Neuron, Vol. 44, 1043–1055, December 16, 2004, Copyright ©2004 by Cell Press

Individual Differences in Trait Anxiety Predict the Response of the Basolateral Amygdala to Unconsciously Processed Fearful Faces

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Summary

Responses to threat-related stimuli are influenced by conscious and unconscious processes, but the neural systems underlying these processes and their relationship to anxiety have not been clearly delineated. Using fMRI, we investigated the neural responses associated with the conscious and unconscious (backwardly masked) perception of fearful faces in healthy volunteers who varied in threat sensitivity (Spielberger trait anxiety scale). Unconscious processing modulated activity only in the basolateral subregion of the amygdala, while conscious processing modulated activity only in the dorsal amygdala (containing the central nucleus). Whereas activation of the dorsal amygdala by conscious stimuli was consistent across subjects and independent of trait anxiety, activity in the basolateral amygdala to unconscious stimuli, and subjects' reaction times, were predicted by individual differences in trait anxiety. These findings provide a biological basis for the unconscious emotional vigilance characteristic of anxiety and a means for investigating the mechanisms and efficacy of treatments for anxiety.

Introduction

During conscious processing, emotional content is readily reportable by subjects, while during unconscious processing, stimulus evaluation takes place, but in a manner that is not accessible for explicit report (Merikle, 1992; Shevrin et al., 1996). The fact that unconscious processing occurred is inferred by the consequences that unconscious processes exert on observable behavior such as reaction times. The conscious and unconscious processing of information about threat represent distinct processes that are thought to be associated with different neural responses (Morris et al., 1998; Phillips et al., 2004) and to produce different behavioral and emotional responses (Beck and Clark, 1997; Mathews, 1990; Shevrin et al., 1996; Wong, 1999). These modes of information processing presumably reflect complementary mechanisms for processing danger signals (Beck and Clark, 1997; Mathews, 1990; Shevrin et al., 1996; Wong, 1999).

Neurobiological studies of fear and anxiety have identified the amygdala as a central component in the processing of threat in both people and experimental animals (Aggleton, 2000; LeDoux, 2000) and as one of the most prominent sites of change in anxiety disorders (Rauch et al., 2003). Moreover, stimuli with different objective levels of threat lead to differential activation of the amygdala (Aggleton, 2000). For example, fearful facial expressions are powerful signals of danger in one's environment that are evolutionarily and culturally conserved (Darwin, 1872; Ekman et al., 1969). Conscious presentation of these faces consistently results in activation of the amygdala (Aggleton, 2000; Phan et al., 2002; Wager et al., 2003). By contrast, the capacity for unconscious processing of these stimuli by the amygdala is controversial. Early studies suggested that the amygdala may process emotional stimuli unconsciously (Morris et al., 1998; Whalen et al., 1998), but more recent work has obtained contradictory results (Japee et al., 2004; Phillips et al., 2004).

All of these earlier studies assumed that all subjects were responding similarly to these stimuli. The starting point of our research was the well-established fact that even healthy subjects can differ dramatically from one another in their sensitivity to threat (Mathews, 1990; Spielberger et al., 1970). We therefore report preexisting, stable individual differences in the degree of apprehension and feeling of tension that subjects experience in response to a given threatening stimulus using the quantitative trait measure in Spielberger's State-Trait Anxiety Inventory (STAI-T) (Spielberger et al., 1970).

We investigate the effects of threatening information presented consciously or unconsciously to normal individuals differing widely in their levels of trait anxiety with the purpose of testing two ideas. (1) Is the amygdala differentially activated by the degree to which a given individual is sensitive to threat as measured by that individual's trait anxiety? (2) Does an individual's response to fearful faces differ depending on whether information is processed consciously or unconsciously?

Anxious individuals show an increased sensitivity to threat-related cues, presumably resulting from increased unconscious vigilance for these threatening stimuli (Beck and Clark, 1997; Fox, 2002; Mathews, 1990; Mogg and Bradley, 1999; Wong, 1999). The recruitment of attentional resources for the evaluation of even unconsciously processed threat in anxious individuals can be seen as an advantageous way of appraising threat for a faster and more effective defensive response. Interestingly, these earlier behavioral studies found that the capture of attention by threat-related facial stimuli is most effective during unconscious rather than conscious processing (Fox, 2002; Mogg and Bradley, 1999).

We have sought to extend this analysis to the biological level by exploring whether individual differences in

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trait anxiety modulate the degree to which the amygdala in general and specific subregions of the amygdala in particular are activated by unconsciously processed stimuli. To resolve subregions of the amygdala, we acquired neuroimaging data at a high spatial resolution (1.5 × 1.5 × 4.5 mm voxels; the amygdala is ~1000 mm^3) capable of distinguishing between activations in two subregions of the human amygdala: (1) the basolateral amygdala and (2) the dorsal amygdala (Merboldt et al., 2001). This basolateral subregion is anatomically consistent with the basolateral complex of the amygdala in humans (Mai et al., 1997), which in rodents is the primary input site of the amygdala, receiving sensory information from thalamic nuclei and sensory association cortices (Amaral et al., 1992), and also provides the majority of the thalamic and cortical projections from the amygdala. By contrast, the central nucleus, an output region that projects to brain stem, hypothalamic and basal forebrain targets (Paxinos, 1990), is located in the dorsal amygdala of humans (Mai et al., 1997). In rodents, the basolateral amygdala encodes the threat value of a stimulus, while the central nucleus is essential for the basic species-specific defensive responses associated with fear (Davis and Whalen, 2001).

Since most neuroimaging studies have not employed sufficient spatial resolution to distinguish between the dorsal and basolateral subregions of the amygdala, little is known about their functions in humans. Several lines of evidence, however, suggest that the basolateral complex and central nucleus in nonhuman primates and people may be functionally analogous to those in rodents. Extra-amygdalar connectivity of these nuclei are similar in primates and rodents, both in terms of which region targets thalamic and cortical regions compared to the brain stem, basal forebrain and hypothalamus, but also with respect to the identity of specific target cortical regions and thalamic nuclei (Amaral et al., 1992; Pitkanen, 2000). Likewise, the basolateral amygdala projects to the central nucleus in both species (Amaral et al., 1992; Pitkanen, 2000). In addition, differential intra-amygdalar distribution of anxiety-related gene expression is similar across species. For example, benzodiazepine receptors are enriched in the basolateral amygdalae of both humans and rodents (Niehoff and Kuhar, 1983; Niehoff and Whitehouse, 1983).

Results

We elicited conscious emotional perception by presenting faces with clear and reportable expressions of fear (denoted F) drawn from a set of normalized images (Ekman and Friesen, 1976). We employed the commonly used control of neutral expression faces (N) to control for the viewing of faces and procedural aspects of the task (Japee et al., 2004; Morris et al., 1998; Pessoa et al., 2002; Phillips et al., 2004). Although angry faces have been previously reported to elicit amygdala activation (Adams et al., 2003), we chose not to use them because their effect on the amygdala is less reliable across studies than activation by fearful faces (Phan et al., 2002). For unconscious processing, we presented the same fearful expression faces in a backward masking paradigm, which renders masked stimuli unreportable (Esteves and Ohman, 1993; Morris et al., 1998; Phillips et al., 2004; Whalen et al., 1998). Backward masking was achieved by briefly displaying a fearful face, immediately followed by a neutral face as a "mask" (FN), with two neutral faces (NN) presented with the same timing as a control. Masked faces were presented in the same color and with the same gender as the neutral face mask. Masking was deemed to have occurred when subjects were unable to explicitly report the emotional content of masked fearful faces in a post-scan interview and was verified with a forced choice test (see Experimental Procedures and below). Subjects viewed all of these faces in the context of a color identification task (see Figure 1). They were asked to judge the color of each face (pseudocolored in either red, yellow, or blue) and to indicate the answer by a keypad button press.

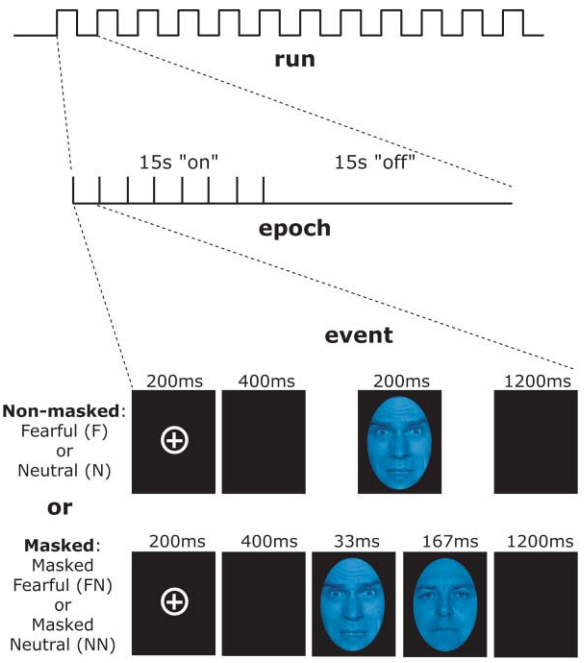

Figure 1. Experimental Paradigm for the Interaction of Attention and Affect

Stimuli were either fearful (F) or neutral (N) expression faces, pseudocolored in red, yellow, or blue. Faces were presented either nonmasked (200 ms for each face; F or N) or masked (33 ms for a fearful or neutral face, followed by 167 ms of a neutral face mask of the same gender and color, but different individual; FN or NN, respectively).

Assessment of the Success of Backward Masking

The existence and characteristics of unconscious processing have been controversial in the behavioral and neuroimaging literatures, largely because different authors have adopted different thresholds for defining processing as occurring outside of conscious awareness (Pessoa, 2005; Snodgrass et al., 2004). At any given luminance and duration of the target (masked) face, backward masking may not work for all subjects (Pessoa, 2005; Snodgrass et al., 2004). Differential masking effects may explain the discrepancies between previous studies with regard to whether the amygdala is activated by masked fearful faces (Pessoa, 2005). To ensure that

backward masking was adequate in our study, we excluded those subjects from the analysis for which we were not confident that masking succeeded.

In general, three distinct criteria for determining the success of masking have been proposed in the literature. The first, the *subjective threshold*, involves asking subjects whether they had perceived any fearful faces when shown masked fearful (and neutral) stimuli (Merikle, 1992). This is the oldest criterion for demonstrating perception without awareness (Pierce and Jastrow, 1884) and is attractive because it directly assesses the conscious experience of subjects (Merikle, 1992), has produced many demonstrations of behavioral effects of unconscious stimuli (Cheesman and Merikle, 1986; Merikle, 1992), and was used by the earliest neuroimaging study of backwardly masked fearful faces (Whalen et al., 1998). Of the 26 subjects scanned, 17 met the subjective threshold.

One criticism raised of the subjective threshold is that it does not provide an easily quantifiable, potentially objective numerical index of masking success (Merikle, 1992; Snodgrass et al., 2004). The most commonly used method for addressing this problem, the *objective identification threshold*, involves showing subjects masked fearful and masked neutral stimuli and asking them to make an explicit forced-choice decision for each stimulus with regard to whether they see a fearful face. The vast majority of behavioral studies examining unconscious affect, including those testing for effects of anxiety measures, have used the objective identification threshold of chance (50% accuracy) identification of masked fearful faces. In addition, this threshold has been used by an early study (Morris et al., 1998) which reported amygdala activation to masked fear-conditioned angry faces and a more recent report (Phillips et al., 2004) which found that masked fearful faces failed to elicit amygdala activation. All of our 17 included subjects performed at or below chance level, which differed significantly from the accuracies of the excluded subjects ($p < 0.001$), who performed above chance. Furthermore, accuracy of nonmasked fearful face identification was well above chance for all subjects and did not differ between the two groups ($p = 0.33$).

In examining only masked fearful face identification, the objective identification threshold method leaves out an important aspect of each subjects' responding—how likely they are to judge a neutral stimulus as fearful, which would be reflected in their false alarm rates. Absence of a false alarm measure can be a particular problem under conditions of low signal to noise, but is dealt with within the framework of signal detection theory (Green and Swets, 1966). One useful signal detection theory approach relevant for the assessment of masking (determining the *objective discrimination threshold*) is the discriminability index (denoted d′). Calculation of d′ is independent of the internal criteria the subject uses for distinguishing signal from noise, and values refer to how strong or discriminable a signal is from no signal. We calculated d′ values for each subject for both the masked and nonmasked forced-choice results. We found that for nonmasked stimuli, both groups had d′ scores significantly different from 0 (one-sample t test, $p < 0.001$ in both cases), and there was no difference between groups (two-sample t test, $p = 0.87$), demonstrating that both groups showed high discriminability for nonmasked fearful compared to neutral faces. By contrast, for masked stimuli our included subjects did not have d′ scores significantly different from 0 ($p = 0.11$), while the excluded subjects did significantly differ from 0 ($p = 0.001$). Furthermore, there was a significant difference between the d′ values of the included and excluded groups for masked faces ($p = 0.01$). These data therefore demonstrate that masking was successful only for the included group. In addition, the objective measures were consistent with the results of the subjective threshold initially used to select subjects for fMRI data analysis.

One potential concern with our inclusion criteria is that at the very beginning they may have led to selective inclusion of subjects with skewed anxiety scores. It may be argued that more anxious subjects were better able to see through the masking and thus were preferentially excluded from our analysis. We explored this issue in several ways. We correlated discriminability index (d′) values with trait anxiety scores for all 26 subjects, but found no significant correlation ($r = -0.3$, $p = 0.15$). Likewise, there was no correlation between masked fearful identification accuracy and trait anxiety ($r = -0.16$, $p = 0.44$). We also compared the trait anxiety scores of the groups after splitting into included and excluded subjects and found anxiety scores to be essentially identical (included 32.7 ± 1.5, excluded 32 ± 1.2; $p = 0.72$).

Different Amygdalar Subregions Process Information during Masked and Nonmasked Presentations, and Relate Differentially to Trait Anxiety

We examined amygdala activity during masked and nonmasked presentations of fearful faces in a population of 17 healthy volunteers who varied in trait anxiety (from the 6th to 85th percentile of undergraduate norms; Spielberger et al., 1970). Trait anxiety in our subjects was very stable across the scanning session (pre versus post scan, $r = 0.94$, $p < 0.0001$). To be certain that we were analyzing data from the appropriate subregions of the amygdala and to reduce the risk of false positive findings, we took a region-of-interest (ROI) approach to identify the dorsal and basolateral subregions of the amygdala separately for each individual based on their high-resolution anatomical scans (see Experimental Procedures). We also distinguished these regions from the hippocampus (see Figure 2). Finally, signal was extracted from nonsmoothed functional scans to take full advantage of the high-resolution data and to prevent blurring of activity across ROIs. We report data only for the right amygdala, as the left amygdala gave no significant activation in any comparison. To isolate the effects of emotional content of stimuli from other aspects of the stimuli and the task, we subtracted neutral or masked neutral activity (N, NN) from fearful or masked fearful activity (F, FN), respectively. The conscious perception of fearful faces is denoted as *nonmasked fear* (F-N) and the unconscious perception of fearful faces as *masked fear* (FN-NN).

As shown in Figure 3A, significant activation was observed in response to *nonmasked fear* in the dorsal amygdala ($p = 0.0006$), but not in the basolateral amyg-

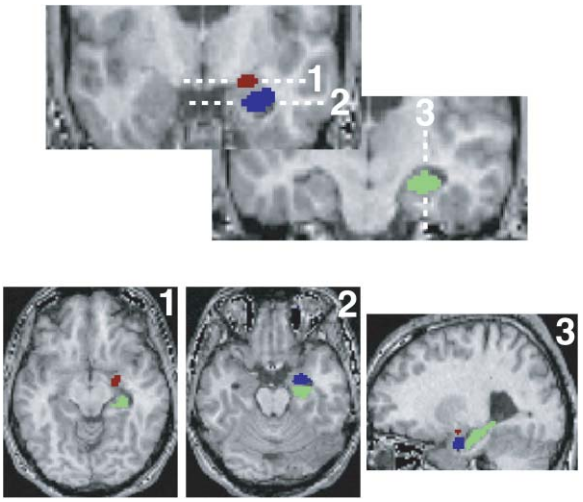

Figure 2. Regions of Interest Used in the Analysis

Regions of interest were drawn for the dorsal amygdala (red), basolateral amygdala (blue), and hippocampus (green) for each subject based on their anatomical scans, illustrated here for one representative subject. Data were extracted from nonsmoothed functional scans to ensure that signal originated from the identified ROIs.

dala ($p = 0.49$) or in the hippocampus ($p = 0.3$). Activation of the dorsal amygdala could be dissociated from the other two ROIs by significant expression × region repeated measures ANOVA interactions (versus basolateral amygdala, $p < 0.0001$; versus hippocampus, $p = 0.002$). Selective activation of the dorsal amygdala by *nonmasked fear* is consistent with a recent meta-analysis showing that the peak of amygdalar activation by conscious threat-related stimuli is on the dorsal edge of the amygdala (Wager et al., 2003).

In response to *masked fear*, we observed activity in a separate region of the amygdala—the basolateral amygdala. This activity was positively correlated with trait anxiety ($r = 0.74$; see Figure 3B). Variance in neural activity did not contribute to this effect, as there was no relationship between trait anxiety and standard errors in *masked fear*-induced activity in the basolateral amygdala ($r = 0.39$, $p = 0.12$). Furthermore, we carried out a partial correlation of *masked fear*-induced basolateral amygdala activity with trait anxiety controlling for standard errors and still found a significant relationship ($r = 0.68$, $p = 0.004$). Removing selected subjects who may have been potential outliers also did not eliminate the significance of the correlation. In contrast to the effects in the basolateral amygdala, trait anxiety was not corre-

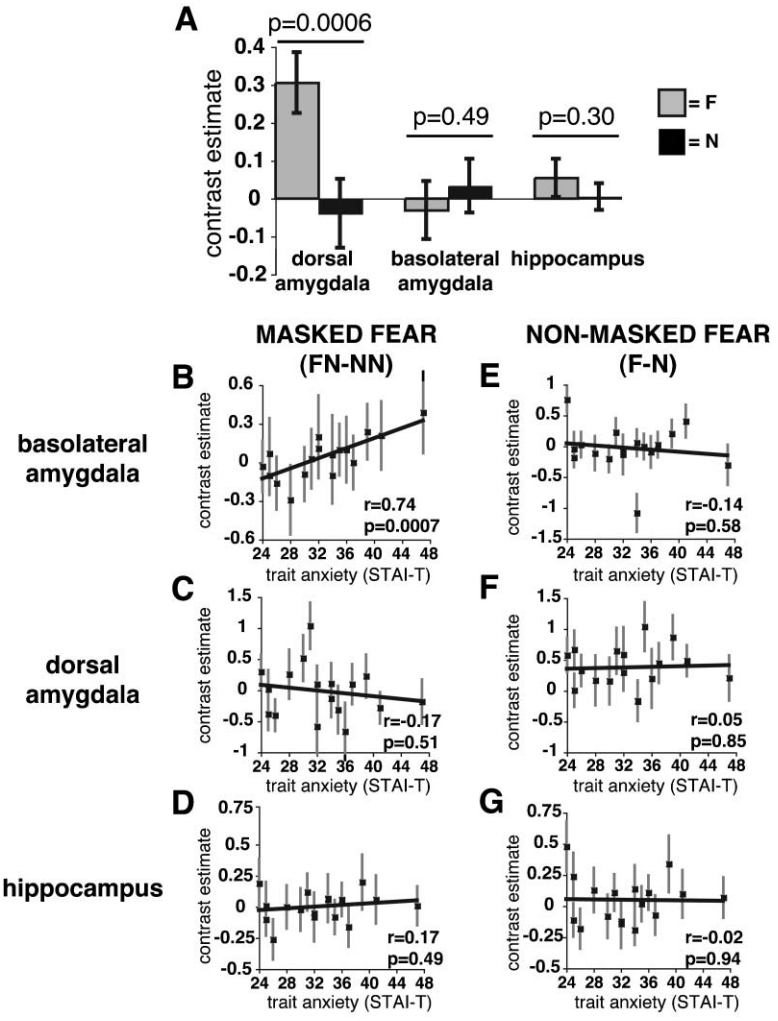

Figure 3. The Basolateral and Dorsal Amygdalar Subregions, as well as the Hippocampus, Are Functionally Dissociable in Emotional Processing

(A) Fearful and neutral epoch main effects during nonmasked presentations from dorsal amygdalar, basolateral amygdalar, and hippocampal ROIs. Activation of the dorsal amygdala by *nonmasked fear* was significantly greater than activation in the other two ROIs.

(B–G) Signal change from dorsal amygdalar, basolateral amygdalar, and hippocampal ROIs for the *masked fear* (FN-NN) and *nonmasked fear* (F-N) comparisons plotted against subjects' trait anxiety (STAI-T) scores and fit to a regression line. The correlation of trait anxiety with basolateral *masked fear*-induced activity was significantly stronger than all other correlations.

lated with *masked fear*-induced activity in either the dorsal amygdala ($r = -0.17$; see Figure 3C) or the hippocampus ($r = 0.17$; see Figure 3D). To complement the regression approach above, we also split our subjects into low and high anxiety groups (based on the median STAI score). This allowed us to verify that there was no activation of the dorsal amygdala in either the low or high trait anxiety group that was not picked up in the regression. Neither group showed significant activation to *masked fear* in the dorsal amygdala (one-sample t test: low anxiety, $p = 0.35$; high anxiety, $p = 0.21$). While we cannot rule out other unexamined latent variables that may predict dorsal amygdala activation by *masked fear*, we found no evidence for modulation by individual differences in trait anxiety. By contrast, the high trait anxiety group showed significant activation of the basolateral amygdala ($p < 0.05$), confirming the results of the regression analysis. Finally, there was no significant relationship between trait anxiety and *nonmasked fear*-induced activity in either region of the amygdala or the hippocampus (basolateral, $r = -0.14$, see Figure 3E; dorsal, $r = 0.05$, see Figure 3F; hippocampus, $r = -0.02$, see Figure 3G).

Direct comparison of the correlations of trait anxiety with *masked fear*-induced activity in the basolateral amygdala, dorsal amygdala, and hippocampus revealed a significant difference in correlation strengths, using Fisher's Z test for correlation coefficients (basolateral versus dorsal, $p = 0.003$; basolateral versus hippocampus, $p = 0.039$). Similarly, comparing the correlation of trait anxiety and *masked fear*-induced basolateral amygdala activity with the correlations of trait anxiety and *nonmasked fear*-induced activity in either amygdalar subregions or the hippocampus also yielded significance (versus basolateral amygdala, $p = 0.004$; versus dorsal amygdala, $p = 0.017$; versus hippocampus, $p = 0.01$). Finally, when individual differences in trait anxiety were not included in the analysis, no ROI showed significant activation during *masked fear* (basolateral amygdala, one-sample t test, $p = 0.3$; dorsal amygdala, $p = 0.89$; hippocampus, $p = 0.95$). We verified that the dissociations we reported in the amygdala were not due to outlier effects by employing robust regression, a correlation approach that objectively differentially weights possible outliers (Rousseeuw and Leroy, 2003). Using this method, we found correlation coefficients nearly identical to those using the ordinary least-squares method above, with Fisher's Z test significance for all planned comparisons (basolateral: FN-NN, $r = 0.74$, F-N, $r = -0.12$; dorsal: FN-NN, $r = -0.15$, F-N, $r = 0.075$).

In summary, we found that *nonmasked fear* activated the dorsal subregion of the amygdala (but not the basolateral amygdala or hippocampus) in a manner that was independent of subjects' trait anxiety. By contrast, *masked fear* led to activity in the basolateral subregion of the amygdala (but not the dorsal amygdala or hippocampus) that was predicted by subjects' trait anxiety. Since we scanned at a high spatial resolution and MRI signal to noise is proportional to voxel volume (Macovski, 1996), it is possible that lack of activation in the basolateral amygdala or correlation in the dorsal amygdala may be due to inadequate signal to noise. This possibility seems unlikely, however, since we do find robust effects in one amygdalar subregion under each condition, and voxel size-related signal-to-noise issues would be expected to be the same across the amygdala.

Reaction Times and Trait Anxiety

If anxiety-related neural activation, seen only during masked presentations, relates to the underlying evaluation and response biases of individuals with different levels of trait anxiety, then a relationship would also be expected between trait anxiety and behavioral performance during masked presentations. We therefore recorded reaction times (RT) for color identification of all four stimulus types and calculated difference scores as a measure of the allocation of attentional resources for emotional processing (see Experimental Procedures) (Mathews, 1990). Consistent with the neuroimaging findings in the amygdala, we observed a significant relationship between trait anxiety and individual reaction time differences during *masked fear* presentations ($r = -0.56$, $p = 0.02$), but not during *nonmasked fear* presentations ($r = -0.07$, $p = 0.79$). Importantly, elevated trait anxiety was associated with enhanced performance—faster identification of the color of neutral masks when preceded by masked fearful faces than when preceded by masked neutral faces. Variance in reaction times did not contribute to this effect, as there was no correlation between standard errors of the *masked fear* presentation RT difference scores and trait anxiety ($r = 0.11$, $p = 0.67$). Furthermore, error rates were not significantly correlated with trait anxiety, suggesting that there was no speed/accuracy tradeoff. Finally, when trait anxiety was not considered as a variable, emotional content did not affect reaction times (*masked fear* difference = 1.4 ms, one sample t test, $p = 0.91$; *nonmasked fear* difference = −5.1 ms, $p = 0.58$).

A Neural Circuit Sensitive to Trait Anxiety during *Masked Fear*

To explore brain regions in addition to the basolateral amygdala that contribute to modulation of behavior by trait anxiety during *masked fear* we took a whole-brain voxel-wise analytic approach. First, we sought to verify that this approach would also be able to identify two dissociable regions of the right amygdala. In response to *nonmasked fear*, there was consistent activation only in the dorsal amygdala (see Figures 4A and 4B and Table 1, line 4). In response to *masked fear*, activity was restricted to a basolateral amygdala cluster, and this was positively correlated with trait anxiety (see Figures 4C and 4D and Table 2, line 7). By contrast, in the *nonmasked fear* comparison there was no relationship between trait anxiety and activity in any region of the amygdala, even at a very lenient statistical threshold ($p = 0.05$, uncorrected). The two amygdalar clusters were separated by approximately 16 mm, a distance that was greater than the spatial resolution of the functional data. Additionally, both of these clusters also passed a small volume correction for multiple comparisons for the right amygdala ($p < 0.05$, corrected).

Knowledge of a subject's trait anxiety level was again found to be critical for the detection of activation in the amygdala in response to *masked fear*, but not for the response to *nonmasked fear*. As indicated in Figure 5A, trait anxiety-independent activation could be detected

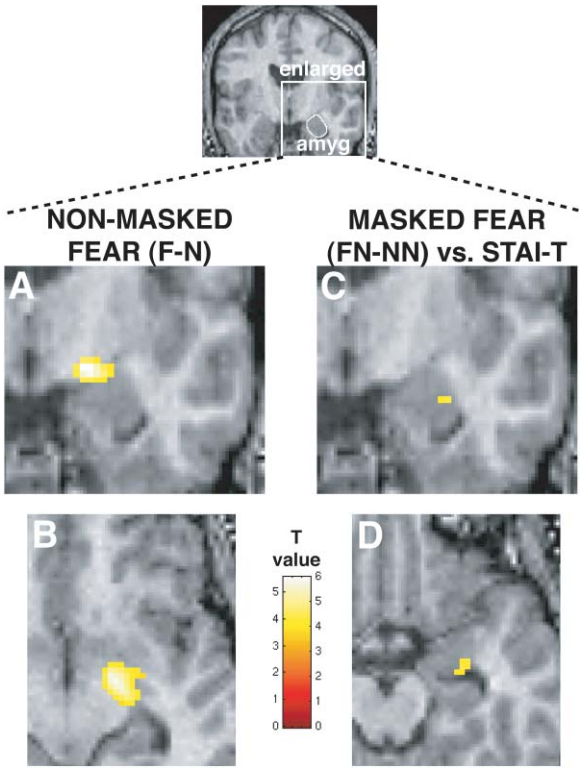

Figure 4. Unconscious Amygdala Activity Reflects Subjects' Trait Anxiety Levels While Conscious Amygdala Activation Is Consistent across Subjects but Independent of Trait Anxiety

Enlarged views of the right amygdala illustrating (1) the dorsal amygdalar cluster from the *nonmasked fear* (F-N) comparison (coronal view at y = −8 [A] and axial view at z = −16 [B]) and (2) the basolateral amygdalar cluster from the correlation of *masked fear*-induced activity (FN-NN) with trait anxiety (coronal view at y = −8 [C] and axial view at z = −28 [D]). The color bar indicates the significance (t value).

in the right amygdala to *nonmasked fear*, even at the most stringent statistical threshold examined ($p = 0.0001$, uncorrected), whereas no significant voxels were detected in the amygdala to *masked fear* unless an unacceptably lenient threshold was used ($p = 0.01$, uncorrected). We also obtained identical results using empirical Bayesian inference, a statistical approach less susceptible to false positives associated with making multiple comparisons (see Figure 5B) (Friston and Penny, 2003). Thus, averaging over the predictive value of individual differences in trait anxiety, a common approach in neuroimaging studies, prevents detection and interpretation of activity in the basolateral amygdala.

To explore brain regions outside the basolateral amygdala that contribute to unconscious perception of fearful faces, we examined the clusters positively correlated with trait anxiety during *masked fear* presentations (see Figure 6 and Table 2). These areas included (1) bilateral dorsolateral prefrontal cortex (DLPFC), (2) the right posterior cingulate gyrus, (3) regions in the left fusiform gyrus, and (4) the cortex around the calcarine fissure. As in the case of the basolateral amygdala, these cortical areas showed no differential activation by *masked fear* unless trait anxiety was incorporated as a variable (data not shown). Furthermore, no cortical clusters covaried with trait anxiety during *nonmasked fear* presentations using our statistical criteria, but activation in a variety of cortical regions was found during *nonmasked fear* presentations independent of trait anxiety (see Table 1). Compared to *masked fear*, *nonmasked fear* perception [i.e., (F-N) − (FN-NN)] leads to greater activation in two attentional areas, the left intraparietal sulcus (−28, −40, 40; z = 4.02) as well as the left precuneus (−16, −76, 50; z = 3.66), consistent with previous studies showing that conscious perception differs from unconscious perception in that it leads to activation of a frontoparietal network (Lumer et al., 1998; Marois et al., 2004).

Discussion

We have discovered a double dissociation between two anatomically distinct subregions of the human amygdala—the dorsal and basolateral regions of the amyg-

Table 1. Activations in the *Nonmasked Fear* Contrast

Region	Side	MNI Coordinates x	y	z	Z	Voxels
Medial frontal gyrus	L	−20	−10	48	5.55	72
		−10	−10	50	3.72	
Subgenual anterior cingulate, caudate	L/R	−6	6	−6	5.51	47
		4	8	−4	3.49	
Precuneus	R	14	−74	30	5.15	22
		6	−78	28	3.39	
Dorsal amygdala	R	16	−8	−12	4.02	94
Inferior parietal lobe	R	−26	−40	40	3.57	9
Inferior frontal gyrus	R	58	−18	28	3.52	29
Dorsolateral prefrontal cortex	L	−54	−6	44	3.42	12
Superior temporal gyrus	R	62	−8	0	3.19	5
Inferior frontal sulcus	L	−36	36	16	3.14	7
Fusiform gyrus	L	−20	−76	−20	2.97	37

A priori regions of interest were evaluated at $p = 0.001$ and >5 contiguous voxel spatial extent, while activations outside of these regions were evaluated at $p < 0.05$, whole-brain corrected for spatial extent. Additionally, as an a priori region of interest, the fusiform gyrus was evaluated at $p < 0.005$ (uncorrected) and a 10 contiguous voxel spatial extent (yielding the same false positive rate). Z scores indicate the significance of peak voxels.

Table 2. Areas with *Masked Fear*-Induced Activity that Is Positively Correlated with Trait Anxiety

Region	Side	MNI Coordinates x	y	z	Z	Voxels
Dorsolateral prefrontal cortex	R	38	20	30	5.49	61
		32	30	30	3.23	
Dorsolateral prefrontal cortex	L	−32	40	18	4.21	32
Posterior cingulate gyrus	R	12	−28	38	4.19	46
Fusiform gyrus	L	−24	−44	−20	5.55	34
Fusiform gyrus		−46	−54	−22	3.45	14
Fusiform gyrus		−30	−70	−16	3.26	7
Basolateral amygdala	R	28	−10	−22	3.41	9
Calcarine fissure	L	−14	−76	6	3.4	29

Statistical criteria are the same as in Table 1. Additionally, as an a priori region of interest, the calcarine fissure was evaluated at $p < 0.005$ (uncorrected) and a 10 contiguous voxel spatial extent. Z scores indicate the significance of the correlation (r values) between peak voxel *masked fear* activity and subjects' STAI-T scores.

dala. These two regions can be dissociated based on whether threat-related stimuli are processed consciously or unconsciously, as well as by individual differences in threat sensitivity (trait anxiety). Consciously processed fearful faces consistently engage only the dorsal amygdala, and in a manner that is independent of trait anxiety. By contrast, unconsciously processed fearful faces engage only the basolateral amygdala, with activity positively correlated with subjects' trait anxiety levels. Consistent with these effects in the basolateral amygdala, trait anxiety levels also predicted reaction times during unconscious processing, but failed to predict it during conscious processing. Greater anxiety was associated with enhanced performance during unconscious processing on this behavioral measure. Furthermore, we found that trait anxiety levels also predicted activity during unconscious processing in frontal, cingulate and ventral visual areas important in attention, consistent with the behavioral findings. Finally, unless individual differences in trait anxiety were accounted for, no differential effects could be detected in the amygdala, behavior, or in the cortex during unconscious processing.

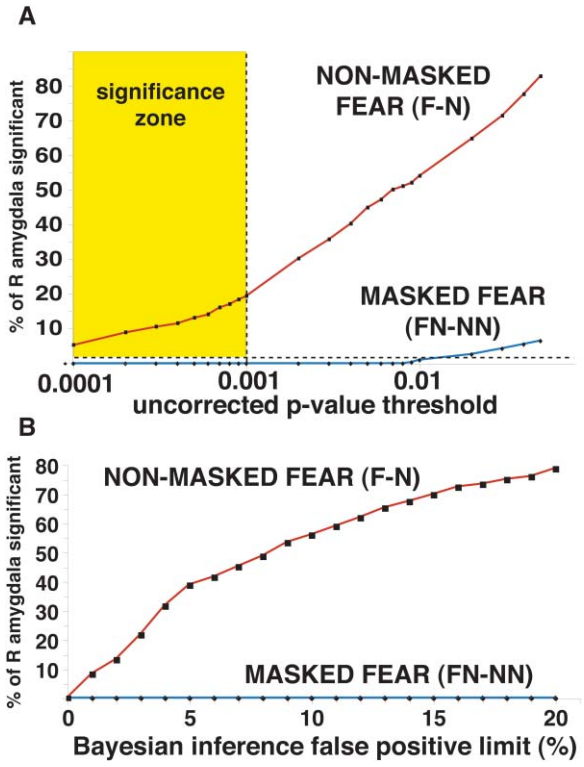

Figure 5. Individual Differences in Trait Anxiety Are a Critical Variable for the Detection of Amygdalar Activation during *Masked Fear*

(A) Percent of the right amygdala exceeding various statistical thresholds is plotted for the contrasts indicated. The significance zone corresponds to the statistical criteria employed for a priori regions of interest.

(B) The same as (A), except that the contrasts indicated were evaluated using Bayesian statistical inference (activation threshold 0%), a method less susceptible to false positives associated with multiple comparisons.

Individual Differences Are Critical in Emotional Processing

In this study, we have examined the neural fate of threat-related stimuli processed outside of subjects' awareness. This issue has been a matter of considerable debate in recent years, with most studies focusing on whether amygdalar activation can be seen in response to undetected fearful faces. Early backward masking studies suggested that the amygdala may be consistently activated by unconsciously processed fearful faces (Whalen et al., 1998) or unconsciously processed angry faces that had been paired with a shock in a fear-conditioning paradigm (Morris et al., 1998). Using similar masking parameters, Phillips et al. (2004) recently found that, while consciously presented fearful faces readily elicited amygdala activity, unconsciously processed fearful faces failed to do so. More recently, another study explicitly divided its subjects by whether or not masking was successful. They found that the activation of the amygdala during conscious processing of fearful faces was eliminated when masking was successful, but could still be detected when masking failed (Japee et al., 2004). These results led to the proposal that different efficacies of masking across studies, perhaps due to different masking thresholds used (subjective, objective identification, objective discrimination), may account for differences in whether amygdala activity was reported (Pessoa, 2005). It may be, for example, that amygdala activity

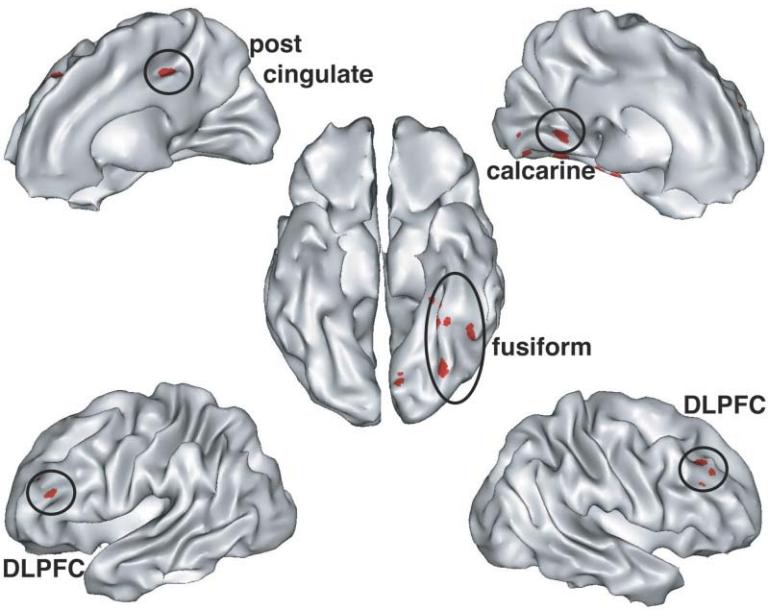

Figure 6. 3D Rendering of Cortical Regions Positively Correlated with Trait Anxiety in Response to *Masked Fear* across the Group of Subjects

Clusters were identified bilaterally in the dorsolateral prefrontal cortices (DLPFC), in the right posterior cingulate, and in left ventral visual areas (fusiform gyrus), as well as around the left calcarine fissure.

is only detected when processing is unconscious by the subjective threshold, but not by objective thresholds (Pessoa, 2005). This view leads to the prediction that successful masking by objective thresholds would result in a lack of activation in the specific amygdalar subregion activated by consciously processed fearful faces. Conversely, it predicts that failure to mask well enough would be reflected in significant activation of that subregion.

We report that consciously processed fearful faces selectively activate the dorsal but not the basolateral amygdala. This is consistent with most previous fMRI studies of conscious perception of threatening stimuli (Japee et al., 2004; Pessoa et al., 2002; Phillips et al., 2004; Wager et al., 2003), although these earlier studies did not have sufficient spatial resolution to confirm the distinction between the dorsal and basolateral subregions of the amygdala. When masked fearful faces are presented and amygdala activation is seen (potentially due to masking only at the subjective threshold), activation is also consistent with localization to the dorsal amygdala (Rauch et al., 2000; Whalen et al., 1998). Since even at a very lenient statistical threshold we find no differential activation of any part of the amygdala (including the dorsal region) when trait anxiety is not considered as a variable and have masked successfully at the most stringent objective threshold, we support the view that completely successful masking eliminates activation in this region.

The capacity for automatic (i.e., attentional resource-independent) processing of threat information, and by extension automatic amygdala activation, however, is an important component of current theories of anxiety and its disorders (Beck and Clark, 1997; Mathews, 1990; Mogg and Bradley, 1998). Beck's cognitive model of anxiety, for example, calls for an initial unconscious step in threat processing, which he terms the "orienting mode" (Beck and Clark, 1997). In this model, differences between anxious and nonanxious individuals already exist in the orienting mode, which serves to bias subsequent steps in stimulus processing and evaluation, including the allocation of attentional resources. Previous studies of unconscious amygdala activation, whether or not they found that subjects activated the amygdala, are thus inconsistent with models of anxiety like Beck's because they do not account for differences in the orienting modes of anxious and nonanxious individuals. Additionally, because an "anxious" orienting mode biases later attentional processes, anxiety-related activation in cortical attentional areas connected with the amygdala would also be predicted from cognitive models of anxiety.

Importantly, we discovered that individual differences in trait anxiety predict activity in the basolateral subregion of the amygdala during unconscious processing, which is a subregion distinct from that which is activated by consciously processed stimuli. These findings rely on having acquired our data at a sufficiently high spatial resolution to dissociate between amygdalar subregions and focusing on individual differences in trait anxiety. Likewise, we found correlations between anxiety and activation in attentional areas as well as behavior. These individual differences were not considered in previous neuroimaging studies but were predicted by cognitive theories of anxiety such as Becks's (Beck and Clark, 1997; Mathews, 1990; Mogg and Bradley, 1998). Thus, we propose that amygdala activity (but only in the basolateral subregion) accounts for individual differences in threat sensitivity under masking conditions stringent enough to eliminate dorsal amygdalar activation (which responds to conscious threat). This trait anxiety factor is therefore an important latent variable across all previous studies that explains aspects of unconscious processing not related to the success of the masking procedure. Were, for example, anxiety related to the discriminability of masked stimuli (a consideration we had earlier ruled out), we would expect that anxiety correlations with amygdala activity would also be seen in the dorsal

amygdala, but we found no evidence for this. Finally, unconscious effects on behavioral response and the activation of a widespread cortical attentional network were eliminated when analyzed independently of trait anxiety.

Support for the finding that individual differences in anxiety are an important predictor of amygdala activity has been recently obtained independently in a study from Bishop et al. (2004) that examined the activity of the amygdala during an attentional distraction task in which the processing of unrelated images that were shown along with face stimuli siphon away attentional resources from the processing of emotional content. Using this protocol, they found that individual differences in state anxiety predicted amygdala activity under conditions of distraction. Amygdala activation to attended faces, however, was not related to anxiety. These results therefore parallel our findings that trait anxiety only influences unconscious amygdala activity.

A Basolateral Amygdalar-Cortical Network for Unconscious Emotional Vigilance

Few previous imaging studies have acquired data at a spatial resolution sufficient to identify functions specifically attributable to the basolateral amygdala. Considerable evidence exists, however, to support a functional similarity between the basolateral complex of the amygdala in humans, primates, and rodents. There are interspecies similarities both in terms of the intra- and extra-amygdalar connectivity of the basolateral amygdala in primates and rodents (Amaral et al., 1992; Pitkanen, 2000), as well as in the distribution of anxiety-related gene expression in the basolateral nucleus (Niehoff and Kuhar, 1983; Niehoff and Whitehouse, 1983). Differential functions of amygdalar nuclei have been studied more thoroughly in rodents than in primates, making knowledge about the function of the amygdala in rodents particularly useful in interpreting our results. Thus, by examining our findings in the comparative context of the rodent basolateral amygdala, we believe we can enhance current understanding of the functional specifications within the human amygdala, an exciting but as yet little-explored area of emotion research.

Since the basolateral complex is the primary input site of the rodent amygdala (Amaral et al., 1992) and encodes the objective threat value of a stimulus in rodents (Davis and Whalen, 2001), we propose that in humans it may also reflect an individual's sensitivity to threat (i.e., perceived threat value), as indicated by trait anxiety. Furthermore, most of the neocortical and thalamic projections of the rodent and primate amygdala originate from the basolateral complex (Amaral et al., 1992). Thus, anxiety-related activation of the basolateral amygdala during unconscious processing may account for the coactivated ventral visual areas through direct projections, and the coactivated dorsolateral prefrontal cortices and posterior cingulate cortex likely through indirect projections via the mediodorsal thalamus or medial prefrontal cortex (Amaral et al., 1992). Recruitment of the dorsolateral prefrontal cortices and posterior cingulate cortex may enhance the allocation of attentional resources for threat stimulus processing (Funahashi, 2001; Small et al., 2003). Enhancement of attention would be reflected by increased early sensory processing in visual cortical areas, ranging from very early visual areas along the banks of the calcarine fissure to regions in the fusiform gyrus (Pessoa et al., 2003), which specialize in the processing of human faces (Kanwisher, 2000). The prefrontal, cingulate, and ventral visual cortical connections of the basolateral amygdala may therefore represent a neural circuit for the enhanced unconscious perceptual sensitivity to ambiguous or subthreshold threat information observed in individuals with high levels of anxiety (Beck and Clark, 1997; Mathews, 1990). This behavioral sensitivity was evidenced in our study by the anxiety-related enhancement of behavioral performance during unconscious processing. Supporting this view is the finding that patients with posttraumatic stress disorder, a severe anxiety disorder, show a heightened response in the amygdala to masked fearful faces (Rauch et al., 2000; Whalen et al. 1998).

These findings extend prior work with the dot probe attentional paradigm (Fox, 2002; Mogg and Bradley, 1999), which found that unconsciously processed threat-related expression faces preferentially attracted the spatial attention of more anxious individuals. Healthy individuals with high levels of trait anxiety respond faster to dot probes at the location of unconsciously processed threat-related expression faces than subjects low in trait anxiety (Fox, 2002; Mogg and Bradley, 1999). Enhanced attention at a spatial location has been reported to result in an enhancement in the processing of a variety of basic visual features, such as orientation, motion, and color (Di Russo and Spinelli, 1999; Haenny and Schiller, 1988; McAdams and Maunsell, 2000; Motter, 1994; Treue and Martinez Trujillo, 1999). Our color identification task likely tapped into these consequences of enhanced anxiety-related attention, producing faster color identification reaction times in more anxious individuals.

We are also in agreement with previous reports that anxiety-related modulation of attention is detected most readily when stimuli are processed unconsciously (Fox, 2002; Mogg and Bradley, 1999). That unconscious processing reflects trait anxiety more strongly than conscious processing highlights another important point—that conscious and unconscious emotional processing recruit distinct neural circuitry that produces qualitatively different effects. The double dissociation within the amygdala is a clear demonstration of this fact. Similarly, parietal attentional areas are activated more powerfully during conscious processing than unconscious processing, consistent with their role in the conscious awareness of nonemotional stimuli (Lumer et al., 1998; Marois et al., 2004). Nonetheless, these processes are not wholly separable; several key cortical regions—the dorsolateral prefrontal cortex and fusiform gyrus—were modulated by both conscious and unconscious processing. Thus, inasmuch as differential sensitivity to individual differences in trait anxiety reflects the qualitative differences between conscious and unconscious processing, these differences are most likely attributable to differential recruitment of amygdalar circuitry.

Our results also raise another interesting question—why was there basolateral amygdalar modulation by unconscious processing but no activation during conscious processing, since the first 33 ms of masked and

nonmasked fearful faces is the same. We speculate here on two possibilities. It may be that the unconscious stimulus recruits the basolateral amygdala initially and this activity persists due to the uncertain nature of the masked stimulus. During conscious processing, the basolateral amygdala may be only transiently recruited because the emotional content of the stimulus becomes rapidly clear. Alternatively, there may be active top-down inhibition of the basolateral amygdala during the conscious but not the unconscious condition (Rosenkranz et al., 2003). The magnitudes of fMRI signals would be the same for both of these possibilities, but the magnitudes and time courses of the neural signals may not be. Resolving these issues requires the use of methods with much better temporal resolution than fMRI, such as in vivo recordings in monkey basolateral amygdala and prefrontal cortices.

In our study, amygdalar activity was right lateralized, which may be consistent with lateralization theories of emotional processing (Wager et al., 2003). However, we never formally tested for laterality-specific effects, and two recent meta-analyses found evidence suggesting greater left than right amygdalar activation to negative emotional stimuli (Baas et al., 2004; Wager et al., 2003). Unfortunately, no experimental parameter has been found that reliably predicts laterality (Baas et al., 2004), so we cannot fairly speculate on which factor led to right lateralization in our study.

An Unconscious Basis for Behavior

From a theoretical point of view, the relationship between trait anxiety and unconscious neural processes provides a biological validation for the idea that unconscious mental processes represent part of the underlying information processing biases of the individual. These biases may then undergo additional regulation by conscious processes, a view broadly consistent with both psychoanalytic and cognitive psychological theories (Beck and Clark, 1997; Gabbard, 2000a). During unconscious processing in the task, subjects could not explicitly compare their anxiety-related vigilance for threatening stimuli with the realistic context-specific threat value of the unconscious stimulus. Instead, subjects responded to the stimuli as a threat with a magnitude determined by their individual level of trait anxiety. When, by contrast, emotional content was clear, subjects responded in a strikingly consistent manner that was independent of trait anxiety. Thus, once the potential source of anxiety was recognized, subjects responded similarly and independent of trait anxiety.

Unconscious neural processing biases associated with elevated trait anxiety may reflect hard-wired differences that cannot be altered. Alternatively, they may be modifiable by plastic processes, such as those presumably engaged by psychotherapy. One goal of psychotherapy is to make maladaptive, often unconscious, biases conscious, reality-based, and thus controllable (Brewin, 1996; Gabbard and Westen, 2003). Interestingly, for anxiety disorders the decrease in trait anxiety scores with psychotherapy is considered a standard measure of success (Fisher and Durham, 1999). It is not known, however, whether different forms of psychotherapy act primarily by modifying unconscious processes or only the conscious regulation of behavior. Furthermore, very little empirical evidence exists about the neural mechanisms of the different forms of psychotherapy and the relationship of psychotherapy to the mechanisms of the underlying psychopathology (Gabbard, 2000b). This study provides an experimental probe for unconscious processes that is sensitive to an individual's information processing biases, which may be useful for understanding how anxiety disorders are maintained and how the anxious outlook of these patients can be modulated by different forms of therapy. Specifically, in the future it may be possible to address whether psychotherapy modulates conscious or unconscious processes and to dissociate functional changes in amygdalar circuits from the modulation of cortical circuits that regulate behavior.

Experimental Procedures

Subjects

Twenty six healthy, nonclinical volunteers (13 females, 13 males; 20–33 years old; 24/26 right handed) took part in the study after giving their informed consent according to institutional guidelines for protection of human subjects (Columbia University). Based on a postscan interview to confirm the effectiveness of the masking (see below), 17 of the 26 subjects (8 females, 9 males; 20–33 years old; all right handed) were included for analysis. The color identification task was explained to the subjects, and they were told that they would see faces, some of which may have expressions, but were naive to the experiment's goal of studying expression-related neural activity and behavior. A standardized questionnaire was administered to the subjects to determine their self-reported state and trait anxiety levels both before and after scanning (Spielberger et al., 1970). To ensure that the top of the range of trait anxiety scores didn't represent individuals with unreported anxiety disorders, subjects were screened using a structured clinical interview tool, and no evidence of anxiety disorders was found (First et al., 1996).

Stimuli

For the experimental task, black and white pictures of male and female faces showing fearful and neutral facial expressions were chosen from a standardized series developed by Ekman and Friesen (1976). Faces were cropped into an elliptical shape that eliminated background, hair, and jewelry cues and were oriented to maximize interstimulus alignment of eyes and mouths. Faces were then artificially colorized (red, yellow, or blue) and equalized for luminosity. For the training task, only neutral expression faces were used and were derived from an unrelated set available in the lab. These faces were also cropped and colorized as above.

Experimental Paradigm

Each stimulus presentation involved a rapid (200 ms) fixation to cue subjects to fixate at the center of the screen, followed by a 400 ms blank screen and 200 ms of face presentation. Subjects then had 1200 ms to respond with a key press indicating the color of the face. Nonmasked stimuli consisted of 200 ms of a fearful or neutral expression face, while backwardly masked stimuli consisted of 33 ms of a fearful or neutral face, followed by 167 ms of a neutral face mask belonging to a different individual, but of the same color and gender (see Figure 1). Each epoch consisted of eight trials of the same stimulus type, but randomized with respect to gender and color. The functional run had 12 epochs (three for each stimulus type) that were randomized for stimulus type. To avoid stimulus order effects, we used two different counterbalanced run orders. Stimuli were presented using E-prime (version 1.0; Psychology Software Tools, Inc.; Pittsburgh, PA) on an IFIS system (MRI Devices; Waukesha, WI), and were triggered by the first radio frequency pulse for the functional run. The stimuli were displayed on VisuaStim XGA LCD screen goggles (Resonance Technology, Northridge, CA). The screen resolution was 1024 $\times$ 768, with a refresh rate of 60 Hz. Behavioral responses were also recorded on the IFIS system.

Prior to the functional run, subjects were trained in the color identification task using unrelated neutral face stimuli that were cropped, colorized, and presented in the same manner as the nonmasked neutral faces described above in order to avoid any learning effects during the functional run. After the functional run, subjects were shown all of the stimuli again, alerted to the presence of fearful faces, and asked to indicate whether they had seen fearful faces on masked epochs. Additionally, subjects were administered a forced-choice test under the same presentation conditions as during scanning and asked to indicate whether they saw a fearful face or not. These data were used to determine masked fearful accuracies and d′ values.

Data Acquisition

Functional data was acquired on a 1.5 Tesla GE Signa MRI scanner, using a gradient-echo, T2*-weighted echoplanar imaging (EPI) with blood oxygen level-dependent (BOLD) contrast pulse sequence. Twenty-one axial slices were acquired along the AC-PC plane, with a 128 × 128 matrix and 19 cm field of view (voxel size 1.5 × 1.5 × 4.5 mm). A total of 134 volumes were acquired during the functional run (TR 3 s, TE 40 ms). Structural data were acquired using a 3D T1-weighted spoiled gradient recalled (SPGR) pulse sequence with isomorphic voxels (1.5 × 1.5 × 1.5 mm) in a 24 cm field of view (256 × 256 matrix, 124 slices, TR 34 ms, TE 3 ms).

Data Analysis

All images were analyzed using SPM2 (Wellcome Department of Imaging Neuroscience, London, UK; see http://www.fil.ion.ucl.ac.uk/spm/spm2.html). After excluding the first four "dummy" volumes, all images were motion corrected and coregistered with subjects' SPGR scans. ROIs were constructed and data were extracted for the basolateral and dorsal amygdalae and the hippocampus using MarsBar (Brett et al., 2002) (see http://marsbar.sourceforge.net/). ROIs were drawn for these regions on each individual's anatomic scans, based on an atlas (Mai et al., 1997). Signal across all voxels in these ROIs was averaged and evaluated for the *masked fear* and *nonmasked fear* comparisons. Low-frequency signal drift was corrected by applying a high-pass temporal filter with a 120 s cutoff. Statistical analyses were carried out using the general linear model implemented in SPM2 (Friston et al., 1995b). Regressors were created for each event type in an epochal design with 15 s "activity on" periods. These regressors were then convolved with a canonical hemodynamic response function (HRF). We indicate contrasts of regressors (e.g., fearful epochs > neutral epochs) using a shorthand (e.g., F-N). Error bars signify SEM.

For the voxel-wise analysis, coregistered functional scans were spatially normalized to a standardized template, resampled at a voxel size of 2 × 2 × 2 mm, and spatially smoothed using a Gaussian kernel with a full width at half maximum of 8 mm (Friston et al., 1995a). Fixed-effects single subject level contrast images were entered into a group-level one-sample t test for a random effects analysis of the 17 subjects. Single subject level contrast images were also entered into a simple regression group level analysis with trait anxiety scores as a covariate of interest. The mask for the entire right amygdala was created using WFU PickAtlas (Maldjian et al., 2003), by dilating the standard amygdala mask by a factor of 1, to ensure that it contained the entire amygdala. Reported voxels correspond to standardized Montreal Neurological Institute (MNI) coordinate space. For all displayed images, the right side of the subject is the right side of the image.

A priori regions of interest were determined based on areas activated in other neuroimaging studies of emotional processing or attentional control, as well as areas hyperactivated in anxiety disorder patients. For these a priori regions of interest, a statistical threshold of $p = 0.001$ (uncorrected) and five contiguous voxel spatial extent was applied, yielding an appropriate false-positive rate of <1/100,000 (Eisenberger et al., 2003; Forman et al., 1995). Areas that were not a priori regions of interest were evaluated at a threshold of $p < 0.05$, whole-brain multiple comparison corrected for spatial extent. Confirmatory small volume corrections were applied to the right using the right amygdala mask, and both the basolateral and dorsal amygdalar clusters passed these criteria ($p < 0.05$) (Worsley et al., 1996). Group-level statistical parametric mapping results were displayed at a threshold of $p = 0.001$ and >5 voxel spatial extent, overlaid on the structural scan of an example subject using a color scale to indicate the significance of each voxel. Three-dimensional rendering of activations on a surface mesh of one subject's brain was done with Brainvisa (www.brainvisa.info). Clusters were displayed in regions described in Table 2 at $p = 0.005$ and >10 voxel spatial extent for clarity. Bayesian inference was also conducted on the *nonmasked fear* and *masked fear* group level one-sample t test analyses and was also implemented in SPM2 (Friston and Penny, 2003).

Reaction time data for each stimulus type were determined only for trials where subjects correctly identified the color of the faces. The average accuracy (±SEM) for all stimuli was 98% ± 1%. Reaction time difference scores were calculated by subtracting the average reaction time for NN or N trials for each subject from their corresponding FN or F average reaction times, respectively. Prior to any additional analyses, all ROI, behavioral, and trait anxiety data were confirmed to be normally distributed. In this study, we report results for correlations with trait anxiety. State anxiety correlations were not reported because trait anxiety is considered to be a more stable measure of baseline anxiety (Spielberger et al., 1970), and to accurately measure state anxiety one would have to administer the survey during or between scans, which could not easily be done. Prescan state anxiety scores were also highly correlated with prescan trait anxiety scores, such that none of the results of the analyses reported for trait anxiety changed when prescan state anxiety was used instead (data not shown).

Acknowledgments

We would like to thank Kevin LaBar, Daniel Salzman, Jonathan Polan, Tor Wager, and Brad Peterson for their helpful discussion of the data or the manuscript. This work was funded (in part) by the Howard Hughes Medical Insittute, the Kavli Institute for Brain Sciences, the Neurobiology and Behavior Research Training Program (NICHD HD 07430), an NIMH MD/PhD NRSA fellowship to A.E., NSF graduate research fellowships to K.C.K. and J.T.D., and by Johnson & Johnson (J.H.). The authors of this paper have declared a conflict of interest. For details, go to http://www.neuron.org/cgi/content/full/44/6/1043.

Received: September 18, 2004
Revised: November 14, 2004
Accepted: December 2, 2004
Published: December 15, 2004

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