Interactions Between Transient and Sustained Neural Signals Support the Generation and Regulation of Anxious Emotion

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Interactions between transient and sustained neural signals support the generation and regulation of anxious emotion

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Anxious emotion can manifest on brief (threat-response) and/or persistent (chronic apprehension and arousal) timescales, and prior work has suggested these signals are supported by separable neural circuitries. This fMRI study utilized a mixed block-event-related emotional provocation paradigm in 55 healthy participants to simultaneously measure brief and persistent anxious emotional responses, testing the specificity of, and interactions between, these potentially distinct systems. Results indicated that components of emotional processing networks were uniquely sensitive to transient and sustained anxious emotion. Whereas the amygdala and midbrain showed only transient responses, the ventral basal forebrain and anterior insula showed sustained activity during extended emotional contexts that tracked positively with task-evoked anxiety. States of lesser anxiety were associated with greater sustained activity in the ventromedial prefrontal cortex. Further, ventromedial prefrontal recruitment was lower in individuals with higher scores on intolerance of uncertainty measures, and this hyporecruitment predicted greater transient amygdala responding to potential threat cues. This work demonstrates how brain circuitries interact across temporal scales to support brief and persistent anxious emotion, and suggests potentially divergent mechanisms of dysregulation in clinical syndromes marked by brief versus persistent symptoms of anxiety.

Keywords: amygdala, fMRI, insula, BNST, unpredictability, emotion, intolerance of uncertainty

Theoretical accounts have motivated a division between manifestations of anxious emotion based on the source and timescale of affective experience (Barlow 1988). On one hand, the classically characterized "fear" response entails a transient alarm reaction to environmental inputs that predict or embody threat (LeDoux 1998). By contrast, "anxiety" describes a persistent and diffuse mood state marked by defensive preparedness, sustained arousal, and vigilance (Barlow 2000; Lang et al. 2000; Davis et al. 2010). Prior work has asserted that fearlike and anxietylike emotions are subserved by distinct but interactive neural circuitries (Davis et al. 2010; Alvarez et al. 2011). Human imaging research has broadly supported this distinction. Whereas fearlike paradigms have focused on the amygdala's role in processing salient environmental cues (LaBar et al. 1998; Phelps et al. 2001; Etkin et al. 2004), lengthy anxietylike contexts tend to engage prefrontal regions and regions of the basal forebrain neighboring the amygdala (Chua et al. 1999; Simpson et al. 2001; Hasler et al. 2007; Somerville et al. 2010).

Though these findings are generally consistent with a division of the neural representation of fear and anxiety, a number of outstanding issues remain. Studies to date targeting anxious emotion tend to assay fearlike or anxietylike emotion separately, precluding assessment of the specificity of, or interactions between, these neural circuitries. Functional imaging designs such as the mixed-block-event-related design and analysis scheme enable transient neural responses to be decomposed from neural responses that are sustained persistently across lengthy contexts (Visscher et al. 2003). Using this design, the present study targeted both event-related neural responses engaged by brief emotional cues as well as neural responses that persist throughout contexts in which participants are made to feel anxious. This enables analysis of the specificity of systems that are sensitive to brief emotional triggers, maintain greater engagement during anxious states, and maintain greater activity during states of lesser anxiety.

The driving hypothesis, based on animal and human work, was that the representation of brief emotional responses would be supported by an amygdala-hypothalamic-periaqueductal gray network biologically suited to modulate rapid "fight-or-flight" behavior via descending glutamatergic projections (McNaughton and Corr 2004; Kober et al. 2008). Conversely, persistent neural signals observed throughout anxiogenic contexts would manifest in sustained signaling in the insular cortex and ventral basal forebrain (including the bed nucleus of the stria terminalis [BNST]), key modulatory structures for stress and arousal maintenance (Davis 1988; Paulus and Stein 2006; Somerville et al. 2010). In addition, the present work assesses biased sensitivity of these systems in individuals with high trait-anxious characteristics.

On a behavioral level, brief and persistent emotions are not experienced in isolation. Extended states of heightened anxiety potentiate sensitivity to emotion cues (Seligman 1968; Grillon et al. 2004), especially in individuals with high trait anxiety (Fox E. et al. 2001; Robinson et al. 2011). However, the neural mechanisms by which persistent affective states influence brief emotional responding remain largely unaddressed. This experiment assesses interactions between brief and persistent affective responding, evaluating how sustained engagement of "anxiety" systems influence transient "fear" responses. Understanding the specificity, interactions and individual differences-based biases amongst these circuitries holds potential to specify novel contributors to the pathophysiology of psychiatric illnesses characterized by isolated fear (e.g., specific phobias) versus anxiety (e.g., generalized anxiety disorder; GAD; (Davis and Whalen 2001; Craske et al. 2009).

Materials and Methods

Participants. Sixty-one healthy adult participants completed this experiment. Participants were right handed, reported no abnormal neurological history, and were native speakers of English. Participants were verified to be absent of clinically diagnosable levels of current anxiety

disorders and current or past mood disorders using the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al. 1995) and no participant was using psychotropic medications. The potential for covarying mood effects was minimized by excluding any participant scoring greater than 10 on the Beck Depression Inventory (Beck et al. 1961). One participant's response data during fMRI scanning was lost due to technical error, leaving a final sample of *n*=60 for behavioral analyses (*n*=36 female, mean age=19, standard deviation=1.2 years). FMRI data from six participants were excluded for movement of more than 2mm and/or signal artifacts, leaving a final sample of *n*=55 for imaging analyses (*n*=32 female, mean age=19, standard deviation=1.2 years). All participants provided informed consent for their participation in accordance with the Committee for Protection of Human Subjects at Dartmouth College.

Individual Differences in Anxiety. Participants completed several scales assessing a range of anxiety symptoms, including the Spielberger State-Trait Anxiety Inventory (STAI) (Spielberger et al. 1988), Behavioral Inhibition/Activation Scale (BIS/BAS) (Carver and White 1994), NEO Personality Inventory Neuroticism and Extraversion subscales (Costa and McCrae 1991), Intolerance of Uncertainty Scale (IUS) (Buhr and Dugas 2002), Penn State Worry Questionnaire (PSWQ) (Meyer et al. 1990), and Anxiety Sensitivity Index (ASI) (Peterson and Reiss 1987). Individual difference analyses focused on the total scores on the Intolerance of Uncertainty (IU) scale. IU, defined as, "a tendency of an individual to consider it unacceptable that a negative event may occur, however small the probability of its occurrence ((Buhr and Dugas 2002) p. 932)," This construct holds particular relevance to tonic or long-lasting anxiety, a primary target of this study. The mean (*M*=57.5) and standard deviation (*SD*=15.3) of IU scores in the present sample are consistent with published normative data (Buhr and Dugas 2002) and represents a wide range of scores (sample: 29 to 97; possible scores: 27 to 135). IU also demonstrated substantial overlapping variance with other individual difference measures targeting different

facets of trait anxiety (correlations with: Trait STAI r(60)=0.32, p=0.01; BIS r(60)=0.57, p<0.001; NEO neuroticism r(60)=0.44, p<0.001; PSWQ r(60)=0.45, p<0.001; ASI r(60)=0.26, p<0.05).

Stimuli. Negative and neutral images were primarily drawn from the International Affective Picture System (Lang et al. 1997). Negative images included pictures of overt threats (attacking animals, weapons), disasters (bombings, plane crashes), graphic depictions of sick or injured individuals, and other arousing negative imagery whereas neutral images depicted innocuous social and nonsocial scenes. As in prior work (Dolcos et al. 2004, 2005), we sought to equate negative and neutral pictures on certain higher-order features that may engage the circuitry of interest (i.e., presence of humans). To attain a sufficient sample set of neutral images depicting people, fifty supplementary neutral images featuring people were compiled by experimenters and shared from prior work (Yamasaki et al. 2002), which were normed for valence and arousal by a separate group of n=45 participants and added to the pool of images (data and images available on request). Two sets of 30 negative pictures with comparable valence and arousal, and two sets of 30 neutral pictures with comparable valence and arousal were selected as stimuli for the four experimental conditions. In addition, all four sets were matched for relevant aspects of scene content [proportion of images depicting people with visible faces, peoples' bodies without visible faces, proportion of pictures taking place indoors and outdoors (p's > 0.3)], see Supplementary Table 1.

Task structure. A mixed block-event related design and optimized analysis specifications enabled independent detection of transient and sustained BOLD responses within a single experiment and modeling analysis (Visscher et al. 2003; Dosenbach et al. 2006). This design incorporates both brief and persistent experimental manipulations to evoke transient and sustained neural responses, respectively. Comparison of brief responses to negative versus neutral images isolated transient emotional responses (Hariri et al. 2003; Britton et al. 2006;

MacNamara and Hajcak 2009), serving as generators of cued affect that experimentally model the timecourse of fearlike emotion (Figure 1B, top panel for schematic version).

Design provisions additionally permitted the independent detection of sustained neural signals that remained persistently active throughout task blocks (see Figure 1B, bottom panel for schematic version). Historically, the mixed design approach has targeted neurocognitive representations of "task sets"- long-duration upregulation of localized BOLD signals that maintain a cognitive state superordinate to moment-to-moment stimulus features (Logan and Gordon 2001; Dosenbach et al. 2006). The present study targeted cognitive sets related to the persistent subjective experience of anxiety by isolating components of the BOLD response that were engaged and remained active throughout task blocks with varying levels of task-evoked anxiety.

To evoke differential levels of anxiety across blocks, two affective manipulations were crossed, each of which has been validated to evoke heightened anxiety. One manipulation was presenting either negative or neutral images in a given task block, based on evidence that blocked exposure to negative images induces state anxiety and psychophysiological responses that persist superordinate to image presentations (Bradley et al. 1996; Smith et al. 2005). Thus, we predicted heightened anxiety during lengthy blocks containing only negative image presentations relative to blocks in which participants knew they would view only neutral pictures.

The second manipulation involved modulating temporal properties of picture presentations, presenting the images within either predictable "countdowns" or with unpredictable, random timings (Figure 1A). This design feature was chosen based on prior work demonstrating the inherent anxiogenic properties of temporal uncertainty (Ladouceur et al. 2000; Carleton et al. 2007; Herry et al. 2007) and its contribution to the pathophysiology of anxiety disorders (Dugas et al. 1998; Ladouceur et al. 2000). We predicted that persistent task-evoked anxiety would be induced by temporally unpredictable picture presentations, particularly when the pictures are negatively valenced. According to participant reports, the 2x2 crossing of

valence and unpredictability manipulations effectively induced significant differences in task-evoked anxiety across blocks (see Results, Figure 2).

Functional Imaging. During fMRI scanning, participants viewed negative and neutral images embedded within contexts of predictable or unpredictable timings (Figure 1A). Half of blocks contained only negative pictures; the remaining half contained only neutral pictures. A total of fifteen pictures were presented per block. The valence manipulation was crossed with a predictability manipulation. Half of the blocks contained predictable timings, consisting of a one to eight second "countdown" in which numbers were consecutively presented, accurately representing the number of seconds remaining until picture onset (Figure 1A, top panel). The other half of blocks contained unpredictable timings consisting of random numbers that provided no predictive information with regard to picture onset (Figure 1A, bottom panel). For all blocks, number presentations varied pseudorandomly with one to eight seconds between pictures. Each picture was presented once and the assignment of pictures to unpredictable and predictable blocks was pseudorandomized across participants.

Scan runs consisted of two 118-second blocks interleaved with 60-second resting fixation periods. Blocks began with a 3-second start cue alerting participants of the forthcoming block type to isolate activity associated with transitions into a task set (Konishi et al. 2001), to ensure sustained activity estimates were constrained to maintenance functions, and to mitigate the need for the participant to gradually decipher the block type. Following the on-cue, stimulus presentation continuously alternated between number (1 second per number; jittered one to eight numbers) and picture presentations (3 seconds).

Participants were instructed to passively view the number stimuli, being mindful of the information provided by the numbers. For each picture presentation, participants were instructed to press one of two buttons indicating whether the picture took place indoors or outdoors (Lane et al. 1997; Ochsner et al. 2004), a low-level task chosen to interfere minimally with affective

responding (Lieberman et al. 2007) while providing a behavioral metric for assessing event-related response latency as a function of valence and predictability.

Each block ended with a three-second stop cue to isolate transient task set release related activity (Konishi et al. 2001). Two such blocks were presented per run, with block order pseudorandomized within and across participants resulting in eight blocks presented across the experiment (two repetitions of each type). Stimuli were presented using Cedrus Superlab 4.0.2 (San Pedro, CA), interfaced with the Lumina Response box, which recorded participant key presses and fMRI triggers. During scanning, visual stimuli were displayed onto a back projection screen at the head end of the scanner bore with an Epson (model ELP-7000) LCD projector.

Task-evoked anxiety. Immediately after the task, participants remained in the scanner and provided verbal answers to post-test questions assessing the subjective levels of anxiety evoked by different task blocks. Specifically, participants were asked to rate on a one to nine Likert scale how nervous they felt throughout each of the four block types (negative pictures predictable timings; negative pictures unpredictable timings; neutral pictures predictable timings; neutral pictures unpredictable timings). Ratings effects were evaluated with a 2x2 mixed ANOVA with repeated effects of predictability (unpredictable, predictable) and valence of pictures within the block (negative, neutral), with individual differences in IU input as a mean-centered covariate. Reaction times were z-scored and submitted to the analogous group analysis.

fMRI acquisition. Imaging was performed on a Philips Intera Achieva 3.0 Tesla scanner with a SENSE head coil (Philips Medical Systems, Bothwell, Washington). Four T2* weighted scans sensitive to the blood oxygenation level-dependent (BOLD) response (repetition time=2000 msec, echo time=35 msec, flip angle=90°, 3x3 in-plane resolution, SENSE factor=2) were used to acquire 832 whole-brain volumes (36 slices, 3.5mm slice thickness, 0.5mm gap, anterior

commissure-posterior commissure plane). A T1-weighted high-resolution image of the brain was acquired with a magnetization-prepared rapid gradient echo sequence (160 sagittal slices, echo time=4.6 msec, repetition time=9.9 msec, flip angle=8°, voxel size=1x1x1mm).

fMRI analysis. Processing of fMRI data took place in SPM2 (Wellcome Department of Cognitive Neurology, London, UK (Friston et al. 1995)). Preprocessing steps included slice time correction, motion correction, correction of movement-by-susceptibility interactions with unwarping routines, and spatial normalization. Normalized functional data were spatially smoothed (6 mm full-width at half maximum Gaussian kernel).

Transient and sustained components of the BOLD signal were detected using first-level general linear modeling (GLM) at the individual participant level, optimized to independently and simultaneously identify brief and persistent neural signals. The present design permitted transient responses to be disentangled from sustained responses due to specific design components including sufficiently variable jitter between transient stimuli, sufficient time spent in a sustained state and not experiencing a transient event (>60% total block time), and modeling of transients using a finite impulse response (FIR) basis function rather than a canonical response to ensure that sustained condition estimates are truly maintained and not aliased by high frequency components of the signal. These design features enabled simultaneous modeling of transient and sustained signals for the present purposes of identifying common and distinct networks subserving emotional responses that manifest over fearlike and anxietylike timescales.

For each participant, a general linear model incorporated regressors for the four transient event conditions, the four sustained block conditions, start cues, stop cues, and regressors of non-interest (session mean, linear trend, run regressor, and six movement parameters) to compute parameter estimates (β) and contrast maps (weighted parameter estimates). Trials with incorrect indoor/outdoor judgments or no response were not modeled

separately due to their infrequent occurrence (~6% of all trials). Transient conditions (neutral pictures within predictable blocks, negative pictures within predictable blocks, neutral pictures within unpredictable blocks, neutral pictures within unpredictable blocks, on cues, and off cues) were modeled with a Finite Impulse Response function over 10 TRs (20 seconds)[1]. Four sustained regressors served as predictors of signal changes that were recruited and remained persistently engaged throughout the blocks, independent of transient picture presentations. Sustained regressors (blocks of predictable timings with neutral pictures, predictable timings with negative pictures, unpredictable timings with neutral pictures, and unpredictable timings with negative pictures) consisted of a boxcar function beginning after the conclusion of the start cue and lasting until the onset of the stop cue.

Group fMRI statistical analyses. Voxelwise random effects group analyses were used to test targeted hypotheses regarding transient and sustained responses as a function of valence, predictability, and IU. All voxelwise analyses were thresholded at $p<0.05$ whole-brain corrected, using a p-value and cluster size threshold combination stipulated by Monte Carlo simulations to maintain a whole-brain $\alpha=0.05$. Regions showing transient responses as a function of valence were identified with a truncated area under the curve (AUC) analysis of finite impulse response parameter estimates (as in (Wig et al. 2009)) focused on the expected hemodynamic peak 4 to 11 seconds following picture onset given a 3-second event duration. Truncated AUC estimates for negative relative to neutral images were statistically compared with a voxelwise paired t-test to identify brain regions transiently sensitive to emotional picture content.

Two complementary group statistical analyses were used to identify regions showing differential sustained emotional modulation. First, participant ratings were used to identify areas of the brain showing sustained activity titrating positively with heightened task-evoked anxiety.

[1] Though the FIR model does not assume a response shape, inspection of timecourses from regions of interest (ROIs) validated that the reported transient responses conformed to standard hemodynamic properties.

Parameter estimates (β) for the each of the four sustained conditions were input to a linear contrast per participant that identified the extent to which sustained neural activity tracked faithfully with the differential self-reported anxiety experienced across conditions (i.e., Figure 2). Z-scored, demeaned values of self-reported anxiety ratings were generated and used as contrast weightings for each of the four sustained conditions (the sustained predictable neutral β weighted -0.98, the sustained unpredictable neutral β weighted -0.54, the sustained predictable negative β weighted +0.38, and the sustained unpredictable negative β weighted +1.14). The resulting value per participant indicates to what extent sustained neural responses titrated faithfully with self-reported anxiety across the four conditions, with positive values representing increasing sustained activity with greater anxiety and negative values representing greater sustained activity proportional to states of lesser anxiety. A group statistical map was subsequently generated using a voxelwise group one-sample t-test, inputting the contrast estimates described above for each participant. Regions of the brain with positive t-values are candidates for the maintenance of lengthy states of heightened anxiety independent of discrete stimulus detection. Negative t-values identified regions with sustained responses that were strongest for experimental blocks during which lower level of anxiety was experienced.

To validate this approach, a second set of analyses was conducted that did not rely on self-report. Specifically, whole-brain paired t-tests were generated to isolate sustained neural responses as a function of picture valence (sustained activity in blocks containing negative versus neutral pictures) and predictability (blocks containing unpredictable versus predictable timings).

A final objective was to target the potential interactions between tonic and phasic systems. We predicted that individual differences in persistent engagement in contexts in which participants experienced heightened anxiety would predict brief responses to affective cues. We explored this possibility with bivariate correlation analyses across amygdala and vmPFC ROI

data, testing whether individual differences in the magnitude of sustained network recruitment in unpredictable contexts predicted the magnitude of transient neural responses to cued events.

Though a whole-brain statistical analysis was performed, region-of-interest (ROI) selection was constrained to loci within *a priori* affective circuities of interest (Kober et al. 2008; Davis et al. 2010). Regional specificity to transient versus sustained responses, interactions between regions, and modulated activity with individual differences in intolerance of uncertainty (IU) were evaluated within these ROIs during offline analyses. Four-millimeter radius spherical regions of interest about activation peaks were generated with the MarsBaR 0.41 toolbox, and signal estimates were extracted for each condition for offline statistical analysis using SPSS Statistics 18.0 and 19.0 software. Visualization of cortical activity was aided with surface reconstructions generated by Computerized Anatomical Reconstruction and Editing Toolkit (Caret) v5.5 software (Van Essen et al. 2001). Key findings beneath the cortical surface are presented on a representative spatially normalized T1-weighted image. All reported coordinates have been converted to Talairach atlas space (Talairach and Tournoux 1988).

Results

Accuracy & Reaction Times. Participants performed with 93.9% mean accuracy on indoor/outdoor judgments. Accuracy was worse for negative than neutral pictures ($F(1,58)=4.10$, $p=0.047$), were not affected by predictability of timings ($p>0.8$), and did not interact with individual differences in IU (p's > 0.1; main effect of IU $p>0.9$). Reaction times were significantly slowed by negative picture valence ($F(1,58)=162.5$, $p<0.001$; z_{neg}-$z_{neu}=0.41$) and by unpredictable contexts ($F(1,58)=13.50$, $p<0.001$; z_{unp}-$z_{pre}=0.16$). In addition, there was a significant three-way interaction between valence, predictability, and IU ($F(1,58)=4.35$, $p=0.041$). Posthoc comparisons revealed that individuals with above-median IU showed a particular slowing to negative pictures regardless of predictability (negative picture RT for above

median IU versus below median IU: t(58)=2.02, p=0.048; z_{above}-z_{below}=0.09), and trend toward a greater influence of unpredictability on neutral picture RTs (unpredictable vs predictable neutral picture RT: t(58)=1.6, p=0.1; z_{above}-z_{below}=0.18).

Task-evoked anxiety. Blocks containing negative pictures elicited higher task-evoked anxiety than blocks containing neutral pictures (main effect of valence: F(1,58)=204.72, p<0.001; Figure 2). Blocks containing unpredictable timings also evoked heightened anxiety (main effect of predictability (F(1,58)=61.79, p<0.001), and individuals with greater IU trended toward endorsing task-evoked anxiety (main effect of IU: (F(1,58)=2.72, p=0.1). There was also a significant valence by predictability interaction (F(1,58)=19.63, p<0.001), driven by a stronger effect of unpredictability on task-evoked anxiety for negative than neutral image blocks [(unpredictable negative – unpredictable neutral) versus (predictable negative – predictable neutral) t(59)=4.46, p<0.001)]. Finally, we observed a valence by IU interaction (F(158)=6.33, p=0.015), with greater IU predicting higher nervousness ratings for negative image blocks (p's<0.04) but not neutral blocks (p's>0.6).

Transient Responses to Pictures. Brain regions transiently active to negative pictures (relative to neutral pictures) are listed in Table 1. Active regions included the left (x=-21, y=-7, z=-17) and right amygdala (x=24, y=-1, z=-20; Figure 3A-B) and an area of the midbrain consistent with the periaqueductal gray (midbrain/PAG; x=6, y=-30, z=-13; Supplementary Figure 1). Timecourse data (derived from FIR signal estimates) was extracted from 4mm ROIs centered on activation peaks and evaluated offline for independent effects of predictability and anxiety. Effects of predictability on the right amygdala response were not significant (main effect, valence by predictability interaction p's > 0.3) but were qualified by a significant valence by predictability by IU interaction (F(1,53)=4.02, p=0.05). Specifically, peak transient responding to negative pictures embedded in unpredictable timings was selectively exaggerated with greater IU (r(54)=0.31, p=0.019, R^2=0.1; Figure 3C), whereas IU did not predict amygdala responses to the

other conditions (*p*'s>0.5). Left amygdala activity was not modulated by predictability (*p*'s>0.2), though yielded a trend toward overall larger responses with higher IU (F(1,53)=3.57, *p*=0.06). Midbrain/PAG activity did not differ by predictability or individual differences in IU (*p*'s>0.3).

To test for temporal specificity, the left and right amygdala and midbrain/PAG ROI values were evaluated for possible sustained responses - signals that remained continuously engaged throughout task blocks. Sustained beta estimates from the left and right amygdala showed no evidence of persistent responding relative to resting baseline (mean parameter estimate for sustained activity, left: -0.039; right: -0.178) or modulated sustained activity as a function of valence or predictability (*p*'s>0.5). The midbrain/PAG did not yield evidence of greater sustained responding relative to baseline (*p*>0.7) though it did show a trend toward greater sustained responding to unpredictable relative to predictable states (F(1,53)=3.31, *p*=0.07). This effect was substantially weaker than other sustained effects and would not survive multiple comparisons correction, and thus was not considered further.

Anxiogenic state maintenance. A whole-brain analysis incorporated subject reports to identify regions of the brain that remained continuously active throughout task blocks proportionally to task-evoked anxiety, which was heightened via negative valence and temporal unpredictability manipulations (e.g., Figure 2). Positive neural predictors of sustained anxiety included the left and right inferior frontal gyrus (BA47m in the (Price 2007) nomenclature and Area 12 in the primate; left x=-36, y=20, z=-14; right x=33, y=29, z=-12; Figure 4B) that extended to the insular cortex (x=30, y=3, z=10), as well as the right ventral basal forebrain/bed nucleus of the stria terminalis (VBF/BNST; x=-6, y=-3, z=-2[2]; Figure 4A; see Table 2 for full list of activations). In

[2] The VBF/BNST and midbrain/PAG reported earlier are anatomically small structures. These activations achieved the cluster size/p-value combination required to preserve *p*<0.05 corrected thresholding in part due to spatial contiguity with a more posterior cluster outside of the VBF/BNST-proper, and in the midbrain/PAG, with a more dorsal cluster within the midbrain. For completeness, we note that the VBF/BNST cluster, alone, consists of 108mm^3 at *p*<0.001, uncorrected and the midbrain/PAG region consists of 702mm^3 at *p*<0.0005, uncorrected.

addition, the right BA47m/insula cluster showed an interaction between responsivity to unpredictable states and IU, with greater IU associated with higher sustained activity in unpredictable blocks ($F(1,53)=5.36$, $p=0.024$; see Supplementary Figure 2). Aside from a trend for which the left BA47m showed marginally greater responding for negative blocks in high IU individuals ($p=0.08$), individual differences in IU did not further modulate sustained activity in these regions (p's>0.18).

We evaluated temporal specificity by evaluating whether these regions also showed transient responses to picture events by testing transient beta estimates within these ROIs. Notably, all regions also showed some degree of transient activation relative to resting fixation (p's<0.02). However, unlike the transient responses reported earlier, responses in these regions showed no main effects of predictability or valence, and no modulation of activity by IU (p's>0.1).

A second approach to evaluating sustained emotional responses was to test for replication of 47m/insula and BNST engagement using basic contrasts that did not rely on self-report. Whole-brain paired t-tests targeting differential sustained effects of valence (sustained activity in blocks containing negative versus neutral pictures) and predictability (blocks containing unpredictable versus predictable timings) also identified the regions described above at $p<0.05$, whole brain corrected thresholding. Specifically, greater activity was observed in area 47m/insula and VBF/BNST to negative relative to neutral sustained states, and to unpredictable relative to predictable sustained states (Table 2).

Sustained responses to states of lesser anxiety. The current design permitted identification of regions showing maximal sustained activity in contexts of low anxiety. To do so, we queried for greater sustained activity with lesser task-evoked anxiety by using subject ratings of task-evoked anxiety as inverse contrast weightings in a group whole-brain analysis (see Methods).

Results yielded a single region located in the ventral anterior cingulate cortex bordering on ventromedial prefrontal cortex (vACC/vmPFC; x=3, y=29, z=-12; BA25/32; Table 2, Figure 5A). Signal estimates derived from ROI analyses of the vACC/vmPFC (4mm sphere surrounding peak activation) are plotted in Figure 5B for descriptive purposes. Testing ROI parameter estimates yielded a significant predictability by IU interaction ($F(1,52)=11.28$, $p=0.0014$)[3], such that greater IU predicted sustained vACC/vmPFC hyporecruitment during unpredictable contexts ($r(53)=-0.30$, $p=0.03$, $R^2=0.087$; Supplementary Figure 3), an effect not observed for predictable contexts ($p>0.4$).

Sustained-transient interactions in emotion response and regulation. Prior research assaying limbic/prefrontal circuitry (Milad and Quirk 2002; Phelps et al. 2004; Johnstone et al. 2007; Soliman et al. 2010) suggests an inverse relationship between the vACC/vmPFC and amygdala. The design of the current study permitted testing for inverse interactions between sustained signals and the magnitude of stimulus-wedded transient responses. Bivariate correlation analyses of parameter estimates from previously-defined amygdala and vmPFC ROIs (see above) evaluated whether the degree of sustained vACC/vmPFC engagement predicted the magnitude of transient amygdala response to negative events. Because the construct of IU isolates uncertainty-related anxiety, we focus analyses on interactions between these regions in the unpredictable phases of the experiment. Results indicated that sustained underrecruitment of the vACC/vmPFC during unpredictable states predicted the degree of exaggeration of transient amygdala responses to negative pictures in unpredictable contexts ($r(53)=-0.39$, $p=0.004$; $R^2=0.15$; Figure 5C). This relationship was specific to transient negative picture responses, as the inverse correlation was not evident when considering neutral pictures ($p>0.1$).

Discussion

[3] One participant with questionable signal coverage was excluded from all vmPFC analyses.

Anxious emotion can be experienced along brief and persistent timescales. Using an fMRI paradigm sensitive to both transient “fear”-like and sustained “anxiety”-like neural responses, we demonstrate that these components of anxious emotion are spatially and temporally distinct at the neurobiological level, and organized in a manner consistent with regional downstream modulatory effects on behavior. Rapid glutamatergic output centers including the amygdala and midbrain/PAG were uniquely sensitive to transient emotional provocation. Conversely, brain regions critical to modulating sustained arousal maintenance such as the BNST and insula remained continuously engaged throughout lengthy emotional contexts, showing greater activity during states of heightened anxiety. Further, downregulation of transient amygdala responses was predicted by heightened sustained activity in ventromedial prefrontal regions, suggesting regulation of momentary emotional responses may be accomplished via sustained prefrontal engagement. These results provide evidence supporting the specificity of “fear” and “anxiety” systems, and offer a mechanism by which persistently anxious states influence brief emotional sensitivity.

Behavioral Findings

During fMRI scanning, participants viewed negative and neutral images, embedded within predictable and unpredictable timings. Negative (versus neutral) image presentations constituted a cued threat manipulation, which selectively slowed reaction times in individuals with high IU, the anxiety measure we focused on given the relationship of this metric to unpredictable anticipatory states ((Buhr and Dugas 2002); see Methods). This response profile is consistent with assertions that trait anxiety, which correlates with IU (Buhr and Dugas 2002, 2006), enhances attention toward potential threats (Bar-Haim et al. 2007; Bishop 2009) perhaps due to compromised capacity to engage in attentional control during threat processing (MacLeod and Mathews 1988; Bishop 2007).

Sustained emotional modulation was achieved by blocking the valence of images such that a given task block contained only negative or neutral pictures, and by embedding the images within contexts varying in temporal unpredictability. Task-evoked anxiety was greater during blocks containing negative pictures relative to neutral pictures and in contexts involving temporal unpredictability. Though the present findings rely on participant report, they are consistent with studies reporting that temporal uncertainty elicits startle responses (Grillon et al. 2004) and physiological upregulation (Grupe and Nitschke 2011) in humans, as well as anxiety-like behavior in rodents (Herry et al. 2007). Further, blocks in which negative pictures were presented within unpredictable timings elicited an exaggerated rise in anxiety relative to either condition alone. Thus, the effects of unpredictability are especially evident when uncertainty relates to negative outcomes (Whalen 1998; Grillon and Baas 2003).

Transient emotional responses

As in prior work (Hariri et al. 2003; Britton et al. 2006; Urry et al. 2006), we observed transient amygdala responding to negative relative to neutral images. Further, the peak height of this response was positively predicted by greater IU. This is consistent with other findings documenting the influence of healthy variation in anxiety on amygdala activity (Etkin et al. 2004; Somerville et al. 2004; Bishop et al. 2007; Stein et al. 2007). Here, amygdala response upregulation with IU was constrained to unpredictable temporal contexts. Other work has shown heightened amygdala response to emotional cues when the contingency schedule between a cue and aversive stimulus is made uncertain by being partially reinforced or ambiguous (Belova et al. 2007; Sarinopoulos et al. 2010). The specificity of IU effects to temporally unpredictable contexts also suggests that event-related fMRI designs with variable duration jittering, due to the less predictable temporal presentation, may be incidentally more suited to evoke biased amygdala recruitment with greater trait anxiety.

Responses in the amygdala and midbrain/PAG were constrained to transient "fear"-like responses, as signal change maintained across lengthy blocks did not differ from rest, and largely were not modulated by valence, predictability, or individual differences in anxiety. Anatomically, the PAG is a key convergence point for rapid amygdala and hypothalamic signaling of environmental salience, serving to orchestrate defense responses via downstream reflex modulation (Fanselow 1991; Brandão et al. 1999). Recent human neuroimaging work documents a role for the human PAG in mediating physiological responses during social anxiety (Wager et al. 2009), and representing imminent danger relative to less threatening phases of exposure to a simulated predator (Mobbs et al. 2007; Mobbs et al. 2009). The response specificity to brief threat events in the present study constrains the role for the human PAG to transient signaling.

Sustained anxious responses

This study applied design and analysis parameters from validated studies of task set maintenance to target circuitry demonstrating persistent neural activity during anxious states. The inferior frontal gyrus (BA47m) and anterior insula, subpeaks of a larger activation increasing in activity with more anxiogenic states, are heavily interconnected with one another as well as with a number of structures in the temporal and orbital cortex (Saleem et al. 2008). The insula is physiologically suited to maintain sustained changes in arousal given its role in supporting the continuous updating of a subjective sense of "feeling" (Critchley et al. 2002; Craig 2003) and has been implicated in prior studies measuring brain activity during anxiogenic contexts (Chua et al. 1999; Hasler et al. 2007; Alvarez et al. 2011; Carlson et al. 2011). The present study demonstrates temporal persistence of insular signals during contexts involving heightened anxiety, with additionally exaggerated activity observed in high IU individuals during contexts of temporal unpredictability (see also (Simmons et al. 2008)). A role for the insula in maintaining states of anxious arousal fits well with its proposed role as an integrator of body state

information, which is thought to support physiological upregulation associated with anxiety (Paulus and Stein 2006). Evidence that antianxiety medications such as benzodiazepines reduce insular activity in a dose-dependent fashion (Paulus et al. 2005), and that symptom reduction in GAD correlates with lesser insula sensitivity (Hoehn-Saric et al. 2004) supports this conceptualization. Interestingly, the inferior frontal gyrus has been implicated in cognitive task set maintenance in nonemotional contexts (e.g., (Dosenbach et al. 2006)). Further research is needed to determine the exact nature of these adjacent regions' relative contributions to emotional and nonemotional task sets, and to further probe whether the cognitive operations subserved by this region are anxiety-specific or domain-general processes drawn on during anxious states.

The present study identified the bed nucleus of the stria terminalis (BNST) as maintaining persistent states of heightened anxiety. The BNST has been widely implicated in sustained anxiety using animal models (Davis et al. 1997; Hammack et al. 2004), though has remained elusive in human imaging results until recently. Human (Straube et al. 2007; Somerville et al. 2010; Alvarez et al. 2011) and non-human primate (Fox A. S. et al. 2008; Oler et al. 2009) studies have begun to target this structure, finding increased activity during sustained anticipatory states. Our prior work has demonstrated that VBF/BNST and insula activity tracks sustained threat monitoring using an event-free paradigm in which threat level slowly and continuously fluctuated (Somerville et al. 2010). It is notable that VBF/BNST and insula recruitment in a prior study (Somerville *et al.* 2010) was exaggerated in individuals with high trait anxiety, whereas the present study observed IU modulation in the insula, but not the VBF/BNST. It is possible that the experimental context contributed to these differential effects. Whereas the present study utilized subtle valence and predictability manipulations, Somerville et al. (2010) reported findings from a shock-threat paradigm, likely a more powerful and salient manipulation of sustained anxiety. Thus, while both studies report engagement of insula and VBF/BNST circuitry, potential physical threat evoked further VBF/BNST upregulation in more

anxious individuals. Taken together, these findings constitute accumulating evidence that the inferior frontal gyrus (47m), insula and VBF/BNST support the maintenance of anxious emotion via tonic signaling.

Sustained-transient interactions as a function of trait anxiety

In contrast to circuitry showing greater task-evoked anxiety, we observed greater activity with lesser anxiety along the cortical midline spanning the ventral anterior cingulate cortex and ventromedial prefrontal cortex. The vmPFC as been implicated in the extinction of conditioned fear (Milad and Quirk 2002; Phelps et al. 2004) and in predicting positive interpretations of ambiguous information (Kim et al. 2003). The present findings suggest that the vmPFC may also accomplish regulation of brief affective responses via greater sustained signaling throughout contexts in which environmental cues instruct an individual that nothing negative will happen (e.g., neutral blocks) and that nothing unexpected will happen (e.g., predictable blocks). Indeed, predictive knowledge relating to current and future environmental events is thought to buffer individuals from experiencing state anxiety by blunting cognitions associated with intolerance of uncertainty, a key generator of anxious arousal (Ladouceur et al. 2000).

Accordingly, individuals with higher IU showed reduced engagement of the vACC/vmPFC during unpredictable contexts. This reduction may influence or reflect anxious individuals' inherent sensitivity to ambiguity (Eysenck 1992; Ladouceur et al. 2000), such that signals of 'safety' are weakened in contexts in which environmental inputs are temporally ambiguous. This idea converges with work demonstrating differential engagement of this circuitry in clinically anxious adolescents while processing uncertainty (Krain et al. 2008). Further, Nitschke and colleagues reported that treatment response efficacy in individuals with clinically significant anxiety is positively predicted by ventral anterior cingulate cortex responsivity (Nitschke et al. 2009). The present results suggest that blunted sustained

vACC/vmPFC activity may be one mechanism by which individual differences in IU can influence tonic anxiety signals.

Several studies have documented an inverse relationship between the vACC/vmPFC and amygdala, with greater vACC/vmPFC involvement predicting a reduction of amygdala response (Kim et al. 2003; Urry et al. 2006; Johnstone et al. 2007) and comprising a functional network supported by direct reciprocal anatomical projections (Amaral 1986; Ghashghaei and Barbas 2002). In the present study, lesser sustained vACC/vmPFC recruitment predicted the degree of exaggeration of amygdala response to negative pictures with greater trait anxiety, which converges with other recent findings (Indovina et al. 2011). It is notable that in the current study, the level of *sustained* vACC/vmPFC response negatively predicted *transient* amygdala sensitivity. This suggests that tonic signals of safety may orchestrate moment-to-moment emotional sensitivity, analogous to tonic-phasic interactions thought to coordinate cognitive operations such as sustained attention (Posner and Petersen 1990) and cognitive control (Carter et al. 1998; Botvinick et al. 1999; Dosenbach et al. 2008). Though outside of the core "default mode" network demonstrating spontaneous and correlated activity at rest (Fox M. D. and Raichle 2007), the locus of vACC/vmPFC activity has recently been identified as a subsystem of the default mode network with strong intrinsic connectivity with regions of the medial temporal lobe (Andrews-Hanna et al. 2010). This network is thought to support self-oriented cognitions and the constructive representation of future events, though its precise role in emotional or anxiogenic contexts remains unexplored. In the present study, persistent vACC/vmPFC activity is suspended in contexts in which participants are made to feel anxious. Future research incorporating affective tasks and resting state connectivity data may further specify affective contributions to "default-mode" activity.

The current findings may hold particular relevance to the neural substrates of mood and anxiety disorders. Whereas the symptoms of some anxiety disorders are thought to be modulated by exaggerated sensitivity of the threat-detection system (Barlow 1988), the

symptomatology of other anxiety disorders (such as GAD) are marked by chronic and higher-order apprehension of future threats that are not necessarily triggered by an external threat cue (Ohman 1993; Lang et al. 2000). Though the present study only assesses variation within the healthy range of trait anxiety, these data may inform predictions regarding the specificity of transient and sustained affective networks on ”fear”-based and “anxiety”-based features of mood and anxiety dysregulation. For example, a meta-analysis (Etkin & Wager, 2007) reported exaggerated amygdala responses in anxiety disorders with core “fear”-like features (e.g., specific phobias, social anxiety disorder). However, such a clear pattern of hyperresponse is less evident in other anxiety disorders such as GAD. Rather, the literature reports mixed results, with some showing exaggerated amygdala responses in GAD participants to threat cues (McClure et al. 2007; Monk et al. 2008), some showing diminished responses (Blair et al. 2008), some reporting no differences (Whalen et al. 2008), and others demonstrating comparable responses to aversive cues but indiscriminate and exaggerated responses in GAD to anticipatory cues (Nitschke et al. 2009). Etkin and colleagues (2009) have suggested that the connections between subregions of the amygdala and areas of the frontal and parietal cortices show selective aberrant patterns of activity in GAD, implying a broad network of pathophysiology in disorders such as GAD marked by chronic and less cue-driven symptoms of anxiety. The present findings offer the suggestion that the heterogeneity of anxiety symptoms seen across disorders may predict selective dysregulation of (or interactions between) brief and persistent affect circuitries identified in the present study. More generally, this work demonstrates that evaluating affective processes across broader timescales offers new insights into how emotional brain systems can support the wide variety of emotional behaviors humans exhibit, in healthy and pathological states.

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Table 1. Transient neural responses modulated by emotion.

Label	BA	X	Y	Z	t
Negative > Neutral Pictures					
Visual Cortex	18	-12	-96	-2	7.50
Amygdala		-21	-7	-17	6.39
Amygdala		24	-1	-20	6.23
Extended Amygdala/Insula*		-42	-1	-15	3.42
Extended Amygdala/Insula*		36	2	-20	5.57
Inferior Frontal Gyrus	47	-45	32	7	5.64
Midbrain		6	-29	-6	5.41
Midbrain/Periaqueductal Gray*		-6	-29	-13	4.21
Inferior Frontal Gyrus	9	50	13	24	5.05
Inferior Frontal Gyrus	46/10	53	38	-1	4.80
Middle Frontal Gyrus	6	-48	4	25	4.58
Neutral > Negative Pictures					
Inferior Parietal Lobule	40	56	-53	39	6.36
Medial Prefrontal Cortex	6	-6	15	60	6.32
Medial Prefrontal Cortex	10/11	18	43	-12	6.03
Superior Frontal Gyrus	9	-15	45	20	4.48
Superior Frontal Gyrus	9	36	37	29	4.14
Superior Parietal Lobule	7	12	-62	47	3.96
Medial Prefrontal Cortex	8	3	36	29	3.88
Middle Temporal Gyrus	21	-57	-14	3	3.57

Threshold $p<0.05$, whole-brain corrected. Areas with * denote subclusters encompassed within larger functional activation clusters. BA=Brodmann Area.

Table 2. Sustained emotional responses modulated by task-evoked anxiety and replication with direct contrasts.

Label	BA	X	Y	Z	t
Increasing Responses with Increasing Task-Evoked Anxiety					
Anterior Insula/Inferior Frontal Gyrus	47m	-36	20	-14	4.38
Anterior Medial Insula*		-30	2	-10	4.37
Ventral Basal Forebrain/BNST*		6	-3	-2	3.62
Inferior Frontal Gyrus	47m	33	29	-12	4.00
Visual Association Cortex	37	27	-55	3	4.33
Visual Cortex	18	-15	-67	9	3.89
Superior Occipital Gyrus	19	-45	-60	14	4.73
Superior Frontal Gyrus	6	-9	-11	61	3.89
Superior Temporal Gyrus	20	42	-27	-9	3.85
Increasing Responses with Decreasing Task-Evoked Anxiety					
Ventral ACC/vmPFC	32	-6	37	-17	3.51
Ventral ACC/vmPFC*	32	3	29	-12	3.38
Replication: Whole-Brain Contrasts					
Sustained Negative vs Sustained Neutral					
Anterior Insula	11	-30	11	-3	4.09
Inferior Frontal Gyrus	11	48	17	-16	3.83
Ventral Basal Forebrain/BNST		6	-3	-2	4.05
Sustained Unpredictable vs Sustained Predictable					
Anterior Insula/Inferior Frontal Gyrus	47	30	26	-13	4.33
Ventral Basal Forebrain/BNST		12	0	3	3.66

Threshold $p<0.05$, whole-brain corrected. Areas with * denote subclusters encompassed within larger functional activation clusters. BA=Brodmann Area. BNST=Bed Nucleus of the Stria Terminalis. ACC=Anterior Cingulate Cortex. vmPFC=Ventromedial Prefrontal Cortex.

Figure Captions

Figure 1. Experimental design and analysis schematic. (A) Within a task block, negative or neutral pictures are presented (3 sec duration) embedded within variable-duration predictable or unpredictable timings. (B) Schematic images depicting canonical transient event responses (top) that are estimated separately from sustained responses that remain persistently active throughout the blocks (bottom). This figure is for illustration purposes only and is not intended to accurately represent the actual experimental parameters.

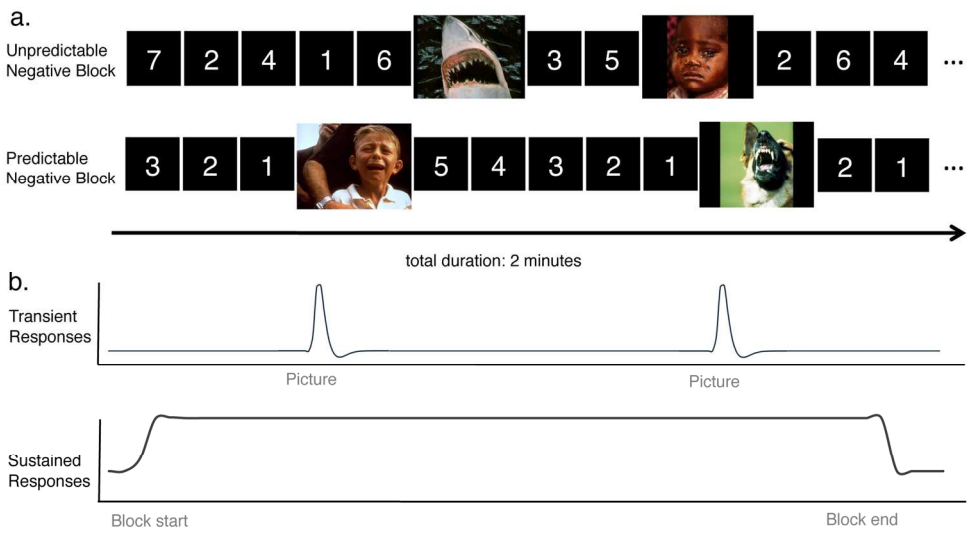
Figure 2. Task-evoked anxiety. Blocks containing only negative pictures evoked greater anxiety than blocks containing neutral pictures, and blocks with unpredictable timings evoked greater anxiety than blocks containing predictable timings. The effect of unpredictability was exaggerated when presented with negative pictures. Error bars denote standard error of the mean.

Figure 3. Mean transient responses to Negative versus Neutral pictures. (A) The left and right amygdala responded more strongly to negative than neutral pictures. Image threshold $p < 0.05$, whole brain corrected. (B) Timecourse of right amygdala (x=24, y=-1, z=-20) response to pictures as a function of valence and predictability. Timecourse values were derived from FIR parameter estimates; error bars denote standard error of the mean. (C) Greater trait anxiety predicts exaggerated right amygdala response to negative pictures when embedded within unpredictable timings. Gray curves denote 95% confidence interval.

Figure 4. Sustained responses that increase as a function of greater task-evoked anxiety. (A) The right VBF/BNST, left (B) and right insula increased in sustained activity with greater task-evoked anxiety. Image threshold $p < 0.05$, whole-brain corrected. (C) Mean signal estimates in

right insula (x=33, y=29, z=-12) plotted for the four sustained conditions. Error bars denote standard error of the mean.

Figure 5. Greater responding in less anxious states in the right ventral anterior cingulate/ventromedial prefrontal cortex (A). Image threshold $p < 0.05$, whole-brain corrected. (B) Signal estimates in (x=3, y=29, z=-12) plotted for the four sustained conditions. Error bars denote standard error of the mean. (C) Greater recruitment of vACC/vmPFC to unpredictable states predicts lesser transient amygdala response to unpredictable negative events. Gray curves denote 95% confidence interval.



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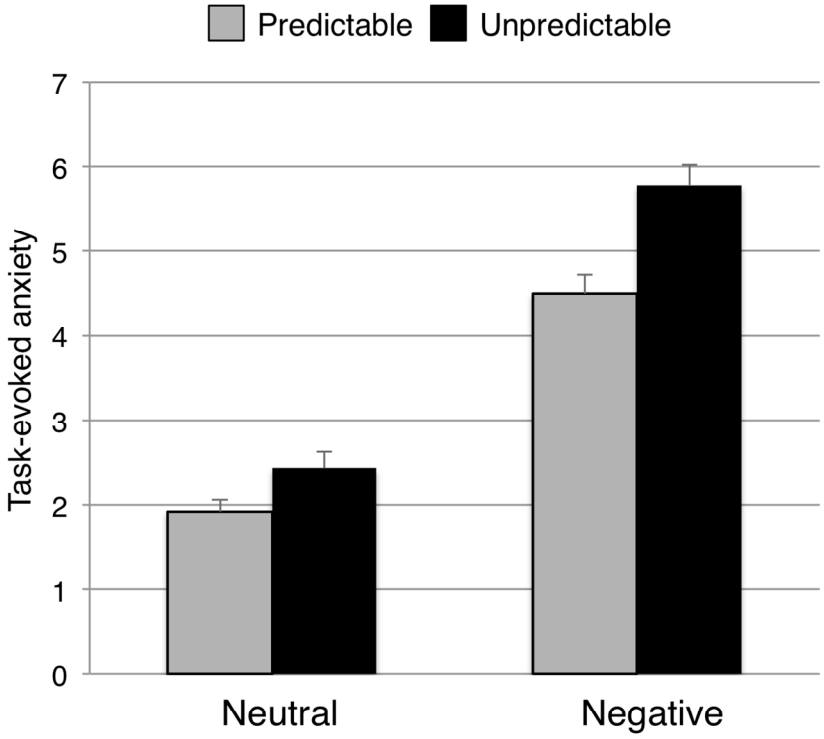

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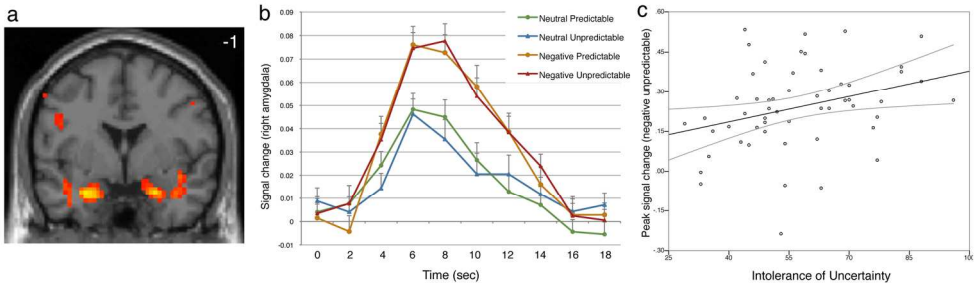

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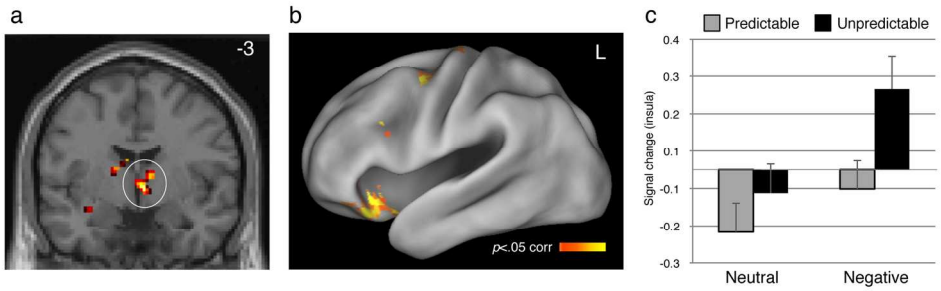

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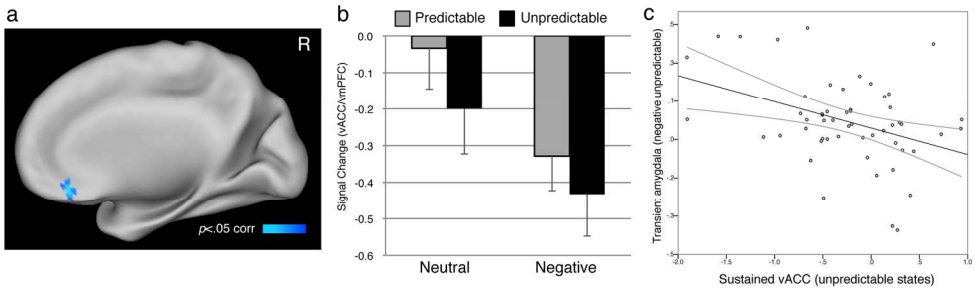

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180x55mm (300 x 300 DPI)

Supplementary online material for

Interactions between transient and sustained neural signals support the generation and regulation of anxious emotion

by

Somerville, Wagner, Wig, Moran, Whalen, & Kelley

Figure Legends

Supplementary Figure 1. A region of the midbrain consistent with the periaqueductal gray (A) demonstrated significantly greater transient responses to negative relative to neutral pictures. Image threshold $p < 0.05$, whole-brain corrected. (B) Timecourse of midbrain/PAG (x=6, y=-30, z=-13) response to pictures as a function of valence and predictability. Timecourse values were derived from FIR parameter estimates; error bars denote standard error of the mean.

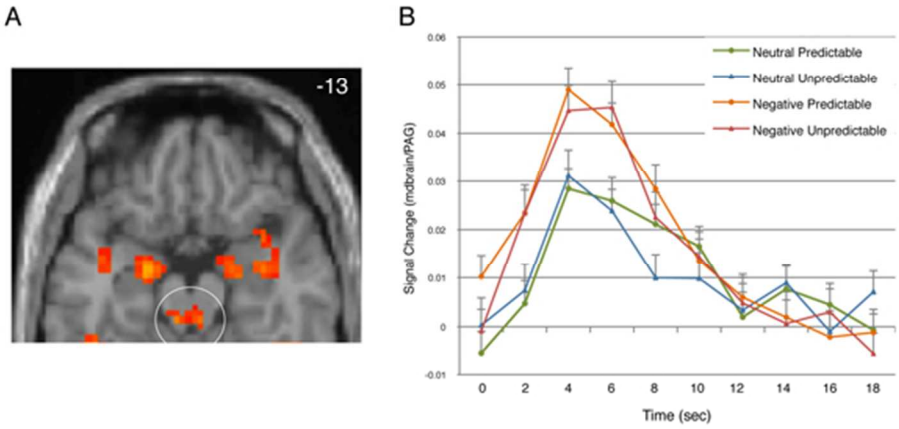
Supplementary Figure 2. Predictability by Intolerance of Uncertainty interaction in right insula activity. Individuals with high IU show a selective exaggeration of sustained insular recruitment during unpredictable contexts. IU groups defined by median split for presentation purposes.

Supplementary Figure 3. Greater Intolerance of Uncertainty (IU) predicts sustained underrecruitment of the vACC/vmPFC during states of unpredictability. *Note*: Outliers are not excluded from analyses, though exclusion of the two participants with lowest vACC/vmPFC activity results in a more robust correlation (p=0.004). Gray curves denote 95% confidence interval.

Supplementary Table 1. Stimulus characteristics.

	Valence	Arousal	Proportion depicting human	Proportion depicting human with visible face
Set 1: Negative valence	M=2.14 SD=0.38	M=6.17 SD=0.76	0.87	0.70
Set 2: Negative Valence	M=2.10 SD=0.40	M=6.09 SD=0.77	0.90	0.67
Set 3: Neutral valence	M=5.41 SD=0.7	M=3.41 SD=1.14	0.90	0.67
Set 4: Neutral valence	M=5.46 SD=0.68	M=3.38 SD=1.08	0.93	0.70

Negative and neutral stimuli were selected to differ maximally on valence ($t(118)=32.58$, $p<0.001$ and arousal ($t(118)=15.86$, $p<0.001$; sets 1&2 versus 3&4) while remaining balanced within each valence category (set 1 versus 2; set 3 versus 4). Sets 1 and 3 were embedded within predictable timings, and sets 2 and 4 were embedded within unpredictable timings during the experiment. Valence and arousal values were taken from IAPS normative ratings and supplementary norming data described in the main text. Valence ratings ranged from 1 to 9 (1=very negative, 9=very positive); arousal ratings ranged from 1 to 9 (1=low arousal, 9=high arousal). To control for low-level features that might engage the circuitries of interest, all four sets were matched on the proportion of images depicting humans, and humans with visible faces.



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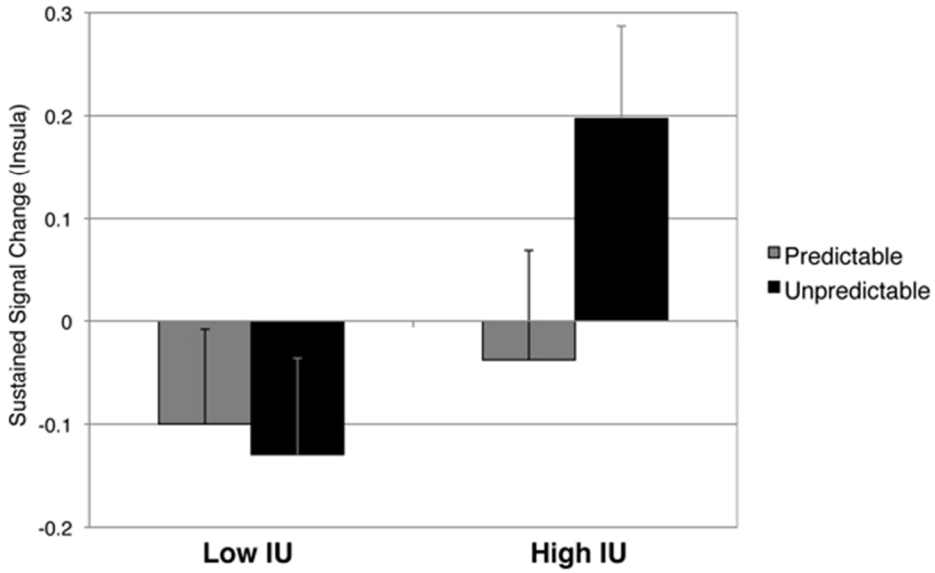

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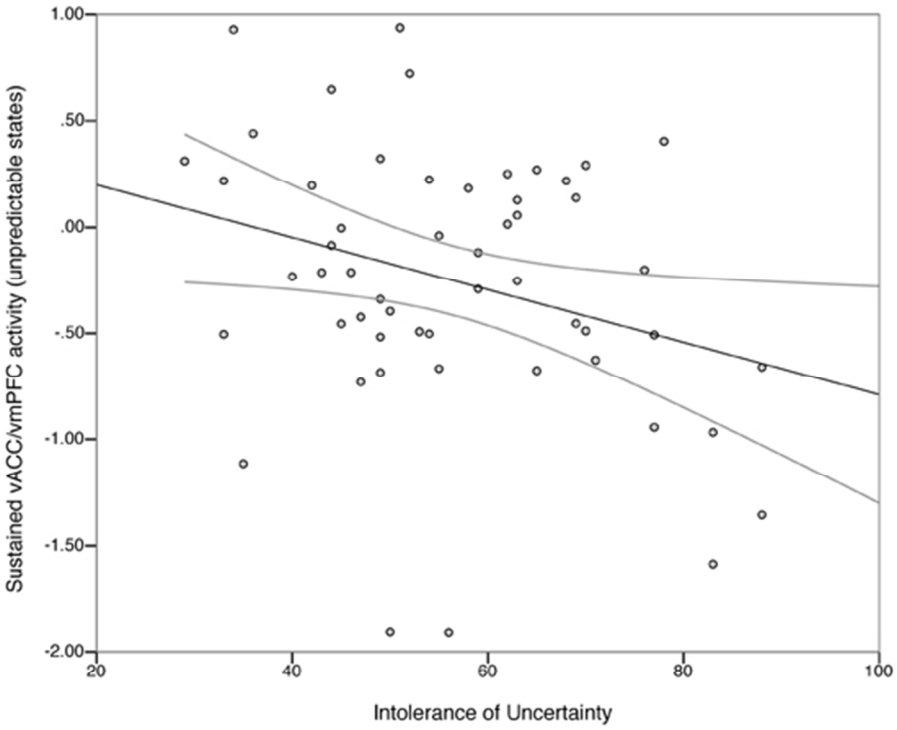

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216x169mm (72 x 72 DPI)