Increased Amygdala Response to Masked Emotional Faces in Depressed Subjects Resolves with Antidepressant Treatment: An fMRI Study

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Background: *The amygdala has a central role in processing emotions, particularly fear. During functional magnetic resonance imaging (fMRI) amygdala activation has been demonstrated outside of conscious awareness using masked emotional faces.*

Methods: *We applied the masked faces paradigm to patients with major depression (*n *= 11) and matched control subjects (*n *= 11) during fMRI to compare amygdala activation in response to masked emotional faces before and after antidepressant treatment. Data were analyzed using left and right amygdala a priori regions of interest, in an analysis of variance block analysis and random effects model.*

Results: *Depressed patients had exaggerated left amygdala activation to all faces, greater for fearful faces. Right amygdala did not differ from control subjects. Following treatment, patients had bilateral reduced amygdala activation to masked fearful faces and bilateral reduced amygdala activation to all faces. Control subjects had no differences between the two scanning sessions.*

Conclusions: *Depressed patients have left amygdala hyperarousal, even when processing stimuli outside conscious awareness. Increased amygdala activation normalizes with antidepressant treatment.* Biol Psychiatry 2001;50:651–658 © *2001 Society of Biological Psychiatry*

Key Words: Amygdala, depression, functional magnetic resonance imaging, unconscious, emotion, antidepressant

Introduction

The amygdala is a brain structure located in the medial temporal lobe that is known to process emotionally valenced stimuli (Aggleton 1992). A large literature points to the central role of the amygdala in processing emotions, particularly fear (reviewed by Davis 1994; LeDoux 1996; Phelps and Anderson 1997). In normal subjects, functional studies have shown the amygdala to be involved in the generation of a response to emotional stimuli, such as fearful faces (Breiter et al 1996; Morris et al 1996). Bilateral damage to the amygdala impairs the processing of fearful facial expressions (Adolphs et al 1994). Functional neuroimaging studies demonstrate that the amygdala is activated during negative affective states, such as sadness and anxiety (Davidson and Irwin 1999). Functional abnormalities have also been found in the amygdala in depression. Depressed subjects have impaired production of emotional facial expressions and abnormal recognition of facial expression (Gur et al 1992). In addition, positron emission tomography (PET) studies have shown increased resting blood flow of approximately 6% in the amygdala in patients with major depression or bipolar disorder (Drevets et al 1992).

Using the technique of backward masking (Esteves and Ohman 1993) stimuli can be manipulated to be presented outside of the subjects' conscious awareness. This technique was combined with functional magnetic resonance imaging (fMRI) in human subjects and demonstrated the role of the amygdala in nonconscious processing of emotion (Whalen et al 1998). In the current investigation, we used this technique to compare the amygdala responses to masked emotional faces in depressed and control subjects. Part of the syndrome of major depression frequently involves rumination on negative thoughts. An advantage of using a nonconscious paradigm is that it avoids confounding interpretation by the presence of other cognitive processing during the scan. It thereby lessens the problem of a potential difference between depressed and control subjects resulting from perseveration on a fearful face. In addition, we compared amygdala activation before and after antidepressant treatment in depressed subjects. We predicted that depressed subjects would have greater bilateral amygdala activation to masked fearful faces than comparison subjects and that this increased activation would resolve with antidepressant treatment.

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Methods and Materials

Subjects

Eleven right-handed subjects (6 women and 5 men) aged 18–55 (mean, 40.3 years) meeting DSM-IV criteria for major depression were recruited by advertisement to participate in the study and were matched with a control group of 11 comparison subjects (6 women and 5 men) aged 20–55 (mean 39.8 years). A total of 12 control subjects needed to be recruited, because one subject was excluded (as described below). Subjects were also matched on educational level with a mean of 15 years of education in each group. All subjects provided written informed consent in accordance with criteria established by the Washington University Human Subjects Committee. Inclusion criteria for depressed subjects were an acute episode of unipolar recurrent major depression by DSM-IV criteria. All subjects were free of psychotropic medication for a minimum of 4 weeks. Psychiatric diagnosis was determined by DSM-IV criteria (American Psychiatric Association 1994). In addition, all subjects were administered a 17-item Hamilton Rating Scale for Depression (HRSD) (Hamilton 1960) to determine depression severity. Depressed subjects were included with HRSD scores > 17 and control subjects were included with HRSD scores < 8. Right-handedness was also necessary for inclusion and was determined by the Edinburgh Inventory (Oldfield 1971). Exclusion criteria were any history of neurologic trauma resulting in loss of consciousness, any current neurologic disorder, any lifetime psychiatric disorder other than major depression in depressed subjects, or any lifetime psychiatric disorder in control subjects. Careful attention was paid to excluding co-existing anxiety or substance abuse disorders. Acute physical illness was also an exclusionary criterion. Subjects were told that they would see pictures of faces and they would respond by pressing a button to identify gender. All subjects were naïve with regard to the hypotheses of the experiment.

Treatment

Depressed subjects were treated with the antidepressant sertraline, a selective serotonin reuptake inhibitor (SSRI). Doses were titrated according to clinical response at subsequent biweekly visits and on average were approximately 100 mg. Following 8 weeks of antidepressant treatment, a second fMRI scan was obtained. On the same visit, an HRSD score was recorded. Control subjects also received a second scan after 8 weeks.

Masked Faces Paradigm

During fMRI, subjects were presented with masked fearful (F) masked happy (H) and masked neutral (N) faces, which were organized in a block design. Interspersed with masked emotional faces in each block were masked neutral faces in a pseudorandom order. This ensured the emotional faces occurred unpredictably. Each masked face stimulus consisted of a 40-msec presentation of an emotional face (either F, H, or N) followed by a 160-msec presentation of a neutral face (See Figure 1). Within the happy and fearful blocks the subject saw 20 masked emotional faces (either F or H) and 20 masked neutral faces in a predetermined random order. The computer-generated pseudorandom order was constrained to prevent more than three of any given face emotional type sequentially. Within the neutral face block the subject saw 40 masked neutral faces. Masked face stimuli were presented at a rate of one per echo planar image (EPI) sequence (repetition time [TR] = 2.16 sec). Following each face block there was a block of 30 cross-hair stimuli fixation points (+), presented at the same rate as the faces, to serve as a control period.

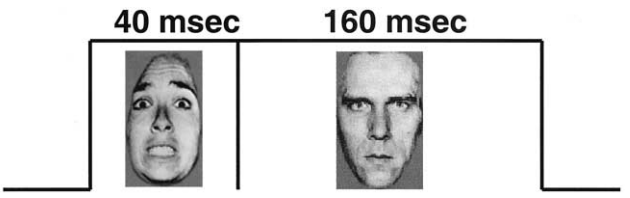

Figure 1. Subjects were presented with masked fearful (F), masked neutral (N), and masked happy (H) faces (courtesy J. Morris and D. Perrett). The masking paradigm consisted of an emotional face (either F, H, or N) stimulus presented for 40 msec followed by a 160-msec presentation of a neutral face. These masked faces were arranged in a block design consisting of 20 masked emotional faces (either F, H, or N) and 20 masked neutral faces in a computer-generated pseudorandom order. There were three blocks of faces separated by fixation cross-hairs for each run and four runs per subject.

The order of the masked faces presentation was the same for all subjects across runs. The first run consisted of N,+,F,+,H,+. The second run was H,+,F,+,N,+. The third run was N,+,H,+F,+. The fourth run was F,+,H,+,N,+. Each run lasted 7.7 min. Each subject viewed four runs. For one subject, data from two runs was utilized due to loss of data.

Stimuli and Equipment

Face stimuli consisted of fearful, happy, and neutral expressions (Ekman and Friesen 1976) which had undergone computer gray-scale normalization (Morris et al 1996), courtesy of J. Morris and D. Perrett. Stimuli were presented using PsyScope on a G3 Macintosh computer (Apple, Cupertino, CA, USA), in which each stimulus onset (masked face or cross hair) was triggered directly by a pulse from the scanner. The images were projected onto a computer screen behind the subject's head within the imaging chamber. The screen was viewed by a mirror positioned approximately 8 cm above the subject's face.

Image Acquisition

All scanning was performed on the 1.5T Siemens VISION system (Erlanger, Germany) at the Research Imaging Center of the Mallinckrodt Institute of Radiology at the Washington University Medical School. Head stabilization was performed using foam padding. Both structural and functional scans were acquired during each scan session. To transform MR data into standard atlas space (described below) and to aid in anatomic localization, we obtained T1-weighted magnetization prepared rapid gradient echo (MP-RAGE) and T2-weighted fast spin echo (FSE) anatomical images in all subjects. The T1-weighted

images were acquired using a sagittal MP-RAGE three-dimensional sequence (TR = 10 msec, echo time [TE] = 4 msec, flip = 8°; voxel size = 1 × 1 × 1.2 mm) and the T2-weighted images were acquired in 8-mm-thick axial slices, and in plane voxel size of 1.8 mm × 0.9 mm with no interpolation using TR = 3800 msec, TE = 22 msec, flip angle = 180°. The functional images were collected using an asymmetric spin-echo echo-planar sequence sensitive to blood oxygenation level-dependent (BOLD) contrast (T2*). During each functional run, sets of 16 contiguous, 8-mm-thick axial images were acquired parallel to the anterior–posterior commissure plane (3.75 × 3.75 mm in plane resolution), allowing complete brain coverage at high signal-to-noise ratio (Conturo 1996). In each run, 216 functional images were acquired, with a TR of 2.16 sec (TE = 50 msec, field of view = 24 cm, flip = 90°).

Data Analysis

Magnetic resonance data were reconstructed into images, and then normalized across runs by scaling whole-brain signal intensity to a fixed value. The MR data were aligned to correct for head motion using a six-parameter, rigid-body rotation and translation correction, which mutually registers all frames in all runs for each subject (Friston et al 1994; Snyder 1996; Woods et al 1992). Between-subjects analysis was conducted by co-registering participants' structural images to a reference brain using an algorithm almost identical to automated image registration (AIR) (Woods et al 1992, 1993), registering the functional images to these structural images, and then blurring the images with an 8-mm full width half maximun (FWHM) Gaussian filter (Barch et al 1997, 2001).

Regions of Interest

Based on our a priori hypothesis of amygdala activation, as described in the introduction, we used a region of interest (ROI) analysis of variance (ANOVA) block analysis treating subjects as a random effect. The predetermined regions of interest were the right and left amygdala. These regions of interest were drawn onto the combined and averaged MRI scans from the subjects. As shown in Figure 2A, the regions were drawn with 3 × 3 × 3-mm voxels, producing blocklike edges. Definitions of amygdala boundaries were the same as in previous studies (Sheline et al 1998, 1999). Visualized in coronal section, the anterior boundary of the amygdala was the first section in which the white matter connecting the frontal and temporal lobes became continuous. Dorsally, the border was defined in anterior sections by the endorhinal sulcus and posteriorly in sagittal sections by a horizontal with the temporal horn of the lateral ventricle. Ventrally, visualized in sagittal section, the amygdala was bounded by a horizontal line connecting to the ventral/anterior edge of the hippocampus and proceeding posteriorly in subsequent sections to the actual border with the hippocampus. Medially, seen in coronal section, the amygdala was bounded by subarachnoid space. Laterally, seen in coronal section, the amygdala was bounded by white matter. To assure that the amygdala ROIs did not fall into regions of susceptibility artifact, we overlayed the amygdala ROIs on an average EPI image generated from the first frame of each BOLD run from all participants. Visual inspection of the images demonstrated the ROIs did not involve any brain areas with substantial susceptibility artifacts (see Figure 2B).

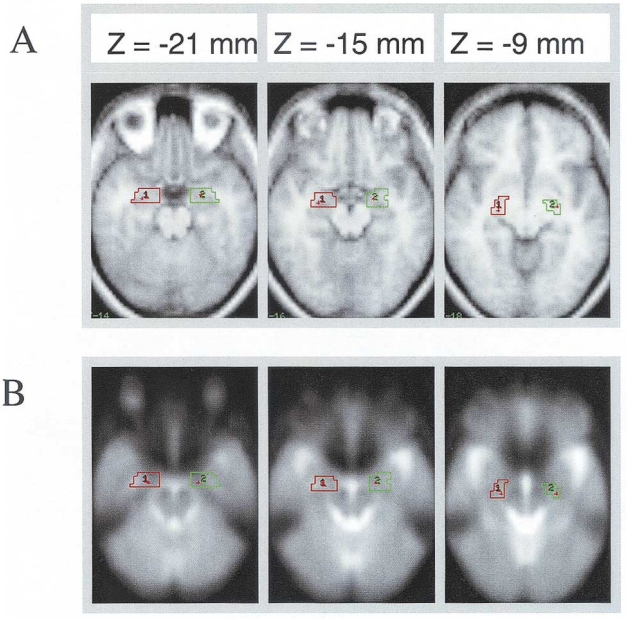

Figure 2. (**A**) Magnetic resonance imagine scans were combined and averaged for all the subjects. A priori left and right amygdala regions of interest were drawn on this combined image, as shown above in axial sections, for use in analysis of variance comparisons of the effect of emotional faces on amygdala activation in depressed and control subjects. (**B**) Functional data, and the first blood oxygenation level-dependent (BOLD) run were combined from all subjects, and the regions of interest (ROIs), left and right amygdala, were superimposed. The area of susceptibility artifact can be seen anterior to, but not including, the amygdala ROIs.

Results

Subject Debriefing

As soon as subjects finished the experiment they were asked to describe what they had seen of the presented faces. All but one control subject described the faces seen as having neutral expressions. One subject described seeing a face with teeth (which occurred in a masked fearful face). Five subjects noticed a "flicker" in the faces. Subjects were then asked if they had seen happy or fearful faces and all subjects denied having seen either happy or fearful faces. The data from one subject was excluded because she called back to the office after the scan to volunteer the information that the task made her very anxious, the reason being that the faces reminded her of nuns she had encountered as a child in parochial school. The magnitudes of her responses were higher than mean for all masked faces.

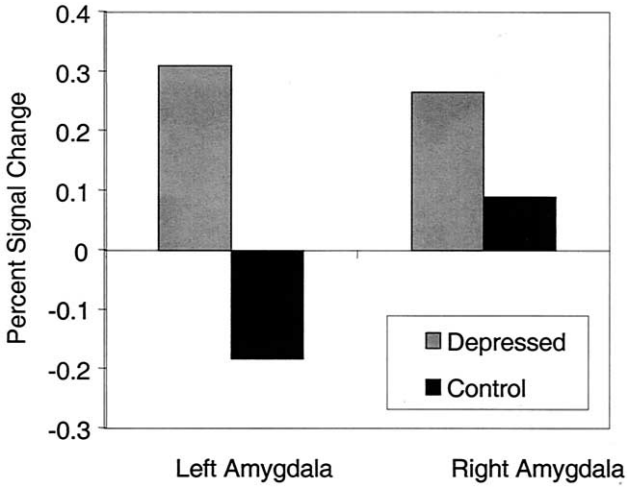

Figure 3. Lateralized differences in amygdala activation were observed in response to face presentation. Depressed subjects had significantly greater left amygdala activation to face presentation than control subjects, but did not differ significantly in right amygdala activation from control subjects. Percent change in magnetic resonance (MR) signal was determined for the left and right amygdala for each subject by calculating the MR signal magnitude with the following formula: (Masked Emotional Face − Crosshair Fixation)/Crosshair Fixation × 100. In this calculation all faces were combined.

fMRI Data: Time 1

EFFECT OF FACE PRESENTATION. We began our analysis of the fMRI data by examining amygdala activity during the presentation of faces, irrespective of emotional type. We analyzed these data using two-factor ANOVAs (one for right and one for left amygdala), with group (depressed, control) as a between-subject factor and condition (face presentation, fixation) as a within-subject factor. As shown in Figure 3, this ANOVA revealed that depressed participants had significantly greater left amygdala activation to face presentation compared to control subjects [group × condition interaction; $F(1,20) = 7.6$, $p < .05$]. In right amygdala, depressed patients demonstrated numerically greater activation to faces presentation than control subjects (Figure 3); however, the group × condition interaction was not significant in right amygdala.

EFFECT OF EMOTION TYPE. We next examined the specificity of the right and left amygdala responses to fearful faces as opposed to happy or neutral faces. To do so, we calculated percent changes for left and right amygdala activation for each subject for each emotional condition (i.e., fear, neutral, happy) using the following formula: (((task − fixation)/fixation)) × 100). Planned contrasts (using one-tailed t tests) indicated that across groups, activation in right amygdala (see Figure 4) was significantly greater for fearful as compared to either neutral [$t(21) = 2.04$, $p < .05$] or happy faces [$t(21) = 1.75$, $p < .05$]. This fear effect was similar in depressed patients and control subjects. In the left amygdala, depressed patients demonstrated greater activation to fearful as compared to either happy or neutral faces, but these differences were not significant. Additional planned contrasts indicated that depressed patients demonstrated significantly greater left amygdala activation than control subjects for both fearful faces [$F(1,20) = 4.68$, $p < .05$] and happy faces [$F(1,20) = 4.60$, $p < .05$], but not for neutral faces [$F(1,20) = 2.64$, $p > .10$].

fMRI Data: Time 2

We began our analyses of the time 2 data by examining the stability of activation among control subjects, to provide a comparison against which to interpret any activation changes found in depressed patients as a function of treatment. To examine any changes in amygdala activation to faces as a function of time, we used three-factor ANOVAs with time, condition (face presentation, fixation) and emotion (fear, neutral, happy) as within-subject factors. In control subjects for both right and left amyg-

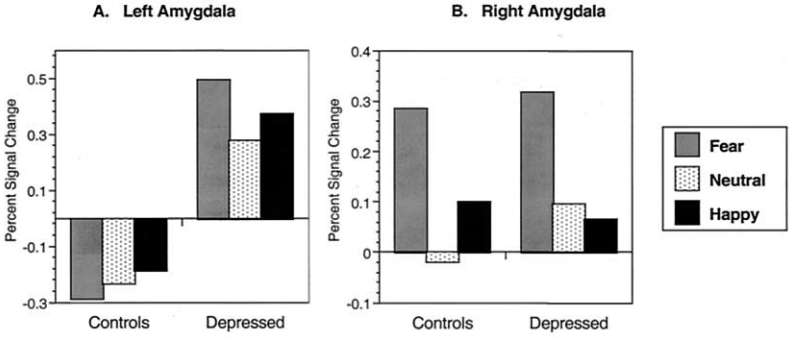

Figure 4. Lateral differences in amygdala activation were observed in response to masked fearful face presentation. Depressed subjects had significantly greater left amygdala activation to fearful face presentation than control subjects. Percent change in magnetic resonance (MR) signal was determined for the left and right amygdala for each subject by calculating the MR signal magnitude for each emotional face type.

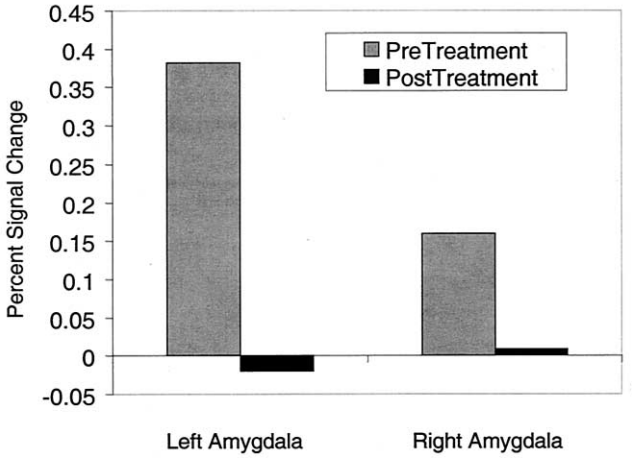

Figure 5. Depressed subjects had significant reduction in both left and right amygdala activation in response to all face presentations following antidepressant treatment.

dala, these analyses indicated no significant interactions of time with either condition or emotion (all $p > .50$).

Following treatment, the mean HRSD scores decreased from 23.3 to 9.7. To examine changes in amygdala activation among depressed participants as a function of treatment, we again used three-factor ANOVAs with time, condition (face presentation, fixation) and emotion (fear, neutral, happy) as within-subject factors. For the right amygdala, this ANOVA indicated a significant time × condition interaction [$F(1,10) = 5.74$, $p < .05$]. As shown in Figure 5, depressed patients demonstrated a significant reduction in right amygdala activation following treatment. Planned contrasts indicated that the reduction in right amygdala activation was significant even when fearful faces alone were considered [$F(1,10) = 5.8$, $p < .05$] (Figure 6). For the left amygdala, the ANOVA also indicated a significant time × condition interaction [$F(1,10) = 5.5$, $p < .05$]. As shown in Figure 5, depressed patients also demonstrated a significant reduction in left amygdala activation following treatment. Planned contrasts again indicated that the reduction in left amygdala activation was significant even when fearful faces only were considered ($F(1,10) = 3.13$, $p = .05$) (Figure 6). Further, planned contrasts indicated that following treatment, depressed patients and control subjects no longer differed in either left or right amygdala. For left amygdala, there was no difference in overall amygdala activation [$F(1,19) = 2.23$, $p > .10$] or in activation to fearful faces [$F(1,19) = 2.9$, $p > .10$]. For right amygdala there was also no difference in activation to all faces [$F(1,19) = .41$, $p > .10$] or in activation to fearful faces [$F(1,19) = .41$, $p > .10$]. Because of loss of scanner data, only 10 control subjects had data included at time 2.

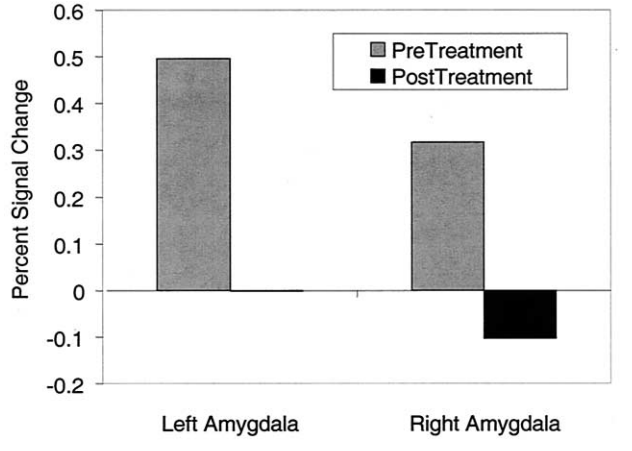

Figure 6. Depressed subjects had significant reduction in both left and right amygdala activation in response to masked fearful face presentation following antidepressant treatment.

PERFORMANCE DATA. Depressed and control subjects did not differ in latency of response for total faces 723 (66) msec and 705 (86) msec, mean (SD), respectively, [$t(19) = 0.55$; $p = .59$], nor for any of the masked face subtypes (fear, neutral, happy). Depressed and control subjects also did not differ on accuracy of responses. Overall percent accuracy was 86% (9.5%) and 90% (11%), mean (SD), respectively, [$t(16) = -0.92$; $p = .37$], and subjects did not differ for masked fear, neutral, or happy face types. Note that the df reflects that some of the comparisons are based on missing data.

MOVEMENT DATA. Inspection of the estimated movement parameters generated by the image movement correction algorithms did not indicate any statistically significant differences between groups in the amount of movement (using absolute values) (all ps $> .05$); however, there was a trend for depressed patients to show greater movement on the Pitch parameter [$t(20) = 2.13$, $p = .06$], primarily due to higher movement in two depressed subjects. Removing the two highest-moving depressed subjects from the analyses produced depressed and control groups equal in movement and still revealed a significant group × condition interaction for left amygdala df (1,18) ($F = 4.35$, $p = .05$), despite the reduction in the patient sample size.

CORRELATION ANALYSES. Post hoc analyses were conducted in the ROI that showed a significant group difference (left amygdala). There was no correlation between fMRI signal intensity change within the left amygdala and the severity of depressive symptoms, as measured by the HRSD ($r = -.04$; $p = .90$). In addition, using the three-item anxiety subscore (mean score = 4.9) from the 17-item HRSD, there was no correlation with fMRI signal intensity ($r = .09$; $p = .80$). This was true at time 2 as well for the total HRSD score ($r = -.23$, $p = .50$ and the anxiety subscore (mean = 2.7) ($r = -.13$; $p = .70$).

Discussion

The primary result of this study is the demonstration in depressed patients of greater left amygdala activation to fearful faces, even when these faces were masked and presented outside of conscious awareness. In addition, depressed patients demonstrated greater left amygdala activation for all faces, although the magnitude of this effect for happy and neutral faces was not as strong as the effect for fearful faces. In contrast, in right amygdala both depressed patients and control subjects demonstrated significantly greater activity to fearful as compared to either neutral or happy faces; however, there were no group differences in right amygdala activity. These results provide interesting suggestions of a lateralizing effect. Our finding of significantly greater left amygdala activation in depression is consistent with PET studies of increased resting metabolism and blood flow in left but not right amygdala of depressed compared with control subjects (Drevets et al 1992). In contrast, Abercrombie et al (1998), using PET regional cerebral glucose metabolic rate (rCMRglu), found no group differences in resting metabolism but found that in depressed subjects increasing resting metabolic rate in the right amygdala but not the left correlated with negative affect. Recently, Wright et al (2001) studied repeated presentations of emotional faces using fMRI in normal subjects and observed that, unlike our study, the left amygdala was significantly more activated than the right to fearful versus happy faces and that there was more habituation on the right than the left. Phillips et al (2001) also found lateralizing effects, with more left amygdalar reponse to fearful faces and increasing magnitudes of right amygdalar responses to neutral faces with time. One possibility that we considered to explain the decreased activation to masked neutral faces relative to fearful faces in our experiment was the greater number of neutral faces seen by subjects, potentially producing accommodation; however, this would argue for higher and equal fMRI magnitudes to both fearful and happy faces, which was not seen, and would also not explain the group differences.

Other fMRI studies have found increased amygdala activation in anxiety disorders including social phobia (Schneider et al 1999) and posttraumatic stress disorder (PTSD) (Rauch et al 2000). To our knowledge, however, this is the first fMRI report of increased amygdala activation in major depression. It will be important to replicate these findings in future studies with a larger sample size. Although Rauch et al reported findings in PTSD, it should be noted that half of their sample had comorbid depression—three of their eight subjects had comorbid major depression and one had dysthymia. It may be that there are commonalities among anxiety disorders and major depression in producing increased amygdala activation, or some of the findings may have resulted from comorbid depression; however, it should be noted that Rauch et al did not find a correlation between fMRI magnitudes and depressive symptom severity. In post hoc analyses we examined whether there was a correlation between magnitude of anxiety symptoms (using the anxiety items on the HRSD) and left amygdala fMRI magnitudes; however, we found no correlations either pre- or posttreatment. It is possible that using a more sensitive instrument to detect anxiety symptoms would yield a greater correlation with fMRI response. Furthermore, we found no correlation between the overall HRSD score and fMRI magnitude in the left amygdala, the region of interest in which group differences were identified. This may be owing to the small sample size. In addition we were interested in those subjects who did not respond to treatment to aid in confirming that amygdala findings in depressed patients were a function of illness and not of a nonspecific factor; however, we had only one nonresponder and two partial responders (who had less than 50% reduction in HRSD but achieved final scores of 12 and 14) compared with eight responders. We therefore could not meaningfully compare fMRI magnitude in the responders and nonresponders.

The reason why depressed patients might have greater left amygdala activation in response to masked fearful and happy faces is not clear. Artifactual reasons for these findings we considered and believe to be highly unlikely are that greater movement in depressed subjects accounted for a false positive result, that depressed and control subjects differed in performance, and that areas of signal drop-out in the amygdala ROI might differ in depressed subjects. The former two possibilities are addressed by data presented in the results section, with neither possibility being confirmed. The third possibility, that there may be differences in susceptibility artifacts, is difficult to address quantitatively. As noted in the Methods section, we overlayed the amygdala ROIs on an average EPI image and found that the ROIs did not fall into areas of major susceptibility artifact. (Figure 2B). One might argue, however, that given the findings of a trend toward smaller amygdala volumes in depression (Sheline et al 1998), the amygdala ROIs might have been more likely to fall into regions of susceptibility artifact in patients as compared to control subjects; however, such a hypothesis would predict decreased activation magnitudes among patients, rather than the increased left amygdala response that we found.

Another possibility is that the findings can be explained by an increased propensity to negative ruminations, which is part of the syndrome of depression, with depressed individuals demonstrating enhanced attribution of negative emotions to neutral faces (Bouhuys et al 1999) and a negative cognitive bias in their explicitly articulated description of negative simulated situations (White et al

1992). Both of these findings, however, reflect conscious mechanisms. In contrast, our findings were in response to stimuli presented for only 40 msec, and appeared to act outside of the subjects' awareness. Thus, it appears more likely that hypersensitivity to fearful stimuli is occurring at an early processing level, or what LeDoux (1996) calls the "emotional unconscious," too quickly to be modified by conscious thought processes.

The neuroanatomical pathway in the auditory system for an immediate reaction to perceived danger has been shown in lesion studies to involve a direct connection to the amygdala that bypasses the cortex and responds automatically to perceived threats (LeDoux et al 1986). In our study, not only masked fearful faces but masked happy and masked neutral faces also produced greater amygdala activation, suggesting that in depression perhaps all faces represent potential threats, assuming that a direct pathway holds for the visual system as well as for the auditory system. Although we emphasize the automaticity of the amygdala response by using a standardized masked faces paradigm, we did not make any distinction in our data analysis regarding each individual subject's level of awareness of the emotional stimuli. Five of the 22 subjects noticed some "flickering" during the face presentation. Future studies of depression will need to assess the benefits of a masked emotional faces paradigm versus explicit presentation of emotional stimuli.

Antidepressant treatment significantly decreased amygdala activation to masked emotional faces in depressed patients compared with control subjects—similar to PET studies of Mayberg et al (1999) finding treatment-induced decreases in other ventral limbic regions, including subgenual cingulate. Depressed and control subjects in our study differed significantly at time 1 (pretreatment) in the magnitudes of response to masked fearful faces, whereas at time 2 (posttreatment) they did not differ. Although control subjects also had lower magnitude responses at time 2, the difference was minimal and not statistically significant, whereas in depressed subjects the reduction between pre- and posttreatment activations was significant. It is important to have a control group that receives two studies for adequate comparison at the time of the second study.

Given that not just fearful faces but all faces resulted in amygdala over-activation in depressed subjects, we cannot rule out the possibility that this occurred as a result of a nonspecific factor. As above, we do not believe that this effect resulted from anxiety, because the anxiety subscores on the HRSD changed no more than overall depression scores from baseline to posttreatment and were not correlated with fMRI magnitude; however, the treatment effects could still be nonspecific; our study reflects the rudimentary state of our present knowledge concerning these mechanisms.

Our finding that the increased amygdala activation resolved with treatment is consistent with evidence in preclinical studies for an inhibitory effect of chronic antidepressants on amygdala function. Tricyclic antidepressants suppress kindled seizure activity in the amygdala (Schmitt 1966) and ameliorate poor performance in the forced swim test (a rat model of depression) when microinjected into the amygdala (Duncan et al 1986). In autoradiography experiments examining competitive binding of antidepressants to various brain sites, it was concluded that the amygdala was the most important site for antidepressant action (Ordway et al 1991). An associated finding indicating postsynaptic activation related to long-term antidepressant treatment changes, is the induction of c-fos expression in the amygdala following the administration of either imipramine, a tricyclic antidepressant, or citalopram, an SSRI similar to sertraline (Morrelli et al 1999). A significant decrease in corticotrophin-releasing factor, which modulates a number of behavioral, neuroendocrine, and autonomic responses to stress, has also been localized to the amygdala following chronic antidepressant treatment (Aubry et al 1999). These findings implicate the amygdala as a key site for the action of antidepressants. In summary, our study suggests that during depression there may be an over-activation of the amygdala, which is suppressed by chronic antidepressant treatment.

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