Anticipation of Aversive Visual Stimuli Is Associated With Increased Insula Activation in Anxiety-Prone Subjects

Alan Simmons, Irina Strigo, Scott C. Matthews, Martin P. Paulus, and Murray B. Stein

Background: Anticipation is a critical component of affective processing in general and for anxiety in particular. Prior research suggests that the right insula plays an important role in anticipation of affective processing during aversive images. This study aimed to test the hypothesis that individuals with increased anxiety-related temperamental traits (anxiety-prone [AP]) relative to anxiety-normative (AN) subjects would show an exaggerated insula response during anticipation of an aversive image.

Methods: 16 AP and 16 AN individuals performed a task in the functional magnetic resonance imaging scanner, during which they viewed pictures of spiders and snakes. Subjects were prompted 4–6 sec before the onset of each aversive image. Blood oxygenation level-dependent signal was contrasted during cued anticipation of images versus non-anticipatory task performance as well as viewing images.

Results: As hypothesized, AP subjects showed greater response than AN subjects in the bilateral insula during anticipation. In addition, these individuals had lower activity within the superior/midfrontal gyrus. During the image presentation phase, AN subjects showed greater activation than AP subjects in the bilateral temporal lobes and left superior frontal gyrus. Moreover, bilateral temporal lobe activation during image presentation was inversely correlated with bilateral insula activation during anticipation both within groups and in the combined group.

Conclusions: These data suggest that greater activation of the insula during visual anticipation is associated with visual processing of aversive stimuli in AP individuals. Insula hyperactivity might be a common feature in persons with elevated trait anxiety and, as such, might be a neuroimaging marker for anxiety proneness.

Key Words: Anticipation, anxiety, avoidance, fMRI, imaging

Anticipation of future harm is a key aspect of anxiety (Bradley et al 1997; Eysenck 1997). Specifically, phobic anxiety occurs during both the expectation and presence of the phobic object or situation (Tillfors et al 2002). Greater anticipatory anxiety might be associated with avoiding phobic stimuli, which limits extinction and might serve to further maintain phobic responses. Therefore, exaggerated anticipatory processing might be a vulnerability factor for the acquisition of phobias (Ost 1987) and, perhaps, other anxiety disorders.

The insula, medial prefrontal cortex (MPFC), and anterior cingulate cortex (ACC) seem to be critically involved in both anxiety and anticipatory processing. Functional imaging studies suggest the MPFC/ACC (Chua et al 1999; Sawamoto et al 2000; Simpson et al 2001a) and insula (Chua et al 1999; Plooghaus et al 1999) activate during anticipation of an electric shock or of noxious thermal stimulus. Similarly, activation in the MPFC/ACC and insula has been observed during anticipation of feedback in a decision-making task (Critchley et al 2001). In a prior functional imaging study (Simmons et al 2004), we examined in healthy volunteers anticipation of images of spiders and snakes, which are among the most commonly reported phobic stimuli (Kendler et al 2001). In that study, 20 of 23 subjects rated some images as at least moderately distressing and showed an increase in activation within the right insula during the anticipation phase of these images.

The insula, a part of the extended limbic system, has afferent and efferent connections to medial and orbitofrontal cortex, anterior cingulate, and several nuclei of the amygdala (Augustine 1996). Although insula activation has been frequently associated with disgust (Phillips et al 1998), there is increasing evidence of a broader role for this brain structure in emotion processing (Phan et al 2002). Insula activation is thought to be involved in many emotional processes, including differential positive versus negative (in particular, fear) emotion processing (Buchel et al 1998; Morris et al 1998), pain perception (Gelnar et al 1999; Peyron et al 2000), anticipation and viewing of aversive images (Phan et al 2006; Simmons et al 2004), and the making of judgments about emotions (Gorno-Tempini et al 2001). Activation in the insula correlates with anxiety indices during a risk-taking task (Paulus et al 2003), is greater in subjects with specific phobia when viewing fearful faces (Wright et al 2003), and seems responsive to treatment with anti-phobia drugs (Paulus et al 2005). These properties make this region a good candidate for showing the combined effects of anxiety and anticipation.

Altered anticipation of aversive events might be based on altered cognitive and/or perceptual processes. Riskind (1997) has suggested that some people have a pervasive “looming maladaptive style” that makes them susceptible to developing anxiety disorders and, in particular, subjects with this style and fear of spiders have perceptual distortions that the spider is approaching them (Riskind et al 1995). This suggests the possibility that anxiety-(or phobia-) prone individuals might differ in the way they process visual information about potential phobic stimuli.

The aim of this study was to examine how the neural circuitry involved in anticipating potentially aversive affective stimuli differs in normal and anxiety-prone (AP; i.e., defined on the basis of high trait anxiety) subjects. We hypothesized that AP individuals would show heightened insular activation compared with anxiety-normative (AN) individuals. Support for this hypothesis...
would link a specific neural substrate, the insular cortex, with a psychological process, anticipation, toward the development of a vulnerability marker for individuals who are at elevated risk for anxiety disorders.

Methods and Materials

Subjects
This study was approved by the University of California San Diego and San Diego State University (SDSU) institutional review boards, and all subjects provided written informed consent to participate. Initially, approximately 3000 undergraduate SDSU students participated in screening with the Spielberger Trait Anxiety Questionnaire (Spielberger et al 1983). Subsequently, subjects who scored high in trait anxiety (in the upper 15th percentile of the distribution) and subjects who had normative levels of trait anxiety (from the 40th to 60th percentile of the distribution) were selected for further screening. Of these, approximately one in three expressed a willingness to participate in distribution) were selected for further screening. Of these, approximately one in two of these proved eligible.

Sixteen healthy AP subjects (13 women and 3 men), age 18.8 ± 1.3 years (range 13–15), and 16 healthy AN subjects (12 women and 4 men), age 18.7 ± .7 years (range 18–22) with an average education level of 13.4 ± .7 years (range 13–15), and 16 healthy AN subjects (12 women and 4 men), age 18.7 ± .7 years (range 18–20) with an average education level of 13.5 ± .8 years (range 13–15), were included in the study.

All subjects underwent a structured diagnostic interview (SCID; First et al 1997) that was modified to enable us to document the presence of subthreshold (i.e., not fulfilling full DSM-IV criteria because of insufficient number of symptoms and/or below diagnostic threshold for distress and/or interference) anxiety and mood disorders. The AP subjects could have a DSM-IV diagnosis (full or subthreshold) but were not currently seeking or had ever in the past sought treatment for their anxiety symptoms. In the AP group, seven subjects had no DSM-IV diagnosis (six of these subjects had subthreshold generalized anxiety disorder [GAD] and/or social anxiety disorder [SAD]), five subjects had GAD only, three subjects had GAD with SAD, and one subject had GAD, SAD, panic disorder, and obsessive-compulsive disorder. The AN subjects were those who were determined to have no DSM-IV disorders, even at the subthreshold level. None of the subjects had taken any psychotropic medications in the prior 12 months. Subjects habitually consumed < 400 mg of caffeine daily. All subjects gave their informed written consent and performed an anticipatory anxiety task during fMRI.

Procedure
Before scanning, subjects gave a subjective rating of fear of snakes and spiders with the following two self-ratings (with 7 point Likert scales): “If I saw a snake now, I would feel very panicky” and “If I came across a spider now, I would leave the room.” These two items were taken from the Fear of Snakes Questionnaire (Szymanski and O’Donohue 1995) and the Fear of Spiders Questionnaire (a modified version of the Fear of Spiders Questionnaire). In a non-published dataset of 83 college-age students we found that the scores on these two questions correlated very highly with the total score on the Fear of Snakes Questionnaire (r = .932, p < .001) and Fear of Spiders Questionnaire (r = .913, p < .001), respectively.

Stimulus and Apparatus

The task (described in detail in Simmons et al 2004) combined a continuous performance task during fMRI similar to a task described in a study by Huettel et al (2002) with the intermittent presentation of aversive affective stimuli (Figure 1). During the continuous performance task, subjects were asked to press a LEFT mouse button whenever they saw a circle and a RIGHT mouse button whenever they saw a square on the screen. Stimuli were presented at an visual angle of 4° a rate of .5 Hz. Simultaneously, a 250-msec 500-Hz tone was presented at a rate of 2 Hz. Subjects were instructed that during the task the pitch of the tone would change (from 500 to 1000 Hz) 4–6 sec before the appearance of a picture of a spider or a snake on the screen. Thirty images of spiders or snakes that were largely taken from the International Affective Picture System (Lang et al 1998) were presented during the duration of the task. The total duration of the task was 512 sec. Behavioral data were collected and scored for accuracy and latency of response during the continuous performance task. No response was required when an image of a snake, spider, or fixation cross was presented on the screen.

Response accuracy and response latency were obtained for the continuous performance task during the low tone (baseline), high tone (anticipation), and post-stimulus (as depicted in Figure 1). To examine the behavioral effect of anticipation, we examined the difference between behavioral measures during the low and high tone.

Image Acquisition

During the task, an fMRI run sensitive to blood oxygenation level-dependent contrast was collected for each subject with a 1.5 Tesla Siemens scanner (T2* weighted echo planar imaging, repetition time [TR] = 2000 msec, echo time [TE] = 40 msec, 64 × 64 matrix, 20 4-mm axial slices, 256 scans). The fMRI acquisitions were time-locked to the onset of each trial. During the same
experimental session, a high resolution T1-weighted image (MPRAGE, TR = 11.4 msec, TE = 4.4 msec, flip angle = 10°, field of view = 256 × 256, 1 mm³ voxels) was obtained for anatomical reference.

Three regressors were constructed to quantify the neural substrates contributing to the different components of the task: 1) the low tone regressor, measuring the baseline performance during the processing of the continuous performance task; 2) the high tone regressor, capturing the anticipatory phase; and 3) the stimulus regressor, which assesses the processing of aversive visual stimuli (as depicted in Figure 1). In particular, the difference in activation between the high tone and the low tone regressors was interpreted to represent the activation due to anticipation.

Data were preprocessed and analyzed with the Analysis of Functional NeuroImages software package (Cox 1996). Preprocessed time series data for each individual were analyzed with a multiple regression model. Regressors of interest included three task-related regressors described earlier. In addition, five nuisance regressors were entered into the linear regression model: three movement-related regressors used to account for residual motion (in the roll, pitch, and yaw direction), and regressors for baseline and linear trends used to eliminate slow signal drifts. A Gaussian filter with full width at half maximum 6 mm was used to smooth the time series data. The time series data for each individual were analyzed with a multiple regression model. Regressors of interest included three task-related regressors described earlier. In addition, five nuisance regressors were entered into the linear regression model: three movement-related regressors used to account for residual motion (in the roll, pitch, and yaw direction), and regressors for baseline and linear trends used to eliminate slow signal drifts. A Gaussian filter with full width at half maximum 6 mm was applied to the voxel-wise percent signal change data to account for individual variations in the anatomical landmarks. Data of each subject were normalized to Talairach coordinates.

Voxel-wise percent signal change data for whole brain were entered into an independent samples t test for activation (separately during anticipation and image presentation) between AP and AN. A threshold adjustment method on the basis of Monte-Carlo simulations was used to guard against identifying false positive areas of activation (Forman et al 1995). A priori voxel-wise probability of p < .05 in a cluster of 1024 µL resulted in an a-posteriori probability of p < .05. Finally, the average percent signal difference was extracted from regions of activation that were found to survive this threshold-cluster method. All analyses for the behavioral data were carried out with SPSS 10.0 (Norusis 1990).

To examine the relationship between anticipation and aver- sive image processing, we correlated the activation magnitudes of the clustered areas during anticipation with those obtained during aversive stimulus presentation. Moreover, to examine the relation- ship between behavioral performance and brain activation, we correlated response accuracy and latency with activation magnitude during both anticipation and aversive stimulus presentation.

Activation levels acquired from the functionally defined areas that result from the whole brain analysis during the anticipation and image presentation were correlated with response accuracy and latency data.

### Results

#### Behavioral Measures

The AP individuals did not differ from AN subjects on the response latency or the ratings on the fear of spiders and fear of snakes questions (see Table 1 and Figure 2).

#### fMRI Data: Task-Related Activation Differences

The AP subjects showed greater activation than did AN subjects during the anticipation phase (i.e., during the high tone trials relative to the low tone trials) in the right (x = 42, y = −6, z = 3) and left insula (x = −44, y = 0, z = −2). In comparison, AN subjects activated more in the right superior/medial frontal gyrus (BA 9; x = 6, y = 53, z = 30) than did the AP individuals (see Table 2 and Figure 3). There were no differences between AP and AN subjects during the actual presentation of the aversive images. However, the right middle temporal gyrus (BA 39; x = 49, y = −65, z = 23), right superior temporal gyrus (BA 22; x = 46, y = −29, z = 12), left middle temporal gyrus (BA 39; x = −39, y = −67, z = 23), and left superior frontal gyrus (BA 10; x = −29, y = 56, z = −3) were more active in the AN than the AP group during the image presentation phase (Figure 4A and Table 3). Figures 4B and 4C show the average hemodynamic responses from these regions and corresponding average hemodynamic responses in the primary visual cortex extracted to examine whether the groups also differed in visual processing. Unlike the differences observed in the temporal lobes during image presentation, however, the percent signal change within the primary visual cortex was not different between the groups (Figure 4C), suggesting that basic visual processing was similar between the two groups.

#### fMRI Data: Activation by Behavior Correlations

To determine whether neural activation differences between the groups were associated with behavioral performance, we extracted percent signal change in anticipation and image pro-

### Table 1. Fear of Spider and Fear of Snake Questionnaire Responses and Behavioral Response Latencies in AN and AP Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Spider Mean</th>
<th>Spider SD</th>
<th>Snake Mean</th>
<th>Snake SD</th>
<th>CPT Mean</th>
<th>CPT SD</th>
<th>Ant Mean</th>
<th>Ant SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN</td>
<td>2.5</td>
<td>2.27</td>
<td>2.9</td>
<td>2.17</td>
<td>763</td>
<td>73</td>
<td>732</td>
<td>61</td>
</tr>
<tr>
<td>AP</td>
<td>2.5</td>
<td>2.12</td>
<td>3.6</td>
<td>2.22</td>
<td>767</td>
<td>75</td>
<td>750</td>
<td>60</td>
</tr>
</tbody>
</table>

Fear questionnaire data on a likert scale from 1 (not at all), to 7 (very much). Response latencies are in milliseconds.

AN, anxiety-normative; AP, anxiety-prone; CPT, continuous performance task; Ant, CPT during High Tone (signaling impending onset of aversive image).

### Table 2. Differences in AN and AP Groups During Anticipation

<table>
<thead>
<tr>
<th>Group</th>
<th>Volume</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Side</th>
<th>Location</th>
<th>BA</th>
<th>t Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP &gt; AN</td>
<td>2176</td>
<td>42</td>
<td>−6</td>
<td>3</td>
<td>R</td>
<td>Insula</td>
<td>13</td>
<td>3.27</td>
</tr>
<tr>
<td>AN &gt; AP</td>
<td>1664</td>
<td>−44</td>
<td>0</td>
<td>−2</td>
<td>L</td>
<td>Insula</td>
<td>2.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1088</td>
<td>6</td>
<td>53</td>
<td>30</td>
<td>R</td>
<td>Superior frontal gyrus</td>
<td>9</td>
<td>−3.15</td>
</tr>
</tbody>
</table>

AN, anxiety-normative; AP, anxiety-prone.
cessing phases. There were no correlations between activation differences and response latency or the ratings on the fear of spiders and/or fear of snakes questions (data not shown).

**fMRI Data: Activation Correlations During Anticipation and Visual Processing**

One approach to evaluating the role of different neural systems during the processing of anticipation and during visual processing of aversive stimuli is to examine whether the amount of activation during anticipation is related to the amount of activation during visual processing. For example, increased activation during anticipation might correspond to preparatory affective or cognitive processes that are aimed at minimizing the impact of the aversive visual stimulus. Thus, we performed correlation analyses between the average percent signal changes in areas that differed across groups, as shown in Table 4. We found that the positive activation in the bilateral insular cortex during the anticipation phase correlated significantly with negative activations (i.e., deactivations) in the temporal and frontal lobes during the image presentation phase (see Table 4 and Figure 5). These correlations were significant in the whole group as well as in the AP and AN groups individually. There was no correlation between response latency during anticipation and brain activation during anticipation.

**Discussion**

This investigation yielded three main results. First, during the anticipation phase before the presentation of aversive images, AP relative to AN subjects showed greater activation in bilateral insula and less activation in superior/medial frontal gyrus. Second, during the actual presentation of the images AN subjects showed greater activation in the bilateral temporal lobes and left superior frontal gyrus than AP subjects, but both groups showed similar activation in the visual cortex. Third, the bilateral temporal and left superior frontal gyrus activation during the image presentation phase was inversely correlated with the bilateral insula activation during the anticipation phase both within groups and in the combined group. The inverse correlation between activation in the insula during anticipation and activation in secondary visual areas during image presentation is consistent with the idea of a neural circuit that is important for avoidance of unpleasant visual stimuli. Specifically, increased insula activation during the anticipation phase might attenuate processing of subsequently presented aversive images.

Lesion studies in both humans and animals support the notion that the insular cortex is important for affective processing (Adolphs et al 2000). Therefore, it is not surprising that numerous imaging studies show activity in this area during presentation of stimuli associated with high emotional distress (e.g., pain) in normal subjects (Eisenberger et al 2003; Price 2002) and increased activation across multiple anxiety populations (Rauch et al 1997). Recent studies also suggest that the insula plays an important role in anticipation of aversive stimuli; both when subjects anticipate aversive painful stimuli (Ploghaus et al 1999; Porro et al 2002) and when they expect the delivery of potentially aversive visual stimuli (Simmons et al 2004).

Even though insular activation during anticipation of aversive stimuli would be expected in both groups studied here, higher insular activation in AP subjects is consistent with other studies in groups of anxious subjects. For example, phobic individuals were found to show increased insular cortex activation relative to non-phobic comparison subjects in paradigms that use pictures (Dilger et al 2003) or words (Straube et al 2004b) of spider-related stimuli. Moreover, social phobic individuals showed

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**Figure 3.** Cluster (A) and average percent signal change (B) in bilateral insula and right superior frontal gyrus (R SFG) during anticipation that differs significantly between the anxiety-prone (AP) and anxiety-normative (AN) groups. The average extracted hemodynamic responses within the insula clusters are shown (C) where the yellow block indicates anticipation phase. ROI, region of interest; TR, repetition time.
increased activation to fearful faces in the right insula (Wright et al 2003) and to angry faces in the bilateral insula (Straube et al 2004a). These and other results of healthy volunteers processing aversive sensory stimuli (Simmons et al 2004) are consistent with the notion that insular activity might not only underlie the affective process of emotional distress in normal and phobic individuals but might also be involved in the action planning and selection related to these stimuli (i.e., might mediate phobic behavior).

Furthermore, Critchley et al (2003, 2004) have found strong and consistent correlations between insula activation and autonomic arousal (e.g., heart rate and heart rate variability), anxiety, and visceral changes associated with facial emotion processing (Critchley et al 2005). Aversive physiological reactions are key in avoidant behavior in the development of phobias (e.g., agoraphobia) (Langs et al 2000; Starcevic et al 1993). Therefore, it is reasonable to conclude that the insular cortex provides the neural substrate that links emotional distress, anticipatory processing, and autonomic arousal with action-planning aimed at reducing exposure to the aversive stimuli. These processes take place in full and sometimes painful awareness, which is consistent with the role of the insula in mediating self-awareness (Karnath et al 2005). The area within the insular cortex that

**Table 3. Differences in AN and AP Groups During Image Presentation**

<table>
<thead>
<tr>
<th>Group</th>
<th>Volume</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Side</th>
<th>Location</th>
<th>BA</th>
<th>t Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP &gt; AN</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AN &gt; AP</td>
<td>2432</td>
<td>49</td>
<td>-65</td>
<td>23</td>
<td>R</td>
<td>Middle temporal gyrus</td>
<td>39</td>
<td>-3.26</td>
</tr>
<tr>
<td></td>
<td>2176</td>
<td>46</td>
<td>-29</td>
<td>12</td>
<td>R</td>
<td>Superior temporal gyrus</td>
<td>22</td>
<td>-3.01</td>
</tr>
<tr>
<td></td>
<td>1920</td>
<td>-39</td>
<td>-67</td>
<td>23</td>
<td>L</td>
<td>Middle temporal gyrus</td>
<td>39</td>
<td>-3.40</td>
</tr>
<tr>
<td></td>
<td>1024</td>
<td>-29</td>
<td>56</td>
<td>-3</td>
<td>L</td>
<td>Superior frontal gyrus</td>
<td>10</td>
<td>-3.47</td>
</tr>
</tbody>
</table>

AN, anxiety-normative; AP, anxiety-prone.

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**Figure 4.** Differential activation between the AP and AN groups during image presentation. (A) The AN had greater activation in the left middle temporal gyrus (left solid oval), right superior temporal gyrus (right solid oval), left superior frontal gyrus (left dotted oval), and right middle temporal gyrus (right dotted oval). The average extracted hemodynamic response within the cluster is displayed for the left middle temporal gyrus and the right superior temporal gyrus (B) and the left and right occipital lobes, including the fusiform gyrus (C) where the yellow block indicates image presentation phase. ROI, region of interest; TR, repetition time.
During aversive image presentation we found decreased activity in AP compared with AN subjects in the areas of temporal cortex thought to be involved in secondary visual processing. Studies on visual attention (Beauchamp et al 1997) and visual working memory, especially imagery (Baddeley 2003), indicate that this area is important in attending to a visual stimulus. The medial temporal lobe has been shown to have a direct connection to visual nodes of the thalamus in primates (Sincich et al 2004) and to down-modulate other visual areas (Friston and Buchel 2000). Furthermore, lesions near the superior temporal gyrus (BA 22) result in “spatial neglect”—a failure to explore the contralateral side of space (Karnath et al 2001). In a recent study, Paquette et al (2003) examined the effect of cognitive behavioral therapy on neural responses to images of spiders in subjects with spider phobias and found increased activation in occipital areas directly inferior to temporal gyrus areas after treatment, implicating these regions in avoidance behavior. Although this interpretation is somewhat speculative, we would expect this area to play a significant role in avoidance-related processing. Nevertheless, future studies will need to replicate this finding with other emotional picture tasks.

Riskind et al (1995) suggest that the fear of spiders actually involves a perceptual misinterpretation that the spider is aggressively moving toward them and that this fear of imminent danger is part of a generalizable anxiety syndrome that they discuss as a “looming maladaptive style.” This looming style is highly correlated with other continuous measures of anxiety as well as anxiety disorders (Williams et al 2005). This gives a potential theoretical framework for the reduced temporal response we observed in AP in our study, which could reflect activity within a system geared toward avoidance of the imagined aggressive approach of the image on the screen, similar to the process seen in phobic individuals by Paquette et al (2003). Furthermore, the inverse relationship between activity in insular and secondary visual areas during image presentation found here suggests that the insula might have a “top-down” modulatory effect, as has been suggested for spatial neglect (Karnath et al 2004).

**Table 4. Correlation Between Anticipatory Clusters and Image Presentation Clusters**

<table>
<thead>
<tr>
<th>Anticipation Cluster</th>
<th>RMTG</th>
<th>RSTG</th>
<th>LMTG</th>
<th>LSFG</th>
</tr>
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<tbody>
<tr>
<td>Right Insula</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>$-0.638^{a}$</td>
<td>$-0.843^{a}$</td>
<td>$-0.758^{a}$</td>
<td>$-0.612^{a}$</td>
</tr>
<tr>
<td>AP</td>
<td>$-0.502^{a}$</td>
<td>$-0.832^{a}$</td>
<td>$-0.758^{a}$</td>
<td>$-0.558^{a}$</td>
</tr>
<tr>
<td>AN</td>
<td>$-0.525^{a}$</td>
<td>$-0.680^{a}$</td>
<td>$-0.535^{a}$</td>
<td>$-0.265^{a}$</td>
</tr>
<tr>
<td>Left Insula</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>$-0.638^{a}$</td>
<td>$-0.832^{a}$</td>
<td>$-0.791^{a}$</td>
<td>$-0.626^{a}$</td>
</tr>
<tr>
<td>AP</td>
<td>$-0.522^{a}$</td>
<td>$-0.800^{a}$</td>
<td>$-0.803^{a}$</td>
<td>$-0.526^{a}$</td>
</tr>
<tr>
<td>AN</td>
<td>$-0.528^{a}$</td>
<td>$-0.742^{a}$</td>
<td>$-0.610^{a}$</td>
<td>$-0.452^{a}$</td>
</tr>
<tr>
<td>RSFG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>$0.043$</td>
<td>$0.040$</td>
<td>$0.13$</td>
<td>$0.042$</td>
</tr>
<tr>
<td>AP</td>
<td>$-0.235$</td>
<td>$-0.302$</td>
<td>$-0.250$</td>
<td>$-0.461$</td>
</tr>
<tr>
<td>AN</td>
<td>$-0.375$</td>
<td>$-0.539^{b}$</td>
<td>$-0.438$</td>
<td>$-1.118$</td>
</tr>
</tbody>
</table>

RMTG, right middle temporal gyrus; RSTG, right superior temporal gyrus; LMTG, left middle temporal gyrus; LSFG, left superior frontal gyrus; RSFG, right superior frontal gyrus.

$^{a}p < .001$.  
$^{b}p < .05$.  

differed across groups was more anterior than those areas that showed common task-related activation. Because AN individuals did not show significant activation in this area, we hypothesize that AP subjects recruit a larger and more anterior part of the insular cortex during anticipation. Together, insular hyperactivity, particularly in the anterior part of this cortical structure, in AP individuals might reflect an increased self-awareness of impending aversive stimuli, which is subsequently associated with attenuated processing of this stimulus by secondary visual areas.

The AN individuals showed greater activation than AP individuals during the anticipatory period in the superior/medial frontal gyrus. Several studies have implicated this area in affective processing (Hutcherson et al 2005; Simpson et al 2001b; Stein et al 2002). Furthermore, activation in this region was observed when subjects were rating their own emotions compared with rating the emotions of others (Ochsner et al 2004) and during attending to emotions over not attending (Gusnard et al 2001; Northoff et al 2004; Taylor et al 2003), suggesting that this region might be important in self-relevant processes (Wicker et al 2003) such as understanding one’s own affective state (Castelli et al 2000). Activation of this area during anticipation of aversive stimulation in AN but not AP individuals observed in this study might indicate that AN subjects have a greater degree of self-monitoring capacity in this regard, which might be important in order to properly regulate emotional responsiveness in this context.

**Figure 5. Correlation between increased anticipatory response and decrease image presentation response.** The bilateral insula response to anticipation was significantly negatively correlated with all areas that showed differential activation between anxiety-prone and anxiety-normative group during image presentation; two representative correlations are displayed.
been suggested in prior research (Damasio et al 2000; Rauch et al 1997).

This study has several limitations. Primarily, we studied the behavior of high trait anxious individuals and not clinically anxiety-disordered or phobic subjects. This means that our current findings might not extend to individuals with phobic or anxiety disorders. For example, one might speculate that once a clinically significant anxiety disorder has developed, different neural pathways might operate to control the behavioral response. However, the study of subjects with high trait anxiety, some of which might exhibit subsyndromal anxiety disorders, can give insight into the brain behaviors of those who, because of their temperament, are prone to the development of anxiety disorders (Riskind 1997; Riskind et al 1995; Williams et al 2005). Another limitation is that images of spiders and snakes are not highly aversive to all individuals. This might decrease both the emotional and the statistical power of the current study. If more aversive images were selected, a greater insula response might be found. We did not examine anticipation to positively valenced images; thus it is not possible to determine whether the groups differ in the anticipation of a positive event or of a negative event, or both.

The focus of this investigation is the neural substrates underlying aversive anticipatory processing. Future investigations might need to include positive and neutral valence images to examine whether AP individuals show altered modulation of anticipatory processing as a function of valence. It is important to note that we did not find differences in the fear ratings between groups, which supports the idea that the brain pattern differences do not merely reflect a phobic response by the AP subjects. It is important to note that the concept of “looming anxiety” does not necessitate that individuals have greater fear of the object, instead it poses that individuals have a heightened anxious reaction when anticipating an approaching aversive stimulus. The significant neuroimaging differences between groups were observed in the absence of differences in the behavioral task data. One reason for this discrepancy, particularly as far as the response latency data are concerned, could be the repetitive pacing of the task. Thus, future studies might need to include a randomized temporal jitter during the continuous performance task response to better detect response time differences during anticipation. Because the conditions of interest (anticipation and stimulus) related in this paper were theorized to be psychologically related, main effect conditions from two different epochs were compared. Voxel-based temporally linked correlations that are commonly used in neuroimaging (Penny et al 2004) are more appropriate when the conditions are linked neurophysiologically. Because a less common methodology was used, replication of these findings is required.

Future studies should look at the activation patterns in patient populations to see whether greater insula activation is associated with reduced secondary visual activation in clinical samples (e.g., specific phobia, social phobia, posttraumatic stress disorder). It would also be of critical interest to see if the insula of anxiety-disordered subjects is differentially affected by psychopharmacological interventions that are commonly used to treat anxiety disorders. The use of multiple anticipation conditions might help determine to what degree the insula effect is due to the unpleasantness of the images versus the impact of anticipation itself. One approach to examine altered processing of aversive stimuli in secondary visual areas in AP individuals would be to present individuals with an approaching threat object via video when there is no prior information available, which would make cognitive avoidance difficult.

To summarize, the current findings support the notion that greater activation of the insula during anticipation of a visual stimulus might lead to reduced secondary visual processing of the stimulus and that this process is altered in anxiety prone subjects. The enhanced anticipatory response in AP subjects within the insula might lead to reduced engagement of potentially aversive visual stimuli, evident from reduced activity in areas of visual attention. These findings are the first step to uncover a cognitive/affective process and neural circuitry for formation/maintenance of phobic reactions in individuals who are prone to the development of anxiety disorders.

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