SUPPLEMENT 1

SUPPLEMENTARY METHODS

Stimulus line generation

Values representing line height were generated by experimenters and adhered to several constraints. First, each line needed to exceed the value of nine for a total of 30 seconds, which represented the accumulation of five shocks over the trajectory of the line. Second, each line started and ended in the lowest third of the graph. Third, the line was artificially split into three levels (low: values 3-5, medium: values 5-7, high: values 7-9) and 'shock' periods were added in during which the line exceeded the value of nine. Though less overall time was spent in 'shock' periods than the other levels, the duration of each instance of line height was roughly balanced across levels (low mean duration 15.67 seconds +/- standard deviation 5.01, medium: 9.44 seconds +/- 5.29, high: 10.37 +/- 5.35, shock: 10.56 +/- 5.44) and did not differ significantly across conditions (p = 0.1). Finally, the line trajectory was created such that all fluctuations in the line occurred in a physiologically plausible manner without straight lines or sharp angles. Two lines were generated, and were inverted to create backwards-going versions of the same line, with specific line stimuli counterbalanced across participants (for example of one entire stimulus line, see Figure S1).

To create a believable viewing context, numerical line values were imported into ADInstruments Chart 5.0 (Colorado City, CO) physiological recording and analysis software, which displayed the values as though they were actual data. Data within the Chart window were saved as a movie file where the line advanced at a rate of 1 frame per second from the right hand side of the screen. Output movie files were imported into iMovie software where the line stimulus movies were centered on a black background. Added to the screen was a blue horizontal line, which remained stationary, representing the shock threshold. To the left of the stimulus line, the words "Subject" or "Other" were displayed to remind the

participant whether the line represented ostensibly their own or someone else's physiological state. To the right of the stimulus line, a tally was displayed to represent the number of shocks that had been accumulated during the task, which updated each time the value of nine was exceeded with additional shocks accumulated based on the duration of time spent above the line (see Figure 1). Regressors were coded based on the height of the line at the newest (rightmost) point. Each line accumulated a total of five shocks. Movie files were exported and embedded within a Superlab 4.0.2 stimulus presentation script.

Procedures to facilitate believability

Additional procedures were carried out to facilitate believability in shocks and the physiological recording. When participants entered the fMRI control room, they initially underwent a shock workup procedure, whereby they received actual electrical shocks and titrated the intensity of shocks to what would supposedly be delivered after the scan was complete. This was performed using ADInstruments psychophysiological recording equipment, including a PowerLab 8/30 physiological recording unit, Bio Amplifier, stimulus isolator, shock bar, and a Dell Inspiron laptop running ADInstruments Chart 5.0 software. A very mild train of shocks was delivered to the inner wrist (10 shock train, 5 ms intershock interval, 400 mA intensity), followed by shocks increasing in intensity by 100 mA increments until the participant reported achieving a level that was 'uncomfortable but not painful' (mean 930 mA).

Following the shock titration, participants were set up in the fMRI scanner. During set-up, a finger clip pulse oximeter was placed on their left index finger and was described as a device to 'measure information about your internal state, representing how calm or nervous you are, for use during the experiment'. The experimenters also attached a faux shock bar that closely resembled the real shock bar in look and feel to the participant's left wrist which they believed would be used to deliver earned shocks after the task was complete.

Psychophysiological methods: Skin conductance sample

Equipment. Skin conductance data were acquired using ADInstruments psychophysiological recording equipment, including a PowerLab 8/30 physiological recording unit, Galvanic Skin Response (GSR) Bio

Amplifier, metal paddle finger electrodes, and a Dell Inspiron laptop running Chart 5 software. Skin conductance data were sampled continuously at 100 Hertz in standard units of microSiemens (μS).

Set-up. Immediately prior to skin conductance recording, participants washed their hands with warm water but no soap. Participants were then seated comfortably in a chair at a desk in front of the stimulus presentation computer in a dimly lit room. The two metal paddle skin conductance electrodes were placed on the inner distal phalanges of the middle and index fingers of the nondominant hand, attached with a Velcro strap. Finally, the stimulation shock bar was attached to the right inner wrist, which was only used during calibration (identical to what was described in fMRI methods) but remained on the wrist throughout the experiment. Participants were instructed to find a comfortable place to rest their hand during recording and were reminded to keep their hand very still and to avoid holding their breath, sniffling or coughing. To reach a stable baseline prior to experiment onset, participants rested for 2 minutes between setup and task onset.

Task delivery was identical to the fMRI session with the exception of the final seconds when shock delivery would supposedly occur. After the last video stimulus was complete, a thirty second fixation rest period was presented, followed by a 10 second instruction screen stating, “Next, we are going to deliver the number of shocks you earned during this task.” Then, over the next ten seconds, the program presented successive screens made to appear that the computer was tallying the number of shocks earned and initializing the connection between the shock device and computer. Then, a yellow square appeared on the screen with the words “Shocks delivering …” for ten seconds in duration, which constituted the measurable ‘shock delivery’ period. At the conclusion of this period, participants were told that the task was over and the experimenter expressed surprise when the participants told them that they did not feel any shocks. The experimenter then said there must have been a wire that wasn’t connected properly, stated that there was no point to try again and the experimenter led them to the adjacent testing room to complete the post-test.

Skin conductance analysis. Nonspecific skin conductance responses (NS-SCRs) are phasic skin conductance response waveforms that represent nonspecific arousal and increase in frequency during

contexts evoking anxiety or autonomic arousal (1), and in people more psychologically sensitive to stressors (2). Due to the continuous nature of the stimulus in this experiment, SCRs cannot be temporally linked to a short-duration phasic event and thus are termed 'nonspecific'. The rate of NS-SCR responding per minute was calculated for each level of line height and was the primary measure of interest in the present analysis.

Skin conductance data were exported from the Chart recording program at 20 Hz sampling rate and were visually inspected for movement artifacts. NS-SCRs were detected in accordance with set guidelines (1) as increasing response waveforms of at least 0.05 microsiemens in amplitude (measured as the difference between the peak height and mean of 'baseline period' of one second before waveform onset) and were required to reach their peak within 4 seconds of waveform onset. NS-SCRs were mapped to the timecourse of the line stimulus by timeshifting the line stimulus by 1 second to account for the delay in response onset characteristic of skin conductance data (1, 3) and subsequently categorized by the condition that was viewable when the NS-SCR was evoked. NS-SCR counts were then standardized by converting response counts into response rate per minute. Rates of NS-SCR responding were evaluated statistically using a 2 x 4 ANOVA, with line type (SELF, OTHER) and line height (low, medium, high, shock) as within subjects factors and anxiety scores as a continuous between-subjects covariate.

Heart rate (fMRI participants). Following fMRI scans, the experimenter explained that the shocks would be delivered in a few seconds. During the next ten seconds, the participant's heart rate was logged through a Philips pulse oximeter clip attached to the index finger of the nondominant hand. The experimenter then asked them to rate, on a 1 to 9 scale, how painful the shocks were. Participants typically (correctly) stated that they had not felt any shocks. The experimenter then told the participant they would check the equipment and try again. Heart rate was logged again, after which the experimenter stated they were experiencing technical problems and removed the participant from the scanner. This was performed as an objective manipulation check in the fMRI cohort to verify physiological upregulation to the possibility of being shocked.

Heart rate data was not available for five participants due to measurement error. Heart rate during the two sampling windows was converted to beats per minute and averaged to generate a mean heart rate measurement during the possibility of being shocked. Two ten second control measures were also calculated- one from the first fixation rest period of the fMRI run (e.g., a resting control condition), and a second while the experimenter was reading instructions to the participant (e.g., an 'active' control condition while interacting with the experimenter). The heart rate measurement taken during the supposed shocks was compared to both control conditions using paired-samples *t*-tests. Anxiety effects were evaluated by inputting anxiety scores as a continuous between-subjects covariate.

Experimental post-test. Data from the following post-test items were reported in the main manuscript:

1) When your own internal state was controlling the line, how much did the physiological response line reflect how you felt internally? *[1: not at all, 9: perfectly]*
2) When your own internal state was controlling the line, how successful were you at altering your physiological response in the intended way? *[1: very unsuccessful, 9: very successful]*
3) When your own internal state was controlling the line, how much effort were you putting into trying to change your physiological response? *[1: none, 9: maximally]*
4) When your own internal state was controlling the line, how concerned or nervous were you about receiving the electric shocks? *[1: not at all, 9: extremely]*
5) When someone else's internal state was controlling the line, how much did the physiological response line reflect how you felt internally? *[1: not at all, 9: perfectly]*
6) When someone else's internal state was controlling the line, how successful were you at altering your physiological response in the intended way? *[1: very unsuccessful, 9: very successful]*
7) When someone else's internal state was controlling the line, how much effort were you putting into trying to change your physiological response? *[1: none, 9: maximally]*
8) When someone else's internal state was controlling the line, how concerned or nervous were you about receiving the electric shocks? *[1: not at all, 9: extremely]*

SUPPLEMENTARY RESULTS

Supplementary psychophysiological results

Heart rate (fMRI participants). Although the GSR cohort supplied evidence that changes in line height modulated physiological arousal, its applicability to the fMRI cohort is indirect. To assess the salience of the task within the fMRI cohort directly, we collected heart rate measurements at the conclusion of the experiment, when participants believed they would receive the shocks they had accumulated during the fMRI task. When expecting to be shocked, participants generated a significant increase in heart rate response relative to both a passive control condition (i.e., rest) ($t(44) = 5.39$, $p < 0.001$) and an active control condition (i.e., during task instruction) ($t(44) = 2.08$, $p < 0.05$). In addition, high anxious participants generated a marginally larger heart rate response to supposed shocks than low anxious participants ($t(43) = 1.71$, $p = 0.047$, one-tailed), indicating greater task-elicited physiological reactivity in these individuals.

Supplementary fMRI results

Analysis of time effects. We performed exploratory analyses to test whether line height effects in the ventral basal forebrain (VBF)/bed nucleus of the stria terminalis (BNST) were more pronounced in the first versus second fMRI scan run. Line height effects were tested as a function of time in the VBF/BNST ROI and in skin conductance data (NS-SCRs) using 2 (time: early, late) x 4 (line height: low, medium, high shock) repeated ANOVAs. It should be noted that each fMRI run also represented SELF or OTHER conditions, but this difference is controlled for as order was counterbalanced across participants. We observed a significant time by line height interaction in the VBF/BNST ($F(3,144) = 2.97$, $p = 0.03$), such that the second scan generated more polarized line height responding than the first (see Figure S5A). GSR data converged in the same direction, with the second scan generating greater skin conductance changes as a function of line height (time by line height interaction: $F(3,141) = 2.80$, $p = 0.042$; see Figure S5B).

Parametric line height analysis. An additional general linear model was performed for each participant, and subsequently submitted to group analyses, in which the numerical values representing the stimulus

line height were entered as a single parametric regressor. The purpose of this analysis was to determine whether fluctuations in magnitude of signal in the observed brain regions showed concordance with the fluctuations in the height of the line stimulus. When treating the stimulus line as a continuous modulator of neural activity, the same regions were identified, though at a more liberal threshold ($p < 0.001$, uncorrected, 5 voxel minimum cluster size).

Testing for functional dissociation between VBF/BNST and amygdala. Neither the left or right amygdala demonstrated line height or anxiety-modulated activity. A dissociation in response pattern between the left VBF/BNST and the left and right amygdala were evaluated formally with a series of 2 (region) x 4 (proximity to threat) ANOVAs with anxiety input as a continuous covariate. Results supported a functional dissociation between the amygdala and the VBF/BNST (right: region x anxiety x proximity interaction $F(3,144) = 2.77$, $p < 0.05$; left: region x anxiety interaction $F(1,48) = 3.48$, $p = 0.068$).

SUPPLEMENTARY DISCUSSION

Time effects. Though not the focus of the current study, the present design was capable of testing whether the VBF/BNST response modulation based on line height was further modulated by time. This question is particularly relevant when considering a current debate regarding the particular role of the VBF/BNST in representing tonic threat. On one hand, it has been proposed that BNST activity is more pronounced early during threat processing, while the potential threat is initially recognized as temporally distant (4). Others have proposed that BNST activity should become greater as threats become more and more diffuse, or as time passes (5). As participants believed they would receive the electric shocks after the two scans, greater responding in the early scan would support the notion that the BNST is selectively engaged when the threat is perceived as distant. If greater responding is seen in the later scan, it would support the notion that BNST responding becomes over engaged over time in contexts of tonic, but not imminent, threat.

The current data were evaluated as a function of time in order to inform these competing hypotheses, as line height responses in the first scan (e.g., early time) and second scan (e.g., late time)

could be compared. Skin conductance findings suggested that the later stages of the experiment were more arousing than early stages. Converging neuroimaging findings support the notion that BNST responses become more responsive to environmental threat cues at temporally later timepoints, perhaps because the threat cues had become more tonic in nature. It is also possible that BNST responses were more pronounced at later stages because participants were aware that the actual threat (electric shocks) was becoming temporally nearer or that the cumulative number of accumulated shocks continued to increase as time passed. These interpretations are not mutually exclusive and cannot be dissociated with the available data. However, these findings support the general conception that BNST responses to environmental threat become stronger over time, rather than decay.

Some limitations in interpreting these effects should be acknowledged. First, evaluating differences between early and late scan runs is a crude representation of time effects, as it is difficult to examine time-dependent responses within runs due to covarying noise properties of the fMRI signal. In addition, one should consider the context in which shocks would supposedly be delivered. Participants were aware that shocks would be delivered after the two scans were complete but participants were told that they would be warned prior to shock delivery. Therefore, the temporal properties of the threat were somewhat fuzzy from the participant's point of view. This relative ambiguity runs counter to the highly specified contexts in which tonic and phasic threats are trained and delivered in experimental setups involving nonhuman samples.

Line controllability effects. In addition to the line height manipulation, this experiment contained a manipulation of the source of the line. Psychologically, these conditions can be described as differing in locus of control, with the SELF line believed to represent the subject's own physiological state and the OTHER line thought to represent someone else's physiological state recorded previously, which the participant had no control over. Line height and anxiety effects were collapsed across these two conditions, because all regions of interest demonstrated equivalent activity to both lines. When directly comparing neural responses to the two lines, a region of the right insular cortex showed greater activation during the SELF condition relative to the OTHER condition. The locus of this activation was more

posterior but partially overlapped with the right insula activation observed to track threat proximity and anxiety. Though speculative, this finding offers intriguing convergence with brain regions implicated in controlling actual autonomic states (6-8). The right anterior insular cortex is anatomically the final convergence point in the cortex for the afferent signals relating to one's body state (6). In support of this idea, activity in the right insula is observed when volitionally controlling one's own skin conductance (9). The present data are in line with these results, though it is less clear how the false feedback paradigm used in the present experiment might engage autonomic control centers in the same way as in cases involving real or no feedback. However, Gray and colleagues recently observed that activity in the right insular cortex increased as a function of the mismatch between real and perceived autonomic states in light of false feedback, implicating the insula as a comparator serving to detect mismatches between an individual's physiological state and the present environmental context (10). It is possible then that our observation of consistently enhanced insular activity while viewing the SELF line reflects a continuous mismatch signal, representing the discrepancy between the false physiological state feedback and the participant's actual internal state. Alternatively or concomitantly, the insula may serve to code arousal associated with mental effort while attempting to modulate the line height, a possibility that has also received some empirical support (11).

Table S1. Rotated component loading structure of self-report scales from Principal Components Analysis.

	Component 1 "Anxiety"	Component 2 "Extraversion"
NEO – Neuroticism	0.86	-0.21
STAI – Trait	0.82	-0.33
Penn State Worry Questionnaire	0.74	-0.11
STAI – State	0.68	-0.14
Behavioral Inhibition	0.68	-0.30
Beck Depression Inventory	0.65	0
Anxiety Symptom Inventory	0.61	0.06
Intolerance of Uncertainty	0.58	-0.23
Behavioral Activation	-0.02	0.89
NEO – Extraversion	-0.18	0.82

STAI, State-Trait Anxiety Inventory

Table S2. Results of whole-brain conjunction analysis. Identified regions demonstrate an exaggerated linear response based on line height in anxious individuals ($p < 0.05$, corrected), within an inclusive mask of regions demonstrating linear increasing response based on line height (mask $p < 0.05$, FDR corrected).

Region	**BA**	**x**	**y**	**z**	**t**
Left ventral basal forebrain (BNST)		-9	0	-3	4.33
Left ventral basal forebrain (BNST)		-9	-2	8	3.42
Right insula		56	12	-1	4.02
Right insula		42	14	-16	3.30
Left lateral prefrontal cortex	44	-56	13	30	4.24
Left lateral prefrontal cortex	6	-27	-7	45	4.09
Left lateral prefrontal cortex	6	-48	2	33	4.05
Right lateral prefrontal cortex	6	30	-1	47	4.52
Right lateral prefrontal cortex	10	48	46	-5	3.62
Right lateral prefrontal cortex	9	30	45	28	3.26
Dorsal anterior cingulate cortex	23	6	-19	29	5.43
Medial frontal gyrus	6	6	34	34	3.78
Left superior parietal cortex	7	-15	-56	55	3.96
Right superior parietal cortex	7	18	-59	36	3.04
Left inferior parietal cortex	40	-48	-33	40	3.03
Right inferior parietal cortex	40	45	-42	35	4.39
Left inferior temporal gyrus	37	-48	-56	-15	3.90
Left superior temporal gyrus	22	-62	-43	5	2.96
Left cerebellum		-30	-60	-25	3.77
Thalamus		-15	-14	6	3.25
Thalamus		12	-17	4	3.45

BA, Brodmann area

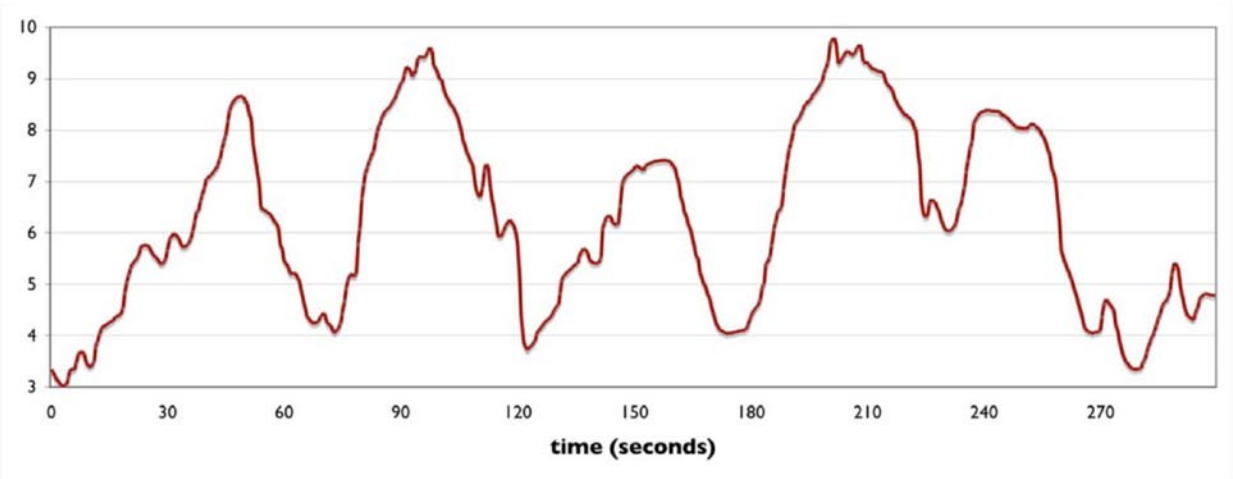

Figure S1. Timecourse of an entire stimulus line.

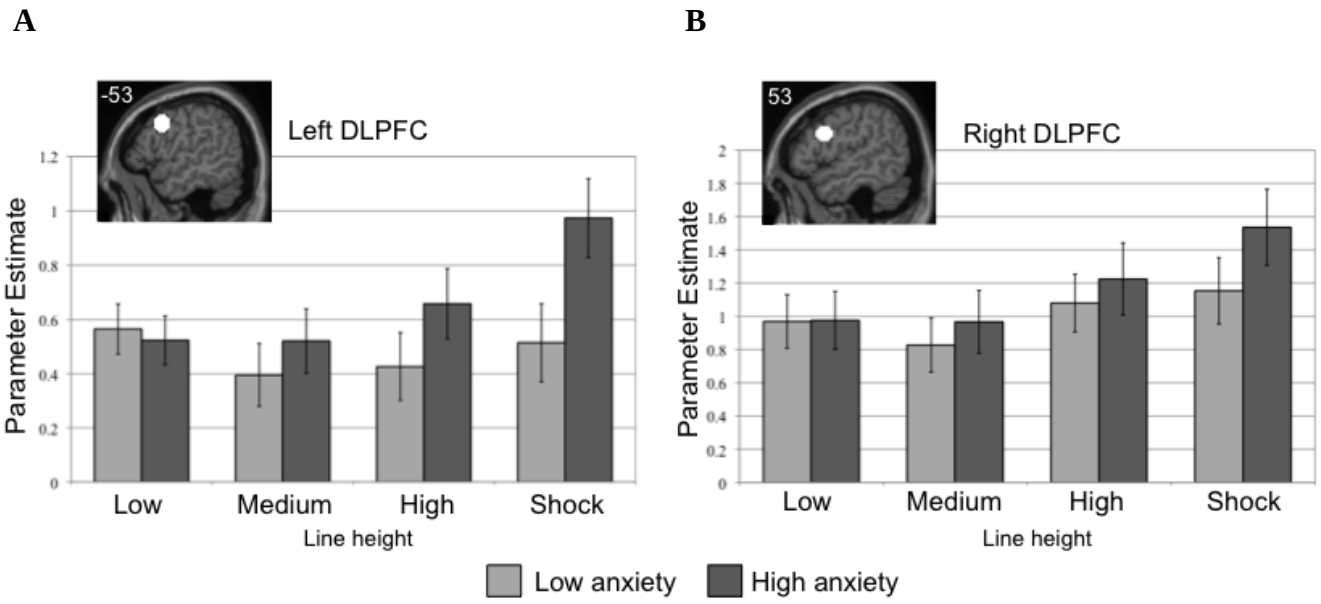

Figure S2. The left (**A**) and right (**B**) dorsolateral prefrontal cortex (DLPFC) demonstrate a significant line height by anxiety interaction. Line height represents a continuous stimulus that fluctuated in height, with greater height representing subsequent risk for receiving electric shocks. 'Shock' is the maximum height, indicating a shock has been accumulated, to be received later. Spherical regions of interest (white) were defined by an unbiased Task > Rest contrast and are depicted on a representative high-resolution anatomical image. Number in image represents the in-plane slice number in Talairach and Tournoux atlas space. Image presented in left = left coordinate space. Anxiety groups are based on a median split of component scores for ease of presentation, though all statistical tests treat anxiety as a continuous variable. More anxious participants demonstrate a significantly steeper sloped response as line height increases. Error bars represent standard error of the mean.

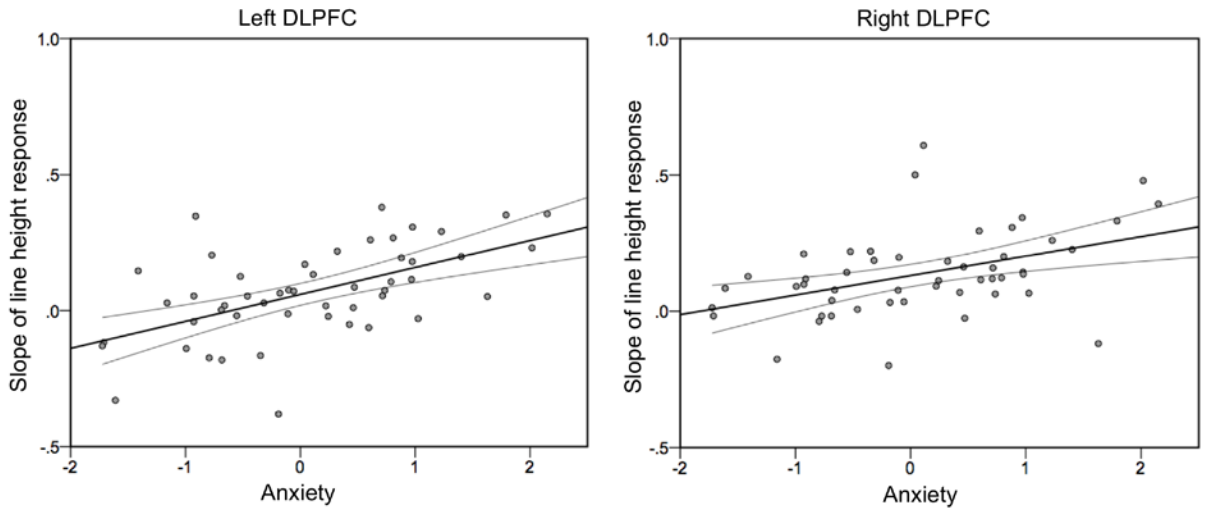

Figure S3. Anxiety predicts line height response in the left and right DLPFC. The x-axis represents anxiety based on component scores and the y-axis represents the slope of the regression line representing increasing fMRI responses with increasing proximity to threat. Black line denotes regression fit line and gray lines represent 95% confidence intervals of the mean.

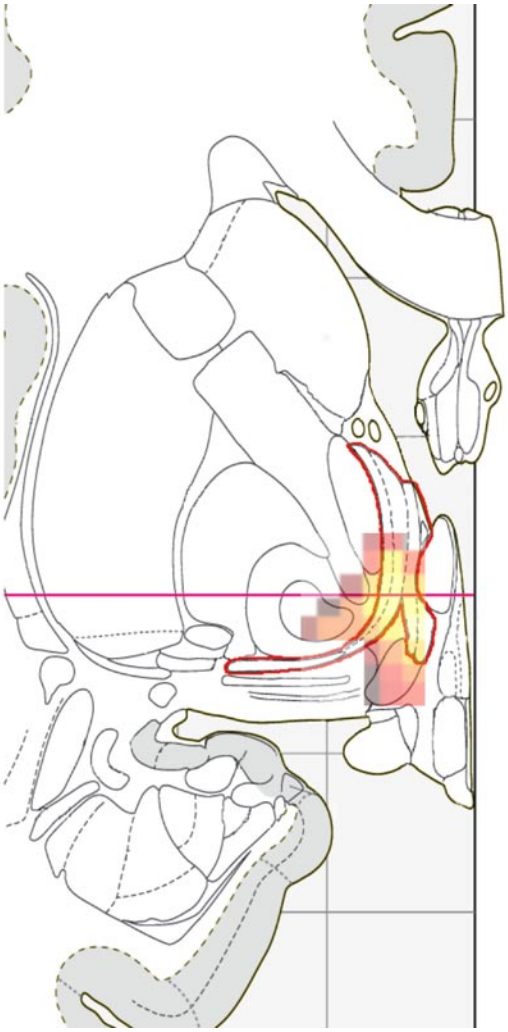

Figure S4. Localization of ventral basal forebrain effects. Ventral basal forebrain activations (depicted in Figure 4) overlaid on a detailed subcortical plate from the Mai atlas (12) to depict anatomical location within small forebrain nuclei. The BNST complex is outlined in red and activations observed in the present study are depicted in semi-transparent shading. Placement of activation shading is approximated based on atlas grid spacing. While the maximum activated peak voxel and its associated cluster map directly to the BNST, to obviate bias, we note that activated voxels also extended into the lateral hypothalamus and medial globus pallidus.

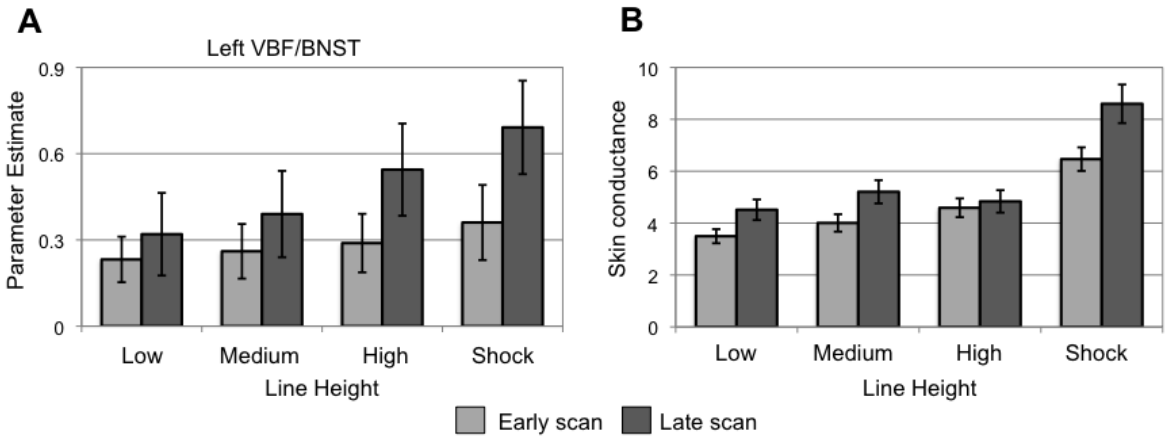

Figure S5. Time effects in VBF/BNST and skin conductance responses. (**A**) The right VBF/BNST (see Figure 2 for region) shows more robust line height responding in the late scan relative to the early scan. (**B**) Skin conductance responses parallel the BNST findings. Line height is represented on the x-axis and rate of nonspecific skin conductance responding, a measure of autonomic arousal in continuous stimulus paradigms, is represented on the y-axis in response rate per minute.

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