidence. If both novelty detection (and/or priming) and associative encoding (or early consolidation) cause activation of the medial temporal lobe region, it may be that they involve activity in distinct medial temporal lobe regions. Although this need not be true, if it were, then the location of the activation we observed should be distinct from that found by Gabrieli et al. [1997], provided their activation effect was caused by novelty detection alone (and/or priming), and should be at least partially distinct from the activations reported by Tulving et al. [1994b] and Stern et al. [1996], which may have been caused by novelty detection (and/or priming) as well as associative encoding. Whereas Stern et al. [1996] found bilateral activation in both the posterior hippocampus and adjacent parahippocampal gyrus, and Gabrieli et al. [1997] found it in a posterior region focused on the parahippocampal gyrus, Tulving et al. [1994b] found a right-sided activation in posterior regions of both the hippocampus and parahippocampal gyrus. Unlike all three of these studies, we only found weak evidence of a right-sided activation, although the left-sided activation included both the hippocampus and parahippocampal gyrus. The activations we found also seemed to be slightly anterior to those of the other three studies (Fig. 3a). Comparison of the four studies, therefore, weakly suggests that novelty detection and/or priming of associations may involve slightly more posterior medial temporal lobe structures than does the encoding or consolidation of associations. However, any conclusions drawn from this type of comparison must allow for differences

between the studies in scanner resolution (fMRI, PET, and SPECT) and methods of data analysis (i.e., data averaging). For example, the two emission studies (Tulving et al. [1994b] and the present study) used averaged data, whereas the two fMRI studies (Gabrieli et al. [1997] and Stern et al. [1996]) reported individual subject data.

This comparison's value is also limited by two further factors. The first factor is the ability of current imaging techniques to localize and resolve distinct patterns of activation. Confidence in the above conclusion regarding the relative position of the medial temporal activities, would be stronger if a double dissociation were shown in a single study between an activation associated with novelty at encoding and an activation associated with associative encoding/consolidation. If this were shown using an ROI approach, problems with warping distortions could be avoided. The second factor is that the exact nature of the processes involved in the tasks being compared is likely to be critically important, and differs across the four studies. For example, our baseline condition involved a matching task which was difficult and probably involved some kind of spatial associative encoding, the performance of which could have activated the right medial temporal lobe region almost as much as the encoding task. In contrast, the baseline condition in Tulving et al. [1994b] and Stern et al. [1996] involved relatively spontaneous processing of familiar pictures. Subjects may not spontaneously process familiar pictures in a spatial associative manner, so that more right-sided activation is seen with novel compared to familiar pictures. These problems would be minimized if a double dissociation between a novelty effect and an associative encoding/consolidation effect were shown in a single study and replicated in further studies that used subtle theory-driven variations in the comparison conditions.

Our results and those of Kapur et al. [1996] provide preliminary support for the view that the encoding/consolidation of picture and word-related associations activates the medial temporal lobe region. Future work will have to address four issues that have not been resolved by the current work.

First, it will have to identify exactly which medial temporal lobe regions are activated, possibly using the greater localization and resolution abilities that fMRI is likely to have in the future. At present, it is hard to know whether the hippocampus, parahippocampal gyrus, or both are implicated.

Second, future work will need to identify exactly what kind of information needs to be encoded to activate different parts of the medial temporal lobe

region. Although our left-sided activation may have reflected verbal and semantic associative encoding and the hint of right-sided activation may have reflected spatial associative encoding, this remains to be proved. In order to specify precisely what kinds of associative encoding or consolidation activate the medial temporal lobe region, encoding conditions that differ in a more fine-grain fashion will have to be compared. Our active encoding condition differed from the matching condition in requiring particular kinds of semantic and item-location associative encoding, but it needs to be shown convincingly that these specific kinds of associative encoding or consolidation activate the medial temporal lobe structures. One might, for example, compare semantic encoding conditions that either link two items semantically or that semantically encode the two items separately in order to identify the activation produced by associative semantic encoding.

Third, future work will need to determine whether novelty detection and/or associative priming also activates this region. There is some support for this, although the evidence needs to be strengthened. Future studies must attempt both to control encoding, matching it between familiar and novel materials, and to differentiate between novelty detection and associative priming by using appropriate procedures.

Finally, neuroactivation studies of amnesics with selective damage to parts of the hippocampal circuit that lie outside the hippocampus (e.g., to the fornix) will need to be performed to see whether medial temporal lobe activation is caused by the encoding or the early consolidation of associative information. There is some evidence that amnesics encode normally [e.g., Mayes et al., 1993], but this could be independently demonstrated. If this proves possible and the patients still fail to show a normal activation in their structurally intact medial temporal lobe regions when encoding associative information, then this would strongly support the view that this activation is caused by the consolidation of associative information.

ACKNOWLEDGMENTS

We thank Mary-Theresa Hansen and Marion Cresswell for their assistance in the administration of SPECT scans. This study was funded by the Neuroscience Foundation.

REFERENCES

- Arndt S, Cizadlo T, Andreasen NC, Zeien G, Harris G, O'Leary DS, Watkins GL, Ponto LLB, Hichwa RD (1995): A comparison of approaches to the statistical analysis of (O^{15}) H_2O PET cognitive activation studies. *J Neuropsychiatr Clin Neurosci* 7:155–168.

- Arndt S, Cizadlo T, Oleary D, Gold S, Andreasen NC (1996): Normalizing counts and cerebral blood flow intensity in functional imaging studies of the human brain. *Neuroimage* 3:175–184.
- Baddeley AD, Emslie H, Nimmo-Smith I (1994): “The Doors and People Test,” Bury St. Edmunds: Thames Valley Test Co.
- Barnes A, Wyper DJ, Montaldi D, Patterson J (1996): Effect of restricted image data on automated co-registration algorithms: A method of investigation. *Neuroimage* 3:48.
- Barnes A, Dai D, Montaldi D, Patterson J, Wyper D (1997): Image quality versus statistical power. *Nucl Med Commun. Vol. 18*:1155–1160.
- Beversdorf D, Metzger S, Nelson D, Alonso R, Knight J (1995): Single-word auditory stimulation and regional cerebral blood flow as studied by SPECT. *Psychiatry Res* 61:181–189.
- Bruce D, Fagan RL (1970): More on the recognition and free recall of organized lists. *Journal of Experimental Psychology* 85: 153–154.
- Buckner RL (1996): Beyond HERA: Contributions of specific prefrontal brain areas to long-term memory retrieval. *Psychonomic Bull Rev* 3:149–158.
- Crosson B, Williamson DJG, Shukla SS, Honeyman JC, Nadeau SE (1994): A technique to localize activation in the human brain with technetium-99m-HMPAO SPECT: A validation study using visual stimulation. *J Nucl Med* 35:755–763.
- Demb JB, Desmond JE, Wagner AD, Vaidya CJ, Glover GH, Gabrieli JDE (1995): Semantic encoding and retrieval in the left inferior prefrontal cortex: A functional MRI study of task difficulty and process specificity. *J Neurosci* 15:5870–5878.
- Fletcher PC, Frith CD, Grasby PM, Shallice T, Frackowiak RSJ, Dolan RJ (1995): Brain systems for encoding and retrieval of auditory-verbal memory: An in vivo study in humans. *Brain* 118:401–416.
- Friston KJ, Frith CD, Liddle PF, Frackowiak RSJ (1991): Comparing functional (PET) images: The assessment of significant change. *J Cereb Blood Flow Metab* 11:690–699.
- Gabrieli JDE, Brewer JB, Desmond JE, Glover GH (1997): Separate neural bases of two fundamental memory processes in the human medial temporal lobe. *Science* 276:264–266.
- Grady CL, McIntosh AR, Horwitz B, Maisog JM, Ungerleider LG, Mentis MJ, Pietrini P, Schapiro MB, Haxby J (1995): Age-related reductions in human recognition memory due to impaired encoding. *Science* 269:218–221.
- Grasby PM, Frith CD, Friston KJ, Simpson J, Fletcher PC, Frackowiak RSJ, Dolan RJ (1994): A graded task approach to the functional mapping of brain areas implicated in auditory-verbal memory. *Brain* 117:1271–1282.
- Habib R, Wheeler M, Cabeza R, Houle S, Tulving E (1996): Brain regions associated with encoding of novel information: A positron emission tomography study. *Soc Neurosci Abstr* 22:1865.
- Kapur N, Friston KJ, Young A, Frith CD, Frackowiak RSJ (1995): Activation of human hippocampal formation during memory for faces: A PET study. *Cortex* 31:99–108.
- Kapur S, Craik FIM, Jones C, Brown GM, Houle S, Tulving E (1994): Neuroanatomical correlates of encoding in episodic memory: Levels of processing effect. *Proc Natl Acad Sci USA* 91:2008–2011.
- Kapur S, Tulving E, Cabeza R, McIntosh AR, Houle S, Craik FIM (1996): The neural correlates of intentional learning of verbal materials: A PET study in humans. *Cogn Brain Res* 4:243–249.
- Martin A, Lalonde FM, Wiggs CL, Weisberg J, Ungerleider LG, Haxby JV (1995): Repeated presentation of objects reduces activity in ventral occipital cortex: An MRI study of repetition priming. *Soc Neurosci Abstr* 21:1497.
- Martin A, Wiggs CL, Haxby JV, Weisberg J (1997): Modulation of hippocampal activity by stimulus type and experience. *Neuroimage* 5:628.
- Mayes AR (1988): *Human Organic Memory Disorders*, Cambridge: Cambridge University Press.
- Mayes AR, Downes JJ, Shoqeirat M, Hall C, Sagar HJ (1993): Encoding ability is preserved in amnesia: Evidence from a direct test of encoding. *Neuropsychologia* 31:745–759.
- Nelder JA, Mead R (1965): A simplex method for function minimization. *Comput J* 7:308–313.
- Nelson HE, Willison JR (1991): *National Adult Reading Test (NART)*, Windsor: NFER-Nelson.
- Nyberg L, McIntosh AR, Houle S, Nilsson LG, Tulving E (1996): Activation of medial temporal structures during episodic memory retrieval. *Nature* 380:715–717.
- Pardo JV, Temporini HD, Pardo PJ, Lee JT (1996): Encoding of memory in medial temporal cortex: I. Word list memorization. *Neuroimage* 3:553.
- Robert PH, Migneco O, Benoit M, Darcourt J, Ellul E, Benoliel J, Bonhomme P, Aubin V, Bussier F, Darcourt G (1995): A single photon emission tomography study of cerebral regional perfusion changes induced by a learning task in subjects with mild memory impairment. *Psychiatry Res* 61:231–241.
- Shallice T, Fletcher P, Frith CD, Grasby P, Frackowiak RSJ, Dolan RJ (1994): Brain regions associated with acquisition and retrieval of verbal episodic memory. *Nature* 368:633–635.
- Sharp PF, Smith FW, Gemmell HG, Lyall D, Evans NRS, Guozdanicov D, Davidson J, Tyrrell DA, Pickett RD, Neirinckx R (1986): Technetium-99m HMPAO stereoisomers as potential agents for imaging regional cerebral blood flow in human volunteer studies. *J Nucl Med* 27:171–177.
- Squire LR, Ojemann JG, Miezin FM, Petersen SE, Videen TO, Raichle ME (1992): Activation of hippocampus in normal humans: A functional anatomical study of memory. *Proc Natl Acad Sci USA* 89:1837–1841.
- Stern CE, Corkin S, Gonzalez RG, Guimaraes AR, Baker JR, Jennings PJ, Carr CA, Sugiura RM, Vedantham V, Rosen BR (1996): The hippocampal formation participates in novel picture encoding: Evidence from functional magnetic resonance imaging. *Proc Natl Acad Sci USA* 93:8660–8665.
- Stoddart HA, Stoddart HF (1992): New multi-dimensional reconstructions for the 12-detector, scanned focal point, single-photon tomograph. *Phys Med Biol* 37:579–586.
- Talairach J, Tournoux P (1988): *Co-Planar Stereotaxic Atlas of the Human Brain*, New York: Thieme Medical Publishers, Inc.
- Tulving E, Kapur S, Markowitsch HJ, Craik FIM, Habib R, Houle S (1994a): Neuroanatomical correlates of retrieval in episodic memory: Auditory sentence recognition. *Proc Natl Acad Sci USA* 91:2012–2015.
- Tulving E, Markowitsch HJ, Kapur S, Habib R, Houle S (1994b): Novelty encoding networks in the human brain: Positron emission tomography data. *Neuroreport* 5:2525–2528.
- Tulving E, Kapur S, Craik FIM, Markowitsch HJ, Houle S (1994c): Hemispheric encoding/retrieval asymmetry in episodic memory: Positron emission tomography findings. *Proc Natl Acad Sci USA* 91:2016–2020.
- Tulving E, Markowitsch HJ, Craik FIM, Habib R, Houle S (1996): Novelty and familiarity activations in PET studies of encoding and retrieval. *Cereb Cortex* 6:71–79.
- Ungerleider LG (1995): Functional brain imaging studies of cortical mechanisms for memory. *Science* 270:769–775.
- Worsley KJ, Evans AC, Marrett S, Neelin P (1992): A three-dimensional statistical analysis for CBF activation studies in human brain. *J Cereb Blood Flow Metab* 12:900–918.