Rapid Communication

Encoding activity in anterior medial temporal lobe supports subsequent associative recognition

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Received 14 April 2003; revised 22 September 2003; accepted 23 September 2003

The ability to bind information together, such as linking a name with a face or a car with a parking space, is a vital process in human episodic memory. To identify the neural bases for this binding process, we measured brain activity during a verbal associative encoding task using event-related functional MRI (fMRI), followed by an associative recognition test for the studied word pairs. Analysis of the encoding data sorted by the associative recognition accuracy allowed us to isolate regions involved in successfully creating associations. We found that encoding activity in bilateral anterior medial temporal lobe (MTL) regions was greater for successfully bound pairs, that is, those later recognized as intact, than for all other pairs. These findings provide evidence that the anterior medial temporal lobes support the successful binding of information in memory.

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Keywords: Magnetic resonance imaging; fMRI; Memory; Hippocampus; Medial temporal lobe; Associative recognition; Binding

Introduction

Patients with damage to the medial temporal lobes (MTLs), including the hippocampus and parahippocampal regions, exhibit severe deficits in episodic memory (Scoville and Milner, 1957), the ability to remember information embedded in a specific context (Tulving, 1983). Such impairments are present even in cases of damage limited solely to the hippocampus, suggesting that this structure plays a key role in the episodic memory system (Cave and Squire, 1991; Squire, 1992). Further studies have proposed that these regions may be involved in associating or binding the multiple elements that comprise a learning experience (Eichenbaum and Bunsey, 1995). While these data support a role for the MTL in the episodic memory system, data from studies of neuropsychological lesions typically do not permit distinctions between encoding and retrieval processes within episodic memory.

Functional neuroimaging provides a noninvasive means of observing the neural correlates of episodic memory function in humans that allows researchers to isolate activity to either encoding or retrieval stages of memory processing. Such studies have begun to illuminate the role of MTL in specific aspects of encoding and retrieval (for review see Schacter and Wagner, 1999). Previous neuroimaging studies have focused on the process of associative encoding by comparing associative encoding conditions with those that placed reduced demands on associative processes, such as single word encoding or rote rehearsal. Overall, the pattern of results from these studies suggests greater hippocampal activity during associative encoding than during nonassociative encoding (Sperling et al., 2001; Zeineh et al., 2003). However, the associative conditions in these studies include both successfully “bound” items and “unbound” items, for which no association was remembered, raising the question of whether the accompanying neural responses contribute to successful binding, or simply reflect the attempt to create associations regardless of outcome.

Based on the suggestion that the hippocampus is engaged by tasks requiring participants to relate multiple stimuli (Bussey and Eichenbaum, 1996; Henke et al., 1997), recent subsequent memory studies have employed associative encoding conditions in attempts to more directly engage the hippocampus and related structures in the MTL. Some studies have failed to report hippocampal activation during individual item encoding (Otten and Rugg, 2001), while others have found some evidence during the encoding of multiple items (Davachi and Wagner, 2002). Importantly, the memory tests employed in these studies have focused on the retrieval of individual items. Thus, one possible reason for the observed inconsistencies in hippocampal and MTL activity is that these regions are specifically involved in the creation of durable links between individual items of information; therefore, activity in this region should support successful performance on a task that requires the retrieval of inter-item associations.

In the present study, we used functional MRI (fMRI) to isolate the neural activity corresponding to associative encoding. We set out to identify brain regions involved in the creation of associations that can later be retrieved. Using event-related fMRI in a subsequent memory paradigm allowed us to contrast brain activity during encoding trials that support successful associative recognition on a subsequent test with those that do not. This study represents the first application of the fMRI subsequent memory paradigm to associative recognition. The employment of an asso-
cative recognition task affords the opportunity to observe discrete binding processes in the brain.

Materials and methods

Participants

Participants were 12 healthy, right-handed, native English speakers (7 women, 5 men; ages 18–25 years) who received $50 each for participation. Informed consent was obtained in a manner approved by the Human Studies Committee of Massachusetts General Hospital.

Stimuli and cognitive task

A list of 1200 nouns of 3 or fewer syllables was used to construct 4 lists of 150 word pairs that were matched for frequency and length (Kucera and Francis, 1967). The test stimuli consisted of 5 lists of 120 pairs. Each test list included 45 critical pairs from the study lists distributed onto the test lists intact and unaltered, 45 rearranged pairs constructed from separate study pairs, and 30 entirely novel pairs. Each participant received three functional scans during the study phase. Across the three scans, 450 study pairs were presented for 4 s each. Periods of visual fixation lasting between 2 and 10 s were pseudo-randomly interspersed between the experimental trials to maximize the efficiency of the design matrix (Dale, 1999). During each 4-s trial, participants performed an associative encoding task that required them to form a mental image incorporating the concepts represented by both words in the pair. They were asked to indicate the quality of the image they formed by pressing one of two keys while the stimuli were on the screen. Due to equipment malfunction, imagery responses and reaction times were only collected for 8 of the 12 participants. Response options included (a) a poor image or no image and (b) a fair to excellent image. The location

Fig. 1. Average number of participant responses to intact pairs sorted by response category and confidence rating.

Fig. 2. Proportion of low and high imagery task responses to intact pairs sorted by test response.

Fig. 3. Results from left hippocampal region of interest. (A) Coronal section from anatomical template showing SPM defining hippocampal ROI. (B) Averaged event-related responses in left hippocampus from 12 participants plotted with standard error. Responses for successful associative binding (“intact”/green) were statistically greater than unsuccessful associative binding responses (“rearranged”/red and “single”/blue).
of each word in a pair and the order of list presentation were counterbalanced between participants. Participants were not told that there would be a subsequent memory test.

Following the three encoding scans, participants were removed from the scanner and taken to a separate room for administration of the associative recognition test. Test pairs were presented for 4 s, during which participants indicated their memory for the pairing with a button press. In this task, we presented participants with pairs from three categories. Intact pairs \( (N = 225) \) were presented unaltered from the study phase; rearranged pairs \( (N = 225) \) included two words from separate study pairs. Pairs of novel words \( (N = 150) \) were also presented. Participants pressed one of four keys to indicate their memory for each presented pair: “intact” (i.e., a pair of words they had seen together in the previous phase), “rearranged” (words from separate study pairs), “single” (a word from the study phase and a novel word), or “new” (two novel words). Successful recognition performance required memory for the specific pairing of words from the encoding task (Donaldson and Rugg, 1998; Glenberg and Bradley, 1979; Humphreys, 1976). After each test pair, participants were also asked to rate confidence (high or low) in their answer. It should be noted that while the test included a “single” response option, there were no test pairs of this type.

All stimuli were presented on a Macintosh G4 desktop computer using PsyScope software (Cohen et al., 1993). Stimuli were projected into the scanner using a rear mounted LCD projector in conjunction with a mirror mounted approximately 2 in. in front of the subject.

**Preprocessing and data analysis**

Data were preprocessed using SPM99 (Wellcome Department of Cognitive Neurology, London). Images were first corrected for differences in slice acquisition timing by resampling all slices in time to match the first slice, followed by motion correction across all three runs (using sinc interpolation). Data were then spatially normalized to an EPI template based upon the MNI305 stereotactic space. Images were resampled into 3-mm cubic voxels and then spatially smoothed with an 8-mm FWHM isotropic Gaussian kernel. Statistical analysis was performed using the general linear model in SPM99.

The subsequent memory analysis involved sorting encoding trials into bins depending on participants’ responses in the associative recognition test. Specifically, trials were divided into two categories depending on how the words were later presented at test...
(intact or rearranged). Trials that were used to construct rearranged test pairs were excluded from the subsequent memory analysis because test responses to rearranged pairs corresponded to multiple encoding trials. Encoding trials for intact test pairs were further sorted by the subject’s associative recognition response into Associative Hit (correct “intact” response), Associative Miss (“rearranged” response), and Single bins. There were too few trials categorized as “new” to permit meaningful analysis. All trials were modeled using a canonical hemodynamic response and temporal derivative. These effects were estimated using a subject-specific fixed-effects model, with session-specific effects treated as confounds. Low-frequency signal components were filtered using the SPM99 default high-pass filter cutoff of twice the maximum time between two events of the most frequent condition. Linear contrasts were used to obtain subject-specific estimates for the contrast of Associative Hits versus Associative Misses. These estimates were entered into a second-level analysis treating subjects as a random effect, using a one-sample t test against a contrast value of zero at each voxel. Statistical parametric maps were created for the Associative Hits versus Associative Misses contrast, and were subsequently characterized using an uncorrected voxel level height threshold of $P < 0.001$ that kept clusters of five or more voxels. In addition to the subsequent memory analysis, we conducted a comparison of all encoding trials to the fixation baseline task to characterize general encoding-related activity. This analysis was identical to the subsequent memory analysis except that the linear contrast consisted of all trials versus fixation.

To further explore the nature of activation associated with each associative recognition outcome condition, regions of interest (ROIs) were identified from clusters that survived the thresholding criteria. The hemodynamic responses were extracted from each ROI on a subject-by-subject basis, adjusted by condition and selectively averaged. The response estimates were then subjected to repeated measures analyses that included factors for condition (Associative Hit, Associative Miss, and Single) and peri-stimulus time (from stimulus onset to 14 s poststimulus onset). Because ROIs were obtained from a direct contrast of Associative Hit and Associative Miss conditions, in the ANOVA, these conditions were compared separately against the Single condition. Percent signal change for functionally defined ROIs was determined using the SPM ROI Toolbox (http://spm-toolbox.sourceforge.net). An additional ROI analysis was conducted using hand-drawn hippocampal regions from individual participants’ high-resolution structural images. Because this analysis yielded similar results to the original ROI analysis, only the original results are reported here.

Results

Behavioral data

The distribution of associative recognition responses across trial types is presented in Table 1. On average, participants correctly recognized nearly two-thirds of all intact pairs (64%) and mistook very few rearranged pairs for intact (12%). To assess levels of associative recognition, we conducted a paired sample t test between the proportion of “intact” responses to intact test items (associative hits) and the proportion of “intact” responses to rearranged test items (associative false alarms). The t test revealed that participants made more “intact” responses to intact pairs than to rearranged pairs ($t_{11} = 10.87, P < 0.01$). “Intact” responses to intact pairs represent accurate associative recognition, while “intact” responses to rearranged pairs represent participants’ willingness to make an “intact” response in the absence of any associative information. Thus, this measure characterizes the difference between participants’ ability to correctly identify intact pairs and their bias to respond “intact” when a pair includes two old items regardless of their original pairing, thereby allowing us to assess participants’ memory for pairings above and beyond memory for items. In addition to significant associative memory, significant recognition of individual items was evidenced by the fact that nearly half of all rearranged pairs (47%) were correctly identified, while few novel pairs were called “rearranged” ($t_{11} = 7.03, P < 0.01$). Participants’ performance was also assessed using signal detection measures of sensitivity ($D’\ mean = 1.55, SE = 0.05$) and bias ($C\ mean = 0.30, SE = 0.03$). In the absence of associative recognition for intact pairs, participants were more likely to make a “rearranged” than a “single” response ($t_{11} = 2.05, P < 0.05$), suggesting that these responses were also driven by successful item memory.

One concern is that when presented with a test pair from which both items were previously encountered, participants might recognize two items, only one item, or neither item. If a response of “single” were not allowed, then participants could begin to make “rearranged” responses after recognizing only a single item in the pair, although they fail to recognize the other member. Thus, we included a “single” response option to prevent contamination of the “rearranged” response category with responses based solely on the recognition of a single item. The range of total “single” responses to all pairs, from 89 to 191 (15–32%) across 12 participants, suggests that participants relied on this option and were unaware of the lack of single test pairs. Fig. 1 shows participant’s responses to intact pairs sorted by response and confidence rating.

Although we were not able to sort functional data by imagery success due to data loss, we sorted the existing participant data by imagery to explore task performance. The proportion of responses for these eight subjects, sorted by imagery and test category, is shown in Fig. 2. Overall, participants formed better images for intact pairs that were subsequently recognized as intact. Participants were also faster at responding to these pairs in the imagery task, as reflected in their reaction times shown in Fig. 3.

Imaging data

Analysis of the imaging data reveals that compared to the fixation baseline, activation during the verbal associative encoding task was primarily left lateralized and included left prefrontal, medial temporal, inferior–posterior temporal, and bilateral occipital
Table 2
Peak voxel coordinates within regions showing greater responses during the associative encoding task compared to fixation baseline

<table>
<thead>
<tr>
<th>Region</th>
<th>Coordinates (MNI)</th>
<th>Max T</th>
<th>P &lt; 0.001 uncorrected</th>
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<td></td>
<td>X</td>
<td>Y</td>
<td>Z</td>
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<tr>
<td>Brainstem</td>
<td>–27</td>
<td>–36</td>
<td>–6</td>
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<tr>
<td>Left superior frontal gyrus</td>
<td>–33</td>
<td>51</td>
<td>18</td>
</tr>
<tr>
<td>Left cerebellum</td>
<td>–6</td>
<td>–57</td>
<td>–21</td>
</tr>
<tr>
<td>Right insula</td>
<td>45</td>
<td>–18</td>
<td>18</td>
</tr>
<tr>
<td>Left putamen</td>
<td>–18</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Right putamen</td>
<td>21</td>
<td>9</td>
<td>–6</td>
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<tr>
<td>Left cerebellum</td>
<td>–3</td>
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<td>–39</td>
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<td>Left middle temporal gyrus</td>
<td>–48</td>
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Table 2 (continued)

<table>
<thead>
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<th>Region</th>
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<td></td>
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<td>0</td>
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cortices. These results are consistent with those of prior neuroimaging studies of verbal episodic encoding (Davachi and Wagner, 2002; Lepage et al., 2000; Nyberg et al., 1996; Wagner et al., 1998).

A list of these regions is included in Table 2.

A contrast of encoding scans for word pairs with differing levels of subsequent associative recognition isolated brain regions that were active during the successful forming of associations. An “intact” response to an intact pair was interpreted as successful associative memory in combination with memory for the items. A “rearranged” response was interpreted as an associative miss with spared item memory. A direct comparison between encoding trials that were subsequently recognized with varying degrees of associative memory—associative hits versus associative misses—revealed activation related to the successful binding of word pairs in memory. Left anterior hippocampus and entorhinal cortex bilaterally showed differential encoding-related activity that predicted successful associative binding. That is, during study pairs later correctly endorsed as “intact” demonstrated significantly greater activation in these regions than pairs that were mistakenly called “rearranged”. The average event-related responses within the left hippocampal region identified from this contrast are shown in Fig. 4. Within the left hippocampal region, repeated measures ANOVA revealed that activity for Associative Hits was greater than activity for single responses ($F_{1,11} = 6.4, P < 0.05$), while activity for Associative Misses was not significantly different from activity for single responses ($F_{1,11} = 0.84, P = 0.38$). All regions showing significant activity in this contrast are listed in Table 3. A region in anterior left inferior prefrontal cortex also demonstrated a successful binding pattern at a slightly relaxed threshold. This region is shown in Fig. 5, with coordinates included in Table 3. A repeated

Table 3
Peak voxel coordinates within regions showing greater responses for successful associative binding than unsuccessful associative binding

<table>
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<th>P &lt; 0.001 uncorrected</th>
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<tbody>
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<td>X</td>
<td>Y</td>
<td>Z</td>
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<tr>
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<td>–27</td>
<td>0</td>
<td>–36</td>
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<tr>
<td>Left entorhinal/perirhinal cortex</td>
<td>–33</td>
<td>6</td>
<td>–27</td>
</tr>
<tr>
<td>Left entorhinal/perirhinal cortex</td>
<td>–39</td>
<td>15</td>
<td>–27</td>
</tr>
<tr>
<td>Right central sulcus</td>
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<td>–21</td>
<td>39</td>
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<tr>
<td>Right cingulate gyrus</td>
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<td>42</td>
<td>0</td>
</tr>
<tr>
<td>Left hippocampus</td>
<td>–18</td>
<td>–6</td>
<td>–21</td>
</tr>
<tr>
<td>Brain stem</td>
<td>12</td>
<td>–24</td>
<td>–15</td>
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<tr>
<td>Left middle temporal gyrus</td>
<td>–57</td>
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<td>–18</td>
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<tr>
<td>Right entorhinal/perirhinal cortex</td>
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measures ANOVA on the responses from this region revealed a marginally significant difference between Associative Hit activity and single activity ($F_{1,11} = 4.64, P = 0.05$), and no difference between Associative Misses and single responses ($F_{1,11} = 0.09, P = 0.77$). An additional contrast conducted to identify regions demonstrating greater activity for associative misses than associative hits revealed no significant activity.

**Discussion**

In the current study, we identified encoding-related activation that is differentiated between successful and unsuccessful binding, allowing us to identify regions that are important for the successful creation of associations in memory. Our observation of left anterior hippocampal and medial temporal activation during successful binding is consistent with the proposal that anterior MT regions are specifically involved in relating or binding multiple elements of an experience (Cohen and Eichenbaum, 1993; Mitchell et al., 2000; Schacter and Wagner, 1999).

The associative recognition paradigm emphasized the retrieval of associative information over individual item information. Correct “intact” responses were presumably driven by a combination of item and associative memory (Hockley and Cristi, 1996; Hockley and Consoli, 1999). However, the use of rearranged pairs at test negates the utility of item information for task performance. Thus, it can be argued that the correct “intact” responses are based primarily on associative information, that is, participants are unlikely to base successful judgments on familiarity of the items alone. Because associative hits likely involve a mix of item and associative information, while associative misses consist of item memory alone, the contrast of associative hit and associative miss conditions, that is, “intact” versus “rearranged”, should isolate regions responsible for the successful formation of associations.

Our findings converge with those from prior neuroimaging studies of episodic encoding. Earlier studies demonstrated that greater hippocampal activity is recruited during associative encoding conditions (Henke et al., 1999; Sperling et al., 2001). It was suggested that this activity reflected the binding of items together in memory. Further studies indicated that the greater hippocampal activity evident under associative encoding conditions was correlated with successful subsequent memory for items (Davachi and Wagner, 2002). The current results extend these prior findings to show that under associative encoding conditions, hippocampal activity predicts memory for inter-item associations beyond memory for individual items.

Our findings also converge with those from recent neuroimaging studies of memory retrieval. Yonelinas et al. (2001) used fMRI to scan participants during two recognition memory tests. In an item recognition test, participants made old and new judgments about previously studied and novel drawings of objects. In an associative recognition test, participants made judgments about the study color of previously presented objects. Retrieval activity was greater for associative recognition than item recognition in bilateral hippocampal and parahippocampal regions. Eldridge et al. showed similar results using a remember–know paradigm, where “remember” responses indicate specific recollection of episodic details and “know” responses indicate familiarity without recollection (Gardiner and Java, 1993). This study demonstrated greater hippocampal responses for items associated with correct remember responses than for those associated with correct know responses or unrecognized items (Eldridge et al., 2000). The findings of these studies support the hypothesis that hippocampal regions are involved in the retrieval of episodic–contextual details. The similarity of these regions to those found in the current study suggests that the role of these regions in associative binding may cut across standard encoding–retrieval lines (see Schacter and Wagner, 1999).

One question with respect to the current findings is whether successful associative recognition depends solely upon successful employment of the imagery strategy at encoding. For example, it may be that only those pairs that included items for which subject formed good images were successfully recognized later. Interestingly, prior fMRI subsequent memory studies have shown MTL activation during encoding that required visual imagery, and not during encoding in which the participants read words backwards (Davachi et al., 2003). In conjunction with the present results, these findings suggest that successful imagery may play some role in eliciting MTL activity. However, if successful associative recognition was confounded with imagery, and thus item encoding in the present study, we might expect to see a graded pattern of encoding activity in hippocampus. That is, we should see the greatest activity for associative hits, with less activity for associative misses (item responses), and even less activity for pairs that received a “single” response indicating that participants remembered only one item. The absence of such a pattern suggests that the observed hippocampal responses reflect a process distinct from imagery or item memory.

Although not apparent in our primary contrast, anterior left inferior prefrontal cortex also demonstrated an activity pattern predictive of successful binding at a slightly lower statistical threshold ($P < 0.002$ uncorrected). LIPC has previously been shown to be involved in the successful encoding of single items (Wagner et al., 1998). In addition, similar regions have been shown to play a role in word pair learning. For example, this region was found to be more active during learning of distantly related pairs than closely related pairs (Fletcher et al., 2000). In a more recent fMRI study, Wagner et al. (2001) observed greater anterior LIPC activity during semantic relatedness judgements when the associative strength between items was weaker. These results demonstrate a role for LIPC in controlled semantic retrieval. Thus in the present study, left inferior prefrontal activity may reflect the processing of semantic attributes of the pairs (Tulving et al., 1994).

Forming episodic memories involves multiple processes from the retrieval and manipulation of prior semantic information to the linking of new and prior information into a memory trace. Recent reviews of the neuroimaging literature (Mayes and Montaldi, 1999) have highlighted the need to assess the role of MTL regions during the encoding of associations. Interestingly, many prior imaging studies employing the subsequent memory paradigm have failed to demonstrate MTL activity that predicted later memory. This result is surprising because this region has long been suspected to play a key role in episodic memory formation. In view of our results, one possible reason for these null findings is the failure of previous studies to isolate those processes that tap hippocampal and anterior medial temporal lobe function. The data presented here suggest that the role of the hippocampus and anterior medial temporal regions in episodic memory may be the creation of lasting links or associations between elements of an experience.
Acknowledgments

This work was supported by NIMH Grant MH60941. O. Jackson was supported by a Ford Foundation Predoctoral Fellowship.

References


