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Brief report

Amygdala activation in the processing of neutral faces in social anxiety disorder: Is neutral really neutral?

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Abstract

Previous research has suggested that Social Anxiety Disorder (SAD) is associated with a tendency to interpret ambiguous social stimuli in a threatening manner. The present study used event-related functional magnetic resonance imaging to examine patterns of neural activation in response to the processing of neutral facial expressions in individuals diagnosed with SAD and healthy controls (CTLs). The SAD participants exhibited a different pattern of amygdala activation in response to neutral faces than did the CTL participants, suggesting a neural basis for the biased processing of ambiguous social information in SAD individuals.

Keywords: Amygdala; Social phobia; Social anxiety disorder; Functional magnetic resonance imaging (fMRI)

1. Introduction

Social Anxiety Disorder (SAD) is a prevalent and debilitating disorder characterized by intense fear of social situations that significantly impairs quality of life (Cramer et al., 2005). Negatively biased processing of social information has been posited to play a critical role in this disorder; indeed, individuals diagnosed with SAD have been found to have better memory for critical than for accepting faces (Lundh and Ost, 1996) and to selectively attend to negative faces and other threatening social stimuli (Amir et al., 2003; Clark and McManus, 2002).

Recently, investigators have begun to examine the neurobiological bases of the processing of social stimuli in SAD and have found that individuals with SAD exhibit elevated activation to negative faces (specifically anger and disgust) in the anterior cingulate cortex, insula, parahippocampal gyrus, and amygdala when their responses are contrasted with activations to neutral faces (Straube et al., 2004; Amir et al., 2005). In interpreting these findings, researchers have suggested that, in particular, amygdala activation to threatening social stimuli plays an important role in SAD (Phan et al., 2006; Stein et al., 2002). It is important to note, however, that behavioral studies suggest that participants with SAD are likely to interpret neutral and other emotionally ambiguous facial expressions negatively (Winton et al., 1995). This is consistent with brain imaging studies that demonstrate that individuals diagnosed with SAD, compared with control participants, exhibit different patterns of amygdala activation when presented with neutral faces that are paired with an aversive stimulus (Birbaumer et al., 1998; Schneider et al., 1999; Veit et al., 2002). Given these behavioral and imaging finding indicating that SAD individuals may perceive

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neutral faces as threat-related, it is critical to assess the neural responses of SAD individuals to neutral faces alone. Indeed, the possibility that SAD participants are characterized by threat-related neural activations to neutral faces would have important implications for how one interprets results of contrasts of activations to negative versus neutral faces. The present event-related functional magnetic resonance imaging (fMRI) study was designed to examine whether SAD participants exhibit activation to neutral faces in areas of the brain that have been found to be associated with the processing of negative faces. Specifically, we hypothesized that individuals diagnosed with SAD, compared with nonpsychiatric controls, would exhibit increased amygdala activation in response to neutral faces versus an oval fixation and that the strength of amygdala activation within the group of SAD participants would be correlated with level of state and trait anxiety.

2. Methods

2.1. Participants

Ten participants with SAD (mean age=28.7 years, S.D.=8.46; 6 female; all right-handed) and ten healthy control participants (CTL; mean age=28.8 years, S.D.=5.33; 7 female, 9 right-handed) were recruited from the Department of Psychiatry at Stanford University and from the community. Participants were diagnosed using the Structured Clinical Interview for DSM-IV (First et al., 1996). Nine of the ten SAD participants received a diagnosis of Generalized Social Anxiety Disorder. SAD participants were excluded if they had comorbid Major Depressive Disorder or Panic Disorder, or if they reported alcohol or substance abuse symptoms in the last 6 months; we did not exclude SAD participants for lifetime Axis-I comorbidity. Control participants were free of current or lifetime diagnoses of any Axis-I disorder. Five of the ten SAD participants were taking selective serotonin reuptake inhibitors (or Wellbutrin) at the time of scanning. All participants were fluent in English and free of head trauma. Participants completed the State-Trait Anxiety Inventory (STAI), State (STAI-S) and Trait (STAI-T) versions (Spielberger et al., 1970), immediately before being scanned.

2.2. Procedure

Color stimuli were selected from the MacArthur Network Face Stimuli Set (http://www.macbrain.org/faces/index.htm). Fearful, happy, sad, angry, and neutral faces from 20 actors, and an oval with a cross-hair in the middle that was the same size as the actors' faces, were presented in the scanner in an event-related design (because of the specific question being addressed in the present study, only the neutral faces and the oval figures are compared here). Each actor's neutral pose was presented no more than four times over the course of the experiment. Participants were instructed to use a button box to make valence ratings (negative, neutral, or positive) and to indicate 'neutral' when they saw an oval. Faces and ovals each were presented for 2 s separated by a fixation cross (jittered, mean of 4 s). Data were collected from two runs (approximately 337 TRs and 11 m each).

Functional scans were acquired on a 1.5T GE Signa scanner using a T2* in-/out-spiral pulse sequence (TE=40 ms, flip angle=90; Glover and Law, 2001) consisting of 24 4-mm interleaved slices (axial in-plane resolution 3.75×3.75 mm, no gap) at a temporal resolution of 2 s (1.00 TR). All preprocessing and analyses were conducted using Analysis of Functional Neural Images (AFNI; Cox, 1996). Time series data were concatenated, slice time and motion corrected, excluding subjects who moved more than 1 mm. Data were spatially smoothed with a 4-mm Gaussian kernel, high-pass filtered, converted to percent signal change and coregistered to anatomical images.

Preprocessed time series data for each individual were analyzed with multiple regression. The neutral face vs. oval contrast was convolved with a canonical hemodynamic gamma-variate function response (Cohen, 1997), including terms for residual motion, trend, and the neutral face vs. oval contrast regressors. Resulting individual *t*-statistic maps were transformed into *z*-scores and warped into Talaraich space (Talairach and Tournoux, 1988). The CTL and SAD group maps were analyzed with a two-sample *t*-test to compare neutral face vs. oval contrast activations, and the *t*-scores were then converted into *z*-scores.

For the *a priori* regions of interest (ROIs; left and right amygdala), we used a Monte Carlo simulation (1000 iterations) to determine a joint voxel-wise and cluster size threshold protected at $P<0.05$, corrected. Percent signal change to the neutral faces vs. oval contrast was extracted from 2-mm radius spherical masks at the peak voxel between groups in the bilateral amygdala ROIs and was correlated with STAI scores.

3. Results

The CTL participants correctly identified a nonsignificantly higher proportion of neutral faces than did the

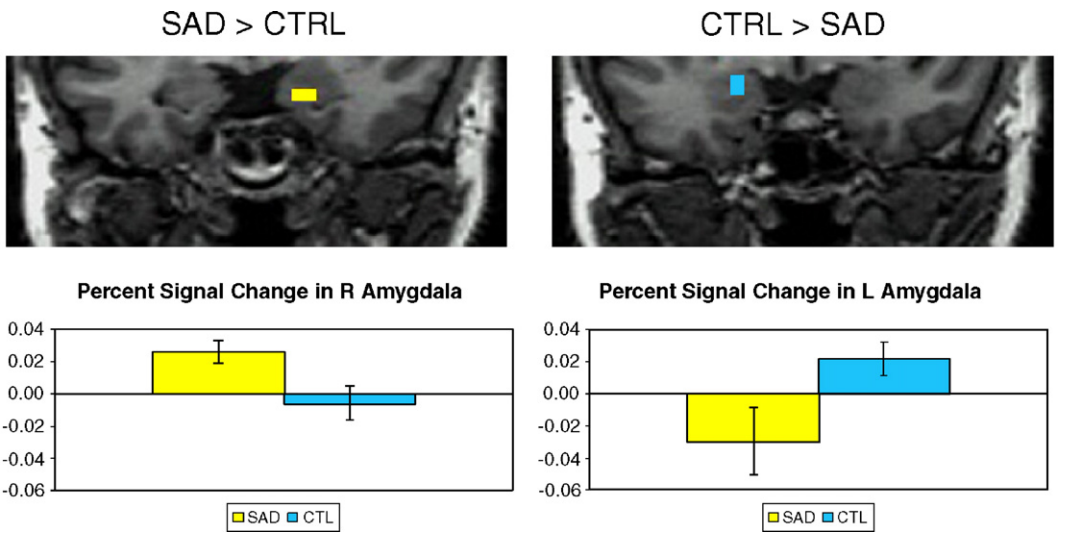

Fig. 1. (Left) Yellow activation in the right amygdala represents Social Anxiety Disorder (SAD)>Control (CTL). Bar graph depicts significant group differences in mean percent signal change for the neutral faces vs. ovals contrast in the right amygdala for SAD and CTL groups ($P=0.005$). (Right) Results of between-groups t-test for neutral faces vs. ovals. Blue activation in the left amygdala represents CTL>SAD. Bar graph depicts significant group differences in mean percent signal change for the neutral faces vs. ovals contrast in the left amygdala for CTL and SAD groups ($P=0.003$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

SAD participants (CTL: 90.95%; SAD: 84.37%), who were nonsignificantly more likely than controls to misidentify neutral faces as negative (CTL: 5.7%; SAD: 9.6%). Analyses of activations in the bilateral amygdala ROIs for the neutral face vs. oval contrasts revealed that whereas SAD participants demonstrated significantly greater activation in the right amygdala [Tal., 19, −7, −19] than controls ($z=2.80$, $P=0.005$; Fig. 1 — left), CTL participants exhibited significantly greater activation in the left amygdala [Tal., −19, −3, −17] than SAD participants ($z=-2.98$, $P=0.003$; Fig. 1 — right). Importantly, the SAD and CTL groups did not differ significantly in the time course to ovals, suggesting that the group differences in amygdala activations in the neutral face vs. ovals contrasts were not driven by increased activation to ovals in the SAD group.

To assess whether this pattern of differential neural responding to neutral faces extends to positive faces, we also examined the happy faces vs. oval contrast in the SAD and CTL groups. There was no group difference in either the left amygdala ($z=1.22$, $P=0.222$) or the right amygdala ($z=0.564$, $P=0.573$) for this contrast. Finally, there was also no group difference for the contrast of happy vs. neutral faces in either the left amygdala ($z=0.148$, $P=0.882$) or the right amygdala ($z=0.125$, $P=0.901$), indicating that the group difference in amygdala activation is a function of how SAD and CTL participants differentially activated to the neutral faces.

As expected, the SAD and CTL groups differed significantly in levels of both state and trait anxiety (STAI-S: SAD $m=28.0$, CTL $m=45.0$, $t(18)=3.84$; STAI-T: SAD $m=27.2$, CTL $m=54.2$, $t(18)=6.50$, both P's<0.001). Within the SAD group, mean percent signal change in the right amygdala for the neutral face vs. oval contrast was correlated significantly with scores on both the STAI-S ($r=0.69$, $P<0.03$) and the STAI-T ($r=0.74$, $P<0.02$); neither STAI-S nor STAI-T scores were correlated with mean percent signal change in the left amygdala (STAI-S: $r=-0.46$, $P=0.19$; STAI-T: $r=-0.18$, $P=0.62$). In contrast, within the CTL group, neither right nor left amygdala mean percent signal change was correlated significantly with either STAI-S or STAI-T scores (all P's>0.05). Finally, to examine whether medication status was associated with activations within the SAD group, we compared activation to neutral faces vs. ovals in the medicated ($n=5$) and the unmedicated ($n=5$) SAD participants. There were no significant differences between medicated and unmedicated SAD participants in activation in either the left or right amygdala (left amygdala: $z=0.067$, $P=0.50$; right amygdala: $z=1.44$, $P=0.15$).

4. Discussion

This is the first study to examine specifically neural correlates of the processing of neutral faces in individuals diagnosed with SAD. Previous studies investigating the neural bases of SAD have shown amygdala and associated limbic hyperactivity in response to threat-related facial expressions. These studies, however, have used "neutral"

expressions as a baseline in contrast analyses (e.g., Straube et al., 2004; Amir et al., 2005). In the present study we employed an event-related design to clarify and elucidate the pattern of amygdala activation in response to faces with neutral expressions in SAD and CTL participants. The present results suggest not only that neutral faces actually elicit affective appraisals in SAD and CTL participants, but more importantly, that they do so differentially. Whereas SAD individuals exhibited right amygdala activation in the contrast of neutral faces vs. ovals, CTL participants exhibited left amygdala activation. These findings have important implications for interpreting the results of studies that have examined neural responses to negative faces in individuals diagnosed with mood and anxiety disorders by using neural activations to neutral faces as a contrast condition (e.g., Thomas et al., 2001; Cannistraro et al., 2004). Indeed, these results could explain discrepancies in the findings obtained both within and across these studies. Specifically, it is not clear to what degree previous findings of differential amygdala activation in SAD compared with control participants are actually due to differential processing of the negative emotional expressions or to differential processing of the neutral faces that are commonly used as the baseline or contrast condition.

Interestingly, the differential activation of the right amygdala in SAD individuals and of the left amygdala in CTL participants in response to neutral faces parallels findings obtained in studies investigating the laterality of the neural response to emotionally salient stimuli. The fact that neutral faces elicited emotional processing in both groups of participants indicates that neutral faces are best conceptualized as emotionally ambiguous stimuli instead of as neutral stimuli. These findings suggest that control and SAD participants process these emotionally ambiguous faces differently. Indeed, in studies of healthy participants, left amygdala activation has been observed more consistently than right amygdala activation during tasks involving the processing of affective stimuli (Baas et al., 2004). Combined with the present results in the CTL participants, this pattern of findings offers further support for the formulation that neutral faces are perceived as having some level of affect. Previous findings that left amygdala activation is implicated in more sustained cognitive appraisals of emotional stimuli, whereas right amygdala activation is implicated in rapid orienting responses or when stimuli are ambiguous (Glascher and Adolphs, 2003; Wright and Liu, 2006) suggest that SAD individuals are quickly attending to, and evaluating, the neutral faces to a greater degree than are the CTL participants.

Importantly, the elevated right-sided amygdala activation found in the SAD sample in response to neutral facial expressions replicates findings of studies that have investigated the processing of threatening stimuli in anxiety disorders, specifically social anxiety (Fredrikson and Furmark, 2003; Straube et al., 2004; Phan et al., 2006). Similarly, in a recent study of healthy controls, Somerville et al. (2004) found that self-reported state anxiety correlated positively with right amygdala activation to neutral faces. Consistent with these results, in the present study we found a significant correlation between right amygdala activation in response to the neutral faces and levels of both state and trait anxiety within the SAD group, further highlighting a possible link between anxiety and right-lateralized amygdala functioning.

It is important to point out that the relatively small sample size in our study and the possibility of signal drop-out in regions near the sinuses (e.g., the amygdala) may limit the reliability of our findings. We should also note that the laterality effect in the processing of emotional stimuli in anxiety disorders is not a consistent finding; indeed, there are reports of increased left amygdala activation in anxiety disorders (e.g., SAD: Stein et al., 2002; PTSD: Bryant et al., 2005). Clearly, therefore, further research is required to corroborate the presence of a right-lateralized association in individuals diagnosed with anxiety disorders during emotional face processing. Equally important, the present findings strongly indicate that future studies must include baseline conditions other than neutral faces to elucidate more systematically abnormal patterns of neural response to emotional stimuli.

In closing, the present findings of right amygdala activation to neutral faces in participants diagnosed with SAD, but not in controls, suggest a hyperactivity in the threat-detection and emotional evaluation system of SAD individuals when they are confronted with ambiguous interpersonal stimuli. These results clearly underscore the importance of selecting adequate baseline or control conditions in studies investigating neural correlates of the processing of emotional stimuli in individuals experiencing emotional disorders.

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