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Hippocampal, but not amygdala, activity at encoding correlates with long-term, free recall of nonemotional information

(parahippocampal gyrus/declarative/memory/consolidation/deoxyglucose)

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ABSTRACT **Participation of two medial temporal lobe structures, the hippocampal region and the amygdala, in long-term declarative memory encoding was examined by using positron emission tomography of regional cerebral glucose. Positron emission tomography scanning was performed in eight healthy subjects listening passively to a repeated sequence of unrelated words. Memory for the words was assessed 24 hr later with an incidental free recall test. The percentage of words freely recalled then was correlated with glucose activity during encoding. The results revealed a striking correlation ($r = 0.91$, $P < 0.001$) between activity of the left hippocampal region (centered on the dorsal parahippocampal gyrus) and word recall. No correlation was found between activity of either the left or right amygdala and recall. The findings provide evidence for hippocampal involvement in long-term declarative memory encoding and for the view that the amygdala is not involved with declarative memory formation for nonemotional material.**

Since the seminal report of Scoville and Milner (1), neurobiological investigations of explicit (or "declarative") memory have focused on the role of medial temporal lobe (MTL) structures in encoding processes. Within the MTL, the hippocampal region and amygdala are believed to play distinct roles in long-term memory consolidation (2–7). Substantial evidence suggests that the hippocampal region (including cortical regions such as the parahippocampal gyrus) is critically involved with declarative memory formation. In contrast, substantial evidence suggests a more selective role for the amygdala in declarative memory formation, namely, modulation of memory storage for emotionally arousing events (5–7). A necessary corollary of this view is that amygdala activity is not related to long-term memory for relatively nonemotional material.

Many studies have examined hippocampal function in human memory with brain imaging techniques, primarily using verbal stimuli. Evidence from these studies has been equivocal. Although some found hippocampal activation in relation to memory (8–13), others have not (14–17). As noted recently by Buckner and Koutstaal (18), "The absence of consistent findings relating hippocampal formation activity to verbal encoding is a puzzle." In one effort to explain the puzzle, Schacter and colleagues (10) presented evidence that hippocampal activation may be more closely related to the successful recollection of an event than to the attempt to recollect.

Several other potential explanations for inconsistent hippocampal findings exist. For example, most studies to date used relatively short retention intervals (typically a few minutes). If the hippocampus functions primarily to consolidate information into long-term storage, investigations using longer- term (e.g., 24 hr) retention intervals may yield more consistent results. Second, most studies have examined hippocampal activity during retrieval. Neuropsychological evidence has long suggested a preferential role for the hippocampal region in encoding, rather than retrieval of long-term declarative memory. Imaging studies focused on hippocampal participation in long-term memory encoding therefore also may yield more consistent results. Finally, most investigations have used group-average "subtraction" analyses to identify hippocampal participation in memory. Although these techniques are powerful, a growing number of studies suggest that analyses of individual differences between subjects (for example, by using correlations between activity in a specific structure and memory performance) reveal aspects of brain function not detected by standard subtraction techniques (19–23). For example, in an earlier study we reported preliminary evidence correlating activity in the parahippocampal cortex at encoding with long-term (3-week) recall of both relatively emotional and nonemotional information (20).

With these considerations in mind, we examined hippocampal and amygdala involvement in long-term (24 hr) memory by using positron emission tomography (PET) of regional cerebral glucose. The stimuli used were words with no apparent emotional content. Two primary hypotheses were tested. First, if the hippocampal region is involved with consolidation of the words into long-term memory, then correlational analyses should reveal a relation between the degree of hippocampal activity at encoding and the amount of word retention 24 hr later. This relationship may be especially apparent for the left hippocampal area, which has been implicated to a greater degree in verbal as opposed to nonverbal memory (2, 3, 13). Second, if the amygdala specifically functions to modulate declarative memory for emotionally arousing material, its activity should not be related to memory in these conditions.

METHODS

Subjects. Eight healthy right-handed male volunteers were recruited through campus advertisements and were paid $100 for participating in this experiment. Their average age was 25 yr (SD 5 yr, range 18–32 yr). Subjects were screened for previous medical or psychiatric history, and they fasted at least 8 hr before undergoing PET scan procedures. All gave informed consent in accordance with the University of California Irvine Institutional Human Subjects Review Committee.

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Abbreviations: PET, positron emission tomography; MTL, medial temporal lobe; GMR, glucose metabolic rate; rGMR, relative GMR; BA, Brodmann's area.

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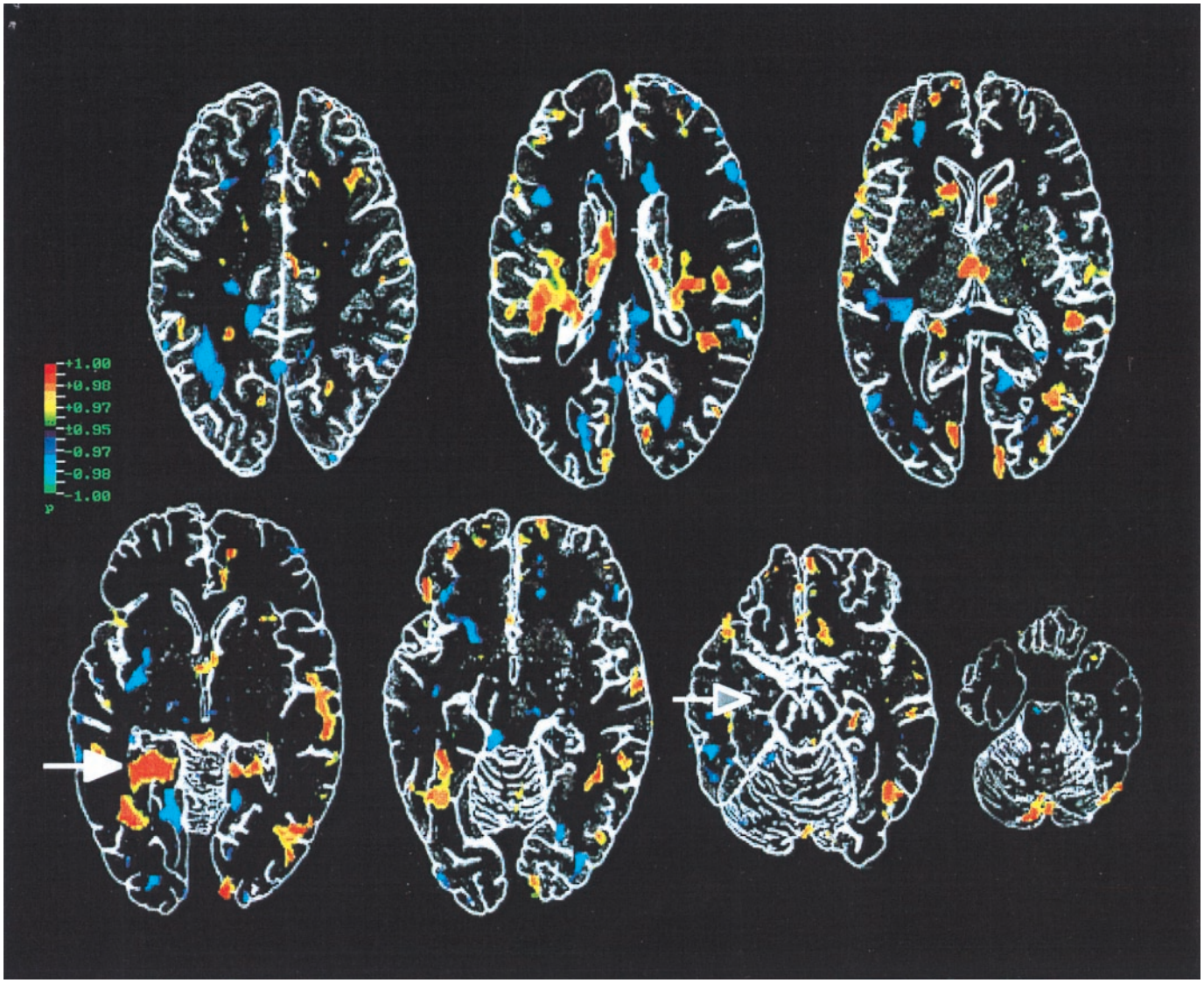

FIG. 1. Brain regions in which rGMR while subjects listened to a word list correlated significantly with number of words freely recalled 24 hr later. The scale bar represents the *P*-values ranging from $P < 0.05$ to $P \leq 0.001$. Only areas of statistically significant correlation ($P < 0.05$) are shown. Positive correlations are depicted in yellows/reds, negative correlations in blues. Axial brain sections (29) are taken from 61%, 47%, and 41% (*Upper*, left to right), and 34%, 28%, 21%, and 14% (*Lower*, left to right) of head height above the canthomeatal line. The front of the brain is toward the top of each image, and the left side of the brain is on the left of each image. Solid arrow (*Lower*, left) identifies the primary hippocampal region finding. The shaded arrow (*Lower*, second section from right) identifies the amygdala. [Reproduced with permission from ref. 24. Copyright 1996, JKB Sutherland.]

All subjects were participants in a broader study of conscious versus unconscious learning mechanisms (24).

Materials. The test stimuli were words selected after the model of Squire *et al.* (9). Sixty words were selected from the *Computational Analysis of Present-Day American English* (average word frequency: 40) (25). All words (which are listed in Table 1) contained 4–8 letters and were devoid of any apparent ability to induce an emotional reaction (e.g., spot, screen, excess, blanket). Six lists of 10 words each were made from this pool of words. Words in each list were chosen to minimize both phonological and semantic similarities. Average word length was balanced between lists. Each list of words was recorded with flat affect onto audio tape according to two randomly assigned patterns such that on playback a subject heard the same 10 words presented repeatedly at the rate of one word every 5 sec, alternating between two randomly ordered presentations of the 10-word list.

Procedure. A standard PET scan procedure was followed as described (19, 20). Presentation of an audio tape to each subject began 30–60 sec after the subject was injected with 5 millicuries of ^{18}fluorodeoxyglucose, a glucose analog tracer used to determine regional glucose metabolic rate (GMR). Subjects lay on a gurney in a darkened room while wearing blindfolds to minimize any visual input and listened through headphones to the audio-taped word list for approximately 32 min. Subjects were instructed to listen passively to the words and were not told that their memory for the words would be tested later.

Memory for the words was tested in a free recall test 24 hr later. Subjects returned to the laboratory and were asked to freely recall the words presented on the previous day. No scanning was conducted during the memory test. The responses were recorded by the experimenter, who was blind to the word list heard. Each subject was allowed 5 min to recall as many words as possible.

PET scans were performed by using a NeuroECAT head-dedicated scanner (CTI, Knoxville, TN). The PET scanner has a single ring with shadow shields and septae to achieve 7.6-mm resolution (full-width-half-maximum) in plane and 9.9 mm resolution in the Z-dimension. Subjects were positioned by

Table 1. Words used in this study

1) accept	31) lane
2) advocate	32) league
3) afford	33) male
4) allotment	34) magic
5) approval	35) marine
6) band	36) merit
7) beard	37) pastor
8) blanket	38) pension
9) breadth	39) plenty
10) bride	40) porch
11) burden	41) purple
12) capable	42) quantity
13) chapter	43) reflect
14) cloud	44) region
15) crack	45) revenue
16) curve	46) scarcely
17) defeat	47) screen
18) delight	48) sight
19) destroy	49) silly
20) draft	50) slipped
21) embassy	51) soldier
22) empty	52) spare
23) excess	53) spot
24) garden	54) stone
25) glance	55) swept
26) grand	56) swing
27) heaven	57) supper
28) immediate	58) temple
29) impact	59) tension
30) invited	60) weak

using laser guidance, and a thermosetting plastic face mask was used to hold each subject's head stationary during the period of image acquisition. PET data were corrected for attenuation and background activity, and reconstructed with a 4.5-mm Hanning filter. For each PET scan session, 13 image slices were obtained parallel to the canthomeatal line. Scanning began at the 85% level of head height (vertex to canthomeatal line, approximately 12–14 cm) and proceeded downward in steps of 10 mm. GMR (mg/100 gm/min) was calculated by using established PET methodology using arterialized venous blood sampling (26, 27). Relative GMR (rGMR) was calculated by dividing the GMR of each pixel by whole-slice GMR as described (28). rGMR corrects for wide individual differences typically found in whole-brain GMR.

Each subject's PET data were realigned and normalized into standardized stereotactic coordinates based on the Matsui and Hirano (29) atlas by using a bounding box method. Memory scores (percent of the total word list recalled) were correlated with normalized rGMR values on a pixel-by-pixel basis using Pearson's product moment correlations with Fisher's r to z conversion. Correlation maps were thresholded to show correlations significant at 0.05, uncorrected for multiple comparison because of the specific *a priori* hypotheses regarding the parahippocampal gyrus and amygdala (20). An anatomist (J.H.F.) confirmed the location of all findings. Some of the findings reported in the results were included in a previous study focused on investigating differential involvement of thalamic mechanisms in conscious versus unconscious learning (24).

RESULTS

Subjects recalled an average of 60% (± 5%) of the words (range 20–90%) at the 24-hr retention test. Fig. 1 demonstrates correlation maps in which statistically significant positive and negative correlations between rGMR and word recall were found. The solid arrow indicates a region of significant correlation in the left hippocampal area overlapping the width of the parahippocampal cortex in the region. It includes the dorsal parahippocampal gyrus and possibly subiculum, but does not appear to include Ammon's Horn (CA1-CA4 or dentate gyrus) immediately anterior or ventral to this region, or the entorhinal cortex. Modified Talaraich and Tournoux (30) coordinates for the center of this region of correlation are as follows: $X = -25$, $Y = -40$, $Z = -4$. Pixels of correlated activity in the parahippocampal region also were found in slices immediately adjacent to the primary region of correlation indicated by the solid arrow (see Fig. 1). Additionally, pixels of correlated activity were found, although to a lesser extent, in the right hippocampal region.

The correlation found in the left hippocampal region, which coincided with the dorsal parahippocampal gyrus as defined *a priori* by our anatomist (J.H.F.), was further examined with a region of interest analysis. For each subject, rGMR in the left hippocampal region was determined stereotactically by positioning a 3 × 3-pixel-wide box template centered over the region of correlation identified by the solid arrow in Fig. 1. Fig. 2 shows the results of this analysis. Relative metabolism in the left hippocampal region correlated highly with word recall ($r = 0.91$, $P < 0.001$). In contrast, activity in the amygdala region on both sides of the brain did not correlate significantly with recall. The open arrow in Fig. 1 indicates the amygdala area.

In addition to the predicted effects in the hippocampal region and amygdala, the correlation images revealed areas of activity significantly correlated with memory in several other brain regions including: the frontopolar cortices (Brodmann's area 9, 10), dorsolateral prefrontal cortex (BA 46), Broca's area (BA 44, 45), Wernicke's area (BA 22), the thalamus (centering on the mediodorsal nucleus), the caudate bilaterally, the ideational speech area of the supramarginal gyrus (BA 40) and angular gyrus (BA 39) in the inferior parietal lobule (BA 40), the ventral precuneus (BA 31), the posterior cerebellar vermis, as well as perirhinal cortex (BA 35) bordering the temporal parahippocampal region. Other smaller and less obvious regions found in this exploratory analysis included bilateral regions of the auditory cortex (BA 41, 42), inferior temporal gyrus (BA 20, 21), perisylvian language area (BA 43), posterior ventral-inferior frontal gyrus (BA 47), and in the region of the temporal tip (BA 38).

DISCUSSION

PET scanning of regional cerebral glucose in healthy subjects was used to test *a priori* hypotheses about the role of two MTL structures, the hippocampal region and the amygdala, in long-term declarative memory formation. The most striking finding was a highly significant correlation between activity of the left hippocampal region (centered on the dorsal parahippocampal gyrus) during encoding and long-term (24 hr) free recall of nonemotional words. Amygdala activity was not related to recall in these conditions, providing additional evidence for a necessary corollary of the view that the amygdala preferentially functions to modulate declarative memory for emotionally arousing events (5–7).

This study differs from previous imaging studies of the hippocampal role in memory in a combination of respects. First, hippocampal activity at encoding, rather than retrieval, was measured. Second, memory was assessed with free recall, as opposed to recognition memory tests. Third, long-term (24 hr) memory was tested. Fourth, incidental, rather than intentional, memory processes were studied. Fifth, hippocampal activity was assessed by measuring glucose metabolism, rather than blood flow. Finally, a correlational analysis was used relating brain activity to subsequent memory performance across individual subjects. This combination of parameters makes comparisons with previous imaging studies somewhat difficult. However, the very high correlation found between

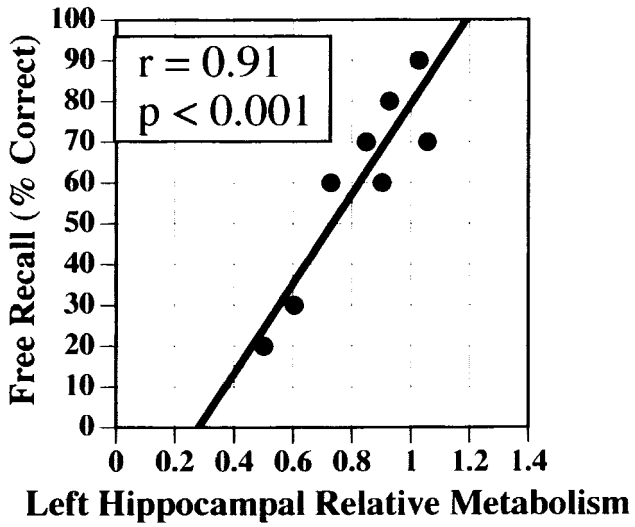

FIG. 2. Scatterplot of rGMR versus recall for the left hippocampal region identified by the solid white arrow in Fig. 1.

left hippocampal activity and 24-hr retention suggests that the parameters were well suited to the identification of a hippocampal role in verbal memory encoding.

The present findings confirm preliminary findings from an earlier study suggesting a relationship between the degree of activity in the parahippocampal gyrus at encoding and subsequent memory (20). A few other studies to date also used correlational analyses in investigations of hippocampal memory functions. For example, Nyberg and colleagues (22) examined blood flow in relation to recognition memory for words and found that blood flow in the left hippocampal region correlated significantly with recognition success. However, although correlations between hippocampal activity and memory retrieval may reflect hippocampal participation either in retrieval or re-encoding processes, the correlation found in the present study must reflect a hippocampal role in encoding/consolidation processes.

One recent study examined the relationship between hippocampal activity (assessed with functional MRI) at encoding and recall. Fernandez *et al.* (23) found significant correlations between activity of posterior regions of the hippocampus and word list recall. This study differs from the present study in at least two important respects. First, Fernandez *et al.* (23) assessed short-term (15-sec) recall; second, they tested intentional, as opposed to incidental, memory. Still, taken together with the present findings, the results of Fernandez *et al.* (23) underscore the power of incorporating individual subject performance differences when investigating brain memory function.

Findings from two other recent imaging studies strongly confirm the present findings regarding the parahippocampal gyrus (31, 32). In both studies, activity of the parahippocampal gyrus (assessed with functional MRI) was greater on average for material remembered versus not remembered shortly after completion of scanning. Those studies differ from this study in several important respects (e.g., retention interval, imaging technique, type of analysis). Despite these differences, the three studies, together with preliminary evidence from an earlier study (20), strongly support the conclusion that activity of the parahippocampal gyrus at encoding in healthy humans is related to the degree to which information is stored and provide what we feel is among the most compelling support in the human brain imaging literature for a specific mnemonic function of a particular brain region.

Although hippocampal activity correlated highly with long-term declarative memory for verbal information in our study, amygdala activity did not. Many investigators have argued that the amygdala plays no role in declarative memory (1, 4, 33). However, substantial evidence from animal studies and recent studies involving human subjects suggest a specific role for the amygdala in declarative memory. The amygdala appears selectively involved with enhanced long-term declarative memory associated with emotionally arousing (sympathetic nervous system activating) events. On the basis of such evidence, McGaugh and colleagues (5–7, 20) propose that the amygdala modulates memory storage occurring in other brain regions during and after emotionally arousing events. A necessary corollary of this proposal is that amygdala activity generally should be unrelated to declarative memory formation for nonarousing material or events. The results of the present study, in which nonemotional stimuli were used, therefore provide additional support for the modulation hypothesis of amygdala function in declarative memory (6, 7).

Besides the hippocampus, activity in several other brain regions correlated significantly with recall. Although *a priori* predictions in this experiment were made only regarding the hippocampus and amygdala, some discussion of these other brain regions is warranted. Of particular interest are significant correlations between activity and recall found in the left dorsolateral prefrontal cortex (BA 46), another brain region often implicated in verbal memory encoding (2, 3). Activity correlated with memory also occurred in several brain regions thought to be involved with auditory verbal memory, including the frontopolar cortices (Brodmann's Area 9, 10), Broca's area (BA 44, 45), Wernicke's area (BA 22), angular gyrus (BA 39), and the mediodorsal thalamic regions (15, 16, 35, 34). Collectively, these findings support the view that verbal memory encoding likely requires an interaction between hippocampal activity and a diverse network of associated cortical and subcortical structures (36).

In summary, activity of the left hippocampal region (centered on the dorsal parahippocampal gyrus) correlated very highly with long-term, free recall of nonemotional verbal information. Amygdala activity in these conditions was not significantly related to recall. The findings provide additional support for two hypotheses regarding mnemonic functions of the MTL. First, the hippocampal region (in particular the left parahippocampal gyrus) participates in long-term declarative memory encoding of verbal information in the healthy human brain. Second, the amygdala plays a more selective role likely involving memory for emotionally arousing material. Human brain imaging experiments using emotionally provocative verbal stimuli should further define the contribution of these MTL structures to declarative memory storage.

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