

# Interaction between the Amygdala and the Medial Temporal Lobe Memory System Predicts Better Memory for Emotional Events

Florin Dolcos,<sup>1,2,3,\*</sup> Kevin S. LaBar,<sup>1</sup> and Roberto Cabeza<sup>1</sup>

<sup>1</sup>Center for Cognitive Neuroscience  
Duke University

Durham, North Carolina 27708

<sup>2</sup>Centre for Neuroscience

University of Alberta

513 HMRC

Edmonton, Alberta T6G 2S2

Canada

## Summary

Emotional events are remembered better than neutral events possibly because the amygdala enhances the function of medial temporal lobe (MTL) memory system (modulation hypothesis). Although this hypothesis has been supported by much animal research, evidence from humans has been scarce and indirect. We investigated this issue using event-related fMRI during encoding of emotional and neutral pictures. Memory performance after scanning showed a retention advantage for emotional pictures. Successful encoding activity in the amygdala and MTL memory structures was greater and more strongly correlated for emotional than for neutral pictures. Moreover, a double dissociation was found along the longitudinal axis of the MTL memory system: activity in anterior regions predicted memory for emotional items, whereas activity in posterior regions predicted memory for neutral items. These results provide direct evidence for the modulation hypothesis in humans and reveal a functional specialization within the MTL regarding the effects of emotion on memory formation.

## Introduction

Scientific and anecdotal evidence shows that emotionally arousing events tend to be better remembered than nonarousing neutral events (Bradley et al., 1992; Christianson, 1992). According to the modulation hypothesis (McGaugh, 2000; McGaugh et al., 1996, 2002), the beneficial effect of emotion on memory is due to modulatory influences of the basolateral amygdala (BLA) on encoding and consolidation processes occurring in medial temporal lobe (MTL) memory structures, including the hippocampus and associated parahippocampal regions (i.e., entorhinal, perirhinal, and parahippocampal cortices; Squire and Zola-Morgan, 1991). Early evidence supporting the modulation hypothesis identified the hippocampus as a putative target for the modulatory influences from BLA (McGaugh et al., 1996), but more recent studies have also identified anterior parahippocampal regions, particularly the entorhinal cortex, as

brain sites sensitive to amygdalar modulatory influences (McGaugh et al., 2002; Roesler et al., 2002).

Although the modulation hypothesis is supported by a considerable amount of evidence from nonhuman animals (Cahill and McGaugh, 1998; McGaugh, 2000, 2002; McGaugh et al., 2002), direct evidence from humans is scarce. Neuropsychological studies of patients with amygdalar lesions have shown that these patients are impaired in some explicit tests of emotional memory (Adolphs et al., 1997, 2000; Cahill et al., 1995; LaBar and Phelps, 1998; Phelps et al., 1998). However, it is unclear if these deficits reflect a lack of amygdalar modulation or the damage of neighboring MTL regions. Also, lesion studies cannot easily distinguish whether memory difficulties reflect deficits during encoding, consolidation, or retrieval (LaBar and Phelps, 1998). Functional neuroimaging studies provide a greater spatial resolution and the ability to distinguish between encoding and retrieval, but they have not yet provided complete evidence for the modulation hypothesis.

The ideal neuroimaging method to investigate the modulation hypothesis is the subsequent memory paradigm (Paller et al., 1987; Paller and Wagner, 2002), which has been applied to both event-related potentials (ERP) and event-related functional magnetic resonance imaging (fMRI). In this paradigm, brain activity while items were encoded is analyzed according to whether the items were remembered or forgotten in a subsequent memory test. Greater activity for subsequently remembered than for subsequently forgotten items is known as Dm (difference due to memory; Paller et al., 1987) and is assumed to reflect successful encoding processes. In the present study, we partitioned the Dm (remembered stimuli – forgotten stimuli) according to the emotional content of stimuli involved. More specifically, we measured an emotional Dm (remembered emotional stimuli – forgotten emotional stimuli) and a neutral Dm (remembered neutral stimuli – forgotten neutral stimuli). Using this method, the effects of emotion on successful encoding activity can be revealed by identifying regions where the emotional Dm is greater than the neutral Dm. We identified several significant differences between emotional and neutral Dms in a previous ERP study (Dolcos and Cabeza, 2002), but this method did not provide a precise localization of the neural generators of the observed differences. Thus, in the present study we compared the emotional Dm and the neutral Dm using event-related fMRI.

The main goal of the study was to test the hypothesis that better memory for emotionally arousing than for nonarousing neutral events is due to an effect of the amygdala on the MTL memory system during memory formation (modulation hypothesis). From this hypothesis we derived two predictions: (1) the emotional Dm (emotional remembered – emotional forgotten) should be greater than the neutral Dm (neutral remembered – neutral forgotten) in both the amygdala and the MTL memory system, and (2) the correlation between activity in the amygdala and the MTL memory system should be greater for the emotional Dm than for the neutral Dm.

\*Correspondence: fdolcos@duke.edu

<sup>3</sup>Present address: Brain Imaging and Analysis Center, Duke University, P.O. Box 3918, Durham, North Carolina 27710.

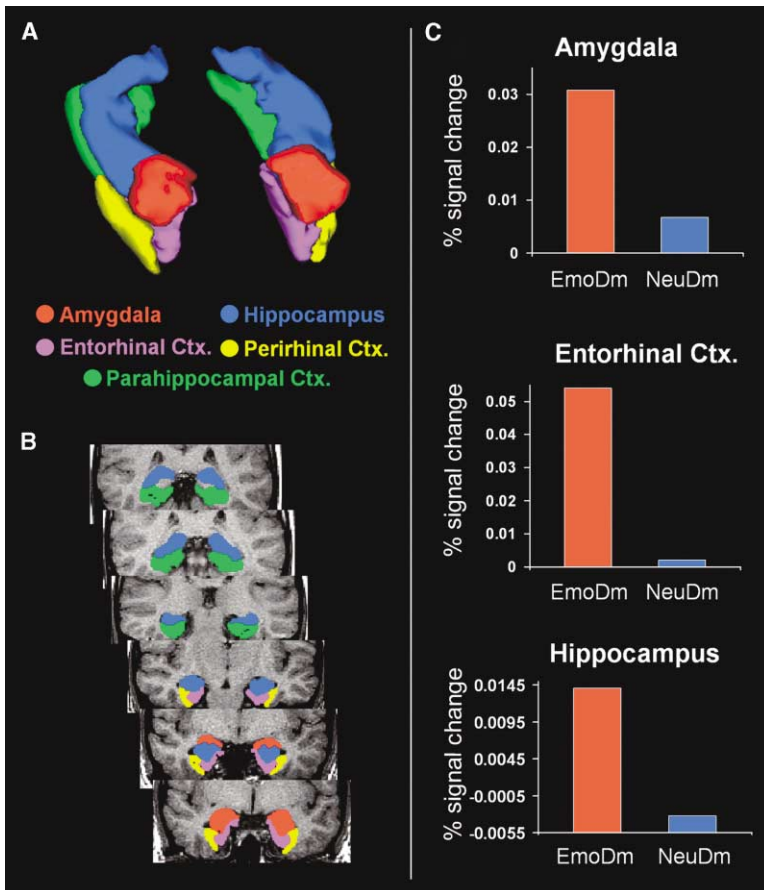


Figure 1. Greater Emotional Dm than Neutral Dm in the Amygdala and MTL Memory System

(A) Three-dimensional view of the anatomically defined regions of interest (ROIs) from one representative subject.

(B) Coronal view of six representative slices showing the location in the brain of the medial temporal lobe (MTL) regions.

(C) The bar graphs compare the percent signal change for emotional and neutral Dms, as extracted from the peak activation slice/subregion in the amygdala (BLA), hippocampus (the head/left hemisphere), and anterior PHG (the entorhinal cortex; see also Table 1). Unless specified, the graphs are based on the fMRI signal averaged across hemispheres and emotion conditions (pleasant and unpleasant collapsed). Ctx, cortex; Emo, emotional; Neu, neutral; Dm, remembered – forgotten.

Additionally, a second goal of the study was to explore the possibility of anatomical specialization within the MTL memory system for effects of emotion on memory formation. Although little evidence is available, reasonable candidates are anterior MTL memory regions, particularly the hippocampus and the entorhinal cortex, which are not only richly interconnected with the amygdala (Amaral et al., 1992; Pitkanen et al., 2000) but also have been identified as potential targets of amygdalar modulatory influences in pharmacological and lesion studies (McGaugh et al., 2002; Roesler et al., 2002). Thus, we investigated the possibility that the effect of emotion on Dm activity would be more pronounced in anterior than in posterior MTL memory regions.

To investigate these ideas, encoding-related activity from MTL regions was obtained using event-related fMRI while participants rated high-arousing emotional (both pleasant and unpleasant) and low-arousing neutral pictures for pleasantness. Cued recall for the pictures was assessed following scanning and used to identify the emotional Dm and the neutral Dm, which were then compared to each other. Correlations between the Dm in amygdala and the MTL memory regions were also calculated for emotional and neutral stimuli. Emotional Dm was calculated by collapsing both categories of high-arousing pictures to form the emotional category. Activity in the amygdala and the MTL memory regions was identified using manually traced anatomical regions of interest (ROIs). The ROI approach was used because

it provides a precise localization of the fMRI signal recorded from neighboring brain regions, such as those in the MTL. In the amygdala, the main focus was on separating the signal from BLA, which has been identified as the main site of amygdalar modulatory influences on memory-related activity in other brain regions. In the MTL memory system, ROI tracing first identified the main regions (i.e., the hippocampus and the parahippocampal gyrus), which were then further subdivided into their major subregions. Thus, following a rostro-caudal organization, the hippocampus was subdivided into head, body, and tail, and the parahippocampal gyrus (PHG) was subdivided into anterior and posterior PHG. Posterior PHG included the parahippocampal cortex. Finally, anterior PHG was further subdivided into entorhinal and perirhinal cortices.

## Results

### Behavioral Results

#### Valence Ratings

The average valence scores (1 = unpleasant, 2 = neutral, 3 = pleasant) as rated by the participants in the scanner were 1.14 for unpleasant pictures (SD = 0.16), 2.18 for neutral pictures (SD = 0.40), and 2.64 for pleasant pictures (SD = 0.26). All pairwise comparisons were significant ( $p < 0.0001$ ). Thus, the subjects' rating scores were consistent with the normative scores provided by the International Affective Picture System

Table 1. MTL Regions Showing Significantly Greater Emotional than Neutral Dm and Greater Amygdala-MTL Correlations for Emotional than for Neutral Dm

MTL Regions	EmoDm > NeuDm (F Scores)	Amygdala-MTL Correlations (R scores – EmoDm)	Amygdala-MTL Correlations (R scores – NeuDm)
Amygdala	5.09* (BLA)	N/A	N/A
Hippocampus (whole)	-	0.67**	0.54*
Hippocampus (head)	6.67* (L)	0.78****	0.60*
Hippocampus (body)	-	0.75*** (R)	0.46
Hippocampus (tail)	-	-	-
Anterior PHG (whole)	6.63*	0.77*** (BLA/L)	0.03
Anterior PHG (entorhinal ctx.)	6.43*	0.77*** (BLA/L)	0.02
Anterior PHG (perirhinal ctx.)	-	0.59* (BLA/L)	-0.05
Posterior PHG (parahippocampal ctx.)	-	0.75*** (R)	0.62*

Unless specified, the results are based on the averaged signal from both hemispheres. BLA, basolateral amygdala; PHG, parahippocampal gyrus; Dm, remembered – forgotten; EmoDm, emotional Dm; NeuDm, neutral Dm; L, left; R, right.

\* $p < 0.05$ , \*\* $p < 0.005$ , \*\*\* $p < 0.0005$ , \*\*\*\* $p < 0.0001$ .

(IAPS; Lang et al., 1997), which is the source of stimuli used in the present study. Further validating this consistency, the correlation between our subjects' average scores and the normed IAPS scores of the pictures used in the present study was highly significant ( $R = 0.90$ ,  $p < 0.0001$ ).

#### Memory Performance

As expected, mean recall ( $\pm$ SD) was better for high-arousing emotional pictures (pleasant,  $52\% \pm 4.5\%$ ; unpleasant,  $53\% \pm 4.8\%$ ) than for low-arousing neutral pictures ( $38\% \pm 4.8\%$ ). An ANOVA yielded a significant picture type effect [ $F(2,15) = 41.21$ ,  $p < 0.0001$ ]. Post hoc contrasts showed that recall of pleasant and unpleasant pictures was similar ( $p > 0.05$ ), and recall for both high-arousing categories was higher than that for neutral pictures ( $ps < 0.0001$ ).

#### fMRI Results

##### Effect of Emotion on Dm in the Amygdala and MTL Memory System

Confirming our first prediction, the emotional Dm was greater than the neutral Dm in both the amygdala and the MTL memory system. As seen in Figure 1 and Table 1, ANOVAs computed on data extracted from anatomically defined ROIs at peak time points and activation slices/subregions yielded a significant emotion effect on Dm in the amygdala, hippocampus, and anterior PHG but not in the posterior PHG. Interestingly, in the amygdala this difference occurred in the basolateral aspect (BLA) of the amygdalar ROI, in the hippocampus it occurred in its most anterior part (the head), and in the anterior PHG it occurred in the entorhinal cortex.

##### Correlation between Emotional Dm in Amygdala and MTL Memory System

Confirming our second prediction, greater correlations between the amygdala and MTL memory system were found for the emotional Dm than for the neutral Dm. Correlations were calculated for both the regions showing the greatest differences between the emotional Dm and the neutral Dm (i.e., BLA, head of the hippocampus, and the entorhinal cortex) and for those whose Dm activity was not modulated by emotion (i.e., the body and tail of the hippocampus, and the parahippocampal cortex). As illustrated by Table 1, Pearson coefficients calculated across subjects showed that the correlation be-

tween the emotional Dm in the amygdala and the MTL memory regions were highly significant and greater for emotional than for neutral pictures. In other words, the subjects showing greater emotional Dm in the amygdala also showed greater emotional Dm in the MTL memory regions. Correlations between the Dm in amygdala and hippocampus were significant and greater for emotional than for neutral pictures when they involved the whole hippocampal structure or when the correlations were calculated separately for the head and the body, but not when Dm activity from the tail was involved. The maximum difference in correlation between the Dm for emotional and neutral stimuli occurred for the amygdala-entorhinal cortex correlation, which was highly significant for the emotional pictures ( $R = 0.77$ ,  $p < 0.0003$ ) but not for the neutral pictures ( $R = 0.02$ ,  $p > 0.9$ ; see Table 1 and Figure 2), and the difference between these two correlations was also significant ( $p < 0.01$ ). Taken together, the present results suggest that the amygdala and the MTL memory regions are coactivated more consistently during successful encoding of emotional pictures than during successful encoding of neutral pictures.

##### Double Dissociation between Emotional and Neutral Dm along the Longitudinal Axis of MTL Memory System

Consistent with our expectations, we found that the emotional Dm was greater in the anterior than in the posterior MTL memory regions. Additionally, the reverse pattern was found for the neutral Dm, demonstrating a double dissociation between the anterior and the posterior regions. To test for this double dissociation in the hippocampus, we conducted an ANOVA comparing the Dm for emotional and neutral stimuli in the most anterior (head) and most posterior (tail) hippocampal regions, calculated at the peak time point and averaged across hemispheres. Confirming the double dissociation, this 2 (region: head versus tail) by 2 (emotion: emotional versus neutral) ANOVA yielded a significant interaction [ $F(1,15) = 5.7$ ,  $p < 0.05$ ]. To test for this double dissociation in PHG, we conducted a similar ANOVA comparing the Dm for emotional and neutral stimuli in anterior versus posterior PHG regions. Again, confirming the anterior-posterior double dissociation, a 2 (region: anterior versus posterior) by 2 (emotion: emotional versus neutral) design yielded a significant interaction [ $F(1,15) =$

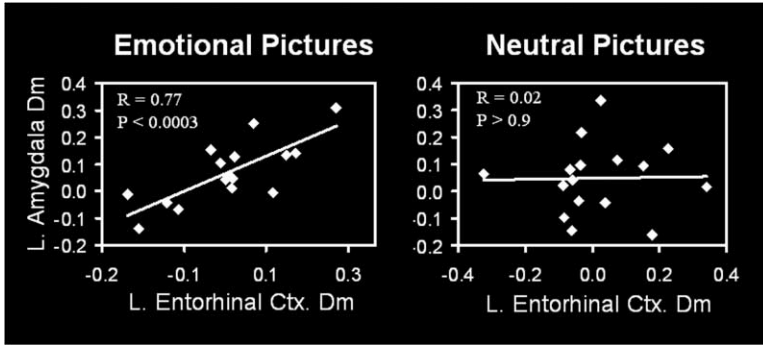


Figure 2. Stronger Amygdala-Entorhinal Correlation for the Emotional Dm than for the Neutral Dm

The plots are based on the emotional Dm (left) and the neutral Dm (right), as extracted from left amygdala (the peak activation slice in BLA) and left entorhinal cortex. Ctx, cortex; Dm, remembered – forgotten; L, left.

11.25,  $p < 0.005$ ], which was more significant when the entorhinal [ $F(1,15) = 10.9$ ,  $p < 0.005$ ; see also Figure 3] rather than the perirhinal cortex was compared to the posterior PHG [ $F(1,15) = 5.8$ ,  $p < 0.03$ ].

**Discussion**

The present study yielded three main findings. The first two findings are consistent with the modulation hypothesis: (1) the emotional Dm was greater than the neutral Dm in both the amygdala and the MTL memory system, and (2) the correlation between the amygdala and the MTL memory system was greater for the emotional Dm than for the neutral Dm. The third finding was a dissociation between anterior and posterior MTL memory regions: whereas anterior regions showed a greater emotional Dm, posterior regions showed a greater neutral Dm.

**Evidence for the Modulation Hypothesis**

Successful encoding activity in the amygdala and MTL memory structures was greater and more strongly correlated for emotionally arousing than for neutral pictures. Initial positron emission tomography (PET) studies exploring the neural correlates of emotional encoding suggested a link between amygdala activity at encoding and memory for emotionally arousing material (Cahill et al., 1996; Hamann et al., 1999). However, these studies used blocked designs that cannot accommodate the subsequent memory paradigm to distinguish brain activity for successfully versus unsuccessfully encoded stimuli within participants. More recent fMRI studies by Canli and colleagues (Canli et al., 2000, 2002) used event-related designs to examine emotional memory formation, but they focused primarily on the amygdala and did not report correlations between the amygdala and the MTL memory regions. Moreover, these previous PET and fMRI studies used voxel-wise analyses, which, because of the normalization of individual variations in human anatomy to a standard brain template and because of image smoothing, do not provide an accurate localization in neighboring MTL regions. The extant human literature thus implicates involvement of the amygdala in emotional memory but has not revealed the mechanisms by which the amygdala interfaces with other memory processing areas of the brain. By addressing these limitations, the present study provides more direct evidence for the modulation hypothesis in humans.

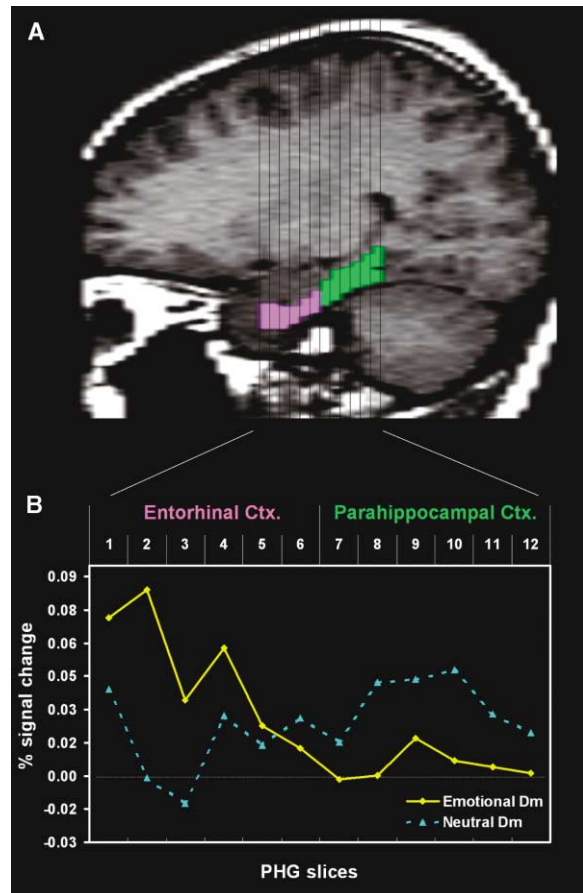


Figure 3. Functional Dissociation along the Longitudinal Axis of the PHG

Anterior PHG, particularly the entorhinal cortex, was associated with subsequent memory for emotional pictures, whereas posterior PHG (i.e., parahippocampal cortex) was associated with subsequent memory for neutral pictures.

(A) Sagittal view of the entorhinal and parahippocampal cortices as traced on the anatomical brain image of one representative subject. (B) Comparison of the emotional Dm and the neutral Dm as extracted slice-by-slice from anatomical ROIs traced in the entorhinal and parahippocampal cortices. The numbers from the upper part of the graph correspond to the PHG slices as they are shown in (A). PHG regions are color-coded as follows: Entorhinal cortex, magenta; parahippocampal cortex, green. PHG, parahippocampal gyrus; Ctx, cortex; Dm, remembered – forgotten.

The use of anatomical ROIs also allowed us to identify the specific subregions of the amygdala and MTL memory system sensitive to emotion effects during encoding. Within the amygdala, the emotional memory effects (i.e., emotional Dm > neutral Dm) were largest in its basolateral aspect (BLA), which is anatomically interconnected with the fronto-temporal neocortex (Amaral et al., 1992) and has been identified by animal research on emotional memory as the main site through which the amygdala modulates memory-related activity in other brain regions (Cahill, 1998; McGaugh, 2000, 2002; McGaugh et al., 2002). Within the MTL memory system, the effect of emotion on Dm was most pronounced in its anterior portion, comprising both hippocampal and parahippocampal regions. The involvement of these regions in successful encoding is consistent with the results of fMRI studies using the subsequent memory paradigm (Brewer et al., 1998; Davachi and Wagner, 2002; Kirchoff et al., 2000; Otten et al., 2001; Otten and Rugg, 2001; Strange et al., 2002; Wagner et al., 1998; for a review, see Paller and Wagner, 2002) and with animal studies that places the PHG, particularly the entorhinal cortex, in a key position within the MTL memory system (Eichenbaum, 2000; Squire and Zola-Morgan, 1991). The fact that the emotion effect on Dm activity in the MTL memory system was maximal in the anterior hippocampus and entorhinal cortex fits well with anatomical evidence that these regions are recipients of rich feedback projections from the amygdala (Amaral et al., 1992; Pitkanen et al., 2000). Moreover, these regions have been also associated with the effects of emotion on memory in pharmacological and lesion studies (McGaugh et al., 2002; Roesler et al., 2002).

In addition to the finding that the emotional Dm was greater than the neutral Dm in both the amygdala and the anterior MTL memory regions, the present results showed that the correlation between Dm activity in these regions was greater for the emotional than for the neutral stimuli. This finding suggests that these regions interact more intimately during the encoding of emotional stimuli than during the encoding of neutral stimuli. Although correlations do not imply causation, our findings are consistent with the hypothesis that the amygdala enhances processing of emotional stimuli in MTL memory regions, thereby leading to better memory for emotional than for neutral information (McGaugh, 2002). Thus, the combined finding that successful encoding activity in the amygdala and MTL memory regions were both greater and more strongly correlated for emotional than for neutral stimuli is strong evidence for the modulation hypothesis in humans. The left-sided bias of the amygdala-entorhinal correlation is consistent with previous studies of emotional memory in female participants (Cahill et al., 1996; Canli et al., 2002) and may reflect deeper semantic processing or other verbal strategies (Funayama et al., 2001).

It is unclear why the effect of emotion on successful encoding activity was stronger for the entorhinal cortex than the hippocampus. This difference may be related to factors influencing the detection of Dm with fMRI. In fact, several fMRI studies found the Dm in parahippocampal regions but not in the hippocampus (Brewer et al., 1998; Otten and Rugg, 2001; Wagner et al., 1998), and it is uncertain why other studies found it in both

regions (e.g., Davachi and Wagner, 2002; Kirchoff et al., 2000; Otten et al., 2001; Strange et al., 2002). Alternatively, larger effect of emotion on entorhinal cortex versus hippocampus may be related to the timing of the modulatory influence. That is, the neurohormonal influences of the amygdala on the consolidation processes occurring in the hippocampus, as reported in animal studies, are typically found after longer retention intervals (i.e., several hours or days; McGaugh and Roozendaal, 2002; Roesler et al., 2002), whereas the present results are based on a shorter retention interval (less than an hour). Thus, the present entorhinal findings may reflect short-term neuronal effects rather than long-term neurohormonal effects. At any rate, the present results are consistent with the notion that emotional arousal exerts its beneficial effect on explicit memory through interactions between the amygdala and memory-related MTL regions.

#### Functional Dissociation along the Longitudinal Axis of MTL

The second goal of the study was to investigate the existence of MTL memory regions differentially sensitive to the effects of emotion on successful encoding. Confirming this idea, we found that the emotional Dm was greater in anterior than in posterior sectors of the MTL memory system. Additionally, we found that the neutral Dm was greater in posterior than in anterior MTL memory regions. In other words, we observed a double dissociation along the longitudinal axis of the MTL (see Figure 3). The emotional Dm was greater in the anterior hippocampus (the head) and anterior PHG (particularly in the entorhinal cortex), whereas the neutral Dm was greater in the posterior hippocampus (the tail) and posterior PHG (i.e., parahippocampal cortex). The finding that the emotional Dm was greater in anterior MTL memory regions is consistent with anatomical evidence that these regions are richly interconnected with the amygdala (Amaral et al., 1992; Pitkanen et al., 2000) and with animal evidence specifically identifying amygdalar modulation of emotional memory formation in both hippocampal and entorhinal regions (McGaugh et al., 2002; Roesler et al., 2002). This finding is also consistent with a neuroimaging study that found activity related to emotional memory in anterior PHG (Alkire et al., 1998), although this evidence was not conclusive since activity in this region was also related to neutral memory. Even though greater neutral Dm in the posterior than in the anterior MTL was not predicted, it is consistent with the results of several neuroimaging studies of successful encoding of neutral stimuli (Alkire et al., 1998; Brewer et al., 1998; Wagner et al., 1998; for a review, see Paller and Wagner, 2002).

It could be argued that the greater neutral Dm in posterior MTL, particularly in the posterior PHG, reflected a confound with the amount of scene information in neutral pictures, as compared to emotional pictures. Given that posterior PHG ("parahippocampal place area") is activated by scene perception (Epstein and Kanwisher, 1998) and during the encoding of complex scenes (Brewer et al., 1998; Gabrieli et al., 1997), it is important to determine that scene content did not differ between emotional and neutral stimuli. To investigate this idea,

Table 2. Example of Scoring Criteria for Cued Recall Procedure Conducted outside the Scanner

Actual Picture Description	Picture Cue	Subject's Description	Raters' Decision
Green snake on black background; open mouth facing viewer	snake	open mouth	not recalled
Brown snake on tree branch; open mouth facing left	snake	open mouth/brown/on tree branch	recalled

we asked 10 participants to rate our stimuli using a scene content scale (1 = no scene content, 4 = very high scene content). The ratings for emotional (1.86) and neutral (1.93) pictures were similar ( $T = 0.64$ ,  $p > 0.5$ ), suggesting an equivalent amount of scene information in the two classes of stimuli. Nevertheless, we analyzed Dm activity in PHG using a 3-way ANOVA with scene content (low versus high), PHG region (anterior versus posterior), and emotion (emotional versus neutral) as the factors. A nonsignificant 3-way interaction [ $F(1,15) = 0.62$ ,  $p > 0.44$ ] confirmed that neutral Dm in posterior PHG was not differentially greater for pictures with high scene content. This nonsignificant interaction was also confirmed when the same ANOVA was performed separately for the entorhinal [ $F(1,15) = 2.5$ ,  $p > 0.13$ ] and perirhinal [ $F(1,15) = 0.53$ ,  $p > 0.47$ ] cortices versus parahippocampal cortex and when anterior and posterior hippocampal regions (head versus tail) were compared to each other [ $F(1,15) = 0.67$ ,  $p > 0.42$ ]. These results, together with the fact that emotional and neutral pictures were equated for complexity, presence of human figures, and other lower-level visual features (Yamasaki et al., 2002), suggest that scene content or perceptual factors cannot account for the differences between anterior and posterior PHG activity. Thus, the present double dissociation represents direct evidence that different regions of the MTL memory system are differentially involved in the successful encoding of emotional versus neutral stimuli.

Several hypotheses with respect to possible rostro-caudal segregations of MTL function have been proposed. One model suggests that anterior MTL regions may be more important for relational aspects of encoding, whereas posterior regions are more important in item-related aspects of encoding (Schacter and Wagner, 1999). Another dissociation following the functional organization along the ventral visual pathway (Ungerleider, 1995) proposes that posterior regions, closer to the primary visual cortex, are more associated with lower-level perceptual processes, whereas more anterior regions are associated with higher-level mnemonic processes. Our results are consistent with both kinds of models. One possibility is that the greater emotional Dm in the anterior MTL memory regions reflects enhanced semantic and relational processing for emotional stimuli, whereas the greater neutral Dm in the posterior MTL reflects enhanced perceptual processing for neutral stimuli. This idea is compatible with evidence that anterior MTL regions are associated with memory for semantic information and posterior MTL regions with memory for perceptual information (Cabeza et al., 2001). Although future research is needed to clarify the specific mechanism involved, the double dissociation revealed by the present study clearly indicates that different MTL

sectors are specialized to encode emotional and neutral information into long-term memory.

## Conclusions

Remembering emotionally arousing events entails concomitant activity in an amygdala-based emotional processing system and in MTL regions that support various mnemonic functions. Results from the present study provided novel insights into the neural mechanisms associated with the formation of explicit emotional memories in the intact human brain. Successful encoding activity in the amygdala and MTL memory structures was greater and more strongly correlated for emotional than for neutral pictures. Furthermore, a double dissociation was observed along the longitudinal axis of the MTL memory system for successful encoding of emotional and neutral stimuli. The results support the modulation hypothesis of emotional memory as developed from animal models and provide clear neuroimaging evidence that emotionally arousing stimuli exert their beneficial effect on episodic memory by enhancing activity in both the amygdala and the MTL memory system in the human brain. The present study also highlights the key position of the entorhinal cortex in this process. Taken together, the findings advance an understanding of the MTL mechanisms underlying emotional memory formation and their conservation across species.

## Experimental Procedures

### Subjects

Sixteen young (mean age =  $25 \pm 4.6$  yrs, all female), right-handed, healthy adults participated in the study. Female participants were chosen because previous studies showed that, compared to men, women are physiologically more reactive to emotional stimuli (Lang et al., 1993) and are more likely to report intense emotional experiences (Shields, 1991). All subjects provided written informed consent for a protocol approved by the Duke University Institutional Review Board.

### Materials

Stimuli consisted of 120 high-arousing emotional (60 pleasant and 60 unpleasant) and 60 low-arousing neutral pictures selected from the International Affective Picture System (IAPS) series (Lang et al., 1997), based on their standard scores for emotional arousal and emotional valence. Pleasant and unpleasant pictures were equally arousing, and both were more arousing than the neutral pictures. The mean arousal score was 6.0 for pleasant ( $SD = 2.2$ ), 6.15 for unpleasant ( $SD = 2.2$ ), and 3.15 for neutral pictures ( $SD = 2.0$ ). The mean valence score was 7.1 for pleasant ( $SD = 1.7$ ), 2.3 for unpleasant ( $SD = 1.5$ ), and 5.2 for neutral ( $SD = 1.4$ ). To equate the emotional and neutral categories for visual complexity and content (e.g., human presence), the IAPS pictures were supplemented with neutral pictures from other sources (Yamasaki et al., 2002).

### Procedure

The pool of 180 pictures was divided into six sets of 30 pictures (10 pleasant, 10 unpleasant, and 10 neutral), which were randomly

assigned to six study blocks. Six different block orders were randomly assigned to the participants. To avoid the induction of long-lasting mood states, the pictures within each block were pseudo-randomized so that no more than two pictures of the same valence were consecutively presented. Functional MR images were recorded while subjects viewed the emotional and neutral pictures. Participants were instructed to experience any feelings or thoughts the pictures might trigger in them and to rate each picture for pleasantness using a 3-point scale (1 = unpleasant, 2 = neutral, 3 = pleasant). Nothing was mentioned about the subsequent memory test, before or during the encoding task (incidental learning). The pictures were presented, using an LCD projector, to a screen located behind the subjects' crown that subjects could see via an angled mirror. Each picture was presented for 3 s and followed by a 12 s fixation cross. This long interstimulus interval (ISI) was used to allow the fMRI signal to come back to baseline and avoid elevated baseline activity prior to the onset of the next stimulus (Yamasaki et al., 2002). This is an important issue to consider, particularly in the context of comparing emotional and neutral stimuli using selective averaging of fMRI signal relative to ISI baseline. That is, it is possible that experiencing the emotion might continue into the fixation period, and thus selective averaging relative to ISI baseline may reduce the magnitude of the response for these trials. Therefore, the use of long ISI and pseudo-randomization stimulus presentation, as well as the use of stimulus onset, rather than ISI, as baseline (see below) helped us avoid a possible confound of the baseline with the trial types.

Forty-five minutes after the scanning session, subjects performed a surprise cued-recall test conducted outside the MRI suite. Subjects were provided with one- or two-word written cues for each picture (e.g., snake, building, skydivers) and had to describe in writing, and in as much detail as they could, the pictures they remembered. Participants were asked to provide enough relevant details (e.g., about the number of elements, color, action, etc.) so that an outsider could identify each picture and discriminate it from similar studied pictures (e.g., a brown snake facing viewer versus several small green snakes). The test lasted until participants could not recall any additional pictures or until a maximum of 50 min had elapsed. Two raters were involved in the evaluation of the written descriptions provided by the subjects, and only the pictures whose description was detailed enough to allow both identification and discrimination were classified as remembered. To illustrate this, let us consider the example of two similar pictures presented in Table 2. In this example, both pictures contain a snake with open mouth and both can be recalled based on this single cue. If the subjects only remember that they saw a snake with an open mouth, the raters can identify several possible pictures that fit the description, but they cannot distinguish among them. Hence, none of these pictures would be considered "recalled" without additional distinguishing details.

Functional MRI signal recorded during encoding was selectively averaged based on the emotion category (i.e., emotional versus neutral) and on subsequent memory performance (i.e., remembered versus forgotten = Dm). Since the main goal of the study was to compare the emotional Dm with the neutral Dm, the Dm was first calculated for both emotional and neutral pictures, separately. Then, emotional and neutral Dms were directly compared to each other. The basic assumption was that activity in brain regions showing greater emotional Dm than neutral Dm reflects emotional arousal modulations of encoding mechanisms.

Given the fact that subjects' rating scores were highly consistent with the IAPS standard scores (see Behavioral Results), the latter were used to separate the emotional and neutral categories. Also, behavioral (Bradley et al., 1992) and neuroimaging (Anderson et al., 2003; Hamann et al., 2002) evidence suggests that the emotional intensity rather than the emotional valence is the main factor determining amygdalar activity and long-term explicit memory for emotional stimuli. Therefore, the pleasant and unpleasant pictures were collapsed to form the emotional category. This procedure was methodologically suitable since pleasant and unpleasant pictures were equated for emotional arousal.

#### MRI Acquisition

Neuroimaging was performed using a 1.5 Tesla GE scanner. Two series of high-resolution T1-weighted structural images were ac-

quired relative to the anterior commissure-posterior commissure (AC-PC) plane. 34 axial (parallel to AC-PC plane) and 46 coronal (perpendicular to AC-PC plane) were acquired using the following parameters: TR = 450 ms; TE = 9 ms; FOV = 24; matrix size = 256<sup>2</sup>; slice thickness = 3.75 mm. 34 contiguous functional images (gradient-echo echoplanar images sensitive to BOLD contrast) were acquired parallel to the AC-PC plane (TR = 3000 ms; TE = 40 ms; FOV = 24; matrix size = 64<sup>2</sup>; FA = 90°; one radio frequency excitation; slice thickness = 3.75 mm, resulting in cubic voxels).

#### fMRI Data Analysis

Region of interest (ROI) analyses were performed using in-house software developed at Duke University's Brain Imaging and Analysis Center (Jha and McCarthy, 2000; Yamasaki et al., 2002). In this analysis, the fMRI signal is extracted from ROIs that are manually traced on each subject's anatomical brain image. Consequently, it can provide a precise localization of the fMRI signal recorded from anatomically proximal brain regions, such as those from MTL.

#### Preprocessing and ROI Tracing

Image preprocessing involved slice timing correction and coregistration. ROIs were manually drawn on each participant's coronal brain images, based on corroborated guidelines for MTL tracing (Brierley et al., 2002; Duvernoy et al., 1999; Insausti et al., 1998; Pruessner et al., 2000, 2002). In-house software packages were used for tracing ROIs in the following MTL regions: amygdala, hippocampus, and parahippocampal gyrus (PHG; see Figures 1A and 1B). The amygdalar ROIs were additionally divided into four quadrants. Particularly, we were interested in activity from the basolateral quadrant (BLA), since this region has been identified by animal research as the main site through which the amygdala modulates the memory-related activity in other brain regions (McGaugh, 2002; McGaugh et al., 2002). The hippocampal and PHG ROIs were also subdivided into their main anatomical components. Following a rostro-caudal organization, the hippocampus was subdivided into head, body, and tail, and the PHG was subdivided into anterior and posterior PHG. This anterior-posterior separation was employed as a result of evidence suggesting possible functional dissociations along the longitudinal axis of the MTL memory system (Amaral et al., 1992; Pitkanen et al., 2000; McGaugh et al., 2002; Roesler et al., 2002). The anterior PHG was further subdivided into medial and lateral portions corresponding to its anatomical subregions (i.e., entorhinal and perirhinal cortices, respectively). The boundaries among the hippocampal and PHG subregions were identified in each subject using anatomical landmarks (Duvernoy et al., 1999; Insausti et al., 1998; Pruessner et al., 2002). Consistent with anatomical nomenclature for MTL regions, in the present article the term "parahippocampal" refers generically to PHG regions. If it is associated with more specific terms (e.g., anterior versus posterior), it refers either to both entorhinal and perirhinal cortices (anterior PHG) or to parahippocampal cortex proper (posterior PHG). The terms entorhinal and perirhinal both refer to specific subregions of the anterior PHG.

#### Statistical Analyses

ROI analysis employed two levels: individual and group analyses. For individual analyses, the fMRI signal extracted from all voxels contained in the ROIs was selectively averaged in each subject as a function of stimulus condition (e.g., remembered versus forgotten), hemisphere, slice, and time point (one prestimulus and four post-stimulus onset time points were employed). For group analyses, ANOVAs were performed on the percent signal change relative to stimulus onset for each effect of interest (e.g., emotional Dm versus neutral Dm) and MTL region. Furthermore, the ROI approach also involved correlation analyses. Across-subject pairwise Pearson correlation tests between the Dm in the amygdala and in memory-related MTL regions (PHG and hippocampus) were performed on the average data extracted from each subject's ROIs and hemispheres and compared for emotional and neutral pictures. For all analyses involving the fMRI signal extracted from a priori anatomically defined ROIs, the significance threshold was set at  $p < 0.05$ , uncorrected.

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