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RAPID COMMUNICATION

Sex-Related Difference in Amygdala Activity during Emotionally Influenced Memory Storage

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We tested the possibility suggested by previous imaging studies that amygdala participation in the storage of emotionally influenced memory is differentially lateralized in men and women. Male and female subjects received two PET scans for regional cerebral glucose — one while viewing a series of emotionally provocative (negative) films, and a second while viewing a series of matched, but emotionally more neutral, films. Consistent with suggestions from several previously published studies, enhanced activity of the right, but not the left, amygdala in men was related to enhanced memory for the emotional films. Conversely, enhanced activity of the left, but not the right, amygdala in women was related to enhanced memory for the emotional films. These results demonstrate a clear gender-related lateralization of amygdala involvement in emotionally influenced memory, and indicate that theories of the neurobiology of emotionally influenced memory must begin to account for the influence of gender.

Converging evidence from both animal and human subject investigations strongly indicates that the amygdala is critical for enhanced explicit memory associated with emotional arousal. The evidence suggests that the amygdala interacts with endogenous stress hormones released during and after emotionally charged events to modulate memory storage occurring in other brain regions (Cahill, 2000; Cahill & McGaugh, 1998; McGaugh, Cahill, & Roozendaal, 1996).

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Several recent human brain imaging studies have provided strong additional support for this view (Cahill et al., 1996; Canli, Zhao, Brewer, Gabrieli, & Cahill, 2000; Canli, Zhao, Desmond, Glover, & Gabrieli, 1999; Hamann et al., 1999). In each, activity of the amygdala while subjects viewed emotionally provocative stimuli correlated with the degree to which the stimuli were retained in long-term memory. However, in each study, lateralization of the amygdala effects was apparent, and unexplained. One potential explanation concerns gender. In the two studies involving only male subjects, the amygdala effect was either exclusively (Cahill et al., 1996) or predominantly (Hamann et al., 1999) right lateralized. In the two studies involving only female subjects, the amygdala effect was predominantly (Canli et al., 1999) or exclusively (Canli et al., 2000) left lateralized. However, many other major differences between these studies, such as the type of imaging technique (glucose PET, blood flow PET, fMRI) and stimuli used (films, pictures), make it impossible to confidently attribute the lateralization differences to the gender of the subjects.

In this study we explicitly examined whether amygdala involvement in emotionally influenced memory exhibits gender-specific lateralization. The relationship between amygdala activity and memory for relatively emotionally arousing versus emotionally neutral films was examined using an equal number of men and women tested in identical conditions. The paradigm was the same as that in our earlier report (Cahill et al., 1996). Additional male and female subjects, as well as a different specific analysis of the data, are presented here.

METHODS

Subjects

Twenty-two healthy, right-handed subjects (11 male, 11 female, average age 6 SEM 5 22.1 6 0.60) participated in the study. The average age of the female subjects was 22.7 6 0.81 years, and for the males it was 21.5 6 0.88 years. All were students at the University of California at Irvine. Data from 8 of the male subjects were taken from our earlier report (Cahill et al., 1996). All were recruited through campus advertisements and paid $200.00 for their participation. Exclusionary criteria included any major medical or psychiatric illness, substance abuse, or history of head injury. All gave informed consent in accordance with the University of California Irvine Institutional Review Board.

Materials

The materials were those described by Cahill et al. (1996). Briefly, two videos, each composed of 12 film clips, were used as stimuli during the PET sessions. One was composed of relatively emotionally neutral films, the other of relatively emotionally arousing (negative) films. The two sets of films were matched as much as possible for length, style, and comprehensibility. The average length of the clips for both videos was slightly over 2 min. The film clips were all taken from commercially available, documentary-style videos. Independent judges rated the emotional (E) films as significantly more emotional than the neutral (N) films, but rated the two sets of films as equally understandable (Cahill et al., 1996). The emotional films were described as arousing negative emotions such as fear or disgust. The order of the film clips within a single video was the same for each subject.

Procedure

Each of the 22 subjects completed two PET sessions. The sessions were separated by 2–7 days. During one session, they viewed the E video; during the other session, they viewed the N video, with the order of video presentation counterbalanced across subjects. Subjects were asked to watch each film clip and then rate how emotional they found the clip to be on a scale of 0 (“not emotional at all”) to 10 (“extremely emotional”). They were instructed that their rating should reflect only their personal emotional reaction to viewing the film. They were told they would have approximately 10 s at the end of each film clip to write down their rating before the start of the next clip. Subjects were not told which video they were viewing nor was any mention of a memory test made. Video viewing took place in a darkened, sound-attenuated room.

Three weeks after the second session, the subjects were asked to freely recall as many film clips as possible for both film sessions. They were told to take as much time as they wished and to continue until they felt they could recall no more. The subjects took 5–15 min to complete their responses. In the vast majority of cases, the films referred to by the subject were clearly identified. In those few cases where one was not, the subjects were asked to give some more detail about the film until the film referred to was made clear to the investigator. After the recall session, the subjects were debriefed as to the intent of the study. All subjects indicated that they were unaware that their memory would be tested.

PET scan procedures. A standard procedure was followed as described in Haier et al. (1992). Image acquisition and processing were identical for all subjects. Each video began about 30 s before the subject was injected with 18F-fluoro-2-deoxyglucose (FDG), a glucose analog tracer used to determine regional brain glucose metabolic rate (GMR). Subjects then watched the video for approximately 32 min while 80–90% of the FDG was taken up by the brain. Following the uptake period, the injected FDG remains metabolically fixed for several hours, with the highest concentrations occurring in the brain areas that were the most metabolically active during the 32 min. Scanning was done after the FDG uptake and brain tracer labeling with a GE2048 head-dedicated scanner (full-width half maximum resolution about 4.5 mm in plane). Transmission scans obtained for each subject were used for attenuation correction. Thirty overlapping axial slices parallel to the canthomeatal (CM) line were obtained at 6-mm intervals (15 slices simultaneously). Each subject wore an individually modeled thermoplastic mask to locate the CM line and hold the head still during the scanning. This results in head placement errors between the two scans of less than 2 mm, which is well within the spatial resolution of the scanner. GMR was calculated following Sokoloff et al. (1977) in milligrams of glucose per 100 g of brain tissue per minute.

Statistical Analysis

Data were processed using the statistical parametric mapping (SPM-99) software from the Wellcome Department of Cognitive Neurology, London, United Kingdom, implemented in Matlab (Mathworks, Sherborn, MA). This process determined regionally significant effects for every voxel in standardized space using the generalized linear model (Friston, Frith, Liddle, & Frackowiak, 1991; Friston et al., 1989). Individual data were realigned, normalized into stereotactic space, and then smoothed with a 4-mm FWHM gaussian filter. Global metabolic rates were normalized to the same mean value using proportional scaling. A design matrix was specified using a conjunction analysis that could relate the difference in memory performance for the E versus N sessions to the difference in glucose metabolism for those sessions. The two conjuncted components consisted of (1) the brain regions where regional relative glucose metabolism was significantly higher during the viewing/encoding of the emotional films compared with the viewing/encoding of the neutral films, and (2) the brain regions where the increase in memory performance (emotional–neutral) significantly correlated with viewing/encoding of the emotional films. The data for the first component were derived by first determining activity averaged across subjects voxel-by-voxel separately for the E and N sessions, and testing for significant differences between the voxels. The second component is derived from a subject-by-subject correlation of brain activity and memory score. This conjunction reveals those brain regions whose increase in activity during viewing of the emotional films was related to the subsequent increase in memory performance. This analysis was performed twice, once using data only from the male subjects and once again using data only from the female subjects. Given the clear *a priori* hypotheses derived from previous studies (Cahill et al., 1996; Canli et al., 1999, 2000; Hamann et al., 1999) regarding the laterality of the amygdala's involvement in memory for emotionally significant material, we performed an exploratory analysis at the $p < .05$ level uncorrected for the whole brain. This analysis revealed two clusters in the amygdala region (one in men on the right, one in women on the left). We then analyzed the spatial extent of these two clusters using the small volume correction procedure in SPM-99 with a 7-mm diameter sphere correction volume (after Morris, Ohman, & Dolan, 1998). Location of the effects was confirmed by an anatomist (J.F.) with extensive expertise both in the anatomy of the human amygdala and in the interpretation of localization of amygdala effects from glucose PET data.

Analysis of the ratings of emotional reaction to the films and the recall scores was conducted using paired (for within-subject comparison) and unpaired (for between-subject comparison) *t*-tests. Significance level was set at $p < .05$.

RESULTS

The primary results are shown in Fig. 1, displayed on the SPM-99 PET template. In men, the conjunction analysis revealed a large area of activity in the right amygdala region for which increased activity between the neutral and emotional PET sessions was significantly related to increased recall of the emotional compared to the neutral films. This area was contiguous over at least three consecutive coronal sections (including $Y = 0$ in men, not shown). No similar activity was detected on the left side for the men at any anterior/posterior level of the amygdala or extended amygdala, even when a more liberal statistical threshold ($p < .10$) was used.

An identical conjunction analysis revealed a very different pattern of results in the women. For a large area of left amygdala region activity, increased glucose utilization between the neutral and the emotional scans was significantly related to increased recall of the emotional versus the neutral films. As for the men, this area extended over at least three consecutive coronal sections (including $Y = 4$ in women, not shown). This area of

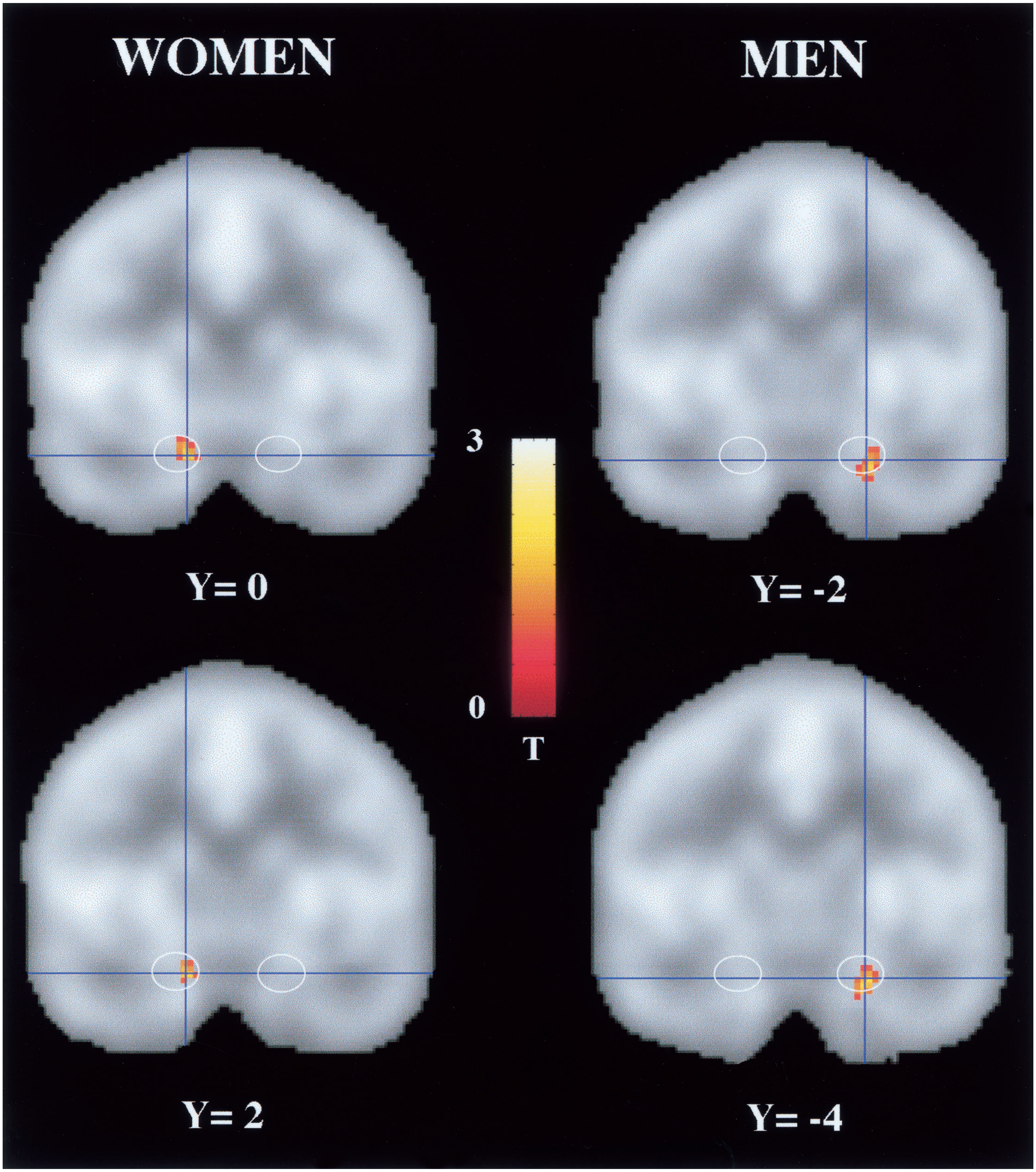

FIG. 1. Results of the SPM-99 conjunction analysis relating increased activity to increased recall of the E versus the N films in both men and women. The white circle indicates the approximate amygdala region. Given the hypothesis at issue, only significant activity in the amygdala and adjacent regions is shown. In this representation, the left side of the brain is located on the left side of the figure. Y values in white correspond to the anterior/posterior coordinate of the respective slice, taken from the atlas of Talairach and Tournoux (1988). The data are displayed on the SPM-99 PET template. Crosshairs represent the horizontal and sagittal coordinates of the most significant voxel identified in Table 1.

activation was clearly more anterior than that for the men, and included not only portions of amygdala proper but substantia innominata sectors that may contain the extended amygdala, ventral striatum, pallidum, and nucleus basalis as well. No significant activity was detected on the right side for the women at any anterior/posterior level of the amygdala or extended amygdala, even when a more liberal statistical threshold ($p < .10$) was used. Table 1 shows for both men and women the coordinates according to the atlas of Talairach and Tournoux (1988) of the maximally activated voxel, as well as the number of voxels per cluster, and the relevant Z scores and P values for those voxels. For both men and women, there were voxels within the cluster that were within the amygdala, as well as voxels outside the amygdala as defined by Talaraich space.

The self-assessed emotional reactions of the men and women to the films did not differ. The mean ($\pm$ *SEM*) rating for the N films was 2.8 ($\pm$ 0.53) for the men, and 3.3 ($\pm$ 0.42) for the women [$t(20) = 0.69$, *ns*]. The ratings for the E films were 5.2 ($\pm$ 0.53) for the men, and 5.4 ($\pm$ 0.65) for the women [$t(20) = 0.24$, *ns*]. As expected, the subjects rated the E films as significantly more emotional than they did the N films [$t(21) = -4.10$, $p < .005$].

The recall of the films by men and women was similar, as was the advantage in recall of the E versus N films. Men recalled on average 5.73 ($\pm$ 0.8) of the E, and 2.1 ($\pm$0.6) of the N films ($p < .001$). Women recalled on average 5.36 ($\pm$0.6) of the E, and 2.5 ($\pm$0.51) of the N films ($p < .001$). There were no significant differences between the men and women in their recall of either the E or the N films ($p >> .10$).

DISCUSSION

Recent human brain imaging studies have confirmed the hypothesis derived from animal research that amygdala activity is especially related to enhanced explicit memory for emotionally arousing events (Cahill, 2000; Cahill and McGaugh, 1998; McGaugh et al., 1996). However, they have also suggested that the left and right amygdalae are not equally involved in emotionally influenced memory. In the present study, we examined the possibility that sex-related lateralization of function accounts for at least some of the variability in amygdala activation seen in previous studies. Amygdala activity was directly compared in men and women in identical conditions. The results reveal a clear sex difference in amygdala function in emotionally influenced memory. Increased activity of the right, but not left, amygdala in men was significantly related to increased recall of the emotional compared to neutral films in these conditions. In striking contrast, increased

TABLE 1
Conjunction Results for Memory Performance Related to Amygdala Regions in Men versus Women

SEX	Coordinates[a] (x, y, z mm)	Voxels	Z score	P value
Men (right amygdala)	22, 4, −24	23	2.93	0.02
Women (left amygdala)	−14, 2, −22	17	2.59	0.05

[a] Coordinates of the maximally activated voxel and the number of voxels per cluster are shown.

activity of the left, but not right, amygdala in women related significantly to increased recall of the emotional compared to the neutral films. The effect on the left in women appeared to be more anterior, and to include more of the “extended amygdala” regions, than did the effect on the right in men. The degree of lateralization in each case is made clear by the fact that no involvement of the contralateral amygdala (left in men, right in women) was detected at any anterior/posterior level even when a very liberal significance threshold was employed. Clearly, long-term memory storage of the films did not occur identically in the brains of the men and women vis-à-vis the function of the amygdala and neighboring regions.

While these findings point strongly to the existence of sex-related differences in amygdala participation in emotionally influenced memory, they do not explain why these differences exist. Several theoretical possibilities can be considered, some derived from more general studies of lateralized processing of emotional material, but none of these possibilities appear capable of accounting for the present results. For example, substantial work has suggested differential involvement of the left and right sides of the brain depending on the valence (positive or negative) of the experimental material or situations (Canli, Desmond, Zhoo, Glover, & Gabrieli, 1998; Davidson & Irwin, 1999). This possibility cannot account for the present findings, since the study involves only emotionally negative stimuli. Another possibility is that conscious versus unconscious learning situations differentially engage the left and right amygdalae (Morris et al., 1998; but see Whalen, Rauch, Etcoff, & McInerney, 1998). This possibility also appears incapable of accounting for the present findings, which involve only conscious memory. Finally, the fact that the self-assessed emotional reactions of the men and women to viewing the E and N films did not differ makes it unlikely that the differential amygdala activity reflects strongly differing emotional reactions of the men and women.

It should be noted that while some might consider the significance levels of the effects reported here to be low relative to some findings from blood flow PET studies of amygdala function, such studies are subject to several large sources of variability (Drevets & Raichle, 1998) that do not apply to the present findings. Furthermore, the vast majority of these studies have employed a “subtraction” analysis, not the conjunction analysis used here. Thus, directly comparing statistical results of this study to those of blood flow PET studies using simple subtraction analyses can be problematic. It is also important to keep in mind that the primary conclusion of these experiments concerns a *differential* participation of the amygdalae in long-term memory for emotionally arousing material in men and women under identical experimental conditions and identical statistical analysis.

It is possible that the laterality of amygdala function is indirectly, rather than directly, related to subject gender. That is, the differential lateralization of amygdala function may reflect generally different cognitive processing strategies in men and women. For example, some evidence suggests hemispheric lateralization of the processing of local versus global features of stimuli (Fink, Marshall, Halligan, & Dolan, 1999). Men and women could conceivably differ in their reaction to the global versus local aspects, spatial versus verbal aspects, or some other, as yet unidentified, dimension of the stimuli used in this study. If this is true, then studies in which male and female subjects are matched along these dimensions should not detect sex-related lateralization of amygdala function. We are presently conducting follow-up studies to identify such potential sex-related differences in our paradigm.

The purpose of this study was specifically to examine potential lateralities in amygdala function suggested by prior work. However, the amygdala likely interacts with other brain regions to influence memory. In fact, the "memory modulation" hypothesis of amygdala function requires such interactions (McGaugh et al., 1996). Although our focus for this study is on the amygdala, our analyses revealed several interesting patterns of activity that warrant some discussion. As can be seen in Fig. 1, the area of activity within the amygdala appears to be more medial in women (in addition to being more anterior), and thus to involve to a greater extent the central and/or medial nuclei and extended amygdala. The effect in men appears to involve more the basolateral nuclei. Interestingly, the conjunction analysis indicated that activity in hypothalamic regions related to enhanced memory for emotional films in women, while activity in several striatal and cortical regions related to enhanced memory in men (data not shown). The medial/central amygdala nuclei have well-established connectivity to hypothalamic and brain stem regions, while the basolateral nuclei connect strongly to striatal and cortical, but not hypothalamic areas (Swanson & Petrovich, 1998). Thus, these preliminary findings suggest the intriguing possibility that memory-related processing of the emotional stimuli involves a more "visceral," hypothalamic-related circuitry in women, and a more "cognitive," cortical-related circuitry in men. Confirming such a possibility will require additional studies involving, for example, a greater range of emotional stimuli than used in the present study.

Human brain imaging studies of both memory in general, and amygdala function in particular, have developed rapidly in recent years (e.g., Cahill, 2000; McIntosh, 1999; Whalen, 1998). Sex-related differences detected with these methods have been reported in several cognitive domains, including language (Shaywitz et al., 1995), navigational ability (Groen, Wunderlich, Spitzer, Tomaczak, & Riepe, 2000), defensiveness (Kline, Allen, & Schwartz, 1998), mathematical ability (Haier & Benbow, 1995), attention (Mansour, Haier, & Buchsbaum, 1996), and both resting glucose metabolism (Gur et al.,1995) and the relationship of resting metabolism to verbal memory (Ragland, Coleman, Gur, Glahn, & Gur, 2000). The present findings add to these demonstrations that neurobiological mechanisms underlying cognitive processing can differ sharply between men and women. They further indicate that theories about the neurobiology of emotionally influenced memory storage should begin to actively take into account the evident influence of gender.

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