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Striatal-Limbic Activation is Associated with Intensity of Anticipatory Anxiety

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Abstract

Anxiety experienced in anticipation of impending aversive events induces striatal-limbic activation. However, previous functional magnetic imaging (fMRI) studies of anticipatory anxiety have utilized post-test measures of anxiety, making a direct association between neural activation and distress problematic. This paradigm was designed to assess the BOLD response to an aversive conditioned stimulus while simultaneously measuring subjective anxiety. Fifteen male healthy subjects (45.5±8.5 years old) were studied. A high threat conditioned stimulus (CS) was paired with either an unpredictable, highly aversive (painful) or a non-aversive (non-painful) unconditioned stimulus and compared to a low threat CS paired with a predictable, non-aversive stimulus. Neural response was assessed with fMRI, and subjective anxiety (1 to 4) was recorded upon the presentation of each CS. High subjective ratings of real-time anticipatory anxiety (2, 3, and 4), relative to low anticipatory anxiety (1), elicited increased activation in the bilateral striatum, bilateral orbital frontal cortex, left anterior insula, and anterior cingulate cortex (ACC) and decreased activation in the posterior cingulate cortex (PCC). The amplitude of BOLD signal change generally paralleled the subjective rating of anxiety. Real-time measures of anticipatory anxiety confirm previous reports, using post-test measures of anxiety, of striatal-limbic activation

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during anticipatory anxiety while simultaneously demonstrating an increase in BOLD response in parallel with heightened anxiety.

Keywords

striatal-limbic system; striatum; anticipatory anxiety; functional magnetic resonance imaging

1. Introduction

Anxiety is “a future-oriented mood state in which one is ready or prepared to attempt to cope with upcoming negative events” (Barlow, 2000). The anxiety experienced during the anticipation of impending aversive stimuli serves an important adaptive function, allowing an individual to make emotional, cognitive, and physical preparations (Nitschke et al., 2002). Pathological anxiety states occur when the anticipatory anxiety becomes excessive and/or an individual incorrectly predicts future aversive events (Paulus and Stein, 2006; Phil, 2009). A sizable literature has evolved demonstrating distinct neural signatures of anxiety disorders relative to healthy participants (Blair et al., 2008; Monk et al., 2006; Nitschke et al., 2009). However, pathological anxiety is not an all-or-none phenomena but a disorder that often progresses from more mild/moderate symptoms to a disease state (Karsten et al., 2011). This is best exemplified by the gradual shift in DSM-V to approaching psychopathology from a dimensional, rather than categorical, perspective (Andrews et al., 2008). Imaging paradigms that allow the concurrent assessment of neural activity and variable levels of subjective anxiety would therefore be highly informative in exploring this dimensional relationship.

In preclinical studies the expectation of aversive events is commonly assessed with cue conditioning paradigms. In a classical learning conditioning paradigm, a neutral conditioned stimulus (CS) is paired with an unpleasant unconditioned stimulus (US) that evokes a strong negative emotional response. After training, a conditioned response can then be elicited by the original neutral CS in animals (Delgado et al., 2006). To understand the neural processes that occur during this period of expectation, investigators have explored neural responses to CS in human subjects with brain imaging techniques. Ploghaus et al (1999) reported that the expectation of a noxious thermal stimulus activated areas in the mesial prefrontal and anterior insular cortex close to, but distinct from, areas activated by the painful stimulus itself. Subsequent studies assessing the response to CS signaling the certain expectation of painful stimuli (e.g. heat, electric shock, loud noise) also showed activation of the anterior insula and/or the anterior cingulate cortex (ACC) (Carlson et al., 2011; Chua et al., 1999; Marschner et al., 2008; Ploghaus et al., 2003; Straube et al., 2009). Perhaps more ecologically relevant to the experience of anxiety is the assessment of neural responses during a period of uncertain expectation. That is, the subject is unsure whether or not a noxious stimulus will be administered. In this paradigm, the anticipation period preceding the unpredictable application of painful stimuli increased BOLD response in the medial orbitofrontal cortex (mOFC) and ACC (Porro et al., 2002; Schiller et al., 2008). In addition, the striatum, a key area in the processing of reward and aversive prediction (Bayer and Glimcher, 2005; Delgado et al., 2008), also showed activation (Jensen et al., 2003; Schiller et al., 2008).

Critical to the interpretation of studies of anxiety, however, is the demonstration that assumed anxiety levels are indeed experienced during the appropriate CS; specifically, that high levels of anxiety are experienced during the CS signaling a highly noxious stimulus and that low levels of anxiety are experienced during the CS signaling a non-noxious stimulus. In the previous studies noted, anxiety either was not assessed (Chua et al., 1999; Jensen et

al., 2003; Marschner et al., 2008; Ploghaus et al., 1999; Porro et al., 2002; Schiller et al., 2008) or was rated after the fMRI session (Straube et al., 2009) was completed. Recently, Carlson et al. (2011) assessed real-time measures of subjective anxiety in anticipation of a loud noise (100 dB), although ratings of anticipatory anxiety were obtained following administration of each US. In order to extend the previous findings of anticipatory anxiety by affirming subjective responses to each CS in real-time and avoiding the potential contaminate of US presentation (both by obtaining ratings prior to US presentation and individualized US intensity), we adapted a paradigm originally developed by Ploghaus et al. (Ploghaus et al., 1999). The adapted approach allowed the neural response to both an aversive (painful) and unpredictable high threat CS (whether a CS would be followed by a painful or non-painful US coupled with uncertainty about the US time of onset) (Carlsson et al., 2006; Grillon, 2002) and a nonaversive (non-painful) low threat CS to be measured while simultaneously assessing subjective anxiety upon presentation of each CS. This approach allowed real-time, variable levels of subjective anxiety to be assessed concurrent with the associated neural response.

Based on studies of anticipation of aversive events and cued conditioning learning, we hypothesized that the high threat CS would result in real-time reporting of high anxiety and the low threat CS would result in real-time reporting of low anxiety. Since our primary interest was in assessing the relationship between the subjective rating of anticipatory anxiety and BOLD response, we predicted that high levels of subjective anxiety (irrespective of the CS) would activate striatal-limbic areas, including the anterior insula, ACC, mOFC, and striatum, compared with low levels of subjective anxiety. Further, we hypothesized that the increases in striatal-limbic BOLD responses would increase in tandem with the increasing levels of subjective anxiety.

2. Methods and Materials

2.1. Participants

Healthy participants were recruited by advertisements in local papers. Informed consents were obtained after the study was fully explained. Subjects were financially compensated for their participation. Participants with active DSM-IV (American, Psychiatric Association, 1994) Axis I psychiatric disorders, significant medical disorders, or a history of major head trauma were excluded using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID)-Lifetime (First et al., 1996). One subject had a single major depression episode at age 48 (2 years prior to the study). Participants also underwent a medical history interview and physical examination, routine clinical laboratory testing, electrocardiography, urine drug screening and a self-report of state anxiety (Spielberger, 1971). Participants with active use of medications that interfered with neural, autonomic, or hypothalamic-pituitary-adrenal (HPA) axis functioning (e.g. psychotropics, anti-hypertensives other than thiazides, hypoglycemic agents) were excluded. Sixteen subjects were studied. Data from one subject was not included in analyses due to technical problems during scanning. The final sample of fifteen men, 12 Caucasians and 3 African-Americans, was 45.5 ± 8.5 (mean $\pm$ SD) years old. Due to the marked differences in stress responses between genders, particularly in functional imaging studies, only men were studied to limit variability (Adinoff et al., 2003; Wang et al., 2007; Yang et al., 2007).

2.2. fMRI Task

Threshold Testing—Sensory threshold calibration was performed with a computerized thermal stimulator (Pathway Pain & Sensory Evaluation System, Medoc Ltd., Haifa, Israel) that administered stimuli through a non-magnetic CHEPS (Contact Heat Evoked Potentials) circular 27-mm diameter thermode secured to participants' left ventral inner forearm,

approximately 5-cm proximal to the wrist. Sensitivity to heat was determined using a Method of Limits program (Heldestad et al., 2010; Yarnitsky, 1997) within five days prior to the fMRI session. For this sensitivity calibration, the thermode temperature was increased from 32°C by 1.5°C per sec and participants clicked a mouse button when they felt that if the thermode got any hotter, it would burn them. This process was repeated four times; the mean threshold temperature of the four measurements defined the participant's pain threshold to be used to determine individualized temperature settings for the USs in the subsequent fMRI experiment. The maximal allowable threshold temperature for the CHEPs system is 50°C; no participant exceeded this threshold.

Anticipatory Anxiety Task Paradigm—The paradigm was designed to induce anticipatory anxiety using a combination of pain (the CS warned of an impending painful US) and unpredictability (the participant did not know either if a painful US would occur following any given CS or how long following the CS onset the US would occur). Two CSs were, therefore, presented: a high threat CS (square) denoting the possibility of a painful US and a low threat CS (triangle) denoting a non-painful US. Visual stimuli were generated using Presentation software (version 10.0; Neurobehavioral Systems, Albany, CA) and projected with an LCD projector (NEC LT260) via a back projection system. For each trial, a triangle or square was presented as a CS and followed by an US: low heat (5°C below threshold) or high heat (1°C above threshold). A triangle (low threat CS) signaled the impending and certain application of a low heat US; a square (high threat CS) signaled the impending uncertain application of either a low or high heat US. The CS remained on screen throughout the trial. The trial was separated into two periods: Anticipatory Anxiety (10 to 18 seconds) and Heat Pulse (8 seconds). During the first four seconds of the Anticipatory Anxiety period, the CS was accompanied by the question “How anxious are you now” and a rating scale (1 thru 4) (see Fig. 1). Participants were to rate their anxiety regarding the impending heat stimulus during this four-second period. The CS (without the rating question) then remained on the screen for an additional 6–14 sec (pseudo-randomized 6, 8, 10, 12, and 14 sec) for the duration of the Anticipatory Anxiety period. During the subsequent (and final) 8 sec of each trial (Heat Pulse period), the heat stimulus increased from a baseline temperature of 32°C by 10°C/sec to the low or high temperature (using a Ramp and Hold program), remained at the peak for 3 sec, and then returned to baseline temperature. Forty trials, 20 squares (10 low heat and 10 high heat) and 20 triangles (all low heat), were presented over 23 minutes. Trial order was pseudo-randomized with the stipulation that neither triangles nor squares were presented more than twice consecutively. A pseudo-randomized interstimulus interval consisted of a circle shown between each trial for 9, 10, or 11 sec. A 90-sec rest period separated the paradigm into halves during which “Break” was presented on the screen. After the fMRI scan the subjects were asked to rate how anxious they felt when they saw a square or triangle during the fMRI task (“How much anxiety did you feel when you saw a square/triangle?”; 1 = not anxious to 5 = very anxious).

Mock Scan—To familiarize subjects with the paradigm and the MR scanner environment, a mock scan was performed on the same day as threshold testing. Participants were instructed that the triangle signaled the onset of a (predictable) warm stimulus and a square signaled the onset of either a warm or a hot (unpredictable) stimulus. The high heat stimulus was set 2°C lower than threshold to avoid needless exposure to pain, compared to 1°C higher than threshold during fMRI, but the design was otherwise the same as described for the fMRI paradigm. The task was presented for 10 minutes. Subjects who did not endorse higher ratings for the high threat CS (square) relative to the low threat CS (triangle) were queried regarding their understanding of the paradigm. To avoid demand characteristics, there was no attempt to encourage or advise subjects to rate the square (high threat) CS

differently than triangle (low threat) CS. All subjects described an understanding of the paradigm and no further instructions were provided.

fMRI—Subjects arrived for testing in the late afternoon. Scans were obtained on a Siemens 3T Tim Trio scanner, equipped with 12-channel receiver array head coil. fMRI scans were obtained using gradient echo planar imaging (GRE-EPI): TR = 2000 ms, TE = 20 ms, flip angle = 90 degrees, base resolution (matrix size) = 64×64, voxel size = 3.3×3.3×3.0 mm, field of view (FOV) = 210 mm, A-P phase encode, bandwidth = 2442 Hz/pixel. After 3 discarded acquisitions to establish magnetization equilibrium, 690 image volumes of 40 interleaved sagittal slices with 3 mm slice thickness and no gap were obtained to cover whole brain. Anatomical scans using a T1-weighted multi-planar reformatted MPRAGE sequence (TR = 2250 ms, TI = 900 ms, TE = 2.9 ms, flip angle = 9 degrees, base resolution (matrix size) = 256×256, FOV = 230 mm, voxel size = 0.9×0.9×1.1 mm) facilitated localization and coregistration of fMRI data.

2.3. Statistical Analyses

Measures of Anticipatory Anxiety—To verify CS-induced changes of subjects' anticipatory anxiety, we compared mean rating levels between low- and high-threat CS cues by standard paired *t*-test. A positive finding would support our use of subjective anxiety ratings as factor levels in a linear model of BOLD change.

fMRI analysis—The fMRI data were analyzed with FEAT (FMRI Expert Analysis Tool) Version 5.98, a tool of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Preprocessing included motion correction, slice-timing correction, spatial smoothing with a 5mm full-width-half-maximum Gaussian filter, intensity normalization using a grand mean scaling factor, and high-pass temporal filtering (sigma=30.0s) to remove low frequency drift. Brain extraction and registration to high resolution and template (MNI152) images were carried out by BET (Brain Extraction Tool) (Smith, 2002) and FLIRT (FMRIB's Linear Image Registration Tool) (Jenkinson et al., 2002; Jenkinson and Smith, 2001), respectively.

The pre-processed data were analyzed in two levels, as is standard practice for functional imaging analyses. Level one consisted of voxel-level estimation of %BOLD amplitude (and its variance) for each subject and each anxiety-rating category. Specifically, the epochs consisting of the Anticipatory Anxiety period for each of the four rating levels were convolved with a canonical gamma hemodynamic response function (HRF) and its temporal derivative. Each time series was regressed on the resulting convolution, and regression parameter estimates representing %BOLD amplitude were obtained.

Level two consisted of entering the estimates of %BOLD amplitude and their variances in a second linear model with the four anticipatory anxiety rating categories as factor levels to assess mean %BOLD change within subjects as a function of anticipatory anxiety. First-level parameter estimation and second-level repeated measures were performed by FMRIB's Improved Linear Model (FILM) and FMRIB's Local Analysis of Mixed Effects (FLAME), respectively. One benefit of a repeated measures model is that contrasts of anxiety ratings directly test within-subject effects of those ratings on mean %BOLD change, assuming only that the subjects are from a single population. Unequal variances were accounted for in the second-level model by a weighted analysis; i.e., %BOLD amplitudes for each subject and rating category were weighted by the reciprocal of the variances of those estimates to assess, in a statistically optimal manner, within-subject changes due to anxiety level. Cognitive and motor responses during the rating period were presumed similar between the high and low anticipatory periods.

Four contrasts of primary interest were tested from the second-level model. These corresponded to tests of mean %BOLD change at each rating level 2, 3, and 4 vs. the mean change at the lowest anxiety rating level 1. In addition, we tested the average of mean %BOLD change across the rating levels 2, 3, and 4 vs. the mean change at rating level 1, which we refer to as 2/3/4 vs. 1, to improve power in the event that rating levels 2, 3, and 4 had similar effects on mean %BOLD amplitude. Adjustments to observed significance probabilities (p-values) were obtained using the Bonferroni inequality to account for multiple contrasts. Inference was at the cluster level, using a cluster-defining threshold of $Z = 2.3$. Inference was at the cluster level, using a cluster-defining threshold of $Z = 2.3$. At this threshold, corrected cluster-level p-values were less than 0.01 given the smoothness estimates of our Z-statistic maps (Worsley, 2001).

To assess the contributions of each anxiety rating level to mean %BOLD change within a significant cluster, we summarized selected cluster-level results by averaging %BOLD amplitudes at each rating level over the voxels within the clusters using Featquery tool of FSL. Anatomical identification of our functionally-derived clusters was aided by the FSL atlas (http://www.cma.mgh.harvard.edu/fsl_atlas.html) and an in-house template (for ACC; (Bush et al., 2000)).

Given the correspondence between subjective anticipatory anxiety levels with low- and high-threat CS cues, we also ran a separate set of two-level linear models using the CS cues as factor levels in place of the anxiety rating categories as confirmation that anxiety rating categories correlate well with CS cues with respect to their effects on mean %BOLD change. The contrast of interest compared mean %BOLD change between the high-threat and low-threat CSs.

3. Results

3.1. Temperature Threshold and Anxiety Ratings

The average threshold temperature in all subjects was $45.4 \pm 1.4^\circ\text{C}$ (SD). Compared with low threat CS (triangles, 1.1 ± 0.2), high threat CS (squares, 2.7 ± 0.8) induced significant anxiety (paired t-Test, $t_{(14)} = 7.68$, $P < 0.001$) (Fig. 2a). Mean subject ratings were relatively stable over the 20 trials (Fig. 2b). All subjects rated the low threat CS (mean score) as less than two. Three participants rated the mean high threat CS as less than two, three participants rated it as between two and three, and nine participants rated it as more than three. Distributions of the 20 low and 20 high threat CS subjective responses in the 15 subjects (600 responses total) are shown in Fig. 2c and 2d. There was relatively little intra-subject variability in the real-time anxiety response to either low (range of SEM 0.0 to 0.11) or high (0.0 to 0.16) threat CS. Post-session anxiety ratings for low (1.6 ± 0.51) and high (3.0 ± 0.82) threat CSs significantly correlated with real-time measures (low-threat: $r = 0.90$, $df = 11$, $P < 0.001$; high threat: $r = 0.89$, $df = 11$, $P < 0.001$) (post-session anxiety ratings were not obtained on two participants). Real-time high minus low threat anxiety ratings did not significantly correlate with Spielberger State Anxiety Inventory scores ($r = 0.4$, $df = 13$, $p = 0.89$), suggesting that responses to the CS were not reflective of basal anxiety.

3.2. BOLD Response to High vs. Low Anticipatory Anxiety

Rating 2 vs. 1 elicited significant activation only in the bilateral occipital cortex (Figure 3a.). Rating 3 vs. 1 elicited significant BOLD activation in the bilateral caudate and thalamus and in the right dorsal anterior cingulate cortex (dACC), superior frontal cortex (SFC), putamen, pallidum, brainstem, occipital cortex, and cerebellum were also activated (Figure 3b). The 4 vs. 1 comparison showed significant BOLD activation in the left orbital frontal cortex (OFC), inferior frontal gyrus (IFC), insula, putamen and in bilateral caudate and thalamus, plus deactivation in left posterior cingulate cortex (PCC) and precuneus (Figure 3c).

Anticipatory Anxiety 2/3/4 vs. 1 showed significant BOLD activation in striatal-limbic areas, including the bilateral OFC, IFG, insula, caudate, putamen, pallidum, dACC, SFC, and deactivation in left PCC and precuneus. Increased BOLD responses were also observed in the bilateral thalamus, brainstem, occipital cortex, and cerebellum, but no significant response was found in the nucleus accumbens (Figure 3d). Comparisons of high vs. low threat CS (Fig. 3e) revealed relatively similar BOLD activation as observed in 2/3/4/ vs. 1 comparison, except PCC and precuneus deactivation was not present in the high vs. low threat comparison (Figure 3e).

To assess the hypothesized progressive relationship between anticipatory anxiety and striatal-limbic BOLD activation, we explored the BOLD response in clusters from the 2/3/4 vs. 1 contrasts for each subjective rating of anxiety. Selected clusters defined by the 2/3/4 vs. 1 contrast (e.g. left anterior insula and left putamen) showed the predicted tandem increase at each level of anxiety (Figure 4). In contrast, right and left caudate and the ACC all showed an increase in BOLD activation from Anticipatory Anxiety ratings of 1, 2 and 3 but remained constant or decreased at the highest level of anxiety (Figure 4). The PCC showed a decrease in activation from rating 1 to 2 and then remained relatively similar for ratings 2, 3 and 4, albeit with a maximal response at 4 (Figure 4).

4. Discussion

The cued conditioning paradigm employed allowed variable levels of real-time anticipatory anxiety to be correlated with associated BOLD activation. Higher ratings of Anticipatory Anxiety were associated with increased BOLD activation in striatal-limbic areas, including the bilateral caudate, bilateral OFC, IFC, and the left anterior insula, relative to the lowest anxiety rating. Generally, BOLD activation in these regions paralleled the increase in subjective anxiety. These findings confirm the results of previous studies with the addition of real-time assessment of anxiety, providing a clear association between subjective anxiety and BOLD response.

A novel aspect of this study allowed the assessment of variable levels of anxiety to be associated with the mean BOLD response within subjects. Although some studies measured real-time anxiety or fear induced by a scream (Lau et al., 2011) or the expectation of a snake (Nili et al. 2010) or tarantula (Mobbs et al., 2010; Nili et al., 2010), these studies did not assess the BOLD response with variable lower vs. higher levels of anxiety or fear. This unique aspect of our paradigm provided the opportunity to assess, within individuals, variability in BOLD response at variable levels of anxiety. Although the real-time assessment of anxiety may impact a participant's subjective response to the CS as well as the associated brain responses neural, our findings of increased striatal-limbic BOLD response during anticipatory anxiety was consistent with that reported by others using post-test measures of anxiety (Chua et al., 1999; Jensen et al., 2003; Marschner et al., 2008; Schiller et al., 2008; Straube et al., 2009). In addition, we found a low within-subject variability of real-time ratings as well as a persistence of similar within-subject rating levels over time and a high correlation of real-time and post-stress measures. This suggests that real-time ratings of anticipatory anxiety can be conducted without interfering with either the psychological state or the neural response. We also found a similar activation in the BOLD response to Anticipatory Anxiety ratings 2/3/4 vs. 1 relative to high threat vs. low threat contrasts. These findings indicate that (1) the intensity of an individual's anticipatory anxiety remains relatively stable throughout a task, (2) real-time measures of anticipatory anxiety can be reliably obtained in post-test interviews, and (3) there is little, if any, anxiety experienced during low threat CSs. Thus, querying control subjects' anxiety during the anticipatory epoch on every trial may not be necessary to evaluate real-time anxiety. Nevertheless, real-time anxiety measures may be unstable in clinical populations

experiencing extreme dysfunctional responses to stress and anxiety, such as panic, generalized anxiety, post-traumatic stress, and addictive disorders. The inclusion of real-time anticipatory anxiety measures during fMRI, therefore, may provide useful in non-healthy populations.

As noted, the BOLD activation of striatal-limbic areas during trials associated with higher ratings of anticipatory anxiety was consistent with previous studies using cued conditioning paradigms involving both similar and different unconditioned noxious stimuli (Chua et al., 1999; Jensen et al., 2003; Marschner et al., 2008; Schiller et al., 2008; Straube et al., 2009). Previous clinical imaging studies have reported that the ventral striatum, a key element of the brain reward system, was activated in anticipation of uncertain aversive physical stimuli (Jensen et al., 2003; Schiller et al., 2008). Thus, the striatum is activated not only during reward anticipation (Berns et al., 2001; Knutson et al., 2001) but also during the anticipation of punishment (Jensen et al., 2003). Activation in the bilateral dorsal striatum (dorsal caudate) during high, relative to low, anticipatory anxiety, as well as the correspondence between ventral striatal activation and anxiety level in the present study further confirms and extends this observation. The inclusion of the dorsal striatum is important, as this region has been implicated in decision-making and learning processes that support goal-directed action (Balleine et al., 2007). In contrast to the ventral striatum, which assesses the likelihood of future rewards and punishments, the dorsal striatum more actively engages behavioral change in response to these same predictors (O'Doherty et al., 2004). The inclusion of both active responses (subjective ratings of anxiety) and outcome predictors (CSs) in our paradigm may have combined to induce both ventral and dorsal striatal activation.

Anterior insula activation is consistently observed during the certain expectation of unpleasant events (Buchel et al., 1998; Chua et al., 1999; Ploghaus et al., 1999) and is involved in evaluating potentially distressing cognitive and interoceptive sensory information during high anticipatory anxiety (Reiman et al., 1997). As also observed by Carlson et al. (2011), we found the anterior insula increased activity in tandem with ratings of subjective anxiety. Lateral OFC was also activated during higher anticipatory anxiety, possibly due to the uncertain expectation of pain or financial loss (Critchley et al., 2001). Both O'Doherty et al. (2001) and Nitschke et al. (2006), for example, have reported that the lateral OFC was activated during monetary loss and the anticipation of negative pictures.

ACC activation during cued conditioning paradigms (Buchel et al., 1998; Chua et al., 1999; Critchley et al., 2001; Knight et al., 1999) is likely associated with vigilance to the environment and readiness for action (Straube et al., 2009); more dorsal-caudal regions of the ACC and medial PFC, consistent with the clusters identified in the 2/3/4 contrast, have been implicated in the appraisal and expression of negative emotion (Etkin et al., 2011). While our results confirmed the expected increase in BOLD response in the striatal and insular regions at progressively higher levels of anxiety, dACC activation appeared to maximally increase at a moderate level (rating 3) of anxiety. The finding of maximal dACC activation in the Anticipatory Anxiety 3 vs. 1 contrast is similar to the inverted U-function of activation Straube et al. (2009) observed in the pregenual ACC in a paradigm assessing anticipatory anxiety. In the Straube et al. (2009) study, ACC (as well as insular) activation were heightened during a moderate threat but decreased during a strong threat.

A decrease in BOLD activation was observed in the PCC at even mild levels of anxiety relative to the lowest rating. The PCC (as well as the precuneus, which also decreased) is a component of the default mode network (DMN), a network of brain regions that are most active when an individual is awake, resting, and not engaged in a cognitive task (Raichle et al., 2001). It is well known that during task-specific fMRI experiments the DMN is suppressed, and the more demanding the task the stronger the deactivation in DMN

(McKiernan et al., 2006; Singh and Fawcett, 2008). In our healthy subjects, even low levels of anxiety, coupled with the cognitive appraisal required during anxiety rating, induced substantial reductions in PCC activity. A study of processing words with emotional connotations found evidence that the PCC evaluates the emotional salience of external stimuli (Cato et al., 2004), consistent with an earlier postulate about the role of the PCC (Heilman et al., 1993). The function of the PCC in the DMN and emotional processing requires further exploration.

There are several methodological issues that require comment. First, a combined epoch, the Rating and Anticipatory period, was used to assess the neural response during anticipatory anxiety. As the Anticipatory Period always immediately followed the Rating Period and subjects were considered to be anticipating the upcoming stimulus during the entire epoch, these events were combined into a single epoch variable to avoid the problem of co-linearity and improve the design efficiency of the paradigm. Although the Rating Period also included a brief cognitive appraisal of anxiety accompanied by motor movement, these neural signals would be expected to be similar on every trial and would thereby be excluded from the contrasts. Second, our paradigm utilized an uncertain onset as a key attribute of our high threat CS. Thus, our paradigm was unable to assign possible contributions of distal vs. proximal threats upon brain activity (Mobbs et al., 2007). Third, although the range of within-subject anxiety ratings is limited, this work provides preliminary evidence for graded neural correlates of anticipatory anxiety. Replication of this work and extension of the paradigm to include different levels of CS threat would provide further evidence for graded neural correlates of anticipatory anxiety. Fourth, only subjective anxiety was recorded during anticipatory anxiety. Objective indicators of anxiety previously used to model anxiety and fMRI response, such as heart rate or galvanic skin conductance (Critchley et al., 2001; Phelps et al., 2001; Somerville et al., 2010), were not obtained.

Although subjects were trained in the paradigm prior to the fMRI session, the endorsement of variable levels of anxiety during the high threat CS supports the absence of training-induced demand characteristics (i.e. subjects did not recognize or learn that they were expected to endorse high subjective ratings for the high threat CS). Although both high and low- threat CSs involved duration uncertainty, the duration uncertainty likely did not appreciably affect the anxiety level in the low-threat CS, since the subjects knew with certainty to expect a non-painful US stimulus. The shapes (triangle, square) were not counter-balanced between the high and low stress CS. Although previous research suggests that a triangle might be interpreted as more threatening than a square (Larson et al., 2009), this was not found to be the case in our study. The absence of a trait anxiety measure did not allow us to determine whether a participant's response to anticipatory anxiety was reflective of a persistent level of anxiety. The inclusion of older adults (all but two were over 30 years old) extends the previous observations in anticipatory anxiety studies conducted in young adults only (Jensen et al., 2003; Schiller et al., 2008; Straube et al., 2009). However, the absence of women in the study limits the interpretation of our findings to men only.

This study demonstrated the feasibility of utilizing an ecologically relevant, individually calibrated, universally noxious, and uncertain stimulus to successfully induce intense anxiety during the assessment of BOLD response while simultaneously assessing the subjective level of anxiety upon the presentation of each CS. The differential responses of various striatal-limbic regions in response to higher levels of anxiety may suggest that qualitatively different neural responses may be evident in different striatal-limbic regions in extreme emotional states. Thus, this paradigm may prove useful in assessing clinical populations experiencing extreme dysfunctional responses to stress and anxiety.

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Figure 1. Anticipatory anxiety paradigm. Presentation of the triangle conditioned stimulus (low-threat CS) predicted a warm unconditioned stimulus (US), whereas presentation of a square (high-threat CS) predicted either a hot or a warm US. A circle was presented for each inter-trial interval.

Figure 2.

2a) Mean subjective ratings to low and high threat CSs (mean $\pm$ S.E.) and 2b) mean subjective ratings to each low and high threat CS based on the trial position (1 to 20). Distributions of individual Anticipatory Anxiety ratings for 2c) low- and 2d) high-threat CSs. CS = conditioned stimulus.

Figure 3.

Contrasts between BOLD response of high vs. low anxiety ratings. (3a) BOLD response induced by Anticipatory Anxiety 2 vs. 1. (3b) Anticipatory Anxiety 3 vs. 1. (3c) Anticipatory Anxiety 4 vs. 1. (3d) Anticipatory Anxiety 2+3+4 vs. 1. (3e) Anticipatory Anxiety high vs. low. Red areas denote voxels with increased BOLD activity during high vs. low Anticipatory Anxiety, and blue areas denote voxels with decreased BOLD activity during high vs. low Anticipatory Anxiety. MNI coordinates (x and z axis) are noted at the bottom left of (3a). L= left; R= right; P = posterior; A = anterior; IOFC= lateral orbital frontal cortex; dACC = dorsal anterior cingulate cortex; PCC = posterior cingulate cortex; SFC = superior frontal cortex.

Figure 4.

Mean BOLD responses with standard error bars in relevant clusters from the contrasts for each subjective rating of Anticipatory Anxiety (1, 2, 3, and 4). Selected clusters defined by the Anticipatory Anxiety 2/3/4 vs. 1 in the left caudate, right caudate, anterior cingulate, left anterior insula, putamen, and posterior cingulate. The BOLD responses for each rating are presented for illustrative purposes and are not intended to depict results of an additional statistical test.

Table 1

Clusters Identified in Contrasts between Levels of Anticipatory Anxiety

2 vs. 1	MNI Coordinates (mm)			Volume (mm ³)	Max Z scores [*]
	X	Y	Z		
Increased Activation					
R occipital cortex	10	-94	-12	3224	3.57
L occipital cortex	-16	-76	-24	4088	3.32
No deactivation					
3 vs. 1					
Increased Activation					
R superior frontal cortex	16	4	68	5384	3.60
R dorsal anterior cingulate cortex	4	22	36	2656	3.33
R thalamus	2	-18	10	3200	3.34
L thalamus	-14	-20	12	2752	3.23
R caudate	10	4	8	1336	3.46
L caudate	-10	-2	14	544	3.17
R putamen	16	8	-4	600	3.15
R pallidum	18	2	-6	456	2.98
L brain stem	-4	-30	-4	376	2.92
R occipital cortex	10	-94	-12	5048	4.37
L occipital cortex	-8	-92	-18	1488	4.01
R cerebellum	18	-50	-28	4864	3.23
L cerebellum	-26	-66	-28	12976	3.84
No deactivation					
4 vs. 1					
Increased Activation					
L thalamus	-8	-4	10	872	2.81
R caudate	10	2	10	856	3.54
L caudate	-14	6	20	392	2.88
L putamen	-24	6	-4	1872	3.31

2 vs. 1	MNI Coordinates (mm)			Volume (mm ³)	Max Z scores ^a
	X	Y	Z		
L insula	-44	8	-6	344	3.09
L lateral orbital frontal cortex	-34	24	-12	1400	3.11
L inferior frontal gyrus	-50	18	2	376	2.89
<i>Decreased Activation</i>					
L posterior cingulate cortex	-2	-54	32	1080	3.44
L precuneus	-4	-56	32	3280	3.67
2/3/4 vs. 1					
<i>Increased Activation</i>					
R superior frontal cortex	4	32	52	4000	3.77
L superior frontal cortex	-2	34	56	1224	3.05
R dorsal anterior cingulate cortex	6	12	50	2480	3.61
R thalamus	10	0	8	3544	4.06
L thalamus	-16	-16	16	3248	3.49
R caudate	10	2	10	1576	4.09
L caudate	-10	-2	14	1640	3.19
L putamen	-22	8	-6	1344	3.12
R pallidum	18	4	0	480	2.96
R brain stem	6	-28	-6	520	3.17
R insula	44	12	-4	408	3.14
L insula	-32	24	2	1232	3.51
R lateral orbital frontal cortex	48	24	-10	1032	3.84
L lateral orbital frontal cortex	-42	24	-8	1328	3.41
L inferior frontal gyrus	-50	18	2	408	2.96
R occipital cortex	10	-94	-12	7672	4.76
L occipital cortex	-6	-72	-10	3680	2.63
R cerebellum	6	-76	-20	2448	3.77
L cerebellum	-36	-62	-24	1312	3.58
<i>Decreased Activation</i>					
L posterior cingulate cortex	-8	-56	10	4960	3.72

2 vs. 1	MNI Coordinates (mm)			Volume (mm ³)	Max Z scores [*]
	X	Y	Z		
L precuneus	-16	-60	16	2992	3.82

^{*} Corrected cluster significance threshold P=0.01.