The timing of associative memory formation: frontal lobe and anterior medial temporal lobe activity at associative binding predicts memory

J. B. Hales and J. B. Brewer

Departments of 1Neurosciences and 2Radiology, University of California, San Diego, California

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Hales JB, Brewer JB. The timing of associative memory formation: frontal lobe and anterior medial temporal lobe activity at associative binding predicts memory. J Neurophysiol 105: 1454–1463, 2011. First published January 19, 2011; doi:10.1152/jn.00902.2010.—The process of associating items encountered over time and across variable time delays is fundamental for creating memories in daily life, such as for stories and episodes. Forming associative memory for temporally discontinuous items involves medial temporal lobe structures and additional neocortical processing regions, including prefrontal cortex, parietal lobe, and lateral occipital regions. However, most prior memory studies, using concurrently presented stimuli, have failed to examine the temporal aspect of successful associative memory formation to identify when activity in these brain regions is predictive of associative memory formation. In the current study, functional MRI data were acquired while subjects were shown pairs of sequentially presented visual images with a fixed interitem delay within pairs. This design allowed the entire time course of the trial to be analyzed, starting from onset of the first item, across the 5.5-s delay period, and through offset of the second item. Subjects then completed a postscan recognition test for the items and associations they encoded during the scan and their confidence for each. After controlling for item-memory strength, we isolated brain regions selectively involved in associative encoding. Consistent with prior findings, increased regional activity predicting subsequent associative memory success was found in anterior medial temporal lobe regions of left perirhinal and entorhinal cortices and in left prefrontal cortex and lateral occipital regions. The temporal separation within each pair, however, allowed extension of these findings by isolating the timing of regional involvement, showing that increased response in these regions occurs during binding but not during maintenance.

functional magnetic resonance imaging; human; perirhinal; lateral occipital; encoding

WHEN NAVIGATING THROUGH THE WORLD, people encounter a stream of information. Items that are deemed important will be attended to, and associations will be made between these related items to create more robust memory for the event. What factors predict which associated items will be later remembered? Prior studies using concurrently presented stimuli focused mainly on which regions are involved in forming associations. This approach, however, represents a very limited view of associative memory formation in the real world and misses an important aspect of encoding the stream of information one encounters. Thus recent studies have delved more deeply into associative memory formation to examine how items are linked and encoded across a delay (Hales and Brewer 2010; Hales et al. 2009; Konkel et al. 2008; Murray and Ranganath 2007; Qin et al. 2007, 2009; Sommer et al. 2005a,b; Staresina and Davachi 2009; Takeda et al. 2005). These studies have reported the involvement of medial temporal lobe (MTL) structures as well as additional neocortical regions, including prefrontal cortex (PFC), medial frontal cortex, parietal cortex, and lateral occipital/inferior temporal regions in forming associative memories for temporally discontinuous items. To address how regions cooperate in and contribute to forming these memories, investigation of the time course of activity across the entire encoding event is essential; in humans, however, examination of these temporal components has only recently gained attention.

Beyond these questions regarding the timing of regional contribution to memory formation, fundamental disagreement remains about the specific involvement of MTL substructures in associative memory formation. Although several neuropsychological and neuroimaging studies have reported the involvement of the parahippocampal gyrus (PHG) in associative memory encoding (Chua et al. 2007; Davachi et al. 2003; Davachi and Wagner 2002; Eichenbaum et al. 2007; Gold et al. 2006; Hales and Brewer 2010; Hales et al. 2009; Kirwan and Stark 2004; Murray and Ranganath 2007; Pihlajamaki et al. 2003; Qin et al. 2007, 2009; Staresina and Davachi 2010; Taylor et al. 2006; Tendolkar et al. 2007), studies have suggested functional distinctions between PHG substructures based on associative versus item encoding (Achim and Lepage 2005; Aminoff et al. 2007; Davachi 2006; Peters et al. 2007; Sommer et al. 2005a; Staresina and Davachi 2008, 2009), novel object perception versus spatial processing (Pihlajamaki et al. 2003), encoding versus retrieval process (Daselaar et al. 2006), and context-dependent learning versus explicit recognition memory (Preston and Gabrieli 2008). These results support the separable contribution of particular MTL substructures to different aspects of memory encoding and retrieval.

Many neuroimaging studies have reported that anterior regions of the MTL, such as perirhinal cortex (PRC), entorhinal cortex (ERC), anterior parahippocampal cortex (PHC), and anterior hippocampus, are involved in the formation of associative memories (Aminoff et al. 2007; Chua et al. 2007; Jackson and Schacter 2004; Mayes et al. 2007; Peters et al. 2007; Pihlajamaki et al. 2003; Rauchs et al. 2008; Sperling et al. 2003; Staresina and Davachi 2006, 2009, 2010; Taylor et al. 2006), whereas more posterior regions of the PHC and hippocampus are involved in visual item memory (Kirchhoff et al. 2000; Peters et al. 2007; Rauchs et al. 2008). Such findings, however, are not universal. Some studies have suggested that the locus for associative memory formation is the hippocampus, whereas item memory formation preferentially involves PRC (Chua et al. 2007; Diana et al. 2007; Eichenbaum et al. 2007; Staresina and Davachi 2009).
Recently, studies have started addressing this discrepancy by looking more closely at the specific types of associations being made. In a recent review, Mayes et al. (2007) provided support for PRC involvement in within-domain associative encoding and hippocampal involvement in between-domain associative encoding based on human psychological and functional imaging studies as well as human and animal lesion studies. Additional studies have also supported this distinction in PRC and hippocampal contribution to associative encoding, where PRC is involved in forming associations that are unitized or regarding item-related details (such as item-color associations) and the hippocampus is involved in forming domain-general or item-context associations (Diana et al. 2007; Staresina and Davachi 2008, 2010).

Extensive anatomical research of the cortical projections to MTL substructures also supports a functional dissociation within PHG. Tracing studies in the macaque monkey have indicated that PRC receives cortical inputs that are distinct from inputs to PHC (Suzuki and Amaral 1994). ERC receives the majority of its inputs from PRC and PHC but also receives projections from additional neocortical regions, including superior temporal gyrus and orbitofrontal cortex. Similar results have been reported using retrograde tracing in the rat, where PRC and postrhinal cortex each receive distinct cortical and subcortical inputs (Furtak et al. 2007). The anatomical evidence of distinct cortical inputs to PRC and PHC suggests and supports functional differences between anterior and posterior regions of the PHG.

Results from electrophysiological studies in monkeys provide further support for involvement of anterior parahippocampal regions, such as PRC and ERC, in associative memory. Neurons in inferotemporal cortex showed “associative” responses, while monkeys performed a visual paired-associates task (Higuchi and Miyashita 1996; Sakai and Miyashita 1991). In these studies, neurons were identified as “pair coding” if, after training, they showed a preferential response to a stimulus and to its associated pair. Neurons were identified as “pair recall” if, having shown a strong response to a stimulus, the neuron also fired strongly in the period following the presentation of the pair of the stimulus. These responses were identified only in monkeys with an intact entorhinal and perirhinal region. Although anterior PHG lesions ablated the associative memory responses, they did not diminish neuronal responses to individual stimuli (Higuchi and Miyashita 1996).

Electrophysiological studies in monkeys have also examined delay-period activity in PFC and MTL regions during associative encoding of temporally discontiguous stimuli. Foster et al. (2000) recorded extracellularly from dorsolateral prefrontal cortex (DLPFC) while monkeys performed a sound-color associative encoding task. They reported that cells in DLPFC exhibited correlated firing for associated colors and tones and that some of these cells also showed increased firing during the delay between tones and their associated colors (Deco et al. 2005; Foster et al. 2000). Electrophysiological results in rats and monkeys are inconsistent, however, regarding MTL activity during short-delay maintenance. Some studies have reported MTL activity during short-delay maintenance (Caehusac et al. 1989; Watanabe and Niki 1985; Young et al. 1997), whereas others have reported very rare or no MTL activity during the delay period (Hampson and Deadwyler 2003; Vidyasagar et al. 1991). Human lesion and imaging studies looking at working memory also report mixed results of MTL involvement in delay period maintenance (Axmacher et al. 2007; Cave and Squire 1992; Ezzyat and Olson 2008; Grady et al. 1998; Habeck et al. 2005; Hannula et al. 2006; Hartley et al. 2007; Kessler and Kiefer 2005; Monk et al. 2002; Nichols et al. 2006; Olson et al. 2006a; Petit et al. 1998; Picchioni et al. 2007; Piekema et al. 2006; Ranganath et al. 2004; Ranganath and D’Esposito 2001; Shrager et al. 2008; Stern et al. 2001). Although delay period activity has been examined in functional magnetic resonance imaging (fMRI) studies of working memory, the few studies that have looked at associative encoding of temporally discontiguous stimuli have focused on subsequent memory effects during the encoding of the items (Hales and Brewer 2010; Hales et al. 2009; Konkel et al. 2008; Murray and Ranganath 2007; Qin et al. 2007; Qin et al. 2009; Sommer et al. 2005a,b; Staresina and Davachi 2009; Takeda et al. 2005). One exception is a study that examined associative and item encoding of temporally discontiguous stimuli, which showed increased PFC activity during the delay between paired items relative to the delay between unpaired items (Hales et al. 2009). This study, however, only examined successfully encoded paired and unpaired items; therefore, intrapair delay period activity has yet to be explored in relation to subsequent associative memory.

The current study examines the time course of activity across the entire associative encoding event of two temporally discontiguous items. MTL and PFC activity during this associative encoding task were examined using rapid event-related fMRI, and the subsequent associative and item memory for the visual stimuli were determined using a postscan recognition test. By presenting each item individually and controlling for item memory strength, brain activity in response to successful associative binding could be isolated. Based on previous findings, the prediction was that anterior MTL regions, such as PRC, ERC, and anterior hippocampus, and PFC regions would show increased activity for successful associative binding. In addition, subsequent associative memory effects were predicted to occur in frontal regions during both maintenance (following presentation of the first item of the pair) and binding (once the second item of the pair was presented); however, subsequent associative memory effects were predicted to occur in anterior MTL regions only during binding with no difference during maintenance.

**MATERIALS AND METHODS**

**Subjects.** Fifteen healthy volunteers (mean age 26.6 ± 3 yr, 7 males) were recruited from the University of California, San Diego (UCSD) community and the surrounding area. All subjects gave informed consent approved by the UCSD Institutional Review Board and had normal or corrected vision.

**Stimuli.** Two hundred ninety color images of everyday objects were used as stimuli in this experiment. While subjects were in the scanner, 250 of the images were presented sequentially; a plus sign was presented during the interitem delay to link each set of two images and reduce cross-pair binding. During the postscan recognition test for item memory, the remaining 40 stimuli were included as foils. Images were acquired from Rossion and Pourtois color Snodgrass images (Rossion and Pourtois 2004) and the Hemera object library (Hemera Technologies).

**Experimental procedure.** During the associative encoding task in the scanner, subjects were shown pairs of sequentially presented

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individual images, with each image presented for 2 s (Fig. 1A). All items were paired pseudorandomly to remove obvious semantic relationships between pairs. Between the two images of a pair, a fixed interitem delay of 5.5 s was used with a plus sign presented in the center of the screen for the first 0.5 s of the delay followed by a blank screen for the remaining 5 s. Between pairs were jittered intertrial intervals (ITIs) ranging between 0.5 and 10.5 s. The ITIs were calculated to optimize the study design for modeling the hemodynamic response to trials (Dale 1999; Dale and Buckner 1997). Subjects were told to remember all individual images. Subjects were also instructed to associate the image that preceded the plus sign (1P) with the image that followed the plus sign (2P) and to remember the items as a pair. By separating the 2P item from the instruction to associate (plus sign), this design allowed for isolation of the response to associative binding. Subjects were given a button box and asked to press a left or right button if the image represented a living or nonliving object, respectively, to make sure that subjects were attending to each image. One hundred twenty-five image pairs were presented to subjects in the scanner across five 383-s runs. Each image was presented once, and objects in each pair were unrelated.

After the encoding task in the scanner, subjects completed a self-paced postscan recognition test to examine subsequent item and associative memory. Subjects were shown all stimuli previously viewed during the encoding task that followed the plus sign (2Ps) as well as 40 novel stimuli that were used as foils for the item memory question. For each of the 165 stimuli, subjects were asked to rate their confidence that the picture was new or that it was shown during the scan (old) on a “1, definitely new” to “6, definitely old” scale (Fig. 1B). For trials in which the object was previously viewed during encoding, subjects were given an immediate follow-up question in which they were shown two choice stimuli, A and B (both of which were previously shown during encoding), and asked to rate their confidence that the picture was paired with image A or B on a “1, definitely A” to “6, definitely B” scale (Fig. 1B). All 125 2P images from the encoding task were judged in this manner; the 40 novel items were also judged in the same manner, but without a follow-up question. This recognition test lasted ~30 min.

**fMRI parameters.** Subjects were scanned using a 3T GE scanner at the Keck Center for Functional MRI at UCSD. Functional images were acquired using gradient-echo, echo-planar, T2*-weighted pulse sequence (repetition time = 2.5 s; one shot per repetition; echo time = 30; flip angle = 90°; bandwidth = 31.25 MHz). Forty slices covering the brain were obtained perpendicular to the long axis of the hippocampus with 4 × 4 × 4-mm voxels. Field maps were acquired to measure and correct for static field inhomogeneities (Smith et al. 2004). A T1-weighted structural scan was acquired in the same plane and with the same voxel size as the functional scans. A high-resolution structural scan was also acquired sagittally using a T1-weighted (1 × 1 × 1-mm) inversion recovery prepared fast spoiled gradient recalled sequence.

**Data analysis.** After functional data from each run were field map corrected (Smith et al. 2004), slices were temporally aligned and coregistered using a three-dimensional image alignment algorithm, voxels outside the brain were eliminated using a threshold mask of the functional data, and functional runs were corrected for motion and concatenated, all using the AFNI suite of programs (Cox 1996). A 4.0-mm FWHM Gaussian filter was also applied to smooth the functional data from each run. A general linear model was constructed using multiple regression analysis; six motion regressors obtained from the registration process were included along with eight behavioral regressors based on subsequent memory performance. Subjects’ behavioral trials were sorted on the basis of accuracy and subject ratings of item memory confidence and associative memory confidence. On the basis of item memory confidence, trials were divided into four outcomes: high-, medium-, and low-confidence hits (“definitely,” “probably,” or “maybe old,” respectively) and misses (“definitely,” “probably,” or “maybe new,” together). Associative memory was defined as successful (associative) or unsuccessful (item only) on the basis of testing responses. Associative trials were those in which the subject indicated the correct pair with responses of “definitely” or “probably.” Item-only trials were those in which the subject indicated the incorrect pair with responses of “definitely” or “probably” or made any “maybe” judgment. For each outcome, a hemodynamic response function was derived from the fMRI data using signal deconvolution with TENT basis functions and a defined time window of 22.5 s following the onset of each 1P stimulus (Cox 1996). Multiple linear regression analyses were used to examine activity only during the encoding of items that were later remembered with high confidence, with separate measures for when targeted associative information was remembered (associative) or forgotten (item only). These were the only two conditions used for analysis; therefore, all discussions of associative and item-only trials are referring to trials with high-
Table 1. Behavioral results

<table>
<thead>
<tr>
<th>Memory Question</th>
<th>Memory Outcome</th>
<th>Percentage of Trials</th>
<th>Average Trials/Subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>High confidence</td>
<td>61 ± 5*</td>
<td>76.1 ± 6.1</td>
</tr>
<tr>
<td>Association</td>
<td>Associative</td>
<td>76 ± 5†</td>
<td>59.9 ± 7.3</td>
</tr>
<tr>
<td>Item only</td>
<td></td>
<td>24 ± 5†</td>
<td>16.2 ± 3.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Value is a percentage of all trials. †Value is a percentage of high-confidence item trials.

confident memory with associative memory or high-confidence item memory without associative memory.

Structural and functional data were transformed into Talairach space (Talairach and Tournoux 1998) by AFNI using nearest-neighbor interpolation (Cox 1996) after standard landmarks, including the anterior and posterior commissures, were manually defined on the anatomical scans. Whole brain voxelwise t-tests (2-tailed) carried out across all 15 subjects were conducted to examine which brain regions showed more activity for associative versus item-only memory encoding. Difference during each time period of the event (i.e., 1P, 2P) was examined separately. To correct for multiple comparisons and yield a whole brain significance value of P < 0.01 corrected for all comparisons (based on Monte Carlo simulations), functional clusters of least five contiguous voxels were identified in this condition. The average hemodynamic response function was extracted for each cluster of interest.

To improve MTL alignment between subjects, the region of interest large deformation diffeomorphic mapping (ROI-LDDMM) alignment technique (Miller et al. 2005) was applied. Bilateral hippocampus and subregions of PHG, including PRC, ERC, and PHC, were defined for each subject on Talairach transformed images. Previously described landmarks were used to define PRC and ERC (Insausti et al. 1998) and PHC (Stark and Okado 2003). These defined anatomical regions of interest for each subject were normalized using ROI-LDDMM to a modified model of a previously created template segmentation (Kirwan et al. 2007). Functional imaging data, after being corrected for spatial distortions with the use of field maps acquired during each subject’s scanning session (Smith et al. 2004), underwent the same ROI-LDDMM transformation as was applied to the anatomical data. Active voxels in the associative minus item-only condition, P < 0.05, that were located in the MTL were identified using a mask of the anatomically defined MTL substructures.

RESULTS

Behavioral analysis. Analyses were focused on trials in which 2P stimuli were recognized with high confidence [61 ± 5% (SE) of all trials]. Of these strongly remembered stimuli, the correct associative pair was identified with medium to high confidence at a rate of 76% (±5% SE; associative condition), and the correct associative pair was not identified or was identified with low confidence at a rate of 24% (±5% SE; item-only condition). Subjects’ memory performance was generally accurate, leading to a large number of associative trials, with a mean of 59.9 (±7.3 SE) trials per subject. There were fewer comparison trials in which the subject confidently remembered the item but forgot the association; this item-only condition had a mean of 16.2 (±3.0 SE) trials per subject. Behavioral results are summarized in Table 1.

fMRI analysis. By holding item memory strength constant, brain regions with selective involvement in the successful formation of associative memory could be isolated. These associative memory binding regions were identified where the size of the blood oxygen level-dependent (BOLD) response was greater during the encoding of the 2P stimulus when the association was remembered than when it was forgotten (associative minus item-only trials). Regions identified by this contrast (P < 0.01, corrected) are listed in Table 2. Left frontal regions, including DLPFC, ventrolateral prefrontal cortex (VLPFC), middle frontal cortex, and medial frontal cortex, as well as left lateral occipital/fusiform cortex, showed increased activity during associative trials relative to item-only trials; this increase was present not during the encoding of the 1P stimulus or during the interitem delay but only once the 2P stimulus was presented and the two items could be associated (Fig. 2, A–D). Left DLPFC, medial/middle frontal cortex, and lateral occipital/fusiform cortex each showed a response to both the 1P and 2P stimulus for the associative and item-only trials, with a larger response to the 2P stimulus only in the associative trials. Left VLPFC, however, only responded to the 2P stimulus during associative trials, with no response during item-only trials. No significant clusters were identified in the reverse contrast during binding (item-only trials > associative trials, P < 0.01, corrected). Associative memory analyses were also performed focusing on the 1P and delay time periods of the encoding event, and there were no regions showing greater activity for associative trials relative to item-only trials during either time period. The only region showing significant subsequent associative memory effects during the delay period was located in right superior temporal gyrus, which showed greater suppression during associative trials relative to item-only trials; no regions showing significant subsequent associative memory effects were identified during the encoding of the 1P item.

To examine the specific contribution of MTL regions to the successful binding of associative information, we isolated active voxels from the associative memory contrast (associative minus item-only trials, P < 0.05) that overlapped with the anatomically defined MTL substructures. These active voxel clusters were located in left PRC and left ERC (Fig. 3, A and B, and Table 2). Because of the size of the active voxel clusters in these small anatomical regions, these clusters did not survive cluster size-based correction for multiple comparisons and there-

Table 2. Significantly active brain regions for associative vs. item-only trials

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Cluster Volume (mm$^3$)</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>t Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left medial/middle frontal (BA 6)</td>
<td>1,984</td>
<td>-18</td>
<td>-1</td>
<td>56</td>
<td>4.6394</td>
</tr>
<tr>
<td>Left lateral occipital (BA 19)</td>
<td>1,216</td>
<td>-46</td>
<td>-57</td>
<td>-4</td>
<td>4.0167</td>
</tr>
<tr>
<td>Left dorsolateral prefrontal (BA 46)</td>
<td>704</td>
<td>-38</td>
<td>31</td>
<td>12</td>
<td>4.3769</td>
</tr>
<tr>
<td>Left ventrolateral prefrontal (BA 47)</td>
<td>512</td>
<td>-42</td>
<td>35</td>
<td>-4</td>
<td>4.7694</td>
</tr>
<tr>
<td>Right superior parietal/postcentral (BA 7)</td>
<td>320</td>
<td>14</td>
<td>-45</td>
<td>72</td>
<td>4.3278</td>
</tr>
</tbody>
</table>

P values indicate significantly active brain regions for associative vs. item-only trials. Coordinates correspond to the voxel of maximum intensity for each cluster (see text). *Values are corrected for multiple comparisons. †Values are uncorrected for multiple comparisons (active voxels for associative vs. item-only trials overlap with anatomically defined medial temporal lobe substructures).

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fore were reported as uncorrected values. Similar to the activity reported in left frontal regions and lateral occipital/fusiform, left PRC and ERC showed a greater response during the encoding of 2P stimuli only when associative binding was successful. There were no voxels in MTL regions during the delay period showing greater activity for associative trials relative to item-only trials.

General item subsequent memory effects have been extensively explored in prior studies (beginning with Brewer et al. 1998 and Wagner et al. 1998), and such analyses were not the

Fig. 2. Activity in left frontal and lateral occipital regions predicts the successful associative binding of items. Statistical activation maps for regions showing increased activity during binding ($P < 0.01$, corrected for multiple comparisons) for associative trials compared with item-only trials are overlaid on sagittal and coronal slices of mean anatomical scan images across all 15 subjects. Functional clusters located in left (L) dorsolateral prefrontal cortex (DLPFC; A), ventrolateral prefrontal cortex (VLPFC; B), medial/middle frontal cortex (C), and lateral occipital/fusiform cortex (D) were used for time-course analyses. Graphs depict the time course of percent signal change in these regions for each condition beginning with the onset of the first stimulus of each pair, 1P. The blue bar represents the time of stimulus presentation, and the green bar represents the time of associative instruction presentation (plus sign). Error bars represent the standard error of the mean.

Fig. 3. Activity in left perirhinal and entorhinal cortex predicts the successful associative binding of items. Statistical activation maps for regions showing increased activity during binding ($P < 0.05$, uncorrected) for associative trials compared with item-only trials are overlaid on sagittal and axial slices of mean anatomical scan images across all 15 subjects. Graphs depict the time course of percent signal change in left perirhinal (A) and entorhinal (B) cortices for each condition beginning with the onset of the first stimulus of each pair, 1P. These clusters were isolated from voxels functionally defined in the contrast of associative memory trials relative to item-only memory trials ($P < 0.05$) that were located in anatomically defined MTL regions, left perirhinal and entorhinal cortices. The blue bar represents the time of stimulus presentation, and the green bar represents the time of associative instruction presentation (plus sign). Error bars represent the standard error of the mean.
focus of the current study. Nevertheless, noted is the single activation predictive of high-confidence subsequent item (only) memory ($P < 0.01$) in right lateral occipital/fusiform cortex. These findings of both item and associative memory effects in lateral occipital/fusiform cortex complement results from a previous study (Hales and Brewer 2010) that found this area to be the only region of overlap between subsequent associative and item memory contrasts.

**DISCUSSION**

The current study identified subsequent memory effects in the MTL, PFC, and lateral occipital/fusiform cortex during associative encoding of temporally discontiguous images. Left frontal and lateral occipital cortices, like left PRC and ERC, showed increased activity during successful associative binding. Activity in these regions during the interitem delay, however, did not predict subsequent associative memory.

**PRC/ERC involvement in associative memory formation.** When item memory strength was controlled for, subsequent associative memory effects for image pairs were found in left PRC and ERC in the present study. These results complement multiple studies that have reported the involvement of PRC, ERC, and other anterior regions of the MTL in successful associative encoding (Aminoff et al. 2007; Chua et al. 2007; Haskins et al. 2008; Jackson and Schacter 2004; Mayes et al. 2007; Peters et al. 2007; Pihlajamaki et al. 2003; Rauchs et al. 2008; Sperling et al. 2003; Staresina and Davachi 2009, 2010; Taylor et al. 2006). A recent study examining MTL activity for a visual associative memory task in which subjects saw objects presented against one of two backgrounds (providing source information) found increased right PRC activity for correct source encoding (Peters et al. 2007). The present finding of increased PRC activity during associative encoding has been supported in other studies examining memory for source information (Tendolkar et al. 2007), picture pairs (Pihlajamaki et al. 2003), word pairs (Jackson and Schacter 2004), and visual landmarks and their specific contexts (Rauchs et al. 2008), and the current study extends the involvement of the PRC to include the formation of associative memory for temporally discontiguous items. Some studies, however, have reported PRC involvement only in item, and not associative, memory and instead highlight a separable role of the hippocampus in associative memory formation. A study examining the encoding of face-name pairs reported increased activity in anterior hippocampal formation for associative memory, whereas PRC activity was only increased for successful memory for the face items (Chua et al. 2007). Staresina and Davachi (2008) examined the function of PRC and the hippocampus during the encoding of item/color associations with or without additional associated context information. Both PRC and the hippocampus showed increased activity for subsequently remembered item/color associations, whereas the hippocampus showed an additional increase in activity when the context was also remembered in the association. The authors concluded that PRC may contribute to item-level associative encoding, whereas the hippocampus may be responsible for domain-general, including contextual, associative encoding (Staresina and Davachi 2008). Nevertheless, the interpretation of PRC involvement in item-level encoding is complicated, since this activity may also represent increased response to associative encoding within a domain; such findings are consistent with the results of the current study, which reports greater activity in PRC and ERC during associative encoding of two visual objects, which are of the same domain.

A recent study, in which subjects were shown two words and instructed either to encode the two words as a single novel compound word or to encode the two words in a sentence, has provided a possible explanation for the seemingly different roles attributed to PRC (Haskins et al. 2008). Increased activity was seen in left PRC during the encoding of the words as a single unit compared with encoding the words as two separate words in a sentence. The results of Haskins et al. (2008) suggest that PRC is involved in the associative encoding of items that can be represented as a single unit. This concept of PRC involvement when associated items are unitized has also been discussed in a recent review (Diana et al. 2007). To further examine the process of unitization in associative memory encoding, another recent study used fragmented objects that needed to be unitized when forming memory for the object and for the association between the object and its color (Staresina and Davachi 2010). PRC showed increased activity when the object was remembered relative to forgotten and even greater activity when the object-color association was subsequently remembered. A recent study described a possible model for how temporally discontiguous items could be associated, where neocortical working memory regions maintain the percept for an item across a delay period, allowing for concurrence between that active representation and a later one for associative binding (Hales and Brewer 2010). This model provides a possible mechanism for how a unitized association could be formed for temporally discontiguous items based on concurrent percepts of the two items at the time of binding. Nevertheless, the present study only suggests left PRC and ERC involvement in successful associative encoding; future investigation is needed to examine whether this involvement specifically predicts memory for a unitized percept that includes both objects or for a more flexible association of two separate items. In addition, these findings do not exclude the involvement of PRC in successful item encoding, but rather provide further evidence for PRC and ERC activity predicting successful associative encoding.

In addition to human imaging studies, the importance of PRC and ERC in associative encoding has been explored and supported by electrophysiological and lesion studies in nonhuman primates (Buckley and Gaffan 1998; Buckmaster et al. 2004; Fujimichi et al. 2010; Higuchi and Miyashita 1996; Miyashita and Chang 1988; Miyashita et al. 1998; Murray et al. 1993; Murray and Richmond 2001; Sakai and Miyashita 1991; Yanike et al. 2009; Yoshida et al. 2003). Sakai and Miyashita (1991) examined neuronal activity in anterior inferotemporal (IT) cortex in macaque monkeys while they performed a paired-associates task. After memorizing pairs of Fourier descriptors, monkeys were shown one image of a pair (cue) and were then shown two simultaneous patterns, one of which was the cue’s pair. During this memory task, the authors conducted extracellular recordings of single neurons in anterior IT cortex and discovered the presence of associative memory-coding neurons (Sakai and Miyashita 1991). A follow-up study examined the importance of intact connections between PRC/ERC and IT cortex for the formation of the associative memory...
representation in IT (Higuchi and Miyashita 1996). Monkeys received anterior commissural transection and were then trained on the previously described visual paired-associates task. After the PRC and ERC were unilaterally lesioned, there was no longer evidence of associative memory coding in anterior IT cortex. The authors concluded that the integrity of PRC and ERC is necessary for the formation of associative memory representations for picture pairs in IT cortex (Higuchi and Miyashita 1996).

The presence of increased activity in PRC and ERC in humans during the encoding of visual paired-associates reported in the present study is in line with such electrophysiological and lesion data from nonhuman primates; however, despite all of the support from primate research for the function of PRC in associative encoding, determining the timing of PRC involvement in associative encoding events would be difficult, because monkeys require multiple presentations of the event to learn the task and association. Yanike et al. (2009) recorded from PRC in monkeys learning new associations of a scene and an eye-movement location, and particular location-scene associations were selected in which a significant difference in cell firing rate was measured between the first 5–10 trials and the last 5–10 trials. The authors found PRC cells involved in learning this association that changed their firing rate during the scene, the delay period, or both; however, regardless of the time period of firing rate change, the monkey had already learned the association. In the present study, since all stimuli are presented only once, the only time period at which the association can be known is after the second stimulus is presented and the associated items can be bound. Therefore, the current study allows examination of the temporal component of PRC involvement in associative encoding that cannot be addressed in primate studies that involve repeated presentation of events during learning.

Recent studies have reported prestimulus MTL activity that is predictive of subsequent recollection of accidentally or intentionally encoded words (Gruber and Otten 2010; Guederian et al. 2009; Park and Rugg 2010). Although the current study did not find delay period MTL activity differences between the conditions of interest (associative and item-only trials), these prior studies would suggest the presence of increased MTL activity just before the onset of subsequently remembered 2P items. It should be noted that since the 2P item was subsequently remembered in both conditions, this activity might be expected to be similar in these trial conditions of interest.

Subsequent associative memory effects in left PRC and ERC were only seen in the current study at the time that the 2P stimulus was presented and the association could be formed; there was not a subsequent associative memory effect during the delay period between the 1P and 2P stimuli. Whether the MTL is involved in the maintenance of stimuli over a short delay is an active area of research without consensus. Human lesion and imaging studies looking at working memory report mixed results of MTL involvement in delay period maintenance; however, there is a common distinction across most of these studies. MTL involvement in delay period maintenance is often reported in studies that used nonverbal stimuli, such as faces or abstract pictures (Axmacher et al. 2007; Ezzyat and Olson 2008; Grady et al. 1998; Hannula et al. 2006; Hartley et al. 2007; Monk et al. 2002; Nichols et al. 2006; Olson et al. 2006a; Picchioni et al. 2007; Piekema et al. 2006; Ranganath et al. 2004; Ranganath and D’Esposito 2001; Shrager et al. 2008; Stern et al. 2001), whereas MTL involvement in delay period maintenance is not often reported in studies that used verbal stimuli, such as words or nameable objects (Cave and Squire 1992; Habeck et al. 2005; Hales et al. 2009; Kessler and Kiefer 2005; Petit et al. 1998; Shrager et al. 2008; Talmi et al. 2005), although exceptions to this dissociation exist (Cabeza et al. 2002; Campo et al. 2005; Mencl et al. 2000; Olson et al. 2006b; Oztekin et al. 2009; Tesche and Karhu 2000). Although some researchers argue that the presence of MTL activity during delay period maintenance suggests MTL involvement in working memory, it is also possible that there is a categorical difference between maintaining verbalizable and nonverbalizable stimuli over a short delay and that working memory load capacity for these two types of stimuli is different. Therefore, maintenance of nonverbalizable stimuli may engage brain regions involved in long-term memory encoding, such as MTL regions, even for short delays.

This reasoning has been supported in studies examining working memory processing during a delayed match-to-sample task and subsequent long-term recognition memory (see Hasselmo and Stern 2006 for review; Schon et al. 2004, 2005). These studies have shown that the involvement of MTL structures in active maintenance is correlated with subsequent long-term memory recognition. A recent study has additionally probed this effect by showing that MTL activity is further modulated by working memory load in a task involving the maintenance of two or four unfamiliar, trial-unique complex visual outdoor scenes (Schon et al. 2009). Stern and colleagues (2001) also provide an alternative explanation for the presence of MTL activity during short delays in some studies, but not in others, as a distinction between the maintenance of familiar information versus novel information. Although PFC and parietal regions are commonly isolated for maintaining familiar representations during working memory delays, additional structures, including PRC/ERC, are recruited for creating a novel representation for maintenance (Hasselmo and Stern 2006). The current results, using verbalizable stimuli depicting simple common objects and showing no maintenance activity in the MTL, are in line with studies that have provided distinctions regarding MTL activity during short-delay maintenance of verbalizable (or possibly familiar) and nonverbalizable (or possibly novel) stimuli.

Frontal involvement and functional dissociation in associative memory formation. In the current study, left DLPFC, VLPFC, and medial/middle frontal cortex all showed increased activity during the encoding of 2P stimuli that were subsequently recognized along with their corresponding associative pair (associative trials) compared with subsequently recognized 2P stimuli with forgotten associative information (item-only trials; Fig. 2, A–C). This finding of increased frontal activity for successful associative encoding is consistent with previous imaging, electrophysiology, and patient studies (Achim and Lepage 2005; Davachi and Wagner 2002; Dolan and Fletcher 1997; Geuze et al. 2008; Jackson and Schacter 2004; Kapur et al. 1996; Montaldi et al. 1998; Murray and Ranganath 2007; Pihlajamaki et al. 2003; Qin et al. 2007; Sperling et al. 2003; Staresina and Davachi 2006, 2010; Weyerts et al. 1997). Although these separate regions of the left frontal lobe all showed a subsequent associative memory effect, the response time course across the entire encoding
memory formation has been well established in primates. Lesion of PRC and ERC is important for the associative encoding of temporally discontiguous visual object pairs. Although the importance of PRC and ERC in associative encoding of temporally discontiguous visual object pairs has been seen selectively for successful associative encoding in the present study. Lateral occipital cortex is commonly cited for its involvement in object recognition (Doehrmann et al. 2010; Grill-Spector et al. 2001; Malach et al. 1995; Murray et al. 2004), and some recent studies have described its specific role in visual imagery (Deshpande et al. 2010; Kaas et al. 2010; Lacey et al. 2010; Schendan and Stern 2008) and in object maintenance (Ferber et al. 2005; Harrison and Tong 2009). Lateral occipital cortex has also been found to play a role in the encoding of object-location source information (Cansino et al. 2002). In addition, a study examining lateral occipital-hippocampal correlations found increased functional correlations during rest following an associative encoding task with high subsequent memory performance (Tambini et al. 2010). An extension of these findings of lateral occipital involvement in associative memory, the current study showed that increased lateral occipital activity during encoding selectively predicted subsequent associative memory for object pairs, even when controlling for the memory strength of the item being encoded. Increased lateral occipital activity at the time of associative binding might reflect the creation of a newly unitized percept that, when accessed at retrieval, supports associative memory performance; however, further investigation is needed to test such a putative underlying mechanism.

The current study confronts missing information regarding the time course of regional involvement in the associative encoding of temporally discontinuous visual objects pairs. Although the importance of PRC and ERC in associative memory formation has been well established in primate lesion and electrophysiology studies, such studies could not have answered questions about when these regions are involved in the successful encoding event. By temporally separating the subjects' exposure to each item of a pair and by showing subjects each pair only once, the current study extends prior studies, demonstrating that increased activity in left PFC, lateral occipital cortex, and anterior MTL happens once the pair is completed and predicts successful associative encoding of temporally discontinuous visual object pairs when item memory strength is controlled. Although some of these regions showed delay activity suggestive of object maintenance, this activity is simply part of attempting to encode the association and is not sufficient to show subsequent associative memory effects. The increase of activity in these frontal, lateral occipital, and MTL regions might represent binding and mnemonic storage of the new percept that incorporates the pair of stimuli or a conceptual or verbal association that links the objects.

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No conflicts of interest, financial or otherwise, are declared by the author(s).

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