Functional activation of the left amygdala and hippocampus during associative encoding

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The human hippocampus is critical to episodic encoding, but the role of the amygdala in memory is less clear. Animal research suggests a role for the amygdala in associative memory, but this has not been examined systematically in humans. Using fMRI, we compared amygdala and hippocampus activation for seven healthy subjects during two visual encoding tasks: serially presented single faces and faces presented as pairs. Single faces activated bilateral hippocampi, but not the amygdala. Paired faces activated bilateral amygdala, but only the left hippocampus. Subtraction of the two conditions revealed greater activation within the left amygdala and hippocampus during paired face encoding, suggesting that associative encoding activates a left-lateralized limbic network including the hippocampus and amygdala. *NeuroReport* 11:2259–2263 © 2000 Lippincott Williams & Wilkins.

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INTRODUCTION

The encoding of explicit information into long-term memory depends critically on the medial temporal lobes (mTL) [1,2], which comprise a complex network of interconnected nuclei including the amygdala, hippocampus, and surrounding structures. Neuroimaging studies support the role of the hippocampus and posterior mTL during memory encoding and retrieval [3–6], and lesion studies have demonstrated that memory performance is significantly affected by the integrity of mTL structures [7]. Compared with the hippocampus, the role of the amygdala in memory processing is poorly understood at present. It is well established that the amygdala is involved in affective processes such as fear recognition [8,9] and aversive conditioning [10,11], leading some authors to suggest that the amygdala may directly enhance or strengthen biologically relevant episodic memories by giving them emotional salience [12,13]. For example, the amygdala may directly facilitate rewarding or aversive associations to individual stimulus cues [14], or it may modulate the activity of the hippocampus and its cortical projections by enhancing long-term potentiation, which in turn, facilitates storage of memories throughout the brain [12,15]. To date, however, most studies have examined the role of the amygdala in emotional associative memory by evaluating specific stimulus–emotion, stimulus reinforcer (e.g. taste aversion) [16,17], or cross-modal sensory (e.g. touch–vision) associations [18]. Although evidence from animal research suggests that the amygdala may serve to form stimulus–stimulus memory associations [19], this has not yet been demonstrated conclusively in humans.

Given that recent neuroimaging studies suggest that one principal role of the mTL is to form associations in memory [20], we hypothesized that we could elicit greater activation in the hippocampus and nearby structures during associative than during non-associative encoding. Healthy subjects performed two similar face memory tasks while undergoing fMRI to evaluate blood oxygenation dependent (BOLD) changes in the amygdala and hippocampus during encoding. During the non-associative memory task, subjects were required to encode a series of individual face photographs for later recognition. By contrast, in the paired-associative memory task, subjects were presented with pairs of mixed gender face photographs and were required to form a memory association to remember that each pair of faces 'go together' (i.e. were a pair).

MATERIALS AND METHODS

Subjects: Functional neuroimaging data were collected from seven healthy participants (two males, five females) who provided written informed consent and were each paid $20. The mean (± s.d.) age was 24.7 ± 5.1 years (range 21–34). Six subjects were right-hand dominant and one was left-hand dominant by self-report, and all had normal

or corrected-normal vision. Participants were recruited via an Internet advertisement placed on the University of Pennsylvania newsgroup web-site. All subjects were students or trainees at the university (five undergraduates, one graduate student, and one post-doctoral fellow). Subjects had no known history of psychiatric or severe medical problems. The University of Pennsylvania Institutional Review Board approved all research prior to data collection.

Imaging methods: Imaging data were collected on a 1.5 T GE Signa MRI scanner equipped with a standard quadrature RF head coil fit with a fast gradient echo, echoplanar imaging system using BOLD contrast (TR = 2 s, TE = 50 ms) for functional imaging. Head motion was minimized by comfortable placement of foam padding around the head. Functional images were collected in 16–18 axial slices with a 24 cm field of view and a 64×64 acquisition matrix, with an in-plane resolution of $3.75 \times 3.75 \times 5$ mm. BOLD activation data were collected during a single 240 scan run consisting of six alternating control/task cycles of 80 s duration. Functional images were corrected for motion and static susceptibility effects [21], and matched T1-weighted images were collected at the outset of the scanning session.

Face encoding paradigms: Two visual activation tasks were used: a single-face encoding task and a paired-face encoding task. The stimuli were randomly drawn from a large database of University of Pennsylvania ID photographs. Consent was obtained from all volunteers whose photographs were used. The photographs were matched for image quality and general size proportions, and included fully visible hair and facial features (see Fig. 1). The stimuli were back-projected onto a screen placed at the foot of the scanning bed and were viewed via a mirror mounted on the head coil.

Single-face encoding task: While undergoing functional scanning, subjects viewed a total of 60 individual color face photographs at a rate of one every 4 s (3.5 s projection, 0.5 s ISI). Face stimuli were presented during 40 s blocks (10 stimuli/block) that alternated with a matched control task over six task/control cycles. To control for the effects of luminance and color stimulation, the control task consisted of matched presentations of a randomly degraded photograph. Prior to scanning, subjects were informed that they would view a series of faces that they would be required to recognize during a post-test at the completion of the scan. During the post-test, subjects viewed a series of 120 face photographs (60 target faces and 60 foils). Subjects were required to make a 'yes' or 'no' response to indicate recognition of previously seen faces using a hand held keypad. A discriminability index was calculated for each subject (the proportion correct minus the proportion of false positives). As a manipulation check, the mean ($\pm$ s.d.) discriminability index (0.54 ± 0.12) was found to be significantly above zero ($t(4) = 9.87$, $p < 0.001$), suggesting that subjects were attending to and encoding the stimulus items.

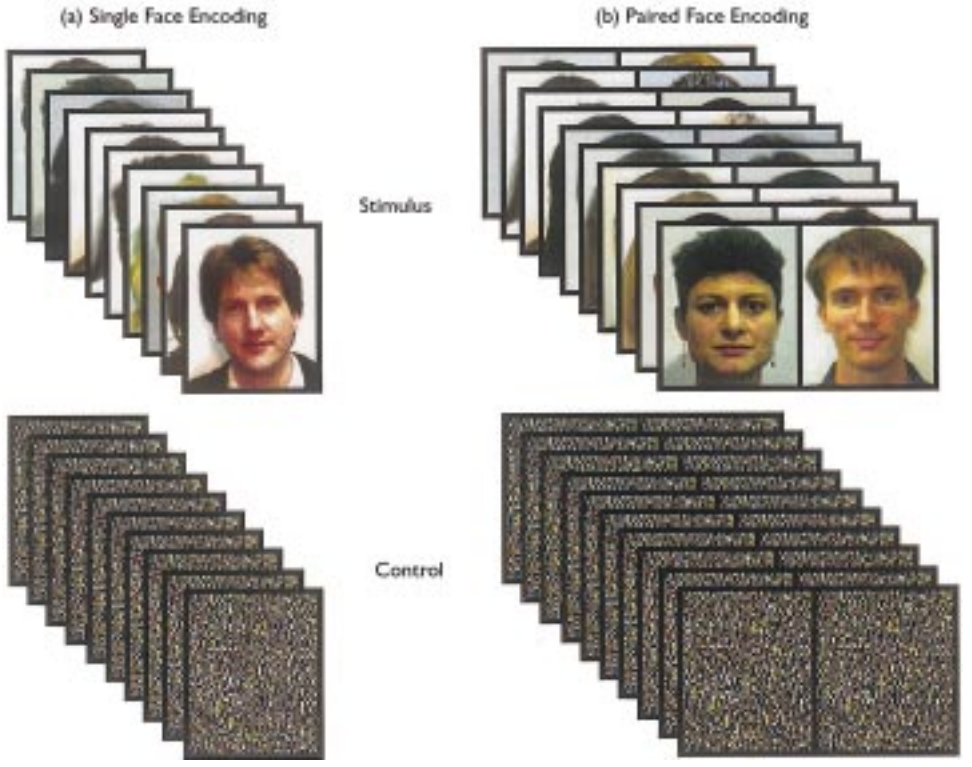

Fig. 1. Representative face photographs used during functional activation studies. During single face encoding (**a**) subjects viewed blocked series of individual photographs that alternated with blocks presenting only a randomly retiled control image. During the paired face encoding condition (**b**) subjects viewed pairs of faces designated as couples which alternated with blocks of paired control images.

Paired-face encoding task. Subjects also viewed another face encoding task consisting of paired face photographs (see Fig. 1). The order of the tasks was varied pseudorandomly across subjects. The paired-face task retained the same overall timing parameters as the single-face task. For this task, however, a single face was presented individually on the screen for 3.5 s, and was then joined by a second, opposite-sex face for an additional 3.5 s, with a 1 s ISI. Thus, subjects viewed the same number of faces per block as during the single face condition. Subjects were told that they would be viewing photographs of heterosexual couples and were asked to remember that the two faces constituted a pair or couple that should be remembered together. The control task consisted of pairs of degraded images presented in the exact manner as the paired face photographs. Subjects were informed that there would be a recognition test following the scan. During the post-test, half of the stimulus faces were presented with their correct mates, and the other half were incorrectly paired with either a novel face or an incorrectly matched though previously seen face. Subjects were required to make a button press response indicating whether the two faces had been seen previously as a pair. The mean discriminability index (0.34 ± 0.15) was significantly above zero ($t(5) = 5.60$, $p < 0.002$), suggesting that subjects were encoding the item pairs.

Image processing and analysis: Functional images were corrected for motion and static susceptibility-induced distortions and convolved into three-dimensional space using a nonisotropic gaussian kernel (full width half maximum (FWHM) $= 11.25 \times 11.25 \times 15$ mm). A statistical parametric map was generated for each subject using a two-condition regression model in SPM97 [22], and a multisubject SPM ($\alpha = 0.05$) was constructed in Talairach space [23]. Two a priori defined anatomical regions of interest (ROIs) comprising only the amygdala and hippocampus were defined for each hemisphere using a standardized atlas of brain anatomy [24]. The number of active voxels within each lateralized search region exceeding a threshold of statistical significance ($\alpha = 0.05$, mapwise) was determined and the proportion of suprathreshold voxels within each ROI was calculated [3]. To evaluate laterality of activation, the proportion of active voxels within each lateralized ROI was compared using a z-test for the difference between independent proportions.

RESULTS

Figure 2 shows the fMRI data for the single and paired face memory conditions and the results of the subtraction of the two data sets. The images displayed in Fig. 2 are presented with a mask restricting the view only to the significant activation within the ROIs. The total voxels included with-

(a) Single Faces

	Left	Right	Sig.
Amygdala	1	0	*ns*
Hippocampus	11	4	*ns*

(b) Paired Faces

	Left	Right	Sig.
Amygdala	12	12	*ns*
Hippocampus	15	1	$p < 0.001$

(c) Pair-Single Subtraction

	Left	Right	Sig.
Amygdala	16	1	$p < 0.0001$
Hippocampus	8	1	$p < 0.05$

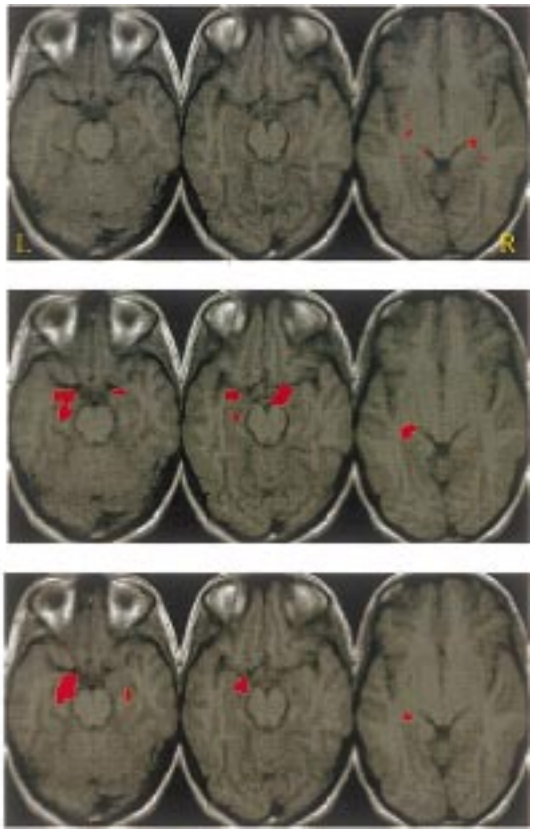

Fig. 2. Masked functional activation maps showing significant suprathreshold voxels within the amygdala and hippocampal ROIs. (**a**) During the encoding of single faces, there is no significant difference between the proportion of activation within the right and left ROIs. (**b**) During encoding of paired faces there is bilateral activation within the amygdala and left-lateralized activation within the hippocampus. (**c**) Compound subtraction (paired faces minus single faces) resulted in significantly lateralized suprathreshold activation within a network of mesial temporal lobe structures including the amygdala and hippocampus.

in each search region are as follows: left amygdala = 50, right amygdala = 60, left hippocampus = 72, right hippocampus = 62. Single face encoding produced limited activation within the amygdala, with only a single voxel (2% of ROI voxels) exceeding the statistical threshold ($\alpha = 0.05$) in the left amygdala and no (0%) suprathreshold activation in the right amygdala (Fig. 2a). In contrast, greater suprathreshold activation was observed within the hippocampus, but the proportions of active voxels within the left (15%) and right (7%) ROIs were not significantly different ($z = 1.62$, $p = 0.11$), suggesting limited bilateral hippocampal activation. Table 1 presents Talairach coordinates for local maxima of ROIs with significant suprathreshold activation.

When subjects encoded face photographs as pairs, there was significant ($\alpha = 0.05$) bilateral amygdala activation (Fig. 2b). The proportion of activation within the ROIs was not significantly different between the left (24%) and right (20%) sides ($z = 0.51$, $p = 0.61$). In contrast, there was a significantly greater proportion of active voxels within the left relative to the right hippocampus ($z = 3.42$, $p = 0.0006$) during paired face encoding, with 21% of the voxels exceeding threshold in the left hippocampus, and only 2% exceeding threshold in the right.

To test the role of the amygdala in visual associative encoding, it was of interest to remove the influence of facial perception to highlight the brain activity required for making the paired associations. Thus, we subtracted the single-face activation from the paired-face activation using SPM97. The resulting activation map is presented in the bottom row of Fig. 2. The subtraction revealed significant ($\alpha = 0.05$) suprathreshold activation within the ROIs. Moreover, the activation within the amygdala was almost exclusively left lateralized, with 32% of the voxels within the left amygdala showing supradireshold activation, compared to only 2% within the right ($z = 4.38$, $p = 0.00001$), suggesting a significant lateralization of activation favoring the left amygdala. Similarly, the subtraction revealed a significantly greater proportion of suprathreshold voxels within the left hippocampus (11%) in contrast to negligible activation (2%) within the right ($z = 2.19$, $p = 0.03$).

DISCUSSION

The aim of this study was to identify whether amygdala and hippocampal structures are preferentially activated during associative compared to non-associative face encoding. We contrasted the functional brain activity during an associative encoding task using paired face photographs with activity during a similar non-associative memory task requiring only the encoding of individual face photographs. This contrast revealed preferential activation of the left amygdala and hippocampus during the associative encoding task. Because both tasks engaged similar processes of perception and complex visual analysis of facial expressions, the between-task subtraction was expected to highlight the brain activity that correlated with the associative memory component. While these findings lend themselves to a number of potential explanations, we believe that our results suggest a specific role for the left amygdala in the process of associating paired visual stimuli in memory. Given that there was only minimal amygdala activation during the single face encoding condition, the activation that we observed appears to be exclusive of the established role of the amygdala in recognizing individual faces and facial affect [9,25]. Memory research involving the amygdala has, to date, focused primarily on the role of the amygdala in modulating the consolidation and storage of memories through emotional association and the enhancement of long-term potentiation [12,15]. The amygdala is important in forming learned associations between stimuli and the emotional experience of pleasure or discomfort [14,16,17]. Our findings are consistent with those of the animal literature suggesting that the amygdala is involved in associative memory for paired visual stimuli [19] and that the amygdala serves to modulate hippocampal memory functioning [15]. Of course, since ours is the first study of its kind to specifically examine functional MRI activation in the mTL during paired association of faces, the foregoing conclusions about the role of the amygdala in associative memory must remain tentative and open to other interpretations.

While one explanation of the data suggests that the amygdala plays a specific role in forming associations in

Table 1. Talairach coordinates and Z-scores of the local maxima within each ROI.

Region	x	y	z	Total volume	Active volume	Mean Z	Maximum Z
Single faces							
Left amygdala	−27	−7	−9	50	1	1.94	1.94
Left hippocampus	−19	−39	5	72	11	2.00	2.71
Right amygdala	–	–	–	60	0	–	–
Right hippocampus	24	−39	0	62	4	1.93	2.09
Pair faces							
Left amygdala	−23	−3	−19	50	12	1.95	2.31
Left hippocampus	−31	−31	−9	72	15	2.00	2.34
Right amygdala	24	−11	−29	60	12	2.21	2.90
Right hippocampus	32	−15	−24	62	1	1.72	1.72
Single−pair subtraction							
Left amygdala	−19	−7	−19	50	16	2.22	3.06
Left hippocampus	−27	−15	−24	72	8	2.03	2.86
Right amygdala	32	−11	−24	60	1	2.10	2.10
Right hippocampus	32	−15	−24	62	1	2.43	2.43

Mean Z score indicates the average of all suprathreshold voxels within the ROI. Active volume represents the number of voxels within the ROI exceeding the significance threshold ($p \leq 0.05$).

memory, there are several alternative explanations that are also compatible with the present data. First, given the demonstrated role of the amygdala in the processing of facial affect [8,9], it is possible that its increased activation during the paired face condition represents the recruitment of emotional processes in the formation of memories. For example, during the post-scan debriefing, most subjects admitted that they had used affective strategies to associate the pairs (e.g. imagining the paired individuals in a sexual situation or judging the 'compatibility' of the couple). Thus, the ability to form memory associations between the two human faces may have been enhanced by imbuing the pair with affective salience. Such a possibility is in line with evidence suggesting that the amygdala modulates hippocampal functioning [15], perhaps by increasing arousal within the memory system for emotionally relevant stimuli [12,13]. Second, it is conceivable that the experience of being visually confronted with two unfamiliar faces may have created an analog situation representing social dominance, which may have potentially activated primitive threat responses. The left amygdala is particularly important to processing the emotion of fear [9], and its activation when presented with a pair of unfamiliar faces may represent a simple limbic fear/dominance response to perceived threat. Because we did not include a paired face condition that did not require memory association, it is impossible to rule out such an explanation. The fact that the paired encoding of face photographs produced significant left-lateralized hippocampal activation, however, suggests that the paired face condition specifically engaged the mTL memory system and not just the affect perception system. Finally, the greater amygdala activation during the associative memory condition may have been due to the more challenging or interesting nature of paired relative to the single face condition. The observed activation may, therefore, have simply resulted from greater cognitive effort or increased emotional arousal secondary to performing a difficult or engaging task. These possible interpretations cannot be ruled out based on the present data and additional research will be required for definitive clarification. The observed activation differences are significant, however, as they provide evidence regarding the functional role of discrete limbic structures during similar encoding tasks that draw upon different cognitive processes.

CONCLUSION

Our data suggest that associative encoding of paired face photographs differs from non-associative encoding of single faces, resulting in preferential activation of a left-lateralized network of limbic structures including the amygdala and hippocampus. These findings support previous animal research suggesting that the amygdala is involved in the formation of associations in memory, most likely through emotional facilitation of hippocampal activity. It is not yet clear, however, whether the pattern of amygdala activation is specific to the associative nature of the task or whether it represents the involvement of affective processes in the formation of memory associations. The present findings extend previous research by suggesting that the amygdala is not only involved during emotional stimulus-response and stimulus-stimulus associations in animals, but may also be important to the formation of stimulus-stimulus associations in humans.

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