Probing the transformation of discontinuous associations into episodic memory: An event-related fMRI study

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Introduction

The ability to associate and remember different aspects of an experience as one coherent episode is a critical feature of episodic memory (Tulving, 1983, 2002; Eichenbaum, 2004). In particular, it is crucial to bind and remember aspects of an experience that do not overlap in time as belonging to one episode. However, little is known about the neural underpinnings that support the encoding of discontinuous associations (i.e., non-overlapping in time or across temporal associations), although the neural correlates of successful memory formation for single items (Brewer et al., 1998; Fernández et al., 1998; Wagner et al., 1998; Otten et al., 2001; Ranganath et al., 2005) and simultaneously occurring inter-item associations (Henke et al., 1999; Davaichi and Wagner, 2002; Sperling et al., 2003; Jackson and Schacter, 2004; Prince et al., 2005) are well-established.

Generally speaking, the medial temporal lobe (MTL) and the prefrontal cortex play a critical role in episodic memory formation, including associative memories (Buckner et al., 1999; Fernández and Tendolkar, 2001; Paller and Wagner, 2002). Numerous neuroimaging studies have shown that different substructures of the MTL and the prefrontal cortex are more active while encoding single items that are later remembered than those forgotten (Brewer et al., 1998; Fernández et al., 1998; Wagner et al., 1998; Kirchhoff et al., 2000; Otten et al., 2001; Kao et al., 2005). Specifically, activity in the anterior MTL including the hippocampus and the inferior or medial prefrontal gyrus correlated with successful formation of associative or relational memories when two or three simultaneously presented items were bound together (Dolan and Fletcher, 1997; Henke et al., 1997, 1999; Sperling et al., 2003; Jackson and Schacter, 2004; Mitchell et al., 2004; Prince et al., 2005; Uncapher et al., 2006). The parahippocampal region was also thought to play a role in contextual associative processing, such as forming spatial and non-spatial associations (Aminoff et al., 2006).

Nevertheless, there is still controversy over the specific contribution of different regions within the MTL to episodic memory...
formation, especially for associative memory (Schacter and Wagner, 1999; Davachi et al., 2003; Ranganath et al., 2004a,b; Squire et al., 2004). Moreover, in the studies mentioned above, information – either an item or an inter-item association belonging to one episodic experience – was presented simultaneously. Thus, there is no evidence for neural processes that support the storage of discontinuous associations in memory.

Several models have suggested that the hippocampus is critically engaged in bridging discontinuities across time (Rawlins, 1985; Wallenstein et al., 1998; Eichenbaum, 2004). For instance, in trace conditioning, animals with hippocampal lesions show, compared to sham operated animals, impaired formation of discontinuous events when the conditioned stimulus and the unconditioned stimulus do not overlap in time (Thompson-Schill et al., 1997). Based on the integration of results obtained in experimental animal and human behavioral work, Wallenstein and colleagues’ developed a computational framework suggesting that the hippocampus plays a critical role in memory formation when discontinuous items have to be associated in terms of their temporal or spatial positioning or both (Wallenstein et al., 1998). Additionally, single-cell recordings from the frontal cortex in monkeys suggest that prefrontal neurons are essential for the temporal integration of visual and auditory stimuli that are separated in time (Fuster et al., 2000). Moreover, a recent event-related fMRI study has shown that, relative to binding word-pairs between constituents simultaneously presented, binding word-pairs between constituents across time (i.e., ‘discontinuity association’) engaged the left hippocampus (Luo and Niki, 2005). However, that study probed neural activity associated with the processing of discontinuous events in general, irrespectively of subsequent memory performance. Hence, it remains unclear whether there are distinct anatomical structures that specifically support the formation of discontinuous associations or whether they overlap with brain regions involved in the formation of associative memories in general.

Here we set out to identify those brain regions that are involved in storing discontinuous associations into episodic memory. Participants were scanned while memorizing item-triplets consisting of a simultaneous association and a discontinuous association (Fig. 1). Afterwards, participants performed single-item and associative recognition memory tests. The single-item recognition memory test is used to ensure whether the center-item of each triplet was remembered, whereas the associative recognition memory test is used to examine whether the associations were formed successfully. This set-up allows us to confirm neural correlates of memory formation for single items and associations. Additionally, and most critically for the question at issue, the contrast between the subsequent memory effects for discontinuous events.
and simultaneous associations allows us to reveal the neural correlates of associative memory formation across time, while memory formation of single items and associations in general is equated.

**Materials and methods**

**Participants**

Twenty-six Dutch native, right-handed healthy university students (mean age=22±4 years; 8 males; 1–5 years of university level education) without any neurological or psychiatric history and normal or corrected-to-normal vision participated in the experiment. All participants gave informed written consent according to the local ethics committee (CMO region Arnhem-Nijmegen, the Netherlands) and the declaration of Helsinki. We excluded data of six subjects, due to poor memory performance on the subsequent memory tasks (the correct rate of remembered simultaneous associations or discontinuous associations <0.55).

**Stimuli**

We selected 396 portraits (half males) from a database of 1000 color photographs of European individuals unknown to the participants (http://www.ftd.de/static/1000europeans). To standardize the stimuli and minimize some potential confounding factors, faces were selected under several criteria such as direct gaze contact, no strong emotional facial expression, no headaddress, no glasses, no beard, and so on. Familiar Dutch first names (n=264; half males) and Dutch city names (n=264) were obtained from the Internet. Length of names and cities ranged from 3 to 10 letters (mean length (SD)=5.6 (1.5) and 5.8 (1.7), respectively). We created 264 face–name–city triplets for the study phase and six triplets for a pre-scan training session. One hundred thirty-two triplets were used as lures for the face item recognition memory test. Triplets were created randomly out of the entire set of 264 faces for each subject individually with gender congruent face–name pairings. Additionally, another six trials were created in order to familiarize the participants with the task prior to scanning.

**Task procedure**

The experiment consisted of a study phase and two subsequent memory tests. At study, participants were scanned while they were memorizing 264 face–name–city triplets, with the face common to two associates (i.e., a first name and a city). Each trial started with one of two associates (either a first name or a city) called ‘Event 1’, which was presented at the center of the screen for 2 s. The first event was followed by a short delay period (3 s) during which a red question mark was presented. Thereafter, the second event (‘Event 2’) was composed of a face and the other associate (either a city or a first name), which was presented at the center of the screen for 4 s, with the face presented above the associate (Fig. 1). To avoid possible bias effects of cognitive process and neural activity related to first names and cities, half of the trials started with a first name and the other half started with a city name, randomly intermixed. To make participants integrate all three items into one association, they were instructed to indicate by appropriate button-presses whether they thought that the face–name–city triplet fitted well together or not. In addition, they were explicitly instructed to memorize each face with the associated name and city name. Therefore, each trial consisted of a discontinuous association (the initial associate and the face separated by the delay period) and a simultaneous association (the associate presented together with the face). Trials were separated by an inter-trial-interval (ITI) of 3 to 6 s (varied in steps of 1 s).

About 20 min after the study phase, subjects were asked to perform two memory tests outside the scanner. The first one was a face–item recognition memory test, in which the participants saw the 264 previously studied faces randomly intermixed with 132 new faces and were asked to judge whether the face was an old (i.e., studied) or a new (i.e., unstudied) face. Participants gave their old/new decision by a confidence rating on a 6-point scale (1=absolutely sure it was a new face; 2= somewhat sure it was a new face; 3= guessing it was a new face; 4= guessing it was an old face; 5= somewhat sure it was an old face; 6= absolutely sure it was an old face). This task was self-paced with a minimal trial duration of 2 s and a maximal one of 6 s. Old faces that received a 6, 5, or 4 rating were included in the subsequent associative memory test. Here, each face was shown with two previously studied names (gender selective) or two studied city names. One of the two names was previously presented together with the face; the other was presented with a different face at study and served as a foil to ensure that the decision about the correct association was not based solely on familiarity. It should be noted that the number of associative recognition memory trials was always limited to 132 face–name and 132 face–city associations, because half of studied names (or cities) should be attributed to foils. Sixteen subjects had more than 132 hit trials with a ‘5’ or ‘6’ rating in the face item recognition memory test. In these subjects, we randomly selected out of these hit trials 132 faces for the associative memory test and the remaining faces were included in ‘the condition of no interest’. Four subjects had less than 132 hit trials in the face item recognition memory test with a ‘5’ or ‘6’ rating. Hence, we randomly selected some studied faces with a ‘4’ rating for their associative recognition memory test, but these trials were included in ‘the condition of no interest’. Each face was presented once for each type of face–name and face–city association. To counterbalance the type and order, when presenting face–name and face–city associations in the associative recognition test, all 132 face–name and 132 face–city trials were randomly divided into two blocks, respectively, and counterbalanced across participants. The position of the correct name was balanced between the right and the left position on the screen. This task was self-paced, limited between 3 and 6 s. Participants were asked to indicate by an appropriate button press, which name was associated with the presented face during the study phase. To exclude trials potentially based on guessing, participants were instructed to give an unsure response when they did not remember which association was the correct one.

**fMRI data acquisition**

During MRI scanning, whole head T2*-weighted EPI-BOLD fMRI data were acquired with a Siemens Sonata 1.5 T MR-scanner using an ascending slice acquisition sequence (33 axial slices, volume TR=2.29 s, TE=30 ms, 90° flip-angle, slice-matrix size=64×64, slice thickness=3.0 mm, slice gap=0.5 mm, field of view=224 mm). Following the experimental session, high-resolution structural images were acquired, using a T1-weighted MP-RAGE sequence (volume TR=2250 ms, TE=3.93 ms, 15°
fMRI data analysis

Image pre-processing and statistical analysis was performed using SPM2 (http://www.fil.ion.ucl.ac.uk/spm). The first five volumes of each participant’s EPI-data were discarded to allow for T1 equilibration. The subject’s structural MR image was coregistered to the mean of the functional images, using mutual information optimization. Subsequently, the functional images were slice-time corrected, spatially normalized and transformed into a common stereotactic space, as defined by the SPM2 MNI T1 template, as well as spatially filtered by convolving the functional images with an isotropic 3D Gaussian kernel (10 mm FWHM). The fMRI data were statistically analyzed using the general linear model and statistical parametric mapping (Friston et al., 1995). In addition, the realignment parameters were included to account for movement-related variability. The data were high-pass filtered (128 s) to account for various low-frequency effects.

Tackling the question at issue, the encoding trials were sorted into several different categories based on the subsequent memory performance (Table 1). Our main interest was the transient neural activity when associative memory formation occurred; therefore, separate regressors of each category were created time-locked to the onset of ‘Event 2’, and convolved with the canonical hemodynamic response function. ‘Event 1’ was separately modeled independently of subsequent memory performance to account for encoding-related neural responses to the first event. According to the recognition memory performance (see Behavioral Results), a ‘1’, ‘2’, or ‘3’ rating for an old face can be regarded as forgotten (i.e., ‘Miss’ condition) and a ‘5’ or ‘6’ rating as remembered. Trials associated with remembered items were subdivided in five different conditions, depending on the subsequent associative recognition memory performance: (1) Hit single item: correct face recognition, incorrect associative recall; (2) Hit simultaneous association: correct face recognition, only correct associative recall for the simultaneously memorized name or city name; (3) Hit discontinuous association: correct face recognition, only correct associative recall for the discontinuously memorized name or city name; (4) Hit all associations (i.e., hit both simultaneous and discontinuous associations): correct face recognition, correct associative recall for the simultaneously and discontinuously memorized name or city name; (5) Hit of no interest: correct face recognition, unsure on both associated name and associated city, miss on either associated name or associated city but unsure on either associated name or associated city, as well as the aforementioned trials in ‘the condition of no interest’ (see Task procedure). Additionally, a low level fixation condition was separately modeled.

The relevant contrast parameter images generated on the single participant level were submitted to the second-level group analysis. At the group level, contrasts between conditions were computed by using one-sample t tests on these images treating participants as a random variable. In the whole brain search, the results from the random effects analyses were initially threshold at \( p<0.001 \) (uncorrected) and the cluster size statistics were used subsequently as the test statistic. Only clusters significant at \( p<0.05 \) (corrected for multiple non-independent comparisons; Worsley et al., 1996) are reported. All local maxima (MNI coordinates) within significant clusters are reported with \( p \) values corrected for multiple non-independent comparisons based on false discovery rate (Genovese et al., 2002), unless otherwise specified. Given that the MTL is an obvious region of interest, the MTL was additionally investigated within a spherical region of interest (radius = 25 mm) centered in the middle of the hippocampus at \([x, y, z] = [±30, −22, −14]\) in combination with a small volume correction (SVC) for multiple non-independent comparisons.

To characterize the patterns of association related to the subsequent memory effects for different types of memory formation within the MTL, we further conducted regions of interest (ROIs) analysis in the anterior and posterior MTL (Schacter and Wagner, 1999; Sperling et al., 2003; Jackson and Schacter, 2004). Using MarsBar (http://www.marsbar.sourceforge.net; Brett et al., 2002), we functionally identified the ROIs based on the average group activation within the MTL. Each ROI included all voxels within a sphere radius of 6 mm centered at a certain local maxima located in the MTL (see Results). Averaged effect sizes of all voxels within the ROIs were extracted by estimating the mean contrast values of all individual subjects.

Results

Behavioral results

Study phase

Participants were more likely to judge the face–name–city association as a good fit than not (0.696±0.143 vs. 0.304±0.143; \( t_{(19)} = 6.01, p<0.001 \)). They performed equally fast in that half of the encoding trials that started with a city name (2062±448 ms) and in the other half of trials that started with a first name (2041±417 ms) \( t_{(19)} = 0.95, p>0.10 \). This suggests that both trial types were similar to each other in difficulty.

Face item recognition memory test

The distribution of averaged response proportions (mean±SE) is shown in Fig. 2. Two separate repeated-measures ANOVAs revealed significant differences of confidence ratings for both old and new faces (\( F_{(5,95)} = 19.24, F_{(5,95)} = 7.10 \), respectively; both \( p \) values <0.001). Paired-sample t tests comparing confidence ratings revealed that the proportion of ‘5’ and ‘6’ ratings for old faces were significantly higher than for new faces (\( t_{(19)} = 6.97, t_{(19)} = 9.07 \), respectively; both \( p \) values <0.001). The proportion of ‘4’ ratings was similar for old and new faces (\( t_{(19)} = 0.66, p = 0.54 \)). Reversely, the proportion of ‘1’, ‘2’, and ‘3’ ratings for old faces was lower than for new faces (\( t_{(19)} = −5.53, t_{(19)} = −6.00, t_{(19)} = −4.79 \) respectively; all \( p \) values <0.001). The performance was independent of ‘fit’ and

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Miss</th>
<th>Single</th>
<th>Simul.</th>
<th>Discon.</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Num.</td>
<td>69.8</td>
<td>14.5</td>
<td>30.3</td>
<td>30.9</td>
<td>39.6</td>
</tr>
<tr>
<td>RTs (ms)</td>
<td>2107</td>
<td>465</td>
<td>2035</td>
<td>499</td>
<td>2038</td>
</tr>
<tr>
<td></td>
<td>423</td>
<td>2026</td>
<td>442</td>
<td>2001</td>
<td>396</td>
</tr>
<tr>
<td></td>
<td>364 (19.5)</td>
<td>14.5 (5.8)</td>
<td>30.3 (5.3)</td>
<td>30.9 (5.3)</td>
<td>39.6 (11.5)</td>
</tr>
</tbody>
</table>

‘Miss’—forgotten faces, ‘Single’—hit single item: correct face recognition and incorrect associative recall, ‘Simul.’—hit simultaneous association, ‘Discon.’—hit discontinuous association, ‘All’—hit both simultaneous and discontinuous associations, ‘Num.’—number of trials. The number is out of 264 in the condition of ‘Miss’. In contrast, the numbers are out of 132 in the remaining conditions (see fMRI data analysis for details).
‘not fit’ responses. These results demonstrate successful discrimination between studied and new faces.

**Associative memory test**

Number of trials and reaction times of the five categories of main interest are shown in Table 2. The rate (mean±SD) of correctly recalled associations was 0.626±0.063 for simultaneous associations and 0.629±0.069 for discontinuous associations. This level of associative memory performance was roughly consistent with previous studies using similar subsequent memory paradigm (Sperling et al., 2003; Chua et al., 2004). More importantly, the performance on both types of associations was significantly above chance level (t(19)=8.98; t(19)=8.34, respectively; both p values <0.001) and did not differ from one another (t(19)=−0.09; p=0.93). Given that the associated name and associated city name were simultaneously or discontinuously presented with the face, we constructed two (presenting types: name and face) repeated-measure ANOVAs to confirm that there was no interaction between these two factors for the performance (F(1,19)<1.0).

Finally, we confirmed that the response in the orientation task had no significant effect on associative memory performance (i.e., ‘Hit simultaneous association’: fit vs. not fit=0.128 vs. 0.117; ‘Hit discontinuous association’: fit vs. not fit=0.128 vs. 0.118; ‘Hit all associations’: fit vs. not fit=0.162 vs. 0.144; all p values >0.13). Thus, we collapsed for all further analysis fit and not fit trials to increase statistical power.

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**Neuroimaging results**

**Neural correlates of the formation of discontinuous associations**

To identify brain regions specifically related to the formation of discontinuous associations, we directly contrasted the conditions ‘hit discontinuous association’ with ‘hit simultaneous association’. Thus, the contrast of two conditions does not differ in the number of associations formed but the type of association formed. A separate set of brain regions was specifically more active for the formation of new discontinuous associations (Table 2; Figs. 3A and B). In more detail, these regions included the left posterior parahippocampal gyrus close to the junction between the fusiform gyrus and the parahippocampal gyrus (local maximum at [−33, −39, −9], Z=4.42; cluster p=0.007 SVC corrected); a large area in the dorsolateral prefrontal cortex including the right superior frontal gyrus (local maximum at [15, 39, 33], Z=4.28; [33, 30, 39], Z=4.04; [15, 48, 30], Z=3.94; all cluster p values <0.05 corrected) and the right middle frontal gyrus (local maximum at [33, 30, 39], Z=3.74; cluster p<0.05 corrected); the basal ganglia including the caudate nucleus and the putamen (local maxima at [−18, 9, 12], Z=4.72; [21, 6, 6], Z=3.98; [27, 9, 15], Z=3.96; [−21, 12, −3], Z=3.87; [−18, 3, −6], Z=3.18; all cluster − values <0.05 corrected); a medial posterior region including the precuneus and the posterior cingulate gyrus (local maximum at [15, −60, 18], Z=3.94; cluster p<0.05 corrected); and the occipital-temporal region from the posterior middle temporal gyrus extending into the superior occipital gyrus (local maxima at [−39, −63, 15], Z=3.85; [−42, −69, 27], Z=3.65; [−33, −81, 36], Z=3.47; all cluster p values <0.05 corrected). There were no significant activations observed for the opposite contrast. The ROI analysis revealed a strong linear increase of the subsequent memory effect in the posterior parahippocampal gyrus from ‘only hit single item’, ‘only hit simultaneous association’ to ‘only hit discontinuous association’ (F(1,19)=5.53, p=0.008). Hence, we identified a set of brain regions that is specifically involved in transferring associations of discontinuous events into episodic memory. This network includes the posterior parahippocampal region, the dorsal–lateral prefrontal cortex, posterior midline structures, and the basal ganglia.

**Neural correlates of the formation of new memories including associations**

To replicate the neural correlates of associative memory formation, we contrasted brain activity associated with successful associative memory formation (i.e., hit simultaneous association, hit discontinuous association, and hit all associations) and non-successful memory formation (i.e., misses). We identified a well-established set of regions that were significantly more active during the successful formation of new associations, including the prefrontal cortex and the occipito-temporal cortex extending into the MTL (Table 3; Fig. 4A). In the MTL, we identified the left posterior hippocampus (local maximum at [−24, −33, −3], Z=4.75; [−18, −36, 3], Z=4.10; p=0.012 corrected), the left anterior hippocampus extending into the amygdala and the parahippocampal gyrus (local maximum at [−21, −6, −18], Z=3.84; p=0.014 corrected), and the right anterior to middle hippocampus extending into the amygdala (local maxima at [24, −6, −18], Z=4.35 and [21, −27, −9], Z=3.58; all p<0.015 corrected). In the prefrontal cortex, we identified the ventral medial frontal gyrus (local maximum at [0, 45, −18], Z=4.08; p=0.012 corrected), the bilateral superior frontal gyrus (local maxima at [3, 60, 24], Z=3.67; [−9, 54, 24], Z=3.54; all p=p=0.017 corrected), and the left and right inferior frontal...
The threshold $p<0.001$ (uncorrected) in the whole brain volume search. Only clusters (20 or more voxels) significant at $p<0.05$ (corrected cluster) were reported. *Small Volume Correction (SVC) used in the MTL activations. L: left; R: right; BA: Brodmann Area; MNI: MNI coordinates (SPM2). The activations in MTL are in bold type.

Table 2
Brain regions associated with the formation of discontinuous associations: Hit discontinuous associations vs. hit simultaneous associations ($n=20$)

<table>
<thead>
<tr>
<th>Brain region</th>
<th>BA</th>
<th>Cluster</th>
<th>$p$ corrected</th>
<th>Z value</th>
<th>MNI x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. superior, medial/middle frontal gyri</td>
<td>8, 9</td>
<td>&lt;0.0001</td>
<td>4.28</td>
<td>15</td>
<td>39</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>L. posterior parahippocampal gyrus</td>
<td>35</td>
<td>0.007*</td>
<td>4.42</td>
<td>-33</td>
<td>-39</td>
<td>-9</td>
<td></td>
</tr>
<tr>
<td>L. caudate, lentiform nucleus, putamen</td>
<td>0.004</td>
<td>4.72</td>
<td>-18</td>
<td>9</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. caudate, lentiform, putamen</td>
<td>0.043</td>
<td>3.98</td>
<td>21</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. precuneus, posterior cingulate cortex</td>
<td>31</td>
<td>0.004</td>
<td>4.05</td>
<td>15</td>
<td>-60</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>L. middle temporal gyrus</td>
<td>19, 39</td>
<td>0.043</td>
<td>3.85</td>
<td>-39</td>
<td>-63</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>L. superior occipital gyrus</td>
<td>39</td>
<td>3.65</td>
<td>-42</td>
<td>-69</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. superior occipital gyrus</td>
<td>19</td>
<td>3.47</td>
<td>-33</td>
<td>-81</td>
<td>36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The trend toward a linear increase of subsequent memory in the bilateral anterior hippocampus from ‘hit single item’, ‘hit simultaneous association’, ‘hit discontinuous association’ to ‘hit all associations’ (Left: $F_{(3,57)}=2.35, p=0.082$; Right: $F_{(3,57)}=2.85, p=0.045$). Hence, these findings confirm previous neuroimaging studies (Dolan and Fletcher, 1997; Henke et al., 1997; Sperling et al., 2003; Jackson and Schacter, 2004; Mitchell et al., 2004; Achim and Lepage, 2005; Prince et al., 2005) showing that specific prefrontal and medial temporal structures including the hippocampus are involved in associative or relational memory formation. It is worthwhile to note that there was an inadequate number of trials in the ‘hit single item’ condition (i.e., mean: 14.5±5.8) for powerful statistical analyses, therefore this condition would introduce an unacceptable level of noise if being compared to other associative memory conditions.

To examine whether the hippocampus was actually activated in each of the three conditions of associative memory formation (i.e., ‘hit simultaneous association’, ‘hit discontinuous association’, and ‘hit all associations’), we constructed three separate contrasts against the ‘misses’. For the formation of simultaneous associations, we found the left posterior hippocampus (local maxima at $p<0.001$ (corrected) $Z=3.78$) and the left posterior parahippocampal gyrus (local maxima at $p<0.001$ (corrected) $Z=3.79$).

Table 3
Brain regions related to associative memory formation: formation of new memories including associations vs. Miss

<table>
<thead>
<tr>
<th>Brain region</th>
<th>BA</th>
<th>Cluster</th>
<th>$p$ (corrected)</th>
<th>Voxel $p_{FDR}$</th>
<th>Z score</th>
<th>MNI x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. middle occipital, lingual and fusiform gyri</td>
<td>18/19</td>
<td>&lt;0.0001</td>
<td>0.012</td>
<td>4.38</td>
<td>36</td>
<td>-72</td>
<td>-9</td>
<td></td>
</tr>
<tr>
<td>R. inferior occipital gyrus</td>
<td>18</td>
<td>0.012</td>
<td>4.58</td>
<td>30</td>
<td>-90</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. lingual gyrus</td>
<td>18</td>
<td>0.012</td>
<td>4.45</td>
<td>30</td>
<td>-87</td>
<td>-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. inferior occipital gyrus</td>
<td>17/18</td>
<td>0.012</td>
<td>4.22</td>
<td>-9</td>
<td>-99</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. hippocampus, parahippocampal gyrus</td>
<td>27/30</td>
<td>0.010</td>
<td>4.05</td>
<td>-30</td>
<td>-93</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. hippocampus, parahippocampal gyrus</td>
<td>35</td>
<td>&lt;0.0001</td>
<td>0.012</td>
<td>4.75</td>
<td>-24</td>
<td>-33</td>
<td>-3</td>
<td></td>
</tr>
<tr>
<td>Ventral medial frontal gyrus</td>
<td>11/10</td>
<td>0.008</td>
<td>0.012</td>
<td>4.10</td>
<td>-18</td>
<td>-36</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>L. fusiform and parahippocampal gyri</td>
<td>37</td>
<td>0.012</td>
<td>0.013</td>
<td>3.96</td>
<td>-36</td>
<td>-51</td>
<td>-15</td>
<td></td>
</tr>
<tr>
<td>L. anterior hippocampus, amygdala</td>
<td>0.007</td>
<td>0.014</td>
<td>3.84</td>
<td>-21</td>
<td>-6</td>
<td>-18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. superior frontal gyrus</td>
<td>9, 10</td>
<td>0.051</td>
<td>0.017</td>
<td>3.67</td>
<td>3</td>
<td>60</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>R. inferior frontal gyrus</td>
<td>46</td>
<td>0.351</td>
<td>0.024</td>
<td>3.64</td>
<td>42</td>
<td>30</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>R. inferior frontal gyrus</td>
<td>47</td>
<td>0.882</td>
<td>0.020</td>
<td>3.53</td>
<td>-42</td>
<td>33</td>
<td>-9</td>
<td></td>
</tr>
<tr>
<td>R. inferior/middle frontal gyrus</td>
<td>47</td>
<td>0.786</td>
<td>0.020</td>
<td>3.53</td>
<td>36</td>
<td>33</td>
<td>-15</td>
<td></td>
</tr>
</tbody>
</table>

The threshold $p<0.05$ (corrected) in the whole brain search. Only clusters (10 or more voxels) significant at $p<0.05$ (corrected cluster) were reported. L: left; R: right; BA: Brodmann Area; MIN: MIN coordinates (SPM2). The activations in MTL are in bold type.
[-24, -33, -3], [-27, -30, -6], all Z = 3.46; cluster p < 0.024 SVC corrected), the left anterior hippocampus (local maxima at [-30, 0, -15], [-24, -9, -21], all Z > 3.28; cluster p < 0.054 SVC corrected), and the right anterior hippocampus/amygdala (local maxima at [24, -6, -18], Z = 4.11; cluster p < 0.008 SVC corrected) to be significantly activated. For the formation of discontinuous associations, we found the left posterior hippocampus extending into parahippocampal and fusiform gyri (local maxima at [-24, -36, -6], [-27, -33, -9], and [-30, -45, -15], all Z > 3.93; cluster p < 0.001 SVC corrected), the left anterior hippocampus (local maxima at [-21, -6, -12], Z = 3.58; cluster p < 0.019 SVC corrected), the right anterior hippocampus/amygdala (local maxima at [21, -3, -21], [33, 0, -15] and [24, -12, -15], all Z > 3.49; cluster p < 0.001 corrected), and the right posterior hippocampus (local maxima at [21, -27, -9], Z = 3.77; cluster p < 0.037 SVC corrected) to be significantly activated. For the formation of simultaneous and discontinuous association, we found the left posterior hippocampus (local maxima at [-27, -30, -9] and [-24, -33, -3], Z > 3.66; cluster p < 0.019 SVC corrected) and bilaterally the anterior hippocampus/amygdala (local maxima at [-21, -6, -18], Z = 3.37; cluster p < 0.060 SVC corrected; [24, -9, -12], Z = 3.90; cluster p < 0.009 SVC corrected) to be significantly activated. Hence, the hippocampal contribution appears independent of the type of association formed. Additionally, to identify the neural activity specifically related to remembering all associations, we conducted the contrast of ‘hit all associations’ vs. ‘hit simultaneous association’ and ‘hit discontinuous association’. When testing the whole brain data (p < 0.001 uncorrected), we found an almost significant effect in the ventral medial prefrontal cortex only (local maxima at [3, 51, -15], Z = 3.90; [-3, 24, -18], Z = 3.83; [-12, 24, -12], Z = 3.83; cluster p values < 0.08 corrected). However, there was not a reliable effect in the MTL; this result indicates that the activity of MTL was not significantly greater in remembering all associations than remembering either simultaneous association or discontinuous association.

Discussion

The goal of this study was to reveal the neural correlates of a specific type of associative memory formation, the binding of events that are separated in time. We show that activity in the left posterior parahippocampal gyrus, the dorsolateral prefrontal cortex, posterior midline structures, and certain parts of the basal ganglia is increased while subjects successfully store discontinuous associations into episodic memory. This finding suggests that there is a distinct (set of) operation(s) that supports specifically an essential function for episodic memory, the integration of inter-item associations across time. Importantly, this operation might be functionally dissociated from operations that form associative memories in general.

To bind two discontinuous events together, it is necessary to keep the initial event online until the second event is perceived and/or to retrieve/reactivate the representation of the initial event while processing the second event. Thus, one probability is that the online maintenance operation might be responsible for our results in part. However, our findings cannot readily be attributed to the online maintenance of the initial event only, a word, because we analyzed the data event-related to the second event and the neural responses of the initial event were accounted for in the design model. Furthermore, active maintenance of words is
typically associated with activity in a left hemispheric set of brain regions including the inferior prefrontal cortex and the temporal-parietal junction (Prabhakaran et al., 2000; Sakai et al., 2002a; Sakai and Passingham, 2004; Narayanan et al., 2005; Hart et al., 2006). Thus, our results can be more readily attributed to an actual binding operation, a retrieval/reactivation operation or both.

The posterior parahippocampal area has often been implicated in actual binding (Davachi et al., 2003; Ranganath et al., 2004a,b; Prince et al., 2005; Sommer et al., 2005a, 2005b), processing contextual associations (Aminoff et al., 2006), and long-term memory formation (Brewer et al., 1998; Wagner et al., 1998; Schon et al., 2004, 2005). Consistently, Eichenbaum (2000) concluded from animal experiments that parahippocampal regions are involved in the encoding of associations into an integrated representation. In non-human primates, the inferior temporal cortex (consisting of two distinct but mutually interconnected areas: area TE and area 36), including the parahippocampal region, has been proposed to be involved in associative long-term memory formation of visual information (Squire and Zola-Morgan, 1991; Murray et al., 1993; Miyashita, 2000; Messinger et al., 2001). However, this does not contradict the well-established view that the hippocampus is critically involved in the formation of associative memories. Our results also confirm that the hippocampus is generally activated when new associative memories including simultaneous association and discontinuous associations are successfully formed. The posterior parahippocampal area seems to play a specific role when discontinuous associations are successfully encoded into episodic memory.

The dorsolateral prefrontal cortex (together with the basal ganglia) has been associated with the maintenance and associative binding of complex information, including across-temporal associations (Fuster et al., 2000; Prabhakaran et al., 2000; Pasupathy and Miller, 2005), and the top-down control of memory retrieval/reactivation (Miyashita, 2004). For instance, lesions including the dorsolateral prefrontal cortex in humans and non-human primates impair performance of specific working memory tasks like the associative learning task (i.e., forming and re-arranging of arbitrary associations) (Petrides, 1985, 1997; Asaad et al., 1998). Numerous functional neuroimaging studies have confirmed that the dorsolateral prefrontal cortex contributes to executive control processes such as selection and active maintenance in working memory (Rowe et al., 2000; Sakai et al., 2002b; Curtis and D’Esposito, 2003, 2006; Curtis et al., 2004). Moreover, some recent studies found that this brain region contributes to long-term memory formation through its role in organizing information in working memory (Wagner, 1999; Blumenfeld and Ranganath, 2006). Several lines of evidence also have suggested that the dorsolateral prefrontal cortex interacts closely with the basal ganglia when complex information has to be maintained in working memory (Levy et al., 1997; Ashby et al., 2005; Pasupathy and Miller, 2005; Chudasama and Robbins, 2006; O’Reilly and Frank, 2006). Hence, our finding of dorsolateral prefrontal and caudate nucleus activity related to the successful
encoding of discontinuous events might be explained by higher organizational demands during associative binding.

An alternative, yet not mutually exclusive explanation for the dorsolateral prefrontal contribution might be related to the top-down control of memory retrieval/reactivation. Previous lesion studies, single-unit neurophysiological, and functional neuroimaging studies have suggested that the dorsolateral prefrontal cortex is the source of top-down signals that reactivate neural representations in posterior cortical areas like parietal and occipital midline structures (Miyashita and Hayashi, 2000; Ranganath and D’Esposito, 2005). In addition, the medial temporal lobe also appears to be involved in top-down control of memory retrieval/reactivation, but by another aspect (Miyashita and Hayashi, 2000; Ranganath and D’Esposito, 2005). For instance, the medial temporal activity spreading backwards reactivates relevant neurons in more posterior inferior temporal regions (Naya et al., 1996, 2001). While signals from the parahippocampal cortex during retrieval may actually trigger the reactivation of relevant information whenever rehearsal and thus active working-memory maintenance were interrupted, signals from the prefrontal cortex are always engaged in the selection of task-relevant information in order to guide behavior (Sakai et al., 2002a; Sakai, 2003; Sakai and Passingham, 2004). Taken together, the dorsolateral prefrontal and posterior parahippocampal activation specifically related to the successful encoding of discontinuous events might reflect that these two areas trigger the reactivation of the representation of the initial event, allowing it to be successfully associated with the second event, in order to form a new association across time into episodic memory.

As shown by our data, posterior midline structures like the precuneus/posterior cingulate cortex are also involved in the formation of discontinuous associations. In the formation of discontinuous associations, when receiving top-down signals this brain region might mediate reactivation/retrieval of the internal representation of the initial event and attention orientations (i.e., directing attention to the internal representation of the initial event, shifting attention between the initial event and the second event) (Cavanna and Trimble, 2006). For example, activity in posterior midline structures is often reported by functional neuroimaging studies to be related to memory retrieval (Fletcher et al., 1995a,b, 1996; Cabeza et al., 1997; Cabeza and Nyberg, 2000; Wheeler et al., 2000, 2006; Wheeler and Buckner, 2003; Wagner et al., 2005) and attention orientation tasks (Nagahama et al., 1999). The activity of these regions could play a role in perceptual reactivation process during remembering and memory retrieval as necessary for episodic memories (Wheeler et al., 2000, 2006). The occipital–temporal region including the middle temporal gyrus and superior occipital gyrus could be related to visual processing of individual aspects of a discontinuous association during associative operations (Yonelinas et al., 2001; Mather et al., 2006). Moreover, one recent neuroimaging study suggests that the posterior brain regions like intra-parietal sulcus, precuneus, posterior cingulate cortex and superior temporal gyrus are also involved in the multifaceted contextual encoding of disparate features of an episode into a common memory representation (Uncapher et al., 2006).

However, several theories suggest that the hippocampus is critically engaged in bridging discontinuities across time (Rawlins, 1985; Wallenstein et al., 1998; Eichenbaum, 2004). The hippocampal coding of local context is thought to be important for accurate sequence encoding (Wallenstein et al., 1998; Eichenbaum, 2004; Kumaran and Maguire, 2006). Similarly, a recent event-related fMRI study confirms that the hippocampus is involved in the process of ‘discontinuity association’, bridging a delay between the constituents of word-pairs in anticipation of a semantic relatedness judgment (Luo and Niki, 2005). However, this finding was not directly related to memory formation. Although our present results replicate that the hippocampus plays a general role in storing new associations (Sperling et al., 2003; Jackson and Schacter, 2004; Prince et al., 2005; as well as reviews, Eichenbaum, 2000, 2004), we did not observe a preference for the formation of discontinuous associations. However, our task differs significantly from typical sequence encoding tasks, in which the order of items and thus contextual coding is most critical. This contextual coding is thought to be mediated by the hippocampus that creates ‘context units’, which do not represent particular items, but instead support the ‘glueing’ together of sequential items (Wallenstein et al., 1998; Eichenbaum, 2004; Kumaran and Maguire, 2006).

In conclusion, our main finding is that there is a (set of) operation(s) that specifically stores discontinuous events into memory, a fundamental supplement to previous studies on episodic memory formation. We suggest that, under goal-directed long-term memory formation, top-down signals from the prefrontal cortex and the MTL may trigger reactivation of internal representations in posterior midline structures of the first event (Tomita et al., 1999; Miyashita and Hayashi, 2000; Naya et al., 2001; Sakai et al., 2002a; Sakai, 2003; Sakai and Passingham, 2004; Miyashita, 2004), thus allowing it to be associated with the second event. At the same time, the dorsolateral prefrontal cortex together with the basal ganglia may support this encoding operation by executive and binding processes within working memory (Prabhakaran et al., 2000; Pasupathy and Miller, 2005). Finally, the posterior parahippocampal area may play a role in binding and actual forming new memory traces. Future studies, however, have to dissociate the functional roles of these brain regions implicated here in the transformation of discontinuous association into episodic memory.

Acknowledgments

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References


